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Evaluation of Protein Profiles, Bioactivity, Allergenicity and Toxicity of Peptides Generated After in silico Digestion of Common Wheat and Einkorn Wheat

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ABSTRACT

The belief that ancient wheat is more beneficial than common wheat has been growing trend in recent years among the consumers. The present study aims to compare bioactive peptide, allergen peptide and toxic peptide generation after gastrointestinal digestion of modern wheat (Triticum aestivum) and ancient wheat, einkorn (Triticum monococcum var. monococcum), using in silico tools. The primary sequences of both kinds of wheat were obtained from BIOPEP-UWM and UniProtKB/Swiss-Prot database. In silico digestion was applied using BIOPEP-UWM online tool. For the simulation of gastrointestinal digestion pepsin (pH 1.3) (EC 3.4.23.1), trypsin (EC 3.4.21.4), and chymotrypsin (EC 3.4.21.1) were selected and analyzed. Homology analysis was performed for each protein sequences using EMBOSS Needle program. Toxic and allergen peptides were predicted using ToxinPred online tool and Allergen FP v.1.0. The results showed that einkorn and common wheat proteins exhibited similar properties including high similarity rate (58.72-87.40%) indicating the percentage of matches between the two sequences and the identical bioactivities for peptides generated after digestion. Most of the bioactive peptides were dipeptides and the majority of them displayed more than one bioactivities including ACE inhibitory, DPP IV inhibitory or antioxidant activity, etc. Allergen peptides generated after in silico digestion were found to be similar for both kinds of wheat. In silico gastric digestion of einkorn and wheat caused toxic peptides production, but they were disappeared after in silico intestinal digestion. In conclusion, although there is a perception related to the Einkorn that is healthier than common wheat, in silico digestion of common wheat and einkorn did not support this perception.

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Exploring the Effects of Information and Communication Technologies in the Marketing of Broiler Birds in Enugu State, Nigeria

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ABSTRACT

Information-intensive and precise knowledge-based marketing approaches is a key aspect in ensuring the long-term viability of agriculture. Unfortunately, the economic potential of information and communication technologies uses in agricultural marketing is not fully utilized. This study, therefore, unravelled the effects of information and communication technologies in the marketing of broiler birds in Enugu state, Nigeria. Primary data collected from 90 marketers were analysed using descriptive and inferential statistics. The result showed that all the marketers accessed and used mobile phones very often to communicate their customers but only a few (40%) used social media platforms while radio and television were rarely used. The gross margin analysis showed that broiler marketing was a viable enterprise with ₦80,972.72 (USD 197.29) gross margin monthly. The degree of use of information and communication technologies, level of education and marketing experience significantly affected the revenue margin of the respondents. High cost of information and communication technology facilities, inconsistence power supply, poor network coverage and connectivity and high cost of airtime and data were among the major constraints faced by the marketers. The study recommends that the problem of inconsistent power supply and poor network coverage should be rectified by the government and the network providers, respectively. The national communication commission should as a matter of urgency regulate, moderate and reduce the high call tariffs and internet data cost to enhance the profit margin of the broiler marketers.

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Introduction

The evolution in the marketing process has continued to unfold; from the traditional product concept through the product and selling concepts to the current marketing concept; in which marketers meet the needs of the target markets at a profit. Increasing communication facilitated by information and technologies (ICTs) is one of the tools of marketing concepts. ICTs now play major roles in the day-to-day management of many businesses and enterprises. The twenty-first-century marketing managers have embraced the continuous innovation and evolutions in the ICTs sectors and have continued to maximize the tools. According to Singh et al. (2015), ICTs include traditional ICTs such as radio, television, and telephone, as well as newer ICTs such as computers, satellite, mobile handsets, and the Internet. These heterogeneous tools can now interact and combine to develop our network world, a vast network of interconnected telephone services, standardized computing gear, the internet, radio, and television that covers every corner of the planet.

Agriculture was driven by bid in previous decades, but today it is driven by demand; nevertheless, all indications are that agriculture will be driven by information in the future. To take advantage of prospective opportunities, new information must reach end-users as quickly and easily as possible. Information-intensive and precise knowledge-based marketing approaches will be key aspects in ensuring the long-term viability of agriculture (Phougat, 2006). Unfortunately, the economic potential of ICTs uses in agriculture and marketing is not fully utilized in terms of precision marketing; however, proper adoption and use of ICTs could help managers of agriculture-related businesses, as well as policymakers, make more efficient

decisions. E-commerce, e-banking, and e-learning are all well-known, but "e-agriculture" is a newer concept, it is also called Digital Agriculture or ICT for Agriculture (ICT4Ag) which is the application of ICTs to solve problems in agriculture. E-agriculture is luring young people to agriculture, and some have recognized prospects for income generation through businesses that provide farmers and other agricultural actors with ICT-enabled services. The introduction of ICT into the agricultural sector is transforming the face of African agriculture and is currently one of the factors driving young people to the enterprise (Okediji, 2016).

According to Chapman and Slaymakers (2002), the internet is rapidly becoming a more affordable and faster means of accessing agricultural information. The emergence of technology, which enabled the automation of many agricultural organizations that provide various services and products; and the introduction of e-mail and the internet, which enabled access to information, are two major trends and developments that are shaping current agriculture. Many agree that knowledge has always been at the heart of development, and vast amounts of knowledge and information have been formed over the years to improve food security and promote long-term development (Food and Agricultural Organization (FAO), (2015). Increased priority for ICTs and their resources for information exchange has the potential to improve rural poor access to and benefit from development activities while also creating a more informed policy environment (FAO, 2003). ICT in agriculture facilitates knowledge and information sharing within and among agricultural channels that include researchers, importers/exporters, extension services, and farmers. The business venture must be able to leverage on ICTs to position its marketing strategies, a step ahead of competitors.

Agricultural marketing encompasses the services required to move farm produce from the farm to the consumer including all activities involved in the value chain (Mukaila et al., 2021). Planning and forecasting, planting and harvesting, grading, packing and branding, transportation, storage, food processing, distribution, advertising, and sale are just a few of the interconnected operations involved. Marketing activities, especially in agriculture cannot take place without the exchange of information, and cannot be carried out effectively without proper communication between the parties involved. Nigeria produces a wide range of agricultural products, and marketing all of these farm products is typically a complicated procedure. Marketing in agriculture entails a variety of operations and processes that move food and raw materials from the cultivated farm gate to the final consumer via middlemen. Agricultural marketing, particularly in developing countries, plays a significant role in long-term poverty reduction and household food security (Katengeza, 2012). Most marketers in Nigeria and other developing countries have little or no means of finding out the true prices of their commodities. Many are sometimes in poor bargaining positions and do not even understand the market negotiation (Nigeria Geography Association, 1996). This may be due to their inability to get accurate, timely, current and relevant market information which could be accessed through ICTs.

The use of ICT has resulted in a fundamental shift in agriculture at all levels, with marketing being one of the key benefactors. It is very possible that introducing an ICT based marketing into the selling of broiler birds will bridge the market information gap that exists between the broiler producers and consumers including the middlemen in agricultural marketing and transaction processes, ICT stands to play a major role in their marketing activities. Proper utilization of ICTs especially mobile phones, radio, social media and television technologies can go a long way in helping agricultural producers who are often unaware of current market prices thereby relying on the information gathered from traders in determining when, where and how much to sell their produce, to have relevant and timely information to this regard. Internet as new technology has created great evolution on marketing methods and selling of agricultural products. In most nations throughout the world, farmers are attempting to take advantage of new technologies and tactics to improve the quality and quantity of their goods while also dealing with new scientific issues (Jalali 2012).

Poultry offers the greatest potential for bridging the available protein gap due to the short-generation intervals (Ahaotu et al., 2016). When compared to other livestock, poultry, particularly broilers, have the quickest and largest turnover rates. The poultry sector also has a significant impact on the Nigerian economy. It is a key source of eggs and meat, both of which are high in nutritional value, especially in terms of protein supply. Additionally, the poultry sector provides job opportunities for the wider populace, as well as a source of revenue. Nigeria's poultry sector can benefit from the advancement of ICTs to increase poultry output. However, to fully leverage this advantage, it is necessary to establish the extent to which poultry farmers have access to ICTs and how they use them for marketing and development. For this study, the ICT considered include mobile phones, television, radio and social media platforms like Facebook and WhatsApp used in facilitating sales and accessing market information.

Most of the previous works on ICT in agriculture focused generally on agricultural marketing (Adejo and Haruna, 2009; Mittal and Mehar, 2016; Okediji, 2016, Kante et al.,2017; Alavion et al., 2017; Hoang, 2020). However, little or no research has been carried out specifically on the role of ICT on broiler marketing especially in the Enugu state, this is the knowledge gap the present study intends to fill. The study aims to examine the effects of ICTs and in the marketing of broiler birds in Enugu State, Nigeria. The specific objectives of the study were to: identify the type of ICTs available to broiler marketers, describe the level of access and usage of ICTs among broiler marketers; ascertain the gross margin in broiler marketing, and determine the effect of degree of usage of ICTs and socio-economic factors on the total revenue of broiler marketers. The benefits of the study cannot be overemphasized especially in the current coronavirus pandemic ravaging the world where physical distancing is being advocated to help curb the menace of the disease. ICTs have become great tools for bridging marketing gaps.

Materials and Methods

Study Area

This study was conducted in Enugu State, Nigeria. The state is one of the 36 states in Nigeria. It has seventeen local government areas with Enugu as the capital. Enugu state has an area of 71,161 square kilometres with a population of about 3,257,298 people (National Population Commission, 2006). It lies between Latitudes 5°55'N and 7°08'N of the equator and longitudes 6°55' E and 7°08' E of the Greenwich meridian. The state shares national boundaries with Anambra State to the West, Benue State to the Northeast, Kogi State to the Northwest, Ebonyi State to the East and Abia State and Imo State to the South.

Sampling Procedure and Data Collection

A multi-stage sampling technique was adopted for the study. In the first stage, three LGAs (Enugu North, Enugu East and Enugu South) located in the Enugu metropolis were purposively selected based on the urban nature of the LGAs and the relatively higher literacy rate compared to other LGAs in the state. In stage two, three popular commodity markets were purposively selected from each LGA. The markets include; Ogbete main market, the New market and Old Artisan market in Enugu North LGA; Abakpa market, Orie Emene market and Kenyatta market in Enugu East LGA and Garriki market, New Artisan Market and Mayor market in Enugu South LGA which gave a total of nine markets. Finally, from each of the nine markets selected,10 broiler marketers were randomly selected. Thus, in all, a total of 90 broiler marketers were selected for the study.

Data Analysis

Descriptive statistics such as percentages, frequencies and mean, gross margin model and inferential statistics were employed in analyzing the data collected. Following Isitor & Ugwumba (2014), Baba et al. (2014), Ibitoye et al. (2016), Ukwuaba et al. (2019) and Onyekuru et al. (2020), the gross margin model was used to ascertain the gross margin of broiler marketers; mathematically, it was expressed as:

$$GM = TR - TVC \quad (1)$$

Where;

GM = Gross Margin

TR = Total Revenue

TVC = Total Variable Cost (transportation cost, cost of feed, cost of vaccines, rent and labour costs).

The Multiple regression model was used to ascertain the effect of the degree of usage of ICTs and socio-economic factors on the total revenue of broiler marketers. The model was adapted following Kainga (2013), Ali et al. (2017), Ukwuaba et al. (2018) and Okpukpara et al.(2021) and implicitly specified as:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_6X_6 + b_7X_7 + b_8X_8 + e \quad (2)$$

Where;

$Y =$ Revenue of broiler marketers

$b_0 =$ Constant,

$X_1 =$ Gender (Female 1, Male 0)

$X_2 =$ Age of marketer (years)

$X_3 =$ Marital status (married 1, single 0)

$X_4 =$ Level of education (in years)

$X_5 =$ Household size (Number of persons

$X_6 =$ Marketing experience (in years)

$X_7 =$ Membership to market association (member 1, otherwise 0)

$X_8 =$ degree of usage of ICTs by the broiler marketers (high degree 1, otherwise 0)

$b_{1-8} =$ Coefficient

$e =$ Error term

The four functional forms were tested and the one with the best fit was chosen as the lead equation

A four-point Likert type scale was used in analysing the constraints of using ICTs in the marketing of broiler in the study area. İt was adapted following Mukaila et al.(2021). The scale was rated as follows; very serious (VS) = 4, serious (S) = 3, less serious (LS) = 2, and not serious (NS) = 1. The model was computed thus; 4+3+2+1 = 10/4 = 2.50 (mean score cut off point). Items ≥ 2.50 mean score was rated as serious constraints, otherwise not significant and thus not constraints to the use of ICTs among broiler marketers.

Results and Discussion

Socio-economic Characteristics of the Respondents

The socio-economic characteristics considered in the study include sex, age, marital status, household size, educational level, marketing experience, membership to market association and monthly income of the respondents Table 1). The result showed that the majority (55.6%) of broiler marketers in the Enugu metropolis were male while 44.4% were females. The relatively higher proportion of men was because men can withstand the rigorous nature of broiler marketing than women. This is in contrast with the findings of Chavula (2013) who was of the view that women undertake 60-90% of broiler marketing. The marketers had a mean age of about 42 years which implied that the majority of the marketers were in their relative economic young and active age and therefore, can withstand the rigorous broiler marketing. This finding supports the study of Oyeyinka and Bello (2013) who found that marketers of agricultural products were in their economic active age of 40-49 years. The majority (80%) of the marketers were married while only 20% were single. The result suggests that the enterprise is an important form of livelihood for the sustenance of a family or household. The result is in agreement with Adejo and Haruna (2009) who found that married couple assists themselves in marketing agricultural products thereby raising adequate income for their family wellbeing.

The result of the socioeconomic characteristics also showed that the majority (57.8%) of the respondents spent about 7-12 years obtaining formal education with a mean age of educational attainment of 10 years.

Table 1. Socio-economic characteristics of the respondents

Socio-economic characteristics	Frequency	Percentage	Mean
Sex			
Male	50	55.6	
Female	40	44.4	
Age (years)			
Less than 31	22	24.4	
31 – 40	23	25.6	
41 – 50	25	27.8	41.51
51 – 60	14	15.6	
Above 60	6	6.7	
Marital status			
Single	18	20.0	
Married	72	80.0	
Educational level (Years)			
1 – 6	27	30.0	
7 – 12	52	57.8	10.04
Above 12	11	12.2	
Household size (Number of people)			
1- 2	18	20.2	
3 – 4	37	41.6	3.81
5 – 6	29	32.6	
Above 6	5	5.6	
Marketing Experience (Years)			
1 – 10	58	64.4	
11 – 20	26	28.9	
21 – 30	3	3.3	10.13
Above 30	3	3.3	
Membership to market association			
Yes	77	85.6	
No	13	14.4	
Monthly Income (₦)			
≤ 50,000	9	10.0	
50,000 – 100,000	33	36.7	
100,000 – 150,000	10	11.1	N102, 096.67
150,000 – 200,000	12	13.3	
Above 200,000	26	28.9	

USD 1 = 410.42; Source: Field surveys, 2019

The relatively high mean educational attainment suggests that the respondents were very knowledgeable and can use ICT tools such as phones, and social media tools in their customer relationship management, retention and general marketing activities. The finding is in line with that work of Oyeyinka and Bello (2013) which reported that the average years of formal education of agricultural product marketers was eight years. The household size indicated that the greater percentage (41.6%) of the marketers had a household size of 3-4 persons while only 5.6% had a household size of above six persons. However, the mean household was four persons in the study area. The mean household size of four persons is very essential as it could support the available labour in the marketing of broilers and thus reduce the expenditure on hired labour. Broiler marketing could be labour intensive; therefore, an average household size of four is in contrast with Ukwuaba et al. (2018) who stated that the average family size of the agricultural marketers was six.

As regards the marketing experience, the result revealed that the majority (64.4%) of respondents had a marketing experience within the range of 1-10 years while 3% had marketing experience above 30 years, with a mean marketing experience of 10 years. The relatively high mean marketing experience of about 10 years indicates that they were very knowledgeable and skilled in the enterprise which are veritable tools for the success of any business.

Results of the market association showed that the majority (85.6%) of the broiler marketers belonged to one form of market association or the other and only 14% of the marketers do not belong to any market association. This result is a clear indication that marketers in the study area are highly socialized and also participate actively in market unionism. Lastly, the result indicated that the larger proportion (36.7%) of the broiler marketers earned ₦50,000 to ₦100,000 monthly, while only about 29% earned above ₦100,000 in a month. The mean monthly income was ₦102, 096.67. Thus, most broiler marketers earn a tangible amount of income monthly in broiler marketing, which is far above the national minimum wage of ₦30,000 for civil servants in Nigeria.

Types of ICTs Used by the Respondents

The type of ICTs available to the respondent include mobile phones and social media which they used to facilitate their marketing as stated in Table 2.

Table 2. Types of ICTs used by the broiler marketers

Variables	Frequency	Percentage
Availability of ICT facilities		
Yes	90	100.0
*Types of ICTs used in broiler marketing		
Mobile phone only	59	61.5
Mobile phone and social media platforms	37	38.5
Social media use		
Yes	37	41.1
No	53	58.9
If yes, do you conduct marketing through it?		
Yes	35	94.6
No	2	5.4.
Aspects of marketing		
Customer outreach	32	91.4
Product advertisement	3	8.60

*Multiple Response; Source: Field surveys, 2019

Table 3. Level of Access and Usage of ICTs among broiler marketers

Variables	Frequency	Percentage
Frequency of ICT Facilities Usage		
Mobile Phone		
Very often	90	100.0
Radio		
Not often	1	1.1
I do not use it in marketing	89	98.9
Television		
Not often	1	1.1
I do not use it in marketing	89	98.9
Social Media Platform		
Very often	36	40.0
Not often	1	1.1
I do not use it in marketing	53	58.9
Access to ICT facilities		
How do you access		
I operate them	90	100.0
Degree of use		
High degree	36	40.4
Low degree	53	59.6
Daily access to facilities		
Yes	80	88.9
No	10	11.1
Customer coverage through ICT		
Yes	90	100.0

Source: Field survey, 2019

All broiler marketers had ICT facilities which they used to reach out to their customers. The majority (61.5%) used only mobile phones to market their broilers while 38.5% adopted both mobile phone and social media platforms. This implies that ICTs, particularly mobile phones, can offer agricultural marketers several avenues to create beneficial networks with other marketers, get vital market information such as agricultural commodity prices, and gain access to reliable knowledge and information. The result is consistent with Tonny et al. (2019) and Haong (2020) who found that mobile phone was the most popular ICTs used by Bangladeshi and Vietnamese agricultural produce marketers. Aside from mobile phones, about 95% of the broiler marketers used social media tools such as Facebook and WhatsApp in marketing and obtaining information about the poultry enterprise.. The result aligns with the findings of Alavion et al. (2017) and Mittal and Mehar (2016) who found that agribusiness entrepreneurs use diverse information sources in marketing agricultural produce in India.

Level of Access and Usage of ICTs among Broiler Marketers

The result in Table 3 revealed that 100% of the respondents accessed and used mobile phones very often to communicate and/or reach out to their customers. The result suggests that the marketers greatly accessed and used ICTs in the study area irrespective of the cost. Mobile

phones were instrumental in collecting most of the information regarding marketing activities. The timely and reliable information accessed by the marketers would help to identify the best markets, reduce price volatility, decline in wastage and achieve higher revenue and profit. This is because using ICTs to market agricultural products helps to eliminate intermediaries, lower transaction costs, and find new customers (Alavion and Allahyari, 2012; Bachaspati, 2018). The result is in line with the findings of Ferris, Engoru and Kaganzi, (2008) which reported that 86 per cent of the agricultural producers and marketers had access to a mobile phone which thus contributed towards developing their linkage with other people including extension experts. This is tan dem with most literatures (Alavion et al., 2017; Abebe and Mammo Cherinet (2018); Haong, 2020) which showed high rate of adoption of ICTs in agricultural related activities and value chain. The result also showed that 99% of the respondents do not use both radio and television in the marketing of broilers. The result indicated a very low degree of usage of the two ICT tools in marketing their products. This could be connected to the high cost of accessing or advertising on these two platforms. On the degree of usage of social media platforms, the result showed that the majority of the respondents (59.6%) do not make use of social media platforms such as Facebook and WhatsApp in conducting their business compared to the frequency of the use of a mobile phone. This indicates that the degree of usage of social media is presently low in the study area. The low use of social media platforms is not unconnected to the high cost of data in Nigeria presently. Lastly, the result showed that all the respondents (100%) operated the ICT tools by themselves, the majority (88.9%) accessed the ICT tools daily and all the respondents (100%) reach out to their customers through ICT tools.

Monthly Gross Margin of the Broiler Marketers

The result in Table 4 showed that the mean variable cost of broiler marketers was ₦513,402.62 while the mean monthly revenue was ₦594,375.34. The gross margin was estimated as ₦80,972.72. This shows that the enterprise is very lucrative as an average broiler marketer earns twice above the national minimum wage of ₦30,000. This finding is in relation with Demo, Mariam and Ueda (2007) in their study of Economics Analysis of Broiler Production at Miango, Plateau State which they reported that broiler production and marketing was a profitable business.

Effect of the Degree of Use of ICTs and Socio-economic Factors on the Revenue of Broiler Marketers

Table 5 shows the effect of the degree of usage of ICTs and socio-economic factors on the total revenue of broiler marketers. The result showed that the linear function had the best fit and was therefore chosen as the lead equation. Results showed that the R^2 was 0.340. This implies that 34.0% variation in the revenue of broiler marketers was jointly explained by the independent variables included in the model. The F-value of 5.095 ($P<0.05$) implied that the overall model had a good fit. Educational level ($P<0.05$), marketing experience ($P<0.05$) and degree of ICT use ($P<0.10$) were the significant variables.

The result showed that educational level, marketing experience and degree of ICT use had a significant influence on the respondents' total revenue. The educational level was positive and significantly influenced the revenue at a 5% probability level. It implied that the higher the educational attainment, the higher the revenue from the sale of broiler birds by the respondents. This could be attributed to the mental alertness and increased knowledge of the respondents on the marketing strategies and as a consequence increases the level of revenue. Marketing experience was also positive and significantly affected the total revenue at a 5% level. Thus, the higher the years of experience, the more revenue is generated in the enterprise. The result is understandable as the marketers continue to sharpen their skills with each passing day and master the arts and strategies effectively to increasing revenue. This could also due to engaging in strategies that minimize costs and maximize revenue. The degree of ICT use was positive and significantly to revenue at 10%.

Table 4. Gross Margin of Broiler Marketing

Cost and return items	Mean value (₦)	Total value (₦)
Revenue (₦)		
Selling price per bird (₦)	1912.22	
quantity sold (Number of birds)	310.83	
Total Revenue		594,375.34
Variable Costs Broiler birds		
The purchase price per bird (N)	1418.33	
Quantity purchased (Number of birds)	320.83	
Cost of birds:		455,042.81
Transportation		10,498.89
Rent		5,594.32
Feeds		20,020.19
Vaccines		4,080.00
Labour		3,063.33
Other expenses (recharge cards for calls and data market levy)		15,103.08
Total Variable Cost (TVC)		513,402.62
Gross Margin (GM)		80,972.72

USD 1 = 410.42; Source: Field Survey, 2019

Table 5. The effect of the degree of use of ICTs and socio-economic factors on the revenue of broiler marketers

Variables	Linear+	Semi-Log	Double-Log	Exponential
(Constant)	-2076347.759 (901793.627) [2.302]	4.436 (.372) [11.925]	2.267 (1.360) [1.667]	5.220 (3.131) [1.667]
Sex	126930.983 (201450.177) [0.630]	0.132 (.083) [1.590]	0.159 (0.082) [1.939]	0.366 (0.189) [1.937]
Age	24379.962 (21697.151) [1.124]	0.014 (.009) [1.556]	1.384 (0.859) [1.611]	1.384 (0.859) [1.611]
Marital Status	-879569.669 (472770.730) [1.860]	0.096 (.195) [0.492]	-0.048 (0.231) [0.208]	0.111 (0.532) [0.209]
Educational level	106841.104* (41793.172) [2.558]	0.028 (.017) [1.647]	0.608 (0.355) [1.713]	0.608 (0.355) [1.713]
Household size	-91631.954 (117179.789) [0.781]	-0.055 (.048) [1.146]	-0.271 (0.384) [0.706	-0.271 (0.384) [0.706]
Marketing experience	41831.519* (19281.791) [2.169]	0.015 (.008) [1.875]	0.432* (0.209) [2.067]	0.432* (0.209) [2.067]
Membership to market association	319876.407 (259736.612) [1.232]	0.140 (.182) [0.769]	0.046 (0.182) [0.253]	0.107 (0.418) [0.256]
Degree of ICT use	1189820.113** (440751.316) [2.699]	0.138 (.107) [1.289]	0.204 (0.108) 1.889]	0.470 (0.249) [1.888]
F-value R^2	5.095 0.340	5.063 0.339	5.229 0.346	5.229 0.346

*and ** denote significance at 5% and 10% Probability levels.; Figures in ( ) are standard errors, figures in [ ] are t-values; Source: Field survey, 2019; + : Lead Equation

Table 6. Constraints to the use of ICTs among broiler marketers

Constraints	Mean	Std. Deviation
Inadequate skill and personnel for handling ICT tools	3.90	0.303
High cost of ICT tools	3.80	0.404
Inconsistence power supply	3.79	0.412
Low network connectivity	3.71	0.482
Long-distance to repair and maintains ICT tools	3.64	0.569
High cost of maintenance	3.70	0.462
High cost of Airtime and data	3.61	0.556
Poor awareness of the benefit of ICTs	3.74	0.512

Source: Field survey 2019

The result implied that respondents' use of ICT tools increased the total revenue. This is expected as the marketers now have convenient ways of reaching out to potential customers via mobile phones, Facebook or WhatsApp messengers without face-to-face meetings. This is made easier as the marketers can easily reach out to thousands of potential customers through social media advertisements which cost little or nothing, unlike traditional media like television and radio. More so, many of the broilers marketers also engage in-home delivery and this also boosts their sales and profit margin. The results affirm the findings of Hoang (2020), who found that the adoption of ICTs especially mobile phones for marketing was positively and significantly associated with income among fruit marketers in Vietnam.

Constraints to the Use of ICTs among Broiler Marketers

The result presented in Table 6 showed the constraints encountered by the broiler marketers with the use of ICT in the study area which include inadequate skill and personnel for handling ICT tools ($\bar{x}$ = 3.90), high cost of ICT tools ($\bar{x}$ = 3.80), inconsistence power supply ($\bar{x}$ = 3.79), low network connectivity ($\bar{x}$ = 3.71), long-distance to repair and maintains ICT tools ($\bar{x}$ = 3.64), high cost of maintenance with the mean of ($\bar{x}$ = 3.73), high cost of airtime and data ($\bar{x}$ = 3.61) and poor awareness of the benefit of ICTs ($\bar{x}$ = 3.74). All the constraints were major limiting factors to the use of ICT in the marketing of broiler birds in the Enugu metropolis. The result implied that any efforts or policies by government or NGOs that will reduce the effects of the variables will enhance the ease of use of

ICTs and also boost the profit margin of the marketers. This finding is in tandem with that of Singh et al. (2014); Kale et al. (2015); Taragona and Gelb (2005); Anoop and Ashok (2015) which reported that high cost of ICT facilities, inadequate skill and personnel for handling ICT tools, limited financial resources, inconsistence power supply among others were the major hindrances in ICTs use among marketers.

Conclusion and Recommendations

The study explored the role of ICTs in the marketing of broiler birds in Enugu state, Nigeria. It can be deduced from the above findings that mobile phone was the most commonly used ICTsin the study area. Findings also indicated that ICT facilities had improved the market information of the respondents, raising their standard of living and has contributed to the growth of their total revenue. Also, it showed that broiler marketing is profitable especially now that the country is facing recession and emphasis are being made on diversification of the economy. The study further showed that educational attainment, marketing experience and use of ICT facilities significantly influence the total revenue of the respondents. However, some constraining factors were identified such as the high cost of ICT facilities, inadequate skills and personnel for handling ICT tools, inconsistence power supply, low network connectivity, high cost of maintenance and airtime, and low awareness of ICT benefits, were among the constraints that demand urgent attention. The study thus recommends that the problem of inconsistent power supply and poor network coverage should be rectified and improved on by the appropriate authorities such as the government and the network providers. The national communication commission should as a matter of urgency regulate, moderate and reduce the high call tariffs and internet data cost to enhance the profit margin of the broiler marketers in the study area.

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Determinants of Choice of Marketing Outlet for Edible Insects among Smallholder Farmers and Traders in Western Kenya

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ABSTRACT

The edible insect sector has, in recent times, gained significant prominence and attention. Particularly, the government of Kenya has made remarkable steps to achieve a potentially large and valuable edible insect market, with a significant milestone being the passing of regulations on edible insects as a new source of proteins. However, research on the edible insect marketing environment is still indistinct. Therefore, the study sought to evaluate the determinants of the market outlets preferred or used by smallholder farmers and traders of domesticated and field-collected edible insects (including cricket, bees, winged termites, lake flies, and dung beetle) in Siaya and Vihiga counties. This cross-sectional study was done among 188 edible insects' farmers and traders. Purposive sampling identified the study area, while snowball sampling reached the study participants. Data was collected using structured questionnaires and analyzed using multinomial logit regression to assess independent-dependent variable relationships, yielding marginal effects. Study findings showed that at 95% confidence interval, the yield was significant to the three outlets used by the farmers. Gender was significant to both institution and open-air markets outlets but insignificant to selling at the farm gate. Age, education level, and experience in marketing were insignificant to all the three market outlets. Marketing training was significant to the institution and open-air markets**.** The study also showed that farmers and traders had limited choices to sell their edible insect produce, which was majorly affected by yield and age variables. In view of these findings, enhancing edible insect marketing and training through initiatives that would increase production among farmers and breaking the attitudes toward open-air marketing among male farmers is pivotal to the thriving of the novel food enterprise towards achieving food security in the region.

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Introduction

The rising human population and food insecurity upsurge, together with increasing concerns related to climate change, has called for an expert reassessment of diets (Durst et al., 2010). As such, the potential of edible insects for food security and commercial farming prospects has gained considerable momentum. Besides being nutritionally and environmentally promising, edible insects have a substantial opportunity to provide income and employment opportunities (McClure & Wynberg, 2020). Insects have been used as food and feed for a long time, but their full commercialization is now being recommended to create a source of livelihood for farmers/traders (Dobermann et al., 2017). It calls for the promotion of the commercialization of edible insects to meet the projected increase in demand for proteins. A wider market creation for edible insects could provide an economic incentive for the commercialization of the emerging enterprise.

The progress in Kenya's edible insect market, particularly within the counties of Vihiga and Siaya, as observed in the study titled "Consumer Acceptance of Edible Insect Foods for Non-Meat Protein in Western Kenya," signifies a noteworthy shift in dietary preferences and sustainability awareness. Drawing from data collected in a 2015 consumer survey involving 234 participants, it is evident that over 75% of respondents have not only embraced edible insects as a viable food source but also as a compelling alternative to conventional meat. This acceptance underscores a significant transition in food choices, driven by factors such as familiarity, convenience, social and environmental responsibility, economic incentives, barriers, risk attitudes, and altruistic concerns. Such high levels of acceptance provide clear evidence of the growing enthusiasm for edible insects in Kenya, reflecting an evolving consumer landscape where

sustainability and protein diversification are paramount considerations, thus paving the way for innovative approaches to address food security and environmental sustainability.

Studies have shown that edible insects bring socioeconomic benefits to farmers/traders (Tao & Yao, 2018; Tanga et al., 2021). In Kenya, Vihiga and Siaya are some of the regions experiencing food insecurity (WFP, 2016). Incidentally, there are instances of insect business with a number of farmers venturing into edible insect enterprises where they have proven to be of benefit both socially and economically (Oloo et al., 2020). However, this region has not portrayed a potentially large and valuable edible insect market. Hence, production is mostly subsistence, with a little amount sold in the local markets. Additionally, limited research has been done on the region's marketing environment of edible insects, particularly the outlets used by farmers in marketing this micro livestock.

Adopting insect farming and marketing as an enterprise is envisaged as a reliable source of income. Good marketing channels can make products made available at the right time, in the right place, and in the right amount. Therefore, convenient marketing channels and outlets help overcome time, place, and position gaps (Qadri, 2018). Subsequently, when insect products can be marketed even to high-end markets, it will most likely help boost farmers' incomes from what they currently earn. Therefore, this study sought to analyze determinants of market outlets used by farmers as a way of strengthening the novel food enterprise.

In Western Kenya's Siaya and Vihiga Counties, income for those involved with the edible insect industry hinges on the selection of the appropriate marketing outlet. This decision carries significant economic consequences as pricing, demand, and payment structure varies among outlets. Smallholder farmers and traders are the focus of this study, and identifying the optimal market outlet choice is of paramount importance. Furthermore, market outlets that are sustainable play a critical role in securing the industry's longevity. Understanding what factors drive these decisions is key to establishing and upholding such outlets. The edible insect value chain also benefits from uncovering the connections between producers and consumers, which can lead to increased efficiency and potential diversification opportunities that promote resilience. Ultimately, this research has policy and development implications, as it can help policymakers and organizations develop interventions that allow smallholders to choose market outlets that best support their financial and livelihood goals.

Materials and Methods

Study Area

The study was conducted in Siaya and Vihiga Counties in the year 2021. The two counties were selected due to their high edible insect occurrences (Alemu et al., 2015). Moreover, communities living in these two counties have traditionally consumed edible insects (Pambo et al., 2016). Further, the areas are uniquely suitable for the study since they have hosted interventions that promote foods from edible insects through projects like the GREEiNSECT and INSFeed (Ayieko et al., 2010). The study areas fall under agro-ecological zones of Lower Midland (LM) and Upper Mid Land (UM), with temperatures ranging between 18°C and 24°C and annual rainfall ranging from 1000mm to 2000mm, depending on the distance from the lake shores. These zones provide favorable conditions for the survival of edible insects and hence the possibility of thriving of such enterprises.

Study Design

This study employed a cross-sectional research design to assess the determinants influencing smallholder farmers' and traders' choice of marketing outlets for edible insects in Siaya and Vihiga Counties, Western Kenya. By collecting data at a single point in time, this de-sign provides a snapshot of the variables affecting marketing outlet choices within the study population.

Data

Both primary and secondary data were collected in this study. The primary data was collected by administering a structured questionnaire and conducting key informant interviews. Secondary data was collected from existing literature and through a review of agricultural reports.

Sampling Procedure

During sampling, a multistage-stage sampling procedure was employed. In the first stage, the two counties, Vihiga and Siaya, were purposively selected due to high edible insect occurrence and the heterogeneous edible insect practices of the occupants. In the second stage, the two areas of Luanda and Bondo sub-counties were purposively selected based on insect trading and farming. In the last stage, the snowballing sampling method was used to identify insect farmers/traders and this was done until the saturation point was reached (Parker et al., 2019). Snowballing was appropriate as the population of insect farmers in the region was not detailed. A sample of 188 farmers who also doubled as traders were selected.

Determinants of Edible Insects Farmers' Choice of Market Outlets

The study identified three major market outlets predominantly used by insect traders in the study region during reconnaissance. The outlets included research institutions, open-air markets, and farm gate. Different variables such as gender, age, marketing experience, education level, and marketing training were hypothesized to influence the market outlet choice of the farmers and were therefore adopted for the study, as illustrated in Table 1. These variables resonated with the major factors that significantly affect market outlet choice as investigated by researchers in agricultural fields (Dessie, Abate & Mekie's, 2018).

Study Variables

In this study, the dependent variable is the choice of marketing outlets for edible insects among smallholder farmers and traders in Siaya and Vihiga Counties, Western Kenya. The study examines how various independent variables influence this choice. These independent variables include gender, age (categorized into four groups: 18-25 years, 26-33 years, 34-41 years, and above

41 years), education level (divided into four categories: no formal education, primary, secondary, and post-secondary), experience in edible insect business (categorized based on the number of years involved in the business: less than a year, between 1 and 5 years, and over 5 years), training in edible insect production or marketing (a binary variable: Yes/No), and yield of edible insects, which was quantified by weighing the produce using a precise weighing scale, measured in kilograms. Gender is categorized as male or female, and the study aims to analyze how these factors influence the selection of marketing outlets, which include farm gates, institutions (such as research institutions), and open-air markets.

Data Analysis

Data analysis was guided by the Multinomial Logit regression model (MNL) represented by equations 1,2, and 3. The Multinomial Logit regression model was considered fit for the analysis in this study because of the nature of the dependent variable and the aim of the study. The dependent variable (choices of market outlets) had three categories, thus, finding the association between the dependent variable and the independent variables was best done by the MNL regression model.

$$Y_I = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_6 X_6 + \varepsilon_I \quad (1)$$
$$Y_{II} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_6 X_6 + \varepsilon_I \quad (2)$$
$$Y_{III} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_6 X_6 + \varepsilon_I \quad (3)$$

Where Y_I, Y_{II}, and Y_{III} represents institution, open-air markets, and farm gate outlets, respectively. X_1 to X_6 represents the explanatory variables as illustrated in Table 1. β_0 is the intercept. β_1 to β_6 represents coefficients associated with each explanatory variable. εi is the error term.

Choosing a specific market outlet is a discrete choice from among the alternative outlets for the farmers. Because only the farmers' choice of a specific market outlet was observed, the following latent structure univariate logit model for the choice of each outlet can be modelled as follows;

$$p_i = \{1\ if\ p_i = x\beta + u_i > 0; 0\ if\ p_i \leq \quad (4)$$

Where p_i is the binary latent variable for outlet choice observed if $p_i > 0\ and\ 0\ otherwise$.x is the specific factor determining the choice of market outlet. However, a producer might choose more than one outlet at a single point. The potential for simultaneous choice across the outlets implies that a multinomial logit regression model would be desirable to ascertain the association, thus combining equations 1,2,3. The model can then be rewritten as:

$$p_{ij} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_n x_n + \varepsilon_i \quad (5)$$

Where p is the market outlet chosen by the farmer, i takes values 1,2,3, each corresponding to the choice. $x_1, x_{2,} x_n$ are factors influencing market choice, and ε_i is a randomized error with j alternative choices.

Results and Discussions

Socio-demographic characteristics of the edible insect farmers'/businesspeople.

Descriptive statistics of demographic and socioeconomic characteristics (Table 2) showed that more than half (57.45%) of the respondents who were dealing in insect farming and trading were female, with their counterparts only contributing 43%. Siaya County had more respondents (male 48.33%, female 51.67%) participating in insect enterprise than Vihiga County (male 32.55%, female 67.65%)) in addition to the highest number of youth participants. This can be linked to the existence of a major research institution in edible insect farming in western Kenya, Jaramogi Oginga Odinga University of Science and Technology, that has endeavored to have a successful edible insect project within its environs. As part of the project's (Insects as Food and Feed) rationale, a number of farmers in the locality of Siaya county were trained to kick start the project's aims. Therefore, many people within this locality ventured into the project relative to their counterparts in Vihiga County.

Table 1. Selection of dependent and independent variables for multinomial logit regression

Variable	Description and Unit of Measurement	Expected sign		
Dependent variable		Farm gate	Institutions	Open-air market
Market outlet				
Independent variable				
Gender	Gender of the farmer (Dummy variable. 0=Male, 1= Female)	+	+	+
Age	Age of the farmer/trader in years Continuous Variable: Age of respondents in years)	-	+	+
Education Level	Education level of the farmer in years (Categorical Variable: 1 if None, 2 Primary and 3 if post-primary)	+	+	+
Experience	Experience in marketing of the farmer Continuous variable: experience in years	+	-	+
Training	Whether the farmer have received training or not regarding insect marketing (Dummy variable Yes=1, No=2)	-	-	-
Yield	Quantity produced or collected by the farmer. Continuous variable: yield in kilograms	+	-	+

Table 2. Socio-demographic characteristics

Characteristics	Sites		
	Siaya	Vihiga	Totals
Gender			
Male	48.33%	32.55%	42.55%
Female	51.67%	67.65%	57.45%
Age			
18-25 years	4.17%	1.47%	3.19%
26-33 years	8.33%	23.53%	13.83%
34-41 years	32.50%	23.82%	32.98%
Above 41	55%	41.18%	50%
Education level			
No formal education	0.83%	0	0.53%
Primary	45%	38.24%	42.55%
Secondary	36.67%	50%	41.49%
Post-Secondary	17.50%	11.76%	15.43%
Experience in insect business			
Less than a year	19.17%	1.47%	12.77%
Between 1 and 5 years	47.50%	30.88%	41.49%
Over 5 years	33.33%	67.65%	45.74%
Training on production and marketing			
Yes	10.83%	2.94%	7.98%
No	89.17%	97.06%	92.02%

Regarding the education level, the majority of the farmers had attained primary level (43%), with only 15% accounting for post-secondary and a paltry 1% having no formal education. Half of the farmers (50%) were above the age of 41, with only 3.2% of the respondents having ages of 18 and 25 years. The results show less youth participating in such enterprises. Youths within the study area view the practice of edible insects as not-so-cool activity.

The majority of the farmers, represented by 45.74%, had the experience of more than five years in the business, with only 12.77% having less than a year of experience. Farmers in Vihiga county showed a remarkable experience in insect trading compared to the Siaya farmers. This is because Vihiga county has the existence of rain forests such as the Kibiri and Guenno Congolian that harbors many insect species (NEMA, 2013). This gives the residents access to insects for a better part of the year than the Siaya occupants, which commonly have intermittent seasonality of edible insects (NEMA, 2013).

Regarding training in the production or marketing of edible insects, only 8% of the farmer had prior training, with the majority (92%) having no training in this novel enterprise. The noticeable- difference in training rate-s between the- two study sites can be attributed to the- presence of a re-search institution within Siaya County. Jaramogi Oginga Odinga University of Science- and Technology plays a crucial role by offering training and capacity-building programs to local farme-rs, providing them with the nece-ssary knowledge and skills for edible-insect farming and marketing. There-fore, the differe-nce in training experie-nces emphasizes how re-search institutions like the one- in Siaya County contribute to promoting better opportunitie-s for education within the edible- insect industry.

Insects' Species Dealt by the Farmers and Sources of Edible Insect

Farmers majorly dealt with five species of edible insects (Winged termites, house crickets, bees, lake flies, and *Carebara vidua Smith*), either domesticated or collected from the wild. Over 80% of the farmers were collecting from the wild, with only 6.38% combining both production and wild collection (Figures 1 and 2). Only 12% of the farmers were engaged entirely in the production of edible insects. It was noted that *Carebara vidua* Smith was mostly seasonal, and only 40% collected it whenever they were available.

Factors Influencing the Choice of the Market Outlet Used by Farmers

The variables included in the multinomial logit regression model explained 26% variation in the dependent variable (choice of marketing outlets) (Pseudo R^2=0.2623). While this is a low R^2 value and may not warrant the preferred goodness of fit, this can be attributed to the few dependent variables used. Also, Onditi (2013) justifies that any research that deals with humans may have a low R^2 as humans are simply harder to predict than physical processes. Further, King (1986) points out that the low R^2 does not show that the model is not fit, and conclusions should be made based on the significance coefficients regardless of the value.

Age, educational level, and experience of edible insect farmers did not have a statistically significant influence on the choice of insect marketing outlet at 0.05 significance level (Table 3). Age did not significantly influence the choice of any outlet, an outcome which two reasons can plausibly explain: First, the respondents indicated that there were no restrictions to the market participants based on age as the young, middle-aged, and old had equal participation in the market.

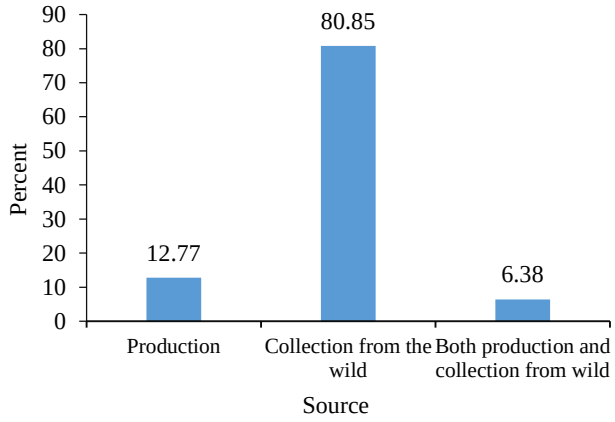

Figure 1. Source of Edible Insect

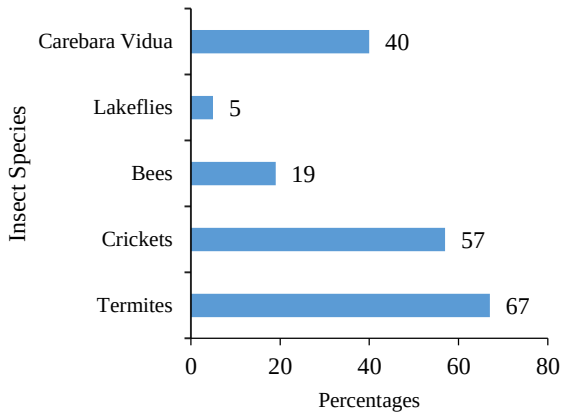

Figure 2. Insects species dealt by the farmers

Table 3. Marginal effect from Multinomial logit on the choice of edible insect marketing outlets.

Market Choice Outlet	Farm Gate		Institution		Open Air Market	
Explanatory Variable	δy/δx	P-Value	δy/δx	P-Value	δy/δx	P-Value
Gender(0-male,1-female)	-0.035***	0.094	-0.149**	0.045	0.184**	0.035
Age	-0.002	0.788	-0.011	0.786	0.014	0.786
Education Level	-0.002	0.795	0.012	0.794	-0.015	0.794
Experience	-0.019	0.204	-0.082	0.166	0.102	0.155
Training	-0.056***	0.052	-0.238**	0.02	0.295**	0.011
Yield (in Kilograms)	0.033**	0.04	0.139*	0.005	-0.173*	0.002

Note: *, **, *** significant at 1%, 5% and 10% respectively. Number of observations=99(Number of active farmers in trading); LR Chi-Square (6) = 37.57; Pseudo R^2=0.2623; Log likelihood= -52.83; Prob > chi^2 = 0.0000.

Secondly, no cultural values inhibit any person, regardless of age, on market participation. Of interest is the negative insignificant influence of age on the institution. An institution requires quality products, and the old are less likely to observe quality standards gained through training. This result is consistent with Jalang'o, Otieno & Oluoch-Kosura's (2016) findings that age did not influence market outlet participation. By contrast, Dessie, Abate & Mekie's (2018) study showed that age directly influenced the decision to sell at the outlet. The trio found out that older farmers often decided to choose better outlets that would give higher pay. Moreover, older farmers preferred rural markets to urban markets due to the complicated logistics of transporting to urban markets, which makes the old shun away (Arinloye et al.,2015).

Conventionally, it would be expected that education would increase one's ability to search for access and process information and thus help in making rational decisions based on the market outlet. However, the result of this study is the contrary. This contrasting outcome can be attributed to the fact that market outlets available for the insects in the region are limited and that farmers do not have more options to evaluate to determine the best. But, of interest is the fact that the increase in the level of education resulted in less likelihood of selling (2%) in the open market. This is further supported by Jalang'o et al.'s (2016) findings that farmers who attained more years of formal education are more likely to participate in high-end value markets that attract considerable prices. Therefore, they are more likely to shun the open-air market that attracts relatively lower prices.

Experience showed insignificant influence in the choice of all the marketing outlets. This can be linked to limited market options. Conventionally, the experience would enable farmers to make rational decisions in choosing the marketing outlet with the highest returns. The result is consistent with a study by Geoffrey et al. (2014) that found a negative relationship between experience in farming and the choice of farm gate as a marketing outlet. It showed that an increase in the farmer's experience would make them less likely to sell at the farm gate. The reason could be that the farmer has learned about the insect market and that he would be sourcing for new and attractive prices with significant output.

The marginal effect of gender at institution (p-value= 0.045) and open-air market (p-value= 0.035) were found significant (Table 3). Implying that being a female decreases the chances of choosing an institution as an insect marketing outlet by about 15% and increases the possibility of choosing an open-air market by 18%. This shows that female farmers were less likely to sell their output in an institution than their male counterparts. Nonetheless, they had a more probability of selling in an open-air market. A plausible explanation for this trend is that male farmers are vibrant and source for high-paying outlets. This is concurrent with Geoffrey et al.'s (2014) findings that male farmers are resource endowed and active in sourcing for high-end markets. Hence, they can produce the required quality produce. Comparatively, female farmers were more active in the open-air markets than men. This could be attributed to the fact that women own most stalls and small businesses in open-air markets in Kenya (Xinhua, 2021), and men view small businesses in the open air as female-oriented, thus shying away from open-air markets. This further explains the low likelihood of females selling to the institutions. However, gender did not influence selling at the farm gate.

Having participated in trainings on insect farming significantly influenced the choice of institution (dy/dx=-0.238, p-value=0.02) and open-air market (dy/dx=0.295, p-value=0.011) as edible insects marketing outlets. Thus, participation in training reduces the chances of choosing an institution as a marketing outlet by about 24% and increases the chances of choosing an open-air market by about 29%. The more the farmer is trained, the more they look for new outlets with attractive prices. The training imparts farmers with marketing strategies and sourcing for attractive prices that farm gate does not give. It can be deduced that training makes farmers knowledgeable of producing quality products and thus could sell even to more demanding yet strict sources like the institutions. Also, because farmers are adept in marketing, they believe they can make informed decisions without the interference of any factor. Jalang'o et al. (2016) contradict these findings by stating that training has no impact on the choice of a marketing outlet

Comparatively, yield positively influenced the choice of the three market outlets. A unit increase in yield increases the chances of choosing a farm gate marketing outlet by 3%, an institution by about 14%, and reduces the chances of choosing an open-air market outlet by 17%. Notably, an increase in the yield would make the farmer more likely to sell to the institutions that source in bulk for further production processes. The results are consistent with Tsougiannis et al. (2008), who reported a positive relationship between output and social institutions, such as cooperatives, with an increase in yield. However, the result contradicts the finding of a study by Mutura et al. (2015), who reported a negative relationship of the choice of the farm gate outlet with an increase in the yield. Interestingly, the yield was negatively significant to the open-air market, implying a unit increase in the yield makes a farmer less likely to sell in the open-air market. High volumes would prompt the farmer to seek high-value markets such as institutions (Boutelle, 2018). Selling in the institution is more remunerative, and significant profits can be attracted with sufficient quantities.

Conclusion and Recommendations

Edible insects play a critical role in improving the smallholders' economic standards and a quantifiable nutritional component in consumers' diets. However, the insights of this study revealed that edible insect production and marketing is yet to gain prominence in Siaya and Vihiga Counties. The study concluded that gender, participation in the training programs and yield are the determinants of choice of marketing outlet for edible insects among smallholder farmers and traders in Western Kenya. Additionally, it is important to note that the study did not explicitly address potential seasonal variations in edible insect farming and trading, which could influence marketing choices, particularly for seasonal insect species.

Based on the findings, the study proposed several recommendations aimed at fostering a thriving edible insect market in Siaya and Vihiga Counties. First, there is a need to encourage the establishment of more institutions and value chain coordination organizations that facilitate marketing edible insects. This could create additional outlets for farmers, enhancing their access to high-value markets. Second, implementing tailored training programs for farmers in edible insect production and marketing is essential. The training should focus on marketing strategies and quality assurance to empower farmers to access higher-paying markets, such as institutions. Third, promoting awareness among farmers about the potential benefits of education and its connection to market opportunities is recommended. Emphasize how increasing educational attainment can open doors to more profitable outlets. Fourth, the farmers should be supported in improving their production techniques to increase yields. This can be achieved through knowledge transfer and best practices, potentially leading to greater access to institutions. Fifth, consumer education campaigns should be initiated to enhance the acceptability of edible insects. Addressing misconceptions and promoting the nutritional and environmental benefits of edible insects can expand market options.

The study also recommends that future research should explore the nuanced gender-related factors impacting marketing choices, investigate the specific content and delivery of training programs that yield the most effective results, and investigate the broader economic implications of edible insect trading within these counties. Such investigations will provide valuable insights to inform policies and strategies aimed at promoting sustainable development within the edible insect industry in the region.

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Declaration of conflict of interests

Authors declare no competing interest.

Ethical Approval

Authors were granted Ethical Approval by Jaramogi Oginga Odinga University of Science & Technology Ethical Review Committee, and the National Commission for Science, Technology, and Innovation (NACOSTI)

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Assessing Vegetable Farmers' Knowledge of Disease and Pests Control Methods in Ghana: A Survey of Tomato (*Solanum lycopersicum* [L]) Farmers in the Mampong Municipality of the Ashanti Region of Ghana

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ABSTRACT

A survey of 200 farmers in the Mampong Municipality in the Ashanti region was conducted between June and October 2016. It was to determine their knowledge of different pests and disease control methods employed, access to extension services, pesticides use and other pests control methods. The study relied on data collected from respondents, 12 key informants (pesticides dealers, vegetable buyers and local chief farmers), field surveillance and observation as well as the reports of the Directorate of Agriculture in the Municipality. Data were captured and analyzed using MS Excel and Statistical Package for Social Sciences (SPPS) version 16 (SPSS Inc., Chicago, IL) and interpreted into simple percentages on tables and charts. The result showed that farmers are above 20 years and 18% were female. The majority (74%) have at least basic education with six (6) or more years' of experience in tomato production. Sixty-four percent (64%) had no access to extension services due to poor contact with the agricultural extension agents (AEAs). Most respondents (70%) lacked education on pesticides and alternative (e.g., integrated pests' management) control. Nine-two percent acknowledged the dangers of pesticides to public health. Sixty-four percent practice bi-weekly calendar spraying while 30 % and 6% practice weekly and occasional spraying respectively. Forty-four percent of farmers throw used containers or leave them on the farm. More education is needed to ensure safe use of pesticides and wholesome tomatoes for the public.

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Introduction

Vegetable production is a traditional activity among Ghanaian farmers. There are several indigenous vegetables such as tomato (*Solanum lycopersicum* [L]), okro (*Abelmoschus esculentus*), ayoyo (*Corchorus sp*), waterleaf (*Tilanum triangulare*), alefo (*Amaranthus sp),* roselle (*Hibiscus sabdarifa*), gboma (*Solanum macrocarpon*), garden egg (*Solanum melongena),* etc., which are used for home consumption. Before the introduction of the exotic vegetables such as cabbage (*Brassica oleracea* var *capitata*), lettuce (*Lactuca sativa*), spring onion (*Allium tricuum*), sweet pepper (*Capsicum anuum*), carrots (*Daucus carota*), etc. local vegetables were also produced for sale at various local markets for income.

Agriculture contributes about 20% of Ghana's Gross Domestic Product (GDP) with 35% of total employment coupled with rising agricultural productivity that contributes to higher agricultural wages, and thus to poverty reduction, especially in rural regions, (Memuna et al., 2015; IMF, 2019; Ghana Statistical Service, 2019). Many farmers especially the youth are engaged in vegetable production such as sweet pepper, tomato, onion, garden eggs, cabbages, carrots, hot pepper, chilli, lettuce, etc. Large-scale vegetable production for export to the European Union (EU) is practised mainly in the southern parts of the country especially the Accra plains, Weija area and some other coastal regions. The bulk of Ghana's vegetables are mostly produced by smallholder farmers with no or little formal education and less food safety knowledge but several years in the business were likely to produced unsafe vegetables compared to other farmers with some level of education (Quansah, et al., 2020). Vegetable production in the northern parts of the country is under irrigation during the dry season for local consumption, while the forest zone (middle belt of Ghana)

produces vegetables mostly during the rainy season and in some areas during the dry season along water bodies for the urban populations (Sinnadurai, 1991; Chagomaka et al., 2015; Nchanji et al., 2017; Chagomaka et al., 2018).

The production and consumption of vegetables contribute to improving the nutrition of the population by providing carbohydrates, vitamins, mineral salts, proteins, fats, antioxidants as well as phytochemicals to protect people from non-communicable disease (Yang and Keding, 2009). Some vegetables serve medicinal purposes such as the traditional use of garlic for lowering blood pressure, cholesterol and glucose concentration and reduction in the risk of prostate cancer (Tsai et al., 2012; Zhou et al., 2013; Baya et al., 2014), and the African eggplant as a cheap and natural anti-ulcer remedy (Chioma et al., 2011). It is also a known fact that tomato is used in almost every home in most dishes in Ghana and has a good market potential in every part of the country. In 2016, tomato contributed GHC294, 449.94 million of household income in the forest area alone (Ghana Statistical Service, 2019). The emergence of supermarkets and high-level hotels and restaurants in urban and peri-urban areas has created an enormous opportunity for increased vegetable production to feed a rapidly increasing middle-class with the quest for healthy eating.

Vegetable production (local and exotic) all over the country apart from meeting the food needs of the people also serve as an income-generating activity for many, especially the unemployed. Despite the importance of this enterprise in the agriculture sector of the Ghanaian economy, the problems it creates in respect of pesticide use in pests control management practices cannot be underestimated. Farmers including tomato farmers are misusing pesticides by spraying too close to harvest (thus contaminating the crop before it is consumed), over-applying the dosage, applying pesticides intended for cash crops to growing food crops or applying pesticides intended for growing crops on stored crops, using obsolete or expired pesticides and mixing different chemical pesticides to have a cocktail that is so potent (Northern Presbyterian Agriculture Services, 2012; Memuna et al. 2015; Afari-Sefa et al., 2015; Amoako et al., 2012; Donkor et al., 2016; Nchanji et al., 2018).

According to Fianko et al. (2011), the number of pesticides imported into the country from 2002 to 2006 increased from 7763 metric tons to 27,886 metric tons. In 2011, Ghana spent over 370 million US dollars on pesticides imports into the country (Food and Agricultural Organization, 2010). The Ghana Statistical Service (2019) indicates that, in 2016 about 1.2 million households purchased herbicides for the farm while one million households purchased insecticides for field activities amounting to GHC16,544.54 million and GHC288.49 million respectively.

Consequently, there have been several mass media reports in recent times of frequent chemical poisoning of farmers and consumers of vegetables in several parts of the country leading to several health challenges such as impotence in men and infertility in women due to the inappropriate use of these pesticides by farmers possibly due to insufficient knowledge of the use of these chemicals (NPAS, 2012; Issahaku, 2012; Asante et al., 2013; Afari-Sefa et al., 2015; Memuna et al., 2015). On the global front, approximately 385 million annual cases of unintentional acute pesticides poisoning (UAPP) are reported with 11,000 fatalities (Boeder, et al., 2020). They indicated that, based on a worldwide farming population of approximately 860 million, about 44% of farmers are poisoned by pesticides every year with the greatest estimated number of UAPP cases. A report by Ghana News Agency (GNA) on 5th October (2015), indicated that the export of vegetables from Ghana to the EU suffered serious challenges in respect of the quality and safety of these vegetables and therefore imposed a temporary ban/freeze of some vegetables and fruits based on sanitary and phyto-sanitary-bacterial contaminants and pesticides. Therefore, concerns have been raised by environmentalists and agriculturalists on the increasing poisoning of farmers and the long-term effects of pesticides on the aquatic and terrestrial ecosystems.

Most of the local tomato varieties cultivated in Ghana are poor in colour, watery, acidic and have a shorter shelf life, making tomato production unprofitable and so some commercial farmers mostly rely on imported (seed) varieties for their high textural qualities (Asante et al., 2020; Melodey et al., 2019). These varieties are however not resistant to local pests and diseases and require rigorous pests and disease control measures. Many farmers rely solely on chemical pest control instead of alternative or integrated pests control methods as over 87 % of Ghanaian vegetable farmers use pesticides (Manu, et al., 2021; Fianko et al., 2011; Amoah et al., 2006). The tomato fruit borer (*Helicoverpa armigera*) has been identified as one of the most serious pests of tomato in Ghana (Youdeowei, 2002; PAN-UK, 2002). It is considered notorious due to its polyphagous nature of having a wide range of alternate hosts such as cabbage, tomato, pigeon pea, and chili (Faqiri, 2016). Unconfirmed reports suggest that though farmers have increased the number of sprays and sometimes the chemical concentrations, the pest (*Helicoverpa amigera)* and leaf spot (*Cercospora sp*) are not effectively controlled as desired.

In Ghana, there is a paradigm shift in the eating habit among the populace especially the middle class where the need to consume more vegetables is fast catching up leading to imports of various vegetables from the Netherlands, South Africa, China and neighbouring countries such as Burkina Faso, Mali and La Cote d'Ivoire (Asante, et al., 2020; Asante et al., 2013). Ghana produces 380 000MT of tomatoes each year and consumes about 480 000MT with deficit being imports from neighbours (Agyenim Boateng, 2021; Asante, et al., 2020; Asante et al., 2013). The country also imports between 109, 513 MT and 120,000MT of processed tomatoes from Europe each year, according to the Chamber of Agribusiness Ghana (FAO STATS, 2013; Agyenim Boateng, 2021). It is against this background that local farmers are doing everything possible to increase production by reducing crop losses to pests both at pre-harvest and post-harvest levels. The use of agrochemicals/pesticides in developing countries including Ghana is said to be on the ascendancy even though it is relatively small compared to developed countries (Duwiejuah, et al., 2019; Demi and Sicchia, 2021). There is however, no published reports on farmers' knowledge of tomato pest control methods in the municipality as vegetable /tomato farming is fast becoming an economic venture that employs many and especially the youth.

The objectives of the study are:

- To assess farmers' knowledge of different types of pests and disease control methods
- To determine whether farmers have access to adequate extension services on various types of pests and disease control methods.
- To determine the most adopted pests control method employed by farmers in the area.

Materials and methods

Description of the study area

The study was conducted in the Mampong Municipality from June 2016 to September; 2016. The Mampong Municipality was split and upgraded from the former Sekyere West District into Mampong Municipal and Sekyere Central District by Legislative Instrument (L.I.) 1908 (Fig. 1). It is one of the 43 administrative capitals in the Ashanti Region. It is bounded to the south by Sekyere South district, to the east by Sekyere Central and to the North by Ejura Sekye Dumasi districts. The capital for the Municipality is Asante-Mampong located within longitudes 0° 05''W and 1° 30'' W and latitudes 6° '55''N and 7° 30''N with a total area of about 23.9km^2. It has a population of 88,051 with an annual growth rate of 1.3% according to the 2010 PHC (Ghana Statistical Service, 2014).

It lies within the Wet Semi-equatorial zone with a bimodal rainfall pattern between March and October with a mean annual rainfall of between 1200 and 1500mm (Hall and Swaine, 1981). There is a short period of drought between December and March marked by the northeasterly winds (harmattan). The average temperature is about 27°C with variations in mean monthlies ranging between plus three to minus five degrees Celsius (+3°C to -5°C) throughout the year. The vegetation of the area has been reduced from its original Moist Semi- Deciduous Forest in most areas to secondary forest as a result of human activities like tree felling, charcoal production and farming. The Municipality is fairly drained by streams and rivers such as the Afram, Kyeremfa and Sasebonso. It exhibits five major soil types according to the Food and Agriculture Organization (FAO) system of classification (Sekyere West District Assembly, 2005). These are the Budewa-Sutawa Association, Ejura-Denteso Association, Nyankpala-Kpelesawgu-Volta Association, Denteso-Sene Association and Dukusen-Bremba Association. The major economic activity is farming, trading and formal employment. With respect to farming the major crops grown in the area are maize (corn), yam, cassava, rice, plantain, cocoyam and vegetables such as tomato, garden eggs, hot and sweet pepper, cabbage, carrots, etc. in areas suitable for these crops.

Selection of communities and interviews with farmers

Sampling sites were in vegetable production parts of the district and determined by use of multi-location purposive sampling. Tomato production is not done in all parts of the district as different soil types are suitable for different crops. Five of the seven communities noted for tomato production were purposively selected. These are Bunuso, Kofiase, Mprim, Adiidwan and Benim. Forty (40) farmers were randomly selected in each community making a total of two hundred (200) farmers. Tools used to obtain information about farmers' knowledge related to tomato pests and disease control methods were semi-structured questionnaires after pretesting was done. All the 200 respondents were interviewed individually at their homes in the local language (Twi).

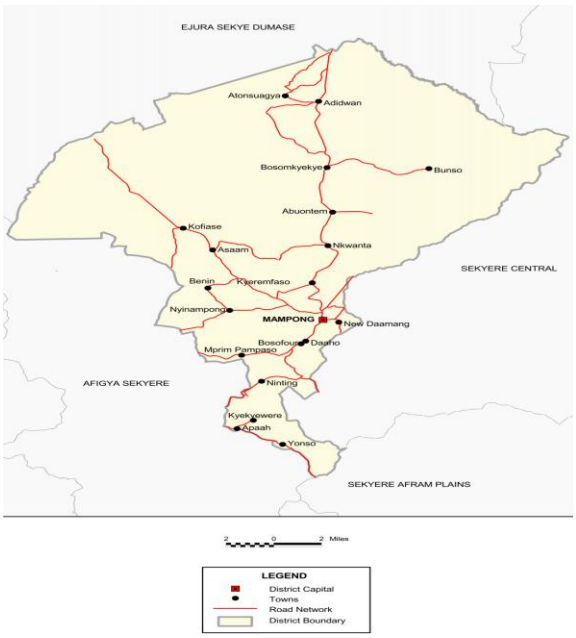

Figure 1. Map of Mampong Municipality
Source: GSS, 2014

Interview with key informants

An interview guide was prepared and used to ensure that all required information was obtained during the interviews. It was conducted on two main groups of stakeholders: opinion leaders and chemical sellers. Twenty key informants (four from each community) were randomly interviewed to gain an insight into the use of pesticides in pests control to triangulate (i.e. getting information from different sources through enquiries, observations, etc. and comparing the information to ensure its reliability) the information obtained from farmers. In most cases the key informants were within the tomato value chain including farmers, drivers, agrochemical/input sellers, market women into tomato buying at the farm level and some, few spraying gang members (a group of young men trained by the government for cocoa pests control but are hired to spray other crops in times of need by farmers). This interview style was used as it relies primarily on the spontaneous generations in the natural flow of an interaction (Cook, 2002).

Secondary data

Secondary data at the District Agriculture Directorate was reviewed to assess the type of pesticides supplied to the farmers in recent years during cropping seasons. The information gathered also included special programmes aimed at providing knowledge to farmers with regards to pests control. In addition, the eight major agrochemical shops at Mampong, Benim, Adidwan and Kofiase were contacted to obtain data on the pesticides sold to farmers, those patronized by farmers and how they (dealers) assist the farmers in acquiring knowledge on pesticide use.

Data analysis

Data were processed and subjected to Microsoft Excel (2010 version) and Statistical Package for Social Sciences (SPSS 16 version). Qualitative information from questionnaires was also used to supplement useful statistical outcomes. Apart from word format presentation, tables and charts were generated by the softwares and presented in the results.

Results

Socio-demography of respondents.

The study revealed that people under 20 years old were not engaged in tomato production. People dominating this activity were above 40 years and made up of 40% of the respondents followed by 31-40 years' group as in Table 1. It was also established that only 18% of the respondents were female as against 82% for their male counterparts. In terms of educational level of the farmers interviewed, only 26% of them had no schooling while a total of 74% had basic, secondary and tertiary education. The study revealed that 66% of the respondents had 6 or more years of experience in vegetable production.

Respondents' access to knowledge and information

The study revealed that only 36% of the tomato farmers claimed to have access to extension services while 64% claimed they did not have access (Table 2). On the frequency of contacts with the Agriculture Extension Agent (AEA), responses varied from weekly to monthly. It showed that 18% of the respondents had fortnightly contacts, while weekly and monthly contacts recorded 10% and 8% respectively, while those without extension was made up of 64%.

When asked whether they felt they had adequate extension education, especially with respect to pests control and pesticides use, 30% responded in the affirmative while 70% responded in the negative as in Figure 2. The respondents (70%) claimed they got information from other farmers and parents whom they worked with for some time before going on their own. Thirty percent (30%) of the respondents had had interaction with the defunct Ghana Organic Agriculture Network (GOAN) in the past through their AEAs in the use of neem extract as pesticide.

Table 1. Socio-demographic Characteristics of Respondents

	No. of respondents	Percent (%)
Age range (Years)		
20-30	48	24.0
31-40	72	36.0
Above 40	80	40.0
Total	200	100
Sex		
Male	164	82
Female	36	18
Total	200	100.0
Level of education of respondents		
No schooling	52	26.0
Basic school	80	40.0
Secondary	56	28.0
Tertiary	12	6.0
Total	200	100.0
Years of experience of respondents		
1-5	68	34.0
6-10	96	48.0
10 +	36	18.0
Total	200	100.0

Source: Field survey, 2016

Table 2. Respondents' Access to Extension Service

	No. of respondents	Percentage (%)
Accessibility to extension		
Yes	72	36.0
No	128	64.0
Total	200	100.0
Frequency of contact to AEA		
Weekly	20	10.0
Fortnightly	36	18.0
Monthly	16	8.0
No Extension service	128	64.0
Total	200	100.0

Source: Field survey, 2016

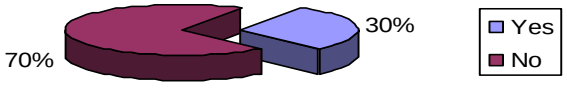

Figure 2. Farmers' response to adequacy of education on pests control and pesticides use
Source: Field survey, 2016

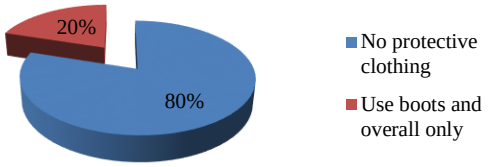

Figure 3. Use and non-use of protective equipment by farmers during spraying
Source: Field survey, 2016

Table 3. Type of Spraying Machine Used and Frequency of Spraying

	No. of respondents	Percent (%)
Spraying machine		
Knapsack	200	100.0
Mist blower	0	0.0
Total	200	100.00
Frequency of spraying		
Weekly	128	64.0
Fortnightly	62	30.0
Occasionally	12	6.0
Total	200	100.0
Pests control method		
Chemical	200	100
Manual	0	0.0
Alternative method (IPM)	0	0.0
Total	200	100.0

Source: Field survey, 2016

Precautionary measures before, during and after pesticides use.

It was established during the field observation and the questionnaire interview that over 80% of the farmers did not use the appropriate gear for their spraying exercises either due to ignorance or lack of resources to purchase them (Figure 3). Only 20% of the respondents wore boots, caps and overalls, while all farmers interviewed said they did not use respirators/nose masks, gloves and goggles during spraying. Handkerchiefs were used as improvised respirators during spraying and eating or smoking was not done when spraying. All respondents washed themselves and the spraying machine with soap after the exercise. Personal interaction with the farmers also showed that they did not observe the wind direction and also sprayed any time of the day provided it was not raining.

Frequency of use of chemical pesticides

The results showed that the knapsack was the sprayer used by all respondents (100 %) in pest control (Table 3). It further showed that all the respondents practiced calendar spraying of weekly (64 %) and fortnightly (30 %). In respect of alternative pests control measures, it was established that all respondents (100 %) depended mainly on chemicals to control insect pests, though some were taught how to use neem extract. The manual use of hand and/ hoe to uproot/weed under the crops is also a common practice among the respondents

Respondents' personal experience of the dangers of pesticides use

All the respondents (100%) according to the study claimed they knew or had had experience about the effects of pesticides on public health. They mentioned some dangers associated with its use such as dizziness, death or sickness if poisoned. Those who had experienced some form of poisoning testified to the agony they went through. (Fig 4a). Examples of death through self-poisoning (suicide) were mentioned in a couple of times. In terms of the effects of pesticides on crops, almost all the respondents acknowledged some effects they had ever observed such as burning/scotching of crops. They also talked of the drift of chemicals on nearby crops and residual effects of some chemicals on certain crops.

Regarding the environment, 82% of the respondents said they had heard on the mass media such as the television and radio that chemicals were polluting certain water bodies in some parts of the country especially around the mining communities, but had not heard much on agrochemical polluting the environment (Figure 4b). Eighteen percent however, indicated they read about chemicals effect on the environment.

Common disposal methods of empty containers

The old practice among vegetable farmers including tomato farmers in Ghana where empty containers after use were thrown away, reused, etc. was confirmed by this study with startling scenes in the study area. Forty-four percent (44%) of respondents said they threw containers away or left them on the farm for other farmers to see. They claimed the practice helped them in information sharing on the chemicals they use, while the rest buried or burnt them (Figure.5)

Chemical poisoning among tomato farmers in the study area

The study results and information from informants showed that the majority of tomato farmers and other farmers who use agrochemicals especially insecticides as the order of the day; had experienced some kind of poisoning. They said symptoms ranged from dizziness, headache, and in some serious cases vomiting and collapse of the applicant/user. Figure 6 indicates that 84% of the respondents experienced occasional poisoning at one time or the other during application while eight percent (8%) experienced poisoning very often. This is attributed to worn out parts of the spraying machine thereby causing leakage and inhaling of the spray (chemical mixture). It was also detected that most of the respondents did not consider the wind direction before undertaking any spraying activity. Therefore, when they are moving against the wind, they unconsciously inhale the spray and by the time they complete the exercise they had taken in a lot of the chemical.

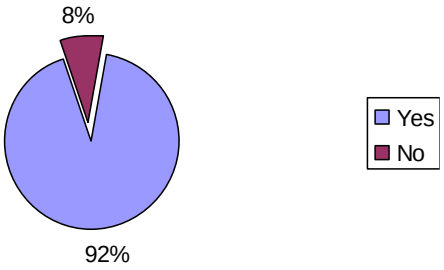

Figure 4a. Respondents' experience of chemical poisoning
Source: Field survey, 2016

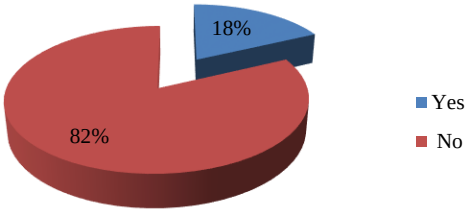

Figure 4b. Respondents' awareness of pesticides effects on the environment
Source: Field survey, 2016

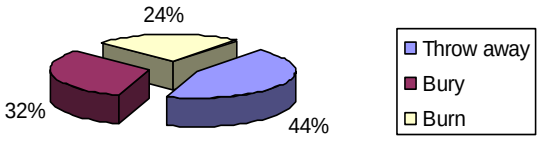

Figure 5. Respondents' disposal methods of empty containers
Source: Field survey, 2016

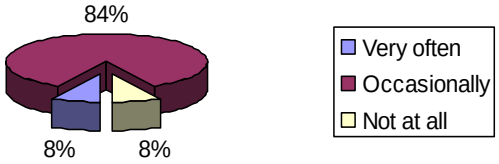

Figure 6. Respondents' frequency of chemical poisoning
Source: Field survey, 2016

Table 4. Farmers' Knowledge and Practice of Integrated Pests Management (IPM)

	No. of respondents	Percentage (%)
Knowledge of IPM		
Yes	10	5.0
No	190	95.0
Total	200	100.0
Practice of IPM		
Yes	0	0.0
No	200	100.0
Total	200	100.0

Source: Field survey, 2016

Farmers' knowledge and practice of IPM

The interview showed that only 5 % of the respondents had heard of Integrated Pests Management (IPM) and attended a workshop on the use of biological pesticides. The rest of the farmers (90%) had not heard of it and did not have any knowledge on IPM (Table 4). On the practice of IPM as an alternative pests control measure the results showed that no farmer among those interviewed practiced it on commercial scale.

Pests control methods used by farmers

Personal field visits to some sampled tomato farms in the five villages were made to cross-check the findings produced from the semi-structured interview. The field visits showed that all the tomato farmers relied solely on chemical pesticides (insecticides and fungicides) in controlling insect pests and diseases.

Observation of safe/re-entry periods before harvest

Safety periods observation among vegetable farmers is a huge problem in the country according to several reports (Wandaat and Kugbe, 2015; NPAS, 2012; Ackerson & Awuah, 2010). It was realized that the re-entry (safety) period after spraying by tomato farmers prior to harvest varied from five (5) days to two weeks (Figure.7). There are reports that some farmers out of the desire for money would harvest after two to three days of spraying especially during the lean season where there is a very high demand for vegetables including tomatoes (Wandaat and Kugbe, 2015; NPAS, 2012). It is also believed that farmers out of fear would tell any interviewer that the last spray was 10 days ago, because some of them adopt calendar spraying of every 3-10 days and could spray as many as times as possible as reported by Osei et al. (2013) and Amoako et al. (2012).

Farmers' Information Sources and Frequency of Contact

The survey showed that the extension services is the second least source respondents get information from. The source of information that respondents often rely on is from other farmers (40 %) while the official source (extension services is only 16 % (Table 5).

Government's interventions in building capacities of farmers

There is a well-organized research institution (the CSIR) mandated to generate agriculture technologies and information for well-structured unified extension services fully funded by the central government Research-Extension Linkage Committees (RELCs), a body comprising research, extension and farmers exist in all the 16 regional capitals of Ghana to build capacities and provide backstopping to agricultural extension officers.

The Agriculture Sub Sector Improvement Programme (AgSSIP) and the West Africa Agriculture Productivity Programme (WAAPP), both World Bank-funded programmes from 2002 to 2016 and recently the modernizing agriculture in Ghana (MAG), a Canadian Government-funded programme since 2017 contributed to building the capacities of extension officers and farmers.

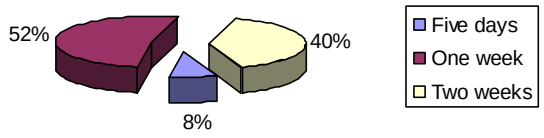

Figure 7. Respondents' observation of safety periods prior to harvest
Source: Field survey, 2016

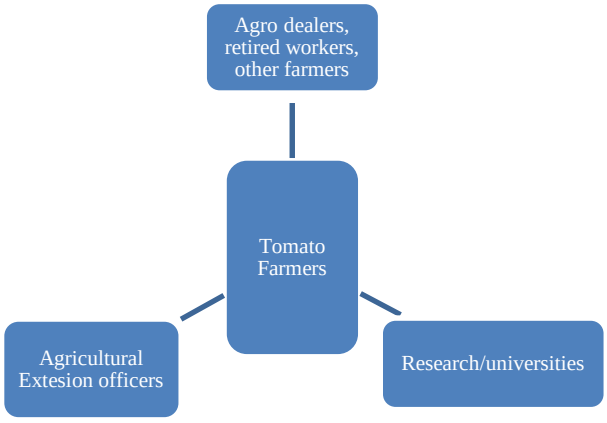

Figure 8. Information flow to tomato farmers in the study area
Source: Field survey, 2016

Table 5. Sources of information and frequency of contact of respondents

SI	FC	NR	%
Extension services	O	32	16.0
Chemical sellers	O	60	30.0
Neigbhours/other farmers	V	80	40.0
Retired workers/family members	V	28	14.0
Total	-	200	100.0

SI: Source of information; FC: Frequency of contact; NR: No. of respondents; O: Occasionally; V: Very often; Source: Field survey, 2016

The Planting for Food and Jobs under the Modernizing Agriculture in Ghana (MAG) programme has strongly supported agriculture knowledge generation (research) and extension delivery in all aspects of agriculture including vegetables. More extension workers were recruited in 2017 that improved the extension officer: farmer ratio that was 1:1500 in previous years. Logistics such as motor-bikes, pick-ups as well as traveling and transport allowances are provided to enhance knowledge and information sharing.

The effects of the enhanced logistics supply for the agricultural extension service delivery increased the annual outputs of some of the targeted crops such as rice, maize, cowpea and soybean by 34 %, 52.7%, 164% and 23% respectively between 2017 and 2020 under the PFJs programme (MOFA, 2021).

The high illiteracy rate among farmers especially female farmers is a serious challenge which makes it difficult for them to access information on pesticide use and source of credit for inputs and other related expenses. The other identified challenge during the study was the fact that many of the input suppliers especially their representatives (sales agents) are not skilled or competent enough to offer informed advice on the proper and safe use of agrochemicals and could mislead the farmers. Trainings organized by the Plant Protection and Regulatory Services Directorate (PPRSD) of MOFA and the Environmental Protection Agency (EPA) are always attended by the suppliers themselves and not the agents. It also showed that apart from the extension services (16%) as the main source of information, respondents get information from other sources such as other farmers, retired agriculture workers, chemical dealers, and family members (Table 5).

Knowledge and information flow from various disseminators to the farmers within the area's agriculture production value chain environment is shown in Figure 8.

Discussion

Socio- economic survey

It is established that 60 % of the respondents fall between the ages of 20 and 40 years and therefore necessary for serious attention to be given to them in terms of education on good agricultural practices to ensure the observance of these practice. This age range is very important for any nation as it is the most economically active group. There is also a high level of gender inequality in terms of tomato production as only eighteen percent (18%) of the respondents are female, an indication that income from this sector is skewed to the male farmers. The revelation relates to a report by the United Kingdom Department for International Development (2010) that total agricultural outputs in Africa could increase by up to 20% if women's access to agricultural inputs was equal to men's.

The educational levels of the farmers are very important as literate farmers are able to read and understand any technology including the use of pesticides. It also showed quite a good picture as majority of the respondents had at least basic education (Table 1). Despite the fact that majority of the respondents have some form of basic education, misuse of pesticides is still a problem among them. A study by Oyekale and Idjesa (2009) showed that the level of education has effect on skills acquisition and technology adoption among farmers. However, as the saying goes that "experience is the best teacher", the results showed a positive situation as 66% of the respondents have six or more years of experience in the industry. Therefore, even if illiterate farmers are into tomato production for up to six years and above, they would be able to learn and gain experience to produce safe and healthy crops. Unfortunately, this is not so as many of the farmers are not using pesticides appropriately. It could be that they do not appreciate the risks and dangers associated with the misuse of pesticides or they have been abusing the use all these years.

Access to knowledge and information about the crop and how to produce it and handle problems associated with the crop is critical in sustainable and environmentally friendly agriculture. It is therefore sad that even though there are extension workers stationed in the operational areas of the study communities the results showed that 64% of the respondents have no access to extension (Table 2). It is further known that those who have access (36%) do not

have regular contact with the extension agent as only 10% and 18% of the respondents have weekly and fortnightly contact respectively with the extension agent. This confirms Tanzubil and Boatbil (2014), Williamson (2003) and Bull (1982) that farmers in developing countries including Ghana do not have adequate knowledge of pests control including pesticides use. The majority (70%) of the farmers relied on other farmers for information which is quite disturbing as wrong practices could be spread to the whole of the farming community. As shown in Figure 2, the majority (70%) of the respondents acknowledge that they need more education on tomato production including chemical pests and disease control. From this revelation, it means that other forms of pests management is not available to the farmers to learn and adopt. It should also be noted that the extension worker: farmer ratio in the country of 1: 1500 and sometimes 1:3000 in some districts (MOFA, 2003), might be an important factor for the inadequate extension delivery for tomato farmers in the district. It has also been established that apart from the low extension worker: farmer ratio, logistical support is woefully inadequate as the majority of the extension workers do not have means of transport to cover the area assigned to them. This may be the reason why the farmer contacts with the AEA are very low (Table 6). The involvement of NGOs in extension delivery as far as vegetable production is concerned has been poor in the study area. It showed that 85% of the respondents have not interacted with any NGO on vegetable production and pests control including the use of pesticides.

Despite the impressive number of farmers with six or more years of experience, respondents did not know the effects of chemicals on the environment (Table 7), as they referred to pollution of water bodies in the mining areas of the country saying they did not know of water pollution caused by agrochemicals. The high incidence of reported poisoning during pesticide use confirms a finding at Akumadan in the Offinso North District and some parts of northern Ghana where pesticide poisoning was common among vegetable farmers (NPAS, 2012; Ntow et al., 2006; Ntow, 2001). The knapsack sprayer is the most popular spraying machine among the farmers probably due to the sizes of their fields and its affordability. Ntow et al. (2006) and Memuna et al. (2015) had similar findings at Akumadan and Ashiaman respectively with the use the knapsack sprayer except where farmers with fields over five acres used motorized sprayers such as the mist blower. According to them these farmers sprayed weekly or every 10 days, whether the level of the pest reached economic injury level or not. This was also established by Haleegoah et al. (2004) where calendar spraying led to the excessive spray of pesticides on vegetables in the Kumasi Metropolis. It is believed that biochemical analysis for pesticides residue in the tomato and other vegetables in the district would be positive.

All respondents depend solely on chemical pesticides for control of pests and diseases especially insect pests. This finding is in keeping with earlier reports that showed that in some parts of Accra, Asante Akim North and Sekyere Kumawu districts in Ashanti region where pesticides use was found to be the most popular pests management strategy as 65%-96% of farmers applied chemicals and in some case more than four times before harvest (Manu et al., 2021; Osei et al., 2013). The report concluded that cabbage like most vegetable farmers abuse the use of synthetic products such as pesticides and chemical fertilizers with its environment implications. The attitude of farmers in using pesticides indiscriminately through harvest has been confirmed by several workers (Amoako et al., 2012; Afari-Sefa et al., 2015; Donkor et al., 2016). They applied pesticides within the last 3 to 7 days before harvest and sometimes do not respect any waiting period as found in this study.

Hand-picking of insect pests such as grasshoppers and caterpillars as a control method is common on backyard gardens and not on large acreage as it is cumbersome and not practicable. Some of the respondents dispose empty containers on the field with the belief that other farmers especially the new entrants would know the chemical they used and termed the practice as information sharing mechanism.

Farmers' knowledge and practice of alternative pests control

There was a clear indication that the majority of respondents did not know of integrated pests management (IPM) and its practices and mostly use pesticides in their quest to fight pests and diseases. This is in keeping with a studies by Tanzubil and Boatbil (2014), Wandaat and Kugbe (2015) in the Upper east region and Ahafo regions of Ghana respectively where farmers did not have adequate knowledge in IPM practices and relied solely on pesticides to control pests and diseases. This may be considered an indictment on the part of the District Agriculture Directorate for failing to at least establish demonstrations on IPM practices in these communities. Precautions before, during and after pesticides use are essential for sound chemical pests control for the applicants and the environment at large. The majority of the respondents do not use the appropriate protective equipment during spraying. This may be due to a high level of ignorance and/or the cost of the protective gear leading to the high incidence of poisoning and intoxication among the farmers. Clarke et al. (1997) encountered a similar situation in the Accra plains where the vegetable farmers were lacking personal protective equipment (PPE) which was causing poisoning among the farmers. Observation of safety periods for re-entry or harvest is crucial in chemical pests control especially when it is insecticides and fungicides. It is alleged that vegetable farmers do not observe actual safety periods and the results of this study have confirmed this ill practice. As indicated above, Clarke et al. (1997) reported in the Accra plains where re-entry to the field few hours to three days after spraying recorded 88.9% with one week recording 11.0 % of the respondents. This malpractice may be due to ignorance coupled with the desire for quick money.

Knowledge generation and information dissemination to farmers

The CSIR-CRI and the CSIR-SARI are institutes of the CSIR-Ghana that are responsible for knowledge generation with respect to crops including vegetables for the forest and savannah agro-ecological areas of the country respectively. This is then passed on to the farmers through the extension services department of the Local Government Service and the Ministry of Food and Agriculture (MOFA). The Agriculture Extension Agents (AEAs) are mostly college-

level graduates holding certificate and/or diploma in general agriculture certificate. There are now many first degree holders in the districts as extension workers. The Ghana Institution of Horticulturists (GhIH), a professional body interested in the promotion of horticultural crops including vegetables and fruits sometimes organizes seminars, workshops, etc. for some vegetable farmers throughout the country.

Conclusion

Chemical pests control is the main control method known and practised by most commercial tomato farmers in the area. It is therefore consistent with the earlier works which indicated that a chemical pesticide is the main control method among vegetable farmers in Ghana and other developing countries (James et al., 2010). Farmers believe chemical pests' control has come at the right time to liberate them from the drudgery and the 'old way' of farming. It is a common practice among tomato farmers to apply chemical pesticides as many times as possible to control pests, some times without regard to the cost and effects on the crop and the environment.

It has been established that vegetable farmers in the area have inadequate knowledge on alternative pests and diseases control methods. The traditional methods of pests control such as the use of ash and hand picking is not feasible in commercial vegetable production. Others such as biological control are not known by the farmers. The use of botanicals like neem extract to control pests is not known and practiced by majority of the respondents. Integrated pests management (IPM) is not practiced mainly due to the lack of knowledge and expertise. The benefits of alternative pests and diseases control are enormous as vegetables are safe for consumers and the farmer is less exposed to poisoning while maintaining a sound environment. There is a huge gap between farmers' quest for knowledge on pests control methods (alternative and pesticides) and their access to knowledge and information. It is interesting to note that extension officers are available in most of the communities but impact is not much realized since their contact with the farmers (especially tomato farmers) is not impressive. This could be due to the fact that they (extension officers) have a lot of farmers to contact, and are also constrained logistically. Many of these farmers therefore, depend very much on their fellow farmers for knowledge and information, which sometimes could be misleading.

The reduction of pesticides use would not be possible in the foreseeable future considering the level of agrochemical influx into the country and the level of education given to the farmers. Unless there is a special intervention by the authorities in launching an alternative pest control programme including biological pesticides, IPM, etc. in the vegetable producing areas of the country. The tomatoes fruit worm *(Helicoverpa armigera*) is said to be one of the most notorious insect pests in the area. The Pesticide Action Network-UK (PAN-UK) once acknowledged the resistance of this pest to many insecticides (PAN-UK, 2002) and has been reported in recent times by Manu et al. (2021) in some parts of Ashanti region Vegetable production including tomato is a very lucrative business and well established in many parts of the country including the study area. The production of vegetables and tomato will be on the increase to meet the growing demands due to the increasing population and people's change in staple foods. This therefore, implies the control of pests and diseases would continue to be a major issue confronting these farmers as they would want to increase production and incomes.

Acknowledgment

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Phenotypic and Pathogenic Characterization of Leaf Fungi of Yam *(Dioscorea spp*) Varieties Grown In Côte D'Ivoire

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ABSTRACT

Yam (*Dioscorea spp*) occupies an important place in the diet of the populations of Côte d'Ivoire. It is a real source of starch and therefore generates enormous commercial potential. However, the decrease in production due to pest attacks represents a real threat to this crop. This study was conducted with the aim of improving yam production in Côte d'Ivoire. To do so, isolations carried out on yam leaves showing symptoms of foliar diseases have allowed us to identify 9 fungal genera. These were *Colletotrichum sp., Fusarium sp., Pestalotiopsis sp., Pestalotia sp., Botryodiplodia sp., Aspergillus sp., Mucor sp., Curvularia sp.* and *Phytophtora sp.* Among these fungi, the genus *Colletotrichum sp.* was the most isolated with a rate of 56% followed by the *Fusarium* and *Pestalotia* genera (8%). Pathogenicity tests performed on healthy leaves of two yam varieties revealed that the *Dioscorea alata* is more susceptible to fungi compared to *Dioscorea rotundata.* The largest average diameter of necrosis was caused by *Pestalotiopsis sp.* (5.97 cm) on the *Dioscorea alata* variety while the smallest was caused by *Colletotrichum sp.*9 on *Dioscorea rotundata* (0.5 cm). Combatting these fungi need to be developed for effective management of leaf diseases of yam in Côte d'Ivoire.

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Introduction

In tropical regions, root and tuber crops (cassava, sweet potato, potato and yam) are important food crops. Among these root and tuber crops, yam (*Dioscorea spp.*), with a global production of 75 million tons in 2021, is the staple diet for more than 500 million people in selected tropical countries in Africa, the Caribbean, Oceania, and Latin America (Onyeka et al., 2006; FAOSTAT, 2021)**.** In West Africa, where the large share of production is carried out, yam is one of the main sources of starch that actively contributes to the food security of the populations (Dansi et al., 2013).

In Côte d'Ivoire, yam occupies an important place in food crop production, with a yield of 7.8 million tons per year, which ranks the country as the second largest yam producing country after Nigeria (FAOSTAT, 2021). This production is mainly based on the species, *Dioscorea cayenensis-rotundata* and *Dioscorea alata*, which are grown on a large scale (Dansi et al.,2013). It is consumed by more than two-thirds of the population in several forms (Digbeu et al., 2009). Apart from the tuber, yam leaves are also used in traditional African pharmacopoeia for the treatment of various health problems (Kouakou, 2004). However, yam production is limited by enormous biotic and abiotic constraints including parasitic attacks such as viruses (Séka et al., 2009; Toualy et al., 2014), nematodes (Anjorin et al., 2014), bacteria (Denis, 2005) and fungi responsible for leaf necrosis (Amusa et al., 1996). Fungal leaf diseases are a real threat to this crop. Among these diseases, anthracnose is the most frequent fungal disease in Côte d'Ivoire and has an impact on yam production as it causes yield losses that can reach more than 90% (Green et Simon, 2007). The aim of this study is to analyze the diversity of fungal pathogens associated with yam leaf symptoms for an effective control approach.

Materials and Methods

Study Site

Samples were collected in localities located in the major yam-producing areas of eastern, northern, central and south-western Côte d'Ivoire (Figure 1). The characteristics of these different localities are shown in Table 1.

Plant Material

Leaves with characteristic fungal disease symptoms were used for fungal isolation and healthy leaves were used for pathogenicity testing of the different fungi isolated. The plant material used is composed of two lots of yam leaves (*Dioscorea alata*) and (*Dioscorea cayenensis-retoundata*). These two yam species were selected for pathogenicity testing after the surveys, taking into account the varieties grown and their susceptibility to foliar diseases.

Surveys and Sample Collection

Surveys were carried out on yam plots at the vegetative stage, 3 to 6 months after planting. Leaves showing different symptoms likes necrosis, spots and burns of fungal diseases observed on the different yam varieties were described, collected, photographed and then classified in envelopes. The samples were then taken to the laboratory for isolation.

Isolation of Fungi Associated with Symptoms

Isolation of fungal strains was done from explants (leaf fragments) taken from leaves showing symptoms of fungal diseases. Symptoms due to fungal attack were identified with reference to studies by (Yao et al., 2017). These explants were collected at the margin of the symptoms and cultured on Potato Dextrose Agar (PDA) medium.

Preparation of the PDA Medium

For the preparation of 1 liter of PDA medium, 20 g of mashed potato, 20 g of glucose and 20 g of agar-agar were weighed on a balance and put in a jar. The volume of the mixture was adjusted to 1 liter by adding distilled water. This medium was autoclaved at 121°C for 30 minutes under a pressure of 1bar. The resulting medium was dispensed into 9 cm diameter petri dishes under a laminar flow hood in the presence of a flame (Camara, 2011; Yao et al., 2017).

Seeding of Explants

Explants were washed with tap water, spread on sterile blotting paper (to remove excess water) and disinfected with 90° alcohol (Camara, 2011). Using sterile forceps, leaf fragments of about 1 cm^2 were cut at the growing front of the symptom and then seeded into petri dishes containing solidified PDA culture medium, with four explants per Petri dish. Two petri dishes were used per symptom. Petri dishes were labeled, sealed, and then incubated for 3 to 4 days at laboratory room temperature (25 ± 2°C).

Purification of Mushrooms

The fungal colonies observed underwent a first purification step from the Petri dish used for seeding the explants. The fungi were individually transplanted on new PDA media. Purification of the fungal strains consisted of transplanting each strain several times onto new media under sterile conditions so that a pure, individualized colony could be isolated (Camara, 2011).

Once the pure fungal strains had been obtained, the cultural characteristics were described in terms of the coloration, appearance and growth pattern of the mycelial colonies. Colony explants were mounted between slide and coverslip and observed under a light microscope at magnification (G × 400). Organs such as mycelia and spores were characterized. The Botton et al. (1990) identification key was used for strain identification.

Frequency of fungi isolation

The isolation frequencies of fungi were calculated according to the formula of Walder (1996):

$$\text{FI}\ (\%) = \frac{\text{NI}}{\text{NTI}} \times 100$$

FI (%): Frequency of isolation in percent

NI: Number of isolations of a fungal genus in all samples

NTI: Total number of isolations of all fungal genera.

Table 1. Characteristics of yam production areas surveyed in Côte d'Ivoire

Areas	Localities	Types of Soil	Pluviometry (mm)	Temperature (°C)	Climate
East	Bondoukou Bouna Tanda	deep sandy-clay	1400-2500	24-29	Topical wet and dry
North	Dabakala; Katiola Kanawolo	ferruginous	1150-1350	26-30	Sec tropical
Center	Bouaké Tiébissou Lolobo Djebonoua	ferrallitic ferruginous	1000-1700	25-38	Topical humide
South-West	Buyo Soubré Gagnoa Agboville	ferrallitic	1300-1600	26-30	Sub-equatorial

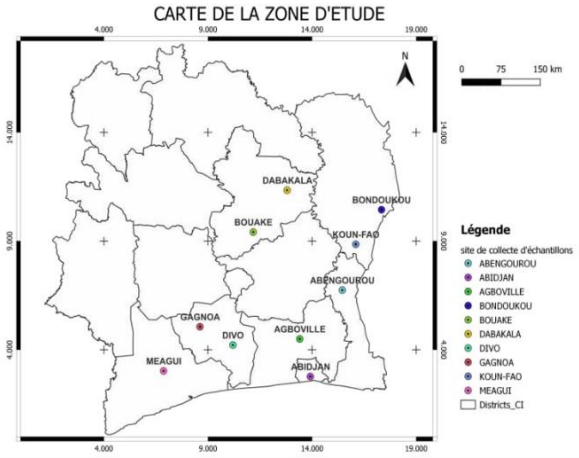


Figure 1. Map of Côte d'Ivoire showing sample collection areas

Spores Quantification and Growth Measurement of Colletotrichum Strains

To obtain sporal suspensions, five mycelial washers were placed in a test tube containing 10 ml sterile distilled water. The spore suspension was homogenized by vortexing, filtered through filter paper and the quantity of conidia per milliliter was assessed per strain under a light microscope using a Malassez cell. Three counts were made for each strain.

To determine the radial growth of the mycelium, daily measurements were taken from two perpendicular lines drawn on the underside of each Petri dish. Average diameters per day were obtained by applying the following formula (Hmouni et al., 2005):

$$\mathrm{Dm} = \frac{\mathrm{D1} + \mathrm{D2}}{2}$$

Dm: average daily growth diameter
D1: growth diameter along axis 1
D2: growth diameter along axis 2

Pathogenicity Test

The pathogenicity test was performed with 11 strains of *Colletotrichum sp.*; 2 strains of *Fusarium sp.* and *Pestalotia sp.* and one strain for the genera *Pestalotiopsis sp.*; *Botryodiplodia sp.*; *Curvularia sp.*; *Mucor sp.* and *Aspergillus sp.*

Healthy leaves of two yam varieties, Bètè Bètè (*Dioscorea alata*) and Assawa (*Dioscorea rotundata*) located between positions 4 and 7 from the stem apex were used for this test. These leaves were collected from three- to four-month-old plants. Three leaves were used per fungus and per variety. Three additional leaves per variety representing the controls were also used.

The leaves were first rinsed with tap water under a hood and placed on sterile blotting paper and disinfected with 90° alcohol. They were then placed in each 90 mm diameter Petri dish containing blotting paper cut into slices and moistened with sterile distilled water. The 12-day-old fungal colonies were used for inoculation (Silué et al., 2018). With sterile punches, mycelial discs of 5 mm diameter were formed. Referring to the work of Touré (2014), inoculations consisted of the deposition of a mycelial disc in the center of the upper surface of the leaf (Figure 2). The blotting papers were moistened with distilled water, every other day if necessary to maintain relative humidity and facilitate inoculum growth. Observations were made daily after inoculation for fourteen days.

Description of Symptoms

At the end of the experiment, the symptoms that appeared on the inoculated leaves were described. The description concerned the shape, contour and color of the symptoms (Yao et al., 2017).

Average Prevalences

To assess the prevalence of the symptom on each leaf, the number of leaves showing necrosis symptoms during the experiment was counted for each fungus and for each species. The percentage of the average prevalence was calculated according to the following formula (Yao et al., 2017).

$$\mathrm{PM}(\%) = \frac{\sum \mathrm{NFM}}{\mathrm{NTFM}} \times 100$$

PM (%): Average prevalence of disease,
NFM: Number of diseased leaves,
NTFM: Total number of diseased leaves,

Average Severity

The average severity of necrosis was assessed. Estimates were made using the Urban and Lebeda (2004) rating scale. For each yam leaf observed, the severity of necrosis was noted.

0: No symptoms on the leaves,
1: less than 25% of the leaf area infected,
2: 26-50% of leaf area infected,
3: 51-75% of leaf area infected,
4: more than 75% of the leaf area infected).

$$\mathrm{SI}\ (\%) = \frac{\sum(\mathrm{xi} \times \mathrm{ni})}{\mathrm{Z} \times \mathrm{N}} \times 100$$

SI (%): Severity index.
xi: Individual note to the disease symptom on each leaf.
ni: Number of times the rating score.
Z: Number of inoculated leaves.
N : Highest score on the scale.

Average Diameters of Necrosis

At the end of the experiment, the diameters of the necrosis induced by the different fungal strains were measured with a graduated ruler along two perpendicular axes drawn on the lid of the petri dish (Yao et al., 2017). The average diameter was evaluated 14 days after inoculation.

***Re-isolation* (koch's postulate)**

In order to verify the responsibility of fungal strains in the induction of necrosis, isolations were made from the observed symptoms. The fungal strains that infected the leaves were isolated from the induced necrosis. Koch's postulate was thus verified.

Statistical Analysis

The data obtained were subjected to an analysis of variance (ANOVA) with Statistica 7.1 software. In case of significant difference between the means, Newman Keul's test at the 5% threshold was used to classify the homogeneous groups.

Results

Observed Symptoms of Leaf Diseases

Various symptoms were observed on the leaves of all yam varieties in the surveyed localities (Figure 3). Three types of symptoms were observed : necrosis, spots and burns.

Isolation and Morphological Characterization of Fungi

Ten fungal genera were isolated from yam leaves showing symptoms of leaf diseases. Among them nine were identified. These were *Colletotrichum sp, Fusarium sp, Pestalotiopsis sp, Pestalotia sp, Botryodiplodia sp, Aspergillus sp, Mucor sp, Curvularia sp and Phytophtora sp* (Figure 3). The greatest species diversity was observed in the fungus *Colletotrichum sp.*

Relationship between Symptomatic Forms and Isolated Fungal Genera

Several fungal genera have been associated with a single type of symptom, just as a single fungus has been responsible for several symptoms. The *Colletotrichum* genus has been associated with several symptoms such as black necroses with yellow halo, brown spots, yellow necroses interspersed with black spots, marginal black and brown necroses, brown necroses with yellow patch, brown necroses with yellow halo, black spots with yellow halo, black necroses without halo, black and brown scorch marks, marginal brown scorch marks and scattered black spots on the leaf with blackening of the veins.

The genus *Fusarium* was isolated from deformed leaves showing black spots and yellow necroses dotted with black spots. The fungus *Pestalotioptis sp.* was associated with black and white necroses. *Botryodiplodia sp.* was associated with brown necrosis with a yellow halo. The fungus *Phytophtora sp.* was isolated from brown necroses with a yellow patch. The *Pestalotia sp.* genus has been isolated from three different symptoms. These are black spots with yellow halo, large black spots and small brown spots with yellow halo. The genera *Aspergillus, Mucor* and *Curvularia* were isolated respectively from brown discoloration, burns with yellow halo and burns without halo.

Isolates of Colletotrichum sp.

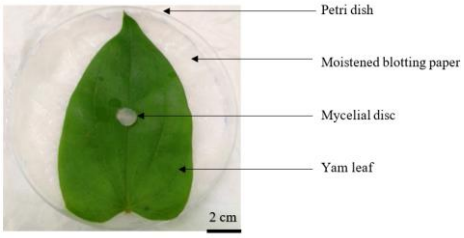
Eleven different strains of *Colletotrichum sp.* were isolated (Figure 4 A à K). The color of the thallus varied from white to black, yellow and gray. Some strains showed a pink or brown thallus mixed with white, not visible on the reverse side of the Petri dish. The thallus was abundant or contuncous in most cases, but also short in some cases. Microscopic examination revealed a wide variety of conidial shapes. Spores were cylindrical, oval, round with rounded or pointed ends. Conidia of various sizes were also observed in other strains. All strains of this genus showed branched mycelia under the light microscope, with the presence of septa for *Colletotrichum* sp. 1, 2, 5 and 6.Growth diameters at day 7 on PDA culture medium vared according to strain. It was maximal for the *Colletotrichum sp.* 7 strain, which reached an average growth diameter of 85 on day 7. he smallest mean growth diameter value (51.5 mm) was obtained with the *Colletotrichum sp.*9 strain. Conidia were predominantly cylindrical (92.85%) and round (7.14%). Spores quantities varied from strain to strain. *Colletotrichum sp.*2 recorded the highest number of spores per milliliter ($26,10^6$), while *Colletotrichum sp.*11 (3.2, 10^6 spores /ml) was the least sporulating strain (Table 2).

Isolates of Fusarium sp.

Two different strains of *Fusarium sp* were isolated :

The thallus of the first strain is yellow-white on the upper surface of the Petri dish and orange-yellow on the underside. Its cultural appearance is cottony. Microscopically, the conidia observed are elongated and curved with a branched, septate mycelium (Figure 4 L).

The thallus of the second isolate is white on the upper surface of the Petri dish and light yellow on the reverse. The cultural appearance is cottony, high in the center and short at the ends of the Petri dish. Under the light microscope, the conidia observed are curved, some with pointed, multi-partitioned ends (Figure 4 M). The mycelium is branched but not septate.



Figures 2. Yam leaf inoculated with a PDA disc (control)

Isolates of Pestalotia sp.

Two different isolates of *Pestalotia sp* were isolated.

The thallus of the *Pestalotia sp* 1 has a white coloration on the upper surface of the petri dish. Black sclerotia appear on the middle surface. The color is light yellow on the reverse side. The sclerotia are much more visible on the reverse side than on the top of the petri dish, but at a low density. The cultural aspect is lined and short. Microscopically the conidia are clavate, light to dark brown in the middle part and hyaline at the ends with three appendages. They have a tapered, transparent tip with dark medial segments and septate mycelium (Figure 4N).

The thallus of the *Pestalotia sp* 2 is yellow-white on the upper surface of the petri dish. In culture black sclerotia appear on the surface of the medium. The color is yellow on the reverse side. The sclerotia appearing on the reverse side have a low density. The cultural aspect is lined, short and radiating. Microscopically the conidia are clavate, dark brown in the middle part with two appendages. They have a tapered, transparent tip, medial segments years are darker (Figure 4O).

Isolates of Pestalotioptis sp.

The appearance of the mycelium is lined and short. In the culture white balls appear. Under the microscope the conidia are clavate, dark brown in the middle and without appendages. They have a tapered, transparent tip and the medial segments are more so mber (Figure 4P).

Isolates of Botryodiplodia sp.

Its cultural aspect is cottony, lined, high. In the culture black balls appear. Microscopic observation gives large oval conidia with rounded ends, surrounded by a sheath in the central part. The black septum is oriented in the direction of the smallest diameter of the conidia (Figure 4Q).

Table 2. Morphometric characteristics of isolated *Colletotrichum sp*. strains

Strains	Growth diameter at 7th day (mm)	Average growth speed (mm/day)	Appearance of the aerial thallus	Average number of conidia per milliliter ($\times 10^6$)
Colletotrichum sp.1	65,50c	8,19c	Cottony	8,0e
Colletotrichum sp.2	73,50b	9,19b	Abundant	26,0a
Colletotrichum sp.3	75,50b	9,44b	Hyalin	13,2c
Colletotrichum sp.4	66,83c	8,35c	Cottony	10,0d
Colletotrichum sp.5	61,27d	7,66d	Cottony	11,2c
Colletotrichum sp.6	81,67a	10,21a	Cottony	17,6b
Colletotrichum sp.7	85,00a	10,63a	Cottony	3,6g
Colletotrichum sp.8	59,17d	7,40d	Fibrous	4,0f
Colletotrichum sp.9	51,50f	6,44f	Cottony	13,6c
Colletotrichum sp.10	61,00d	7,63d	Cottony	10,4d
Colletotrichum sp.11	73,17b	9,15b	Cottony	3,2g

In the same column, values bearing the same letters are statistically identical at the 5% threshold according to the Newmann-Keuls test.

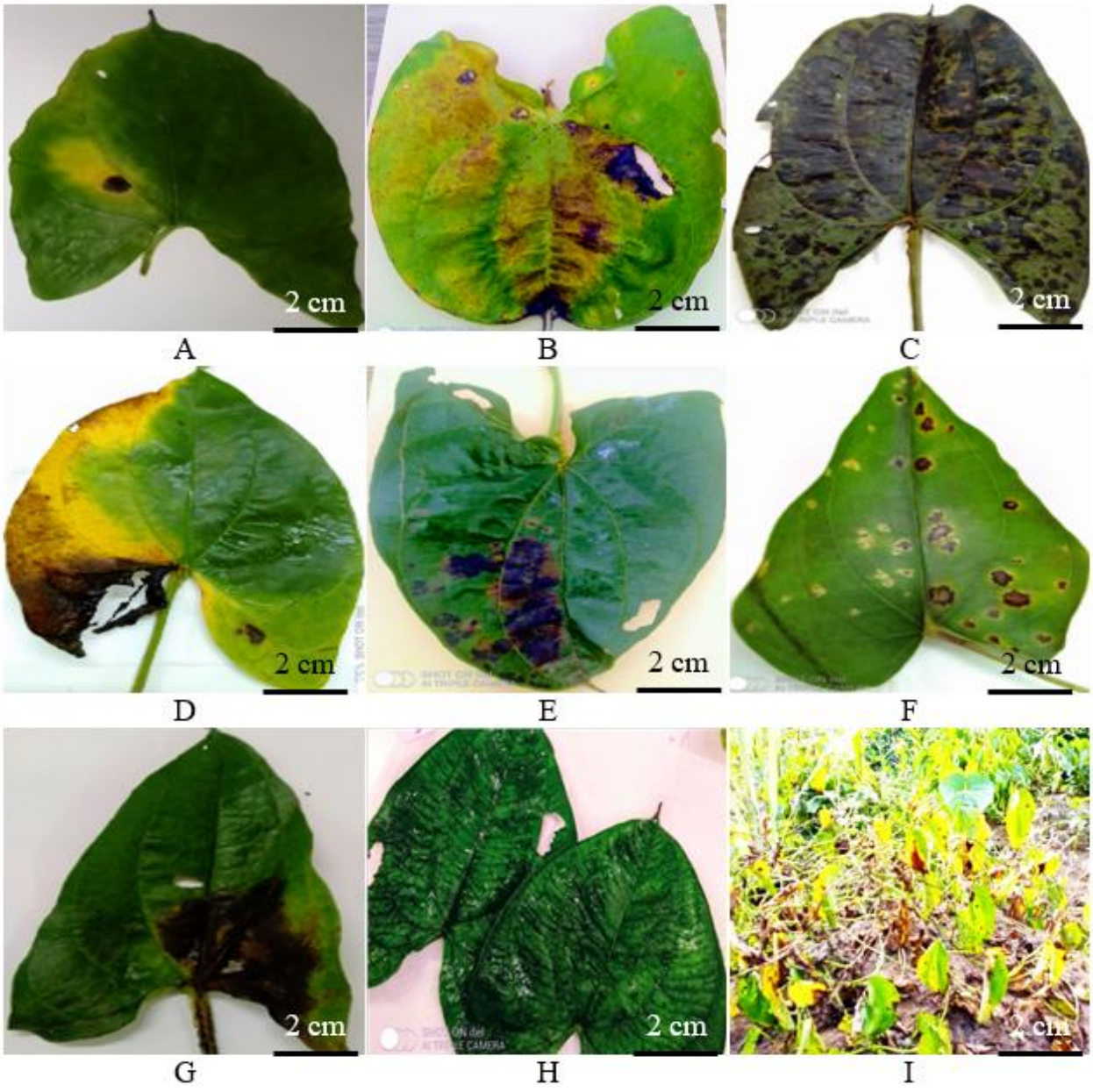


Figure 3. Some leaf disease symptoms observed on the leaves of different yam varieties

A : Brown necrosis with yellow patch; B : Black and brown burns; C : Large black spots all over the leaf; D : Brown and yellow marginal necrosis; E : Black burns; F : Brown necrosis with yellow halo; G: Black and brown necrosis with blackening of veins; H : Black spots + leaf deformations; I : Whole plant wilting.

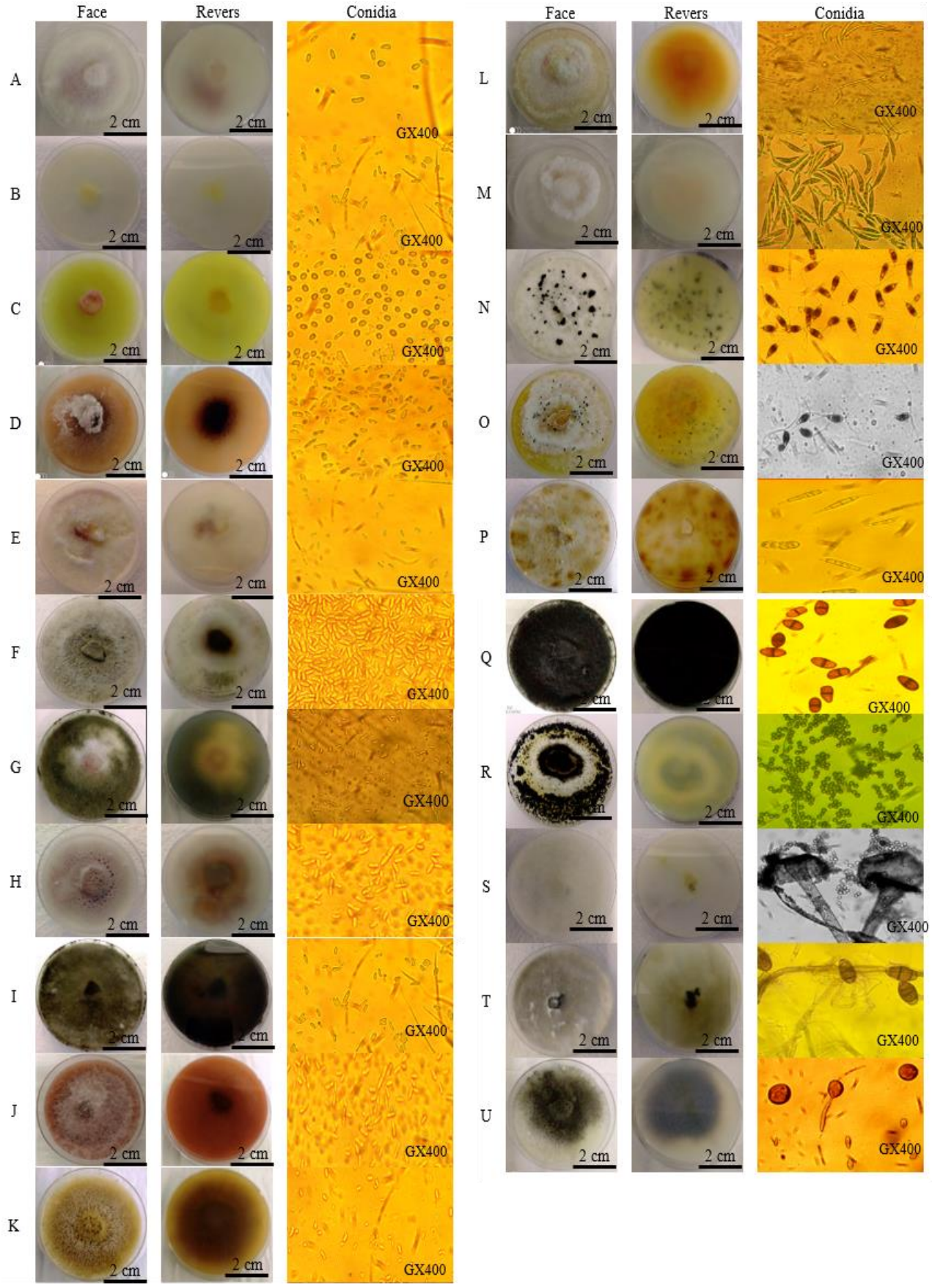


Figure 4. Fungi isolated from yam leaves showing symptoms of foliar diseases

A; *Colletotrichum* sp. 1; B: *Colletotrichum* sp. 2; C: *Colletotrichum* sp. 3; D: *Colletotrichum* sp. 4; E: *Colletotrichum* sp. 5; F: *Colletotrichum* sp. 6, G *Colletotrichum sp 7;* H: *Colletotrichum sp 8;* I: *Colletotrichum sp 9;* J: *Colletotrichum sp 10;* K: *Colletotrichum sp 11;* L: *Fusarium sp. 1;* M: *Fusarium sp. 2;* N: *Pestalotia sp. 1.;* O: *Pestalotia sp. 2 ;* P: *Pestalotioptis sp. ;* Q: *Botryodiplodia sp. ;* R: *Aspergillus sp;* S: *Mucor sp;* T: *Curvularia sp;* U: *Phytophthora sp*

Isolates of Aspergillus sp.

The thallus of the mushroom is white-yellow on the upper side of the petri dish and yellow-brown on the reverse side. The cultural aspect is lined, short, with an irregular growth. Microscopic observation yields circular, tip-attached spores (Figure 4R).

Isolates of Mucor sp.

The thallus of the mushroom has a grayish coloration of face and yellow-grayish on the reverse. The cultural aspect is abundant, high, uniformly growing. Microscopic observation shows oval, grayish-brown conidia. The mycelium is branched (figure 4S).

Isolates of Curvularia sp.

The thallus of the mushroom is of color gray of face and yellow-gray to the reverse. The cultural appearance is lined and concentrically crescent. Microscopically the conidia are curved, penta-septate s, alternating gray and black (Figure 4T).

Isolates of Phytophtora sp

The thallus of the fungus is colored in white-verses of face and white-violet on the reverse. Its cultural appearance is cottony, elevated, growing and irregular. Microscopically the conidia are oval and elongated on one side (Figure 4U).

Frequencies of isolation of fungi

Figure 4 shows the isolation frequencies of the fungi. The genus *Colletotrichum sp* was isolated most frequently with a frequency of 56% **(**Figure 5). It is followed by the genus *Pestalotiopsis sp* and *Fusarium sp* with a frequency of 8%. All other fungi had the same frequency of 4%.

Symptoms Caused by the Fungi after Inoculation

All the fungi inoculated on yam leaves belonging to the *Dioscorea alata* and *Dioscorea rotundata* species induced different types of symptoms compared with the control (Figures 6 and 7), with the exception of the *Colletotrichum sp. 10* and *Botryodiplodia sp*. strains (Figure 8). These two strains did not induce any symptoms on yam leaves of the Assawa variety (*Dioscorea rotundata*). These included brown, black, brown with yellow halo and brown without yellow halo necroses on yam leaves of the Bètè Bètè variety (*Dioscorea alata*), and brown, brown with yellow halo and brown without yellow halo necroses on those of the Assawa variety (*Dioscorea rotundata)*. Symptoms were not specific to a single fungal genus inoculated. Several fungal genera were responsible for a single type of symptom. Thus, brown necrosis with yellow halo was induced by the genera *Colletotrichum Pestalotia* and *Botryodiplodia*. The genera *Aspergillus, Mucor* and *Pestalotiopsis* caused black necrosis. Brown necrosis without halo was caused by the *Curvularia* and *Fusarium* genera.

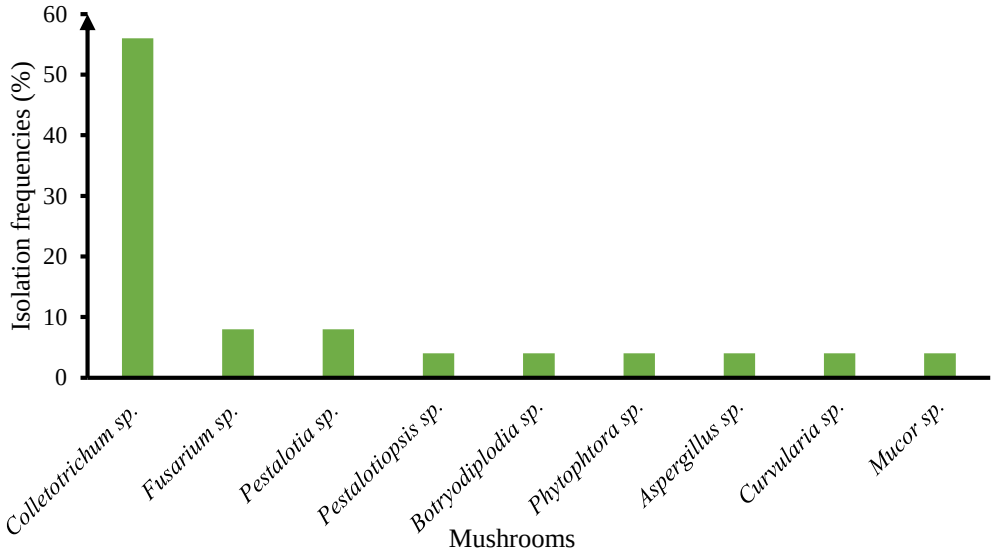


Figure 5. Isolation frequencies of different fungal genera isolated from yam leaves collected in Côte d'Ivoire.

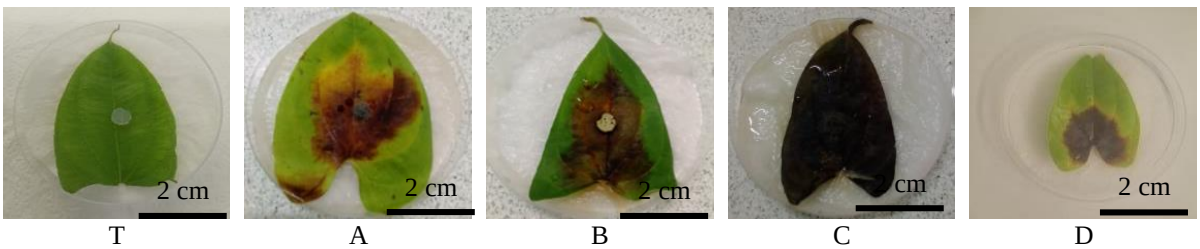


Figure 1. Different types of necrosis induced in vitro by fungal genera deposited on leaves of the Bètè Bètè variety (*Dioscorea alata*) 14 days after inoculation.

A: Brown necrosis with yellow halo ; B: Brown necrosis without yellow halo; C: Black necrosis ; D: Brown necrosis with yellow halo; T: Control leaf

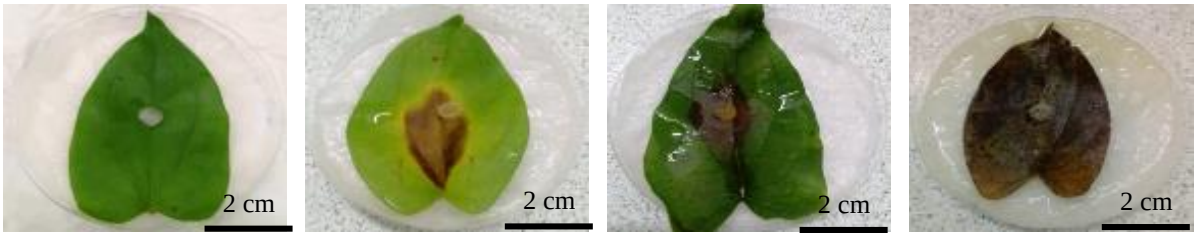


Figure 7. Different types of necrosis induced in vitro by fungi deposited on leaves of the Assawa variety (*Dioscorea rotundata*) 14 days after inoculation.

A: Brown necrosis with yellow halo B : Brown necrosis without halo; C : Black necrosis; T : Control leaf

Figure 2. Fungi that did not induce any symptoms after inoculation on leaves of the Assawa variety (Dioscorea rotundata)

A: *Colletotrichum* sp. 10 ; B: *Botryodiplodia* sp.

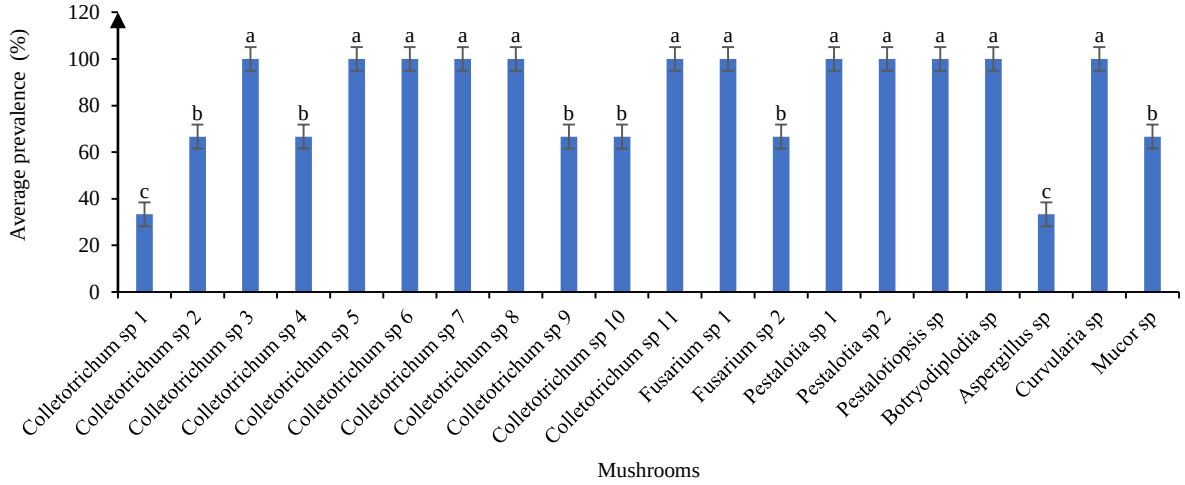


Figure 9. Average prevalences of necrosis obtained 14 days after inoculation of the fungi on the leaves of *Dioscorea. alata.*

Bars with the same letters are statistically identical at the 5% threshold according to the Newmann-Keuls test.

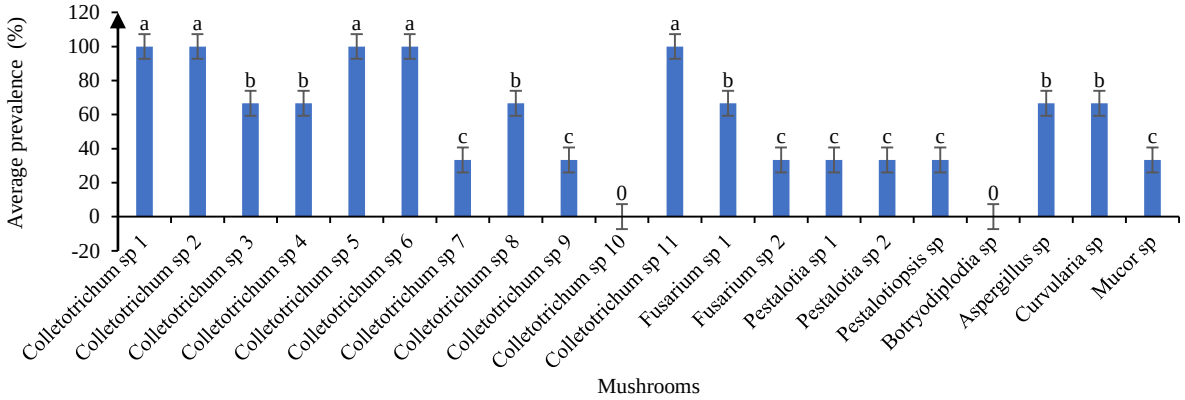


Figure 10. Average prevalences of necrosis obtained 14 days after fungus inoculation on leaves of *Dioscorea. rotundata* species.

Bars with the same letters are statistically identical at the 5% threshold according to the Newmann-Keuls test.

Average Prevalences of Necroses

The average prevalences of necrosis varied according to species and fungi inoculated. The average prevalence of necrosis in *Dioscorea alata* specie ranged from 33.33 to 100%. Rates of 100% were obtained for *Colletotrichum sp.* 3, *Colletotrichum sp* 5, *Colletotrichum sp.* 6, *Colletotrichum sp*. 7, *Colletotrichum sp.* 8, Colletotrichum *sp* 11, *Fusarium sp.* 1 *Pestalotia sp.* 1, *Pestalotia sp.* 2, *Pestalotiopsis sp*, *Botryodiplodia sp*, and *Curvularia sp. Colletotrichum sp*. 2, *Colletotrichum sp*. 4 *Colletotrichum sp*. 9, *Colletotrichum sp.* 10, *Fusarium sp.* 2, and *Mucor sp* induced 66.66%. Finally *Colletotrichum sp1* and *Aspergillus sp., on the other hand,* induced a rate of 33.33%. (Figure 9).

In contrast, the average prevalence of necrosis in *Dioscorea rotundata* species ranged from 0 to 100%. The rates of 100% were obtained with *Colletotrichum* sp.1, *Colletotrichum sp*.2, *Colletotrichum* sp.5, *Colletotrichum sp*.6 and *Colletotrichum* sp.11. *Colletotrichum sp*.3, *Colletotrichum* sp.4, *Colletotrichum* sp.8, *Fusarium* sp.1, *Aspergillus sp* and *Curvularia sp* induced a rate of 66.66%. *Colletotrichum* sp 7, *Colletotrichum sp* 9, *Fusarium sp* 2, *Pestalotia sp.* 1, *Pestalotia* sp. 2, *Pestalotiopsis* sp, and *Mucor sp* induced 33.33%. Finally the fungi *Colletotrichum* sp.10 and *Botryodiplodia sp.* did not induce symptoms. (Figure 10). The results obtained show that the *Dioscorea alata* variety is more susceptible to fungal attacks than the *Dioscorea rotundata* specie.

Average severity of necrosis

The average necrosis severities varied according to the species and the fungi inoculated. The average necrosis severity of the specie *Dioscorea alata* varied from 0 to 100%. The fungi *Fusarium sp* .1, *Pestalotia sp.* 1 and *Botryodiplodia sp* caused the most severe symptoms to 100%. The other fungi had an impact on the leaves, except for the fungus *Fusarium sp.* 2 which did not induce any symptoms on the leaves. (Figure 11). The average necrosis severity of the *Dioscorea rotundata* specie ranged from 0 to 91.66%. *Colletotrichum sp.* 6 and *Colletotrichum sp.* 11 fungi caused the most severe symptoms at 91.66% compared to the control. The other fungi had an impact on leaves, except *Colletotrichum sp*. 7, *Colletotrichum sp*. 9, *Colletotrichum sp.* 10 and *Botryodiplodia sp* induced no symptoms on leaves. (Figure 12). Statistical analysis showed that there was a significant difference between the fungi inoculated in relation to the two yam species used. The fungi *Colletotrichum sp.* 3,6 and 11 ; *Fusarium* sp.1 and *Botryodiplodia sp.* were the most virulent on *Dioscorea alata*, while *Colletotrichum* sp.6 and 11 were the most virulent on *Dioscorea rotundata*.

Average diameters of Necrosis

Average necrosis diameters varied with yam varieties and fungi inoculated. The average diameters of fungus-induced necrosis on the leaves of *Dioscorea alata* species ranged from 0.92 to 5.97 cm. The largest average diameter was obtained with *Pestalotia sp.* 1 (5.97 cm). *Fusarium sp.* 2 induced the smallest mean diameter of necrosis (0.92 cm). Statistical analyses showed a significant difference between the means of necrosis. Eight homogeneity groups were obtained (Table 3).

The average diameters of fungus-induced necrosis on the leaves of the *Dioscorea rotundata* specie ranged from 0.00 to 5.25 cm. The largest average diameter was induced by *Colletotrichum sp*. 6 (5.25 cm). In contrast, *Colletotrichum sp*. 10 and *Botryodiplodia sp. did* not induce any necrosis on the leaves of this variety. Statistical analyses showed a significant difference between the means of necrosis. Eight homogeneity groups were obtained. Statistical analyses showed a significant difference ($P<0.000612$) between the mean necrosis diameters of *Dioscorea alata* and *Dioscorea rotundata* species.

Re-isolation of Fungal Pathogens

Isolations made from the different necroses observed resulted in the same fungi being inoculated. Koch's postulate is thus verified.

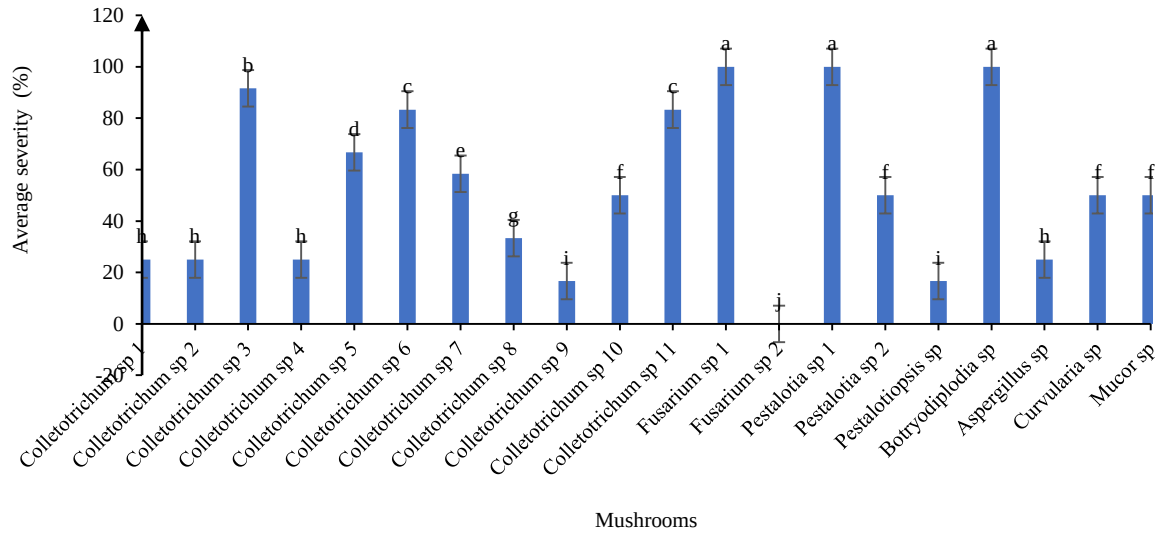


Figure 11. Average severity of necrosis obtained 14 days after inoculation of on Dioscorea alata leaves.
Bars with the same letters are statistically identical at the 5% threshold according to the Newmann-Keuls test.

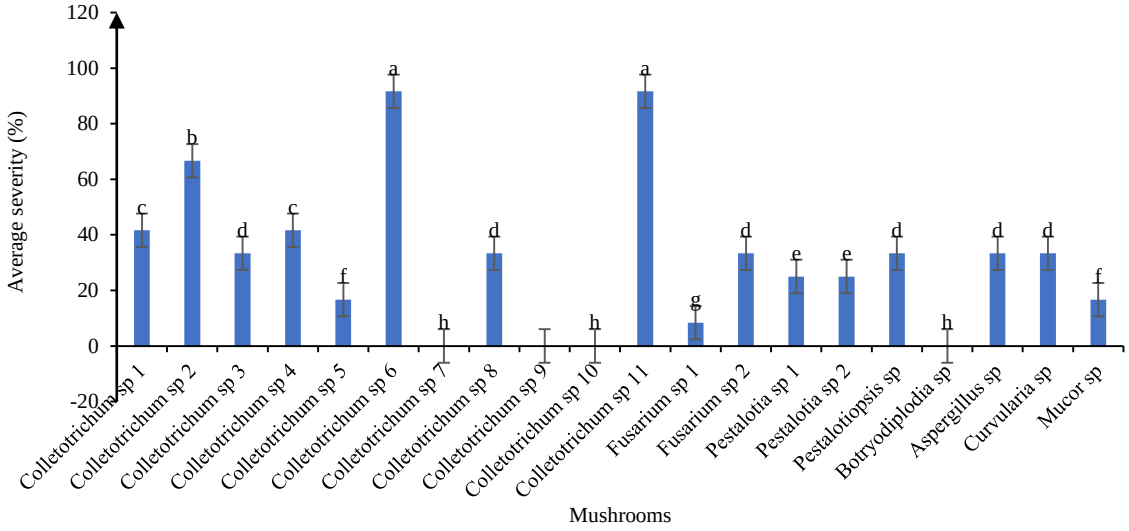


Figure 12. Average severity of necrosis observed 14 days after inoculation of fungi on the leaves of *Dioscorea rotundata*

Bars with the same letters are statistically identical at the 5% threshold according to the Newmann-Keuls test.

Table 3. Average diameters of necrosis induced by fungi inoculated on leaves of *Dioscorea alata and Dioscorea rotundata*

Mushrooms	Average diameter of necrosis (cm)	
	Dioscorea alata	*Dioscorea rotundata*
Colletotrichum sp. 1	1,55 ± 0,25d	4,35 ± 0,81b
Colletotrichum sp. 2	2,58 ± 0,72c	4,47 ± 0,64b
Colletotrichum sp . 3	5.62 ± 0.3a	2.85 ± 1.6c
Colletotrichum sp . 4	2.40 ± 1.35c	2.83 ± 1.55c
Colletotrichum sp . 5	4,07 ± 1,23bc	2,50 ± 0,78c
Colletotrichum sp . 6	5.07 ± 0.63ab	5.25 ± 1.01a
Colletotrichum sp . 7	3.87 ± 1.19c	0.62 ± 0.61e
Colletotrichum sp . 8	3.87 ± 1.16 c	2.02 ± 1.56cd
Colletotrichum sp . 9	2,08 ± 1,4cd	0,50 ± 0,5e
Colletotrichum sp . 10	3,58 ± 1,88c	0,0 ± 0,0e
Colletotrichum sp. 11	3.93 ± 1.2c	4.98 ± 1b
Fusarium sp . 1	5,28 ± 0,25a	1,68 ± 2,3d
Fusarium sp . 2	0,92 ± 0,53e	2,37 ± 2,36c
Pestalotia sp . 1	5,97 ± 1,25a	0,83 ± 0,5e
Pestalotia sp. 2	5.17 ± 0.72a	0.83 ± 0.83e
Pestalotiopsis sp	1.95 ± 0.51d	1.88 ± 1.88d
Botryodiplodia sp	4,72 ± 0,83b	0,00 ± 0,0e
Aspergillus sp	1,27 ± 1,26d	2,57 ± 1,38c
Curvularia sp	4.32 ± 1.1 b	2,25 ± 0,5c
Mucor sp	3,02 ± 0,1c	1.63 ± 1.31d
Probability (p)	P =0,000612	

In the same column, values bearing the same letters are statistically identical at the 5% threshold according to the Newmann-Keuls test.

Discussion

The Diagnosis of foliar symptoms in the production zones surveyed revealed a number of fungal disease symptoms on the yam species *Dioscorea alata* and *Dioscorea cayenensis-rotundata*. These include black, brown, grey and yellow spots, necroses and burns. These results corroborate those of Amusa et al. (1996) whose work in Nigeria on the leaves of *Dioscorea alata* and *Dioscorea rotundata* showed the presence of three types of necrosis from which the fungi were isolated. Similar results were obtained by Touré (2014) during work carried out on the diagnosis of leaf symptoms in four localities in Côte d'Ivoire. The same type of necrosis was observed on the leaves of different varieties of *Dioscorea alata* and *Dioscorea rotundata* species. This similarity in necrosis type could be the presence or attack of the same fungal genus on yam leaves. Studies by Amusa et al. (1996) showed that the main causal agent of anthracnose on *Dioscorea alata* and *Dioscorea rotundata* leaves was *Colletotrichum gloeosporioides*. Different types of symptoms were observed on the leaves of the same yam variety. This diversity of symptoms could be due to the presence of several fungal genera parasitizing the leaves of the same yam variety. Studies carried out in Ghana showed that two types of necrosis were observed only on necrotic

leaves of *Dioscorea alata* (Twumasi, 1986). In addition, studies carried out by Touré (2014) in four localities in Côte d'Ivoire, specifically Nassian, Soubré, Babadougou and Toumodi on *Dioscorea alata* leaves showed the presence of three different types of necrosis on the Bètè Bètè variety.

Several fungal genera were isolated, nine of which were identified. These were *Colletotrichum sp. ; Fusarium sp. ; Pestalotia sp. ; Pestalotioptis sp. ; Botryodiplodia sp. ; Aspergillus sp. ; Mucor sp.* and *Curvularia sp*. These results indicate that these different fungal genera are associated with the symptoms observed on yam leaves collected in production zones. Work carried out on the symptomatology, etiology and incidence of yam leaf disease has highlighted the presence of fungi such as : *Aspergillus sp.; Botryodiplodia sp.; Colletotrichum sp.; Curvularia sp.; Fusarium sp.; Mucor sp.; Pestalotiopsis sp.* (Touré, 2014). The fungal diversity associated with necrotic yam leaves has been confirmed by the work of Achar et al. (2013). These authors isolated *Colletotrichum gloesporioides, Fusarium oxysporum, Pestolatia macrotrichia, Cercospora* and *Cladosporium* fungi from *Dioscorea alata* and *Dioscorea bulbifera* leaves. Isolation frequency was highest for the *Colletotrichum* genus. This fungal genus is widely recognized as responsible for anthracnose on the leaves and fruit of many plants (Freeman, 1998 ; Arauz, 2000). Studies by several authors have shown the preponderance of this fungal genus on yam leaves. Indeed, the work of Touré (2014) in Côte d'Ivoire isolated the *Colletotrichum* genus at 28.9% on yam leaves. Furthermore, the high presence of the *Colletotrichum* genus among the fungi isolated from *Dioscorea alata* leaves was confirmed by the work of Abang et al., (2003) during their study. The work of several authors has shown that this fungal genus is responsible for anthracnose in several crops around the world. Indeed, Silué (2019) isolated this pathogen from various cashew organs with a rate of 63.45%. Also, Ehui et al. (2019) had demonstrated that a very high diversity of this fungus is associated with cassava anthracnose in Côte d'Ivoire. The majority of inoculated fungi infected the leaves of both yam species. The fungi were more virulent on the Bètè Bètè variety of the *Dioscorea alata* species compared with the Assawa variety (*Dioscorea rotundata*). These results corroborate those of Kwasi et al. (2019) whose work in Togo shows that varieties of the species *Dioscorea alata* are highly susceptible to inoculation by strains of *Colletotrichum sp.* Studies carried out by Touré (2014) in Côte d'Ivoire on *Dioscorea alata* leaves showed that the fungi *Colletotrichum sp., Rhizoctonia sp, Rhizoctonia sp., Botryodiplodia sp., Pestalotiopsis sp.* and *Curvularia sp.* were able to induce necrosis on detached leaves. Different types of necrosis have been induced by in vitro inoculation of detached *Dioscorea alata* and *Dioscorea rotundata* leaves with the fungi used. Studies carried out by Amusa et al. (1996) on the leaves of *Dioscorea alata* and *Dioscorea rotundata* showed three types of necrosis induced after inoculation with the fungi. All the symptoms induced by these various inoculated fungi, as well as those observed during field sampling, are generally attributed to anthracnose. This confusion is due to the fact that anthracnose caused by fungi of the *Colletotrichum* genus is the most widely described leaf disease of yam in the literature. However, this study has enabled us to identify the fungi associated with each type of symptom observed. Thus, the genera *Colletotrichum* and *Pestalotia* have been associated with several symptoms. *Colletotrichum* was isolated from symptoms such as black necrosis with yellow halo, brown spots, yellow necrosis with black dots, black and brown marginal necrosis, brown necrosis with yellow patch, brown necroses with yellow halo, black spots with yellow halo, black necroses without halo, black and brown burns, marginal brown burns and scattered black spots on the leaf with blackening of the veins. *Pestalotia* fungi were associated with three symptoms : black spots with yellow halo, large black spots and small brown spots with yellow halo. This result is contrary to that of Mignouna et al. (2001) who attributed these same types of symptoms to yam anthracnose. Touré (2014) isolated the *Colletotrichum* genus from *Dioscorea alata* leaves showing the large black spots.

The fungus *Botryodiplodia sp*. was associated with brown necroses with a yellow halo. This result disagrees with that of Touré (2014), who isolated *Curcularia sp.* from the same type of necrosis in Côte d'Ivoire. Furthermore, brown necrosis with a yellow halo had been described as that caused by *Curcularia sp*. in Guadeloupe (INRA, 2005).

The genus *Fusarium* has been isolated from deformed leaves showing black spots and yellow necroses dotted with black spots. The fungus *Pestalotioptis sp*. was associated with black and white necroses. The genera *Aspergillus, Mucor* and *Curvularia* have been isolated respectively associated with brown discoloration, burns with yellow halo and burns without halo. However, all these symptoms were described as that of yam anthracnose in the work of Yao et al., (2017) in Côte d'Ivoire. These symptoms have also been observed by several authors on yam leaves and seedlings as characteristic symptoms of yam anthracnose around the world, particularly in the Pacific**,** Guadeloupe and Nigeria (Amusa et al., 2003 ; Jacqua et al., 2008 ; Bakayoko et al., 2022). In addition to anthracnose caused by fungi of the *Colletotrichum* genus, this study highlights the diversity of leaf diseases of yam in Côte d'Ivoire. The prevalences and severities obtained after the inoculations depended on the fungi and yam varieties. The fungi *Colletotrichum sp.* 3,6 and 11 ; *Fusarium* 1 and *Botryodiplodia sp.* were the most virulent on *Dioscorea alata*, while *Colletotrichum sp*.6 and 11 were the most virulent on *Dioscorea rotundata*. Studies carried out in Côte d'Ivoire on the leaves of *Dioscorea alata* and *Dioscorea cayenensis-rotundata* species showed a difference in the prevalences and severities obtained following the pathogenicity test (Touré, 2014). The work of Yao et al, (2017) also showed a diversity of necroses caused by different fungi on yam leaves. The average diameters of necroses obtained on leaves of *Dioscorea alata* and *Dioscorea rotundata* species varied during the experiment according to variety and fungus. The largest average diameter for the Bètè Bètè variety belonging to the *Dioscorea alata* species was obtained with the *Pestalotia sp.* 1 fungus. For the Assawa variety of the *Dioscorea rotundata* species, the largest diameter was obtained with the fungus *Colletotrichum sp*. 6. These results show that necrosis size varies according to the degree of aggressiveness of the fungal strains. Inoculations carried

out by Yao et al., (2017) on *Dioscorea alata* leaves with mycelial discs of several fungal genera showed different necrosis sizes and by Rekad (2018) during studies conducted at Algérie on Tomato leaves inoculated with isolates of *Phytophthora infestans*.

Conclusion

This study highlighted the diversity of fungal genera associated with yam leaf disease symptoms in Côte d'Ivoire. Among these fungi, the *Colletotrichum* genus was the most isolated. These fungal genera isolated from diseased yam leaves proved effectively pathogenic on detached feulae of both yam varieties. The fungi *Colletotrichum sp.* 3,6 and 11 ; *Fusarium sp.* 1 and *Botryodiplodia sp* were the most virulent on *Dioscorea alata*, while *Colletotrichum sp*.6 and 11 were the most virulent on *Dioscorea rotundata*. These fungi need to be effectively controlled in order to stem yield losses due to foliar diseases and preserve yam orchards in Côte d'Ivoire and around the world.

Competing Interests

The authors declare that they have no competing interests in the publication of this work.

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A Research on Fertility, Herd Life, Milk Production and Milk Quality Characteristics of Simmental (Fleckvieh) Cows: 2. Milk Quality

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ABSTRACT

The aim of this study was to determine the milk quality characteristics of Simmental (SIM) cows of Austrian origin, which have increased the interest of breeders in Türkiye in recent years. For this aim, the milk analysis results of a farm located in Menemen County, İzmir/Türkiye from 2012 to 2021 were used. Milk fat (MF, %), protein (MP, %), lactose (ML, %), total dry matter (TDM, %) contents and somatic cell count (SCC, cell/ml) were determined. In order to determine the current situation, milk samples were taken from the cows (90 heads) in August 2021, and in addition to the above milk components, the solid non-fat (SNF) and freezing point (FP) were determined. The effects of sampling season, calving month, lactation month, sampling season x calving month and sampling season x lactation month interactions were found to be statistically significant for all traits ($P<0.05$). Parity and calving month effects on Log_{10}SCC were also detected to be statistically significant ($P<0.05$). The mean MF, MP, ML, TDM, FP and SCC of SIM cattle were 3.71±0.018%, 3.42±0.009%, 4.63±0.009%, 12.49±0.03, -0.535±0.003°C and 5.14±0.01 (138.038 cells/ml), respectively. It was concluded that the milk components of Austrian-origin SIM cattle are not very different from the Holstein-Friesian (HF) breed, however, in the low SCC average for many years, besides the important contribution of the measures taken against mastitis in the farm, the resistance against mastitis may be higher in this genotype. This situation is thought to be the reason why breeders in Türkiye prefer Austrian-origin SIM cattle in addition to high milk yield and carcass weight.

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Introduction

Milk quality is examined in two groups as nutritive properties, namely milk components and hygienic properties. While the fat (MF), protein (MP), lactose (ML), mineral, solid-non-fat (SNF), total dry matter (TDM), casein (MC) contents and etc. in milk are taken into account in the nutritional properties of milk, when the hygienic quality of milk is mentioned, total bacterial count, somatic cell count (SCC) and antibiotic residue are understood.

In the studies on the milk components of Simmental (SIM) cattle (Akbulut, 1998; Şekerden et al., 1999; Polanski et al., 1992; Koç and Arı, 2020), MF (3.90-4.1%), MP (3.38-3.90%), ML (4.86%), SNF (8.6-9.09%), TDM (11.18-12.6%), MC (2.5-2.7%), freezing point (FP, -0.577°C), milk urea nitrogen (MUN, 12.07 mg/dL), oleic acid (OA, 0.258 g/100 g) and beta hydroxy butyric acid (BHBA, 0.284 mmol/L) levels were determined. In addition, there were also studies on milk compounds in Holstein-Friesian (HF) and Brown-Swiss (Koç, 2007a), HF (Koç, 2008; Kaya et al., 2014), HF and Montbeliarde (MB) breeds (Koç, 2009; 2011), Red-Holstein (RH) breed (Yılmaz, 2010; Koç, 2015; Koç and Arı, 2020), RH and HF breeds (Koç and Gürses, 2020), HF, SIM and crossbred cattle (Okuyucu and Erdem, 2017) and milk transported to the dairy facilities (Yörükoğlu, 2019).

Hygienic quality characteristics of milk are related to the health of the udder of the cow from which the milk is produced, milking hygiene and storage and transportation conditions of the milk in the process until it is processed into the final products. Mastitis, which is an udder disease and is known as the costliest disease causing significant economic losses in dairy cattle worldwide, is an inflammation of the mammary tissue, which usually occurs due to bacteria, fungi and virus-based factors, causing damage to the udder tissue as well as causing changes in the composition of milk.

Somatic cell count (SCC), as a raw milk quality criterion, provides information about the state of udder health. The SCC level in milk is accepted as a threshold value of 200,000 cells/ml and if the SCC is above this

number, the udder of the cow from which that milk is produced is considered to have mastitis (Dohoo and Leslie, 1991). The increase in SCC in raw milk causes changes in the composition of the milk as well as the deterioration of the udder health and also leads to significant decreases in cow milk yield.

Somatic cells (macrophages, neutrophil cells, lymphocytes and epithelial cells) are the body's main defense mechanisms against diseases and intramammary infections. The main factor affecting SCC in milk is infection of the udder and it is under influence of many factors such as cow genotype, udder morphology, parity, lactation period, teat end hyperkeratosis, cow age, stress condition, season, milking hygiene and equipment and etc.

There are studies to determine the level of SCC in different cattle breeds (Özdede, 2009; Koç, 2006; 2007b; 2011; 2015; Yılmaz, 2010; Kaya et al., 2014; Okuyucu and Erdem, 2017; Koç and Arı, 2020; Koç and Gürses , 2020) and a study to determine the level of SCC in milk transported to dairy facilities (Yörükoğlu, 2019).

In the study of Koç (2016), in which he compiled studies on SIM cattle, while there were many studies on milk yield, fertility, fattening performance and carcass characteristics of Swiss origin SIM cattle raised in Türkiye, it was emphasized the number of studies on milk components and somatic cell count (SCC) of the breed is quite limited. On the other hand, the number of studies conducted on the performance of high yielding Austrian and German origin SIM cattle (Fleckvieh), which breeders have shown great interest in Türkiye in recent years, is almost non-existent.

In this study, it was aimed to determine the milk components and SCC level of Austrian origin SIM cattle (Fleckvieh) raised in a private farm in Menemen District of İzmir province, Türkiye, as well as to investigate the effects of some environmental factors on these traits.

Material and Methods

The study was carried out in a SIM herd brought from Austria in 2008 in Menemen District of İzmir Province, Türkiye. As raw milk components like MF (%), MP (%), ML (%), TDM (%) and SCC (cell/ml), the data of the farm were used. For this purpose, the farm had the milk samples analyzed three times a year from lactating cows between 2012 and 2020. In addition to the milk analyzes that the farm had done in previous years, milk samples were taken from lactating cows during morning milking on August 10, 2021, and analyzed to observe the current situation of the farm when the research was conducted. Since this study is a master's degree study, it is also aimed for the thesis author to gain experience in taking and analyzing milk samples.

Thus, in addition to the above milk traits, SNF (%) and FP (°C) in the milk were determined by analyzing these milk samples taken from 90 heads cows. Approximately 50 ml of milk samples were taken from each cow in sterile containers to represent milking, and the samples were analyzed with a Bentley brand Milk Analyzer in the Laboratory of the Department of Animal Science, Faculty of Agriculture, Ege University, İzmir/Türkiye, on the same day. The necessary distinction was made by placing the letter "c" next to the abbreviation of the traits obtained as a result of the analysis of the milk sample taken in August, in order to express that it is different from the traits obtained from the analyzes made by the farm.

Statistical Analysis

Statistical analysis of the data was made in the SAS (2004) package program. SCC data were analyzed using Log_{10} transformation before statistical analysis.

The lactation periods of the SIM cows whose milk samples were taken to determine the current situation were divided into 4 groups, those on the 5-90th day of lactation were Period-I, those on the 91-240th day were Period-II, those at 241-310 days were Period-III and their lactation day more than 310 days were accepted as Period-IV. In addition, animals with 4 or more parities were included in the 4+ parity.

The following statistical model was used in the analysis of MF, MP, ML, TDM and Log_{10}SCC traits determined from milk analyzes performed by the farm three times a year between 2012 and 2020:

$$y_{ijklmn} = \mu + a_i + b_j + c_k + d_l + f_m + (ad)_{il} + (af)_{im} + e_{ijklmn} \quad (5)$$

Here y_{ijklmn}; observation value of the traits, μ; the mean of the traits, a_i; sampling season effect (j=winter, spring, summer, autumn), b_j; parity effect (j=1, 2, 3, 4, 5+), c_k; calving year effect (k=2012, 2013, …., 2020), d_l: calving month effect (l=1, 2, …., 12), f_m: lactation month effect (m=1, 2, ….., 15), $(ad)_{il}$: sampling season x calving month interaction effect, $(af)_{im}$: sampling season x lactation month interaction effect and e_{ijklmn}; error term.

The following statistical model was used for traits (MFc, MPc, MLc, TDMc, SNFc, FPc and Log_{10}SCCc) to determine the current situation:

$$y_{ijk} = \mu + a_i + b_j + e_{ijk} \quad (6)$$

Here y_{ijk}; observation value of the trait, μ; the mean of the trait, a_i; parity effect (j=1, 2, 3, 4+), b_j; lactation period effect (j=1, 2, 3, 4+) and e_{ijk}; error term.

Results

The milk components and Log_{10}SCC averages and standard errors of SIM cows belonging to the milk analysis results performed by the farm three times a year between 2012 and 2020 are given in Table 1. The overall averages of MF, MP, ML, TDM, and Log_{10}SCC were found to be 3.71±0.018%, 3.42±0.009%, 4.63±0.009%, 12.49±0.03%, and 5.14±0.009 (138.038 cells/ml), respectively. The effects of sampling season, calving year, lactation month, sampling season x calving month and sampling season x lactation month interactions on MF, MP, ML, TDM, and Log_{10}SCC were found to be significant ($P<0.05$), in addition to a significant effect of parity on Log_{10}SCC, and calving month effect on ML and Log_{10}SCC ($P<0.01$).

Milk components

In terms of MF, the mean (3.79±0.08%) of the first sampling season (January-February-March) was similar to the mean (3.94±0.04%) of the fourth season (October, November, December) ($P>0.05$), and these two seasons were determined to be different from the means of the second (3.37±0.06%) and third (3.33±0.05%) seasons ($P<0.05$).

Table 1. LSMEANS and standard errors of milk components and somatic cell counts of SIM cattle

Factor	n	MF, %	MP, %	ML, %	TDM, %	Log_{10}SCC
		$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$
Sampling season		**	**	**	**	**
1 (Winter)	448	3.79±0.08^{a}	3.26±0.03^{a}	4.77±0.03^{a}	12.78±0.12^{a}	5.17±0.03^{a}
2 (Spring)	436	3.37±0.06^{b}	3.14±0.02^{b}	4.24±0.02^{b}	11.59±0.09^{b}	5.14±0.02^{a}
3 (Summer)	577	3.33±0.05^{b}	3.54±0.02^{c}	4.75±0.02^{a}	12.15±0.08^{c}	5.24±0.02^{b}
4 (Autumn)	1122	3.94±0.04^{a}	3.57±0.01^{c}	4.64±0.02^{c}	12.71±0.05^{a}	5.12±0.01^{a}
Parity		NS	NS	NS	NS	**
1	767	3.60±0.04	3.36±0.02	4.60±0.02	12.26±0.06	5.16±0.02^{a}
2	649	3.57±0.04	3.40±0.02	4.59±0.02	12.30±0.06	5.14±0.02^{a}
3	574	3.70±0.05	3.35±0.02	4.61±0.02	12.36±0.07	5.23±0.02^{b}
4	370	3.58±0.05	3.36±0.02	4.58±0.02	12.29±0.08	5.14±0.02^{a}
5+	223	3.59±0.07	3.41±0.03	4.63±0.03	12.34±0.10	5.17±0.03^{ab}
Calving year		**	**	**	**	**
2012	108	3.90±0.10^{ae}	3.72±0.04^{a}	4.84±0.04^{a}	12.95±0.15^{ab}	4.59±0.04^{a}
2013	456	3.95±0.05^{a}	3.61±0.02^{a}	4.87±0.02^{a}	12.94±0.08^{a}	4.85±0.02^{b}
2014	374	3.56±0.06^{bd}	3.40±0.02^{b}	4.82±0.03^{a}	12.86±0.09^{a}	5.00±0.02^{c}
2015	437	3.78±0.05^{ab}	3.45±0.02^{b}	4.81±0.02^{a}	13.46±0.08^{b}	5.07±0.02^{c}
2016	169	3.96±0.08^{ac}	3.45±0.03^{b}	4.84±0.03^{a}	13.53±0.12^{b}	5.20±0.03^{de}
2017	99	3.59±0.10^{bcd}	3.22±0.04^{c}	4.49±0.04^{b}	12.22±0.16^{c}	5.24±0.04^{de}
2018	313	3.38±0.06^{d}	2.91±0.02^{d}	4.20±0.02^{c}	11.37±0.09^{d}	5.27±0.02^{d}
2019	237	3.60±0.07^{bde}	2.89±0.03^{d}	4.11±0.03^{c}	11.35±0.10^{d}	5.09±0.03^{ce}
2020	197	3.35±0.08^{d}	3.43±0.03^{b}	4.56±0.03^{b}	11.60±0.12^{d}	5.51±0.03^{f}
2021	193	3.02±0.08^{f}	3.67±0.03^{a}	4.46±0.03^{b}	10.82±0.11^{e}	5.84±0.03^{g}
Calving month		NS	NS	**	NS	**
1	255	3.56±0.08	3.36±0.03	4.60±0.03^{abc}	12.21±0.12	5.03±0.03^{a}
2	194	3.49±0.09	3.44±0.04	4.66±0.04^{ad}	12.24±0.14	4.98±0.04^{a}
3	206	3.46±0.10	3.39±0.04	4.50±0.04^{be}	11.90±0.15	5.01±0.04^{ac}
4	124	3.51±0.15	3.34±0.06	4.46±0.06^{abe}	11.99±0.22	5.12±0.06^{abc}
5	56	3.64±0.15	3.24±0.06	4.41±0.06^{ab}	12.18±0.23	5.21±0.06^{abc}
6	138	3.86±0.11	3.40±0.05	4.63±0.05^{abc}	12.73±0.17	5.27±0.04^{bcd}
7	274	3.73±0.09	3.36±0.03	4.62±0.04^{abc}	12.44±0.13	5.27±0.03^{bd}
8	251	3.70±0.09	3.45±0.04	4.71±0.04^{cd}	12.58±0.13	5.25±0.03^{bcd}
9	223	3.70±0.08	3.42±0.03	4.68±0.04^{cde}	12.57±0.13	5.34±0.03^{b}
10	309	3.56±0.08	3.39±0.03	4.64±0.03^{abd}	12.34±0.12	5.18±0.03^{cd}
11	269	3.60±0.08	3.36±0.03	4.64±0.03^{abd}	12.35±0.11	5.22±0.03^{bcd}
12	284	3.46±0.08	3.38±0.03	4.64±0.03^{abd}	12.18±0.12	5.10±0.03^{ad}
Lactation month		**	*	*	**	**
1	138	3.41±0.13^{ab}	3.39±0.05^{ab}	4.72±0.05^{a}	12.20±0.19^{ab}	5.14±0.05^{abcdf}
2	144	3.25±0.11^{a}	3.40±0.04^{ab}	4.66±0.05^{ab}	11.94±0.16^{a}	5.02±0.04^{ab}
3	192	3.49±0.09^{abc}	3.42±0.04^{ab}	4.68±0.04^{a}	12.30±0.13^{ab}	5.05±0.03^{abc}
4	179	3.72±0.10^{bc}	3.39±0.04^{ab}	4.61±0.04^{ab}	12.45±0.15^{ab}	5.11±0.04^{abcd}
5	173	3.49±0.09^{abc}	3.28±0.04^{a}	4.58±0.04^{ab}	12.09±0.13^{ac}	4.98±0.03^{a}
6	197	3.58±0.08^{abc}	3.32±0.03^{ab}	4.59±0.04^{ab}	12.26±0.13^{ab}	5.05±0.03^{abc}
7	154	3.54±0.09^{abc}	3.34±0.04^{ab}	4.56±0.04^{ab}	12.18±0.14^{ab}	5.07±0.04^{abcd}
8	184	3.42±0.09^{abd}	3.36±0.04^{b}	4.45±0.04^{b}	11.89±0.13^{a}	5.13±0.03^{abcd}
9	201	3.45±0.09^{abc}	3.43±0.04^{ab}	4.53±0.04^{ab}	12.04±0.14^{ac}	5.15±0.04^{abcdf}
10	174	3.74±0.13^{abc}	3.38±0.05^{ab}	4.58±0.06^{ab}	12.52±0.20^{ab}	5.21±0.05^{bcde}
11	151	3.77±0.17^{abc}	3.24±0.07^{ab}	4.53±0.07^{ab}	12.41±0.25^{ab}	5.29±0.06^{cde}
12	141	4.01±0.13^{c}	3.39±0.05^{ab}	4.65±0.06^{ab}	12.96±0.20^{b}	5.29±0.05^{de}
13	102	3.93±0.12^{cd}	3.48±0.05^{a}	4.70±0.05^{a}	12.79±0.18^{bc}	5.39±0.05^{e}
14	100	3.72±0.11^{bc}	3.40±0.04^{ab}	4.60±0.05^{ab}	12.41±0.17^{ab}	5.32±0.04^{e}
15	353	3.59±0.06^{abc}	3.41±0.02^{b}	4.56±0.03^{ab}	12.20±0.09^{ab}	5.28±0.02^{ef}
Cal. season X Cal. mo	2583	*	**	*	**	**
Cal. season X Lac. mo	2583	**	**	**	**	**
Overall mean	2583	3.69±0.02	3.43±0.01	4.64±0.01	12.48±0.03	5.14±0.009

MF: Milk fat, MP: Milk protein, ML: Milk lactose, TDM: Total dry matter, SCC: Somatic cell count, NS: non-significant, *: P<0.05, **:P<0.01, a,b,c,d,e,f,g: the difference between groups with the same letter is insignificant according to P<0.05.

Table 2. Milk components, freezing point (FP) and somatic cell count (SCC) of SIM cattle

Factor	n	MFc, %	MPc, %	MLc, %	TDMc, %	SNFc, %	FPc, °C	Log_{10}SCC
		$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$
Parity		NS	NS	*	NS	*	**	NS
1	47	4.3±0.20	3.44±0.07	4.73±0.07 ^{ab}	13.14±0.23	8.76±0.09^{ab}	-0.538±0.005^{a}	5.23±0.104
2	20	3.87±0.26	3.50±0.09	4.85±0.08 ^{a}	12.86±0.29	8.99±0.12 ^{a}	-0.540±0.006^{a}	4.99±0.134
3	9	4.64±0.39	3.48±0.14	4.71±0.13 ^{ab}	13.39±0.43	8.75±0.18 ^{ab}	-0.533±0.010^{ab}	4.95±0.201
4+	14	4.01±0.31	3.30±0.11	4.46±0.10 ^{b}	12.40±0.34	8.38±0.14 ^{b}	-0.509±0.008 ^{b}	5.23±0.158
Lactation period		*	NS	NS	NS	NS	NS	NS
1 (5-90 days)	43	4.25±0.22^{ab}	3.43±0.08	4.77±0.07	13.04±0.24	8.80±0.10	-0.536±0.005	4.93±0.11
2 (91-240 days)	15	3.91±0.30 ^{a}	3.34±0.11	4.62±0.10	12.49±0.33	8.58±0.14	-0.519±0.007	5.05±0.15
3 (241-310 days)	9	3.85±0.39 ^{ab}	3.44±0.14	4.78±0.13	12.69±0.43	8.84±0.18	-0.531±0.010	5.15±0.20
4 (>310 days)	23	4.89±0.24 ^{b}	3.50±0.09	4.58±0.08	13.55±0.27	8.67±0.11	-0.534±0.006	5.27±0.12
Overall mean	90	4.32±0.12	3.44±0.04	4.72±0.04	13.09±0.14	8.76±0.06	-0.535±0.004	5.11±0.006

MFc: Milk fat, MPc: Milk protein, MLc: Milk lactose, TDMc: Total dry matter, SNFc: Solid non-fat, PF: Freezing point, SCCc: Somatic cell count, NS: non-significant, *: P<0.05, **:P<0.01, a,b: the difference between groups with the same letter is insignificant according to P<0.05.

In terms of MP, the averages of the third (3.54±0.02%) and the fourth (3.57±0.01%) seasons were found to be similar (P>0.05), while these two seasons had higher averages than the other two seasons (P<0.05). The lowest MP mean (3.14±0.02%) was obtained for the second season (April-May-June) in which the milk yield of the cows was higher in general, this season group was also different from the first (3.26±0.03%) season (P<0.05).

In terms of ML, the first (4.77±0.03%) and third (4.75±0.02%) season were similar to each other and these two seasons were different (P<0.05) from the second (4.24±0.02%) and fourth (4.64±0.02%) season. The difference between the ML means of the second and the fourth seasons were also found to be significant (P<0.05).

In terms of TDM, the second sampling season with the lowest mean (11.59±0.09%) was different from all other seasons (P<0.05), however with the highest mean the first season (12.78±0.12%) was similar to the fourth season (12.71±0.05%). These two seasons means were different from the mean (12.15±0.08) of the third season (P<0.05).

Significant differences were obtained between calving years in terms of MF, MP, ML and TDM (P<0.01), while the difference in calving months was significant only for ML (P<0.01). The mean ML of the cows gave birth in May was the lowest (4.41±0.06%) and this month was found to be different from August (4.71±0.04%), which has the highest mean of ML (P<0.05).

Significant differences were found between lactation months in terms of MF, MP, ML and TDM (P<0.05). The MF mean of the second lactation month was found to be the lowest (3.25±0.11%) and this month was different from the fourth and 12-14th lactation months (P<0.05). The highest MF mean was obtained at the 12th month with 4.01±0.13%. In terms of MP, only the 5th and 13th lactation months were found to be different from the 8th and 15th lactation months, and the other differences were insignificant (P>0.05). In terms of ML, the 8th lactation month with the lowest mean (4.45±0.04%) was different from the first (4.72±0.05%), third (4.68±0.04%) and 13th (4.70±0.05) lactation months (P<0.05) and other differences between the months were insignificant (P>0.05).

The highest TDM mean was obtained for the 12th month of lactation (12.96±0.20%) and this month was determined to be different (P<0.05) from the second (11.94±0.16%), the fifth (12.09±0.13%), the eighth (11.89±0.13%) and the ninth (12.04±0.14%) lactation months.

In order to determine the current situation, the averages and standard errors of the analysis results of the milk samples taken from the lactating animals in the morning milking on 10.08.2021 are given in Table 2. Mean MFc, MPc, MLc, TDMc, SNFc, FPc and Log_{10}SCCc of SIM cattle were 4.32±0.12%, 3.44±0.04%, 4.72±0.04%, 13.09±0.14%, 8.76±0.06%, -0.535±0.003 °C and 5.11±0.06 (128,825 cells/ml), respectively.

While the effect of parity on MLc (P<0.05), SNFc (P<0.05) and FPc (P<0.01) was significant, its effect on MFc, MPc, TDMc and Log_{10}SCCc was insignificant (P>0.05).

In terms of MLc, the mean in the second parity (4.85±0.08%) differed from the mean of the fourth lactation (4.46±0.10) (P<0.05), while the other differences among the parities were insignificant (P>0.05). Similar to MLc, the difference between the second and fourth parities was found to be significant (P<0.05) for SNFc, while other differences were insignificant (P>0.05) among the parities.

In terms of FPc, 4+ parity mean (-0.509±0.008 °C) was detected to be different from the first (-0.538±0.005 °C) and second (-0.540±0.006 °C) parities (P<0.05), other differences between the parities were statistically insignificant (P>0.05).

The effect of lactation period was found to be significant (P<0.05) only for MFc, while the effect of this factor on other traits was insignificant (P>0.05). The only significant difference was between the second lactation period with a mean of 3.91±0.30% and the fourth lactation period with a mean of 4.89±0.24% (P<0.05), other differences between lactation periods were insignificant.

Somatic cell count

According to the results of the analysis of the milk samples performed by the farm, all factors effects on Log_{10}SCC were found to be significant (P<0.01; Table 1). The highest average of Log_{10}SCC was obtained for the third sampling season (5.24±0.02; 173,780 cells/ml) and this season was detected to be different from other seasons and other seasonal differences were insignificant (P>0.05).

The highest Log_{10}SCC mean in terms of parity was obtained for the third parity (5.23±0.02; 169,824 cells/ml) and this parity was similar only to the fifth parity (5.17±0.03; 147.911 cells/ml) (P>0.05), but different from

other parities (P<0.05), other differences between the parities were insignificant (P>0.05). The lowest Log_{10}SCC mean was determined to be 5.14±0.02 (138.038 cells/ml) for the second and fourth parities.

Considering the Log_{10}SCC according to calving years, a regular increase was observed over the years, if the decline in 2019 (5.09±0.03; 123,027 cells/ml) is not taken into account. The mean, which was 4.59±0.04 (38.905 cells/ml) in 2012, when the lowest Log_{10}SCC level was realized, increased to 5.84±0.03 (691.831 cells/ml) in 2021.

According to the calving months, the lowest Log_{10}SCC mean was obtained for February (4.98±0.04 or 95,499 cells/ml) and this month was similar to January, March, April, May and December (P>0.05) but different from other months (P<0.05). On the other hand, while the highest Log_{10}SCC mean was obtained for September (5.34±0.03; 218,776 cells/ml)), this month was different from the first five months and October and December (P<0.05) but, similar to other months (P>0.05).

The Log_{10}SCC level in cows in the first month of lactation was 5.14±0.05 (138,038 cells/ml), maintained this low level in the following months, and decreased to the lowest level of 4.98±0.03 (95,499 cells/ml) in the fifth month of lactation. Towards the end of lactation, as expected, a regular increase occurred and reached its highest level in the 13th lactation month (5.39±0.05; 245,471 cells/ml), and it was around 200,000 cells/ml in the two months following this month (Table 1).

On the other hand, as a result of the analysis of milk samples taken at morning milking on 10.08.2021 to determine the current situation, the mean of Log_{10}SCCc was calculated as 5.11±0.06 (128,825 cells/ml), and the effect of lactation period and parity on Log_{10}SCCc was found to be insignificant (P>0.05; Table 2). With the progression of lactation, the mean of Log_{10}SCCc increased as expected, and the mean in the first period was 4.93±0.11 (85.114 cells/ml) and increased to 5.27±0.12 (186,209 cells/ml) in the fourth lactation period, but 101,092 cells/ml difference between these two periods was found to be is insignificant (P>0.05).

Discussion

Milk components

In this study, the MF mean (3.69±0.02%), which varies significantly according to the sampling season, calving year and lactation months, can be considered low for SIM breed. However, if it is remembered that there is an inverse relationship between milk yield and milk components, it is expected that although the milk yield of SIM cattle used in this study is increased, the fat content in milk will decrease (dilution effect). While it is noteworthy that the MF is low in years when milk yield is high, an increase in the MF towards the end of lactation is an expected situation (Table 1). On the other hand, it is noteworthy that high air temperatures and humidity seen in spring and summer seasons cause a significant decrease in the MF. It should be emphasized that the application of an effective cooling system will provide significant benefits to the business in order to eliminate this negativity.

The average of the MF (3.69±0.02%) determined for SIM cattle is lower than those of the results reported by Polanski et al. (1992) and Akbulut (1998) for the same breed, by Koç (2011) for HF and MB breeds and by Kaya et al. (2014) for the evening milking mean of HF. However, the MF mean calculated in this study was higher than the results reported by Okuyucu and Erdem (2017) for HF, SIM and crosses, the morning milking mean of HF breed reported by Kaya et al. (2014) and the mean determined from the milk samples taken from milk tanks by Yörükoğlu (2019).

The dilution effect similar to MF is also valid for MP. While the decrease in MP average is noteworthy, especially in the summer months, it would be beneficial to make significant changes in the ration as well as an effective cooling system.

The MP mean (3.43±0.01%) of SIM breed found in this study was lower than the mean of Şekerden et al. (1999) for the SIM breed (%3.9±0.41), however similar to the seasonal averages for the same breed reported by Polanski et al. (1992) who reported seasonal averages between 3.41% and 3.46% for the same breed and morning and evening milking (3.41% and 3.44%) means for HF breed reported by Kaya et al. (2014). On the other hand, the MP mean obtained for SIM in this study is higher than the mean of RH breed (3.22±0.029) reported by Yılmaz (2010), the means of HF and MB breeds reported by Koç (2011), the averages reported by Koç and Arı (2020) for RH and SIM breeds (3.38% and 3.40%, respectively), and the average determined by Yörükoğlu (2019) from the mean of milk tanks samples (3.22%) and higher than the average (3.02%) for SA, SIM and crossbred cattle reported by Okuyucu and Erdem (2017).

While the season has a significant impact on ML, it is seen that the ML, which varies relatively less than other milk components, had very low values in 2018 and 2019. It is thought that these lower values may be due to the fact that the sampling in these years coincided with the high productive months. As a matter of fact, the ML in the spring months (4.24±0.02%) was found to be considerably lower than other months (Table 1).

The mean ML of SIM (4.64±0.01%) determined in this study is higher than the averages reported by Koç (2011) for the MB and HF breeds (4.57% and 4.53%, respectively), but lower than the mean of Yılmaz (2010) for RH breed (4.73%), the morning and evening milking means of Kaya et al. (2014) for HF breed (4.77% and 4.79%, respectively), the means of RH and SIM breeds (4.86±0.028 and 4.81±0.019%, respectively) of Koç and Arı (2020) and the mean of Okuyucu and Erdem (2017) for HF, SIM and crossbred cattle (4.19%), but it is close to the mean (4.63%) of Yörükoğlu (2019) from the samples taken from the milk tanks.

The effect of high productivity in spring months on milk components is also clearly seen in TDM. Moreover, it is thought that the significant decrease in the TDM average, especially in recent years, is related to the increase in milk yield of cows in the enterprise in recent years (Table 1). TDM mean determined as 12.48±0.03% for SIM breed in this study was lower than the means of Şekerden et al. (1999) for the same breed (12.6±0.81), Kaya et al. (2014) for the evening milking (13.06%) for HF breed, however TDM mean calculated in this study for SIM breed is higher than means of Kaya et al. (2014) for the morning milking mean (11.99%) for HF breed, Koç (2011) for MB

and HF breeds (11.88±0.103% and 11.47±0.148%, respectively), Koç and Arı (2020) for RH and SIM breeds (11.18%±0.069% and 11.23±0.048%, respectively), Okuyucu and Erdem (2017) for HF, SIM and crossbred cattle (11.76%) and Yörükoğlu (2019) from the mean taken from the milk tanks.

In order to determine the current situation from the analysis of milk samples taken in August 2021, the means of MFc, MLc and TDMc (4.32±0.12%, 3.44±0.04%, 4.72±0.04% and 13.09±%, respectively) were higher than the overall means (3.71±0.018%, 4.63%±0.009% and 12.49±0.03, respectively) found between 2012 and 2020 (Tables 1 and 2). However, when compared with the averages of August 2021 with the years between 2012 and 2020, it was seen that the means of the MP and MPc were almost similar (Tables 1 and 2). It is thought that the higher values in terms of milk components of SIM cattle in the summer months were due to the low milk yield of the cows due to the high air temperature and humidity seen in the region during these months.

The mean MFc found for the SIM (4.32±0.12%) breed in this study was higher than the means reported in the literature for the same breed by Akbulut (1998) and Polanski et al. (1992), and Okuyucu and Erdem (2017) for HF, SIM and crossbred cattle and Yörükoğlu (2019) for the mean of the samples taken from the milk tanks (3.54%).

Şekerden et al. (1999) for SIM (3.9±0.41%), Koç and Arı (2020) for RH (3.38±0.021%) and SIM (3.40±0.015%) breeds, and Okuyucu and Erdem (2017) for HF, SIM and crossbred cattle (3.02%), and Yörükoğlu (2019) for the samples taken from the milk tanks reported higher values than MPc mean (3.44±0.04) found in this study, but Polanski et al. (1992) reported similar seasonal values (range 3.41% to 3.46%) to the MPc mean determined in this study for SIM breed.

The MLc mean determined in this study (4.72±0.04%) was similar to the value reported by Yılmaz (2010) for the RH (4.73±0.024%), but lower than the values reported by Koç and Arı (2020) for the RH (4.86±0.028%) and SIM (4.81±0.019%) breeds, but higher than the means reported by Okuyucu and Erdem (2017) in HF, SIM and crossbred cattle (4.19%), and by Yörükoğlu (2019) in samples taken from milk tanks (4.64%).

The mean TDMc determined for SIM cattle (13.09±0.14%) was higher than all the values determined by Şekerden et al. (1999) for the same breed (12.6±0.81), Okuyucu and Erdem (2017) for HF, SIM and crossbred cattle (11.76%), Koç and Arı (2020) for RH and SIM cattle (11.18%±0.069 and 11.23±0.048%, respectively) and Yörükoğlu (2019) for the samples taken from milk tanks (12.00%).

In this study, the mean SNFc (8.76±0.06%) obtained in this study for the SIM breed was determined to be higher than the values reported by Şekerden et al. (1999) for same breed (8.6±0.32%), Koç (2009) for HF and MB breeds (8.23±0.067% and 8.35±0.047%, respectively), Koç (2011) for MB and HF breeds (8.35±0.047% and 8.23±0.067%, respectively), Koç (2015) for RH breed (8.35±0.047% and 8.23±0.067%, respectively for the morning and evening milkings), Okuyucu and Erdem (2017) for HF, SIM and crossbred cattle (8.32%) and Yörükoğlu (2019) for the samples taken from milk tanks (8.46%). However, Koç (2007a) for HF and Brown-Swiss breeds (9.61±0.048), Koç (2008) for HF breed (9.78±0.024), Yılmaz (2010) for RH breed (8.94±0.036), Kaya et al. (2014) for HF breed (8.83% and 8.80%, respectively for morning and evening milking), Koç and Arı (2020) for RH and SIM breeds (9.09±0.037% and 9.09±0.025%, respectively), Koç and Gürses (2020)) for the first lactating RH and HF cows (9.7±0.09% and 9.9±0.04%, respectively) reported higher SNFc than the mean found in this study for SIM cattle.

The raw milk FP value is used to determine the cheating in milk and the FP of unprocessed bovine milk is between -0.53 and -0.55 ºC and the FP decreases due to the increase in the dry matter content of the milk (Anonymous, 2019). The mean FPc (-0.535±0.003 ºC) for SIM cattle determined in this study was similar to the mean found by Yörükoğlu (2019) from the samples taken from milk tanks (-0.536 ºC), however higher than the value reported by Koç and Arı (2020) for RH and SIM breeds (-0.577±0.0012 and -0.579±0.0009 ºC, respectively).

Somatic cell count (SCC)

The Log_{10}SCC mean (5.14±0.01 or 138,038 cells/ml) detected in this study were lower than the values Özdede (2009) who reported 179,730, 238,899, 267,005 and 204,877 cells/ml, respectively for spring, summer, autumn and winter seasons for the Ankara Cattle Breeders' Association member farms, the values of Koç (2006) who determined the means between 319,448 cells/ml and 497,279 cells/ml for HF breed after conducting a study for two years in four farms () in Aydın Province, Türkiye, the values of Koç (2007b) who reported the means of 218.524 cells/ml and 344,112 cells/ml, respectively, for MB and HF breeds , the value of Koç (2011) for HF breed (199.022 cells/ml), a value (181,339.1 cells/ml) of Okuyucu and Erdem (2017) who conducted a study in small-scale farms rearing HF, SIM and crossbred cattle under semi-intensive conditions in Bafra district of Samsun province, Türkiye, and the mean (586 000 cells/ml) reported by Yörükoğlu (2019) for the samples taken from milk tanks arriving at milk processing facilities in four districts of İzmir Province, Türkiye. In addition, the Log_{10}SCC mean detected in this study was also lower than the means (251,768 cells/ml 261,216 cells/ml) reported by Koç and Arı (2020) for SIM and RH breeds raised together in a private farm in Aydın province, Türkiye. Koç (2011) reported similar value for MB breed (138,644 cells/ml) to the mean found in this study for SIM breed.

On the other hand, Yılmaz (2010) for RH cows (63,753 cells/ml) reared in a farm in Aydın Province, Türkiye, Kaya et al. (2014) for HF cows (67,764 cells/mL and 119,950 cells/mL, respectively for the morning and evening milking), Koç (2015) for RH cows (91,833 cells/mL and 100,462 cells/mL, respectively for the morning and evening milking) and Koç and Gürses (2020) for the first lactating RH and HF cows reared in a farm in Aydın Province, Türkiye, reported lower values than those obtained in this study.

In this study, the Log_{10}SCCc mean (5.11±0.06 or 128,825 cells/ml) determined in this study for SIM cows was lower than the results of Özdede (2009) for the member farms of the Ankara Province Cattle Breeders' Association, Koç (2006) for HF breed raised in four different farms in Aydın Province, Koç (2007b; 2011) for

MB and HF breeds, Okuyucu and Erdem (2017) for HF, SIM and crossbred cattle (181,339.1 cells/ml), Koç and Arı (2020) for RH and SIM breed (261,216 and 251,768 cells/ml, respectively) and Yörükoğlu (2019) for the milk transported to milk processing facilities in İzmir Province, Türkiye. Yılmaz (2010) for RH breed (63,753 cells/ml), Kaya et al. (2014) for the morning and evening milkings means of HF breed (67,764 and 119,950 cells/ml, respectively), and Koç (2015) for morning and evening milking of RH breed (91,833 and 100,462 cells/ml, respectively), and Koç and Gürses (2020) for the first lactating RH and HF breeds (39,811 and 50,119 cells/ml, respectively) reported lower values than those determined in this study.

In this study, the average of Log_{10}SCC (5.14±0.009 or 138.038 cells/mL) obtained as a result of the milk analysis performed by the farm by taking milk samples three times a year between 2012 and 2021 was found to be slightly higher than the average of Log_{10}SCCc (5.11±0.006 or 128.825 cells/mL) obtained from the milk samples taken in the morning milking on 10.08.2021 to determine the current situation. It is thought that the increase in the Log_{10}SCC level in 2020 and 2021, when the Covid-19 pandemic was seen, could be resulted from the disruption of various practices such as health protection, herd and milking management in the enterprise due to various measures taken throughout the country due to the pandemic in these years. Based on this, a high Log_{10}SCC average seen in 2021, when the pandemic was felt intensely, it is possible to talk about a mastitis epidemic in the enterprise during this year.

Conclusion

In this study, in addition to the results of milk analysis performed three times a year between 2012 and 2021 in a farm in Menemen district of İzmir province, Türkiye, which raises Austrian origin SIM (Fleckvieh) cattle, which has increased the interest of dairy cattle breeders in Türkiye in recent years, to determine the current situation on 10.08.2021, the milk samples at the morning milking from the lactating cows were analyzed and some important information was obtained about milk components and SCC level of Austrian origin SIM cows.

The fact that the overall SCC mean (138,038 cells/ml) from 2012 to 2021 years, and the low SCC mean (128,825 cells/ml) determined for evaluating the current situation revealed that the mastitis prevalence in Austrian-origin SIM cattle is quite low. However, especially considering the high SCC level in 2021 and 2020, it is thought that various measures taken throughout all over the world and Türkiye due to the Covid-19 Pandemic led to the disruption of practices such as milking management and hygiene, health protection, etc. in the farm, and accordingly, an increase in mastitis cases in the herd increased as a result of that the hygienic quality of milk decreased in these years.

In conclusion, all the milk analysis results are examined, it has been seen that the milk components of Austrian origin SIM cattle are not much different from those of HF breed which is raised widely in Türkiye and in the world. However, the low SCC mean obtained for Austrian origin SIM cattle in this study revealed that, besides the significant contribution of the measures taken against mastitis in this farm, the resistance to mastitis of this genotype could be higher, and this characteristic of Austrian origin SIM cattle is thought to be among the reasons for preference of high yielding Austrian-origin SIM cattle in Türkiye.

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Rock Samphire (*Crithmum maritimum* L.) as a Functional Food: Awareness, Consumption Habits and Culinary Use

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ABSTRACT

Functional foods are the name given to food groups that, when consumed, have beneficial effects such as promoting and maintaining metabolic health and preventing diseases, rather than just meeting nutritional needs. Rock samphire (*Crithmum maritimum* L.), is a plant that grows naturally in the Mediterranean and Aegean regions of Turkey and on the coasts of Cyprus, has been consumed in these regions for many years. The consumption of the rock samphire, which draws attention with its high iodine and bioactive component content, has been limited to the regions where it grows. In this study, the local consumption habits, recipes of the rock samphire plant and the awareness of its functional properties were determined. In the study, six different recipes were obtained from the local people. Traditional products prepared according to the recipes were photographed by the authors. In addition, twenty local people were interviewed and it was determined that consumers were informed about the functional properties of the rock samphire and that these properties motivated consumers to consume the plant. As a result of the study, it was concluded that the integration of locally-consumed rock samphire into the daily diets by introducing them into non-regional cuisines would contribute positively to the general public health and the economy of the region.

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Introduction

The concept of functional foods is defined as foods with functions that go beyond serving physiological roles (Diplock et al., 1999).

The leading consumer motive for purchasing functional foods is to optimize health, either to help prevent chronic diseases such as Alzheimer's and osteoporosis, or to optimize health by, for example, increasing energy, strengthening the immune system, and creating well-being (Khan et al., 2013). A general classification of functional food can be made as, i-plant origin: Oat, soy, flaxseed, tomato, garlic, broccoli and similar vegetables, citrus fruits, blueberries, tea, wine, and grapes; ii-animal origin: Fish, milk, and dairy products and meat (Guven and Gulmez, 2006). Functional substances with high biological usefulness in these foods are antioxidants, phenolic substances, dietary fibers, oligosaccharides, probiotics and prebiotics, vitamins, PUFA, sulfur-containing compounds, plant sterols, and phytoestrogens (Meral et al., 2012).

The first step in the research and development of functional food is to identify the interaction between one or more components of the food and a function in the organism that is beneficial for health. Increasing, adding, removing or modifying the concentration of a specific functional component of a food with known bioavailability; or more simply, making this food more frequently included in daily consumption, integrating it into diets, creating standard recipes or protecting previously established standard recipes is essential for protecting public health (Roberfroid, 1999). Despite the advances in health practices and technological developments in today's societies, there is an increase in chronic diseases due to intense stress, air pollution, unhealthy foods and many other factors.

Thanks to technological advances, more and more food ingredients are now known to have a positive or negative impact on human health. Consumers worldwide are therefore becoming increasingly aware of the relationship between food and health and are turning to more nutritious diets. In addition, rising health costs and changes in the food industry, which affect product supply and demand,

have led to an increased interest in maintaining a healthy lifestyle through diet and the consumption of foods that are beneficial for the body in addition to basic nutrition (Salmeron et al., 2015). However, the world's population is growing, which necessitates a return to the limited food resources and functional foods that have been consumed for a long time. This need has led to one of the fastest-growing food sectors, with an average annual growth rate of 8.6%. The functional foods portion of this market alone is worth USD 168 billion in global market, 2.5 times the size of vitamins and dietary supplements (Euromonitor, 2010a; Euromonitor, 2010b).

Consumers today are more interested in traditional recipes that have been consumed for many years and are known for their health benefits, rather than modern processed or ready-to-eat packaged foods. Traditional foods are often identified with a particular local area, with specific cooking and consumption methods that are passed down from one generation to the next. If the consumption of such foods is limited to certain regions, they may be forgotten. Developments in the food sector with technology, industrialization and capitalist expansion have led to the introduction of mass-produced and fast-food style foods into diets instead of healthy traditional foods. At this point, a return to healthy traditional foods has become very important in terms of functional nutrition and preserving national culinary cultures. The development of traditional food industries has a positive impact on employment and local economic development. Traditional food sector developments are important in preserving inventories, transferring them to future generations and promoting these foods (Cumhur, 2017; Kocatepe and Tırıl, 2015).

Today, there are widespread problems such as desertification, soil salinization and water scarcity due to various natural and man-made factors, especially in arid regions. Research conducted by the Food and Agriculture Organization of the United Nations (FAO, 2005) estimates that an average of 200 million hectares of agricultural land will be required to produce enough food for the world's population in the coming decades. Therefore, to overcome these problems, there is a need to research and utilize nutritional functional foods that are resistant to harsh conditions and can grow on their own under natural conditions e.g. halophytes. (Atia et al., 2010). Halophytes are specific plants that can develop adaptations to problems such as moisture deficit stress and soil salinization. They can tolerate and reproduce viable seeds at concentrations not lower than 200 mM NaCl (Flowers and Colmer, 2008). Halophytes are salt-tolerant species that can be grown in saline soil or using seawater due to their mechanisms of utilizing inorganic ions such as Na^+ and Cl^-. There is a growing interest in the use of some halophytes as human food and animal feed to address potential future food shortages. In addition, studies showing that high salinity reduces microorganism growth and extends shelf life are among the factors that make halophytes advantageous (Polit, 2013; Varol, 2021). Several halophyte species show high potential and *Crithmum maritimum* L. is one of the promising halophyte species (Atia et al., 2011).

Rock Samphire

The rock samphire is a succulent, aromatic, perennial, erect, or semi-recumbent halophyte that grows naturally on rocks and sometimes on sandy beaches near the sea. This aromatic plant belongs to the *Apiaceae* family of the genus *Crithmum* and is widespread around the Mediterranean and coastal areas of the Atlantic Ocean. It has significant nutritional value and economic potential. Previous studies have described the taste of rock samphire as 'pungent, similar to fennel and anise' (Sarrou et al., 2019). The name *Crithmum maritimum* L. is derived from the Greek words krithe, meaning barley, due to the similarity of its seeds to barley, and maritimum, meaning belonging to the sea, indicating its natural habitat (Renna, 2018). This green-leaved plant, which grows spontaneously on the seashore, in rock crevices and stony sandy areas open to the breeze and wave tides, blooms umbrella-shaped yellow-green flowers from July until the end of October (Clapham et al., 1962). Shakespeare (1623), in his play King Lear, mentioned the dangers of collecting rock samphire on the rocky cliffs by the sea with the phrase "halfway along, there hangs a man gathering grove; what a dreadful business!".

Since rock samphire grows near the sea, it contains high levels of iodine. Its tolerance to salt has caused it to attract high interest in scientific studies. Various studies have been carried out to reveal the potential of rock samphire as a functional food, but its consumption has not reached the desired levels (Sarrou et al., 2019). Rock samphire is also used for medicinal and antimicrobial applications due to its high content of carotenoids, polyphenols and other bioactive components. It is also rich in volatile compounds such as sabinene, γ-terpinene, ᵖ-cymene, thymol methyl ether, dilapiol. The plant also contains water-soluble compounds such as sugar, organic acids and minerals (Özcan et al., 2001). The composition (/100 g) of rock samphire is shown in Table 1.

Table 1. Composition of rock samphire (FAO, 2017)

Component	Amount
Water (g)	88.87
Protein (g)	0.31
Lipid (g)	0.39
Carbohydrate (g)	2.48
Dietary fiber (g)	5.7
Ash (g)	2.25
Ca (mg)	225
Fe (mg)	2.29
Mg (mg)	46
P (mg)	22
K (mg)	313
Na (mg)	368
Zn (mg)	0.26
Vitamin A (µg)	74
ß-carotene (µg)	883
Vitamin C (µg)	10

Iodine is a key component of the hormones produced by the thyroid gland. Thyroid hormones and therefore iodine is essential for mammalian life. Iodine is mostly found in ocean waters. The iodide in the water evaporates into the atmosphere and returns to the soil through rainfall.

This cycle is called the Iodine Cycle. Crops grown in areas where the iodine cycle is weak are low in iodine, and people in these areas suffer from iodine deficiency. Places where iodine deficiency is intense are mostly mountainous areas. Iodine deficiency in populations residing in these areas can be relatively well treated by ensuring that iodine enters the food chain by adding iodine to foods (e.g., iodizing salt) (Woeber, 1991).

Foods of marine origin are richer in iodine than other foods because marine plants and animals concentrate iodine in seawater. Iodine in organic form is found in high amounts in seaweeds. People whose diets include large amounts of seafood and who live in coastal areas have very high iodine intakes. Recent recommendations to reduce table salt for a healthy lifestyle have led to the prediction that iodine intake will also decrease (Teng et al., 2006). Iodine intake status throughout life is a major determinant of thyroid disorders. Severe iodine deficiency causes goiter (abnormal enlargement of the thyroid gland) and hypothyroidism. In these diseases, iodine deficiency prevents the secretion of thyroid hormone, while the activity of the thyroid gland is too high for iodine uptake and cycling. This is seen as a trigger for many diseases such as thyroid cancer. Therefore, optimizing iodine intake for public health is an important component of preventive health care to reduce the prevalence of thyroid disorders (Lind et al., 1998).

The most important reason that emerges in the studies on why rock samphire is not sufficiently preferred by consumers is the characteristic taste of the plant. It has been reported that rock samphire generally has a taste that people either like very much or do not like at all. Other reasons for the lack of industrial and traditional adoption of the plant are studies limited to local areas and local researchers, its presence in niche markets and its limited growing area (Renna, 2018). According to the study carried out by Tan et al. (2017) in the Aegean Region, it was also determined that rock samphire is called sea lettuce, sea grass, island cowpea, genevir and celery grass by local people in the region. In addition, it was determined in the study that the annual per capita consumption of samphire in the region was 3.5 kg on average.

Effects of Rock Samphire on Health

The history of medicinal plants dates back to the beginning of human history. Early humans learned to obtain medicines from the medicinal plants they collected or cultivated with the help of some simple methods. Thus, plants became both the basic food sources and the first sources of medicine (Baydar, 2007). Ethnobotanical practices are among the methods that are still found in many segments of societies today and are frequently used in daily life.

Hippocrates mentioned rock samphire in the 4th century and recommended it as a medicine against urinary tract diseases. Due to the antiscorbutic properties of the plant thanks to its high vitamin C content, sailors took care to use the leaves of rock samphire in their meals (Mucuk, 1999). In Italy, the decoction of rock samphire was used against cystitis, prostatitis and colitis, while its infusion was used in stomach and digestive diseases. In Spain, it was reported that the extract prepared by the decoction method was used to protect liver health (Cornara et al., 2009). Rock samphire is also rich in polyphenols such as chlorogenic acid and flavonoids such as rutin and quercetin (Houta et al., 2015). Essential oil components of the plant have been shown to inhibit the growth of foodborne bacteria such as *E. coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Listeria innocua* by showing antimicrobial effect (Souid et al., 2021).

In general, volatile compounds found in medicinal and aromatic plants have been documented as health-promoting substances. For example, essential oil and phenylpropanoids such as dillapiol, a characteristic volatile compound of rock samphire, have recently been shown to reduce blood pressure by dilating blood vessels and exhibit anticancer activities (Mekinić et al., 2016; Sarrou et al., 2019). Souid et al. (2020) reported that hydro-methanol extracts from rock samphire leaves showed significant in vitro antioxidant activity and exhibited in vivo protective effects in the liver of rats against carbon tetrachloride-induced toxicity. In the same study, it was found that administration of water suspension of rock samphire leaves to rats with liver toxicity decreased the activities of enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) used as markers of liver damage (Karkanis et al., 2022).

Maoloni et al. (2022) reported in their study that artificially acidified rock samphire sprouts in brine can be an excellent vehicle for delivering probiotics to humans. It was determined that the probiotic strains used were attached to the plant tissues of rock samphire and adapted well. The data from this study showed that probiotic-enriched rock samphire can provide the probiotic benefit provided by dairy-based probiotic foods such as yogurt or fermented milk.

This study aims to investigate the awareness of the rock samphire, which has high iodine content and nutritional values, in the region where it grows naturally and to question the local consumption patterns and culinary use to integrate the plant into daily diets.

Material and Methods

This study was conducted between November 1 and December 30, 2022, using the interview technique with people living in Silifke district of Mersin and Alanya district of Antalya, where the rock samphire is frequently consumed (Özcan, 2000). Interview is one of the effective research methods for determining the insights, sensitivity and awareness of people about a subject (Yıldırım and Şimşek, 2006).

Following the literature review, an appropriate list of questions was created in line with the determined purpose. The interview questions were adapted from Halıcı (2001), taking into account the purpose of the research, the participant profile and the main theme of the study. In this context, interviews were conducted with 20 participants, all of whom lived in Mersin and Antalya and familiar with the rock samphire plant. The participants were coded between P1 and P20. In the first stage of the research, the participants were asked 4 demographic questions that examined gender, age, occupation and educational status (Table 2).

Table 2. Descriptive information of participants

Participant	Gender	Age	Occupation	Education
P1	Female	38	Academician	PhD
P2	Female	28	Academician	Master's degree
P3	Male	27	Private sector employee	Undergraduate
P4	Male	23	Private sector employee	High school
P5	Male	23	Private sector employee	High school
P6	Male	37	Private sector employee	High school
P7	Male	38	Private sector employee	High school
P8	Female	45	Private sector employee	High school
P9	Female	55	Civil servant	Bachelor
P10	Male	27	Academician	Master's degree
P11	Female	27	Private sector employee	Master's degree
P12	Female	58	Private sector employee	Primary school
P13	Female	54	Retired	Undergraduate
P14	Female	46	Private sector employee	High school
P15	Female	53	Unemployed	High school
P16	Female	31	Civil servant	Undergraduate
P17	Female	74	Retired	Primary school
P18	Female	62	Retired	Primary school
P19	Female	52	Private sector employee	Primary school
P20	Female	53	Private sector employee	Primary school

Table 3. Open-ended semi-structured interview questions asked to the participants

1	Do you know the rock samphire?
2	Have you ever consumed rock samphire?
3	If so, in what form did you consume it? (e.g. raw, pickled, etc.) Can you share your recipes with us?
4	How often do you consume rock samphire?
5	Do you know in which regions/areas the plant grows?
6	How do you obtain rock samphire?
7	If you collect it yourself, do you have a specific collection period/time?
8	Do you know the health effects of rock samphire consumption?

In the second stage, data were obtained using a questionnaire consisting of 8 interview questions. The research questions asked to the participants are given in Table 3.

The information obtained was interpreted and documented in accordance with qualitative analysis methods. In the last part of the study, different suggestions were made for more consumption of traditional functional foods and especially rock samphire.

Results and Discussion

When the demographic characteristics of the participants were analyzed, it was determined that they were between the ages of 23-74. 25.0% of the participants graduated from primary school, 35.0% from high school and 40.0% from an associate degree or higher education program. The demographic characteristics of the participants are given in Table 4.

After the demographic questions, the research questions on rock samphire were started. Questions 1 and 2 were included in the interview to record in written form the questions about rock samphire awareness that were asked verbally during sampling, to increase the reliability of the subsequent questions and to provide double control. As expected, the responses showed that all participants were familiar with the rock samphire plant and had consumed it at least once before. When the rock samphire consumption of the participants was analyzed, it was found that 50.0% of them consumed rock samphire once a month, 35.0% once a year and 15.0% once a week. In response to the question "Do you know in which regions rock samphire grows?", detailed answers were obtained as "on rocks and coastal edges in the Mediterranean and Aegean regions, bays in the Aegean and Mediterranean, sea slopes and sometimes sandbanks". It was found that 35.0% of the participants obtained rock samphire from their acquaintances, 30.0% bought rock samphire from market, bazaar or roadside vendors, 30.0% collected the plant themselves and 5.0% obtained it from the internet. While 20.0% of the respondents stated that the rock samphire was collected in summer, 10% of the respondents stated that the tips of the plant should be collected without damaging the root during collection.

The answers to question 8 "Do you know the health effects of rock samphire consumption?" are shown in Table 5.

As indicated in Table 5, 85.0% of the participants were informed about the functional properties of rock samphire and were aware of the benefits of the plant.

According to the answers given to the research questions asked about the consumption of rock samphire (each participant reported more than one type of consumption), 60.0% of the participants consumed rock samphire in pickle form. Generally consumed as an appetizer with seafood, it was stated that pickled rock samphire is a great appetizer with its unique taste.

Table 4. Demographic characteristics of participants

Distribution	f	%
Gender		
Female	14	70
Male	6	30
Age		
23-40	10	50
41-58	7	35
59-76	3	15
Occupation		
Private sector employee	11	55
Academician	3	15
Retired	3	15
Civil servant	2	10
Unemployed	1	5
Education		
Primary school	5	25
High school	7	35
Undergraduate	3	15
Bachelor	1	5
Postgraduate	4	20

Table 5. Responses to the question 8

Participant	Answers
P1	“It strengthens the body and has a cell regenerating effect.”
P2	“It is good for intestinal wounds and stomach ailments. High in vitamin C, gives energy. Strengthens immunity.”
P3	“It stimulates appetite and is good for cardiovascular diseases.”
P4	“It's good for the liver.”
P5	“It's good for goiter.”
P6	“I don’t know.”
P7	“It strengthens immunity.”
P8	“It is good for intestinal and stomach diseases and diarrhea.”
P9	“It is good for stomach disorders.”
P10	“I don’t know.”
P11	“I don’t know.”
P12	“It's good for heart health.”
P13	“Diuretic, applied as an ointment on wounds.”
P14	“It's good for goiter and earache.”
P15	“It is good for stomach pains and reflux.”
P16	“It is good for body infections.”
P17	“Ointment of the plant is applied on skin wounds, has a painkiller effect.”
P18	“It is consumed for earache, good for gastritis.”
P19	“It's good for goiter.”
P20	“It is good for thyroid diseases.”

Table 6. Culinary uses of rock samphire

Culinary Use	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Pickled	✓	✓		✓		✓	✓			✓
Salad	✓	✓				✓				✓
With yogurt		✓						✓		
Stewed		✓								
Pastry filling										
Tea		✓								
Culinary Use	**P11**	**P12**	**P13**	**P14**	**P15**	**P16**	**P17**	**P18**	**P19**	**P20**
Pickled	✓		✓		✓		✓	✓		✓
Salad			✓		✓		✓		✓	
With yogurt			✓				✓	✓		
Stewed			✓				✓	✓		
Pastry filling							✓	✓		
Tea								✓		

Picrure 1. Kaya koruğu turşusu (pickled rock samphire)
(Prepared and photographed by the authors.)

Picture 2. Kaya koruğu salatası (rock samphire salad)
(Prepared and photographed by the authors.)

Picture 3. Kaya koruğu yoğurtlaması (rock samphire with yogurt)
(Prepared and photographed by the authors.)

Picture 4. Kaya koruğu yemeği (stewed rock samphire)
(Prepared and photographed by the authors.)

According to the information obtained from the participants, the most delicious presentation of rock samphire pickles is the form served with olive oil, lemon and garlic. The detailed responses regarding the consumption of rock samphire are shown in Table 6.

A pickled rock samphire appetizer prepared according to the recipe obtained from the participants is shown in Picture 1. Select and pick fresh rock samphires carefully. Cut the roots and wash them well. Put them in a pot and add enough water to cover them. Bring the water to a boil and keep them in boiling water for about 10 minutes, then remove it from the stove and rinse with cold water. Prepare the brine by mixing water, rock salt and lemon or vinegar in a deep bowl. Place the plant in a suitable-sized jar. Add the brine to fill the jar completely. Optionally, olive oil, garlic and chickpeas can be added. After fermenting for an average of 1-2 weeks in a cool place with the lid closed, the pickled rock samphire is ready for consumption. Lemon, garlic and olive oil can be preferred for serving. It is a great accompaniment for fish tables.

45.0% of the participants reported that they consume rock samphire by boiling it for a short time to remove the bitterness and then adding it to seasonal salads. Since rock samphire is harvested during the summer season, it was found that salads with olive oil and lemon prepared with cherry tomatoes, cucumber, olives, rock samphire and tulum cheese or halloumi cheese are frequently consumed in the region (Picture 2). After washing and draining the fresh rock samphire, boil them in water for a short time to remove the bitter taste. Then rinse with cold water and combine with peeled and diced tomatoes and cucumbers. After adding green and black olives, walnuts, cheese, etc., flavor with a sauce prepared with crushed garlic, lemon and olive oil and served. It pairs very well with seafood and Turkish raki.

25.0% of the participants stated that they occasionally consume rock samphire with yogurt (in a way known as yoğurtlama in the region). This recipe is shown in Picture 3. After boiling and rinsing in cold water, mix the fresh rock samphire with crushed garlic, strained yogurt and just a little salt due to the natural salty taste of the plant. Pour the mixture into serving bowls. Melt the butter in a separate pan and add ground paprika. When the mixture thickens a little, pour it over the yogurt and rock samphire and serve.

20.0% of the participants stated that they consume rock samphire stewed. They stated that it goes well with slightly hot spices (Picture 4). In a pan, saute finely chopped onion and garlic with olive oil. Then add some tomato paste, spices, chickpeas and hot water. Okra can also be added if desired. Once the ingredients are cooked, a few minutes before removing them from the stove, add the rock samphire. Mix all the products again and let them rest. Serve warm.

10.0% of the participants stated that after boiling the rock samphire for a short time, they cut it into small pieces, mix it with curd cheese and consume it as a pastry filling, and 10.0% of the participants stated that they make tea by boiling the leaves in water (by adding an average of 100 ml of water for each branch) and consume it in this way.

In the studies in the literature, as an example of the use of rock samphire in different countries, a traditional recipe called “rock samphire hash” is prepared by mixing the stems and leaves of rock samphire with pickled cucumber and capers was reported in Britain. In Greek mythology, rock samphire is mentioned as a vegetable offered to Theseus by Hecate (Renna et al., 2017).

In previous studies, it was found that rock samphire was used in pickles, salads and meals in the Aegean Region, similar to the results of this study, but unlike the results of this study, it was also used in omelet making (Tan et al., 2017; Kök et al., 2020). Özbek and Güzeler (2022) examined the elements found in Mersin local cuisine in their study and reported a pickled rock samphire recipe parallel to the recipe obtained in this study. Another example of different uses of rock samphire was reported by Renna et al. (2017). According to the study, dried rock samphire leaves can also be used as an additive with its coloring feature in food preparation. In the study conducted by Aksoy et al. (2019), it was reported that rock samphire leaves are widely consumed as pickles and used as an iodine depot against goiter disease.

Conclusion

Rock samphire is a plant that has been known and consumed for many years in the Mediterranean and Aegean regions of Turkey. In today’s world, the health effects of many new foods are being investigated and the importance of foods with high functional properties is increasing. At this point it is essential to introduce the foods that are consumed locally, together with their consumption patterns and to bring them into gastronomy, in terms of the development of the country’s cuisine, the economic development of the regions and the protection of public health.

Rich in essential nutrients such as vitamins C and A, as well as minerals like calcium and magnesium, rock samphire offers valuable antioxidant properties, supporting overall health and immune function. Beyond its culinary merits, rock samphire has been historically used for its diuretic, digestive, and anti-inflammatory properties, making it a versatile herb with both gastronomic and medicinal appeal. All these properties have been confirmed by local consumers who are familiar with this plant in the regions where rock samphire is frequently consumed and have been pronounced as functional properties within the scope of this study.

In this study, it was aimed to determine the awareness and consumption patterns of the plant in Mersin and Antalya, two provinces of the Mediterranean region, where rock samphire is one of the most widely consumed regions. As a result of the study, three common and three rare consumption patterns were found. The introduction of these consumption patterns is important in terms of introducing the product to the kitchens and increasing its agricultural value. In addition, it was understood that rock samphire consumers are aware of the health effects of the product and the majority of them consume the product frequently for these reasons. Another result of the study is that rock samphire consumption is not so frequent even in the region and only 15.0% consume it once a week.

The geography of Turkey is home to a wide variety of natural functional foods, but the recognition of these foods has generally been limited to the regions where they are grown and consumed. For such reasons, better promotion of region-specific foods will result in functional foods being included in more kitchens. In addition, it is also necessary to facilitate access to less well-known food such as rock samphire. In order to overcome these deficiencies, it is important to establish new food supply chains in the regions where rock samphire grows naturally and to transport raw and processed products to regions where there are public health problems due to iodine deficiency, such as the Central Anatolia Region of Turkey. At this point, it would be appropriate for municipalities, non-governmental organizations and other stakeholders who can take responsibility to take the lead in the use of traditional values such as rock samphire that can contribute to agricultural development both locally and nationwide.

According to the information received from the participants, it is recommended to improve the sales and distribution network of rock samphire, which can rarely be found in markets outside the region, to try the consumption of the product by consumers and chefs using the recipes given in this study or using different recipes, to raise public awareness by explaining and promoting the healthy aspects of such foods more often, and finally to conduct similar research for other natural functional foods. Moreover, food analysis of the rock samphire grown and consumed in the region and the local recipes mentioned in this article can be conducted and the unique active substances they contain can be determined.

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Screening of Promising Maize Varieties Against Maize Weevil (*Sitophilus zeamais* Motschulky) Under Storage Condition

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ABSTRACT

The maize weevil (*Sitophilus zeamais* Motschulsky) causes significant quantitative and qualitative losses during storage. To identify resistant varieties of maize against this pest, an experiment was conducted in a Completely Randomized Design (CRD) with 11 varieties in free and no-choice conditions. The study measured weight loss, mean bored grain, debris, and weevil numbers at 30, 60, and 90 days. The findings showed that BG13Y-POP, Manakamana-7, and RML-19/RML-6 were the most resistant varieties, with weight loss percentages of 1.99%, 1.47%, and 1.74%, respectively, and final weevil numbers of 104, 72, and 73. Ganesh-2 and ZM-401 were the most susceptible varieties, with weight losses of 7.34% and 6.05%, respectively. The maximum debris weight was found in RML-761/RL-105 (1.98 g), while the minimum was found in Manakamana-7 (0.26 g). The highest number of bored grains was observed in Ganesh-2 (81), while the lowest number was observed in Rampur-4 (51). Similarly, ZM-401 (158) and Ganesh-2 (165) exhibited the highest weevil population, while the lowest count was found in Rampur-4 (72). Overall, using resistant varieties, such as Manakamana-7, BG13Y-POP, and RML-19/RML-6, can be an effective approach for reducing post-harvest losses from weevil infestation.

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Introduction

Maize (*Zea mays* L.) is an important cereal crop and ranks as the third most important globally, with an annual production of 1,210 million tons cultivated on 205.8 million hectares, achieving a productivity rate of 5.8 tons per hectare (FAOSTAT, 2021). In Nepal, maize holds the position of the second most important cereal crop, with an annual production of 2.99 million tons grown on 979 thousand hectares, and a productivity rate of 3.06 tons per hectare (MOALD, 2020/21). It contributes 6.83% to the Agricultural Gross Domestic Product (AGDP) in Nepal, making it the second-highest cereal crop after rice (MOALD, 2020/21). Maize cultivation is widespread across Nepal, with the crop being particularly successful in the mid-hills and Terai regions. Nonetheless, maize production is primarily concentrated in hilly areas, and the size of farms tends to be smaller compared to those in the Terai region (Gairhe et al., 2021).

Post-harvest loss due to insect infestation is a major challenge facing maize production worldwide, with losses ranging from 1-5% in developed nations to 20-50% in developing nations (Nukenine, 2010). The maize weevil (*Sitophilus zeamais*), Angoumois grain moth (*Sitotroga cerealella*), and larger grain borer (*Prostephanus truncatus*) are the most prevalent insect species that attack stored maize grains, and they have a rapid rate of reproduction that can lead to significant damage within a single season (Ojo et.al., 2016). Despite improvements in production, this persistent problem continues to impact maize farmers, especially in developing countries.

The maize weevil (*Sitophilus zeamais:* Coleoptera: Curculionidae) is one of the most harmful pests of grains, cereals, and other stored items. It can cause significant qualitative and quantitative damage to untreated stored maize, resulting in grain weight loss ranging from 20% to 90% (Muzemu et al., 2013). Poor storage practices in Nepal are the primary reason for maize seed deterioration, leading to a 10% to 20% quantitative loss during storage (Bhandari et al., 2015). The use of resistant cultivars may be the most effective pest management strategy to mitigate such losses, especially in existing integrated pest management programs (Keba and Sori, 2013).

Maize weevil damages stored maize, making it unsuitable for human consumption and the market, thus reducing pest damage is crucial for grain preservation (Bergvinson and Garcia-Lara, 2004). However, the majority of farmers in Nepal grow hybrid varieties, which are more susceptible to pest infestation and post-harvest loss. Although insecticides have been used to control the maize weevil, their overuse has led to insecticide-resistant populations (Ribeiro et al., 2003). Moreover, botanicals used for biological control have been shown to degrade grain quality. Therefore, there is a pressing need to identify resistant maize varieties that can limit weevil damage. This is crucial for maintaining grain preservation and attaining food security and food safety (Khakata et al., 2018). Therefore, it is essential to select resistant varieties as a long-lasting solution to prevent weevil infestation, taking into account the dual goals of food security and food safety. The use of resistant varieties, in conjunction with other control techniques, could establish an integrated pest management program that is safe, effective, and environmentally friendly.

Materials and methods

Location of the Experiment

The experiment was conducted at the Institute of Agriculture and Animal Science (IAAS), Lamjung Campus, Lamjung, Nepal, during August- November, 2018. The site is situated at an elevation of 610m, with 28.13° N latitude and 84.42° E longitude.

Experimental Design

For this experiment, the treatments were organized using a Completely Randomized Design (CRD) with three replications for each maize variety. The maize varieties used in the research were collected from National Maize Research Program (NMRP) Rampur, Chitwan, as well as from local farmers in Sundarbazar, Lamjung.

Weevil Culture

The starting culture of *S. zeamais* used in the experiment was obtained from the stock at Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur. The weevils were reared on a susceptible maize variety at the entomology lab of IAAS in Lamjung. To obtain a fresh weevil population for the experiment, 500 g of infestation-free maize grains were placed in a plastic jar, and 500 live weevils were added for incubation. The jar was securely covered with muslin cloth during incubation

Experimental Setup

All the maize samples were sun-dried to make them free from insects. The grain moisture content (GMC) of the oven-dried maize samples was determined using a WILE - Moisture Meter and adjusted to 14% moisture for all varieties. The experiment was carried out in both free-choice and no-choice tests under laboratory conditions, at a temperature range of 20-25°C and relative humidity (RH) of 75±5%.

Free Choice Test

The experiment involved testing eleven different maize varieties, with 50 g of grain samples used for each variety. The experiment was arranged in a Completely Randomized Design using polythene bottles with a diameter of 5cm and a height of 7cm, with three replications. Four circular holes were made at the bottom of the bottles on all four sides, and no lids were used to allow the weevils to freely enter the bottle. The bottles were arranged in a circular manner inside a wider circular container with a diameter of 60cm and a height of 20cm. Then, 800 F2-progeny of *S. zeamais* (irrespective of sexes) aged 20 days were released in the center of the container. The wide container was covered with black muslin.

Table 1. Treatment details

S N	Treatment	Varieties
1.	T1	ZM-401
2.	T2	RML-761/RL-105
3.	T3	BG13Y-POP
4.	T4	RML-19/RML-6
5.	T5	DEUTI
6.	T6	RAMPUR COMPOSITE
7.	T7	RAMPUR-4
8.	T8	TLBR-7
9.	T9	MANAKAMANA7
10.	T10	GANESH-2
11.	T11	ARUN-4

No Choice Test

In this experiment, 50 g of maize samples were placed into polythene bottles with a diameter of 5cm and a height of 7cm. Then, 5 pairs of F1-progeny of *S. zeamais* (male and female) aged 20 days were introduced into each bottle as an inoculum. Similarly, 5 pairs of F2-progeny of *S. zeamais* (male and female) aged 20 days were introduced into each bottle as an inoculum. The mouth of the bottles was perforated with a black muslin cloth to ensure free air circulation. The experiment was arranged in a Completely Randomized Design with three replications.

Data Collection

For data collection, 50g of maize sample was used and data were collected at 30-day intervals for three months to determine the total number of damaged grains, weight loss percentage, grain debris, weevil attraction, and weevil emergence. The count and weight method of damaged and undamaged grain as adopted by Gewinner et al. (1996) was used to determine all parameters using a weighing balance. Weight loss is an essential parameter for determining resistance in maize grains as it indicates economic loss for the farmer (Dari et al., 2010; Derera et al., 2014).

Grain weight loss % was determined by using mathematical formula.

$$\text{Weight loss } \% = \frac{(\text{Wu} \times \text{Nd}) - (\text{Wd} \times \text{Nu})}{\text{Wu} \times (\text{Nd} + \text{Nu})} \times 100$$

Where, Wu=Weight of Undamaged grain
Nd=Number of damaged grains
Wd= Weight of damaged grain
Nu=Number of undamaged grains

Statistical Analysis

The data input and tabulation were carried out using Microsoft Excel, while R package was used for statistical analysis. Analysis of variance (ANOVA) was performed at a 0.05% level of significance.

Results

Effect of Maize Varieties on Weight Loss Percentage by S. zeamais

The weight loss percentage was significantly different (P<0.05) among the tested varieties during 30, 60, and 90 days after observations in free-choice conditions (Table 2). In 30 days after treatment, the maximum percent loss was recorded in Ganesh-2 (1.88%) whereas the lowest percent loss was recorded in TLBR-7(0.23%), RML-761/RL-105(0.23%), Rampur composite (0.22%). Similarly, the maximum percent loss in 60 days of observation was recorded in ZM-401(2.72%) followed by Ganesh-2, RML-761/RL-105, and the lowest percent loss in TLBR-7, Rampur composite, and Rampur-4 respectively. However, in 90 days of observation, the grain damage percent was recorded highest in Ganesh-2 (7.34%) followed by ZM-401(6.05%), RML-761/RL-105 (4.91%), Deuti (4.79%) and least loss were recorded in Manakamana-7(1.47%), BG13Y-POP (1.90%), RML-1/RML-6 (1.74%) respectively.

Under no choice condition, a significant difference was observed at 5% level among varieties for weight loss (Table 2). At 30 days, the highest weight loss was seen on Ganesh-2 which showed susceptibility. Low weight loss was seen on RML-761/RL-105, Rampur composite, TLBR-7, while Rampur 4, ZM-401, RML-19/RML-6, Manakamana-7 was statistically par with Rampur composite. At 60 days, the highest weight loss was seen on Ganesh-2 while ZM-401 and RML-761/RL-105 were statistically at par with Ganesh-2. At 90 days, the highest weight loss was seen on ZM-401 and RML-761/RL-105 were statistically par with ZM-401. Manakamana-7, Rampur composite, Rampur-4, and TLBR-7 was less susceptible as they had low weight loss at 90 days.

Table 2. Effect of maize varieties on weight loss % under free choice and no choice by *S. zeamais* in storage, IAAS, Lamjung, 2018/19

SN	Treatment	Mean weight loss (%)					
		Free choice			No choice		
		30 days	60 days	90 days	30 days	60 days	90 days
1.	ZM-401	0.51bcd	2.72a	6.05ab	0.51bcd	2.55ab	6.64a
2.	RML-761/RL-105	0.23d	1.80abc	4.92abc	0.23d	2.45ab	6.03ab
3.	BG13Y-POP	0.82b	1.07cd	1.99d	0.82b	0.49c	1.93cd
4.	RML-19/RML-6	0.46bcd	0.41d	1.74d	0.46bcd	0.76bc	2.17bcd
5.	DEUTI	0.48bcd	1.07cd	4.79abc	0.49bcd	0.83bc	1.30d
6.	RAMPUR COMPOSITE	0.22d	0.69d	2.74cd	0.23d	0.59c	1.21d
7.	RAMPUR-4	0.32cd	0.60d	2.5cd	0.32cd	0.48c	1.08d
8.	TLBR-7	0.23d	0.27d	2.87cd	0.23d	0.55c	1.18d
9.	MANAKAMANA 7	0.25cd	0.77cd	1.48d	0.25cd	1.01bc	1.48d
10.	GANESH-2	1.89a	2.12ab	7.35a	1.89a	3.07a	5.40abc
11.	ARUN-4	0.67bc	1.27bcd	3.89bcd	0.69bc	0.97bc	1.75cd
F-TEST		***	*	**	*	*	*
LSD		0.44	1.14	2.53	0.045	1.63	2.36
CV (%)		47.31	41	40.63793	47.3	27	51.21

Mean separation in columns followed by the same letters are not significantly different at P=0.05 Note: LSD= Least Significant difference, CV= Coefficient of Variation, *= Significant at 5% level of significance, ** = significant at 1% level of significance, ***=significant at 0.1% level of significance.

Effect of Maize Varieties on Grain Damage by S. zeamais

The mean grain damage was significantly different (P<0.01) among the tested varieties during 30, 60, and 90 days after observations in free-choice conditions (Table 3). In 30 days after treatment, maximum grain damage was recorded in Ganesh-2(15) and RML 761/RL-105 (15) varieties whereas lowest percent loss was recorded in Manakamana-7(7), Rampur-4(7) and RML 19/RML-6(8). Similarly, maximum mean grain damage in 60 days of observation was recorded in Ganesh-2(38) followed by ZM-401(34), RML-761/RL-105(38), and the lowest percent loss in RML-19/RML-6(18), Rampur-4 (19), Arun-4(20) respectively. However, in 90 days of observation, maximum mean grain damage was recorded in Ganesh-2(80) followed by ZM-401(74), RML-761/RL-105(73) BG13Y-POP (72) respectively and least grain damage was RML-19/RML-6(44) followed by Rampur-4 (51) respectively.

The mean grain damage was significantly different (P<0.01) among the tested varieties during 30, 60, and 90 days after observations in free-choice conditions (Table 3). In 30 days after treatment, maximum grain damage was recorded in Ganesh-2(15) and RML 761/RL-105 (15) varieties whereas the lowest percent loss was recorded in Manakamana-7(7), Rampur-4(7), and RML 19/RML-6(8). Similarly, maximum mean grain damage in 60 days of observation was recorded in Ganesh-2(38) followed by ZM-401(34), RML-761/RL-105(38), and the lowest percent loss in RML-19/RML-6(18), Rampur-4 (19), Arun-4(20) respectively. However, in 90 days of observation, maximum mean grain damage was recorded in Ganesh-2(80) followed by ZM-401(74), RML-761/RL-105(73) BG13Y-POP (72) respectively and least grain damage was RML-19/RML-6(44) followed by Rampur-4 (51) respectively.

Table 3. Effect of maize varieties on number of grain damage on free choice and no choice by *S. zeamais* in storage, IAAS, Lamjung, 2018/19

SN	Treatment	Mean number of grain damage					
		Free choice			No choice		
		30 days	60 days	90 days	30 days	60 days	90 days
1.	ZM-401	11abc	34ab	74ab	14a	40abc	79 ab
2.	RML-761/RL-105	15a	33abc	73ab	15a	43ab	67 bc
3.	BG13Y-POP	12ab	27bcd	72ab	6b	17d	51 d
4.	RML-19/RML-6	8c	18e	44d	2b	17d	44 d
5.	DEUTI	11abc	32abc	73ab	6b	22d	53 d
6.	RAMPUR COMPOSITE	9bc	23cde	57bcd	3b	23d	48 d
7.	RAMPUR-4	7c	19de	51cd	2b	25cd	53 d
8.	TLBR-7	9bc	25bcde	59bcd	3b	23d	69 ab
9.	MANAKAMANA 7	7c	20de	56bcd	4b	26cd	56 cd
10.	GANESH-2	15a	38a	80a	17a	47a	81 a
11.	ARUN-4	9bc	20de	65abc	6b	30bcd	55 cd
F-TEST		*	***	**	***	***	***
Grand mean		10.57	26.51	64.48	7.57	28.75	59.97
LSD		4.5	8.95	17.29	6.59	13.74	12.20
CV (%)		25.82	19.82	15.742	51.1	28	11.95

Mean separation in columns followed by the same letters are not significantly different at P=0.05 Note: LSD= Least Significant difference, CV= Coefficient of Variation, *= Significant at 5% level of significance, ** = significant at 1% level of significance, ***=significant at 0.1% level of significance.

Table 4. Effect of maize varieties on number of weevil emergence in storage, IAAS, Lamjung, 2018/19

SN	Treatment	Final weevil number (free choice)	Final weevil number (no choice)
1.	ZM-401	158a	105 a
2.	RML-761/RL-105	135b	99a
3.	BG13Y-POP	104c	75 bc
4.	RML-19/RML-6	72f	68 bc
5.	DEUTI	87de	104 a
6.	RAMPUR COMPOSITE	79def	60 c
7.	RAMPUR-4	72f	72 bc
8.	TLBR-7	91cd	68 bc
9.	MANAKAMANA 7	73ef	60 c
10.	GANESH-2	165a	110 a
11.	ARUN-4	89cd	88 ab
F-TEST		***	***
Grand mean		102	83
LSD		13.99	23.32
CV (%)		8.011	16.43

Mean separation in columns followed by the same letters are not significantly different at P=0.05 Note: LSD= Least Significant difference, CV= Coefficient of Variation, ***=significant at 0.1% level of significance.

Effect of Varieties on Number of S. zeamais Progeny Emergence

In no-choice test, there were variations, and significant differences were observed at <0.01% level among the 11 varieties for weevil progeny emergence (Table 4). It ranged from 60 to 110 mean adult emergences, which was low in Rampur composite followed by Manakamana-7 indicating their tolerance to *S. zeamais*. Similarly, the mean number of weevils was high in Ganesh- 2 followed by ZM-401 and Deuti showing their susceptibility to *S. zeamais*. The remaining tested varieties were intermediate types. In free-choice test also, significant differences were observed at <0.01% level among the tested varieties (Table 4). It ranged from 72 to 165 mean weevil emergence. The mean number of progeny emergence was low in Rampur-4 and RML-19/RML-6 followed by Manakamana-7 indicating their tolerance to *S. zeamais*. Similarly, the mean number of progeny emergence was high in Ganesh -2 and ZM-401 followed by RML-761/RL-105 and BG13Y-POP by showing their susceptibility to the *S. zeamais*. The rest of the genotypes were intermediate types.

Effect of Maize Varieties on Grain Debris Release by S. zeamais

In no-choice test, the maize varieties were statistically significant at 1% level for grain debris release (Table 5). It ranged from 0.095g to 1.73g mean grain debris, which was low in BG13Y-POP in followed by Manakamana-7, Arun-4, and RML-19/RML-6 indicating their tolerance to *S. zeamais*. Similarly, the mean amount of grain debris release was high in RML-761/RL-105 followed by ZM-401, and Ganesh-2 showing their susceptibility to *S. zeamais*. The remaining tested varieties were intermediate types.

Under free-choice test, the maize genotypes were statistically significant at 1% level for grain debris release which ranged from 0.21g to 1.24g (Table 5). The amount of grain debris release was low in Arun-4 followed by RML-19/RML-6 showing their tolerance to *S. zeamais*. Similarly, the amount of grain debris release was high in Ganesh-2 followed by Deuti and ZM-401 indicating their susceptibility to *S. zeamais*. The remaining genotypes were intermediate types.

Table 5. Effect of maize varieties on grain debris released in storage, IAAS, Lamjung, 2018/19

SN	Treatment	Final grain debris (free choice)	Final grain debris (no choice)
1..	ZM-401	0.87ab	1.74 a
2	RML-761/RL-105	0.54bc	1.90 a
3.	BG13Y-POP	0.28bc	0.10 c
4.	RML-19/RML-6	0.24c	0.33 c
5.	DEUTI	0.80ab	0.45 bc
6.	RAMPUR COMPOSITE	0.36bc	0.62 bc
7.	RAMPUR-4	0.15c	0.54 bc
8.	TLBR-7	0.28bc	0.43 bc
9.	MANAKAMANA 7	0.44bc	0.26 c
10.	GANESH-2	1.24a	1.47 ab
11.	ARUN-4	0.22c	0.41 c
F-TEST		**	**
Grand mean		0.49	0.75
LSD		0.54	0.89
CV%		65.39	23

Mean separation in columns followed by the same letters are not significantly different at P=0.05 Note: LSD= Least Significant difference, CV= Coefficient of Variation, ** = significant at 1% level of significance.

Table 6. Preference of *S. zeamais* at 30 days on selected maize genotypes in storage, IAAS, Lamjung, 2018/19

SN	Treatment	Preference
1	ZM-401	45.67a
2	RML-761/RL-105	43.67a
3	BG13Y-POP	20.33b
4	RML-19/RML-6	21.00b
5	DEUTI	23.00b
6	RAMPUR COMPOSITE	14.67b
7	RAMPUR-4	16.33b
8	TLBR-7	15.00b
9	MANAKAMANA 7	16.00b
10	GANESH-2	40.67a
11	ARUN-4	16.33b
F-TEST		***
Grand mean		24.79
LSD		15.48
CV (%)		36.67

Mean separation in columns followed by the same letters are not significantly different at P=0.05 Note: LSD= Least Significant difference, CV= Coefficient of Variation, ***=significant at 0.1% level of significance.

Effect of Maize Varieties on S. zeamais Preference

In free-choice test, there was a statistically significant difference at 1% level for the mean number of weevils attracted on tested genotypes at 30 days (Table 6). The mean number of weevils attracted to the different varieties ranged 14.66 to 45.67. The preference was high in ZM-401, RML-761/RML-105, Ganesh-2. Similarly, the preference was low in Rampur composite, Arun-4, TLBR-7. The remaining tested genotypes were intermediate types.

Discussion

The number of damaged grains, grain debris, weight loss, and weevil emergence were all significantly different between maize varieties, and weevil attraction is a crucial signal for determining a variety's vulnerability. According to Abebe *et al.* (2009), the susceptibility index is positively correlated with the number of F1-progeny, percentage of damaged grains, and grain weight loss. These variations in the susceptibility of the maize types reveal a variety's innate capacity to defend itself against *S. zeamais* attack. Physical factors like grain hardness, pericarp surface texture, and nutritional factors like amylose, lipid, and protein content or non-nutritional factors, particularly phenolic compounds, may all contribute to this resistance (Garca-Lara et al., 2004).

Two biochemical substances that take the form of phenolic amides, may operate as antibiosis agents against *S. zeamais* (Muzemu et al., 2013). According to Arnason *et al.* (2004), this phenolic promotes resistance through both structural and antibiosis effects. According to Garcia-Lara *et al.* (2004), resistant maize cultivars have robust pericarps with high concentrations of hydroxycinnamic acids. Additionally, it has been claimed that the effects of antibiotics made insects more agitated, which decreased eating and may have contributed to the low levels of grain damage and weight loss among resistant cultivars (Muzemu et al., 2013). Grain hardiness has been identified as the primary resistance factor for weevils (Bamaiyi et al., 2007). Tryptophan and lysine, two components of proteins that are resistant to the maize weevil, may have a negative impact on feeding behavior, host preference, or growth and development (Arnason et al., 2004). According to Arnason *et al.* (2004), protein content was inversely linked with a maize variety's susceptibility to *S. zeamais*.

The significant difference in the number of weevils that emerged among the varieties may be caused by antibiosis effects, such as the lack of essential nutrients and an unbalanced proportion of nutrients, which cause weevil larvae to die and, in some cases, weevil adults to die before laying eggs (Derera et al., 2001).

Antixenosis mechanisms, such as a smooth pericarp, which could discourage weevils from oviposition and feeding and also hinders mandibles from grabbing maize kernels, may be responsible for the reduced discharge of grain debris. It is also well known that seeds contain attractants. The isolation of many volatiles by including hexanoic acid, that are attractants to maize weevils may account for the variations in weevil attraction between types (Keba and Soli,2013). The flint-like quality of some kinds of grain may also contribute to resistance. Flint maize is resistant to weevil because it is hard, thick with vitreous endosperm, starch collected in the periphery, and poor moisture absorption (Suleiman et al., 2014).

Conclusion

This study aimed to identify resistant varieties of maize against the *S. zeamais* infestation. The results showed that BG13Y-POP, Manakamana-7, and RML-19/RML-6 were the most resistant varieties, while Ganesh-2 and ZM-401 were the most susceptible. The use of resistant varieties such as Manakamana-7, BG13Y-POP, and RML-19/RML-6 could significantly reduce post-harvest losses from weevil infestation. Farmers and other stakeholders can use this information to choose the most suitable maize varieties for storage and reduce losses caused by *S. zeamais*. Further research could explore the genetic and biochemical mechanisms underlying the resistance of these varieties to weevil infestation.

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Presence of Phthalates in Vacuum Packaged Kashar Cheeses Sold Retails in Türkiye

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ABSTRACT

Phthalate esters (PAEs) are synthetic compounds, commonly used as plasticizers and softeners in plastic material production, and they are recognized as endocrine-disrupting chemicals. This study was focused on monitoring the extent of PAEs migration in vacuum-packaged Kashar cheeses and plastic materials used in their packaging. A total of fifteen cheese samples were tested for PAEs, including benzyl butyl phthalate (BBP), dibutyl phthalate (DBP), diisononyl phthalate (DiNP), di-2-ethylhexyl phthalate (DEHP), and diisodecyl phthalate (DiDP). The quantification (LOQ) and detection (LOD) limits varied between 0.197 to 0.619 µg/mL and 0.059 to 0.185 µg/mL for all analytes, respectively. All phthalate ester concentrations in both of the cheese samples and their packaging materials were below the detectable level LOQ of the analytical method. FTIR spectra also confirmed that the packaging materials which consisted of polypropylene and polyethylene.

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Introduction

Plastic materials used in packaging differ in terms of their chemical structures, processing-dependent properties, added additives, and combinations with other polymers (Guerreiro et al., 2018). They are petroleum-based products manufactured by the polymerization of many simple units called monomers. Moreover, plastics are not chemically pure polymers. Therefore, many additives such as lubricants, plasticizers, stabilizers, UV absorbers, antioxidants and antistatic agents, colorants, optical brighteners, etc., are used in the production of plastic materials, that are necessary for improving the quality and the characteristics of the final products (Keleş 2011; Ibarra et al., 2018). These additives and chemicals may migrate from packaging materials to the foods under suitable environmental conditions (Dagdelen, 2016; Korkmaz, 2018).

One of the substances that make the transition from plastic packaging to foods is phthalate esters (PAEs). PAEs, known as di-alkyl or alkyl aryl esters of 1,2-benzene dicarboxylic acid, have been used as plasticizers. Since the 1920s, PAEs have been used as plasticizers to give flexibility, softness, and to increase their durability to plastic materials, especially polyvinyl chloride (PVC) (Ustun et al., 2015; Xu et al., 2020). More than 25 different types of phthalates are used in commercial applications, and each PAE provides a unique quality to the product (Zhang et al., 2021). The most commonly used PAEs in consumer products are dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), di-2-ethylhexyl phthalate (DEHP), diisobutyl phthalate (DiBP), diisononyl phthalate (DiNP), diisodecyl phthalate (DiDP), and di-n-octyl phthalate (DnOP). In addition to their use as plasticizers, phthalates also find applications as fragrances in many personal care products such as cologne, perfume, soap, shampoo and other cosmetic products. The extensive production and application of phthalates have resulted in their ubiquitous distribution in all facets of the environment including air, water, and soil.

PAEs are not bonded to plastics by chemical bonds such as covalent (Mondal et al., 2022). Therefore, they can be released directly or indirectly into food during their manufacture, use, processing, and storage (Du et al., 2016; Hou et al., 2022). It has been stated that this situation causes phthalates to be among the most common chemical contaminants (Adenuga et al., 2020).

PAEs are categorized as endocrine-disrupting chemicals all over the world due to their mimicking nature to human estrogens, and they exhibit mutagenic, teratogenic, and carcinogenic effects on humans (Mondal et al., 2022). As a result of increasing epidemiological

studies on health, long-term exposure to PAEs may cause many adverse effects such as delaying of neuro-development in infants (Jurewicz and Hanke, 2011; Jones et al., 2018), increasing respiratory diseases and allergic reactions in children (Buckley et al., 2018), and these effects, decreasing of lung function and depression in the elderly (Kim et al., 2018). It has also been observed in studies that it is associated with health problems such as low birth weight, autism, attention deficit and hyperactivity (Otero et al., 2015). The use of certain PAEs such as DBP, BBP, and DEHP have been restricted in foods and plastic packages in most countries due to their adverse effects on health, frequent use as plasticizers, and environmental risks. The current codex in Türkiye complies with the legal regulations of the European Union on the Regulation of the "Use of Plastics in Food Contact Materials" (EU No: 10/2011), and the specific limits for BBP, DHEP, and DBP are determined as 30 mg/kg, 1.5 mg/kg and 0.3 mg/kg, respectively.

In the food industry, packaging techniques with plastic films are widely used because of their positive aspects such as flexibility, versatility and increasing the shelf life of food. The best-known example of these packaging techniques is vacuum packaging. By vacuuming the package, that the air is removed from the package and hermetically sealed it (Patil et al., 2020). Thus, removing the oxygen in the packaging minimizes the development of aerobic microorganisms and oxidation problems (Hecer, 2012).Vacuum packaging is frequently used in the packaging of meat products, semi-hard/hard cheeses, olives, and nuts. In addition to plastic films such as polyamide (PA), polyethylene terephthalate (PET), PVC and ethylene vinyl alcohol (EVOH), polypropylene (PP), polyethylene (PE), and PE-copolymers can also be used in vacuum packaging due to their heat-sealing characteristic (Guerreiro et al., 2018). In this type of packaging, plastic films tightly wrap the food, depending on the contact area and time, some components may pass/migrate from polymers to food. There are few studies involving phthalate transitions from vacuum-packaged or wrapped foods with plastic film. Cao et al. (2014) reported that only DEHP was detected in some cheese samples covered with PVC film at levels from 0.29 to 15 mg/g, with an average of 2.8 mg/g. Guerreiro et al. (2018) investigated a mass spectrometry-based application for the detection of several compounds from plastic, directly from vacuum-packaged meat samples. They reported that this application was capable of identifying contaminants in all pieces of beef that were in contact with the vacuum-plastic packaging.

Although there were many studies on phthalates in packaging materials and food, there is a lack of information dealing with the transmission of PAEs from vacuum-packaged cheeses. Also considering the lipophilic nature of phthalates and the high-fat content of cheeses, it is essential to detect the possible presence of phthalates in cheeses. Therefore, the aim of this study was to determine the migration of PAEs which can be widely found in and whose use is restricted in the legislation, from vacuum-packaged Kashar cheeses. At the same time, some physical and chemical properties of cheeses were analyzed to determine whether the composition of the cheeses had an effect on the possible migration of PAEs through plastic films.

Material and Methods

Material and Reagents

Vacuum-packaged Kashar cheese (a pasta-filata type) samples were used in this study. A total of 15 commercial samples were collected from different companies in Türkiye. Descriptive information about each cheese is listed in Table 1. Kashar cheeses were stored at 4°C before analyses and all analyses were carried out at least in duplicate for each cheese sample.

Standards of PAEs, including BBP, DBP, DiNP, DEHP, and DiDP, were obtained from Sigma-Aldrich (Charleston, USA). The stock solution of the PAEs with the concentration of 100 mg/L was prepared in methanol and stored at -20°C. A series of working solutions of PAEs were prepared by appropriate dilution of the stock solution before use. Phthalate quantitation was performed according to a least four-point calibration curve, which was linear for concentrations. The presence of phthalate compounds was initially identified by observing the m/z 149 ion in the GC–MS spectrum. All standards were also injected onto the GC-MS system and retention times are shown in Figure 1.

Table 1. Descriptive information about analysed Kashar cheeses

Sample No	Type of plastic film	Fresh/Ripened	Company	Shape and weight
C1	PET	Fresh	National	Molded 500 g
C2	PP	Ripened	National	Sliced 350 g
C3	LDPE	Fresh	National	Molded 500 g
C4	Unspecified	Ripened	National	Sliced 436 g
C5	LDPE	Fresh	National	Molded 500 g
C6	PE	Ripened	National	Sliced 300 g
C7	PE	Ripened	National	Sliced 350 g
C8	Unspecified	Fresh	Local	Molded 500g
C9	Unspecified	Fresh	National	Molded 600g
C10	Unspecified	Ripened	Local	Sliced 350 g
C11	Unspecified	Ripened	National	Sliced 350 g
C12	Unspecified	Fresh	Local	Molded 1000g
C13	LDPE	Ripened	National	Sliced 400 g
C14	Unspecified	Fresh	Local	Molded 500g
C15	Unspecified	Ripened	Local	Sliced 350 g

Table 2. Main validation parameters for phthalates extraction and analysis

Analytes	Correlation coefficient, R^2	LOQ (µg/mL)	LOD (µg/mL)
BBP	0.9868	0.619	0.185
DBP	0.9855	0.351	0.105
DEHP	0.9958	0.273	0.081
DINP	0.9980	0.197	0.059
DIDP	0.9982	0.478	0.143

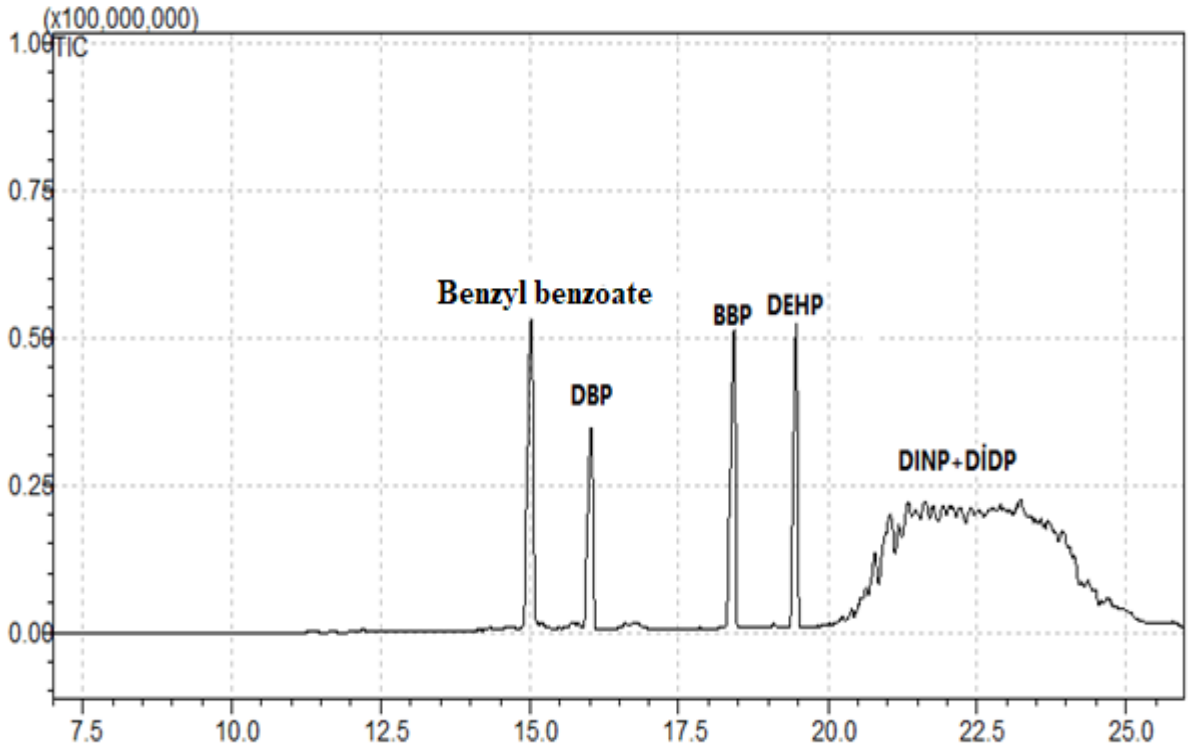

Figure 1. The retention times of PAEs and internal standard

The limit of detection (LOD) and limit of quantification (LOQ) for each phthalate were calculated from eight replicated measurements of low-concentration standard solutions according to the literature data (Kafalı, 2008; Kumawat et al., 2022). LOD and LOQ were calculated from the signal/noise ratios which were multiplied with factor 3 and 10, respectively. Details of quality assurance for phthalates analysis, including the LOD, LOQ and R^2 values are given in Table 2.

Methods

Physicochemical analysis

Cheese samples were analyzed in duplicate by gravimetric method for total dry matter (IDF, 1982), Gerber method for fat, and titration with $AgNO_3$ for salt (Hayaloglu, 2009). Titratable acidity (as % lactic acid) was determined by titration method with 0.1 N NaOH using phenolphthalein as an indicator (Hayaloglu, 2009). For pH measurement, 10 g cheese was softened in 15 mL distilled water and the pH of the slurry was measured using a pH meter (Mettler-Toledo, Seven Compact TM S210, Schwerzen-bach, Switzerland) with combined electrodes (Hannon et al., 2003). The fat (F) rate in dry matter (DM) (%) and the salt (S) rate in dry matter (%) were calculated using the formulas F/DM x100 and S/DMx100, respectively.

Extraction of phthalate esters from cheeses

Cheeses were cut to pieces 1 cm from the surface at different points and the 15 g of homogenised cheese pieces were weighed into a glass vial. Benzyl benzoate (BB) was added as an internal standard and allowed to infuse into the cheeses for 15 min. Then, 30 mL of acetonitrile:dichloromethane (1:1) mixture was added and shaken in an orbital shaker for 4 hours at room temperature. The samples were centrifuged for 5 min at 3000 rpm (Hettich model 320 R, Tuttlingen, Germany) and the supernatant was frozen (–20 °C) to separate the fat layer. Following evaporation to dryness using a rotary evaporator (Buchi, model RV300, Switzerland), the residue was reconstituted in one mL of acetonitrile and transferred to a glass vial for analysis by GC-MS (Bradley et al., 2013).

Extraction of phthalate esters from packaging material

An area of 0.5 dm^2 was cut from each plastic material used in the vacuum packaging of cheeses, and the weight was recorded. The packaging materials were then cut into small particles of approximately 1 × 1 cm and transferred to the glass jar. BB was added as an internal standard and allowed to infuse into the packaging for 15 min. Then, 40 mL of acetonitrile:dichloromethane (1:1) mixture was added and shaken in an orbital shaker for 4 hours at room temperature. The extract was poured into a clean glass bottle and evaporated to dryness on a heating block set at

50°C. The resulting residue was dissolved in 1 mL of acetonitrile before analysis for GC-MS (Bradley et al., 2013).

Gas Chromatography-Mass Spectrometry (GC-MS) operating parameters

The determination of the PAEs in vacuum-packaged Kashar cheese samples and plastic material were performed using a gas chromatography coupled to mass spectrometry (GC-MS) system (Shimadzu, Shimadzu QP–2010, Kyoto, Japan). The injection volume was 1 µL and the split ratio was 10:1. High-purity helium was used as carrier gas and the flow rate of helium was 1 mL/min. The separation of PAEs was achieved using an HP 5-MS (60 m x 0.25 mm x 0.25 µm) column (Agilent, J&W, Santa Clara, CA, USA). The oven temperature was kept at 80 °C for 2 min and increased to 320 °C with 20 °C/min rate and kept at this temperature for 12 min. All samples were analyzed in duplicate.

Fourier transform infrared spectroscopy (FTIR) analyzes of plastic films

The sections (1 cm^2) taken from the plastic materials used in vacuum packaging for FTIR analysis were placed in the reading chamber of the attenuated total reflection (ATR) unit of the Perkin Elmer FTIR (Perkin Elmer Spectrum One FTIR spectrometer, Germany). Absorption or transmittance spectra were taken at a wavelength of 400-4000 cm^{-1}. The type of polymer was determined by comparing the obtained spectra with the electronic certified library (BIO-RAD Sadtler Spectral Databases Library Polymers vol.2) and Polymer Data Handbook (Korkmaz, 2018).

The thickness of the plastic films

The thickness of the plastic films was measured using a digital micrometer (LTF ART 327.13-14).

Results and Discussion

Physicochemical characteristics of cheeses

To determine the effect of chemical composition on the migration of PAEs in vacuum- packaged Kashar cheeses, cheeses were analyzed in terms of some physicochemical properties (Table 3). The dry matter contents of the cheeses ranged from 51.08-63.08%, and it was lower level in fresh Kashar cheese samples. The fat content of most samples of Kashar cheeses was over 30%, and all cheese samples fall into the full-fat cheese (45 ≤ milk fat) category according to Turkish Food Codex Cheese Notification (2015/6). The pH and titratable acidity of the cheese samples varied between 4.98 to 5.94 and 0.351% to 1.359%, respectively. Generally, it was determined that the titratable acidity of the cheeses offered for sale as fresh was lower and the pH values were higher. The chemical compositions of cheeses were similar to with previously reported results for fresh and mature Kashar cheeses (Hayaloglu, 2009; Yılmaz and Dagdemir, 2012).

Method Validation

The phthalates in extracts of cheese samples were identified by matching GC retention times against those of standards and by comparing the mass spectra with standards. The GC chromatogram obtained from five phthalate standards is shown in Figure 1. A successful separation of the five analytes was achieved. DBP, BBP and DEHP peaks were sharp and symmetrical. DiNP and DiDP were present as partially co-eluted peaks due to the presence of many isomers. Phthalates were totally separated in less than 25 min. All calibration curves were linear and correlation coefficients (R^2) were in the range of 0.985–0.998. The detection limits (LOQ and LOD) were separately calculated for each phthalate compound from signal/noise. LOQ and LOD values varied between 0.197 to 0.619 µg/mL and 0.059 to 0.185 µg/mL for all compounds, respectively (Table 2).

Concentration of phthalates in cheeses and packaging materials

The fifteen homogenized cheese samples were analyzed for PAEs (DEHP, BBP, DBP, DiNP, DiDP), and the data obtained are presented in Table 4. All phthalate ester concentrations in the analyzed samples were below the detectable level (LOQ). In this case, it was possible to state that there was no detectable transition from the plastic films used in vacuum packaging, and these cheeses did not have any significant contact with plastic materials during the production stages.

Table 3. Some physcochemical characteristics of Kashar cheese samples

Samples	Total solid (%)	Fat (%)	Salt (%)	Fat in dry matter (%)	Salt in dry matter (%)	Acidty (%)	pH
C1	53.46±0.02	27.75±1.06	2.22±0.16	51.91±1.95	4.15±0.31	0.468±0.05	5.71±0.01
C2	60.99±0.22	35.00±0.00	2.69±0.09	57.39±0.21	4.41±0.15	1.062±0.07	5.28±0.02
C3	58.42±0.02	32.00±0.00	1.61±0.19	54.77±0.01	2.76±0.33	0.540±0.00	5.44±0.00
C4	63.08±0.40	36.00±1.41	2.22±0.16	57.07±1.87	3.52±0.23	0.648±0.05	5.40±0.02
C5	57.56±0.05	33.00±0.70	1.05±0.16	57.33±1.17	1.82±0.28	0.684±0.05	5.52±0.01
C6	62.45±0.07	35.75±1.06	3.39±0.16	57.24±1.63	5.43±0.27	0.693±0.01	5.69±0.01
C7	60.67±0.02	32.25±0.35	3.68±0.08	53.16±0.60	6.06±0.13	1.359±0.03	5.27±0.00
C8	56.70±0.00	31.25±0.35	2.80±0.16	55.11±0.63	4.94±0.29	1.116±0.05	4.98±0.01
C9	51.08±0.18	29.75±0.35	1.57±0.08	58.24±0.90	3.07±0.15	0.540±0.05	5.94±0.03
C10	59.37±0.12	37.50±0.71	3.10±0.08	63.16±1.32	5.22±0.15	0.689±0.03	5.64±0.03
C11	60.03±0.29	39.00±0.00	2.16±0.08	64.97±0.32	3.60±0.15	0.729±0.03	5.50±0.05
C12	55.18±0.08	32.75±0.35	2.74±0.08	59.35±0.73	4.96±0.15	0.351±0.01	5.81±0.00
C13	61.12±0.04	39.50±0.70	2.22±0.16	64.63±1.11	3.63±0.26	0.585±0.01	5.54±0.01
C14	52.44±0.02	26.50±0.70	2.69±0.16	50.53±1.36	5.13±0.31	0.793±0.05	5.59±0.00
C15	57.04±0.21	29.25±0.35	3.15±0.16	51.28±0.43	5.52±0.30	0.828±0.05	5.49±0.02

Table 4. Migration of PAEs in cheeses and plastic packaging materials

Samples	DEHP	BBP	DBP	DiNP	DiDP	Thickness of plastic films (mm)	FTIR
1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.070±0.01	PP
2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.070±0.00	PP
3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.080±0.00	LDPE
4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.080±0.01	PE
5	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.070±0.01	LDPE
6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.060±0.00	PE
7	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.060±0.00	PE
8	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.070±0.00	PE
9	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.110±0.00	PP
10	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.080±0.00	PP
11	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.085±0.01	PE
12	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.065±0.00	PE
13	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.075±0.00	PE
14	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.085±0.00	PE
15	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.090±0.00	PE

Several studies performed in various countries on the migration of phthalates from different food groups, and the variable results are presented. Yang et al. (2019) reported that DEHP and DBP were detected in 218 of 283 convenience food samples in China, and the highest migration content was found in cakes for DEHP and DBP. In Canada, a total of 118 food samples wrapped with PVC film were analyzed for migration of PAEs, and DEHP was detected in only some of the cheese samples (9 out of 40 samples, at 0.29 to 15 mg/g) and other phthalates were not detected in any of the 118 samples (Cao et al., 2014). Oruç (2020) reported that PAEs were found below the detection limit in samples of flavored carbonated beverages, lemonade and drinking water packaged with plastic material. Beltifa et al. (2018) reported that cheeses repackaged in flexible plastic sold in the Tunisian market contained high quantities of DBP and DEHP with concentrations up to 0.46 and 2.339 mg/kg. Van Holderbeke et al. (2014) tested 591 Belgian foods and 30 packaging materials for phthalate levels. In this study, DEHP was the most detected phthalate compound in milk and dairy products (72 of 79), meat and meat products (35 of 37), fish and fish products (18 of 22), snacks (27 of 29), condiments and sauces (40 of 41) and in packaging materials (30 of 30). Pil-Bala et al. (2019) reported that the concentrations of DBP and DEHP in the cheeses packaged in PE or PP packages ranged from 46 to 62 ng/g and 78 to 96 ng/g, respectively.

Phthalate migration depends on numerous factors, such as temperature and storage period, type and characteristics of the package, contact area and duration with packaging material, and chemical composition of the packaged food. It has been reported that PAEs may accumulate especially in foods with high fat content due to their lipophilic nature (Bogdanovicova and Jarosova, 2015). However, there was no detectable transition despite the fact that the analyzed Kashar cheeses had high-fat ratios (ranging from 26.50% to 39.50%). Similarly, it was stated that the moisture content of the cheeses may have an effect on migration. As seen in Table 3, the moisture contents of the cheese samples were low, this might cause an increase in consistency, preventing the passage of phthalates to some extent. Indeed, Goulas et al. (2000) reported that the moisture content in cheese may have an effect on the migration of phthalates used as plasticizers and that high moisture content may reduce the consistency and increase the diffusion rate of plasticizers such as di-(2-ethylhexyl) adipate (DEHA).

Another factor in migration is the properties of the plastic material, especially the type of plastic material from which the packaging material is made. Therefore, to investigate whether packaging material is the primary source of PAEs all packaging materials were analyzed for phthalates. The transition of PAEs from the plastic films to the solvents was below the detectable level (<LOQ). This result was in agreement with Cao et al. (2014) who reported that phthalates were not detected in any of the extracts of the packaging materials. It is seen that this situation is related to the type of plastic films used in vacuum packaging. The descriptive information on the packaging, showed these were made from PE/LDPE, PET, and PP polymers, and in some samples the type of plastic material used in the packaging was not specified (Table 1). As a matter of fact, it has been reported that plasticizers are mostly used for softening and flexibility of hard materials such as PVC, and theoretically, PP contains fewer plasticizer additives than PVC due to the softness and flexibility coming from polypropylene's molecular structure (Fang et al., 2017). Similarly, it was stated that PE was a naturally flexible polymer and less plasticizer was needed (Cao et al., 2010). The fact that a significant part of the analyzed packaging materials is produced from PE/LDPE and PP polymers can be considered as the reason why PAEs are below the detectable level. De Anda-Flores et al. (2021) reported that only one phthalate was detected in 5 of the 15 commercially obtained PVC cling films used for food packaging in Mexico. Korkmaz (2018) reported that the transitions of PAEs from 10 plastic packages made of PET were below the detectable level.

In addition, the ambient temperature is an essential factor in the transitions, and the migration of PAEs can migrate at a higher level at high temperatures. Vacuum-packaged Kashar cheeses were generally stored at refrigerated temperature. This condition may cause to preventing of possible passage of PAEs. Indeed, Yang et al. (2017) reported that PAEs did not show a significant release from PE plastic films to different food simulants at temperatures of 4 °C and -18 °C, and the transition rates were much lower than the transitions at higher temperatures. The characteristics of plastic films used in food packaging such as type, thickness, and permeability of plastic materials are also considered as an effective factor on migration. The thickness of plastic films ranged from 0.060 to 0.110 mm, and there was not much difference between the thicknesses of the plastic films, except for one sample (sample 9) (Table 4). However, because PAEs could not be determined in both cheese and packaging materials analyzed, a relationship could not be established between the film thickness and the passage of these compounds.

FTIR results

The FTIR spectra of the plastic films used in vacuum packaging were compared with the certified electronic library and literature information. FTIR results verified that sample 2 was comprised of PP, and samples 3, 5, 6, 7, 13 comprised PE/LDPE; however, from the descriptive information on the package, it was stated that sample 1 was produced from PET polymer. But it was more compatible with PP according to the FTIR spectra. Also, some plastic films were not specified which type of plastic film was used on the packaging (Table 1). A part of these samples (4, 8, 11, 12, 14, and 15) gave characteristic absorption bands such as C–H stretching vibrations at 2914 and 2847 cm^{-1}, C-H scissoring vibrations at 1470 and long-chain CH_2 rocking vibration at 718 cm^{-1}, and their spectra matched to a large extent with PE. Considering the specific peaks for PP, it was also concluded that the two samples (9 and 10) could most likely be produced from PP polymer.

Conclusions

In this study, a total of fifteen cheeses and their packaging materials were analyzed for the migration of five PAEs including BBP, DBP, DiNP, DEHP, and DiDP. The present results showed that the concentrations of PAEs in cheeses were under the LOQ, and there was no detectable transition from plastic films used in vacuum packaging to cheese. At the same time, it was determined that the cheese did not come into contact with plastic materials during the production or storage periods. However, it should be kept in mind that although they are present in foods in low concentrations, plastic materials in contact with food constitute the primary source of exposure to phthalates, and considering that this exposure may be lifelong, they may have long-term adverse effects on human health. For this reason, it is thought that it would be beneficial to carry out more detailed studies to determine the transitions of PAEs, taking into account the worst conditions of plastic materials used in packaging, including plastic films used in vacuum packaging.

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Application of the Ohmic Heating Process to Make a Semolina Dessert with Milk

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ABSTRACT

Traditional milk desserts are one of the most essential dessert groups that Turkish society consumes. Due to foaming activity, it was aimed to investigate the feasibility of the ohmic heating system to produce a semolina dessert with milk. Hence, an ohmic heating treatment was used to heat the milk, semolina, and sugar mix from 20°C to 100°C using three different voltage gradients (15, 17.5, and 20 V/cm) and then boil for two minutes. It was found that the current value escalated from 20°C to approximately 86°C but decreased after 86°C due to foaming. Since the total consumed energy during the ohmic cooking treatment was inversely proportional to the treatment time, the total consumed energy values decreased based on the rising voltage gradient. As a result, the feasibility of the ohmic heating treatment for making a traditional semolina dessert with milk was determined in this study.

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Introduction

Dairy products, such as milk-based desserts, provide several crucial nutrients to sustain health and avoid chronic diseases (Jouki et al., 2021; Kuriya et al., 2020; Parmar et al., 2018; Silva et al., 2020). Hence, many dairy products can be found in the market, such as desserts, dairy beverages, yogurt, and cheeses (Silva et al., 2021; Viana et al., 2021). Milk-based desserts contribute several nutrients, such as potassium, phosphorus, riboflavin, magnesium, fatty acids, niacin, and proteins, to the diet (Silva et al., 2021). In addition, these products are more attractive to many consumers worldwide due to their functional value (Kuriya et al., 2020). However, conventional heating processes adversely affect the bioactive components, product quality, and functionality of these products (Cappato et al., 2017; Kuriya et al., 2020). Therefore, an alternative heating method such as ohmic heating is needed to diminish the deterioration of these components and retain the functional, sensory, and nutritional quality of milk-based desserts.

Ohmic heating (OH), also known as "joule heating" and "electrical resistance heating," is the process of passing an alternating current through food between two electrodes. Thus, heat generation occurs in the product, and electrical energy is transformed into heat energy (Ariç Sürme and Sabancı, 2021; Rocha et al., 2022). It has been pointed out that this heating process provides a fast, efficient, and homogeneous heating process, especially for liquid foods (Alkanan et al., 2021; Ariç Sürme and Sabancı, 2021; Gavahian et al., 2019). The OH treatment has been applied in many foods for heating (Cappato et al., 2017), evaporation (Icier et al., 2017), drying (Acar et al., 2022), thawing (Cevik and Icier, 2021), cooking (Goksu et al., 2022), and extraction (Çilingir et al., 2021).

There have been a few studies where the OH treatment was applied in milk and dairy products (Kuriya et al., 2020; Parmar et al., 2018; Rocha et al., 2022; Silva et al., 2021). In a study, this treatment was used for ice cream production, and its electrical properties were determined (Suebsiri et al., 2019). In addition, the milk was evaporated using the ohmic heating process at various voltage gradients (Ariç Sürme and Sabancı, 2021). Recently, sweets were produced from whey using an ohmic heating process, and their quality properties and electrical conductivity values were examined (Coimbra et al., 2020). However, throughout the OH treatment at high voltage gradients increasing energy efficiency, foaming activity

was observed in the milk near the boiling temperature (Ariç Sürme and Sabancı, 2021; Rocha et al., 2022). Similar trends were published for various fruit juices and similar products (Icier and Ilicali, 2004; Sabancı and Icier, 2019; Yildiz et al., 2009). Hence, this treatment should be proved whether it is suitable for producing milk-based desserts.

Therefore, the objective of the present study was to explore whether the OH treatment at high voltage gradients was suitable for making a semolina dessert with milk due to the foaming activity, affecting the electrical conductivity. During the heating process, the current and electrical conductivity values were examined, and then the total energy and average power values of the semolina dessert with milk were determined.

Materials and Method

Raw Materials

The semolina (Filiz, Bolu, Turkey), milk (Pinar, Izmir, Turkey), and sugar (Torku, Konya, Turkey) required for the cooking of the milk semolina dessert using ohmic heating were obtained from a local market in Tunceli, Turkey. Until the cooking time, the milk was kept at 4°C and the semolina and sugar in a cool and dark environment.

The Ohmic Heating System

The system image of the ohmic cooking process is demonstrated in Figure 1. The cooking process was achieved by heating the mixture of milk, sugar, and semolina from 20°C to 100°C using 3 different voltage gradients (15, 17.5, and 20 V/cm) and then cooking at 100°C for 2 minutes. The machine was not switched off for 2 minutes during the cooking process.

During the warm-up period, a custom-made microprocessor was run to store electrical properties, voltage, temperature, and current values were recorded every second. The test cell of the system was constituted of polyoxymethylene, and the electrodes are constituted of titanium. A T-type (Cole Palmer, UK) was used to measure the temperature value. Equations 1 and 2 were used to calculate the total energy and average power, respectively, consumed in the system.

$$Q\ (J) = I \times V \times t \quad (1)$$

Q, I, V, and t are the total consumed energy, current, voltage, and time, respectively.

$$P\ (W) = \frac{Q}{\sum t} \quad (2)$$

P is the average power.

Statistical Analysis

All experiments were done three times. SPSS software for Windows (version 20.0; IBM, Chicago, IL, USA) was used for analysis of variance (ANOVA) and the difference ($P<0.05$) between each voltage gradient were evaluated using Duncan`s multiple range test.

Results and Discussion

The time-dependent temperature change in the heating period, which is the first part of the cooking process of the semolina dessert with milk, is given in Figure 2. It was determined that the temperature value of the dessert raised as the processing time raised for all voltage gradients used in the present study (Table 1). It was also found that the high-voltage gradient has a higher temperature value at the same time among three voltage gradients. In addition, the voltage gradient has an impact on the heating rates from 20°C to the target cooking temperature. Furthermore, the first stage of this cooking process was that the mixing of semolina, sugar, and milk was heated up to 100°C, and the second stage was that it was cooked at 100 ˚C for 2 minutes. The total heating time of this mix differed for each voltage gradient. Accordingly, as the voltage gradient raised, the time needed to reach the boiling temperature value was reduced, so the processing time decreased by approximately 35% during the warm-up period. It has been revealed that the processing time was reduced due to the increased voltage gradient in several applications of OH, such as heating, cooking, reaching the required temperature for extraction, and dissolving different samples (Ariç Sürme and Sabancı, 2021; Çilingir et al., 2021; Goksu et al., 2022; Sabancı and Icier, 2019).

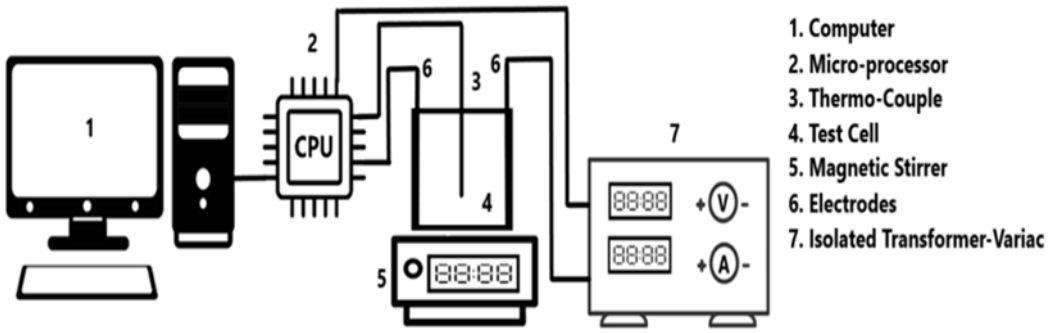

Figure 1. Schematic description of ohmic heating system

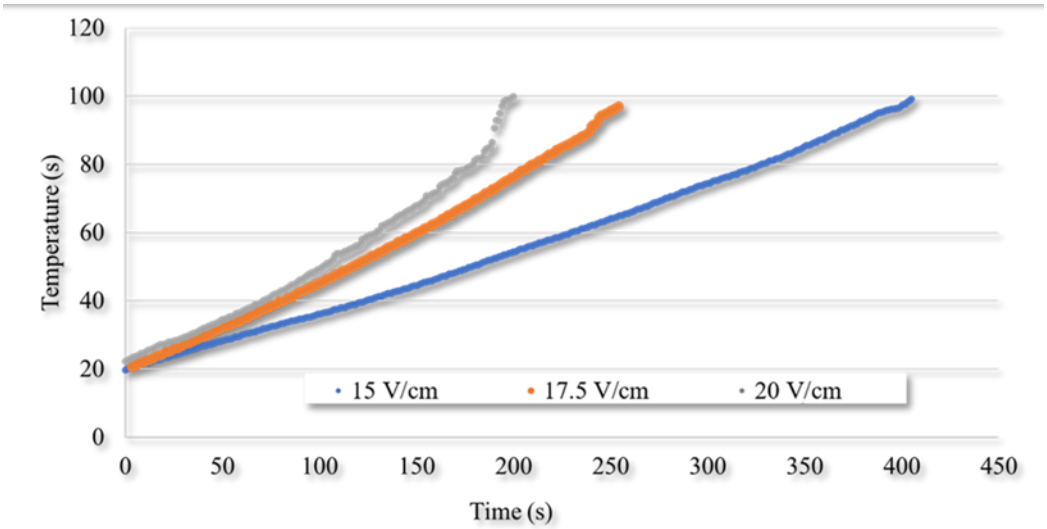

Figure 2. The changes in temperature with time during the heating period

Table 1. The total consumed energy (TCE) and average power (AP) for each voltage gradient

Treatment Period	Voltage Gradient (V/cm)	Process Time (s)	TCE (kJ)	AP (W)
Heating	15	391±13*[a]	27.4±0.8[a]	70±2.1[a]
	17.5	264±7[b]	24.8±0.3[b]	94.0±1.1[b]
	20	203±8[c]	25.4±0.7[b]	125.0±3.5[c]
Cooking	15	120±10[d]	7.0±2.4[c]	45.2±3.7[d]
	17.5		12.9±0.2[d]	107.3±1.7[e]
	20		15.5±0.2[e]	129.6±1.5[c]

[a, b, c, d, e] The values in a column with the same lowercase letter are not significantly different (P>0.05).; * Standard deviation.

In addition, the heating rates for each voltage gradient, 15 V/cm, 17.5 V/cm, and 20 V/cm, were 0.20, 0.30, and 0.39°C/s, respectively. It was reported that during the heating of pomegranate juice from 20°C to 85°C, the heating rate raised with increment in the voltage gradient, and the highest heating rate was reported as 55 V/cm (Sabancı and Icier, 2019).In another study, the voltage gradient influenced the warming rate of sea water during the warming period, and the highest heating rate was acquired at 11.04 V/cm (Assiry et al., 2010). Likewise, the heating rate affected by the voltage gradient was reported for various food process applications in which the ohmic heating process was used for heating (Icier and Ilicali, 2004; Sabancı and Icier, 2019; Yildiz et al., 2009).

The electrical conductivity (EC) value varies depending on the test cell (distance between two electrodes, product contact area) and electrical (current and voltage) properties (Icier et al., 2017; Icier and Ilicali, 2004). Especially during the warm-up period, the properties of the test cell and the voltage value are constant, while the EC value changes with the current value. Therefore, the change in current influences the EC value throughout the process. The variation of the temperature of the semolina desert with milk during the warming period is given in Figure 3. Accordingly, the change in the current for the temperature varied from 20°C and 100°C was altered from 0.41 to 1.15 A, from 0.48 to 1.32 A, and from 0.54 to 1.55 A for 15 V/cm, 17.5 V/cm, and 20 V/cm, respectively. As expected, the current value raised as the temperature raised in the present study.

The formation of foam in milk can hinder or even limit the application of ohmic heating because it reduces electrical conductivity (Gally et al., 2016). The foaming activity was observed at temperature values over 90 ˚C since milk is rich in proteins that behave as foaming agents. The volume of foam formed during the ohmic heating process increased with an increase in temperature, as reported in the previous study (Huppertz, 2010). In addition, as seen in Figure 3, when the foaming process started, there was a decrease in the current value. The decrease in the current value was first observed at 20 V/ cm (Fig. 3). This result may be due to the fact that the soluble salt fraction and water content were reduced at this voltage gradient. A study reported that the electrical conductivity of milk and whey is primarily because of their soluble salt fraction and water content (Mucchetti et al., 1994). Similarly, several studies reported that foaming occurred at the pre-boiling stage during the evaporation of milk or dairy products. Even though the temperature was low, foaming activity due to the high voltage gradients was reported in some studies (Ariç Sürme and Sabancı, 2021; Icier and Ilicali, 2004; Yildiz et al., 2009).

The alterations in the total consumed energy (TCE) and the average power (AP) values in the OH system during the production of the semolina dessert with milk are given in Table 1. At the end of the production of the dessert, the TCE and AP values were examined in two sections, the heating and cooking sections. In the heating part, the total energy spent in the OH treatment was 27.4±0.8 kJ, 24.8±0.3 kJ and 25.4±0.7 for 15 V/cm, 17.5 V/cm, and 20 V/cm, respectively. The results in this study displayed that TCE value decreased as the voltage gradient raised. This result can be pointed out by the shorter processing time and less heat loss due to the increasing voltage gradient.

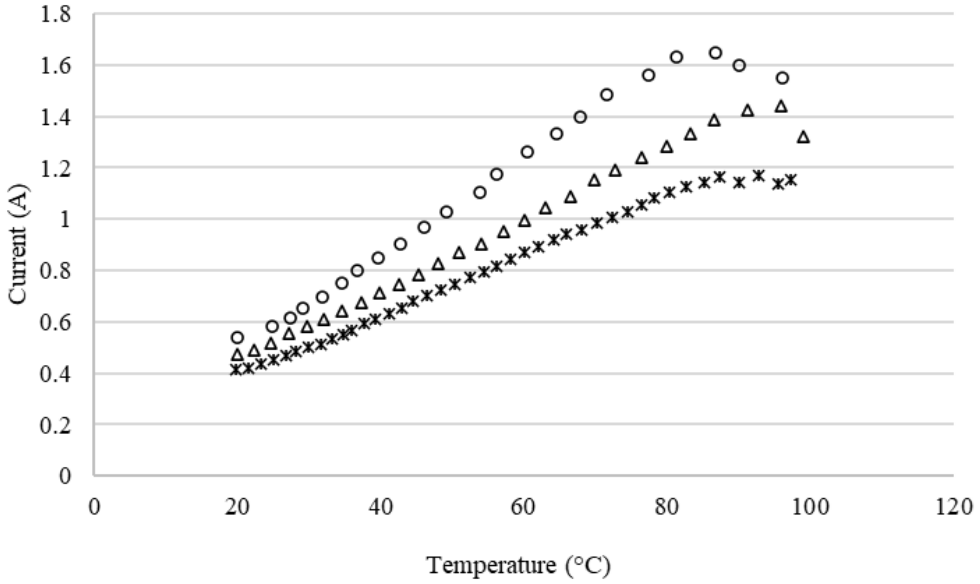

Figure 3. The changes in current with temperature during the heating period

Similarly, it has been reported in different usage of OH in food treatments that the processing time is shortened, and less energy is consumed with an increasing voltage gradient during ohmic heating applications (Çilingir et al., 2021; Goksu et al., 2022; Icier et al., 2017; Sabancı and Icier, 2019).

In addition, the TCE was determined to be 7.0±2.4 kJ, 12.9±0.2 kJ and 15.5±0.2 kJ for 15 V/cm, 17.5 V/cm and 20 V/cm, respectively, in the cooking part. It was discovered that as the voltage gradient raised in the ohmic cooking process, the TCE increased. The main reason for the difference between the two parts can be explained by the high current value at high voltage in the cooking process and, accordingly, more energy consumption at the same time (Goksu et al., 2022; Sabancı and Icier, 2019).

It was verified that the AP value raised when the voltage gradient raised in both, first and second parts. The AP value is a function of TCE, and the process time as seen Eq. 2. Therefore, it varies depending on whether the process time is short or high-energy. In the OH treatment, many studies reported that the average power value increased when the voltage gradient raised in various food process applications including heating, cooking, extraction, and evaporation processes (Çilingir et al., 2021; Goksu et al., 2022; Kuriya et al., 2020; Sabancı and Icier, 2019).

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Conclusions

It was accomplished that the semolina dessert with milk was produced by using the ohmic heating (OH) treatment instead of the conventional heating process. Although the foaming activity was observed, the cooking process was done with the ohmic heating process. It was demonstrated that the treatment time was reduced because of the increased voltage gradient, and the heating rate reached 0.44°C/s from 0.18°C/s due to the increment in the voltage gradient from 15 V/cm to 20 V/cm, respectively. In addition, the TCE in the heating period reduced as the voltage gradient raised, while the TCE in the cooking section increased due to the raising voltage gradient. Furthermore, the AP value escalated with the raise in voltage gradient for both the heating and cooking parts. As a result, this study proved that high voltage gradients can be used for making milk-based desserts, but further studies are needed for the assessment of the quality properties of milk-based desserts treated with ohmic heating at high voltage gradients due to the short processing time.

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Yield and Quality Traits of Black Cumin (*Nigella sativa* L.) Genotypes in Response to the Different Sowing Dates

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ABSTRACT

Black cumin has been used in many countries for the treatment of diseases such as cancer and diabetes, and for thousands of years as a spice, flavoring in products such as bread, and as a food preservative in pickles. Too much delay in the sowing of black cumin has a negative effect on plant growth. In order to determine the most suitable sowing dates for different black cumin genotypes, an experiment was conducted in the open-field conditions of the Eastern Mediterranean region of Türkiye at Çukurova University, for two years, in 2020 and 2021, in three different sowing dates (October 15th, November 01st, and November 15th) with three different black cumin genotypes (Çameli cultivar (G1), Adana population (G2) and Iraq population (G3)). The findings of this research demonstrated significant differences in the agronomic characteristics and overall quality of black cumin. The main components were p-cymene (51.45%-66.33%), trans-4-Methoxythujane (8.40%-11.90%), thymoquinone (0.11%-19.26%), γ-Terpinene (1.28%-9.09%), and limonene (2.93%-4.50%). The main fatty acids were determined as linoleic acid (53.97%-57.56%), oleic acid (20.98-26.40), and palmitic acid (13.73%-15.02%). Consequently, the low number of flowers and the high temperatures observed in May, along with the early spring frosts, negatively affected the fertilization of the flowers. The seed yield was adversely affected because some of the seeds could not mature.

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Introduction

Black cumin (*Nigella sativa* L.) is an annual plant from *Ranunculaceae* family, native to the eastern Mediterranean, northern Africa, the Indian subcontinent, and Southwest Asia (Hannan et al., 2021). Black cumin is also known as black seed, small fennel, Roman coriander, black caraway, and black sesame (Hossain et al., 2021). The name Nigella derives from the Latin word niger, which means "black" (Sadiq et al., 2021). Although the Nigella genus contains 12 species in the Turkish flora, only *N. sativa* and *N. damascena* are cultivated in different regions of Türkiye (Can et al., 2021).

From ancient times to the present black cumin has been included in many beliefs, religions, and holy books due to its medicinal properties. Archaeological evidence for the earliest cultivation of black cumin is insufficient, but studies are reporting that black cumin seeds have been found at various sites in Egypt, including Tutankhamun's tomb (Hannan et al., 2021). Black cumin seeds as a whole, with their extracted forms and fatty and essential oils, have been used as a preventive and therapeutic in many diseases, as well as in cosmetics (Majeed et al., 2021). Today, black cumin seeds are used as herbal medicine in developed countries to treat diseases such as fever, skin diseases, cough, rheumatism, jaundice, headache, paralysis, eczema, and loss of appetite (Haque et al., 2022). For thousands of years, black cumin seeds have been used as spice, and have also been added to coffee, tea, wine, and vinegar (Burdock, 2022).

Black cumin seeds possess significant economic worth and comprise essential oil and fatty oil (Ozyazici, 2020). Its essential oil is yellowish-brown and has a sharp bitter taste, and can be used as a flavouring additive and functional food (Sabriu-Haxhijaha et al., 2020). Furthermore, many studies reported that black cumin has been found that the medicinal value of black cumin is because of the presence of the quinone component, phenolics, and alkaloids (Ojueromi et al., 2022). Therefore, black cumin oil is used for anticancer (Malik et al., 2021), anti-inflammatory (Noël Nyemb et al., 2022), antioxidant (Hwang et al., 2021), antibacterial (Iqbal et al., 2021), antidiabetic (Akhtar et al., 2020) and antimicrobial (Alshwyeh et al., 2022) activities.

Global climate change is a multifactorial stress that has increased in recent years and exposing plants to extreme climatic conditions such as drought, salinity, and flooding, which adversely affect plant growth and developmental processes. In the coming years, an increase in temperature and a decrease in precipitation are expected in Türkiye and especially in the Mediterranean region due to the effects of global warming (Mairech et al., 2021). It is estimated that the annual warming rate in the Mediterranean region will be 20% higher than the global annual average in the coming years (Caretta et al., 2022).

Stress factors such as high temperatures due to global climate change are the major cause of plant failure and yield reduction of more than 50% (Mahajan et al., 2020). Also, winter plants are sensitive to high temperatures during their breeding phase (Moniruzzaman et al., 2015). In Türkiye, black cumin grows with an average temperature of 16.6°C, and monthly precipitation of 122.9 mm (Cahyo et al., 2020). Researchers are working to identify appropriate management options to maintain crop productivity under climate change scenarios and to understand its impact on growth and yield (Kalra et al., 2008). One of the most important factors is the choice of the appropriate sowing dates for agriculture.

The correct sowing date is very important for the successful production of a crop. Sowing date is of great importance for plant growth and controls phenological development that affects seed production (Sharangi and Roychowdhury, 2014). Late sowing shortens the vegetative period of the plants, causing the plant to complete its life cycle in a shorter time and have a short plant height, while early sowing causes higher plant height, especially with a longer vegetative period (Mehmood et al., 2018). Therefore, the benefit of correct timing of sowing is incomparable to any other agronomic management. Achieving high yield values in black cumin, which is cultivated in our country and has economic importance, depends on meeting the temperature and water requirements during planting at the right time. Therefore, the objective of our study was to evaluate the yield and quality characteristics of some black cumin genotypes at different sowing dates under Çukurova conditions.

Material and Methods

Plant Material and Growth Environment

The field study was conducted under field conditions of the eastern Mediterranean Region in Türkiye at Çukurova University in Adana (37°00′55.30″ N, 35°21′26.30″ E) for two years, both in 2020 and 2021, in three different sowing dates (October 15th, November 01st, and November 15th) with three different black cumin (*Nigella sativa* L.) genotypes (Çameli cultivar (G1), Adana population (G2) and Iraq population (G3)) (Figure 1). Seeds of the Çameli cultivar were obtained from the Transitional Zone Agricultural Research Institute, the Adana population from a local producer, and Iraq population from a local grower in Duhok. The climate is typically Mediterranean, with hot, dry summers and temperate, rainy winters (Figure 2).

The soil at the location was clay-loam in texture, with a very low organic matter content (1.10%). The soil was plowed to a depth of 30–40 centimeters and then cultivated with a field cultivator. As plant fertilizers, N and P_2O_5 were applied to the plots at a rate of 25 kg ha^{-1} as diammonium phosphate (DAP) (18–46–0). The field experiment was designed with 3 replicates based on a split-plot design. The size of each plot was 3.6 m2 (3 × 1.2 m) and each subplot occurred in four rows. The seeds of each genotype were sown at a depth of 1-2 cm on October 15th, November 01st, and November 15th, respectively. After the seeds were sown, sprinkler irrigation was applied, and in the absence of rainfall, irrigation was done as needed.

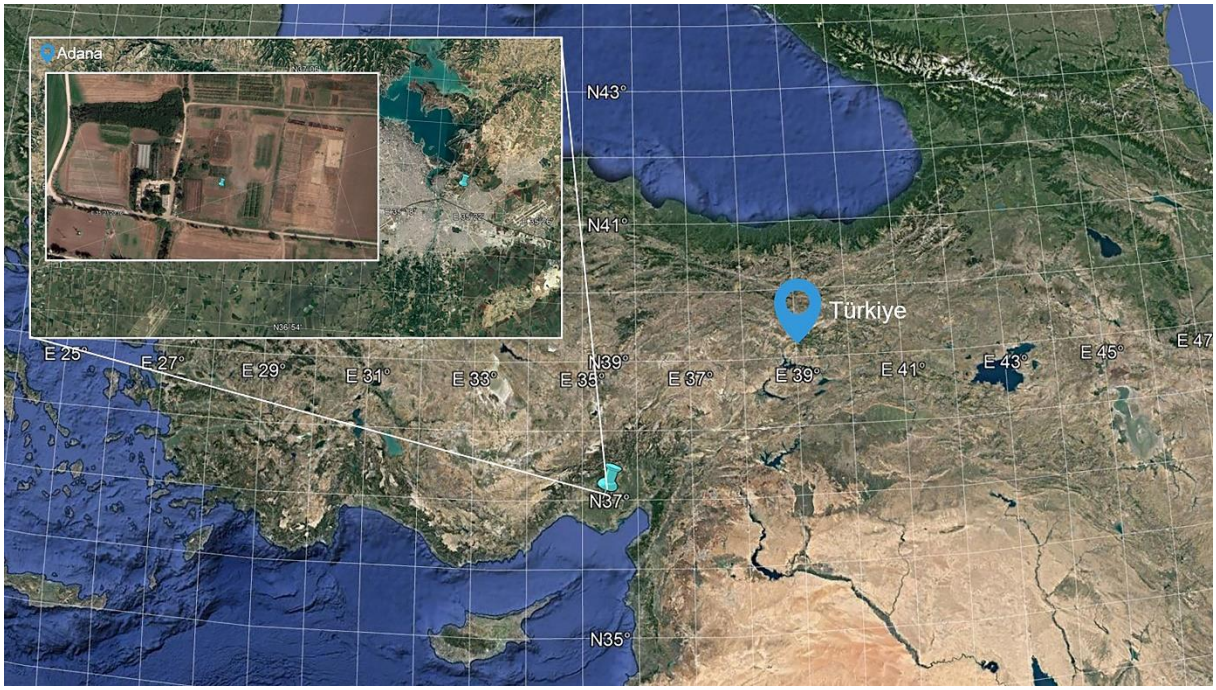

Figure 1. Çukurova University Field Crops Research and Application Area (37°00′55.30″ N, 35°21′26.30″ E)

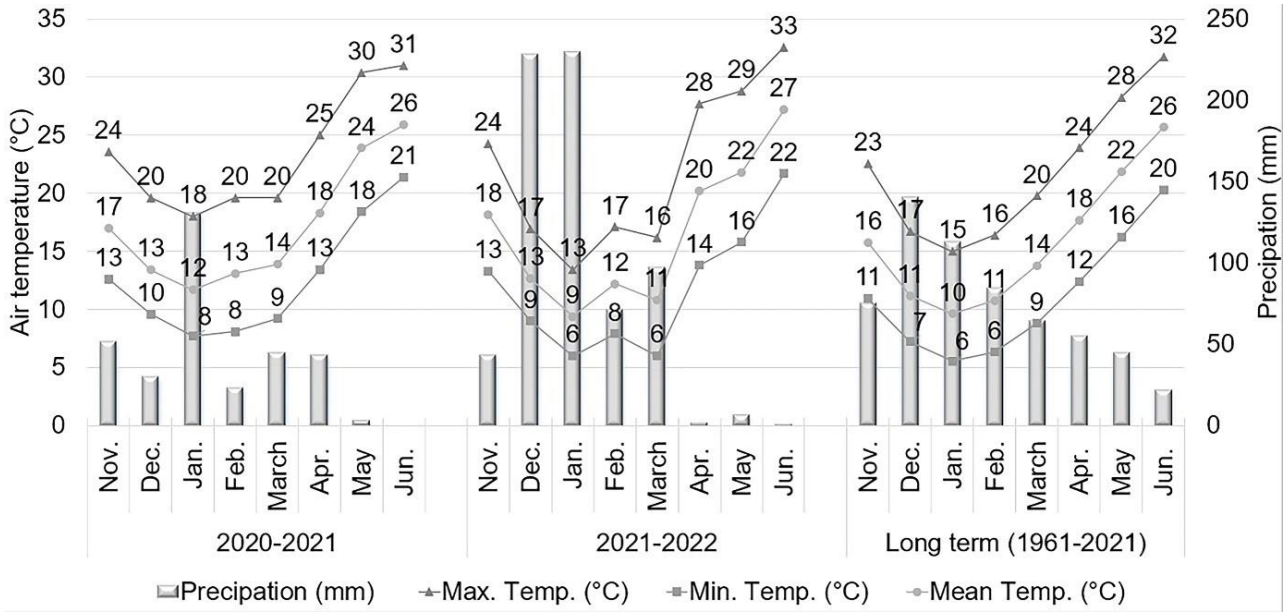

Figure 2. Meteorological data recorded during the field experiment and over the long term (1961–2021). The data were derived from the Meteorology Directorate of Adana, Türkiye

A hoe was used for weed control. During the experiment, no pesticide was applied. In each plot, ten plants were randomly chosen for analysis of agronomic and quality traits. Harvesting was done when the pods began to turn brown and the seeds began to turn black at the beginning of June. In this research, plant height-PH (cm), number of branches-NOB (pcs plant^{-1}), number of capsules-NOC (pcs plant^{-1}), number of seeds-NOS (pcs capsules-1), thousand seed weight-TSW (g), seed yield-SY (kg ha^{-1}), essential oil content-EOC (%), seed fatty oil content-SOC (%), and seed oil yield-SOY (kg ha^{-1}) were investigated as agronomic traits.

Essential oil Extraction

Black cumin seeds were ground, 30 g dried and ground seed samples from each treatment were placed in a glass balloon with 300 ml of distilled water. The Clevenger apparatus was operated for 3 hours, then the amount of extracted sample value was recorded (in mL). The essential oil content was stated as the weight of dry tissue. Chemical analyses were performed three times for each sample.

Seed oil Extraction

Black cumin seeds were ground, and 117 ml of n-hexane was added to 5 g sample, the seed oil was extracted using an ultrasonic bath at 55°C for 45 min, and then the n-hexane was evaporated from the extract at 70°C in a rotary evaporator and the remaining oil was weighed (Barut et al., 2023).

Gas Chromatography-mass Spectrometry (GC-MS) Analysis

GC-MS analyses were carried out in the Department of Biology at Kahramanmaras Sutcu Imam University. Essential oil components, seed oils methyl-esterification, and fatty acid composition protocol were performed as specified in previous studies (Barut et al., 2021; Barut et al., 2022).

Statistical Analysis

Agronomic traits were analyzed based on the split-plot design JMP 14.0 (SAS Institute Inc., Cary, NC, 1989–2019). Sowing dates and the genotypes were organized as the main plot and subplot, respectively. PCAbiplot was performed with the same software. Flourish studio was used to create the heat map. The Corrplot Package of R Studio software was utilized to calculate correlations.

Results and Discussion

Agronomic Traits

To investigate the effects of different sowing dates on different black cumin genotypes, analysis of variance and LSD test were performed on 9 agronomic traits that were recorded (Table 1). The plant agronomic traits studied revealed many variations among the evaluated black cumin genotypes. In our study, three black cumin genotypes were exposed to different sowing dates and statistically significant changes were observed in the studied traits.

There were significant differences in years and genotypes in terms of PH values. When Table 1 is examined, it is observed that 12% higher PH value (53.8 cm) was obtained in the 1st year compared to the PH in the 2nd year (48.0 cm). The higher PH observed in the 1st year compared to the 2nd year is thought to be due to the low temperatures that occurred in January, February, and March 2022. While the average minimum temperature in January 2021 was 8°C, it was lower in 2022 at 5.9°C. Plants that developed earlier in the 2nd year and were taller in the rosette stage were damaged at low temperatures below 0°C (-1.4°C) in January but recovered later on, but this had a negative effect on PH, and 1st year produced taller plants. In terms of genotype, the highest PH was found in the G1 genotype, 85% and 50% higher than the G2 (37.4 cm) and G3 (45.9 cm) genotypes, respectively. The higher PH observed in the G1 genotype compared to the G2 and G3 genotypes is due to the fact that it is a commercially registered cultivar improved through plant breeding.

Table 1. ANOVA and mean comparisons of the yield and yield components of black cumin based on years, the sowing dates, and genotypes.

Factor		PH	NB	NC	NS	TSW	SY	EOC	SFOC	SFOY
2021		53.8a	5.6	20.0	83.6b	3.1a	550.6b	0.4	27.6a	152.0b
2022		48.0b	6.1	18.2	92.9a	2.9b	1069.8a	0.4	24.5b	262.1a
S1		53.8	6.0	19.4	82.4b	2.9	780.0	0.4	25.4	198.1
S2		48.6	5.7	18.0	83.7b	3.0	727.8	0.4	26.2	190.7
S3		50.3	6.0	19.8	98.8a	3.1	922.9	0.4	26.7	246.4
G1		69.3a	7.0a	19.7ab	109.8a	2.7b	978.1a	0.9a	26.9a	263.1a
G2		37.4c	4.7c	15.8b	71.2c	3.1a	538.1b	0.1b	26.8a	144.2b
G3		45.9b	5.9b	21.7a	83.9b	3.1a	914.5a	0.2b	24.5b	224.1a
S1	G1	75.0	7.1	18.3	98.0	2.7	699.9b-e	0.9a	26.4	184.8
	G2	37.2	4.1	13.7	68.3	3.0	451.0de	0.1cd	25.9	116.8
	G3	49.2	6.7	26.3	81.0	3.1	1189.0a	0.1d	23.8	283.0
S2	G1	67.2	7.2	20.1	106.1	2.8	1147.4ab	0.8b	27.5	315.5
	G2	36.4	4.6	15.6	67.8	3.2	367.3e	0.1cd	27.5	101.0
	G3	42.1	5.3	18.3	77.1	3.0	668.8cde	0.2c	23.5	157.2
S3	G1	65.8	6.7	20.8	125.2	2.7	1087.1abc	0.9a	27.0	293.5
	G2	38.6	5.5	18.1	77.7	3.3	796.0a-e	0.1d	27.0	214.9
	G3	46.4	5.7	20.6	93.4	3.3	885.6a-d	0.2c	26.1	231.1
Mean		50.9	5.9	19.1	88.3	3.0	810.2	0.4	26.1	211.5
LSD_Y (%5)		3.8**	ns	ns	4.8**	0.1*	251.0**	ns	2.4*	64*
LSD_S (%5)		ns	ns	ns	5.9**	ns	ns	ns	ns	ns
LSD_G (%5)		3.1**	0.8**	4.1*	6.0**	0.2**	271.0**	0.05**	1.6**	72**
$LSD_{S \times G}$ (%5)		ns	ns	ns	ns	ns	470.0*	0.1*	ns	ns

PH: Plant Height (cm); NB: Number of Branches (pcs plant^{-1}); NC: Number of Capsules (pcs plant^{-1}); NS: Number of seeds (pcs capsules^{-1}); TSW: Thousand seed weight (g); SY: Seed yield (kg ha^{-1}); EOC: Essential oil content (%); SFOC: Seed fatty oil content (%); SFOY: Seed fatty oil yield (kg ha^{-1}); S1: October 15th, S2: November 1st, S3: November 15th, G1: Çameli cultivar, G2: Adana genotype, G3: Iraq genotype, ns: not significant, *: P<0.05, **: P<0.01, *Levels not connected by the same letter are significantly (P<0.05) different according to the LSD test.

Other researchers have also documented variations in PH for black cumin, ranging from 62 to 73 cm (Toncer and Kizil, 2004), 62 cm (Mehmood et al., 2018), 21 to 46 cm (Hosseini et al., 2018), 15 to 45 cm (Golkar and Nourbakhsh, 2019), 24 to 45 cm (Inan, 2020), 35 to 44 cm (Beyzi, 2020), 37 to 44 cm (Ozer et al., 2020), 26 to 60 cm (Varun et al., 2020), and 38 to 43 cm (Kara et al., 2021). When comparing the results of the PHs obtained by the researchers with the results in our study, it is observed that there are low, high, and similar values. The variation in the PH detected across these studies may reflect the genotype of the plant and the environmental influences.

Regarding the NOB, significant differences were found between genotypes. The highest NOB was obtained from the G1 genotype (7.0 pcs plant^{-1}), while it was found to be 49% and 19% higher than the G2 (4.7 pcs plant^{-1}) and G3 (5.9 pcs plant^{-1}) genotypes, respectively. The NOB observed in this study is in agreement with other results, ranging from 4 to 6 pcs plant^{-1} (Toncer and Kizil, 2004), 3 to 6 pcs plant^{-1} (Hosseini et al., 2018), 5 to 10 (Iqbal et al., 2019), 6.5 to 6.9 pcs plant^{-1} (Kara et al., 2021), 4.7 pcs plant^{-1} (Ozer et al., 2020). However, the results herein were lower than 4 to 16 pcs plant^{-1} reported by (Golkar and Nourbakhsh, 2019).

Concerning the NOC, significant differences were observed in genotypes. The highest capsules were obtained from the G3 (21.7 pcs plant^{-1}) genotype, while it was 10% and 37% higher than the G1 (19.7 pcs plant^{-1}) and G2 (15.8 pcs plant^{-1}) genotypes, respectively. Other researchers reported similar results in terms of NOC, ranging from 7 to 9 pcs plant^{-1} (Toncer and Kizil, 2004), 6 to 17 pcs plant^{-1} (Hosseini et al., 2018), 6 to 11 pcs plant^{-1} (Inan, 2020), 16 to 37 pcs plant^{-1} (Varun et al., 2020), 17 to 18 pcs plant^{-1} (Ozer et al., 2020), 16 to 20 pcs plant^{-1} (Sarkar et al., 2022), and 6 to 7 pcs plant^{-1} (Kara et al., 2021). However, the present results were less than 8 to 72 pcs plant^{-1} (Golkar and Nourbakhsh, 2019), and 10 to 65 pcs plant^{-1} (Iqbal et al., 2019).

Statistically significant differences were observed in NOS values between different years, sowing dates, and genotypes. It was found that 11% higher NOS value (92.9 pcs capsules^{-1}) was obtained in the 2nd year compared to the NOS value (83.6 pcs capsules^{-1}) in the 1st year. In terms of sowing dates, the highest NOS value was obtained at S3 sowing date (98.8 pcs capsules^{-1}), while it was 20% and 18% higher than S1 (82.4 pcs capsules^{-1}) and S2 (83.7 pcs capsules^{-1}) sowing date, respectively. The significant decrease of NOS in early sowing processes may be associated with the destructive effect of low minimum temperatures in the period when the number of leaves of the plant is high. Thus, the plants did not have enough opportunities for photosynthesis and produced fewer seeds. In terms of genotypes, while the highest NOS was obtained from the G1 genotype (109.8 pcs capsules^{-1}), which was 54% and 31% higher than the G2 (71.2 pcs capsules^{-1}) and G3 (83.9 pcs capsules^{-1}) genotypes, respectively. Other researchers reported similar results regarding NOS, ranging from (Toncer and Kizil, 2004), 39 to 94 pcs plant^{-1} (Hosseini et al., 2018), 29 to 39 pcs plant^{-1} (Rezaei-Chiyaneh et al., 2018), 56 to 88 pcs plant^{-1} (Bosh et al., 2019), and 73 to 78 pcs plant^{-1} (Ozer et al., 2020). However, the results were lower than 54 to 170 pcs plant^{-1} reported by (Golkar and Nourbakhsh, 2019).

Significant differences were found between years and genotypes with respect to TSW. It is seen that 7% higher TSW value (3.1 g) was obtained in the 1st year compared to the thousand-grain weight value (2.9 g) in the 2nd year. It is thought that the reason for the thick and full grains of black cumin sown in the first year is that the grain filling period coincides with more optimum temperatures. In terms of genotypes, the highest TSW was obtained from the G2 and G3 genotypes (3.1 g), while it was 15% higher than the G1 genotype (2.7 g). Consistent findings regarding the TSW have also been reported by other researchers, with values ranging from 1.8 g (Toncer and Kizil, 2004), 2.3 to 2.8 g (Hosseini et al., 2018), 1.5 to 1.9 g (Rezaei-Chiyaneh et al., 2018), 0.7 to 6.6 (Iqbal et al., 2019), 2.5 to 2.6 g (Ozer et al., 2020), 2.8 to 3.0 g (Sarkar et al., 2022), and 2.3 to 2.6 g (Kara et al., 2021). TSW is a significant yield-contributing trait in the majority of plant. However, the negative correlation between TSW and yield should not be ignored.

In terms of SY values, there were significant differences between years, genotypes, and the interaction of sowing date × genotype. It is observed that 94% higher SY value (1069.8 kg ha^{-1}) was found in the second year compared to the SY value in the first year (550.6 kg ha^{-1}). In terms of genotypes, the highest SY value was obtained in G1 (978.1 kg ha^{-1}) and G3 (914.5 kg ha^{-1}) genotypes, while the lowest was found in G2 genotype (538.1 kg ha^{-1}). In terms of interaction, S1G3 had the highest SY value (1189.0 kg ha^{-1}), while S2G2 had the lowest SY value (367.3 kg ha^{-1}). Other researchers reported similar results regarding the SY, ranging from 564 to 866 kg ha^{-1} (Toncer and Kizil, 2004), 824 to 1866 kg ha^{-1} (Hosseini et al., 2018), 515 to 707 kg ha^{-1} (Rezaei-Chiyaneh et al., 2018), 908 to 1277 kg ha^{-1} (Bosh et al., 2019), 176 to 662 kg ha^{-1} (Bayati et al., 2020), 1129 to 1255 kg ha^{-1} (Ozer et al., 2020), 307 to 542 kg ha^{-1} (Kara et al., 2021), and 556 to 1007 kg ha^{-1} (Moradzadeh et al., 2021). Many open flowers fell off due to late frosts without capsule formation. Deterioration of pollen formation in response to heat stress was the major contributor to yield reduction.

Significant differences in EOC were observed between different genotypes and the sowing date × genotype interaction. In terms of genotypes, the highest EOC was found in the G1 genotype (0.9%), while the lowest was found in the G2 (0.1%) and G3 genotypes (0.2%). In terms of interaction, the highest EOC was obtained from the S1G1 and S3G1 (0.9%) genotypes, while S1G3 and S3G2 had the lowest EOC (0.1%). Similar results based on the EOC were also reported by other researchers, such as 0.31% (Toncer and Kizil, 2004), 0.68 to 1.28% (Hosseini et al., 2018), 0.95 to 1.24% (Rezaei-Chiyaneh et al., 2018), 0.23 to 0.26 (Bayati et al., 2020), 0.04 to 0.19% (Ali et al., 2020), and 0.09 to 0.10% (Kara et al., 2021). The emission of volatiles and the yield and composition of essential oils are significantly influenced by biotic factors such as insects and abiotic factors such as light, temperature, and precipitation.

Significant differences were found in the SOC values between years and genotypes. It is observed that 7% higher SOC value (27.6%) was obtained in the 1st year compared to the SOC value (24.5%) in the 2nd year. The increased SOC in the first year may be a result of improved dry matter production and increased source capacity, which may have enhanced the translocation of photo-assimilates to the sink, thus increasing oil biosynthesis. In terms of genotypes, the highest SOC was obtained from G1 (26.9%) and G2 (26.8%) genotypes, while the lowest was obtained from G3 genotype (24.5%). Variations in the SOC of black cumin have also been reported by other researchers; 25 to 35% (Toncer and Kizil, 2004), 28 to 33% (Hosseini et al., 2018), 8 to 40% (Beyzi, 2020), 32 to 37% (Ozer et al., 2020), 7 to 19% (Bayati et al., 2020), 31 to 33% (Kara et al., 2021), and 34 to 46% (Moradzadeh et al., 2021). There are low, high, and similar values when comparing the SOC results obtained by the researchers with the results of this study. The variation in SOC observed in these different studies may reflect of the genotype of the plant as well as environmental influences.

Significant differences were found in terms of SOY values between years and genotypes. It is observed the SOY value obtained in the second year (262.1 kg ha^{-1}) was %58 higher than the SOY value obtained in the first year (152.0 kg ha^{-1}). Among the genotypes, the highest SOY was obtained from the G1 (263.1 kg ha^{-1}) and G3 (224.1 kg ha^{-1}) genotypes, while the lowest SOY was obtained from the G2 genotype (144.2 kg ha^{-1}). Variations in the SOY of black cumin have also been reported by other researchers; 242 to 568 kg ha^{-1} (Hosseini et al., 2018), 412 to 428 kg ha^{-1} (Ozer et al., 2020), 27 to 133 kg ha^{-1} (Bayati et al., 2020), and 190 to 392 kg ha^{-1} (Moradzadeh et al., 2021). Regarding the subject, it is thought that the differences in the seed oil yield may be due to the genetic differences of the genotypes.

Essential Oil Composition of Black Cumin

The chemical composition of black cumin plant's essential oils was examined using GC/MS analysis, and the results are presented in Table 2. Figure 3 shows a representative GC/MS chromatogram illustrating the essential oil and fatty oil composition. 23 components were identified, representing 89.48%-97.15% of the total essential oil. The major components were *p*-cymene (51.45%-66.33%), trans-4-Methoxythujane (8.40%-11.90%), Thymoquinone (0.11%-19.26%), γ-Terpinene (1.28%-9.09%), and Limonene (2.93%-4.50%), respectively. Based on the heat map shown in Figure 4, the visualization of essential oil components was conducted considering different genotypes and sowing dates.

Compared to the genotypes for *p*-cymene, the highest amounts were found in the G3 genotype. When comparing the p-cymene content with previous studies, a range of diverse results were observed, varying from 25.00% to 25.90% (Botnick et al., 2012), 22.05% (Kazemi, 2015), 34.10% to 39.90% (Al Turkmani et al., 2015), 59.50% (Khalid and Shedeed, 2016), 34.67% (Ndirangu et al., 2020), 25.01% to 26.90% (Kara et al., 2021), 60.20% (Sakdasri et al., 2021), 27.70% to 38.10% (Ghanavi et al., 2022), 49.27% (Ciesielska-Figlon et al., 2022). These variations may have resulted from a range of ecological factors, including as soil fertility, air temperature, radiation, and precipitation, which all affected the formation of components in black cumin. Also, in medicinal and aromatic plants, the composition of essential oil is a key marker that has been altered by a variety of factors, including plant species, plant age, agricultural techniques, extraction methods, plant growth stages, and post-harvest processing (Barut et al., 2021).

Table 2. The essential oil composition of black cumin based on the different sowing dates and genotypes

Components	RT (min)	S1 G1	S1 G2	S1 G3	S2 G1	S2 G2	S2 G3	S3 G1	S3 G2	S3 G3
		Relative Peak Area (%)								
Limonene	11.615	4.00	4.50	3.26	2.93	4.11	3.42	2.96	3.90	3.62
γ -Terpinene	12.143	9.09	7.23	1.28	3.52	6.52	2.34	1.41	5.90	3.53
Terpinolene	12.494	0.28	0.20	0.13	0.23	0.21	0.12	0.12	0.20	0.10
p-Cymene	13.099	55.28	62.78	66.28	51.45	58.97	66.10	51.76	58.10	66.33
trans-4-Methoxythujane	13.675	8.40	10.35	10.10	11.90	9.67	9.90	8.82	9.30	10.20
α-Longipinene	14.838	-	0.76	-	0.76	0.62	2.05	1.22	0.70	2.09
Linalol	16.286	0.20	0.09	-	0.56	0.09	0.11	0.20	0.20	0.11
Valencene	16.624	1.94	2.60	1.35	3.87	3.10	2.45	3.15	3.20	3.95
Longifolene	17.046	0.04	0.05	-	0.04	0.05	-	0.05	0.05	-
2-Caren-4-ol	17.301	0.69	0.65	0.62	1.05	0.69	0.59	0.80	0.53	0.56
3-p-Menthen-7-al	17.598	2.03	1.75	1.84	2.50	1.46	1.87	2.16	1.53	2.02
4-Carvomenthenol	17.877	1.64	0.98	0.88	2.33	0.87	0.88	1.39	0.91	-
Carane	19.509	0.05	0.07	-	0.04	0.08	0.09	0.06	0.09	0.09
Carvone	21.011	-	-	-	0.33	-	-	0.09	-	-
Thymoquinone	21.355	4.88	0.11	-	10.14	0.15	-	19.26	0.18	-
Cyclododecane	21.503	-	0.05	-	-	-	-	-	-	-
cis-cyclooctene oxide	22.079	-	0.38	0.47	-	0.31	0.40	-	0.29	0.32
7-Heptadecene, 1-chloro-	22.328	-	0.06	-	-	0.04	-	-	0.05	-
α-Gurgujene	22.833	0.15	0.19	-	0.13	0.18	-	0.18	0.20	-
Pimaradiene	23.343	-	0.85	0.24	0.36	0.64	0.24	0.13	0.91	0.23
1,7-Dodecadiene	23.860	0.16	0.38	0.21	0.19	0.29	0.29	0.32	0.36	0.36
Wine lactone	24.121	0.21	-	-	0.22	-	-	-	-	-
Carvacrol	24.667	4.07	3.12	2.82	4.39	3.15	2.70	2.72	3.09	2.58
	Total	93.11	97.15	89.48	96.94	91.20	93.55	96.80	89.69	96.09

S1: October 15th, S2: November 1st, S3: November 15th, G1: Çameli cultivar, G2: Adana genotype, G3: Iraq genotype

Table 3. The fatty acid composition according to different sowing dates and genotypes

Components	C 16:0 Palmitic acid	C 16:1 Palmitoleic acid	C 18:0 Stearic acid	C 18:1 Oleic acid	C 18:2 Linoleic acid	C 18:3 Linolenic acid	C 20:2 Eicosadienoic acid	Total
RT (min)	11.876	12.535	13.811	14.541	15.639	17.081	18.494	
S1 G1	14.71	0.16	3.16	20.98	57.18	0.55	3.16	99.90
S1 G2	14.41	0.05	2.95	22.42	57.16	0.60	2.41	100.00
S1 G3	13.89	0.18	3.24	23.52	55.98	0.46	2.70	99.97
S2 G1	13.92	0.19	3.02	24.46	54.94	-	2.63	99.16
S2 G2	14.50	0.22	3.53	24.14	54.53	0.67	2.27	99.86
S2 G3	15.02	0.15	3.34	24.34	53.97	-	2.31	99.13
S3 G1	13.73	-	2.31	26.40	57.56	-	-	100.00
S3 S2	14.96	0.21	2.95	22.03	56.64	-	2.55	99.34
S3 G3	14.00	-	3.47	25.19	54.40	-	2.14	99.20
Mean	14.35	0.17	3.11	23.72	55.82	0.57	2.52	

S1: October 15th, S2: November 1st, S3: November 15th, G1: Çameli cultivar, G2: Adana genotype, G3: Iraq genotype

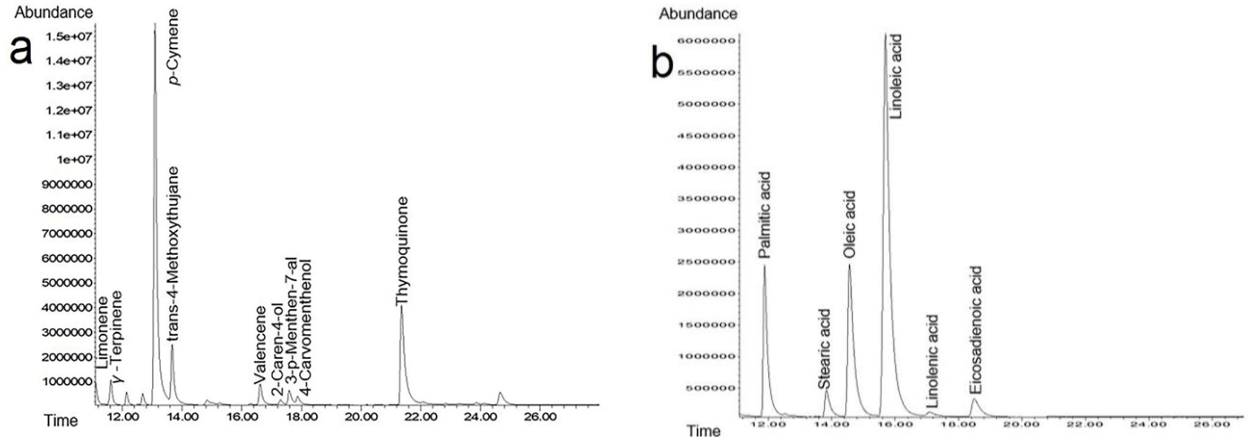

Figure 3. The representative GC/MS chromatogram of essential oil components and fatty acids (a: Essential oil components b: Fatty acids).

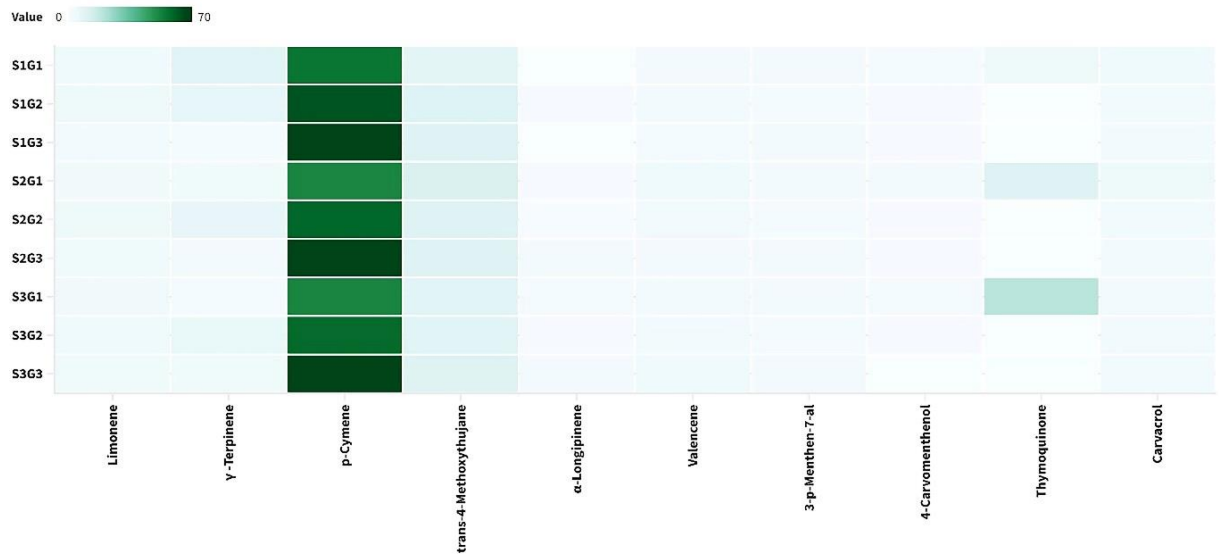

Figure 4. Heat map of main essential oil components according to different sowing dates and genotypes (S1: October 15th, S2: November 1st, S3: November 15th, G1: Çameli cultivar, G2: Adana genotype, G3: Iraq genotype).

p-Cymene, also known as p-cymol or p-isopropyltoluene, is an aromatic chemical with alkyl substitution that occurs naturally in the essential oils of many aromatic plant species.

It is a precursor of the main volatile components like thymol and carvacrol, which are well-known for their broad bioactivity (Bouyahya et al., 2017). Numerous types of studies have revealed the pharmacological effects of *p*-cymene, including anti-inflammatory, antiparasitic, antioxidant, antidiabetic, anticancer, antibacterial, antiviral, and antifungal activities (Balahbib et al., 2021). It has been reported that the anti-inflammatory property of black cumin essential oil is mainly due to *p*-cymene (Ciesielska-Figlon et al., 2022). Similar to the p-cymene content, previous studies have reported varying trans-4-Methoxythujane content; %4.00 (Wajs et al., 2008), 3.80% to 4.00% (Botnick et al., 2012), 5.81% (Ndirangu et al., 2020), 3.40% to 4.80% (Ghanavi et al., 2022), 5.83% (Ciesielska-Figlon et al., 2022). Researchers reported that the aroma of trans-4-methylthujane is intensely camphoric, and woody, with a soil scent (Wajs et al., 2008). The highest amounts of thymoquinone were found in the G1 genotype. Investigations by other researchers were revealed differences in the thymoquinone content of black cumin, with recorded values such as 20.32% (Kazemi, 2015), 17.20% to 25.90% (Al Turkmani et al., 2015), 3.00% (Khalid and Shedeed, 2016), 67.70% (Palabiyik and Aytaç, 2018), 2.39% to 4.41% (Kara et al., 2021), 4.00% (Sakdasri et al., 2021), 19.50% to 40.90% (Ghanavi et al., 2022), 2.26% (Ciesielska-Figlon et al., 2022). Thymoquinone has antioxidant properties and has been shown to induce apoptosis and inhibit cell division in cancer cells, thus supporting its therapeutic potential (Burits and Bucar, 2000; Chehl et al., 2009; Banerjee et al., 2010; Zaid et al., 2012). It is a potent prospective therapeutic candidate for cancer treatment because it successfully suppresses tumor angiogenesis and tumor growth (Yi et al., 2008). Furthermore, the significant negative correlation between *p*-cymene and thymoquinone, which are the main components, was revealed for the first time (Figure 6).

Fatty Acid Composition of Black Cumin

The chemical composition of the fatty oil was examined using GC/MS analysis, and the results are presented in Table 3. The chemical composition of the fatty oil was C 16:0 (Palmitic acid), C 16:1 (Palmitoleic acid), C 18:0 (Stearic acid), C 18:1 (Oleic acid), C 18:2 (Linoleic acid), C 18:3 (Linolenic acid), and C 20:2 (Eicosadienoic acid). Six fatty acids were identified, representing 99.13%-100% of the total fatty seed oils. Palmitic and stearic acids are the most common saturated fatty acids found in plant oils, while linoleic and oleic acids are unsaturated fatty acids. The main fatty acids were determined as C 18:2 Linoleic acid (53.97%–57.56%), C 18:1 Oleic acid (20.98–26.40), and C 16:0 Palmitic acid (13.73%–15.02%). Based on the heat map illustrated in Figure 5, the visualization of fatty acids was performed considering various genotypes and sowing dates.

When comparing the linoleic acid content with previous studies, a range of diverse results were observed, varying from 39% to 44% (Palabiyik and Aytaç, 2018), 53% to 58% (Beyzi, 2020), and 50% (Bayati et al., 2020). The results presented in this study were either comparable or higher than the results reported in previous studies, with a mean value of 56%. Linoleic acid was the main fatty acid in all of these studies. In terms of the nutritional advantages offered by plant oils, a higher concentration of linoleic acid is considered beneficial (Al-Jassir, 1992). When comparing oleic acid with previous studies, varying results were found, such as 33%–38% (Palabiyik and Aytaç, 2018), 19%–31% (Beyzi, 2020), 22% (Bayati et al., 2020) and 24%–28% (Moradzadeh et al., 2021). Upon comparing the palmitic acid content with previous studies, a range of different results was observed, ranging from 10%–20% (Beyzi, 2020), and 16% (Bayati et al., 2020). In the present study, several fatty acids were identified with relatively low levels, including 16:1 (Palmitoleic acid), C 18:0 (Stearic acid), C 18:3 (Linolenic acid), and C 20:2 (Eicosadienoic acid). The reduction in temperature induces changes in the ratio of saturated to unsaturated fatty acids, leading to a transition of plant membrane lipids from a liquid to a solid state (Steponkus, 1984; Murata and Los, 1997).

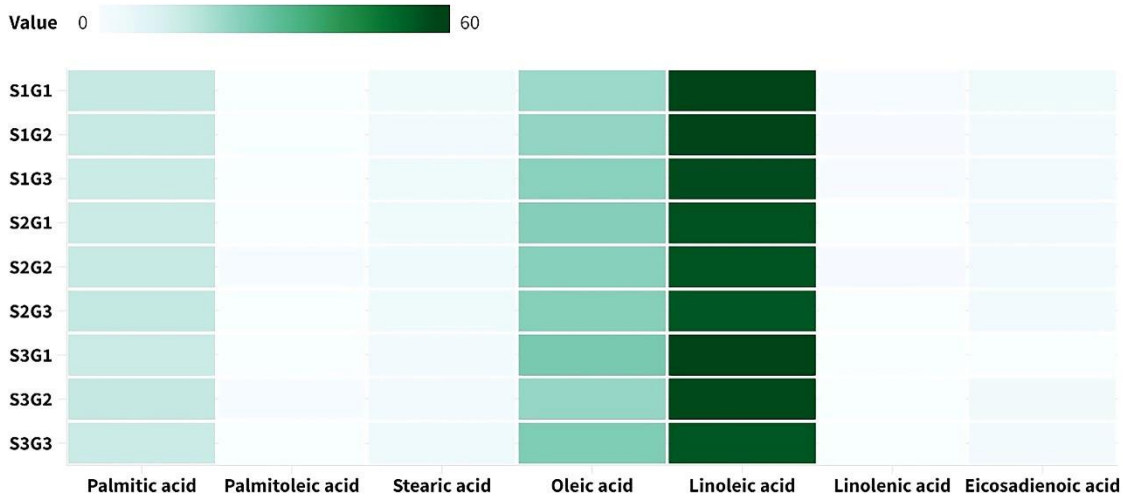

Figure 5. Heatmap of fatty oil composition according to different sowing dates and cultivars (S1: October 15th, S2: November 1st, S3: November 15th, G1: Çameli cultivar, G2: Adana genotype, G3: Iraq genotype).

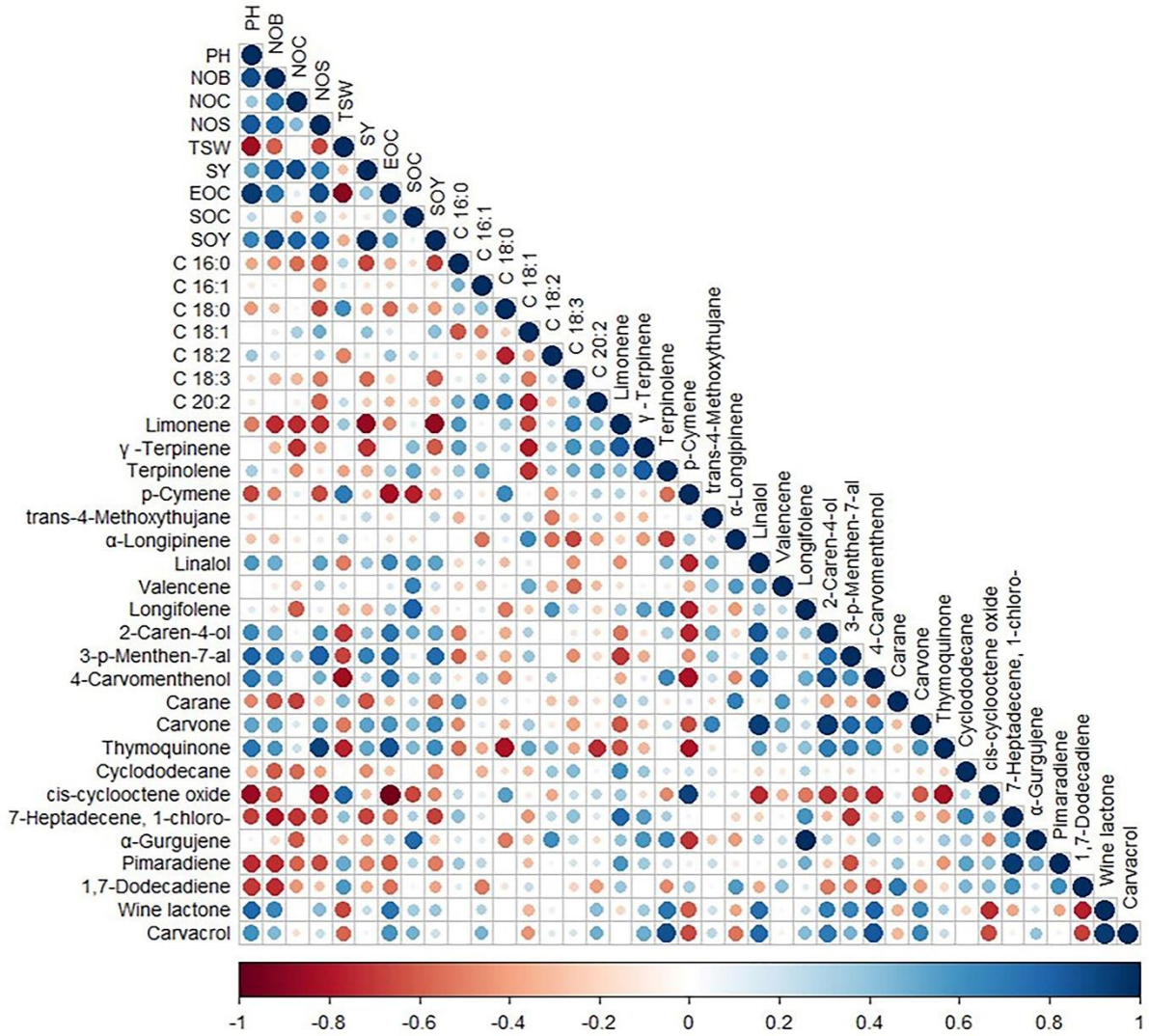

Figure 6. Correlation graph in terms of agronomic and quality traits (S1: October 15th sowing date, S2: November 1st sowing date, S3: November 15th sowing date, G1: Çameli cultivar, G2: Adana genotype, G3: Irak genotype, PH: Plant height, NOB: Number of branches, NOC: Number of capsules, NOS: Number of seeds, TSW: Thousand seed weight, SY: Seed yield, EOC: Essential oil content, SOC: Seed oil content, SOY: Seed fatty oil yield, C 16:0: Palmitic acid, C 16:1: Palmitoleic acid, C 18:0: Stearic acid, C 18:1: Oleic acid, C 18:2: Linoleic acid, C 18:3: Linolenic acid, C 20:2: Eicosadienoic acid.).

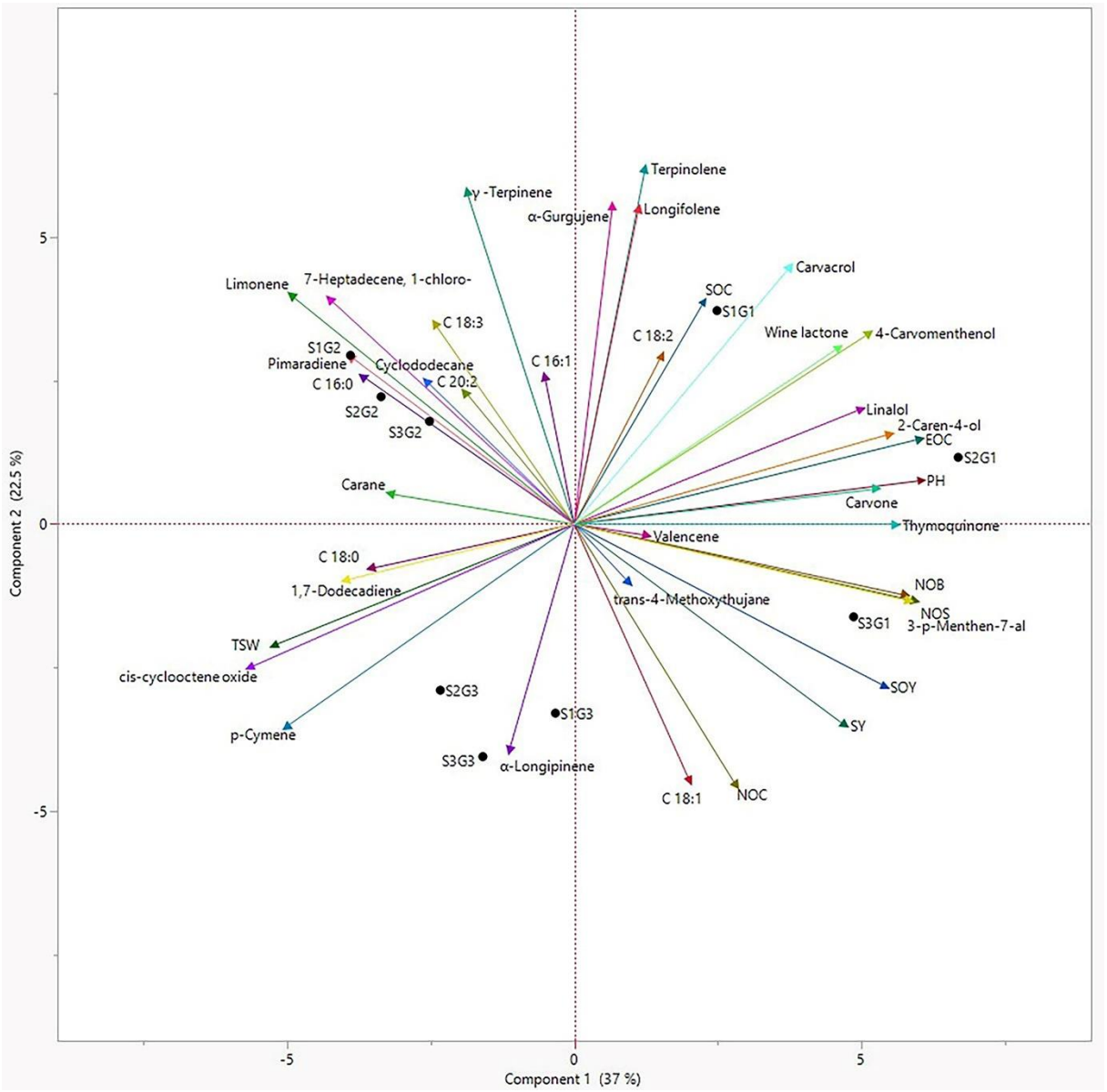

Figure 7. PCA on correlations of the agronomic traits, essential oil components, and fatty acids (S1: October 15th sowing date, S2: November 1st sowing date, S3: November 15th sowing date, G1: Çameli cultivar, G2: Adana genotype, G3: Irak genotype, PH: Plant Height, NOB: Number of Branches, NOC: Number of Capsules, NOS: Number of seeds, TSW: Thousand seed weight, SY: Seed yield, EOC: Essential oil content, SOC: Seed fatty oil content, SOY: Seed fatty oil yield, C 16:0: Palmitic acid, C 16:1: Palmitoleic acid, C 18:0: Stearic acid, C 18:1: Oleic acid, C 18:2: Linoleic acid, C 18:3: Linolenic acid, C 20:2: Eicosadienoic acid).

Moreover, genetic variations, seed production and storage conditions, soil fertility, oil extraction and detection methods, and quantitative methods can all contribute to changes in fatty acid profiles (Barut et al., 2022).

Correlation and Pcabiplot Analysis of the Treatments Based on the Important Traits

The correlation between agronomic traits and quality characteristics is given in Figure 6. Figure 6 shows the correlation of the traits, where the colours represent negative or positive correlations, and the size of the circles signifies the level of importance. The fatty acid composition of plant fatty oils is not always under standard conditions, there are characteristic differences depending on species and varieties, and it may vary depending on many other factors. The distribution and position of the fatty acids in the oil directly affect the quality of the oil, its importance in nutrition, and its technological values. For this reason, it is important in terms of oil quality to know how the fatty acid components of oil plants will change as a result of which conditions and what effect. When Figure 6 is examined, it is observed that there is a negative correlation between TSW and PH, NOB, and NOS, as well as between linoleic acid and stearic acid, which are fatty oil components. In order to evaluate the obtained data as a whole, the data were subjected to principal component analysis (Figure 7). An important part of the variability constituting 59.50% of the total variation, it is observed that they are in a relationship on the Component 1 and Component 2 axes as shown in Figure 7.

Conclusion

The aim of the study was to evaluate the yield and quality characteristics of black cumin, which is a cool season plant, at different sowing dates and in different genotypes in the Mediterranean region, which is highly impacted by global warming and climate change. Differences were observed in the adaptation of the genotypes based on the changing climatic conditions in both years, and Adana and Iraq genotypes showed weak growth compared to Çameli cultivar. Seed yields were adversely affected due to the low number of seed formations and the inability to mature some of the seeds formed due to the low number of flowers with the late spring frosts and the high temperatures observed in May negatively affecting the fertilization of the flowers. While the sowing date had no effect on seed yield and seed oil yield, it was determined that Çameli (G1) and Iraq (G3) genotypes performed better. The expansion of black cumin cultivation in our country is facilitated by the development of improved varieties that exhibit high yield and superior quality traits well-suited to the regional conditions. Black cumin, which occupies an important place among medicinal and aromatic plants obtained from different regions of Türkiye, is compatible with the ecological conditions of our region and resistant to adverse factors, determining the most suitable sowing date and variety recommended below can the conditions of our country and in particular the province of Adana. Identifying resistant, productive, and high-quality genotypes, can contribute to the region's and country's economy. Recognizing that black seed quality is as important as yield, quality criteria for both fatty oil and essential oil components have been proposed. In future studies, it is recommended to conduct appropriate breeding studies to improve high-yielding, region-adapted cultivars and increase insect populations that increase pollination in flowering flowers.

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Mapping the Dispersion Pollution Load of Animal Waste and Investigating its Environmental Effects: The Case of Karaman

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ABSTRACT

Animal wastes are not stored correctly and are used haphazardly without control in agricultural lands. As a result, it causes many irreparable environmental pollution, especially water pollution. These wastes, which are described as diffuse pollution, cause pollution of both underground and surface water resources directly or indirectly and even make them unusable. In this research, waste generation as a result of animal breeding in Karaman province, its districts, neighborhoods and villages and the effects of animal wastes on environmental pollution were evaluated with distributed pollutant load calculations. In the study, the number of 1019277 ovine and 81368 bovine in Karaman in 2022 was used. The total nitrogen (TN) produced annually by the animals has been calculated as 1,723.23 tons/year, and the total phosphorus (TP) amount is determined as 124.23 tons/year. Additionally, for large ruminant animals, the annual total amount of dry manure is 130,305.77 tons, and for small ruminant animals, it is 41,984.27 tons. To prevent environmental pollution, these wastes should be stored in closed areas in compliance with standards, and processes such as composting, drying, and biogas production should be applied. By doing so, not only can environmental pollution be mitigated but also economic value can be obtained. The proper management and utilization of these wastes have high economic potential and can contribute to sustainable development, supporting the country's economy. In addition, this study is a source for researchers working in the field in calculating the pollution load of animal wastes and is thought to be a guide for decision makers and practitioners.

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Introduction

Human beings are consumptive beings and as a result of their consumption, they generate waste. The waste generated as a result of this consumption varies depending on people's consumption habits. With the increase in population, both consumption and waste generation have increased. One of the most important consumption products is animal-based foods (Kocaman et al., 2015; Singh and Rashid, 2017). Looking at the supply-demand balance, livestock farming has increased in our country, as in the rest of the world, in order to meet the increasing demand. This increase naturally leads to a rapid increase in animal-derived waste and its uncontrolled release into the environment, causing disruption of the environmental balance, significant environmental pollution, and threats to environmental health, such as bad odor, flies, insects, and pathogenic microorganisms (Karaman, 2006; Singh and Rashid, 2017). Animal wastes differ from urban and industrial wastes in terms of their polluting nature and content. While the majority of domestic and industrial wastes fall into the category of point source pollutants, animal-based wastes belong to the category of non-point source pollutants and have the capacity to pollute larger areas (Tırınk, 2021). Non-point source pollutants particularly make it difficult to identify the sources of water pollution (Eleroğlu and Yıldırım, 2011).

Indeed, animal waste, which is classified as non-point source pollution, can mix with groundwater or surface water, leading to the deterioration or unusability of water sources' quality (Aydın and Derinöz, 2013; Yetiş et al., 2018). In most livestock farms, especially those operated in small family-style (1-5 and 6-10 cattle) settings, appropriate manure storage areas are lacking, and solid manure is accumulated haphazardly in open environments, while the liquid portion of the manure is discharged into surface water bodies without proper treatment, resulting in water pollution (Boyacı et al., 2011). During the transportation process of these wastes, factors such as the characteristics and quantities of pollutants, as well as important factors like soil structure, land cover, and topography, play a role in their impact on water sources

(Lenzi and Di Luzio, 1997; Akdoğan et al., 2015). When these wastes are not properly stored, they can contaminate surface water sources, and when accumulated in permeable soil areas, they can infiltrate downwards, reaching groundwater sources and causing contamination (Atılgan et al., 2006; Karaman, 2006; Boyacı et al., 2011; Yağlı and Yıldız, 2019). Additionally, animal manure is traditionally used in agricultural fields to improve soil fertility. These wastes contain high levels of nutrients such as nitrogen (N), phosphorus (P), and potassium (K), which provide essential minerals for plant growth, improve the overall structure of the soil, alter the population of microorganisms, and enhance water-holding capacity (Konca and Uzun, 2012). However, improper storage of these wastes on the soil surface or excessive application as fertilizer can result in the leaching of these elements into surface water sources, promoting the growth of algae and causing eutrophication in surface water bodies. This, in turn, reduces oxygen levels, leads to fish stress, and decreases fish populations. Moreover, excessive application of manure can lead to soil compaction and crust formation, negatively affecting the physical properties of the soil (Çayır et al., 2012). Furthermore, the direct application of animal wastes to agricultural fields without proper treatment reduces product quality and disrupts the beneficial properties of soil structure (Yağlı and Yıldız, 2019). Medications and antibiotics are also administered to animals for treatment purposes or to support the growth and protection of animals raised for food. Some of these administered drugs can be metabolized, while others are excreted through feces and urine (Konca and Uzun, 2012; Akdoğan et al., 2015). The manure of animals treated with antibiotics can lead to the proliferation of resistant bacterial species in soil, surface water, and groundwater, posing threats to the environment and human health. These wastes not only contribute to soil and water pollution but also have negative impacts on air quality, thus affecting climate change. Animal manure releases gases such as water vapor (H_2O), carbon dioxide (CO_2), ammonia (NH_3), hydrogen sulfide (H_2S), carbon monoxide (CO), and hydrogen (H_2) into the atmosphere. Moreover, the decomposition of organic matter in manure by anaerobic bacteria results in the release of methane (CH_4) gas. Additionally, the nitrogen (N_2) in manure leads to the formation of nitrous oxide (N2O) during nitrification and denitrification processes. These two gases, in particular, are significant contributors to greenhouse gas emissions, which are known to contribute to global climate change (IPCC, 1996). In the current era, where global warming is an undeniable threat, greenhouse gas emissions play a significant role in the formation of global climate change (Ersoy, 2017). Therefore, it is crucial to take necessary precautions to protect the environment from pollution until these wastes are applied to the land. Practices such as composting, drying, and biogas production applied to animal manure can minimize environmental harm and enable more effective use of organic fertilizers in fields. Ultimately, these practices reduce unpleasant odors, eliminate pathogenic microorganisms, and result in significant reductions in the weight and volume of manure (Karaman, 2006). The aim of this study is to determine the amounts of waste and pollutant loads, specifically total nitrogen (TN) and total phosphorus (TP) concentrations, resulting from animal production in the province, districts, neighborhoods, and villages of Karaman. In this study, the numbers of cattle and small ruminants (sheep and goats) in Karaman province for the year 2022 were determined on a neighborhood and village basis. Then, the TN and TP concentrations resulting from the waste generated by these animals were calculated. Subsequently, the daily amounts of dry manure produced by these animals were determined to evaluate the environmental pollution aspect.

Materials and Methods:

Materials

In this study, data on the number of animals in villages and neighborhoods were obtained from the provincial agriculture and forestry directorate of Karaman province to determine the amount of animal waste. The coordinates of Karaman province, districts, neighborhoods, and villages were marked using the ArcMap 10.5 program. The distribution of animal numbers according to the districts of Karaman province was analyzed. The amounts of dispersion pollutant load and dry manure that could be generated from animal waste were calculated.

The study area is located in Karaman province, which is in the Central Anatolia region of our country, between 37°-11' north latitude and 33°-13' east longitude. Karaman province is surrounded by Mersin and Antalya to the south, and Konya province to the west, north, and east. The surface area of Karaman province is 8,851 km², and the elevation of the provincial center from sea level is 1,033 m. It has a total of 6 districts, 10 townships, and 154 villages, with one being the central district. The northern part of Karaman province is covered with steppe vegetation, while the southern part is covered with forest. Two-thirds of the terrain is mountainous. The provincial center is located in a plain, just south of the Taurus Mountains' extensions (ÇŞB, 2022). The total surface area of Karaman is 885,100 hectares, of which 39% is agricultural land, 23% is meadow pasture land, 27% is forest land, and 11% is other areas. Plant production is carried out on 346,848 ha of our province. Of these areas where plant production is conducted, 62% is allocated to field crops, while 15% is fallow areas. Fruit cultivation is carried out on 8.7% of agricultural lands, viticulture on 1.4%, and vegetable cultivation on 3.9% (TOB, 2022; URL-1). Agriculture and animal husbandry constitute the main livelihood source in the region.

Assumptions made in the study: The production of animal manure and the unit loads of nitrogen (N) and phosphorus (P) released into the environment can vary greatly depending on the feeding habits, types of nutrients, and frequency of water intake of the animals (Kocabey, 2019). In order to calculate the amounts of dispersion pollutants originating from animal waste in Karaman province and its districts, some assumptions were made based on the literature. Estimated unit loads resulting from dispersion pollutants are given in Table 1 according to the values predicted in the literature (Yontar, 2009; Biçer, 2011; Derin et al., 2019; ÇŞB, 2016; Tırınk, 2021). The total amount of dispersion pollutants originating from animal waste in Karaman province was calculated using the following equation (Equation 1).

$$Q_T = Q_{YK} \times A_{CH} \times Y_U \times 365/1000 \quad (1)$$

Here, QT represents the annual total dispersion pollutant load (kg/number of animals/year), QYK represents the dispersion pollutant load varying according to the type of pollutant (kg/ton/number of animals/day), ACH represents the live animal weight according to the animal species (kg); in the literature, these values are taken as 500 kg for BBH, 45 kg for KBH, and 2 kg for KH. The assumptions in Table 1 were obtained using the annual N and P amounts per animal based on these live weights in the literature. Y_U represents the percentage of dispersion pollutants that can reach the receiving environment, assuming that 15% for N and 5% for P can reach the receiving environment (Özalp, 2009; ÇŞB, 2016; Hacısalihoğlu, 2022; Haksevenler and Ayaz, 2021). This value is determined considering the transportation processes and losses of N and P. The calculation of TN load (Equation 2) is as follows:

$$QTN = QT \times NCH/1000 \quad (2)$$

is calculated as. Here, QTN represents the annual total nitrogen load (kg/year), and NCH represents the number of live animals (count). The calculation of TP load (Equation 3) is as follows:

$$QTP = QT \times NCH/1000 \quad (3)$$

is calculated as. Here, QTP represents the annual TP load (kg/year).

After calculating the potential diffuse pollutant loads resulting from animal waste in Karaman province and its districts, the amount of dry manure that can be collected in this province has been estimated. To calculate this manure amount, certain assumptions were made based on the literature, and the range of values predicted in the literature is presented in Table 2 (Köttner, 2002; Omer and Fadalla, 2003; Koçer et al., 2006; Avcıoğlu et al., 2013; Aktaş et al., 2015; Ilgar, 2016; Salihoğlu et al., 2019; Hacısalihoğlu, 2022; Haksevenler and Ayaz, 2021). The total amount of manure resulting from animal waste in Karaman province is calculated using the following equation (equation 4).

$$TYGM = ACH \times YCHA \quad (4)$$

Here, TYGM represents the total daily amount of fresh manure (kg/animal/day), ACH denotes the live animal weight (kg) according to the animal species. The literature suggests that this value ranges from 135-800 kg for BBH, 30-75 kg for KBH, and 1.5-12 kg for KH. YCHA represents the percentage of live animal weight according to the animal species (%). The literature suggests that this value ranges from 5-6% for BBH, 4-5% for KBH, and 3-4% for KH. The amount of fresh manure in animals can vary based on their weight, species, age, gender, feeding type, and climatic conditions of the region they are in. However, in this study, Equation 1 and literature values are considered, assuming that the production of fresh manure will be 27 kg/day for BBH, 2.48 kg/day for KBH, and 0.26 kg/day for KH. The calculation of the collected fresh manure in animal shelters takes into account the duration of animals staying in the shelter. The total available amount of dry manure is calculated using Equation 5.

$$TGM = TYGM \times YKG \times YKM \quad (5)$$

is calculated as. Here, TGM represents the total available amount of dry manure in tons per day, and YKG is the percentage of usable manure (%) based on the animal species. The values used in this study are 65% for BBH, 13% for KBH, and 99% for KH. YKM represents the percentage of dry matter (%) in the animal waste, which varies according to the animal species. In this study, the values used are 15% for BBH, 33% for KBH, and 50% for KH (Tırınk, 2021). The annual total amount of dry manure is calculated using Equation 6.

$$TYGP = TKYGM \times NCH \times 365/1000 \quad (6)$$

It is calculated with the equation. In this equation, NCH is the number of animals.

For the GIS-based mapping of diffuse pollution using the acquired animal population data and the calculated values of dry manure and diffuse pollutant loads, density distribution maps were created using the Kernel Density tool in ArcMap 10.5. The coordinates of the study area were entered into the program to generate the maps (Çakır et al., 2019).

Table 1. Distributed pollutant load acceptances by animal breeds

	Distributed Load Coefficients	BBH	KBH	KH
TN	QYK (kg/ton number of animals/day)	0.3	0.42	0.52
	YU (%)	15	15	15
	QT (kg/number of animals/year	8.213	1.035	0.057
TP	QYK (kg/ton number of animals/day)	0.1	0.06	0.22
	YU (%)	5	5	5
	QT (kg/number of animals/year)	0.913	0.049	0.008

BBH= cattle, KBH= ovine animals, KH= poultry

Table 2. Waste generation acceptances according to animal breeds

Acceptance Parameters	BBH	KBH	KH
Livestock Weight (kg)	135-800	30-75	1.5-12
Wet Fertilizer Formation (%)	5-6	4-5	3-4
Wet Fertilizer amount (kg/day)	6-48	1.2-3.75	0.045-0.48
Availability (%)	25-65	13	99
Dry Matter Content (%)	5-25	30-36	10-90

BBH= cattle, KBH= ovine animals, KH= poultry

Findings and Discussion

In the scope of the study, the number of live BBH and KBH in Karaman province for the year 2022 was determined. As shown in Table 3, Table 4a,b, and Figure 1, the district with the highest presence of BBH in Karaman province is the sum of the villages affiliated with Karaman, with a total of 32,504 animals and a percentage of 40%. The central district follows with a percentage of 35%.

The district with the lowest BBH presence is Başyayla with 1,457 animals and a percentage of 1.79%. The district with the highest KBH presence is the villages belonging to the Center with 499,276 animals and a percentage of 45%. The district with the lowest KBH presence is also Başyayla with 8,540 animals and a percentage of 0.84%. When examining the livestock numbers in Karaman province, its districts, neighborhoods, and villages, it can be observed that KBH farming has a higher percentage. After calculating the livestock numbers in Karaman province, the generated diffuse pollutant loads were calculated. The calculated pollutant loads are presented in Table 5 and Table 6a,b,c.

Table 3. Livestock numbers in Karaman districts for the year 2022

District Name	BBH Total	KBH Total	Total Livestock	Total Livestock Percentage	BBH Percentage	KBH Percentage
Ayrancı	7138	280613	287751	26.14%	8.77%	27.53%
Ermenek	5372	50019	55391	5.03%	6.60%	4.91%
Başyayla	1457	8540	9997	0.91%	1.79%	0.84%
Kazımkarabekir	3541	31047	34588	3.14%	4.35%	3.05%
Sarıveliler	3005	12825	15830	1.44%	3.69%	1.26%
Karaman Villages	32504	466772	499276	45.36%	39.95%	45.79%
Karaman Center	28351	169461	197812	17.97%	34.84%	16.63%

BBH= cattle, KBH= ovine animals

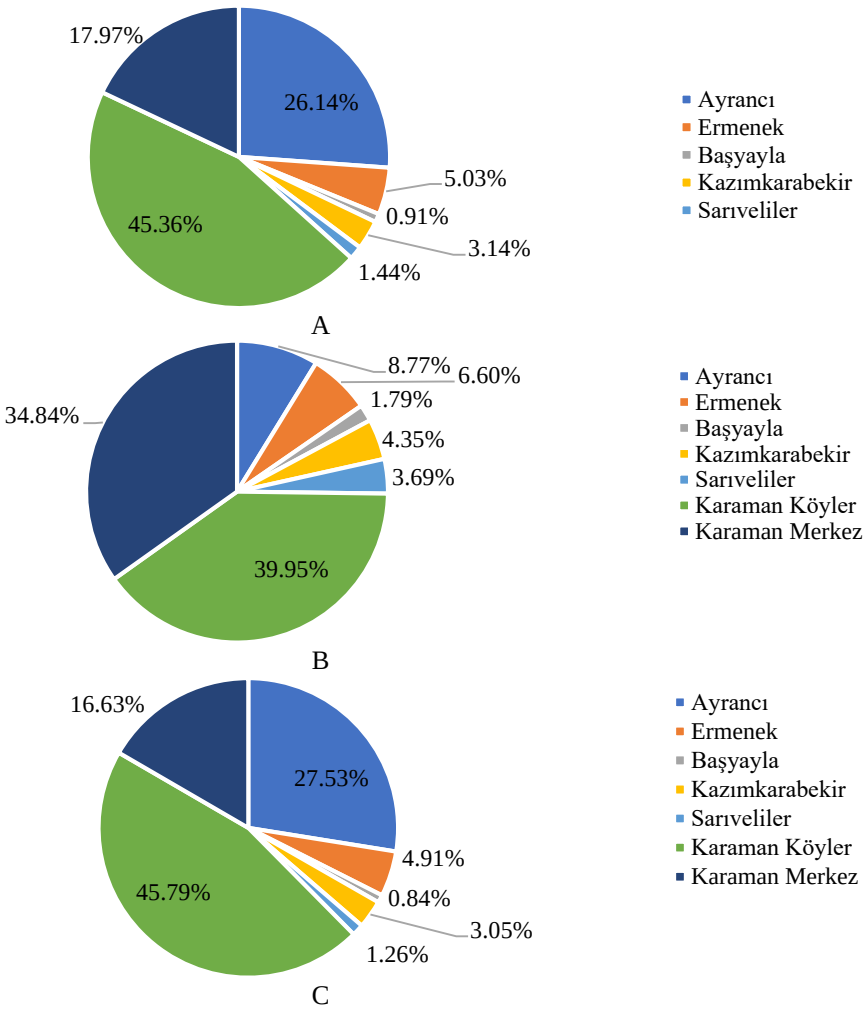


Figure 1. (a) Distribution according to total number of animals, (b) Distribution according to BBH numbers, (c) Distribution according to KBH numbers.

Table 4a. Number of livestock in the villages and neighborhoods of Karaman in 2022

District	Village/Town	CC	SC	GC	District	Village/Town	CC	SC	GC
Ayrancı	Ağızboğaz	562	16199	1813	Sarıveliler	Uğurlu	38	44	442
Ayrancı	Akpınar	12	8895	1290	Sarıveliler	Merkez	70	799	133
Ayrancı	Ambar	0	8244	930	Sarıveliler	Merkez-Başmahalle	85	52	183
Ayrancı	Berendi	147	65404	1236	Sarıveliler	Merkez-Çakıllar	183	762	586
Ayrancı	Böğecik	416	15628	327	Sarıveliler	Merkez-Karakaya	26	112	286
Ayrancı	Buğdaylı	347	2576	12	Sarıveliler	Merkez-Kkarapınar	12	99	34
Ayrancı	Büyükkoraş	0	4727	1377	Sarıveliler	Merkez-Turcalar	348	112	213
Ayrancı	Çat	0	7758	373	Sarıveliler	Merkez-Ulucami	92	1476	395
Ayrancı	Divle	232	6690	839	Sarıveliler	Merkez-Yeşilyurt	41	59	3
Ayrancı	Dokuzyol	372	17403	1100	Merkez	Ada	81	1160	1472
Ayrancı	Höyükburun	245	4037	190	Merkez	Ağaçyurdu	103	292	1924
Ayrancı	Kale	82	1924	167	Merkez	Ağılönü	1850	2314	298
Ayrancı	Karaağaç	0	11084	1288	Merkez	Akçaalan	10	605	2663
Ayrancı	Kavaközü	26	11767	957	Merkez	Akçaşehir	585	31875	4358
Ayrancı	Kavuklar	2067	7468	33	Merkez	Akpınar	73	10964	1813
Ayrancı	Kayaönü	0	12110	1100	Merkez	Alaçatı	745	821	1
Ayrancı	Kıraman	1292	12918	1197	Merkez	Aşağıakın	0	4	1797
Ayrancı	Küçükkoraş	0	5409	919	Merkez	Aşağıkızılca	14	222	1597
Ayrancı	Melikli	168	6046	666	Merkez	Aybastı	282	3854	3346
Ayrancı	Pınarkaya	1	9697	3476	Merkez	Bademli	61	0	1536
Ayrancı	Saray	0	3046	534	Merkez	Barutkavran	223	1145	378
Ayrancı	Yarıkkuyu	51	6505	388	Merkez	Başkışla	277	808	731
Ayrancı	Merkez-Dede	365	1651	420	Merkez	Bayır	60	509	1280
Ayrancı	Merkez-Musa	0	3317	835	Merkez	Beydilli	6	1071	26
Ayrancı	Merkez-Ulu	24	2604	169	Merkez	Bostanözü	0	167	94
Ayrancı	Merkez-Yenimahalle	729	5631	239	Merkez	Boyalı	238	3174	1229
Ermenek	Ağaççatı	4	253	1295	Merkez	Bozkondak	946	454	1560
Ermenek	Ardıçkaya	128	4	1011	Merkez	Bölükyazı	12	1070	248
Ermenek	Aşağıçağlar	258	1094	2473	Merkez	Bucakkışla	0	84	1275
Ermenek	Balkusan	30	2648	19	Merkez	Burhan	71	1405	1083
Ermenek	Boyalık	14	4	183	Merkez	Burunoba	46	5416	386
Ermenek	Çamlıca	4	16	1561	Merkez	Büyükerenkavak	118	858	152
Ermenek	Çatalbadem	89	1247	2880	Merkez	Cerit	134	1379	3525
Ermenek	Çavuş	10	0	285	Merkez	Çakırbağ	1083	8500	1206
Ermenek	Elmayurdu	267	0	121	Merkez	Çatak	299	1364	4222
Ermenek	Evsin	1	0	574	Merkez	Çavuşpınarı	819	3394	1131
Ermenek	Gökçekent	367	232	256	Merkez	Çiğdemli	263	1499	171
Ermenek	Gökçeseki	0	105	671	Merkez	Çimenkuyu	29	124	29
Ermenek	Görmeli	23	775	3303	Merkez	Çoğlu	551	11857	2535
Ermenek	İkizçınar	146	93	352	Merkez	Çukur	6	99	1948
Ermenek	Katranlı	592	1202	1144	Merkez	Çukurbağ	29	1091	1785
Ermenek	Kayaönü	31	0	1135	Merkez	Dağkonak	103	270	1141
Ermenek	Olukpınar	2	0	254	Merkez	Damlapınar	67	965	1211
Ermenek	Pamuklu	41	0	254	Merkez	Değirmenbaşı	24	516	50
Ermenek	Pınarönü	80	14	344	Merkez	Demiryurt	587	3419	200
Ermenek	Sarıvadi	169	46	1428	Merkez	Dereköy	479	5036	1680
Ermenek	Tepebaşı	92	66	226	Merkez	Dinek	129	3578	300
Ermenek	Yalındal	55	77	880	Merkez	Eğilmez	336	9046	1240
Ermenek	Yaylapazarı	13	108	559	Merkez	Ekinözü	666	21334	1161
Ermenek	Yerbağ	70	12	320	Merkez	Elmadağı	58	76	3615
Ermenek	Yukarıçağlar	173	274	807	Merkez	Eminler	204	5643	666
Ermenek	Güneyyurt-Aralık	272	146	530	Merkez	Göcer	0	359	1803
Ermenek	Güneyyurt-Cami	133	236	675	Merkez	Gökçe	34	687	346
Ermenek	Güneyyurt-Habib	39	10	36	Merkez	Göztepe	387	1892	125
Ermenek	Güneyyurt-Kışlacık	136	236	419	Merkez	Güçler	24	3277	252
Ermenek	Güneyyurt-Oda	83	36	25	Merkez	Güldere	20	15285	986
Ermenek	G.Yurt-Ortamahalle	69	82	5	Merkez	Gülkaya	285	7670	1182
Ermenek	Güneyyurt-Pınargözü	91	201	44	Merkez	Hamidiye	38	2640	254
Ermenek	G.Yurt-Yenimahalle	575	266	16	Merkez	İhsaniye	75	3056	3550

CC: Cattle Count; SC: Sheep Count; GC: Goat Count;

Table 4b. Number of livestock in the villages and neighborhoods of Karaman in 2022

District	Village/Town	CC	SC	GC	District	Village/Town	CC	SC	GC
Ermenek	Kazancı-Bucak	42	189	282	Merkez	İslihisar	270	1884	231
Ermenek	Kazancı-Gökceler	184	0	634	Merkez	Kalaba	6	654	1624
Ermenek	Kazancı-Merkez	243	330	684	Merkez	Kameni	2533	4957	441
Ermenek	Kazancı-Tepecik	53	50	160	Merkez	Karacaören	333	6251	1437
Ermenek	Kazancı-Türbeseki	107	4	0	Merkez	Kaşoba	8	893	102
Ermenek	Kazancı-Uluköy	155	38	0	Merkez	Kılbasan	4315	11754	1892
Ermenek	Kazancı-Yukarı	0	18	132	Merkez	Kızık	173	5988	462
Ermenek	Merkez-Akçamescit	7	164	933	Merkez	Kızıllarağini	0	2991	1600
Ermenek	Merkez-Başpınar	5	0	0	Merkez	Kızılyaka	983	1285	0
Ermenek	Merkez-Çınarlısu	0	0	4	Merkez	Kisecik	54	7495	556
Ermenek	Merkez-Değirmenlik	85	334	118	Merkez	Kozlubucak	68	769	249
Ermenek	Merkez-Deliallar	5	0	0	Merkez	Kurtderesi	1013	2375	371
Ermenek	Merkez-Güllük	11	146	307	Merkez	Kurucabel	0	0	338
Ermenek	Merkez-Gülpazarı	0	4	475	Merkez	Küçükerenkavak	132	785	455
Ermenek	Merkez-Karalar	128	6	0	Merkez	Lale	1143	4782	1044
Ermenek	Merkez-Keçipazarı	7	152	318	Merkez	Madenşehri	246	2631	1109
Ermenek	Merkez-Keşillik	39	1196	1647	Merkez	Medreselik	31	1889	109
Ermenek	Merkez-Meydan	74	713	2065	Merkez	Mesudiye	142	1844	134
Ermenek	Merkez-Ortamahalle	3	600	2151	Merkez	Morcalı	759	2465	1336
Ermenek	Merkez-Sandıklı	11	18	643	Merkez	Muratdede	309	2551	3282
Ermenek	Merkez-Seyran	44	84	41	Merkez	Narlıdere	82	995	1803
Ermenek	Merkez-Susaklı	81	295	309	Merkez	Ortaoba	620	10821	347
Ermenek	Merkez-Taşbaşı	31	860	347	Merkez	Osmaniye	86	4857	198
Başyayla	Bozyaka	40	16	324	Merkez	Özdemir	87	299	4073
Başyayla	Büyükkarapınar	164	24	4	Merkez	Paşabağı	271	2821	482
Başyayla	Kışla	720	513	2358	Merkez	Pınarbaşı	57	2334	587
Başyayla	Üzümlü	143	227	1485	Merkez	Salur	158	1880	52
Başyayla	Merkez-Başköy	72	791	576	Merkez	Sarıkaya	6	457	94
Başyayla	Merkez-Göztepe	22	1251	69	Merkez	Sazlıyaka	80	48	101
Başyayla	Merkez-Kirazlıyayla	205	857	39	Merkez	Seyithasan	0	950	220
Başyayla	Merkez-Şirindere	76	2	4	Merkez	Sudurağı	2147	17191	395
Başyayla	Merkez-Yenimahalle	15	0	0	Merkez	Süleymanhacı	697	11570	1586
Kazımkarabekir	Akarköy	525	2036	405	Merkez	Tarlaören	38	236	389
Kazımkarabekir	Karalgazi	0	1371	291	Merkez	Taşkale	95	15467	9198
Kazımkarabekir	Kızılkuyu	1	3137	504	Merkez	Üçbaş	138	0	242
Kazımkarabekir	Mecidiye	42	656	26	Merkez	Üçkuyu	0	1438	533
Kazımkarabekir	Özyurt	71	3721	371	Merkez	Yeşildere	1264	14945	3151
Kazımkarabekir	Sinci	1	2486	171	Merkez	Yılangömü	0	692	980
Kazımkarabekir	Merkez-Boyacı	624	2936	1263	Merkez	Yollarbaşı	271	9093	5732
Kazımkarabekir	Merkez-Eminettin	284	957	240	Merkez	Yukarıakın	0	0	1369
Kazımkarabekir	Merkez-Emsalhayat	69	2675	737	Merkez	Yukarıkızılca	48	1152	4471
Kazımkarabekir	Merkez-Oba	0	694	98	Merkez	Yuvatepe	875	877	219
Kazımkarabekir	Merkez-Pazar	25	838	312	Merkez	Zengen	436	2609	914
Kazımkarabekir	Merkez-Selçuklu	800	492	310	Merkez	Merkez	14437	106907	33595
Kazımkarabekir	Merkez-Subaşı	72	2229	977	Merkez	Merkez-Atatürk	545	883	74
Kazımkarabekir	Merkez-Timsal	1027	600	514	Merkez	Mer.-Bahçelievler	163	2830	114
Sarıveliler	Adiller	268	123	398	Merkez	Merkez-Beyazkent	0	49	110
Sarıveliler	Civandere	116	1239	138	Merkez	Mer.-Cumhuriyet	58	55	0
Sarıveliler	Civler	481	306	614	Merkez	Merkez-Çeltek	543	1468	138
Sarıveliler	Çevrekavak	105	3	62	Merkez	Merkez-Fatih	814	508	154
Sarıveliler	Çukurbağ	108	1	0	Merkez	Merkez-Hacıcelal	0	55	1
Sarıveliler	Daran	59	7	529	Merkez	Merkez-Hisar	298	1084	124
Sarıveliler	Dumlugöze	353	18	1127	Merkez	Merkez-Prireis	2165	3383	363
Sarıveliler	Esentepe	61	47	469	Merkez	Merkez-Siyahser	8	96	22
Sarıveliler	Göktepe	87	75	52	Merkez	Merkez-Sümer	823	1535	13
Sarıveliler	Günder	2	276	10	Merkez	Merkez-Şeyhşamil	0	337	184
Sarıveliler	Işıklı	43	1	98	Merkez	Merkez-Urgan	7484	11739	1632
Sarıveliler	Koçaşlı	102	9	1433	Merkez	Merkez-Yenişehir	1013	1640	336
Sarıveliler	Ortaköy	325	0	0	Merkez	Merkez-Yeşilada	0	18	14

CC: Cattle Count; SC: Sheep Count; GC: Goat Count;

Table 5. Distributed pollutant loads originating from BBH and KBH waste in Karaman province and its districts

District Name	BBH Load TN QTN (ton/year)	KBH Load TN QTN (ton/year)	BBH Load TP QTP (ton/year)	KBH Load TP QTP (ton/year)	Total TN QTN (ton/year)	Total TP QTP (ton/year)
Ayrancı	58.62	290.43	6.52	13.75	349.06	20.27
Ermenek	44.12	51.77	4.90	2.45	95.89	7.36
Başyayla	11.97	8.84	1.33	0.42	20.81	1.75
Kazımkarabekir	29.08	32.13	3.23	1.52	61.22	4.75
Sarıveliler	24.68	13.27	2.74	0.63	37.95	3.37
Karaman Villages	266.96	483.11	29.68	22.87	750.06	52.55
Karaman Center	232.85	175.39	25.88	8.30	408.24	34.19

BBH= cattle, KBH= ovine animals

Table 6a. Distributed pollutant loads originating from BBH and KBH wastes of Karaman neighborhoods and villages

Village/Town	A	B	C	D	E	F	Village/Town	A	B	C	D	E	F
Ağızboğaz	4.62	18.64	0.51	0.88	23.26	1.40	Uğurlu	0.31	0.50	0.03	0.02	0.82	0.06
Akpınar	0.10	10.54	0.01	0.50	10.64	0.51	Merkez	0.57	0.96	0.06	0.05	1.54	0.11
Ambar	0.00	9.50	0.00	0.45	9.50	0.45	Başmahalle	0.70	0.24	0.08	0.01	0.94	0.09
Berendi	1.21	68.97	0.13	3.27	70.18	3.40	Mer.-Çakıllar	1.50	1.40	0.17	0.07	2.90	0.23
Böğecik	3.42	16.51	0.38	0.78	19.93	1.16	Karakaya	0.21	0.41	0.02	0.02	0.63	0.04
Buğdaylı	2.85	2.68	0.32	0.13	5.53	0.44	Kkarapınar	0.10	0.14	0.01	0.01	0.24	0.02
Büyükkoraş	0.00	6.32	0.00	0.30	6.32	0.30	Turcalar	2.86	0.34	0.32	0.02	3.19	0.33
Çat	0.00	8.42	0.00	0.40	8.42	0.40	Ulucami	0.76	1.94	0.08	0.09	2.69	0.18
Divle	1.91	7.79	0.21	0.37	9.70	0.58	Yeşilyurt	0.34	0.06	0.04	0.00	0.40	0.04
Dokuzyol	3.06	19.15	0.34	0.91	22.21	1.25	Ada	0.67	2.72	0.07	0.13	3.39	0.20
Höyükburun	2.01	4.37	0.22	0.21	6.39	0.43	Ağaçyurdu	0.85	2.29	0.09	0.11	3.14	0.20
Kale	0.67	2.16	0.07	0.10	2.84	0.18	Ağılönü	15.19	2.70	1.69	0.13	17.90	1.82
Karaağaç	0.00	12.81	0.00	0.61	12.81	0.61	Akçaalan	0.08	3.38	0.01	0.16	3.46	0.17
Kavaközü	0.21	13.17	0.02	0.62	13.38	0.65	Akçaşehir	4.80	37.50	0.53	1.78	42.31	2.31
Kavuklar	16.98	7.76	1.89	0.37	24.74	2.25	Akpınar	0.60	13.22	0.07	0.63	13.82	0.69
Kayaönü	0.00	13.67	0.00	0.65	13.67	0.65	Alaçatı	6.12	0.85	0.68	0.04	6.97	0.72
Kıraman	10.61	14.61	1.18	0.69	25.22	1.87	Aşağıakın	0.00	1.86	0.00	0.09	1.86	0.09
Küçükkoraş	0.00	6.55	0.00	0.31	6.55	0.31	Aşağıkızılca	0.11	1.88	0.01	0.09	2.00	0.10
Melikli	1.38	6.95	0.15	0.33	8.33	0.48	Aybastı	2.32	7.45	0.26	0.35	9.77	0.61
Pınarkaya	0.01	13.63	0.00	0.65	13.64	0.65	Bademli	0.50	1.59	0.06	0.08	2.09	0.13
Saray	0.00	3.71	0.00	0.18	3.71	0.18	Barutkavran	1.83	1.58	0.20	0.07	3.41	0.28
Yarıkkuyu	0.42	7.13	0.05	0.34	7.55	0.38	Başkışla	2.28	1.59	0.25	0.08	3.87	0.33
Merkez-Dede	3.00	2.14	0.33	0.10	5.14	0.43	Bayır	0.49	1.85	0.05	0.09	2.34	0.14
Merkez-Musa	0.00	4.30	0.00	0.20	4.30	0.20	Beydilli	0.05	1.14	0.01	0.05	1.18	0.06
Merkez-Ulu	0.20	2.87	0.02	0.14	3.07	0.16	Bostanözü	0.00	0.27	0.00	0.01	0.27	0.01
Yenimahalle	5.99	6.08	0.67	0.29	12.06	0.95	Boyalı	1.95	4.56	0.22	0.22	6.51	0.43
Ağaççatı	0.03	1.60	0.00	0.08	1.64	0.08	Bozkondak	7.77	2.08	0.86	0.10	9.85	0.96
Ardıçkaya	1.05	1.05	0.12	0.05	2.10	0.17	Bölükyazı	0.10	1.36	0.01	0.06	1.46	0.08
Aşağıçağlar	2.12	3.69	0.24	0.17	5.81	0.41	Bucakkışla	0.00	1.41	0.00	0.07	1.41	0.07
Balkusan	0.25	2.76	0.03	0.13	3.01	0.16	Burhan	0.58	2.58	0.06	0.12	3.16	0.19
Boyalık	0.11	0.19	0.01	0.01	0.31	0.02	Burunoba	0.38	6.01	0.04	0.28	6.38	0.33
Çamlıca	0.03	1.63	0.00	0.08	1.67	0.08	B.Erenkavak	0.97	1.05	0.11	0.05	2.01	0.16
Çatalbadem	0.73	4.27	0.08	0.20	5.00	0.28	Cerit	1.10	5.08	0.12	0.24	6.18	0.36
Çavuş	0.08	0.29	0.01	0.01	0.38	0.02	Çakırbağ	8.89	10.05	0.99	0.48	18.94	1.46
Elmayurdu	2.19	0.13	0.24	0.01	2.32	0.25	Çatak	2.46	5.78	0.27	0.27	8.24	0.55
Evsin	0.01	0.59	0.00	0.03	0.60	0.03	Çavuşpınarı	6.73	4.68	0.75	0.22	11.41	0.97
Gökçekent	3.01	0.51	0.34	0.02	3.52	0.36	Çiğdemli	2.16	1.73	0.24	0.08	3.89	0.32
Gökçeseki	0.00	0.80	0.00	0.04	0.80	0.04	Çimenkuyu	0.24	0.16	0.03	0.01	0.40	0.03
Görmeli	0.19	4.22	0.02	0.20	4.41	0.22	Çoğlu	4.53	14.90	0.50	0.71	19.42	1.21
İkizçınar	1.20	0.46	0.13	0.02	1.66	0.16	Çukur	0.05	2.12	0.01	0.10	2.17	0.11
Katranlı	4.86	2.43	0.54	0.11	7.29	0.66	Çukurbağ	0.24	2.98	0.03	0.14	3.21	0.17
Kayaönü	0.25	1.17	0.03	0.06	1.43	0.08	Dağkonak	0.85	1.46	0.09	0.07	2.31	0.16
Olukpınar	0.02	0.26	0.00	0.01	0.28	0.01	Damlapınar	0.55	2.25	0.06	0.11	2.80	0.17
Pamuklu	0.34	0.26	0.04	0.01	0.60	0.05	Değirmenbaşı	0.20	0.59	0.02	0.03	0.78	0.05
Pınarönü	0.66	0.37	0.07	0.02	1.03	0.09	Demiryurt	4.82	3.75	0.54	0.18	8.57	0.71

A: BBH Load TN QTN (ton/year); B: KBH Load TN QTN (ton/year); C: BBH Load TP QTP (ton/year); D: KBH Load TP QTP (ton/year); E: Total TN QTN (ton/year); F: Total TP QTP (ton/year)

Table 6b. Distributed pollutant loads originating from BBH and KBH wastes of Karaman neighborhoods and villages

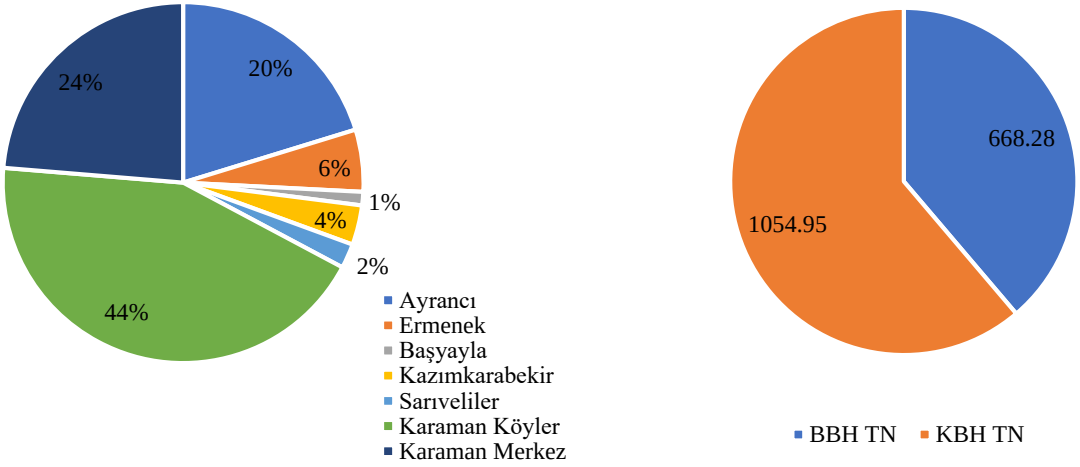
Village/Town	A	B	C	D	E	F	Village/Town	A	B	C	D	E	F
Sarıvadi	1.39	1.53	0.15	0.07	2.91	0.23	Dereköy	3.93	6.95	0.44	0.33	10.89	0.77
Tepebaşı	0.76	0.30	0.08	0.01	1.06	0.10	Dinek	1.06	4.01	0.12	0.19	5.07	0.31
Yalındal	0.45	0.99	0.05	0.05	1.44	0.10	Eğilmez	2.76	10.65	0.31	0.50	13.41	0.81
Yaylapazarı	0.11	0.69	0.01	0.03	0.80	0.04	Ekinözü	5.47	23.28	0.61	1.10	28.75	1.71
Yerbağ	0.57	0.34	0.06	0.02	0.92	0.08	Elmadağı	0.48	3.82	0.05	0.18	4.30	0.23
Yukarıçağlar	1.42	1.12	0.16	0.05	2.54	0.21	Eminler	1.68	6.53	0.19	0.31	8.21	0.50
Aralık	2.23	0.70	0.25	0.03	2.93	0.28	Göcer	0.00	2.24	0.00	0.11	2.24	0.11
Güneyyurt-Cami	1.09	0.94	0.12	0.04	2.04	0.17	Gökçe	0.28	1.07	0.03	0.05	1.35	0.08
Habib	0.32	0.05	0.04	0.00	0.37	0.04	Göztepe	3.18	2.09	0.35	0.10	5.27	0.45
Kışlacık	1.12	0.68	0.12	0.03	1.79	0.16	Güçler	0.20	3.65	0.02	0.17	3.85	0.19
Güneyyurt-Oda	0.68	0.06	0.08	0.00	0.74	0.08	Güldere	0.16	16.84	0.02	0.80	17.00	0.82
Ortamahalle	0.57	0.09	0.06	0.00	0.66	0.07	Gülkaya	2.34	9.16	0.26	0.43	11.50	0.69
Pınargözü	0.75	0.25	0.08	0.01	1.00	0.10	Hamidiye	0.31	3.00	0.03	0.14	3.31	0.18
Yenimahalle	4.72	0.29	0.52	0.01	5.01	0.54	İhsaniye	0.62	6.84	0.07	0.32	7.45	0.39
Kazancı-Bucak	0.34	0.49	0.04	0.02	0.83	0.06	İslihisar	2.22	2.19	0.25	0.10	4.41	0.35
Gökceler	1.51	0.66	0.17	0.03	2.17	0.20	Kalaba	0.05	2.36	0.01	0.11	2.41	0.12
Kazancı-Merkez	2.00	1.05	0.22	0.05	3.05	0.27	Kameni	20.80	5.59	2.31	0.26	26.39	2.58
Kazancı-Tepecik	0.44	0.22	0.05	0.01	0.65	0.06	Karacaören	2.73	7.96	0.30	0.38	10.69	0.68
Türbeseki	0.88	0.00	0.10	0.00	0.88	0.10	Kaşoba	0.07	1.03	0.01	0.05	1.10	0.06
Kazancı-Uluköy	1.27	0.04	0.14	0.00	1.31	0.14	Kılbasan	35.44	14.12	3.94	0.67	49.56	4.61
Kazancı-Yukarı	0.00	0.16	0.00	0.01	0.16	0.01	Kızık	1.42	6.68	0.16	0.32	8.10	0.47
Akçamescit	0.06	1.14	0.01	0.05	1.19	0.06	Kızıllarağini	0.00	4.75	0.00	0.22	4.75	0.22
Merkez-Başpınar	0.04	0.00	0.00	0.00	0.04	0.00	Kızılyaka	8.07	1.33	0.90	0.06	9.40	0.96
Çınarlısu	0.00	0.00	0.00	0.00	0.00	0.00	Kisecik	0.44	8.33	0.05	0.39	8.78	0.44
Değirmenlik	0.70	0.47	0.08	0.02	1.17	0.10	Kozlubucak	0.56	1.05	0.06	0.05	1.61	0.11
Deliallar	0.04	0.00	0.00	0.00	0.04	0.00	Kurtderesi	8.32	2.84	0.92	0.13	11.16	1.06
Merkez-Güllük	0.09	0.47	0.01	0.02	0.56	0.03	Kurucabel	0.00	0.35	0.00	0.02	0.35	0.02
Gülpazarı	0.00	0.50	0.00	0.02	0.50	0.02	K.Erenkavak	1.08	1.28	0.12	0.06	2.37	0.18
Merkez-Karalar	1.05	0.01	0.12	0.00	1.06	0.12	Lale	9.39	6.03	1.04	0.29	15.42	1.33
Keçipazarı	0.06	0.49	0.01	0.02	0.54	0.03	Madenşehri	2.02	3.87	0.22	0.18	5.89	0.41
Merkez-Keşillik	0.32	2.94	0.04	0.14	3.26	0.17	Medreselik	0.25	2.07	0.03	0.10	2.32	0.13
Merkez-Meydan	0.61	2.88	0.07	0.14	3.48	0.20	Mesudiye	1.17	2.05	0.13	0.10	3.21	0.23
Ortamahalle	0.02	2.85	0.00	0.13	2.87	0.14	Morcalı	6.23	3.93	0.69	0.19	10.17	0.88
Merkez-Sandıklı	0.09	0.68	0.01	0.03	0.77	0.04	Muratdede	2.54	6.04	0.28	0.29	8.57	0.57
Merkez-Seyran	0.36	0.13	0.04	0.01	0.49	0.05	Narlıdere	0.67	2.90	0.07	0.14	3.57	0.21
Merkez-Susaklı	0.67	0.63	0.07	0.03	1.29	0.10	Ortaoba	5.09	11.56	0.57	0.55	16.65	1.11
Merkez-Taşbaşı	0.25	1.25	0.03	0.06	1.50	0.09	Osmaniye	0.71	5.23	0.08	0.25	5.94	0.33
Bozyaka	0.33	0.35	0.04	0.02	0.68	0.05	Özdemir	0.71	4.53	0.08	0.21	5.24	0.29
Büyükkarapınar	1.35	0.03	0.15	0.00	1.38	0.15	Paşabağı	2.23	3.42	0.25	0.16	5.64	0.41
Kışla	5.91	2.97	0.66	0.14	8.88	0.80	Pınarbaşı	0.47	3.02	0.05	0.14	3.49	0.20
Üzümlü	1.17	1.77	0.13	0.08	2.95	0.21	Salur	1.30	2.00	0.14	0.09	3.30	0.24
Merkez-Başköy	0.59	1.41	0.07	0.07	2.01	0.13	Sarıkaya	0.05	0.57	0.01	0.03	0.62	0.03
Merkez-Göztepe	0.18	1.37	0.02	0.06	1.55	0.08	Sazlıyaka	0.66	0.15	0.07	0.01	0.81	0.08
Kirazlıyayla	1.68	0.93	0.19	0.04	2.61	0.23	Seyithasan	0.00	1.21	0.00	0.06	1.21	0.06
Şirindere	0.62	0.01	0.07	0.00	0.63	0.07	Sudurağı	17.63	18.20	1.96	0.86	35.83	2.82
Yenimahalle	0.12	0.00	0.01	0.00	0.12	0.01	Süleymanhacı	5.72	13.62	0.64	0.64	19.34	1.28
Akarköy	4.31	2.53	0.48	0.12	6.84	0.60	Tarlaören	0.31	0.65	0.03	0.03	0.96	0.07
Karalgazi	0.00	1.72	0.00	0.08	1.72	0.08	Taşkale	0.78	25.53	0.09	1.21	26.31	1.30
Kızılkuyu	0.01	3.77	0.00	0.18	3.78	0.18	Üçbaş	1.13	0.25	0.13	0.01	1.38	0.14
Mecidiye	0.34	0.71	0.04	0.03	1.05	0.07	Üçkuyu	0.00	2.04	0.00	0.10	2.04	0.10
Özyurt	0.58	4.24	0.06	0.20	4.82	0.27	Yeşildere	10.38	18.73	1.15	0.89	29.11	2.04
Sinci	0.01	2.75	0.00	0.13	2.76	0.13	Yılangömü	0.00	1.73	0.00	0.08	1.73	0.08
Merkez-Boyacı	5.12	4.35	0.57	0.21	9.47	0.78	Yollarbaşı	2.23	15.34	0.25	0.73	17.57	0.97
Eminettin	2.33	1.24	0.26	0.06	3.57	0.32	Yukarıakın	0.00	1.42	0.00	0.07	1.42	0.07
Emsalhayat	0.57	3.53	0.06	0.17	4.10	0.23	Yukarıkızılca	0.39	5.82	0.04	0.28	6.21	0.32
Merkez-Oba	0.00	0.82	0.00	0.04	0.82	0.04	Yuvatepe	7.19	1.13	0.80	0.05	8.32	0.85
Merkez-Pazar	0.21	1.19	0.02	0.06	1.40	0.08	Zengen	3.58	3.65	0.40	0.17	7.23	0.57
Selçuklu	6.57	0.83	0.73	0.04	7.40	0.77	Merkez	118.57	145.42	13.18	6.88	263.99	20.07

A: BBH Load TN QTN (ton/year); B: KBH Load TN QTN (ton/year); C: BBH Load TP QTP (ton/year); D: KBH Load TP QTP (ton/year); E: Total TN QTN (ton/year); F: Total TP QTP (ton/year)

Table 6c. Distributed pollutant loads originating from BBH and KBH wastes of Karaman neighborhoods and villages

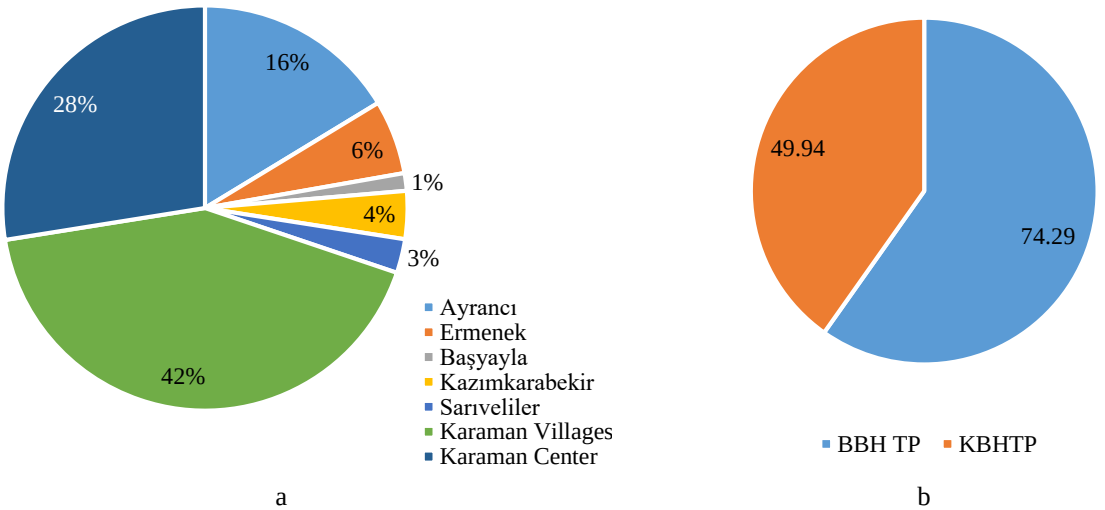
Village/Town	A	B	C	D	E	F	Village/Town	A	B	C	D	E	F
Merkez-Subaşı	0.59	3.32	0.07	0.16	3.91	0.22	Mer-Atatürk	4.48	0.99	0.50	0.05	5.47	0.54
Merkez-Timsal	8.43	1.15	0.94	0.05	9.59	0.99	M.Bahçelievler	1.34	3.05	0.15	0.14	4.39	0.29
Adiller	2.20	0.54	0.24	0.03	2.74	0.27	M.-Beyazkent	0.00	0.16	0.00	0.01	0.16	0.01
Civandere	0.95	1.43	0.11	0.07	2.38	0.17	M.-Cumhuriyet	0.48	0.06	0.05	0.00	0.53	0.06
Civler	3.95	0.95	0.44	0.05	4.90	0.48	M.-Çeltek	4.46	1.66	0.50	0.08	6.12	0.57
Çevrekavak	0.86	0.07	0.10	0.00	0.93	0.10	M.-Fatih	6.69	0.69	0.74	0.03	7.37	0.78
Çukurbağ	0.89	0.00	0.10	0.00	0.89	0.10	M.-Hacıcelal	0.00	0.06	0.00	0.00	0.06	0.00
Daran	0.48	0.55	0.05	0.03	1.04	0.08	Merkez-Hisar	2.45	1.25	0.27	0.06	3.70	0.33
Dumlugöze	2.90	1.19	0.32	0.06	4.08	0.38	Merkez-Prireis	17.78	3.88	1.98	0.18	21.66	2.16
Esentepe	0.50	0.53	0.06	0.03	1.04	0.08	M.-Siyahser	0.07	0.12	0.01	0.01	0.19	0.01
Göktepe	0.71	0.13	0.08	0.01	0.85	0.09	M.-Sümer	6.76	1.60	0.75	0.08	8.36	0.83
Günder	0.02	0.30	0.00	0.01	0.31	0.02	M.-Şeyhşamil	0.00	0.54	0.00	0.03	0.54	0.03
Işıklı	0.35	0.10	0.04	0.00	0.46	0.04	Merkez-Urgan	61.47	13.84	6.83	0.66	75.31	7.49
Koçaşlı	0.84	1.49	0.09	0.07	2.33	0.16	M.-Yenişehir	8.32	2.05	0.92	0.10	10.36	1.02
Ortaköy	2.67	0.00	0.30	0.00	2.67	0.30	M.-Yeşilada	0.00	0.03	0.00	0.00	0.03	0.00

A: BBH Load TN QTN (ton/year); B: KBH Load TN QTN (ton/year); C: BBH Load TP QTP (ton/year); D: KBH Load TP QTP (ton/year); E: Total TN QTN (ton/year); F: Total TP QTP (ton/year)



a b

Figure 2. Distribution of TN load originating from livestock in Karaman province in general (a) According to provinces and districts and (b) Distribution according to animal species



a b

Figure 3. The distribution of TP load originating from livestock in Karaman province in general (a) According to provinces and districts and (b) Distribution according to animal species

The distribution of TN (total nitrogen) load from livestock sources in Karaman province is shown in Figure 2(a) by districts. Additionally, the distribution of TN load by animal species is depicted in Figure 2(b). Moreover, Figure 3(a) displays the TP (total phosphorus) load distribution in the province, calculated for each district. Figure 3(b) illustrates the distribution of TP load by animal species.

According to the provided information, the villages affiliated with Karaman have the highest TN load from diffuse pollutants, with a total of 750.06 tons/year, accounting for 44% of the total. On the other hand, Başyayla district has the lowest TN load of 20.81 tons/year, representing 1% of the total.

The density distribution map of TN load attributed to livestock and the distribution map of TP load calculated for neighborhoods and villages within Karaman province are shown in Figure 4 and Figure 5, respectively. When looking at the pollution load density maps, it can be said that nearly 80% of Karaman province is exposed to widespread pollutants. This is mainly due to the fact that a large portion of livestock activities are carried out on an individual basis. Most of the livestock owners in this area conduct their operations within their own means, indicating that livestock activities are conducted outside of regulated facilities.

When examining the annual TP load formation attributed to BBH and KBH based on animal species in the province, it was calculated that 74.29 tons and 49.94 tons were generated, respectively. Furthermore, the villages affiliated with Karaman have the highest TP load with 52.55 tons per year (42% of the total), while Başyayla district has the lowest TP load with 1.75 tons per year (1% of the total). After calculating the spreading pollutant load originating from animal waste in the province, the amounts of dry manure that can be generated from animal waste were calculated. The calculated amounts of dry manure are presented in Table 7, and the distribution of dry manure is shown in Figure 6. The total amount of dry manure generated from annual animal waste was calculated as 172,290 tons.

As seen in Figure 6 (b), the total amount of dry manure generated in Karaman province is 76% attributed to BBH (bovine-based husbandry). The area with the highest livestock-derived waste in Karaman province is the villages affiliated with Karaman, with 71,279.58 tons per year (41% of the total). The annual potential pollutant loads from livestock activities are estimated to be 1,723.23 tons of TN (total nitrogen) and 124.23 tons of TP (total phosphorus). It is necessary to take appropriate measures to prevent these high pollution loads from contaminating underground and surface water sources. Additionally, Karaman province generates 172,290 tons of dry manure. Proper storage and utilization of commercially valuable animal waste will contribute to the local and national economy.

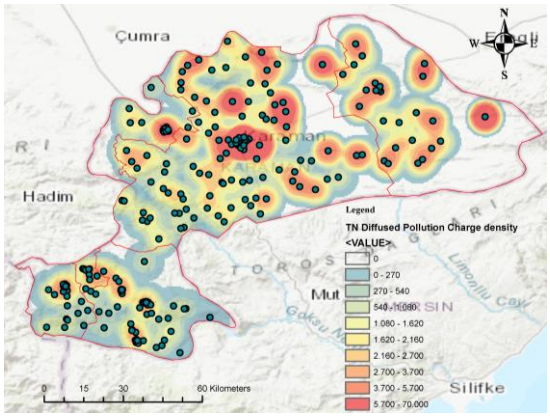


Figure 4. Density distribution map of TN load originating from livestock in Karaman province

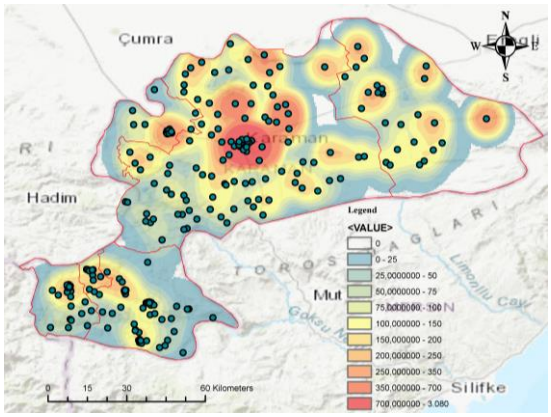
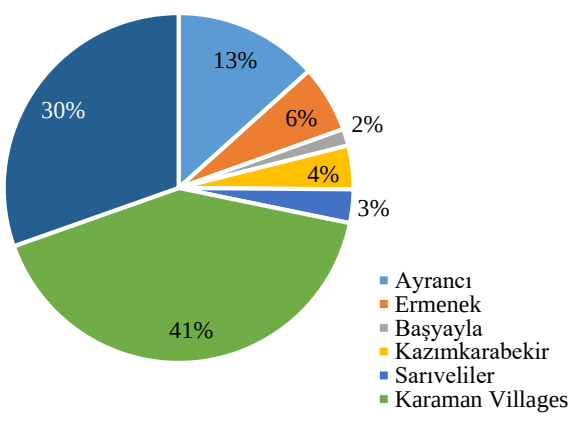


Figure 5. Density distribution map of TP load originating from livestock in Karaman province



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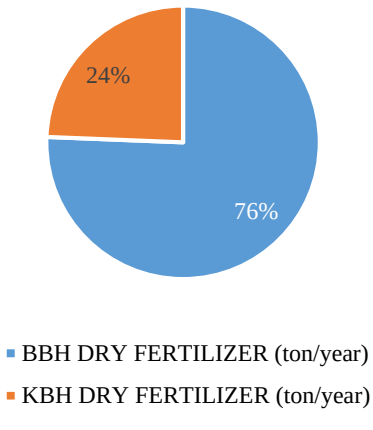

b

Figure 6. The formation of dry manure originating from livestock in Karaman province in general (a) Distribution according to provinces and districts and (b) Distribution according to animal species

Table 7. The amount of fertilizer to be obtained from BBH and KBH wastes of Karaman province and its districts

District Name	BBH Dry Manure (tons/year)	KBH Dry Manure (tons/year)	Total Dry Manure (tons/year)
Ayrancı	11,431.06	11,558.52	22,989.58
Ermenek	8,602.922	2,060.295	10,663.22
Başyayla	2,333.294	351.7647	2,685.059
Kazımkarabekir	5,670.69	1,278.834	6,949.524
Sarıveliler	4,812.32	528.265	5,340.585
Karaman Villages	52,053.12	19,226.46	71,279.58
Karaman Center	45,402.35	6,980.141	52,382.5

BBH= cattle, KBH= ovine animals

Results

Determining the transport processes and quantities of pollutants is important to assess the environmental impacts on underground and surface water sources. Ultimately, it enables the identification of pollutant transport processes, which is crucial for controlling environmental pollution and improving water management practices (Akdoğan et al., 2015). The increasing demand for water resources and the decreasing availability of these resources due to environmental pollutants make the control of diffuse pollutants significant. In this context, the dominant role of agricultural activities in the formation of diffuse pollution necessitates the prevention of pollution originating from these sources (Özalp, 2009; Hacısalihoğlu, 2022; Haksevenler and Ayaz, 2021).

In this study, the diffuse pollutant loads and the amount of collected dry manure were calculated based on the number of livestock in Karaman province in 2022. The livestock population in Karaman province consisted of a total of 1,100,645 live animals (8% beef cattle and 92% dairy cattle). The estimated livestock-related diffuse pollutant loads for TN and TP were 1,723.23 tons/year and 124.23 tons/year, respectively. It was also calculated that approximately 172,290 tons/year of dry manure could be obtained from livestock activities.

Considering that 15% of TN and 5% of TP can reach water sources, it is inevitable that they will cause serious water pollution problems. These livestock wastes not only affect water sources but also contribute to carbon emissions and global warming. When looking at the density map of diffuse pollution in Karaman province, it can be said that nearly 80% of the area is exposed to diffuse pollutants. This is mainly due to the fact that a significant portion of livestock farming is carried out on an individual basis. Many livestock farmers operate within their own means, indicating that livestock farming activities are conducted outside of systematic facilities.

Livestock waste increases the nitrate content in groundwater and surface water sources. High-nitrate content groundwater and surface water, which are used for various purposes, can cause vomiting, cramps, diarrhea, and in severe cases, fatality in animals, as well as immune system disorders and the formation of genetic diseases in humans (Polat and Olgun, 2009). In addition to soil and water pollution, livestock waste also contributes to air pollution. Various gases that contribute to global climate change are released into the atmosphere from these wastes, including carbon dioxide (CO2), carbon monoxide (CO), ammonia (NH3), hydrogen sulfide (H2S), and water vapor (H2O). Furthermore, significant gases that contribute to the greenhouse effect, such as methane (CH4) and nitrous oxide (N2O), are released through certain decomposition and nitrification processes during the storage and transportation of livestock manure (IPCC, 1996). Therefore, it is necessary to store livestock manure in leak-proof and enclosed environments that comply with standards to prevent environmental pollution (Salihoğlu et al., 2019; Tırınk, 2021). In order to prevent environmental pollution from livestock waste, appropriate measures should be taken to store it in environmentally safe conditions before applying it to the land. In this regard, processes such as biogas production, composting, ventilation, and drying can be implemented to mitigate or prevent the environmental issues caused by these wastes, promoting sustainable development (Salihoğlu et al., 2019; Tırınk, 2021). In biogas plants originating from animal waste, electricity, heat and fertilizer are produced as by-products from the waste. Electricity is produced using gas generators in biogas facilities (Seyhan and Badem, 2021). Composting is the process of converting organic matter into a soil-like substance called humus by biodegrading it by bacteria and other microorganisms. The most important factors in the composting process are the C/N ratio, the amount of moisture and the amount of volatile solids (Çataltas, 2013). Disposing of bovine animal waste by reducing the moisture content in the feces by using ventilation, mechanical drying, and heating methods using sunlight, rather than removing it with water, can contribute to reducing environmental problems.

The method presented for calculating pollution loads is expected to provide a roadmap for researchers working in the field. On the other hand, this study, which indicates the source of pollution and the reasons for water contamination, is believed to serve as a guide for decision-makers and implementers.

Conflict Statement

The authors declare that there is no conflict of interest in this study.

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Fortification of Yogurt with Red Dragon Fruit's (*Hylocereus Polyrhizus*) Peel Powder: Effects on Comprehensive Quality Attributes and Sensory Properties

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ABSTRACT

This study was conducted to evaluate the quality features, antioxidant capabilities, microbiological and sensory aspects of yogurt fortified with 2%, 5%, and 7% red dragon (RD) peel powder. The yogurt was formulated using the classical technology adapted to laboratory conditions. The results of the physicochemical properties showed significant differences in pH (4.73–4.36), acidity (0.18–0.16 g lactic acid/100 g), and ascorbic acid (1.17-1.34 mg/100 g) among different yogurt formulations ($P<0.05$). In addition, RD peel powder fortification showed increasing trends in crude fiber (1.53-3.34 g/100 g), ash (5.19-5.29 g/100 g), and moisture (76.70-80.19 g/100 g) content, respectively; while the reversed trend was observed for fat (3.48-2.36 g/100 g), and crude protein (4.49-4.07 g/100 g) contents, respectively. Furthermore, gradual progression of RD peel powder in fortified yogurt manifested an improvement of the overall antioxidant activity (1.30-1.57 µmol TE/mL). The analyses of the sensory properties demonstrated that yogurt with RD peel powder in proportions of 2% received the highest hedonic score for consumer approval. Moreover, no coliform was reported in any of the control and fortified yogurts. Therefore, it could be concluded that RD peel powder can be employed as a functional food constituent in yogurt with improved quality attributes and sensory properties compared to plain yogurt.

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Introduction

Modern food industries generate millions of tons of waste during processing, which poses serious threat to our ecosystem, the economy, and public health. Agricultural wastes are full of bioactive phytochemicals, such as vitamins, phenolic compounds, carotenoids, essential oils, dietary fibers, polysaccharides, antioxidants, and pigments (Dueñas and García-Estévez, 2020). These potential bioactive compounds have the ability to provide numerous health benefits and can be used in food and pharmaceutical industries (Kumar et al., 2020). However, the use of such food wastes or by-products from different agro-industries in product development seemed to be an excellent way to produce secondary food products. Some of the wastes have already been used by the global food markets to improve the nutritional value of some foods. For example, adding soy flour to spaghetti increases the protein and amino acid content, while addition of king palm flour to cookies and gluten-free cookies enhances the yield of nutritious fiber and some nutrients like calcium, magnesium, or potassium. Furthermore, mango peel flour was also used to formulate extruded foods, baked goods, and dairy products (Hernández and Serna-Saldivar, 2019; Tadesse et al., 2019; Kainat et al., 2022). Thus, proper utilization of agricultural wastes rich in functional ingredients and biomaterials are becoming more and more interesting to food scientists.

Red dragon (RD) fruit's peel is one of the potential sources of phytochemicals and natural functional food ingredients among the fruit wastes. It is also rich in several health-beneficial chemicals like polyphenols, anthocyanins, flavonoids, and betalains; in addition to being a plentiful source of essential oils, vitamins, and minerals. Additionally, it has been reported that the peels

of RD fruit contain a significant quantity of dietary fibers (59-90 g/100 g), with the insoluble and soluble fibers (IDF and SDF) accounting for approximately 55-82% and 21-39%, respectively (Jamilah et al., 2011). Furthermore, it has been estimated that peels of RD fruit contain higher nutrients and show greater antioxidant activity compared to its edible portion (Gondim et al., 2005; Le, 2022). However, the peels of RD fruit make up approximately 22–44% of the total fruit weight and are typically thrown away after processing, or used as animal feed on farms, or they are transferred to landfills and incinerators (Liaotrakoon et al., 2013). It is inevitable that dumping these fruit wastes into the environment for an extended period of time causes the emission of greenhouse gases and encourages the growth of certain insects, pests, and microbes (Cheok et al., 2018). Therefore, RD peel can be used in various food product formulations to improve their functional value as well as contributing to a greater reduction in food waste.

Food products with a balanced nutritional composition and additional health benefits have drawn more and more attention in recent years (Klopčič et al., 2020). One of the most popular dairy products, yogurt is far healthier than milk, especially for individuals who are lactose intolerant. This food item is often regarded as a staple by consumers since it provides a significant number of nutrients, including proteins, vitamins, minerals, and numerous health-improving microorganisms (Qiu et al., 2021). Although yogurt has a high nutritional profile, it is not mostly well thought-out as an important source of bioactive phytochemicals. As a result, dairy products that have been supplemented with natural ingredients such as seeds, peels, and plants are considered as a great strategy to increase the overall nutritive value of this food, which can have a significant positive impact on human health (Caleja et al., 2016; O'sullivan et al., 2016). In recent days, growing interest has already been shown in fortifying yogurt with bioactive plant extracts, such as moringa powder, strawberries, chia seeds, and concentrated strawberry pulp, to improve the nutritional benefits of plain yogurt (Jaster et al., 2018; Zhang et al., 2019; Kowaleski et al., 2020). Consequently, this evidence has raised the question to develop a novel dairy product enriched with RD peel powder to offer health benefits as well as to satisfy consumers' sensory appeal.

Thus, considering the health benefits, this study aimed to investigate the feasibility of fortifying red dragon (RD) fruit's peel in yogurt and to analyze its effect on quality attributes, antioxidant properties, microbial, and sensory characteristics of fortified yogurt to increase its popularity among the consumers as well food manufacturers.

Materials and Methods

Materials

Milk and red dragon fruits were procured from local supermarkets, situated in Chattogram, Bangladesh. The raw ingredients were collected in sterile glass bottle and airtight sealed plastic bags, respectively and immediately transferred to the laboratory to avoid contamination and further stored in refrigerated condition. Chemicals such as sodium hydroxide, 2,6-dichlorophenolindophenol, *meta*-phosphoric acid, glacial acetic acid, methanol, agar medium, *n*-hexane, hydrochloric acid, and boric acid were procured from Sigma-Aldrich Co. (St. Louis, Missouri, USA). All chemicals used for the research purposes were of analytical grade.

Preparation of RD peel powder

Fresh fruit was thoroughly washed with distilled water to remove dirt or any extraneous residue. Peels of RD fruit were removed with a hand peeler (OXO Good Grips Prep Y-Peeler) and cut into cube shaped slices. The slices were immediately immersed into potassium *meta*-bisulfite solution (0.02%) for 5 min. Then fruit peels were standardized and blanched at 70 °C for 5 min to avoid enzymatic degradation during storage. Peels were then freeze-dried (Accumax Freeze Dryer, Accumax India, New Delhi, India). Dried peels were ground into fine powders using mixer grinder. Following packing in zipper bags (LDPE Plain Plastic Zipper bags), the finely ground powder was kept in airtight food grade plastic containers with labelling until it was used again.

Preparation of yogurt

The conventional technique of yogurt formulation was used as reported by Dabija et al. (2018). Raw milk was first standardized to contain almost 3.5% fat and 8.5% SNF (solids non-fat) followed by pasteurization (90 °C for 15 min), cooling (41 °C), inoculation with 0.02% (w/v) starter culture obtained from an indigenous yogurt sample containing mixed culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, dosing in yogurt jars with addition of 0%, 2%, 5%, and 7% (w/v) peel powder. All the inoculated milk blends were placed into incubator (43 °C for 6 h) to ease fermentation and further stored in a refrigerator (4 °C) overnight. The whole experimental procedure is presented in Figure 1 and Table 1 shows the formulation of sweetened yogurt with different percentage of dried red dragon peel powder.

Physicochemical characteristics

With a pH meter (model: HI-98107, Hanna Instrument, Italy), the pH of yogurt samples was determined. Titration method from Chouchouli et al. (2013) was used to evaluate titratable acidity of samples with minor changes. Briefly, 20 mL of distilled water was added to 10 g of yogurt before being titrated with standard 0.1 M NaOH in the presence of phenolphthalein indicator. Prior to use NaOH was standardized against potassium hydrogen phthalate. The percentage of lactic acid in the results was then calculated. Vitamin C content was determined using the titrimetric 2, 6-dichlorophenolindophenol method (Ranganna, 1986). Vitamin C in test samples was extracted using a 2% oxalic acid solution followed by titrating it with 2, 6-dichlorophenolindophenol until a light pink color appeared.

Proximate composition

Proximate composition of RD peel powder fortified yogurt samples was determined using the standard methods (AOAC, 2006). Moisture content was determined by hot air oven drying at 105 ºC until a constant mass was produced. The amount of crude protein was estimated by the Kjeldahl procedure. Ash content was measured gravimetrically in a furnace by heating at 550-650 ºC to a constant weight. The soxhlet technique was used to extract fat using n-hexane. The crude fiber content was also determined in this particular effort.

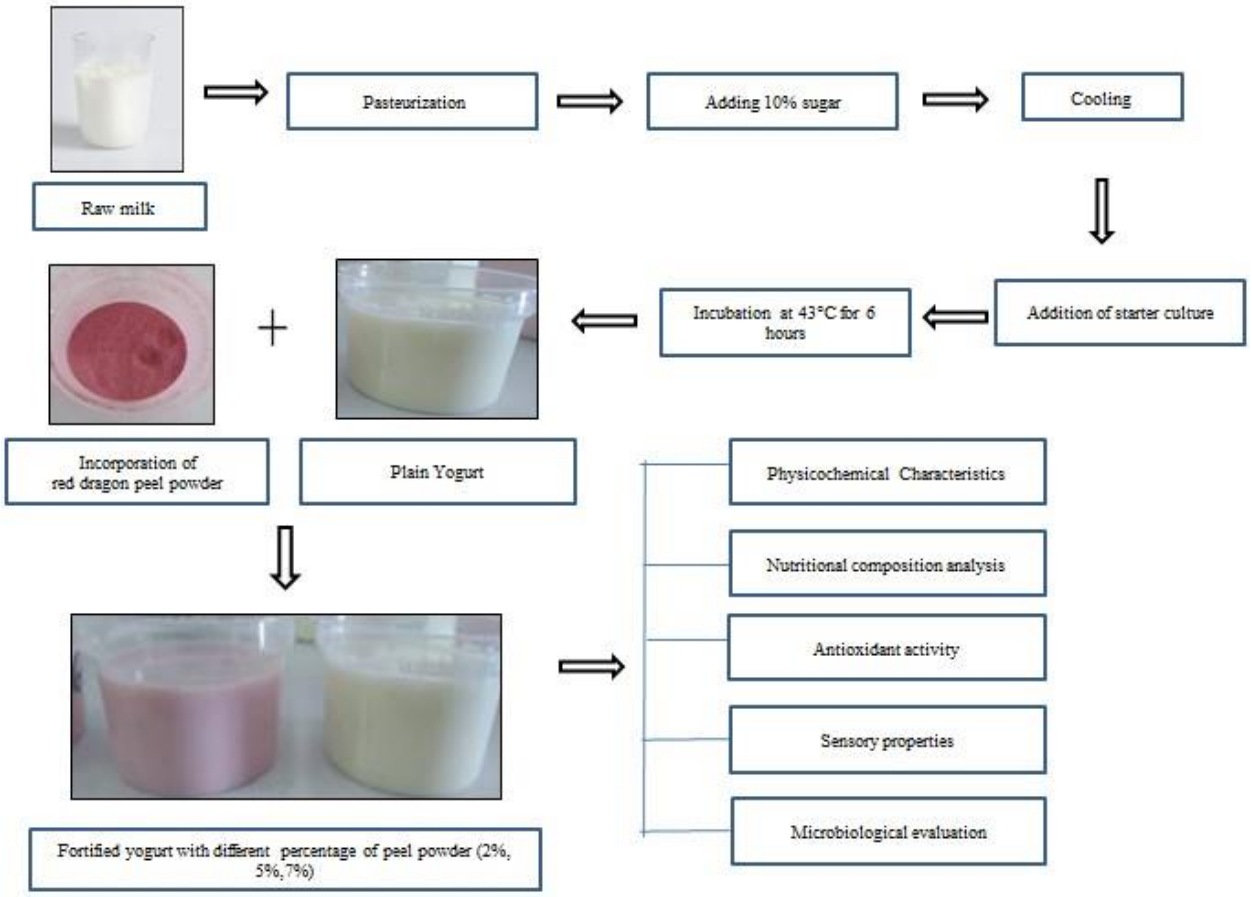

Figure 1. Schematic representation of the experimental procedure

Table 1. Treatments table

Treatments		Levels (%)
T_o	Control	0%
T_1		2%
T_2	RD peel powder (dried)	5%
T_3		7%

Determination of antioxidant activity

Antioxidant activity was calculated through DPPH assay, following the method as stated by Leong and Shui (2002). An aliquot of 1 mL sample extract was added to 2 mL of methanolic DPPH solution and kept in the dark for 30 min. Absorbance was assayed at 517 nm using a UV-visible spectrophotometer (UV-1800 Shimadzu, Japan). The findings were compared with the Trolox standard curve ($y = 0.0894X+0.3145$, $R^2 = 0.997$) and reported in µmol Trolox equivalent per mL sample.

Microbiological Analysis

According to Wang et al., the total viable bacterial count (TVC) in yogurt was determined by serial dilution method using pour plate technique (Wang et al., 2010). Then, one mL of each sample was sequentially added to nine mL of the sterile diluent (peptone water) and shaken vigorously. The serial dilution process was continued up to 10^5 dilutions. On nutrient agar plates, aliquot portions (0.1 mL) of the appropriate dilution were applied. Colony forming units per mL of sample (cfu/mL) were calculated after the plates were incubated at 37 ºC for 48 hours in an incubator.

Using MacConkey agar medium, the total coliforms (TC) number was calculated in accordance with Micanel et al. (1997). The pour plate technique involved transferring 1 mL of each sample into a sterile plate, to which 10–20 mL of agar media were then added. The medium was quickly combined and shaken for the following 5–10 seconds. The plates were put in an incubator and incubated at 37 ºC for 24 hours and visualized pink colonies were counted.

Sensory evaluation

A trained panel of 10 individuals evaluated the sensory acceptability of yogurts using a 5-point hedonic scale (1-Unsatisfactory; 5- Excellent) (Karagul-Yuceer and Drake, 2006). The panelists assessed the yogurt samples that were offered to them at room temperature in terms of their color, taste, consistency, smell, and overall acceptance.

Statistical Analysis

The results were analyzed with one-way ANOVA to identify significant differences between means of samples. SPSS-25 statistical software was used for data analyses and the results were presented as mean ± standard deviation of three replicates. The mean results were compared by the Fisher's LSD test with 95% confidence interval.

Results and Discussion

Physicochemical properties of fortified yogurt

Table 2 shows that, pH decreased and the TA of RD peel powder fortified yogurts at different inclusion level increased. The pH and TA of different treatments (T_0, T_1, T_2, and T_3) ranged between 4.36-4.73, and 0.16-0.18 (g lactic acid/100 g), respectively. Similar results were reported for yogurts enriched with *Spirulina platensis* powder, *Pleurotus ostreatus* extract, and yogurts containing orange fiber (Vital et al., 2015; Agustini et al., 2017; Erkaya-Kotan, 2020). The decrease in pH; conversely increase in TA might be attributed to the acidic nature of dragon fruit peel material as well as improved culture growth attributable to the pre-biotic potential of fruit's peel (Nyamete and Mongi, 2017). The production of lactic acid from the fermentation of lactose by lactic acid bacteria might have an additional effect on this. Resulting lactic acid caused substantial decrease in the pH of yogurt. The production of organic acids increases as additional sources of sugar are digested; hence, the pH will naturally decrease (Jannah et al., 2014). Furthermore, pH level of the fortified yogurts is in accordance with Benedetti et al. (2016) who stated that consumers prefer fermented products with pH on the range of 4.2 to 4.4. Accordingly, vitamin C amount of the yogurts ranged from 1.17 to 1.34 (mg/100 g) with RD fruit's peel powder fortified yogurt having considerably higher ($P<0.05$) vitamin C than control (Table 2). The higher vitamin C content in fortified yogurt might be attributed to the fact that peels of dragon fruit is an excellent source of vitamin C (Nur et al., 2023). Ścibisz et al. (2019) also reported higher vitamin C content for yogurt enriched with straw berry and blue berry fruit extracts as compared to plain yogurt.

Proximate composition of fortified yogurts

Table 3 depicts the proximate composition of control and red dragon peel fortified yogurts. The moisture content, crude protein, fat, ash content, and crude fiber of different treatments (T_0, T_1, T_2, and T_3) varied from 76.70-80.18, 4.49-4.07, 3.48-2.36, 5.19-5.29, and 1.53-3.34 (g/100 g), respectively. The study findings stated that addition of RD peel powder has a profound consequence on the moisture content ($p<0.05$). The moisture content of the control was 78.06 g/100 g, while the lowest and the highest moisture contents were 76.70 and 80.18 (g/100 g) reported in T_1 and T_3 treatments, respectively. This result is in accordance with Iwalokun and Shittu (2007) who reported higher moisture percentage in yogurt samples blended with vegetable extracts. In addition, increased moisture content in yogurt sample with 15% persimmon pulp was also observed by Khatoon et al. (2021). However, presence of complex carbohydrates and fibers in yogurt, which hold the water, might have incremented the moisture content in peel fortified yogurts.

Casein and whey are two of the high-quality proteins found in yogurt (Nyamete and Mongi, 2017). The crude protein content of control used in this study was 4.49 (g/100 g). Addition of RD peel powder up to 7% showed a non-significant declining trend in protein content. Related study findings were also reported by Roy et al. (2015) and Desouky et al. (2018); that protein amount was decreased in fruit-flavored yogurt. As fruits typically have lower protein contents compared to milk, increasing the amount of fruits in fortified yogurt considerably reduced protein content (Khatoon et al., 2021). However, the decreasing trend in protein content might be due to the proteolytic activity of microorganisms which degrade the protein as demonstrated by Han et al. (2012). Furthermore, the fat content in the plain yogurt (control) used in this study was 3.48 (g/100 g). T_2 and T_3 treatments substantially decreased the fat content compared to control. In accordance, Palka and Flis-Kaczykowska (2019) stated that yogurt formulated with different fruit and vegetable extracts showed lower fat content. Another study of Kauser et al. (2011) also reported that addition of apricot lowered fat content in yogurt. Yogurt's lactic acid bacteria may have converted fats into volatile fatty acids, causing a reduction in fat content (Khatoon et al., 2021). Another possible reason behind the reduced fat content in fortified yogurt might be presence of low amount of fat in fruit peel.

Table 2. Physicochemical properties of fortified yogurt

Treatments	pH	TA (g lactic acid/100 g)	Vitamin C (mg/100 g)
T_0	4.73 ± 0.06^a	0.16 ± 0.01^b	0.00^c
T_1	4.36 ± 0.04^b	0.18 ± 0.01^a	1.17 ± 0.01^b
T_2	4.38 ± 0.03^b	0.18 ± 0.02^a	1.34 ± 0.01^a
T_3	4.42 ± 0.03^b	0.18 ± 0.01^a	1.34 ± 0.02^a

*****Values are means of triplicates ± standard deviation. Different superscripts in the same column differ significantly ($p<0.05$). T_0 = Plain yoghurt (control); T_1 = Fortified yoghurt with 2% RD peel powder, T_2 = Fortified yoghurt with 5% RD peel powder, T_3 = Fortified yoghurt with 7% RD peel powder.

Table 3. Proximate composition of fortified yogurts

Treatments	Moisture (g/100 g)	Crude protein (g/100 g)	Fat (g/100 g)	Ash (g/100 g)	Crude fiber (g/100 g)
T_0	78.06 ± 0.04^c	4.49 ± 0.02^a	3.48 ± 0.01^b	5.19 ± 0.01^b	1.53 ± 0.02^d
T_1	76.70 ± 0.03^d	4.05 ± 0.05^b	3.53 ± 0.02^a	5.28 ± 0.01^a	2.37 ± 0.01^c
T_2	79.30 ± 0.02^b	4.02 ± 0.04^b	2.56 ± 0.01^c	5.29 ± 0.01^a	2.43 ± 0.01^b
T_3	80.18 ± 0.02^a	4.07 ± 0.07^b	2.36 ± 0.01^d	5.29 ± 0.01^a	3.34 ± 0.03^a

*****Values are means of triplicates ± standard deviation. Different superscripts in the same column differ significantly ($p<0.05$). T_0 = Plain yogurt (control); T_1 = Fortified yogurt with 2% RD peel powder, T_2 = Fortified yogurt with 5% RD peel powder, T_3 = Fortified yogurt with 7% RD peel powder.

Table 4. Antioxidant activity of fortified yogurts

Treatments	Antioxidant activity (µmol TE/mL)
T_0	1.30±0.003[d]
T_1	1.43±0.004[c]
T_2	1.51±0.003[b]
T_3	1.57±0.005[a]

*Values are means of triplicates ± standard deviation. Different superscripts in the same column differ significantly ($p<0.05$). T_0 = Plain yogurt (control); T_1 = Fortified yogurt with 2% RD peel powder, T_2 = Fortified yogurt with 5% RD peel powder, T_3 = Fortified yogurt with 7% RD peel powder.

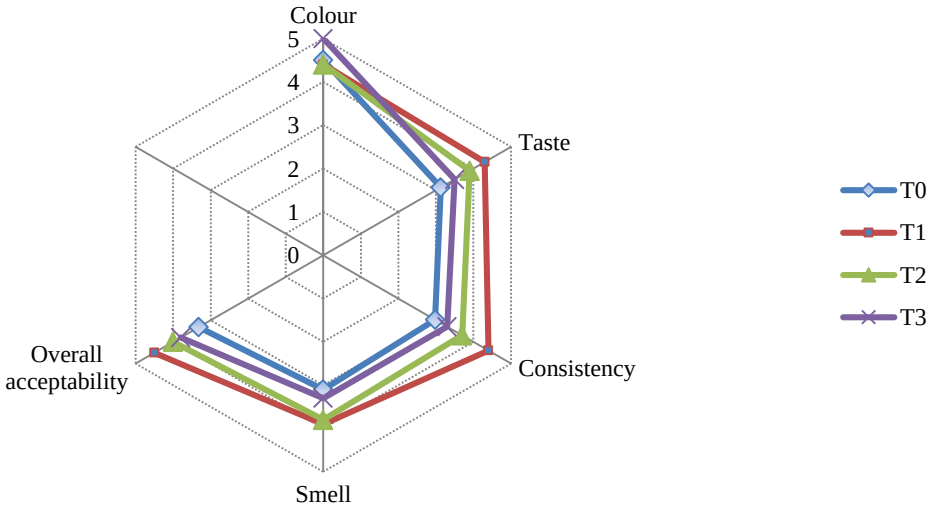

Figure 2. Radar chart of the sensory attributes
*Values are means of ten panelists

The crude fiber content in the plain yogurt (control) used in this study was 1.53 (g/100 g). The findings demonstrated that the amount of fiber increased in fortified yogurt as dragon fruit peel concentration added. This is in accordance with the study of Apriyani (2018); that fiber content of yogurt increases with the addition of dragon fruit skin as dragon fruit peel contain high amount of dietary fiber content (Jamilah et al., 2011). The present study was also found complementary to the report of Mohamed et al. (2014) who reported similar trend in yogurt fortified with dietary fiber and phenolic compounds. The ash content of control used in this study was 5.19 (g/100 g). Addition of RD peel powder up to 7% enhanced the ash contents in fortified yogurts (T_1, T_2, and T_3) compared to control (T_0). In accordance, Ismail et al. (2020) reported that the ash content increased in yogurt developed with the fortification of apricot extract. These reports propose that the ash content is affected by the type of fruits used to fortify the yogurts. The concentration of ash found in red dragon peel extract (16.74 g/100g) is superior to the amount found in orange (1.59 g/100g) and grape (4.24 g/100 g) as reported by Cacatian and Guittap (2018). So, the increasing trend in ash content of fortified yogurt was found owing to the effect of red dragon peel powder associated with higher amount of ash content.

Antioxidant activity of fortified yogurts

The results for antioxidant activity of the fortified yogurts are presented in Table 4 where antioxidant activity of T_1, T_2, and T_3 treatments (1.43-1.57 µmol TE/mL) were significantly higher than that of control (1.30 µmol TE/mL) yogurt ($p<0.05$). This was expected since RD fruit's peel is a rich source of phytochemicals, which might have increased the antioxidant properties in fortified yogurt (Jamilah et al., 2011). The improvement in antioxidant capacity of yogurts enhanced with RD peel is also similar with results previously reported for yogurts enriched with phycocyanin, *Spirulina platensis*, and apple pomace (Mohammadi-Gouraji et al., 2019; Wang et al., 2020). However, the increase might be attributed to the microbial metabolic activity; that may release some of the bounded bioactive materials (Barakat and Hassan, 2017). Rahmawati and Suntornsuk (2016) reported increased antioxidant activity in cow, goat, and buffalo yogurt and attributed this to the release of bioactive (antioxidant) peptides occurring as a result of protein digestion by bacterial fermentation. Milk digestion-produced peptides may function as electron donors, interacting with free radicals to form more stable products (Kullisaar et al., 2002). Hur et al. (2014) also stated that increase in antioxidant activity in fermented plant based foods due to increased release of flavonoids during fermentation. Fermentation induces synthesis of various bioactive compounds as a result of induced structural breakdown of the cell walls (Đorđević et al., 2010). Fermentation is also associated with the modification of bioactive compounds by lactic acid bacteria (Hunaefi et al., 2013; Curiel et al., 2015).

Sensory attributes of fortified yogurts

The scores of sensory evaluation of T_0, T_1, T_2, and T_3, treatments are listed in Figure 2. The degree of likings or acceptance of the sensory attributes such as color, taste, consistency, smell, and overall acceptability varied from 4.40-5.00, 3.13-4.30, 2.98-4.40, 3.10-3.90, and 3.32-4.50, respectively. This result clearly shows RD peel powder fortification considerably affected the sensory scores of formulated yogurts ($p<0.05$). However, T_1 treatment

indicated the maximum sensory scores in taste, consistency, smell, and overall acceptability; while T_3 treatment received the highest hedonic score for color attribute. RD fruit's peel contains betacyanin pigment, which contributes to the reddish coloration in yogurt and is thus favored by most of the panelists (Pamungkas et al., 2020). Similarly, addition of RD peel powder to a certain extent seemed to improve consumer expectations regarding taste and smell, since several volatile and non-volatile bacterial metabolites, as well as carbonyl chemicals, contribute to the flavor of yogurt (Cheng, 2010). In addition, inclusion of certain flavor compounds and other minor constituents such as adjuncts of fruits, vegetables, and nuts also affect the overall flavor (Nyamete and Mongi, 2017). In this study, pasteurization induces the release of more volatile compounds and flavor constituents from RD peel, which might have influenced the improved taste and smell in fortified yogurts. However, addition of higher amounts of RD peel powder (7%) might create complexity between the released compounds and other flavor constituents that could be responsible for lower hedonic score in fortified yogurts. Similar findings were also reported for yogurt fortified with fruits and vegetables extracts (Yu et al., 2014; Barakat and Hassan, 2017). Moreover, the apparent consistency perceived on the yogurt samples fortified with RD peel powder yielded greater sensory score compared to control. Most yogurt usually contain considerable amount of fat and protein, which influence their structure, consistency and textural properties (Routray and Mishra, 2011). In this study, the improved consistency in yogurts fortified with RD peel powder might be due to the presence of fiber in RD peel. Fiber acts as prebiotic and influences the growth of starter culture of yogurt. The consistency of yogurt may increase whether these bacteria grow well and produce the desired texture characteristics (Mousavi et al., 2019). The outcomes of this study also exhibited that the addition of RD peel powder has a substantial impact on the overall acceptability of yogurt. The yogurt sample with 2% fruit peel has the best score for overall acceptability, followed by 5% and 7%, while the control scored the lowest. It is clear from the results that the panelists preferred the RD fortified yogurts to plain yogurt. The higher values of taste, smell and consistency of fruit yogurt might be largely responsible for the incremental trend in the overall acceptability of RD peel fortified yogurt. Similar findings were also observed by Bhat et al. (2018) who investigated the impact of psyllium husk on sweetly stirred yogurt. This suggests that product development efforts should focus on delivering the optimal sensory attributes in order to effectively fortify yogurt with RD peel powder.

Microbial evaluation of fortified yogurts

Table 5 demonstrates reduction in TVC (Total viable count) with RD fruit's peel powder fortification. TVC in yogurt sample usually indicates the total number of live cells (microorganisms) present within different formulated yogurts. The highest TVC was reported in T_0 treatment (65.17×10^4 cfu/mL), while TVC in T_1, T_2, and T_3, treatments ranged from 56.64×10^4 to 64.23×10^4 (cfu/mL). However, the bacteriological status of yogurts was within the acceptable standard ($<10^6$ cfu/mL) for fermented milk products (Lourens-Hattingh and Viljoen, 2001; El-Bakri and El-Zubeir, 2009). In line with our results, Fernandes et al. (2019) demonstrated that in comparison to plain yogurt, yogurt supplemented with apple pomace (3.3%) decreased bacterial counts at the end of fermentation. Furthermore, it is also evident that poly- and oligosaccharides derived from fruit's peel might exhibit a prebiotic effect, improving the vitality and adhesion of various lactic acid bacteria, while simultaneously retards the multiplication of various enteric pathogens (Islamova et al., 2017; Wilkowska et al., 2019). When it came to TC, coliform bacteria were not present in any of the yogurt samples indicating that, hygienic procedures were followed in the collection of raw materials and yogurt formulations.

Table 5. Microbial properties of fortified yogurts

Treatments	TVC (cfu/mL)	Coliform
T_0	$65.17\pm1.15\times10^{4a}$	0
T_1	$56.64\pm0.56\times10^{4d}$	0
T_2	$61.45\pm0.48\times10^{4c}$	0
T_3	$64.23\pm0.27\times10^{4b}$	0

*Values are means of triplicates ± standard deviation. Different superscripts in the same column differ significantly ($p<0.05$). T_0 = Plain yogurt (control); T_1 = Fortified yogurt with 2% RD peel powder, T_2 = Fortified yogurt with 5% RD peel powder, T_3 = Fortified yogurt with 7% RD peel powder.

Conclusion

Red dragon peel has been successfully applied for the formulation of nutrient enriched yogurt. The fortified yogurts were found microbiologically safe with improved quality attributes. The present study showed that yogurt with 7% red dragon fruit peel powder presented most prominent antioxidant activity, while yogurt fortified with 2% RD peel powder revealed the maximum sensory scores with best taste profile among all yogurt samples tested. Therefore, further research should emphasis on the characterization of bioactive compounds to evaluate the mechanisms of antioxidant and anti-diabetic activity of yogurts with added red dragon peel powder.

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The Effects of Seed Infestation by *Fusarium proliferatum* on Root and Crown Rot, Plant Growth and Phenolic Compounds in Roots of Some Pumpkin (*Cucurbita pepo* L.) Cultivars

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ABSTRACT

This study investigates the reactions of four summer pumpkin cultivars (cvs. Çağlayan, Mert Bey, Sena Hanım, TG38) to root and crown rot caused by *Fusarium proliferatum* by taking into account criteria such as disease severity, plant growth (number of leaves, height, dry and fresh weight of shoot) and the accumulation of phenolic compounds in the roots. Seeds of each cultivar were inoculated with the pathogen and left to develop for 1 month at 25°C in a controlled climate room. The content of phenolic compounds in ethanolic root extracts was determined using high-performance liquid chromatography (HPLC). Cv. Sena Hanım had the lowest disease severity (4.40%) among the cultivars, followed by cvs. Çağlayan (10.62%) and Mert Bey (11.07%). Plants developed from inoculated seeds of cvs. Çağlayan and Sena Hanım had no decrease in the number of leaves and in length, fresh and dry weight of shoots in comparison to the control (plants from non-inoculated seeds), while cv. Mert Bey demonstrated a decrease at very low rates in shoot fresh and dry weight (2.24% and 0.77%, respectively). The phenolic compound that exhibited the highest increase in root extracts of cv. Sena Hanım compared to the control among the cultivars was *p*-coumaric acid (6.57-fold). This study demonstrates that *p*-coumaric acid can play an important role in the resistance of pumpkin to seed infestation by *F. proliferatum*.

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Introduction

Pumpkin (*Cucurbita pepo* L.) in Cucurbitaceae family, is one of the most significant vegetables for human nutrition. Previously, seed-borne fungi such as *Alternaria burnsii* and *Didymella bryoniae* were shown to cause seedling rot, although the disease severity rate was not recorded (Lee et al., 1984; Paul et al., 2015). In a recent study, it was reported that seed-borne *Fusarium proliferatum* caused root and crown rot in this plant at a rate of 51.07% (Demir et al., 2023). The improvement of eco-friendly control strategies is critical for long-term sustainability of pumpkin production since there is no information on the control of this disease. The majority of research on resistance to fungal diseases in *Cucurbita pepo* has focused on soil-borne fungi like *Fusarium solani* f. sp. *cucurbitae* (Nagao et al., 1994; Ayala-Doñas, et al., 2022) and *Phytophthora capsici* (Padley et al., 2008; Krasnow et al., 2017; Michael et al., 2019; Ayala-Doñas et al., 2022) as well as airborne fungi like *Sphaerotheca fuliginea* (Cohen et al., 1993, Cohen et al., 2003). However, there is no published information on the reactions of pumpkin cultivars to seed-borne fungi. In this context, it is also necessary to investigate some resistance mechanisms in pumpkin against *F. proliferatum*. Phenolic compounds are among the most important and prevalent secondary products in plants, and they have been shown to contribute to plant disease resistance (Nicholson and Hammerschmidt, 1992; Hammerschmidt and Smith-Becker, 1999; Cheynier et al., 2013).The presence of phenolic compounds such as caffeic acid, chlorogenic acid, ferulic acid, gallic acid, *p*-coumaric acid, protocatechuic acid (3,4-dihydroxybenzoic acid), sinapic acid, syringic acid and vanillic acid in pumpkin fruits has been determined, and their importance for human health was underlined (Dragovi-Uzelac et al., 2005; Kulczyński and Gramza-Michałowska, 2019), although there has been no study about the existence of any phenolics in roots of pumpkin. It has been suggested that the total amount of phenolic compounds in pumpkin leaves or fruits infected

with powdery mildew and yellow vein mosaic virus increased as a defense mechanism against these diseases (Jaiswal et al., 2013; Zhang et al., 2021). It has been proposed that foliar sprays of phenolics like chlorogenic acid may enhance resistance at an earlier stage of *Cucumber mosaic virus* infection in squash, which is a *C. pepo* variety, potentially controlling this viral disease (Abdelkhalek et al., 2022). The objectives of this study were to (a) determine disease severity in plants after inoculating seeds of four different pumpkin cultivars with *F. proliferatum*, (b) investigate the effect of pathogen on some plant growth parameters (leaf number, shoot length, shoot fresh and dry weight), and (c) investigate the role of phenolic compounds in roots in protecting different pumpkin cultivars against root and crown rot caused by this pathogen.

Materials and Methods

Pathogen and Cultivars

AYFA Tarım Limited Company (Bursa/Turkey) and the Trakya Agricultural Research Institute (Edirne/Turkey) provided seeds of a permitted (cv. TG38) and three registered (cvs. Çağlayan, Mert Bey, Sena Hanım) summer pumpkin (*Cucurbita pepo* L.) cultivars, respectively. A *Fusarium proliferatum* (FusP9) isolate was obtained from naturally infected pumpkin seeds. In a previous study, this isolate caused the highest disease severity (51.07%) in summer pumpkin seedlings (Demir et al., 2023). The isolate was cultured for 10 days on potato dextrose agar (PDA) at 25±2°C in the dark.

Pathogen Inoculation, Assessment of Disease Severity and Plant Growth Parameters

First, seeds of each cultivar were sterilized by immersing in a 2% NaClO solution for 7 minutes, subsequently washed three times with sterile distilled water, and air dried for 30 minutes on sterile paper towels. These seeds were pre-germinated in 9 cm petri dishes with filter paper moistened with sterile distilled water for three days at 25°C. Pre-germinated seeds (Erginbas-Orakci vd., 2016; Terna et al., 2022) were then inoculated with FusP9 isolate by immersing them in a suspension of conidia (1×10^6 conidia/ml) containing 0.1% (v/v) Tween 20 for 1 h at 25°C (Aslam et al., 2021; Demir et al., 2023). Sterile distilled water-treated seeds were used as control. Inoculated and non-inoculated seeds were sown separately in pots (12.5 × 10 cm, width x height, with 400 g mixture volume) filled with a mixture of peat (Klasman-Deihmann, Germany) and sand (3:1), which was sterilized in the autoclave. The plants were grown under a photoperiod of 12 h light (25°C) and 12 h darkness in a controlled room for 30 days. The treatments were set up in a randomized plot design with five replications (each pot had eight seeds).

Thirty days after inoculation, plants of the cultivars were gently uprooted from their respective pots and data were recorded in each replication regarding disease severity and some parameters for plant growth such as leaf number, length, fresh weight and dry weight (after drying 72 hours at 50°C) of shoots. Because varied symptoms were detected in developing plants, the severity of the disease was assessed using a 0-4 scale (Figure 1) based on a modified version of the scales reported by Jamiołkowska et al. (2012), Seo and Kim (2017) and Reyad et al. (2021): [0: Healthy, 1: Browning in roots, no browning of stem, 2: Browning in both roots and stems, 3: Post emergence damping-off, 4: Pre-emergence damping off]. The following formula (Townsend and Heuberger, 1943) was used to determine disease severity.

$$\mathrm{DS} = \frac{\sum(\mathrm{DL} \times \mathrm{NPDL})}{\mathrm{TNP} \times \mathrm{HSL}}$$

DS : Disease severity (%)
DL : Disease level
NPDL: Number of plants showing disease at that level
TNP : Total number of plants
HSL : The highest severity level

The pathogen was re-isolated from diseased plant areas and diagnosed using morphological characterization. The decreases (%) in root length, shoot length, shoot fresh and dry weight of each inoculated cultivar (C_p) with FusP were calculated relative to the average values for the non-inoculated (C) respective cultivar by following equation (Alisaac et al., 2018; Cong et al., 2018).

$$\text{The decrease in growth parameter (\%)} = \frac{\mathrm{C} - \mathrm{C_p}}{\mathrm{C}} \times 100$$

Figure 1. The evaluation of disease severity using 0-4 scale for the pumpkin cultivars inoculated with *Fusarium proliferatum*.
0: Healthy 1: Browning in roots, no browning of stem, 2: Browning in both roots and stems, 3: Post-emergence damping-off, 4: Pre-emergence damping-off

Extraction of Phenolic Compounds from Roots and HPLC Analysis

To assess the phenolic component content in roots, 1 g of sample was taken from each replicate and extracted with 32 mL of ethanol (99%) using an ultrasonic water bath for 30 minutes (Kulczyński and Gramza-Michałowska, 2019) and then for 24 hours in the dark. The extracts were sterilized using a 0.22 µm pore size membrane filter (Millipore, Millipore Co., Billerica, Ma, USA). The extracts directly were analyzed through the conditions used by Kulczyński and Gramza-Michałowska (2019) with High-Performance Liquid Chromatography (HPLC) for content of phenolic compounds such as cafeic acid, chlorogenic acid, 3,4-dihydroxybenzoic acid, gallic acid, *p*-coumaric acid, sinapic acid, syringic acid and vanillic

acid. The amounts of phenolic compounds in the roots of inoculated cultivars were compared to their respective controls. The increase (as fold) in each phenolic compound of each cultivar was calculated by dividing the amount of phenolic compound in each replicate of inoculated cultivar by the average amount of its non-inoculated control (Abdelrahman et al., 2016; Özer et al., 2021; Piasecka et al., 2022).

Statistical Analysis

The homogeneity of variances and normality of distribution were initially evaluated with SPSS version 23 (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA) using Levene's and Shapiro-Wilk tests, respectively. The severity of disease in plants, the amounts of chlorogenic acid, vanillic acid, syringic acid and *p*-coumaric acids in the roots of inoculated and non-inoculated plants, and the increase in *p*-coumaric acid completely satisfied the assumptions of two-tailed student T test and the ANOVA test. The two-tailed student T test and two-tailed Wilcoxon Ranks test (at $P < 0.05$ and $P < 0.01$) were used to make comparisons for the amounts of phenolics in roots of inoculated and non-inoculated plants for each cultivar exhibiting parametric or non-parametric data, respectively. Significant differences in disease severity and *p*-coumaric acid increase of the cultivars were assessed using the Tukey HSD test at $P < 0.05$ with Software JMP Version Pro 17 for Windows (Cary, North Carolina, USA). Significant differences for the other non-parametric data such as decreases in plant growth parameters and increases in other phenolics, were analyzed with the Friedman Test ($P < 0.05$)), and the Wilcoxon rank-sum test was utilized for post-hoc analysis with IBM-SPSS. The means based on the decrease (%) in each growth parameter and the increase (fold) in each phenolic compound were compared with disease severity using Pearson's correlation coefficient at $P = 0.05$.

Results and Discussion

Disease Severity of Cultivars and Reductions in Plant Growth by Fusarium Proliferatum

The disease severity varied greatly between cultivars (Figure 2). The cv. Sena Hanım exhibited lowest disease severity (4.40%), and the differences between the disease severity of this cultivar and other cultivars were statistically significant. The highest disease severity with 14.17% was determined in cv. TG38, while the disease severities of cvs. Çağlayan (10.62%) and Mert Bey (11.07%) were in the same statistical group as cv. TG38. Considering the decreases in plant growth parameters measured in this study, the decreases in the number of leaves, shoot length, and the fresh and dry weights of shoots were considerably higher in inoculated TG38 than in other cultivars (Table 1). Although the decreased rates in fresh and dry weights of shoots in cv. Mert Bey were low, they were statistically significant as compared to cvs. Çağlayan and Sena Hanım. In previous studies, pumpkin cultivars or genotypes were only screened for resistance to *F. solani* f. sp. *cucurbitae* (Nagao et al., 1994; Ayala-Doñas et al., 2022), *P. capsici* (Padley et al., 2008; Krasnow et al., 2017; Michael et al., 2019; Ayala-Doñas et al., 2022) and *Sphaerotheca fuliginea* (Cohen et al., 1993, Cohen et al., 2003). It was recently found that the FusP9 isolate isolates used in the present study caused root and crown rot of 51.07% at 10 days after inoculation on seeds of the pumpkin cultivar TG22 (*C. pepo*) in petri dishes by blotter method. (Demir et al., 2023). In the current study, the pumpkin cultivars were tested for their susceptibility to *F. proliferatum* (FusP9 isolate) by seed infestation under pot conditions and the isolate exhibited disease severity percentages of 10.62%, 11.07%, 4.40%, and 14.17% on cvs. Çağlayan, Mert Bey, Sena Hanım and TG38, respectively.

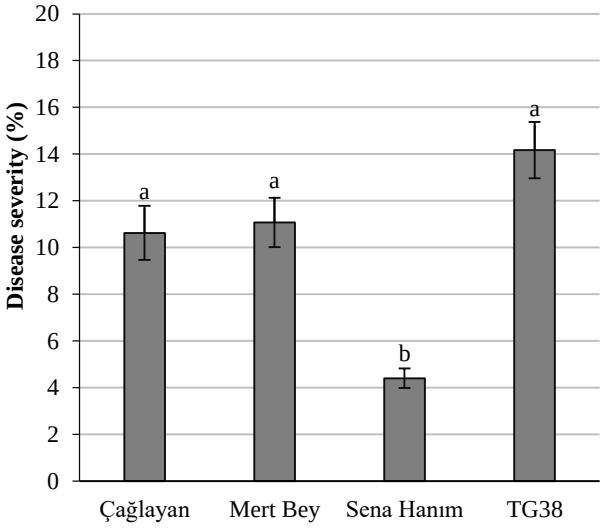

Figure 2. Disease severity caused by *Fusarium proliferatum* (FusP9 isolate) in different pumpkin cultivars. Each value represents the mean and standard error of five replications.
Bars topped by a different letter differ significantly according to the Tukey HSD test at $P<0.05$. $F_{3,16}= 16.4568$, $P<0.0001$

Phenolic Compounds in Roots of the Cultivars

Syringic acid and *p*-coumaric acid were detected in the roots of all cultivars (Figure 3). However, 3, 4-dihydrobenzoic acid was only present in the roots of cv. Çağlayan, exhibiting significantly higher amounts in the roots of inoculated plants (1.72 ppm) than those of non-inoculated plants (1.37 ppm, $P < 0.05$) (data not presented). The presence of chlorogenic acid and vanillic acid varied among cultivars. While the amount of chlorogenic acid (3.51 ppm), syringic acid (25.99 ppm) and *p*-coumaric acid (4.40 ppm) in inoculated cv. Sena Hanım was considerably greater than the control (1.34 ppm, 9.94 ppm and 0.67 ppm, respectively). The amounts of the same phenolic compounds, except syringic acid, in inoculated cv. TG38 were significantly lower (1.43 ppm and 0.82 ppm for chlorogenic acid and *p*-coumaric acid respectively) than its control (2.05 ppm and 1.20 ppm for the same phenolics, respectively). Interestingly, syringic acid also accumulated at higher rate (16.86 ppm) in the roots of inoculated cv. TG38 than in the control (14.55 ppm). Caffeic acid, gallic acid, and sinapic acid, which were detected in pumpkin fruit (Dragovi-Uzelac et al., 2005; Kulczyński and Gramza-Michałowska, 2019), were not found in the roots of the pumpkin cultivars examined in this study.

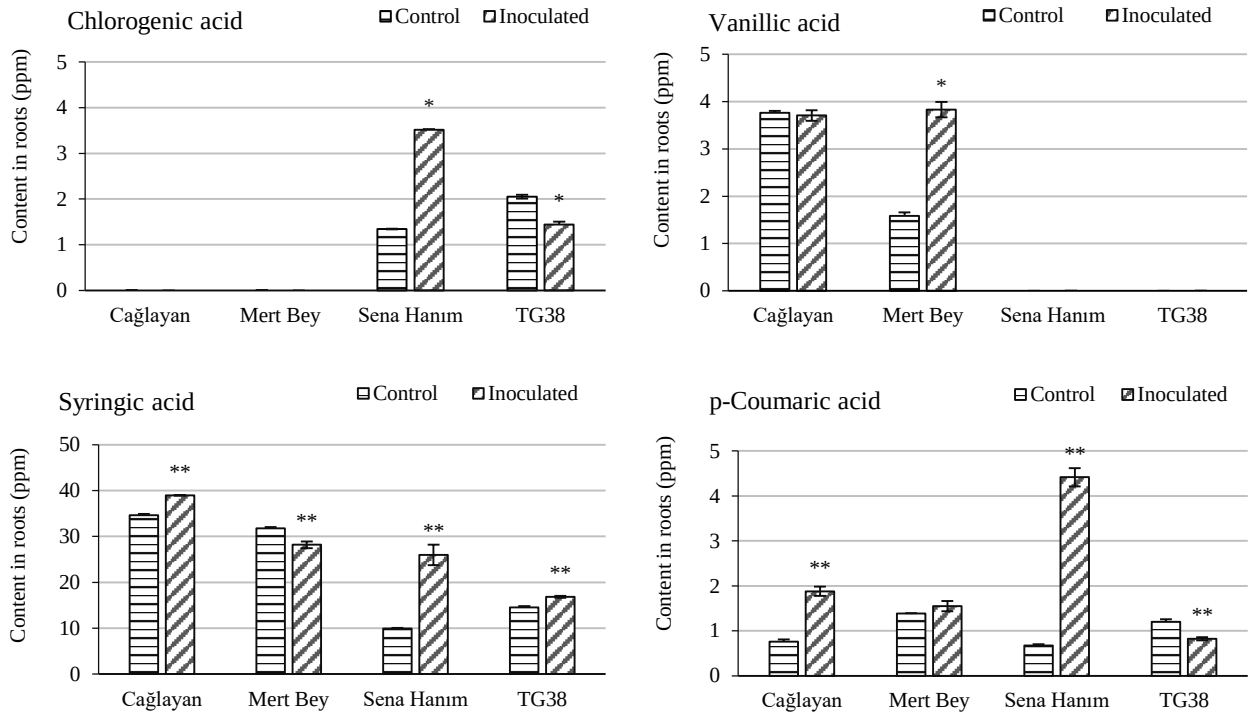

Figure 3. The phenolic compounds and their concentrations (ppm) in root extracts from seeds of different pumpkin cultivars inoculated with *Fusarium proliferatum* (FusP9 isolate) compared to mock-inoculated (control).
Each value indicates the mean and standard error of five replications. Significant differences between control and inoculated plants for each cultivar are indicated with the symbols "*" ($P < 0.05$) "**" ($P < 0.01$).

Table 1. Decreases (%) in some growth parameters of the cultivars from seeds inoculated with *Fusarium proliferatum* (FusP9 isolate) versus plants from non-inoculated seeds

Cultivars	Decrease (%) in[2]			
	Leaf number	Shoot length	Shoot fresh weight	Shoot dry weight
Çağlayan (Ça)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Mert Bey (MB)	0.00±0.00	0.00±0.00	2.24±0.18	0.77±0.06
Sena Hanım (SH)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
TG38	13.13±2.35	5.18±0.42	13.95±3.06	20.45±2.53
P[1]	0.002	0.002	0.002	0.002
Pairwise	Significance of cultivar pairwise[3]			
Ça-MB	ns	ns	*	*
Ça-SH	ns	ns	ns	ns
Ça-TG38	*	*	*	*
MB-SH	ns	ns	*	*
MB-TG38	*	*	*	*
SH-TG38	*	*	*	*

[1]Friedman test; [2]Each value indicates the mean±standard error (SE) of five replications; [3]Based on Wilcoxon rank-sum test, significant differences between cultivar pairwise comparisons for each growth parameter are indicated with the symbol "*" ($P < 0.05$); ns: not-significant

Based on the increases in syringic and *p*-coumaric acid, which were found in the roots of all treated cultivars, versus roots from the control, the highest increase was *p*-coumaric acid (6.57-fold) in the roots of cv. Sena Hanım, followed by cv. Çağlayan (Table 2). An increase in *p*-coumaric acid was not detected in cv. TG38. The increase in syringic acid in the roots of cv. Sena Hanım was considerably greater than in the other cultivars, but it was also significantly higher in the roots of cv. TG38 having the disease severity than in those of cv. Mert Bey. The increases in chlorogenic and vanillic acid were significantly higher in cvs Sena Hanım ($P < 0.05$) and Mert Bey ($P < 0.05$), respectively, than in the other cultivars (TG38 and Çağlayan, respectively), which contain these phenolics. Phenolic compounds serve an important role in plant defense against diseases. They are also induced in response to injury and may play a role as precursors for lignin and suberin production (Matern and Kneusel, 1988; Nicholson and Hammerschmidt, 1992; Dixon and Paiva, 1995; Hammerschmidt and Smith-Becker, 1999). A previous report (Huang and Backhouse, 2005) indicated that total phenolics in roots of sorghum seedlings inoculated with *F. proliferatum* were increased as a defense response. Increase in *p*-coumaric acid only showed significant negative correlation with disease severity ($r = -0.985$, $P < 0.05$). As a result, the increased amount of *p*-coumaric acid in the diseased roots of cv. Sena Hanım, which has the least disease severity, appears to contribute to resistance against *F. proliferatum.* To the best our knowledge, this is the first resistance study of root and crown rot occurred after seed infestation by *F. proliferatum* in summer pumpkin (*C. pepo*) cultivars.

Table 2. Increase in accumulation of phenolic compounds in roots from seeds inoculated with *Fusarium proliferatum* (FusP9 isolate) versus roots from non-inoculated seeds

Cultivars	Increase (fold) in[2]				
	3,4-Dihydrobenzoic acid	Chlorogenic acid	Vanillic acid	Syringic acid	*p*-Coumaric acid
Çağlayan (Ça)	1.25±0.05	-[3]	0.00±0.00	1.12±0.01	2.49±0.14 b[5]
Mert Bey (MB)	-	-	2.42±0.10	0.00±0.00	1.12±0.08 c
Sena Hanım (SH)	-	2.62±0.01	-	2.61±0.22	6.57±0.30 a
TG38	-	0.00±0.00	-	1.16±0.01	0.00±0.00 d
P[1]	-	0.025	0.025	0.003	
Pairwise	-	Significance of cultivar pairwise[4]			
Ça-MB	-	-	*	*	
Ça-SH	-	-	-	*	
Ça-TG38	-	-	-	ns	
MB-SH	-	-	-	*	
MB-TG38	-	-	-	*	
SH-TG38	-	*	-	*	

[1]Friedman test; [2]Each value indicates the mean±standard error (SE) of five replications; [3]The compound is not present; [4]Based on Wilcoxon rank-sum test, significant differences between cultivar pairwise comparisons for each phenolic are indicated with the symbol "*" ($P < 0.05$). [5]The means with a different letter in the column differ significantly according to the Tukey HSD test at $P<0.05$ $F_{3,16}= 280.9147$, $P<0.0001$; ns: not-significant.

Conclusion

The results of our study suggest the importance of the measurement of phenolic compounds and plant growth parameters in resistance tests of pumpkin cultivars, in addition to disease severity. All cultivars of the current study demonstrated relatively low disease severity after seed infestation by *F. proliferatum*. However, in the cultivar with the lowest disease severity, we did not find any decrease in plant growth, number of leaves, shoot length, and fresh and dry weights of shoots. Moreover, *p*-coumaric acid among phenolic compounds increased at the highest amount in this cultivar. These finding may be help establish strategies to develop resistant pumpkin cultivars to *F. proliferatum*. In the future, it will be required to investigate the antifungal action of *p*-coumaric acid on this pathogen.

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Authors'Contributions

This article is a part of Master Science Thesis by Ebru Sevinç. Nuray Özer is a supervisor.

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Orange Peel and Cauliflower Residues Supplementation Induce Morphological and Physiological Tolerance in Common Bean under Drought Stress

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ABSTRACT

Drought is one of the most harmful abiotic stresses affecting the development and yield of the common bean (*Phaseolus vulgaris* L.). The current climate change and the resulting increased drought will worsen the negative impact of water stress on the plant. The powder of orange peel and cauliflower waste were added as soil supplementation at rates of 7 and 15 g/pot to pots of *Phaseolus vulgaris* L. under different drought conditions. The growth and physiological analysis were estimated after flowering period of common bean. In the pots where drought will be applied, irrigation was stopped for 2, 4, 6, 8 and 10 days during the flowering period and irrigation was performed again after water stress application. To measure moisture percentage of pots, first dry pots were weighed and after irrigation, their moisture variation in terms of percent was measured during stress from 2 to 10 days. The highest plant height was obtained from control. Leaf area decreased significantly despite the application of different powder, especially after 4 days of drought conditions. The highest root fresh and dry weight, raw ash were observed under control with the application of 7.5 g orange peel powder. Shoot dry weight decreased as the number of days exposed to drought increased, and the application of 15 g orange peel and cauliflower powder gave the highest results compared to control conditions. The highest dry matter was obtained from the application of 7.5 g and 15 cauliflower powder in the absence of drought. It has been revealed that as the duration of exposure to drought increases, the value decreases and plant powders are effective in increasing this value. Chlorophyll a, chlorophyll b and total chlorophyll values decreased significantly with drought, and the highest value was obtained from control conditions, followed by 15 cauliflower powder applications.

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Introduction

One of the most important factors limiting plant growth and yield is water. It causes reductions of up to 60% in seed yield worldwide. With the changing climatic conditions, it is estimated that heat and drought stress will cause problems in production, especially in regions where high temperature and water are limited. Therefore, considering the increased input costs, drought resistance becomes even more important in the selection of common bean production in regions prone to drought stress (Hussaini et al. 2021; Singh et al., 2022).

Drought is one of the main abiotic stress factors that prevent the growth, development and yield of plants all over the world and it affects germination, vegetative and generative growth differently depending on the frequency and duration of the stress (Anjum et al., 2017). At the germination and seedling stage, it has a negative effect on plant growth by affecting morphological (seedling height and seedling biomass), physiological (water content), biochemical (amylase, protease and lipase activities) and molecular (stress proteins, aquaporins and dehydrins) properties (Yiğit et al., 2016; Hura et al., 2022). Drought in the vegetative period affects important plant processes such as photosynthesis, mineral nutrition, metabolism, translocation, phytohormone, transpiration (Fathi and Tari, 2016; Okunlola et al., 2017; Jyoti et al., 2017). In the generative period, it significantly reduces grain development, grain number, grain weight, yield and yield components and quality (Liu et al., 2018; Kuwayama et al., 2019). In the case of water deficiency in plant cells, morphological changes including limited growth and development, decreased photosynthesis efficiency and yield, and disorders in primary and secondary plant metabolism are observed (Grzesiak et al., 2002; Hura et al., 2016; Gupta et al., 2020; Wójcik. -Jagła et al., 2020). Plants prevent dehydration by performing an effective water uptake with a well-developed root system. They exposed

to water stress synthesize and accumulate preservatives such as dehydrins or carbohydrates that stabilize the phospholipids of their cell membranes (Larcher, 2003). Drought, associated with abscisic acid (ABA) content, decreases transpiration rate, stomatal conductivity by stomatal closure and causes a sudden decrease in photosynthesis and CO_2 assimilation, significantly reducing growth and yield (Aghaee and Rahmani, 2020).

Fruit peel waste accumulates daily in significant amounts at domestic and industrial levels. They are important mineral nutrients such as calcium, iron, potassium, zinc, and are used as natural fertilizers, and also regulate the pH of the soil (Jariwala and Syed, 2016). Banana peel is a source of essential amino acids, fiber, polyunsaturated fatty acids and potassium (Qader, 2019). Citrus peels, like orange peel contain important components such as sugar and acids that affect soil acidity, photosynthesis and plant growth (Shed, 2005). Jariwala and Syed (2016) emphasized that fruit peels (sweet lime peel, orange peel, banana peel, pomegranate peel, citrate peel powder, alkaline peel powder) are effective in regulating the pH of the soil while citrate peel powder is used to reduce the phosphorus content of the soil. In addition, these fruit peels contain high amounts of nutrients such as N, P, K as a natural fertilizer (Jariwala and Syed, 2016). Apart from reducing pollution, banana peels contain nutrients such as potassium, calcium, magnesium, sulfur, phosphate and sodium for plants, increase soil fertility and the number of branches, and help plants to be resistant to diseases (Panwar, 2015; Qader, 2019). Cytokinins in fruit peels cause an increase in root length (Singh and Prasad, 2014). It was observed that the application of banana peels to the okra and the fruit peel to the *Solanum scabrum* positively affected the root length (Sakpere et al., 2018). It has been reported that fruit peel powder applications cause an increase in fenugreek plant height (Mercy et al., 2014), shoot height in *Solanum scabrum* (Kadir et al., 2016) and banana peel plant height in basil (Tan and So, 2018). Altaee (2019) emphasized that 10 g of banana peel powder applied to *Narcissus daffodil* L. significantly increased plant height, number of leaves, leaf length, chlorophyll content, number of flowers and length. In another study, it was stated that banana peel extract provided a maximum increase in chlorophyll a, chlorophyll b, total carotenoids and accordingly total pigments and photosynthetic pigments (Bakry et al., 2016). It was emphasized that foliar application also significantly increased the yield of quinoa. The application of fruit peel powder to the soil increased the leaf area in rye and the application of banana peel increased the leaf area in pea (Mercy et al., 2014; Wazir et al., 2018). Large amounts of peel are produced from oranges in a year. This waste product is a good source of molasses, pectin and limonene. It has been observed that orange peel powder significantly changes wheat dough properties and bread quality in terms of fiber, pectin and polyphenol content (Han et al., 2021). In another study, it was determined that banana and orange peels significantly increased plant height, number of branches, water content, number of pods, chlorophyll a, total chlorophyll content and carotenoids in chickpea (Qader, 2019). Banana peel extract applied at a dose of 500 mg/l increased the yield of quinoa due to its antioxidant properties (Sathya et al., 2014; Bakry et al., 2016). Fruit peel applications increased shoot and root growth rate, leaf pigments, relative water content and membrane stability index in *Schefflera arboricola* L. plant (El-Serafy et al., 2023).

Climate change and agriculture are interrelated processes and drought stress causes serious yield losses (Shafqat et al., 2021). Understanding the physiological and molecular basis of plants' adaptation to drought will provide new avenues for breeders to develop cultivars that are drought tolerant at key stages of growth and therefore better adapted to global climate changes. Another important issue is that fruit peel wastes accumulate in the environment in large amounts every day. This creates a serious problem and environmental pollution. Fruit peels are very rich in macro and micronutrients that are beneficial for plant growth. Therefore, using it as a fertilizer will reduce the amount of waste and the use of chemical fertilizers. Citrus peels are also rich in pectin and polysaccharides. The supportive effect of vegetable and fruit waste on plant growth is due to the presence of natural antioxidants such as vitamins, flavonoids, phenolic compounds necessary for plant growth.

Almost all of our soils are poor in organic matter. Due to the lack of organic matter, soil compaction occurs. It becomes tighter with the use of chemicals. Water permeability decreases. There is a drought problem in our lands, and due to compaction in the event of rainfall, water cannot penetrate deep into the soil. Therefore in this study, acidic orange peel, which is a natural source of antioxidants and an important waste, and cauliflower stems and leaves, which leave a large amount of residue in public markets, were used. It is thought that these wastes will improve the morphological and physiological characteristics of the bean plant by increasing soil moisture and plant water content under drought stress.

Materials and Methods

The research was carried out as a pot experiment in the application area of Aydin Adnan Menderes University, Kocarli Vocational School. The maximum average temperature value of the area where the research was carried out in 2021 is 25.7°C, and the average minimum temperature value is 10.9°C. The total annual precipitation is 482.8 mm. For many years (1970-2021), the average temperature value is 17.7°C, and the precipitation value is 644.7 mm in total. Seeds of Oturak Ayşe bean cultivar were used in this research. The experiment was arranged in a completely randomized design with three replicates

Material supply and processing peels and residues

Orange peels were obtained from the fruit juice factory in Sultanhisar, besides cauliflower leaves and stems were obtained from the public markets. Fruit peels and vegetable stems and leaves collected in February were cleaned and foreign materials were removed. Afterwards, fruit peels and vegetable stems and leaves were cut into small pieces of 1-5 cm and left to dry for 20-25 days. Then, dried fruit and vegetable wastes were pulverized with the help of a grinder. The ground samples were sieved separately using a 2 mm sieve and kept in tightly closed containers at room temperature (El-Bassiouny et al., 2016; Jariwala and Syed, 2016).

Material supplementation and sowing

The pot experiment was created by using 3 different doses (0, 7.5, 15 g/pot) of dried and ground powders obtained from 2 materials (orange peel and cauliflower leaves and stems)] wastes. Fruit peel and vegetable leaf-stem reside powders were filled into 108 pots (24 cm length and 21 cm deep), mixed with 7 kg of sandy-loamy soil sieved with 2 mm sieve. Five seeds were sowed in each pot and then reduced to three plants. Urea and super phosphate fertilizer was added to the pots at a rate of 10 $kg.da^{-1}$ before sowing (Muhummed, 2004). In the pots where drought will be applied, irrigation was stopped for 2, 4, 6, 8 and 10 days during the flowering period and irrigation was performed again after water stress application (Rahimi et al., 2010). To measure soil moisture percentage of pots, first dry pots were weighed and after irrigation, their moisture variation in terms of percent was measured during stress from 2 to 10 days. Plant height, leaf area, shoot and root fresh weight, shoot and root dry weight, dry matter, raw ash, chlorophyll a, chlorophyll b and total chlorophyll content were measured during the development period of the plant.

Measurement indicators

Dry matter

Ten gram (10 g) samples taken from the shoots after flowering were weighed with an accuracy of 0.01 g in a tared evaporation dish. It was left to dry for 3 hours in an oven set at 105 ºC beforehand, then cooled in a desiccator and weighed. It was heated for half an hour until the difference was 0.05, cooled in a desiccator and weighed (He et al., 2005).

Raw ash content

The sample determined after flowering was homogenized by grinding. 5-10 g of homogeneous sample was weighed into a pre-burned and tared porcelain crucible. It was burned in an oven at 550 ºC to constant weight and then cooled in a desiccator and the container was weighed.

Determination of chlorophyll content

After flowering the samples were washed in tap water dried, and then weighed on a sensitive balance. Then 0.5 g of $CaCO_3$ and 80% acetone were added, crushed in a mortar, the extraction obtained was washed with 80% acetone and filtered with the help of ordinary filter paper into 100 cc volumetric flask (Figure 1). The samples taken from here were centrifuged at 3000 rpm for 10 minutes and then the volume of the obtained extract was measured. The crude chlorophyll extract was then calculated as mg.total chlorophyll/L extract by substituting the absorption values at 645 and 663 nm wavelengths in the spectrophotometer in the formula below (Arnon, 1949).

Total chlorophyll (TC)=20.2 × A645 + 8.02 × A663

With this formula, the total amount of chlorophyll in one liter of the extract was found. The amount of chlorophyll per gram of product was obtained by dividing this value by the weight of the product used in the extraction. In the same way, chlorophyll a and chlorophyll b values were determined by using the following formulas (Arnon, 1949; A.O.A.C, 1975, Cevahir, 1991, Deveci and Şalk, 2000).

Chlorophyll a (Ca)=(0.0127×A663)–(0.0026×45.6×A645)
Chlorophyll b (Cb)=(0.0229×A663)–(0.00468×A645)

Statistical analysis

All the data were statistically analyzed with analysis of variance (ANOVA) procedures using the SPSS software (SPSS Inc 10). The experimental data about each study parameter were subjected to statistical analysis using the variance analysis technique, and their significance was tested by the "F" test (Steel and Torrie, 1980). When differences were found in ANOVA, means were compared using Fisher's protected least significant difference (LSD) test at $P \leq 0.05$.

Figure 1. Effect of different dried plant powders on chlorophyll content under drought stress conditions.

Results and Discussion

Table 1 includes data on common bean plant height. As the drought period increased, the plant heights of beans decreased. Orange peel application was more effective on plant height than cauliflower waste. Orange peel powder positively affected plant height in plants with 2 and 4 days of drought. Application of cauliflower waste (7.5 g) to plants until eight days of drought gave similar results to control conditions in terms of plant heights. Increasing the dose of cauliflower waste (15 g) was not effective in alleviating the effects of drought stress, and plant heights decreased. As the drought increased, the leaf area gradually decreased in common beans. After five days of drought stress, it was observed that the leaf water potential of *Plantago ovata* and *Plantago psyllium* decreased significantly, and the relative moisture content was more sensitive to drought stress, therefore it decreased in mild drought stress (Rahimi et al., 2010).

The highest leaf area values were obtained from the control conditions and from the application of 7.5 g of orange peel powder and plants without waste, respectively. The lowest data occurred in the application of cauliflower waste (7.5 and 15 g) under 10-day drought conditions. Low dose orange peel powder application was effective on leaf area under drought stress conditions (Table 2). Drought caused morphological changes in the common bean by reducing plant height and leaf area. Similar results has also been observed in studies conducted by Bañon et al. (2004) and Bhusal et al. (2020). Water increases the turgor pressure in the plant and plays an important role in the elongation of the plant. Drought has a negative effect on plant height (Al-Hayani et al., 2022).

Table 1 Effect of different dried plant powders on plant height (cm) under drought stress conditions.

Drought duration	Orange peel (g)			Cauliflower waste (g)		
	0	7.5	15	0	7.5	15
0	44.33a	44.67a	41.67a	41.00a	38.00a	41.00a
2	43.00ab	41.00b	41.00a	40.67a	37.33a	39.33b
4	43.00ab	41.00b	40.67a	38.33b	37.33a	37.67c
6	42.67bc	41.00b	39.00b	37.67b	37.00a	36.33cd
8	41.33c	38.33c	38.67bc	37.00bc	35.00b	35.33de
10	38.33d	39.33c	37.33c	39.00c	34.33b	34.00e

LSD $_{(AXBXC):}$ 1.446

Table 2. Effect of different dried plant powders on leaf area (cm^2) under drought stress conditions.

Drought duration	Orange peel (g)			Cauliflower waste (g)		
	0	7.5	15	0	7.5	15
0	42.133a	44.053a	39.110a	39.110a	23.040a	26.887a
2	41.707b	41.880b	25.897b	25.897b	21.850b	25.880b
4	40.827c	27.947c	22.880c	22.880c	21.960b	20.053c
6	29.060c	27.827c	21.050d	21.050d	20.827c	19.853c
8	27.037d	24.887d	21.047d	21.047d	21.040c	18.980d
10	21.067e	21.493e	20.040e	20.040e	18.103d	17.950e

LSD $_{(AXBXC):}$ 0.394

Table 3. Effect of different dried plant powders on root fresh weight (g) under drought stress conditions.

Drought duration	Orange peel (g)			Cauliflower waste (g)		
	0	7.5	15	0	7.5	15
0	3.712a	4.195a	3.285a	3.208a	1.983a	3.243a
2	3.658ab	3.300b	3.050b	2.998b	1.942a	2.500b
4	3.602b	3.276b	2.800c	1.965c	1.766b	1.994c
6	3.449c	3.240b	2.100d	1.875d	0.700c	0.642d
8	3.286d	2.144c	1.998e	1.800d	0.688c	0.200e
10	2.108e	2.107c	1.980e	0.796e	0.685c	0.150e

LSD $_{(AXBXC):}$ 0.079

Table 4. Effect of different dried plant powders on root dry weight (g) under drought stress conditions.

Drought duration	Orange peel (g)			Cauliflower waste (g)		
	0	7.5	15	0	7.5	15
0	0.149a	0.151a	0.148a	0.258a	0.082a	0.250a
2	0.140a	0.131a	0.109a	0.113b	0.079a	0.107b
4	0.139a	0.113a	0.108a	0.110b	0.076a	0.080b
6	0.139a	0.110a	0.096a	0.093b	0.062a	0.068b
8	0.118a	0.092a	0.086a	0.079b	0.060a	0.050b
10	0.093a	0.073a	0.080a	0.070b	0.060a	0.048b

LSD $_{(AXBXC):}$ 0.142

A significant decrease in plant height was observed in *Vigna unguiculata* (Abdel Aziz, 2008; Khater et al., 2018) and *Vigna radiata* L. (Bangar et al., 2019) exposed to drought. Dry conditions at the beginning of flowering and pod formation in *Arachis hypogaea* led to a significant decrease in plant height (Muhanna and Saqr, 2016). In other study, drought conditions in the vegetative period caused a decrease in internode length, number of nodes, plant height, first pod height, leaf area, shoot dry weight and root length (Stanak et al., 2023). Similar results were observed in the cotton (Ödemiş and Candemir, 2023). It was determined that low-dose ground plant wastes applied to the common bean together with drought stress for up to eight days were more effective than high-dose, also orange peel waste application. Application of fruit wastes to soil against heat stress increased the growth of *Schefflera arboricola* and the highest value was obtained in orange peel waste compared to banana and pomegranate wastes (El-Serafy et al., 2023).

Root fresh weight decreased significantly with drought conditions. Application of low dose of orange peel powder (7.5 g) alleviated the effects of drought for up to 8 days. It was revealed that the dose increase (15 g) was more effective on only 2-day short-term drought (Table 3). Cauliflower waste application (7.5 g) gave similar results in drought stress lasting longer than 6 days in common beans and was significantly affected by stress. With the increase in drought stress, cauliflower waste applied at high doses could not prevent the decrease in root fresh weight (Table 3). The highest root dry weight of common beans was obtained under control conditions, while the lowest weight was observed after 10 days of drought. Orange peel powder and cauliflower wastes were not effective in increasing root dry weight of beans against drought conditions and root dry weight decreased (Table 4).

The highest shoot fresh weight was obtained under control conditions and decreased significantly with drought. In drought conditions, low dose of orange peel powder (7.5 g) applied did not make a significant difference in shoot fresh weight values compared to the control. The effect of low-dose application of drought on shoot fresh weight was significant and positive. When the application of orange peel powder was increased (15 g), the applications made during 2 and 4 days of drought gave similar results. After eight days of drought, a significant decrease was observed. Application of cauliflower waste in drought conditions gave lower averages than orange peel powder. While low dose cauliflower waste was effective for up to eight days, it showed a high decrease with the increase of drought (Table 5). When Table 6 is examined, it was observed that the shoot dry weight value decreased gradually with drought stress. After 10 days of drought, shoot dry weight of common beans decreased significantly and orange peel powder could not prevent the decrease. Low cauliflower waste (7.5 g) application was more effective than high dose (15 g) in drought conditions. The lowest value was obtained from the application of 15 g cauliflower waste under drought stress conditions for 10 days. Root and shoot fresh weight decreased significantly with arid conditions, 7.5 g of orange peel and cauliflower waste application alleviated the effects of drought in common bean under drought stress up to eight days. Root and shoot dry weight decreased significantly in drought, orange peel and cauliflower wastes could not alleviate the negative effect. The application of oak leaf powder to the tomato in drought conditions affected the root fresh and dry weight positively (Tahir et al., 2022). Application of *Bacillus velezensis* and orange peel to 20 soybean genotypes significantly increased plant height (14.3%), leaf area (11.4%), total above-ground dry weight (13.2%) and root dry weight (12.5%) (Pacheco da Silva et al., 2022). In another study conducted with peanuts, it was observed that the application of orange peel powder against drought stress provided the highest plant dry weight (Bagwell, 2020).

Dry matter value in plant showed a significant decrease in drought conditions. Higher dry matter values were obtained in the application of orange peel powder than in the low-dose application, except for the 10-day drought stress. The highest value after the application of cauliflower waste under stress conditions was obtained in two days of drought stress (Table 7). Dry matter accumulation in common bean decreased in arid conditions. This is due to the destruction of chlorophyll in the plant under stress. The fact that the leaf area is directly related to the photosynthesis activity also affects the amount of dry matter, yield and quality (Ödemiş and Candemir, 2023).

Table 5. Effect of different dried plant powders on shoot fresh weight (g) under drought stress conditions.

Drought duration	Orange peel (g)			Cauliflower waste (g)		
	0	7.5	15	0	7.5	15
0	3.877a	3.093a	3.793a	2.980a	2.560a	3.063a
2	3.810a	3.050a	2.890b	2.663b	1.930b	2.731b
4	3.517b	2.997a	2.710b	2.060c	1.870b	1.820c
6	3.103c	2.973a	1.967c	1.913c	1.740b	1.001d
8	3.160c	2.957a	1.960c	1.158d	0.977c	0.890d
10	2.767d	2.950a	1.957c	1.122d	0.877c	0.783d

$LSD_{(AXBXC):}$ 0.142

Table 6 Effect of different dried plant powders on shoot dry weight (g) under drought stress conditions.

Drought duration	Orange peel (g)			Cauliflower waste (g)		
	0	7.5	15	0	7.5	15
0	0.313a	0.240a	0.284a	0.205a	0.050a	0.159a
2	0.297b	0.232b	0.196b	0.156b	0.042b	0.150b
4	0.272c	0.208c	0.158c	0.050c	0.046ab	0.044c
6	0.250d	0.200d	0.153cd	0.049c	0.046ab	0.044c
8	0.226e	0.155e	0.150d	0.043cd	0.040b	0.030d
10	0.042f	0.042f	0.050e	0.038d	0.040b	0.026d

$LSD_{(AXBXC):}$ 0.008

Table 7. Effect of different dried plant powders on dry matter (g) under drought stress conditions.

Drought duration	Orange peel (g)			Cauliflower waste (g)		
	0	7.5	15	0	7.5	15
0	13.040a	11.880a	13.520a	13.650a	16.410a	14.360a
2	12.791b	11.770b	12.560b	12.160b	13.370b	12.700b
4	12.600c	11.690c	12.330c	11.800c	12.180c	12.220c
6	11.780d	11.601d	11.910d	11.727d	12.210c	12.130d
8	11.600e	11.410e	11.471e	11.600e	11.950d	11.580e
10	10.880f	11.190f	10.570f	11.450f	11.381e	11.480f

$LSD_{(AXBXC):}$ 0.064

Table 8. Effect of different dried plant powders on raw ash value (%) under drought stress conditions.

Drought duration	Orange peel (g)			Cauliflower waste (g)		
	0	7.5	15	0	7.5	15
0	2.380a	3.260a	2.060a	2.470a	4.020a	3.900a
2	2.281b	2.111b	1.987ab	2.280b	2.720b	2.371b
4	2.131c	2.081b	1.961b	2.071c	2.371c	2.100c
6	1.580d	1.340c	1.351c	1.834d	2.021d	1.790d
8	0.500e	0.920d	1.090d	1.530e	1.810e	1.260e
10	0.000f	0.790e	0.720e	1.440f	0.850f	0.880f

LSD $_{(AXBXC)}$: 0.074

Table 9. Effect of different dried plant powders on chlorophyll a content under drought stress conditions.

Drought duration	Orange peel (g)			Cauliflower waste (g)		
	0	7.5	15	0	7.5	15
0	1.110a	0.610a	0.571a	0.690a	0.620a	1.060a
2	0.490b	0.533b	0.510b	0.650b	0.450b	0.590b
4	0.470c	0.490c	0.500c	0.470c	0.410c	0.530c
6	0.350d	0.420d	0.460d	0.450d	0.350d	0.420d
8	0.320e	0.410e	0.330e	0.350e	0.320e	0.380e
10	0.270f	0.280f	0.310f	0.230f	0.201f	0.220f

LSD $_{(AXBXC)}$: 0.001

Table 10. Effect of different dried plant powders on chlorophyll b content under drought stress conditions.

Drought duration	Orange peel (g)			Cauliflower waste (g)		
	0	7.5	15	0	7.5	15
0	0.530a	0.340a	0.290a	0.450a	0.350a	0.520a
2	0.240b	0.260b	0.260b	0.400b	0.220b	0.490b
4	0.220c	0.260b	0.250c	0.240c	0.190c	0.320c
6	0.160d	0.200c	0.220d	0.220d	0.170d	0.270d
8	0.150e	0.190d	0.160e	0.170e	0.150e	0.200e
10	0.130f	0.130e	0.150f	0.110f	0.080f	0.100f

LSD $_{(AXBXC)}$: 0.001

Table 11. Effect of different dried plant powders on total chlorophyll content under drought stress conditions.

Drought duration	Orange peel (g)			Cauliflower waste (g)		
	0	7.5	15	0	7.5	15
0	1.640a	0.950a	0.860a	1.140a	0.970a	1.580a
2	0.731b	0.790b	0.760b	1.050b	0.670b	1.020b
4	0.690c	0.750c	0.760b	0.710c	0.600c	0.910c
6	0.500d	0.620d	0.680c	0.600d	0.520d	0.620d
8	0.470e	0.600e	0.480d	0.520e	0.470e	0.560e
10	0.400f	0.410f	0.470e	0.340f	0.280f	0.320f

LSD $_{(AXBXC)}$: 0.001

Raw ash value decreased with increasing drought stress. In the highest drought application (10 days), this value could not be calculated due to insufficient vegetation and it is shown as zero in the table. It was observed that orange peel powder application was more effective than cauliflower waste in two and four-day drought applications (Table 8). Raw ash content in common bean decreased significantly with drought conditions and it was observed that orange peel powder was more effective than cauliflower waste in short-term drought applications (2-4 days). The highest raw ash content in chickpea was obtained with two irrigations (3.56%) during the 50% flower + 50% pod filling period, while the lowest value was obtained after a single irrigation (1.66%) before flowering (Kırnak et al., 2017). With the decrease in irrigation, the raw ash value decreased. In another study, it was observed that when the vetch was exposed to salt stress the raw ash content increased with the increase in stress (Parlak and Parlak, 2005; Al-Ghumaiz, 2013).

Chlorophyll a content decreased gradually in drought conditions. The decreases were less at low drought degrees and the application of low-dose cauliflower waste (7.5 g) appeared to be more effective (Table 9). After ten days of continuous drought applications, it was observed that the chlorophyll b content decreased significantly. Application of orange peel powder to common bean (7.5 g) in drought stress up to eight days gave similar results and it was determined that the decrease in chlorophyll b was lower than other stress conditions. (Table 10). Total chlorophyll decreased significantly under drought conditions, the highest value was obtained under control conditions. Orange peel powder and cauliflower waste could not prevent the decrease in total chlorophyll value as the drought period increased. The increase in the cauliflower waste dose led to an increase in the total chlorophyll value compared to the low application dose (Table 11). Chlorophyll a, chlorophyll b and total chlorophyll content decreased gradually in drought conditions.

Chlorophyll a was more effective on drought with the application of 7.5 g cauliflower waste. In drought stress up to eight days, application of 7.5 g of orange peel powder to common bean alleviated the effects of drought on chlorophyll b value. The application of orange peel and cauliflower waste powder was ineffective on the total chlorophyll with the increase of drought duration. Cauliflower waste was more effective in high dose. After the application of *Moringa olifera* leaf extract from the leaves to the soybean against arid conditions, shoot and root length, shoot fresh and dry weight, chlorophyll a, chlorophyll b and total pigments increased (Hanafy, 2017). Similar results were also observed in the wheat (Azra et al., 2013). The effect of orange peels on the photosynthetic pigments of the quinoa in drought conditions was positive. Chlorophyll a, chlorophyll b, carotenoid and total pigment content increased significantly after the application in quinoa (El-Bassiouny et al., 2016). In another study conducted with quinoa in drought, it was observed that foliar application of banana peel extract (500 mg/l) significantly increased the fresh and dry weight of the plant and the shoot length increased by 47%. Foliar application of 500 mg/l banana peel also caused an increase in chlorophyll a (61%), chlorophyll b (47%), carotenoid (163%) and total pigment content (55%) (Bakry et al., 2016). Fruit peel waste significantly improved the leaf pigment content, the highest value was obtained from orange peel waste (El -Serafy et al., 2023). In soil pollution the first organelle affected in plants is the root. Potato peel application under stress conditions caused a significant increase in root length, leaf area, chlorophyll a, chlorophyll b, carotenoid and total chlorophyll content of *Vigna mungo* (Askari et al., 2017). Potato peels attract copper and prevent salt from entering the plant due to the high carbohydrate and phenolic compounds it contains (Azadeh et al., 2012).

Conclusion

The potential of orange peel and cauliflower waste to stimulate drought tolerance on common bean was investigated. These wastes applied to the soil allowed growing common beans that are more resistant to drought stress. Fruit peel and cauliflower waste powder improved plant height, leaf area, shoot and root growth, dry matter, raw ash and promoted chlorophyll content in leaves. Against drought stress lasting up to eight days, 7.5 g/pot orange peel application showed better performance compared to cauliflower waste. The use of orange peels on common beans grown in drought conditions during the flowering period not only improved growth and plant tolerance, but also reduced the amount of waste discharged into the environment.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Data Availability

The data analysed in this study have been included in the article and its supplementary information.

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Effect of Fenugreek Gum and Eggplant Peel Extract on Physicochemical, Storage, Bioactive, and Sensory Properties of Dairy Dessert

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ABSTRACT

The objective of this research was to examine the impact of fenugreek gum and eggplant peel extract on multiple characteristics of rice pudding, encompassing parameters such as total soluble solids content, pH, color, syneresis index, storage stability, total phenolic content, antioxidant activity, flavonoid, and anthocyanin content, along with a sensory evaluation using a 5-point hedonic scale. Different concentrations of fenugreek gum (0%, 0.2%, 0.4%) and eggplant peel extract (0%, 0.25%, 0.5%) were prepared and incorporated into the rice pudding formulation at varying levels. Higher concentrations of fenugreek gum and eggplant peel extract in the pudding resulted in improved syneresis percentage (68.25±0.42%). This result shows reduced water release and improved storage stability. The addition of fenugreek gum also positively associated with increased storage stability, while eggplant peel extract had no significant effect. Furthermore, while the content of monomeric anthocyanins, total phenolics, total flavonoids, and antioxidant activity in the rice pudding samples increased with higher concentrations of eggplant peel extract, consumer acceptance scores were reported to be very low.

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Introduction

During production and preparation of food, a large volume of waste is produced by the food industry (Van den Abeele et al., 2017). About one-third of the global food production for human consumption, which is estimated as 1.3-1.6 billion tons of food, is discarded as waste along the entire food supply chain worldwide (Papaioannou et al., 2022). According to the Food and Agriculture Organization (FAO) State of Food and Agriculture (2022) report, after food is harvested in the world, around 14% of it is lost (a value of $400 billion per year). Seeds, peels, pomace, and stems of fruits and vegetables are thrown away even though they possess nutritional compounds such as polyphenols, carotenoids, and dietary fibers. Moreover, for some fruits even the peel contains much more bioactive compounds than the fruit pulp. For example, skins of avocados, lemons, seeds, grapes, jackfruits, and mangoes contain 15% more phenolic compound concentrations than the flesh of the fruit (Gorinstein et al., 2001; Soong and Barlow, 2004; Bhardwaj et al., 2022). Thus, in addition to environmental and economic reasons, recovery of waste in the food industry to obtain useful, cheap, new, high added value and natural products has become increasingly important in scientific research. Moreover, per capita intake of dairy products in Turkey is comparatively lower than that observed in developed nations (Goenenc and Tanrıvermiş, 2008). Thus, there is a pressing need for implementing effective production practices aimed at minimizing additives to augment the demand for this food category in consumer preferences. It is imperative to underscore that this study focuses on a zero waste approach, integrating bioactive nutritional components into the production process.

Eggplant (Solanum melongena L.) is an important vegetable produced in many countries mainly China, India, Egypt, and Türkiye with capacities of 36.6, 12.8, 1.3, and 0.8 million tons, respectively (FAO, 2022). The purple-colored skin of the eggplant is usually peeled off and thrown away in household use and the food industry. Eggplant peel waste was estimated to be around 117,558 tons in Türkiye (Karimi et al., 2021). Eggplant peel is an enormously rich source of anthocyanin with contents varying from 8 to 85 mg per 100 g of peel (Dranca and Oroian, 2016) and pectin (Mauro et al., 2020; Karimi et al., 2021).

Dairy desserts are highly appreciated for their sensory and nutritional properties and are fortified with phenolic extracts in the literature (Kaur et al., 2021; Kaur et al., 2022; Elkot et al., 2022) since they impart a variety of health benefits such as inhibition of cancer and brain cells degeneration, decreasing the risk of inflammatory and circulatory diseases (Yousuf et al., 2015). Blueberry (*Vaccinium myrtillus L.*) and strawberry tree fruits (*Arbutus unedo L.*) were used as a natural ingredient containing high phenolic compounds mainly anthocyanins in ice cream production to increase the nutraceutical potential and nutritive value (Kotan, 2018; Aloğlu et al., 2018). Moreover, when dairy dessert was enriched with beet or ginger or a combination of beet with ginger sensory characteristics and level of acceptance improved as compared to control (Bandyopadhyay et al., 2008).

Rice pudding is one of the most common dairy desserts produced with emulsifiers, and stabilizers like commercial water-soluble gum found in guar seed (Singh and Immanuel, 2022; Toker et al., 2013). Puddings containing carrageenan, alginate, guar and xanthan gums and their combinations were investigated and found that carrageenan was the most effective hydrocolloid on both the steady and dynamic rheological parameters of pudding samples (Toker et al., 2013). Yellow mustard mucilage, fenugreek gum, flaxseed mucilage containing puddings decreased blood glucose and plasma insulin compared to a control pudding, but did not differ from each other (Kay et al., 2017). A creamy mouth feel and smooth texture of a pudding system were achieved by the addition of gums such as xanthan gum (Sanderson et al., 1988). However, in reviewing the literature, no study was found about the investigation of the effect of fenugreek gum and eggplant peel extract in rice puddings in terms of physicochemical, sensory, and bioactive properties. Therefore, in this study, it was aimed to investigate the effect of the addition of fenugreek gum as a thickener, emulsifier and stabilizer and eggplant peel extract as an anthocyanin source in rice pudding. Moreover, the effect of different amounts of fenugreek gum (0%, 0.2% and 0.4%) and eggplant peel extract (0%, 0.25%, and 0.5%) on total soluble solids content, pH, color, syneresis index, storage stability, total phenolic, flavonoid, anthocyanin contents, antioxidant activity and sensory analysis of puddings were investigated. Puddings fortified with eggplant peel extract and fenugreek gum may target consumer groups who generally show interest in healthy eating and natural additives and seek diverse flavor experiences. Particularly, these products could be popular among consumers who prioritize healthy snacks, prefer natural and functional food products, and have an interest in organic and natural ingredients with a consideration for environmental sustainability.

Materials and Methods

Materials

Fenugreek seeds (*Trigonella foenum graecum* L., harvested in 2022) and eggplant (*Solanum melongena* L., harvested in 2023) were purchased from a local store. Folin–Ciocalteau reagent, sodium carbonate, 2,2-Diphenyl-1-picrylhydrazyl, and ethanol, HCl were bought from Sigma-Aldrich Chemie GmbH (Darmstadt, Germany). Eggplant peel was chosen as an ingredient for the study due to its abundant availability as a waste product, making it an environmentally sustainable option. It is rich in anthocyanins and pectin, which have potential health benefits. Fenugreek gum, on the other hand, was selected as it can serve as a thickener, emulsifier, and stabilizer in the rice pudding formulation.

Fenugreek Gum Extraction

Fenugreek gum extraction was formulated according to the method described by Hussain et al. (2022) with minor modifications. An amount of 100 g ground fenugreek seeds was soaked in 1000 ml of distilled water at room temperature (22 °C) for 16 h. Then, the swollen seeds were heated to 100 °C in a water bath until a slurry was formed. Thereafter, the solution was kept at 4 °C for the precipitation of any insoluble particle. The seeds were filtered by cheese cloth then the solution was washed with 400 ml of acetone repeatedly and dried at room temperature for 24 h.

Extraction of Eggplant Peel Bioactive Compounds

Fresh, large, elongated, deep purple-colored eggplants with green calyx were peeled using a peeler. The collected peels were dried at room temperature at dark for 48 h. Deep purple colored eggplant peels (100 g) were extracted with 80% ethanol solution with 1% HCl at 4°C for 24 h (Yong et al., 2019). Then, the solution was centrifuged (5000 g) for 15 min, condensed at 35 °C and dried under vacuum.

Preparation of Pudding Samples

The ingredients for the preparation of fenugreek gum-eggplant peel extract incorporated into rice puddings are shown in Table 1.

Table 1. Formula of pudding preparations fortified with fenugreek gum and eggplant peel extract

Sample	Milk (g/100g)	Sugar (g/100g)	Fenugreek gum (g/100g)	Eggplant peel extract (g/100g)	Rice starch (g/100g)
G0E0	81.8	10	0.00	0	8.2
G0E0.5	81.8	10	0.00	0.5	7.7
G0E1	81.8	10	0.00	1	7.2
G0.2E0	81.8	10	0.20	0	8
G0.2E0.5	81.8	10	0.20	0.5	7.5
G0.2E1	81.8	10	0.20	1	7
G0.4E0	81.8	10	0.40	0	7.8
G0.4E0.5	81.8	10	0.40	0.5	7.3
G0.4E1	81.8	10	0.40	1	6.8

Solid and liquid proportions of the samples were kept constant. Fenugreek gum, eggplant peel extract and rice starch amount in total was 8.2% (w/w) of pudding samples. In the abbreviation in Table 1, the letter "G" stands for gum, the letter "E" stands for extract, and the numbers in between represent the percentages of each component in the formulation, respectively. The preparation method was based on Ares et al. (2009). First dry ingredients except eggplant peel extract were mixed then milk was added. The mixture was heated at 90°C for 15 min by using a water bath with agitation. Thereafter, eggplant peel extract was added after cooling to room temperature (25°C) and then stored in a refrigerator (4°C) for 12 h prior to experiments.

Total Soluble Solids and pH Value

Total soluble solids (TSS) were reported as °Brix using a hand refractometer (Loyka ATC 0–50%, Turkey), at ambient temperature (Lee and Choi, 2020). First, pudding samples (10 g) were homogenized with 20 ml of distilled water by magnetic stirrer at 150 rpm at room temperature for 1 h. Samples were centrifuged at 3000 g for 5 min. The Brix and pH value of the supernatant were determined by refractometer and pH meter (Ohaus ST10, NJ, USA), respectively. The measurements were taken in triplicate and the mean value was reported.

Degree of Syneresis (%)

Syneresis is an important undesired defect observed in starch-containing foods such as desserts due to retrogradation. The method described by Kumar et al. (2022) was used with slight modification. Pudding samples (5 g) at 4°C were transferred into a 15 ml centrifuge tube and centrifuged at 4000 rpm (Elektro-Mag M 815, Turkey) for 15 min. The degree of syneresis was calculated using the equation below.

$$\text{Syneresis (\%)} = \frac{\text{Supernatant weight (g)}}{\text{Product weight (g)}} \times 100 \qquad (1)$$

Storage Stability (%)

Storage stability (SS) was determined according to the method described by Fundagül, (2023). Plastic cups containing 10 g of pudding samples were weighed and sealed with aluminum foil. After 72 h of storage at 4°C samples were brought to room temperature. Free water leached from the pudding was drained and storage stability was calculated by the equation below.

$$\text{SS (\%)} = \frac{\text{WSWL}}{\text{WS}} \times 100 \qquad (2)$$

SS : Storage Stability (%)
WSWL : Weight of the sample after water leaching
WS : Weight of the sample

Color

A colorimeter (TES 135A Color Reader, TES, Taiwan) was used to measure color parameters such as L*, a*, b* values. The L* value represents the lightness ranging from black (L* = 0) to white (L* = 100), the a* value measures the redness ranging from green (−60) to red (60), and the b* value represents the yellowness ranging from blue (−60) to yellow (60). For each pudding sample an average of values obtained from five different points were considered.

Determination of Total Phenolic Content of Pudding Samples

Total phenolic content of pudding samples was determined by Folin-Ciocalteu method (Waterhouse, 2002). Although the Folin-Ciocalteu reagent has the potential to react with interfering substances like ascorbic acid, dehydroascorbic acid, and reducing sugars (glucose and fructose) in plant extracts (Sánchez-Rangel et al., 2013), this method was used as a comparative technique in this research to select the best formulation. Pudding samples weighing 10 g were combined with 20 ml of 99.8% ethanol and subsequently centrifuged at 3000 × g for 10 minutes. 20 µL of the supernatant was mixed with 100 µL of Folin-Ciocalteu reagent and 1.58 mL of distilled water. The mixture was incubated for 6 min. Na_2CO_3 stock solution (20%) of 300 µL was added to the mixture. After 2 h of incubation at room temperature in the dark, the absorbance was recorded at 765 nm by using Spectrophotometer (T80+, UV/Vis. spectrometer, PG Instrument Ltd.) with three replications. Blank was prepared using the same procedure but without the sample.

Determination of Antioxidant Activity of Pudding Samples

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical inhibition method was used to evaluate the antioxidant activity of pudding samples (González-Aguilar et al., 2007). Due to the higher correlation observed between the DPPH method with other commonly employed techniques for assessing antioxidant activity of eggplant, namely CUPRAC, FRAP, and TEAC (Kaur et al., 2014), the DPPH method was utilized for relative calculations rather than determining absolute values. The supernatant of the with a volume of 0.1 mL was mixed with 3.9 mL of a 0.6 mM DPPH 80% ethanolic solution. The solution was left in the dark for 1 h then the absorbance was measured at 515 nm by using the spectrophotometer with ethanol as control. Percent antioxidant activity was calculated using the formula represented below.

$$\text{AOA (\%)} = \frac{A_c - A_s}{A_c} \times 100 \qquad (3)$$

Where, As is the absorbance of the sample and Ac is the absorbance of control.

Determination of Total Flavonoid Content of Pudding Samples

Total flavonoid content was measured according to the study of Adhikari et al. (2019). 250 µL of extract or standard solution was mixed with 1.25 mL H2O and 75 µL 5% NaNO2 solution. After 6 min incubation, 150 µL of 10% AlCl3 -H2O solution was transferred and 0.5 mL of 1 M NaOH and 275 µL ethanol was added. The absorbance of the mixture was measured at 510 nm using a UV-VIS Spectrophotometer (T80+ UV/VIS Spectrophotometer, PG Instruments Ltd., China). The results were expressed as catechin equivalents (CE) per gram of dry weight (mg CE/g sample).

Determination of the Monomeric Anthocyanin Content of Pudding Samples

The monomeric anthocyanin content (MAC) of pudding samples was determined by pH differential method described by Lee et al. (2005). The supernatant (1 ml) was mixed with two buffer solutions at pH 1.0 and 4.5. Thereafter, the absorbance of solutions was recorded at 510 and 700 nm and monomeric anthocyanin content (MAC) was determined by the formula below:

$$\text{MAC} = \frac{\text{A}\times\text{Mw}\times\text{Df}\times 1000}{\varepsilon\times\text{L}} \quad (4)$$

$$\text{A} = [(\text{A}_{520} - \text{A}_{700})_{\text{pH1.0}} - (\text{A}_{520} - \text{A}_{700})_{\text{pH4.5}} \quad (5)$$

Mw is molecular weight of delphinidin-3-glucoside (465 g/mol), ε is the molar extinction coefficient of delphinidin-3-glucoside (26,900 L/mol/cm), L is pathlength (1 cm), Df is the dilution factor, 1000 is factor for conversion from g to mg. The results were expressed as mg of delphinidin-3-glucoside equivalents (D3G) per gram of dry extract.

Sensory Analysis

A voluntary sensory evaluation was performed on pudding samples by 20 semi-trained panelists of ages between 18 and 35. An informatory session was provided to the panelists before the analysis. Samples were coded and presented to the panelists at room temperature in a fluorescent-lit room between 09:00 and 15:00. Panelists were supposed to not eat or chew gum for 1 hour beforehand. Between pudding samples, the panelists were asked to wait one minute and drink water as a mouth rinse. Pudding samples (9 samples) were served in plastic clear cups and the panelists were asked whether they liked the appearance, taste, smell, consistency of the appearance, consistency in the mouth and general acceptability with a 5-point hedonic scale ranging from 1 ("dislike extremely") to 5 ("like extremely").

Statistical Analysis

The analysis of variance (ANOVA) was conducted on data encompassing various responses, including total soluble solids content, pH, color, syneresis index, storage stability, total phenolic content, antioxidant capacity, flavonoid levels, anthocyanin activity, and sensory evaluation. This analysis aimed to assess the impact of factors such as extract content and gum concentration. Subsequently, the means were compared using the Tukey post hoc test in MINITAB, with a significance level set at $P<0.05$.

Results and Discussion

Effect of Fenugreek Gum and Eggplant Peel Extract Concentration on Physicochemical Properties of Pudding Samples

Total soluble solids (TSS), measured in °Brix to determine the concentration of dissolved solids in a liquid solution. Brix is a measurement unit used to quantify the soluble solids in a liquid solution. Brix can also be used to measure the total soluble solids content of a solution that may include other dissolved solids besides sucrose, such as other sugars, acids, and other dissolved solids (Cavalcanti et al., 2008). When the concentration of dissolved solids increases in a liquid, the refractive index also increases, and a refractometer measures higher Brix value of a solution. Table 2 shows the total soluble solids of pudding samples ranged from 6.98±0.11 to 9.25±0.07 °Brix being the highest for the G0E0 and lowest for the G0.4E1 samples. Aronia, acknowledged as a source of anthocyanins and phenolic compounds in the diet, contributed to the increase in total soluble solids in the pudding samples from 3.53 ± 0.06 to 4.40 ± 0.00 (Lee and Choi, 2020). It was seen that gum and extract and their interaction cause significant ($P<0.05$) differences in the Brix values. When 0.4% fenugreek gum and 1% eggplant peel extract were added to the pudding formulas, the TSS decreased from 9.25±0.07 to 6.98±0.11 °Brix ($P<0.05$). Solids and liquids content in the formulations were kept constant, thus as fenugreek gum and eggplant extract content increases rice starch content decreases. This may have something to do with the fenugreek gum and eggplant extract not dissolving as much as the rice starch.

The pH values of pudding samples ranged from 6.35±0.08 to 6.75±0.08 (Table 2.). The addition of fenugreek gum in the formula did not significantly affect the pH value, on the contrary, eggplant peel extract significantly decreased pH value ($P<0.05$). The results were in agreement with those reported by Mirani and Goli (2021). The decrease in pH can be due to the antioxidant activity of the anthocyanin in eggplant and its high phenolic content.

Table 2. Physicochemical and storage properties of different pudding samples

	Total soluble solids	pH value	Degree of syneresis (%)	Storage stability (%)
G0E0	9.25±0.07a	6.75±0.078a	74.08±1.25b,c,d	96.06±1.33c,d
G0E0.5	8.51±0.01b	6.55±0.07a,b,c	72.99±0.12c,d	94.97±0.27c,d
G0E1	7.48±0.04d	6.35±0.078c	73.40±0.50c,d	97.20±0.92a,b,c
G0.2E0	8.13±0.04c	6.65±0.078a,b	75.32±0.59b,c	97.15±0.43b,c
G0.2E0.5	8.05±0.07c	6.45±0.07b,c	78.05±0.78a	96.41±0.73c
G0.2E1	7.48±0.04d	6.45±0.07b,c	76.42±0.45a,b	93.22±0.33d
G0.4E0	6.93±0.11e	6.65±0.07a,b	72.57±0.75d,e	99.07±0.28a,b
G0.4E0.5	7.48±0.04d	6.45±0.07b,c	70.09±0.08e,f	99.69±0.08a
G0.4E1	6.98±0.11e	6.45±0.07b,c	68.25±0.42f	99.21±0.23a,b

Results are given as mean ± standard deviation. Different letters in a column refer to significant differences ($P<0.05$). G0E0 represents 0% fenugreek gum and 0% eggplant peel extract, G0E0.5 represents 0% fenugreek gum and 0.5% eggplant peel extract, G0E1 represents 0% fenugreek gum and 1% eggplant peel extract, G0.2E0 represents 0.2% fenugreek gum and 0% eggplant peel extract, G0.2E0.5 represents 0.2% fenugreek gum and 0.5% eggplant peel extract, G0.2E1 represents 0.2% fenugreek gum and 1% eggplant peel extract, G0.4E0 represents0.4% fenugreek gum and 0% eggplant peel extract, G0.4E0.5 represents 0.4% fenugreek gum and 0.5% eggplant peel extract, G0.4E1 represents 0.4% fenugreek gum and 1% eggplant peel extract.

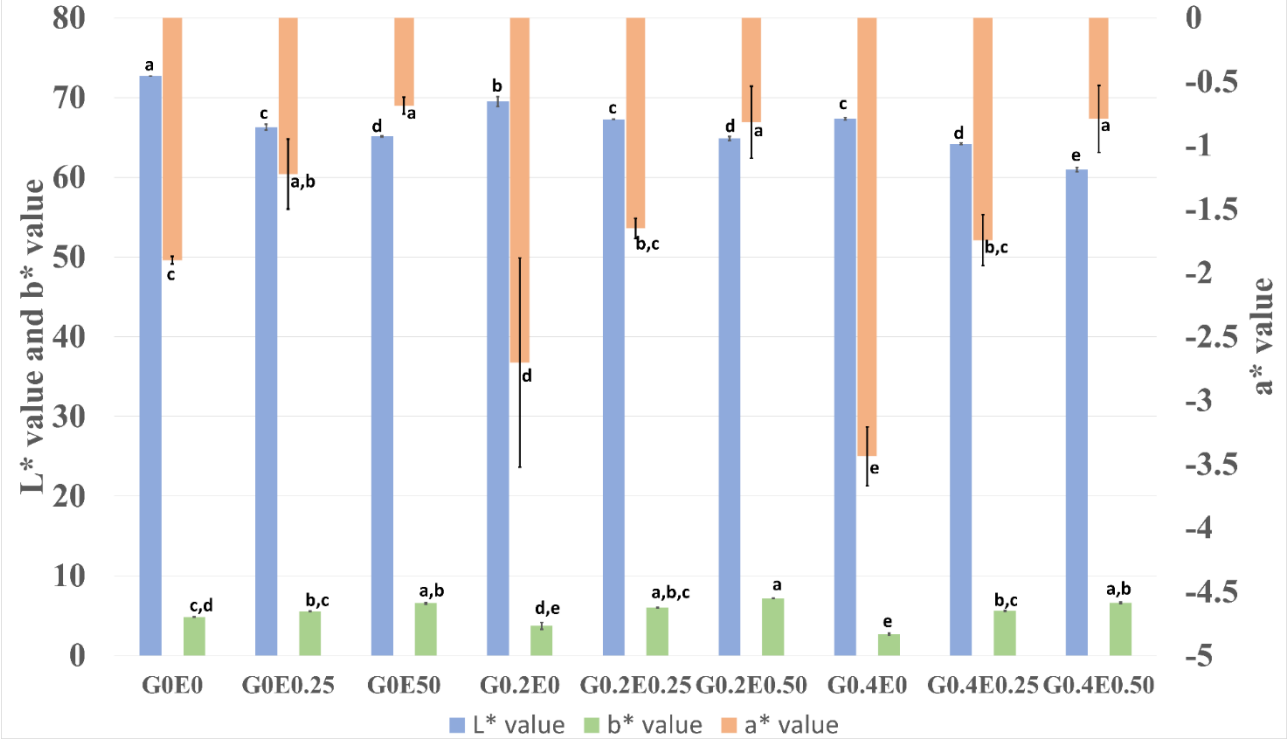

Figure 1. Instrumental color parameters (L*,a*,b*) of pudding samples

Different letters on columns refer to significant differences (P<0.05). G0E0 represents 0% fenugreek gum and 0% eggplant peel extract, G0E0.5 represents 0% fenugreek gum and 0.5% eggplant peel extract, G0E1 represents 0% fenugreek gum and 1% eggplant peel extract, G0.2E0 represents 0.2% fenugreek gum and 0% eggplant peel extract, G0.2E0.5 represents 0.2% fenugreek gum and 0.5% eggplant peel extract, G0.2E1 represents 0.2% fenugreek gum and 1% eggplant peel extract, G0.4E0 represents0.4% fenugreek gum and 0% eggplant peel extract, G0.4E0.5 represents 0.4% fenugreek gum and 0.5% eggplant peel extract, G0.4E1 represents 0.4% fenugreek gum and 1% eggplant peel extract.

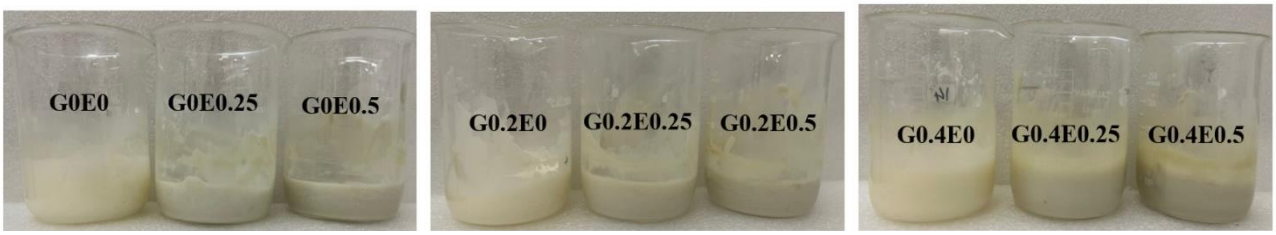

Figure 2. Color of pudding samples

G0E0 represents 0% fenugreek gum and 0% eggplant peel extract, G0E0.5 represents 0% fenugreek gum and 0.5% eggplant peel extract, G0E1 represents 0% fenugreek gum and 1% eggplant peel extract, G0.2E0 represents 0.2% fenugreek gum and 0% eggplant peel extract, G0.2E0.5 represents 0.2% fenugreek gum and 0.5% eggplant peel extract, G0.2E1 represents 0.2% fenugreek gum and 1% eggplant peel extract, G0.4E0 represents0.4% fenugreek gum and 0% eggplant peel extract, G0.4E0.5 represents 0.4% fenugreek gum and 0.5% eggplant peel extract, G0.4E1 represents 0.4% fenugreek gum and 1% eggplant peel extract.

Color is one of the consumer preferred quality parameters for rice pudding. Instrumental color parameters (L*,a*,b*) of pudding samples were presented in Figure 1 and photos of samples were given in Figure 2. The color of pudding samples showed significant ($P \leq 0.05$) variation in color values. The L*values, representing darkness–lightness (from 0 to 100), significantly decrease as fenugreek gum and eggplant peel extract were incorporated into the blends. As fenugreek gum concentration increases and eggplant peel extract concentration decreases, a* and b* values were decreased representing greenness-redness (from negative to positive) and blueness–yellowness (from negative to positive), respectively. However, as there isn't a study specifically addressing the addition of fenugreek gum to dairy products, such comparisons might be done by examining it in relation to a type of bread, chapatti, where fenugreek gum notably affected the lightness value, resulting in a slightly darker color compared to the control sample (Tandon et al., 2021). Mohite and Chandel (2020) utilized the fenugreek mucilage in edible taro starch film packaging and obtained a slightly darker and yellowish-colored film. The chlorophyll pigment leads to the darker color of eggplants whereas the purple color was characterized by the presence of a significant amount of anthocyanins, mainly delphinidin-based pigments (Horincar et al., 2020). A similar change in color was observed as eggplant was introduced into the formula such as the substitution of starch with eggplant flour in edible film decreased L*, while increasing a* and b*, significantly (Nouraddini et al., 2018).

Effect of Fenugreek Gum and Eggplant Peel Extract Concentration on Storage Properties of Pudding Samples

Degree of syneresis refers to the release of liquid or water from a gel or solid, resulting in the contraction or separation of the gel (Ngamlerst et al., 2022) and represents quality change in pudding products. Texture, appearance, and overall quality of the food product can change as a

result of syneresis. Thus, it is generally considered undesirable as it can lead to reduced product quality and shelf life. Syneresis percentage of fenugreek gum-eggplant peel extract incorporated pudding significantly ($P<0.05$) improved as gum and extract concentration were increased. Total syneresis percentage, after 4000 rpm centrifugal spinning for 15 min, ranged from 68.25±0.42% to 78.05±0.78% corresponding to pudding samples G0.4E1 and G0.2E0.5, respectively (Table 2). Sattar et al. (2017) also reported high syneresis value for rice starch puddings. Increasing the concentration of gum in the pudding matrix resulted in reduced syneresis ($P<0.05$). When 0.2% fenugreek gum was added, it exhibited increased syneresis compared to samples without gum. This was attributed to the lower water-holding capacity of 0.2% gum in the matrix than rice starch, whereas 0.4% gum indicated greater water-holding capacity than rice starch. Similar results were observed by da Silva Costa et al. (2020) such as 0.50% guar gum and xanthan gum addition on the starch gels made from the arrowroot inhibit the syneresis. Gums promote the establishment of numerous hydrogen bonds between water molecules and hydrophilic groups of gum molecules leading to a decrease in water activity and more restriction of molecular mobility.

Storage stability shows the stability of pudding samples after 72 h of storage at refrigeration temperature (4 °C). Storage stability of samples were in the range of 93.22±0.33% and 99.69±0.08%. It can be concluded that the pudding samples could be considered to have good storage stability. Moreover, fenugreek gum addition from 0.2% to 0.4% significantly increased storage stability ($P<0.05$) whereas eggplant peel extract concentration had no effect on storage stability. A good negative correlation was found between storage stability and syneresis with a Pearson correlation of 65.4% ($P<0.05$). Although high syneresis values were observed after 4000 rpm centrifuge for 15 min, their effect on storage stability was less. Without centrifugal force, high concentrations of fenugreek gum can create a more rigid structure, which can absorb water and prevent its release leading to high storage stability.

Effect of Fenugreek Gum and Eggplant Peel Extract Concentration on Bioactive Properties of Pudding Samples

In the flavonoid family, anthocyanins are responsible for purple color in eggplants, as well as in many other fruits, vegetables, and flowers. Anthocyanins are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylium salts (Kong et al., 2003). Delphinidin, petunidin, and malvidin are some of the common anthocyanins in eggplants found in the skin and flesh of the fruit. The darker the purple color of the eggplant, the higher the anthocyanin content. Anthocyanins are known for their antioxidant properties, which help to neutralize harmful free radicals in the body. MAC of the pudding samples was significantly affected by eggplant peel extract amount in the formulations ($P<0.05$). The amount of anthocyanin content was increased from 1.23±0.37 to 5.13±0.02 mg CGE/g of sample as eggplant peel amount was increased from 0% to 1%. The presence of slight MAC values in samples without added extract could be attributed to potential interference from certain interfering compounds (antioxidants, reducing sugars, organic acids, minerals, and amino acids) in the MAC analysis (de Oliveira et al., 2017). Eggplant peel anthocyanins were investigated by other researchers. Akhbari et al. (2019) reported that by different extraction conditions anthocyanin content from 6.20 to 434.91 mg CGE/100 g eggplant powder could be extracted, being the highest value was obtained under the following conditions: 90 min extraction time, 37 °C, and solvent/solid ratio of 10 mL/g using 100% acidified solvent. Eggplant peel added food formulations improved the anthocyanin content. The eggplant peel powder was added to the pastry cream in different concentrations (5% and 10%) and MAC of minimum 0.100 ± 0.008 and maximum 0.255 ± 0.017 mg D3G/g DW were obtained (Horincar et al., 2020). Lowbush or wild blueberry and soy protein frozen dessert was found to have an anthocyanin content ranging from 21 ± 4 to 48 ± 6 (mg/100 g) (Teh et al., 2005). Furthermore, MAC had higher Pearson correlation coefficient of 83% and 90.3% with TPC and AOA, respectively ($P<0.05$). G0E0 had minimum TPC among other pudding samples with an amount of 2.47±0.60 µg/mg, whereas G0E1 had the highest with 33.11±0.30 µg/mg. It is evident that even with the addition of 1% extract, the substantial increase in TPC levels is attributed to the high phenolic content in the eggplant peel extract. This is supported by the fact that TPC of eggplant extract were found to be in the range of 851.67 to 3030.25 mg GAE/100 g extract (Akhbari et al., 2019). Gum content of 0.2% had similar effect on TPC whereas 0.4% gum content significantly increased TPC when eggplant peel extract concentration was 0.5%. Eggplant peel extract significantly affected the TPC with a rapid increase as the content increases from 0.5% to 1% ($P<0.05$). The AOA analysis relied on the principle of DPPH radical reduction to DPPH2 by receiving a hydrogen atom from an antioxidant. As observed in Table 3, an increase in the extract amount without gum resulted in a significant rise in AOA values from 21.35 ± 1.56% to 54.96 ± 0.87% and total flavonoid content from 0.20±0.001 to 0.26±0.001 catechin equivalents (CE) per gram of dry weight ($P<0.05$). Similarly, eggplant peel extracts' AOA was found to be in the range of 58.81 ± 1.1% and 63 ± 0.48% (Boulekbache-Makhlouf et al., 2013). The primary source of antioxidant activity within eggplant peel arises from its phenolic compounds, which comprise diverse substances including flavonoids, polyphenols, and anthocyanins. These compounds demonstrate potent antioxidant properties, engaging with free radicals within the body to diminish oxidative stress. Consequently, eggplant peel showcases antioxidant activity, potentially offering health benefits.

Effect of Fenugreek Gum and Eggplant Peel Extract Concentration on Sensory Properties of Pudding Samples

The increasing demand of consumers for healthy and beneficial foods, particularly those with high antioxidant content, has been steadily rising. Nevertheless, the sensory attributes of a food, which greatly impact its acceptance by consumers, remain a crucial factor to consider. As the panelists were unaware of the specific ingredients used in the puddings prior to evaluation, they were unable to correctly identify the flavors of fenugreek and eggplant in the samples.

Table 3. Antioxidant properties of pudding samples enriched by fenugreek gum and eggplant peel extract

Samples	MAC	TPC	TFC	AOA
G0E0	1.27±0.46e	2.47±0.60f	0.20±0.001g	21.35±1.56d
G0E0.5	3.20±0.08c,d	5.95±0.45e	0.23±0.001e	32.72±1.49c
G0E1	5.13±0.02a	33.11±0.30a	0.26±0.001d	54.96±0.87b
G0.2E0	1.87±0.88d,e	7.63±0.15d	0.26±0.001c	20.00±1.74d
G0.2E0.5	3.65±0.14b,c	8.16±0.15d	0.22±0.002f	34.79±1.37c
G0.2E1	5.01±0.16a,b	25.74±0.30b	0.27±0.001a	71.48±0.61a
G0.4E0	1.23±0.37e	7.42±0.15d	0.26±0.001c,d	21.58±1.66d
G0.4E0.5	3.63±0.06b,c	14.58±0.01c	0.23±0.001f	36.28±0.31c
G0.4E1	4.09±0.04a,b,c	26.26±0.30b	0.26±0.001b	52.88±0.002b

Results are given as mean ± standard deviation. Different letters in a column refer to significant differences (P<0.05). MAC is monomeric anthocyanin content in mg of delphinidin-3-glucoside equivalents/g, TPC is total phenolic content in µg of gallic acid equivalent /mg, TFC is total flavonoid content in mg catechin equivalents/g, AOA is antioxidant activity in percent inhibition. G0E0 represents 0% fenugreek gum and 0% eggplant peel extract, G0E0.5 represents 0% fenugreek gum and 0.5% eggplant peel extract, G0E1 represents 0% fenugreek gum and 1% eggplant peel extract, G0.2E0 represents 0.2% fenugreek gum and 0% eggplant peel extract, G0.2E0.5 represents 0.2% fenugreek gum and 0.5% eggplant peel extract, G0.2E1 represents 0.2% fenugreek gum and 1% eggplant peel extract, G0.4E0 represents0.4% fenugreek gum and 0% eggplant peel extract, G0.4E0.5 represents 0.4% fenugreek gum and 0.5% eggplant peel extract, G0.4E1 represents 0.4% fenugreek gum and 1% eggplant peel extract.

Table 4. Results of sensory analysis

	Appearance	Taste	Smell	Consistency of the appearance	Consistency in the mouth	General acceptability
G0E0	4.70±0.47a	4.8±0.4104a	4.80±0.4104a	4.50±0.827a	4.50±0.827a	4.80±0.4104a
G0E0.5	3.45±1.099b,c,d	3.30±1.129c	3.65±0.933b,c	3.50±0.761c,d	3.40±1.046b,c,d	3.55±1.05b,c,d
G0E1	2.80±0.834d,e	2.95±1.191c,d	2.90±1.119c	3.10±0.788c,d	3.10±1.252c,d	2.90±0.641c,d
G0.2E0	4.05±0.887a,b,c	3.65±0.745b,c	3.40±0.94b,c	3.60±0.754b,c	3.40±0.503b,c,d	3.15±0.813c,d
G0.2E0.5	2.90±0.852d,e	2.75±1.07c,d	2.95±1.191c	3.25±0.716c,d	3.00±0.562c,d	3.05±0.759c,d
G0.2E1	2.65±1.268d,e	2.85±1.137c,d	2.85±1.137c	2.75±1.164d,e	2.60±1.046d,e	2.75±1.164d,e
G0.4E0	4.30±0.733a,b	4.55±0.51a,b	4.25±0.639a,b	4.40±0.681a,b	4.10±0.718a,b	4.30±0.657a,b
G0.4E0.5	3.30±1.218c,d	3.35±1.268c	3.40±1.188b,c	3.65±0.933b,c	3.50±1.1b,c	3.60±0.995b,c
G0.4E1	2.00±0.725e	2.00±0.725e	1.80±0.696d	2.15±0.587e	2.00±0.725e	2.00±0.725e

Results are given as mean ± standard deviation. Different letters in a column refer to significant differences (P<0.05). G0E0 represents 0% fenugreek gum and 0% eggplant peel extract, G0E0.5 represents 0% fenugreek gum and 0.5% eggplant peel extract, G0E1 represents 0% fenugreek gum and 1% eggplant peel extract, G0.2E0 represents 0.2% fenugreek gum and 0% eggplant peel extract, G0.2E0.5 represents 0.2% fenugreek gum and 0.5% eggplant peel extract, G0.2E1 represents 0.2% fenugreek gum and 1% eggplant peel extract, G0.4E0 represents0.4% fenugreek gum and 0% eggplant peel extract, G0.4E0.5 represents 0.4% fenugreek gum and 0.5% eggplant peel extract, G0.4E1 represents 0.4% fenugreek gum and 1% eggplant peel extract.

The results of sensory analysis, as presented in Table 4 and Figure 3, revealed significant differences in appearance, taste, smell, consistency of the appearance, consistency in the mouthfeel, and general acceptability of puddings fortified with fenugreek gum and eggplant peel extract. Based on the data, the G0E0 sample exhibited the most favorable ratings in terms of attributes such as appearance, taste, smell, consistency, mouthfeel, and general acceptability, possibly attributed to its natural and familiar flavor. The substitution of rice starch with eggplant extract resulted in significant adverse effects on various sensory attributes, including appearance, taste, smell, consistency, mouthfeel, and overall acceptability, in the samples of pudding. General acceptance scores decreased significantly in a concentration-dependent manner for puddings fortified with eggplant peel extract. Nonetheless, the utilization of fenugreek gum (G0.4E0) as a substitute of rice starch yielded the most favorable ratings for sensory characteristics such as appearance, taste, smell, consistency, mouthfeel, and overall acceptability, which were closest to those of the control sample (G0E0). The intense bitter taste derived from anthocyanins present in eggplant peel extract has become dominant even at low concentrations, rendering it less preferred by consumers.

Conclusions

Food industry generates significant waste during production and preparation, with an estimated one-third of global food production for human consumption, equivalent to 1.3-1.6 billion tons, being discarded as waste along the entire food supply chain. Eggplant peel, a rich source of anthocyanin and pectin, is often discarded despite its potential nutritional value. Dairy desserts, such as rice pudding, are commonly fortified with phenolic extracts, but there is a lack of research on the use of fenugreek gum and eggplant peel extract in rice puddings in terms of physicochemical, sensory, and bioactive properties. The aim of this study was to investigate the effects of fenugreek gum and eggplant peel extract on various characteristics of rice pudding, including total soluble solids content, pH, color, syneresis index, storage stability, total phenolic content, antioxidant, flavonoid, and anthocyanin activity, as well as sensory evaluation. Results showed that the total soluble solids (TSS) content of rice pudding samples varied depending on the concentration of fenugreek gum and eggplant peel extract used, with higher concentrations resulting in lower TSS values. The pH of the samples was also affected, with eggplant peel extract significantly

reducing the pH value, likely due to its antioxidant activity and phenolic content. Increased concentrations of fenugreek gum and eggplant peel extract in the pudding led to improved syneresis percentage, indicating reduced water release and improved storage stability. Furthermore, the addition of fenugreek gum positively correlated with increased storage stability, while eggplant peel extract had no significant effect. Moreover, this study involved a reduction in the product's starch content alongside an augmentation of its bioactive nutrient composition. However, despite the higher content of monomeric anthocyanins, total phenolics, total flavonoids, and antioxidant activity in the rice pudding samples with higher concentrations of eggplant peel extract, consumer acceptance scores were reported to be very low. Interestingly, consumers rated the rice pudding samples with 0.4% fenugreek gum but without eggplant peel extract with similar acceptance scores as the control sample. For future research, the inclusion of encapsulated eggplant peel extract in pudding formulations could be considered as a potential approach to mitigate the issue of bitterness.

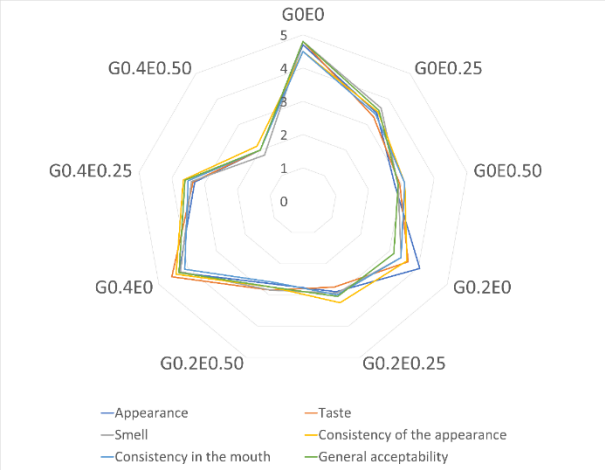

Figure 3. Spider chart of the sensory attributes of pudding samples

G0E0 represents 0% fenugreek gum and 0% eggplant peel extract, G0E0.5 represents 0% fenugreek gum and 0.5% eggplant peel extract, G0E1 represents 0% fenugreek gum and 1% eggplant peel extract, G0.2E0 represents 0.2% fenugreek gum and 0% eggplant peel extract, G0.2E0.5 represents 0.2% fenugreek gum and 0.5% eggplant peel extract, G0.2E1 represents 0.2% fenugreek gum and 1% eggplant peel extract, G0.4E0 represents0.4% fenugreek gum and 0% eggplant peel extract, G0.4E0.5 represents 0.4% fenugreek gum and 0.5% eggplant peel extract, G0.4E1 represents 0.4% fenugreek gum and 1% eggplant peel extract.

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The Rational Use of Oxalic Acid Against to "*Varroa Destructor*"; Regional Scale Pilot Scheme

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ABSTRACT

Varroa destructor mite poses a serious problem for the future of bee populations around the world. Today, there are many commercial drugs with the same and different active ingredients on the market to chemically control over of *Varroa destructor*. More frequent chemical applications for against *Varroa destructor* increases stress resilience, colony losses, loss of yield and residue problems in bee products. The scope of this project is aimed to determine the appropriate control method of Varroa by investigating the efficiency values of the evaporation and dropping methods of Oxalic acid. Experimental area were chosen three different apiaries. 28 colonies were determined in each apiary and equalization studies (area with brood, number of bees with bees, age of queen bees, honey, pollen, etc.) were carried out in these colonies. The determined colonies were randomly divided into 4 groups as 7 colonies. The first group is the control group, the second group is applying 2 g of oxalic acid by vaporizing, the third group is 4% oxalic acid 5 ml of sugar syrup (1:1) is dropped between the frames, and in the fourth group, the fight against a drug that is determined by the beekeeper in the market without interfering with the beekeeper. In order to evaluate the data, samples were taken for four periods, before and after spraying in spring and autumn. While the varroa measurements in the group of syrup, vapor and spraying were found to be statistically less than the control group, the syrup, vapor and spraying groups were statistically similar in terms of varroa measurements. Oxalic acid syrup application showed higher efficiency in spring and autumn than vapor application. There is no statistically difference between both two-application method reveals that it can be used as an effective and safe alternative to chemical control against varroa.

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Introduction

Bee diseases and pests have an important place among the reasons that limit the yield the most. Considering the economic losses, *Varroa destructor* parasite is among the most important bee disease and pest factors. This parasite causes a decrease in the development rate of colonies, winter loss in honey bees, infection in the colony, decrease in the flight efficiency, nectar and pollen collection capacity of forager bees, body deformations and body weight loss in adult bees. In advanced varroa infection, the colony is destroyed and serious economic losses occur in the apiary. Today, there is hardly any apiary without varroa. In the world and in our country, a definite and permanent method of combating such a widespread bee pest has not yet been developed. The aim of varroa control is not to kill all mites in the colony, but to keep the mite population below the level that will harm the colony (Mert et al., 2007).

The parasite was introduced to Turkey in 1976 via Bulgaria to the Thrace region and from there to the apiaries of beekeepers in Anatolia who went to the region to produce sunflower honey. The parasite, which was seen in the Aegean Region in 1977-78, then infected the apiaries of beekeepers who came from all regions of the country to the Aegean Region, especially Muğla province, to produce pine honey, and spread to the whole country in a very short period of 4-5 years after they returned to their own regions. The parasite caused great destruction in our country in the first years, causing the extinction of approximately 600 thousand colonies and 7000-7500 tons of product loss (Akyol et al., 2005). The disease is widespread all over the world except the Hawaiian Islands, New Zealand South Island, Australia and the tropical region of Africa

(Anderson and Truman, 2000; Aydın, 2011). It was detected in Asia in the 1950s and in the Americas and other continents in the 1980s and has covered all countries. This parasite is not found in islands such as Australia, New Zealand (South Island) and Hawaii due to quarantine measures (Aydın 2005, 2011).

There are 4 important species of Varroa, *Varroa jacobsoni, Varroa destructor, Varroa underwoodi and Varroa rindereri*, which are seen in adult and juvenile honey bees. These four species differ in host selectivity and geographical distribution (Anderson, 2000; Anderson et al., 2000). Adult and female Varroa constitute 96% of the Varroa population in the hive. Although the mouth structure of the female Varroa is piercing and sucking, the mouth structure of the males is not suitable for feeding and is shaped to carry sperm to the females (Zeybek, 1991; Aydın, 2012). Female Varroa live 2-3 months during the season (Spring-Summer) and 5-8 months during the off-season (Fall-Winter). Their life is completely parallel to the activation of the queen bee. The legs are short and strong, with 3 pairs in larvae and 4 pairs in nymphs and adults. All of the hairs on their bodies are called ketomes and these hairs allow the parasite to stay on the bee. Although the parasite usually lives between the head and thorax of the bee, it can also be found between the thorax and abdomen and between the abdominal rings (Webster and Delaphane, 2001; Aydın, 2005).

The activity of Varroa in the pupal and larval stages of bees is quite high. Adult female Varroa enter 5-5.5 days old bee larval cells before the cells close. Before laying eggs, it feeds on the larva's hemolymph (juvenile hormone in the blood). After receiving enough juvenile hormone and developing ovaries, Varroa lays the first egg 2-3 days after the eyes close. A female Varroa lays 2-6 eggs at 30 hour intervals and it is reported that the first egg is male (n=7 chromosomes) and the following eggs are fertilized (2n=14 chromosomes) female eggs. Females become adults in 8-10 days and males in 6-8 days. While 3 female adults are formed in a worker's eye, 5 female adults can be formed in the edges of the drone's eye (the temperature is lower) due to more food (drone biology is longer) (Zeybek, 1991; Aydın, 2012). While females mate in the closed chamber, male Varroa die following mating. Female Varroa emerge from the brood cells with the development of the fertilized egg and re-enter the honeycomb cell within 3 to 150 days depending on the season and the brood status of the colony. Varroa, which prefer drone cells more, increase 1.8-2.9 times in worker cells and 2.7-3.7 times in drone cells. Varroa is spread through bees by natural swarming, predation, wind and drones entering new hives. Especially the presence of weak colonies and strong colonies in the same apiary is the most common form. The ability of the drone to move between hives has also been reported as an important factor in the rapid spread of the parasite. The adult bee population has started to decrease, the queen's oviposition performance has decreased, dotted holes in the brood cells, symptoms similar to foulbrood rot, and symptoms such as deceased brood remaining in the 'C' shape in the cells are observed (Aydın, 2012).

Today, various methods, including physical, biological and chemical, are used in the control of Varroa. It is not possible to completely eradicate Varroa with the methods applied today. It is necessary to apply continuous treatment for decreasing Varroa density. For this, chemical control is of great importance. As a result of the widespread use of antiparasitic chemical drugs in the control of *Varroa destructor*, beekeeping has faced two important problems. The first one is the resistance of V. destructor to some of these chemicals. Drug-resistant Varroa populations transferred this hereditary trait from generation to generation in a short time. The second major problem is that some toxic components of chemical drugs accumulate in honey, wax and even propolis (Tutkun 2016). The drugs to be used should be safe for human health and the side effects that may occur in bees should be either absent or minimal. Drugs should not leave any residue or odor in the products to be obtained from bees or should not exceed the highest acceptable residue levels. Nowadays, human health and consequently food safety are being studied intensively. At this point, for the control of varroa in relation to bee products, substances that do not have harmful effects on human health and do not carry the risk of residue in honey have started to be sought. Organic acids, especially formic acid, are used in the control of this parasite because they have not yet developed resistance compared to other chemical synthetic compounds, they are cheap, they do not leave residues in honey above the specified limits and they do not harm adult worker bees. However, the use of these compounds is limited by the variability of the application methods used and the fact that the efficacy obtained varies each time and that they sometimes pose a danger to beekeepers due to their application methods. For example, in the hive where formic acid is applied, its concentration in the air varies according to the application method and ambient temperature. Therefore, the exposure time/concentration ratio is different each time. This creates problems in adjusting the concentration of active and toxic substances (Underwood 2003). For this reason, studies are ongoing on various formulations that will control and facilitate the administration routes of these active substances (Rufinengo et al., 2014). Oxalic acid, an organic acid, is naturally present in the structure of honey. If it is present in honey at very high levels (400-900 ppm), it causes a difference in the taste of honey. If oxalic acid application is made in the fall, it does not have any negative effect on the taste of honey and adult bees and brood (Imdorf et al., 1997). Wehling et al. (2003) emphasized that oxalic acid and formic acid applications are effective against varroa and are not harmful to human health as they are naturally present in honey.

In this study, it was aimed to determine the appropriate control method by investigating the effectiveness values of the evaporation and dripping methods of oxalic acid, which is an organic acid with low residue and resistance risk and will not adversely affect human health, in the control of *Varroa destructor* parasite, which poses a serious problem for the future of bee populations worldwide. With the project outputs, it is aimed to raise awareness among beekeepers and to expand its use throughout the country.

Materials and Methods

Material

Bee Material

The bee material consisted of local bees from Bulancak district of Giresun province, which were equalized in terms of brood area, number of bee frames, queen age and food stock. The experiment was conducted in standard Lanstroth type hives.

Oxalic Acid

Oxalic acid is a compound in the organic acid class with the formula C2H2O4. Oxalic acid used in the study was obtained from a commercial company.

Other Tools and Equipment

In order to determine the level of varroa contamination in adult bees; worker bee samples taken from colonies were taken into glass jars containing 70% ethyl alcohol with the help of a beekeeper's brush. A precision balance with a sensitivity of 0.001 gr was used for the preparation of chemicals for counting. Basic beekeeping materials such as honey pot, partition board, bellows, mask, hand iron were also used for colony maintenance during the study.

Method

Preparation of Colonies and Treatment Groups in Varroa Control

The research was carried out in three separate enterprises engaged in fixed beekeeping in Bulancak district of Giresun province where the risk of contamination was minimized. In each enterprise, 28 colonies were determined and equalization studies (brood area, number of frames with bees, queen age, honey, pollen, etc.) were carried out in these colonies. The colonies were randomly divided into four groups of 7 colonies according to the following experimental design.

Group 1 - Control group without any treatment against Varroa parasite

Group 2 - Application of 2 grams of oxalic acid to colonies by evaporation method

Group 3 - Administration of 4% oxalic acid with sugar syrup (1:1) at 5 ml per frame

Group 4 - Fighting with a commercially available drug

No spraying was done in the first group of colonies. Considering the risk of contamination, the colonies were transferred to a different point where there were no bee colonies around and at least 10 km away from the existing apiary. The second and third group colonies were treated with oxalic acid according to the experimental design given above. Considering the risk of contamination in these colonies, they were transferred to a different point in the opposite direction to the first group colonies, where there were no bee colonies around and at least 10 km away from the existing apiary. The fourth group of colonies were left in the beekeeper's own apiary and treated with a commercially available drug that is widely used in Varroa control for positive control. In the spring and fall periods, no treatment against varroa was performed in the colonies divided into groups, and varroa contamination rates were determined before and after treatment.

Varroa Contamination Rate

In order to detect Varroa pests, ether was sprayed on the live bee samples brought to the laboratory in sealed containers to kill the bees. At least 100 bees each were placed in a jar filled with 70% ethyl alcohol, the lid was closed, shaken for 30 minutes and left to settle for 10-15 minutes. Varroa were collected on the surface while bees and other residues settled to the bottom. After the process, all the material was passed through a narrow mesh strainer that would not allow the passage of varroa and bees, and the bees and parasites remaining on top were taken on a white blotting paper and the parasites were counted and recorded. Varroa parasites were searched for in the bees on the paper, especially between the wing bases, abdominal segments and feathers under a stereo microscope, and the collected parasites were examined and identified under a stereo microscope. These parasites were collected and placed in small capped bottles and the exact parasite load of the colonies was calculated (Kar et al. 2006).

$$\text{Infestation rate (\%)} = \frac{\text{Varroa count}}{\text{Number of bees}} \times 100$$

Efficiency value

The 'Henderson - Tilton Formula' was applied to determine the drug efficacy according to the mite load on the bees sampled before and after the trials (Henderson and Tilton, 1955). According to this formula, the calculation of the efficacy of a drug as a percentage is as follows;

$$\text{AP} = \left[1 - \frac{\text{NMCB} \times \text{NMTA}}{\text{NMCA} \times \text{NMTB}}\right] \times 100$$

AP: Adjusted Percentage
NMCB: Number of mites in the control group before treatment
NMTA: Number of mites in treatment group after treatment
NMCA: Number of mites in the control group after treatment
NMTB: Number of mites in treatment group before treatment

Statistical Analysis

Descriptive statistics of the data obtained were calculated and presented as "Mean ± Std. error". Mixed models were used to examine the significance of group, period main effects and group×period interaction term on varroa measurement results. In the model, establishment was included as a random effect and group, period and group×period interaction were included as fixed effects. For multiple comparisons, simple effects analysis was used with Bonferoni correction. The $P<0.05$ criterion was used in the evaluation of all statistical analyses. All analyses were performed using the SPSS (V22.0) package program.

Results

In the spring period, before the control, all colonies were divided equally into groups and varroa loads were calculated on a group basis. Varroa loads of the first, second, third and fourth groups were 2.97%, 2.99%, 3.26% and 3.26%, respectively. While the varroa loads in the treatment groups decreased after the control treatment, there was an increase of 0.26% in the control group (Table 1).

In the fall period, the varroa loads of the pre-treatment groups were 4.59%, 4.97%, 4.46% and 17.80%, respectively. After the control, varroa load changes were as follows: 3.07% decrease in the first group, 2.56% decrease in the second group and 3.54% decrease in the third group. In the control group, where no control was applied, an increase of 2.85% was observed in varroa load (Table 2). As seen in Tables 1 and 2, an effective control was realized in the treatment groups. In order to determine the effectiveness of oxalic acid applications, applications were made during the periods when the brood area was the lowest (mid-September), while beekeeper applications were made immediately after honey milking (end of August) using long-acting parasitic drugs. In the spring period, the average air temperature was 13.3 centigrade degree in the first treatment and 15 centigrade degree in the second treatment.

In the fall period, the average air temperature was 17.1 centigrade degreein the first application and 17.8 centigrade degree in the second application.

In the model, establishment was included as a random effect and group, period and group×period interaction were included as fixed effects. The random effect of holding was statistically insignificant (P=0.533). This indicates that average varroa measurements were similar across farms. Group, period and group×period interaction terms in the model were statistically significant (P<0.001). In the spring period, the difference between the treatment groups and the control group in terms of pre-treatment varroa loads was not statistically significant. In the autumn period, the difference between the treatment groups and the control group before the control period was statistically significant. In the spring and autumn periods, the difference between the treatment groups was found statistically insignificant, while the difference between the control group and the treatment groups was found statistically significant. When the interaction term in the model was analyzed, it was found that the varroa measurements in the sherbet, steam and own spraying groups were statistically significantly lower than the control group (P<0.05), while the sherbet, steam and own spraying groups were statistically similar in terms of varroa measurements (P>0.05). When the subgroups were analyzed according to the periods within themselves, Sherbet and Steam groups followed a similar trend according to the periods. The lowest varroa was observed after spring, while the highest varroa measurement was observed before fall. When the trend was observed, it was seen that the post-spring and pre-spring periods for these groups were statistically similar in terms of varroa measurements (Table 3).

In the control group, varroa measurements were at the highest point after the fall and statistically significantly higher than the other periods. Post-autumn was followed by pre-autumn, post-spring and pre-spring, and there was no statistically significant difference between pre-spring and post-spring for the control group (P>0.05).

As seen in Table 4, the beekeeper treatments showed the highest efficacy with 92.83% and 82.22% in the spring and fall periods. In the spring period, the efficiency value of oxalic acid slurry treatment was 90.65%, while the efficiency value of steam treatment was 83.58%. In the autumn period, similar results were observed in terms of treatment groups in the spring period. In the autumn period, the efficiency value of oxalic acid syrup application was found to be 71.42%, while the efficiency value of oxalic acid steam application was determined as 58.20%. Oxalic acid slurry application showed higher efficacy than steam application in spring and autumn periods.

Table 1. Varroa contamination rates before and after spring control, %.

Application	Before the spring combat			Mean	After the spring combat			Mean	Alteration
	1.Business	2.Business	3.Business		1.Business	2.Business	3.Business		
Syrup	3.52	2.32	3.08	2.97	0.37	0.34	0.21	0.30	-2.67
Steam	3.25	2.51	3.23	2.99	0.44	0.60	0.56	0.53	-2.46
Beekeper App.	3.85	2.77	3.46	3.36	0.34	0.29	0.17	0.26	-3.10
Control	3.71	2.68	3.40	3.26	3.96	2.90	3.70	3.52	+0.26

Table 2. Varroa infestation rates before and after the fall control period, %.

Application	Before the spring combat			Mean	After the Spring Combat			Mean	Alteration
	1.Business	2.Business	3.Business		1.Business	2.Business	3.Business		
Syrup	4.99	4.37	4.42	4,59	1.39	1.99	1.19	1.52	-3.07
Steam	5.19	5.05	4.68	4.97	2.40	2.60	2.25	2.41	-2.56
Beekeper App.	4.80	3.99	4.60	4.46	1.47	0.64	0.66	0.92	-3.54
Control	16.76	16.00	20.65	17.80	21.34	18.25	22.38	20.65	+2.85

Table 3. Statistical comparison of the periods and control methods of the experimental groups.

Group	Semestr				P			
	Before Spring	After Spring	Before Fall	After Fall				
	Arit.Mean. ± Std. Error	Arit.Mean. ± Std. Error	Arit.Mean. ± Std. Error	Arit.Mean. ± Std. Error	Group	Semestr	Group* Semestr	
Syrup	2.98±0.26 A,ac	0.31±0.12 B,b	4.57±0.34 B,a	1.54±0.25 B,bcd	<0.001	<0.001	<0.001	
Steam	3.02±0.31 A,ac	0.54±0.14 B,b	4.96±0.32 B,a	2.42±0.16 B,bcd				
Own Spraying	3.37±0.25 A,ac	0.27±0.11 B,b	4.45±0.23 B,a	0.9±0.28 B,bd				
Control	3.27±0.29 A,c	3.53±0.26 A,c	17.64±1.64 A,b	20.56±1.58 A,a				

A,B: Different letters in the same column indicate statistically significant difference (P<0.05); a,b: Different letters in the same row indicate statistically significant difference (P<0.05)

Table 4. Efficiency Levels of Treatment Groups, % (Henderson, CF and EW Tilton, 1955)

	Oxalic acid sherbet application	Oxalic acid steam application	Beekeeper application
Spring semester	90.65	83.58	92.83
Fall semester	71.45	58.20	82.22

In varroa control, organic acid is widely used in different doses by dripping method Adjlane et al., (2016); Higes et al., (1999); Del Hoyo et al, (2007); Benfotti and Lucchelli, (1999); Gregorc and Planinc, (2005); Charriere and Indorf, (2002); Gregorc and Planinc, (2001); Gregorc and Planinc, (2004); Marcongeli and Gorcia, (2004); Al Toufaila, (2015), spray method Yücel, (2005); Cengiz, (2012); Girişkin et al, (2010); Çetin, (2010); Al Toufaila, (2015) and evaporation method Radetzki, (2001); Al Toufaila, (2015). Varroa parasite control; Adjlane et al. (2016) achieved 81% efficacy value with 4.2% oxalic acid solution; Al Toufaila, (2015) achieved 93-95% efficacy value with spray, dripping and evaporation methods of oxalic acid; Higes et al. (1999) achieved 94% efficacy value in spring and 73% in autumn with dripping method; Del Hoyo et al, (2007) used 5% oxalic acid solution with dripping method with an efficacy value of 83.8%; Akyol and Yeninar, (2009) dissolved 30 g oxalic dihydrate in 1 liter of sugar water and used 5 ml per frame with 93,40% efficacy value; Benfotti and Lucchelli, (1999) dissolved 100 g oxalic acid in 1 liter of sugar water and used 5 ml per frame with 98-99% efficacy value; Yücel, (2005) 3. 2% oxalic acid solution by spraying 3 ml on each honeycomb surface to 92.1% efficiency value; Cengiz, (2012) 3.2% oxalic acid solution with 5 ml spraying method to 84.90% efficiency value; Girişkin et al, (2010) spraying 4% organic acid solution between the frames with 93.3% efficacy value in fall; Gregorc and Planinc, (2005) using 2.9% oxalic acid sugar syrup solution with 94.42% efficacy value in fall; Nasr et al, (2001) achieved 55% efficacy with 2.8% oxalic acid sugar syrup solution and 90% efficacy with 3.5% oxalic acid sugar syrup solution, 40-50 ml per colony; Charriere and Imdorf, (2002) used 3.4% oxalic acid 47.6% sugar as the first solution; 3.7% oxalic acid/ 26. 1% sugar as the first solution, 3.7% oxalic acid/ 26.9% sugar as the second solution, and 2.9% oxalic acid/ 31.9% sugar as the third solution all had efficacy values above 90%; Gregorc and Planinc, (2004) 2.9% oxalic acid, 31.9% sugar water mixture had an efficacy value of 77.8% in spring and 88.87% in fall; Charriere et al, (1998) reported 98-99% efficacy value of oxalic acid solution in autumn; Çetin (2010) reported 95.56% efficacy value in spring and 92% efficacy value in autumn by using 3% oxalic acid solution as 3-4 ml spray method on each honeycomb surface.

In our study, the efficacy value of the oxalic acid syrup group in the spring period was found to be 90.65%, while the efficacy value of the steam treatment was 83.58%. In the autumn period, the efficiency value of oxalic acid slurry group was 71.45% and the efficiency value of steam treatment was 58.20%. Al Toufaila, (2015); Higes et al., (1999); Del Hoyo et al., (2007); Akyol and Yeninar, (2009): Benfotti and Lucchelli, (1999); Yücel, (2005); Cengiz, (2012); Girişkin et al, (2010); Cornelissen and Blacquiere, (2004); Gregorc and Poklukar, (2003); Gregorc and Planinc, (2001); Gregorc and Planinc, (2004); Marcongeli and Gorcia, (2004); Charriere et al. (1998); Çetin, (2010) reported that the efficacy values were higher than our fall treatment groups and partially similar and partially lower than our spring treatment groups. It is thought that the differences between our study results and the literature data are due to the differences in the type, method and dose of oxalic acid used; the strength of the colonies; in-colony incubation activities; climatic variables, differences in bee races and different treatments applied to the colonies at the beginning of the experiment periodically. Bacandritsos et al. (2007) reported that the varroa efficacy value was 65.3% in colonies with brood activity and 77.3% in colonies without brood activity. In support of the previous literature, Marcongeli and Gorcia (2004) reported that the efficacy value of varroa control was 85.6% in colonies with 3 frames of brood and 75.7% in colonies with 6 frames. Again, Nanetti et al. (2003) reported that the same applications in varroa control gave different results in different locations. In our study, the efficacy value was found to be 90.65% as a result of the application of 4% oxalic acid with sugar syrup (1:1) and 5 ml per frame in the spring period. The efficacy value reported as 90.3% in Italy, where a similar application was made, coincides with the efficacy value we obtained in our study. The efficacy values reported as 94.3% in Germany and 95.6% in Norway were higher than our study values, while the efficacy values reported as 87.8% in Switzerland and 85.0% in Finland were lower than our study efficacy value.

Conclusion

Honey bee populations, which play an important role in the pollination of plants, are decreasing day by day due to internal and external factors, which is a significant threat to the sustainability of foodstuffs. In terms of animal health, Varroa destructor mite poses a serious problem for the future of bee populations in the world. The disadvantages of many drugs with the same or different active ingredients in the market for the chemical control of Varroa destructor are the development of resistance, the residue of some toxic components in honey, beeswax and even propolis, the fact that the main effectiveness occurs only at high doses and the need for frequent dosing. Frequent dosing for an effective control leads to stress development in bees, colony losses, yield loss and residue problems in bee products. For these reasons, the use of oxalic acid, one of the organic acids, has come to the forefront in the control of Varroa destructor, especially in European countries.

In this study, although there were differences between the drug efficacy values of the treatment groups, the change in the varroa load rates of the treatment groups in the spring and fall periods was statistically insignificant. These results revealed that the treatment groups exhibited an effective control against Varroa destructor in the spring and fall periods. It is seen that beekeepers achieved success in the fight against Varroa destructor with their own preferred drugs. The fact that there was no difference between steam and syrup applications of oxalic acid and beekeeper applications reveals that oxalic acid can be used as an effective and safe alternative to chemical control against Varroa destructor. Considering the statistical results of the efficacy values and varroa change rates of the application groups, oxalic acid does not leave any residue on bee products and can be used especially in the late spring period.

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A Research on Fertility, Herd Life, Milk Production and Milk Quality Characteristics of Simmental (Fleckvieh) Cows: 1. Reproduction, Herd Life and Milk Production Characteristics

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ABSTRACT

The aim of this study was to determine the fertility, herd life and milk yield characteristics of Simmental (SIM) cows of Austrian origin, which have increased the interest of cattle breeders in Türkiye in recent years. From the records of the farm between 2011 and 2021, the first calving age (FCA), calving interval (CI), herd life (HL), productive life (PL), lactation length (LL), lactation milk yield (LMY) and 305 days milk yield (305-dMY) were calculated. A total of 307 FCA, 619 CI, 212 HL and PL, 447 LL, 271 LMY and 497 305-dMY data were used. The means of FCA, CI, HL, PL, LL, LMY and 305-dMY for SIM cows were 842.35±5.30 days (28.1 months), 422.98±3.18 days, 75.48±1.72 months, 47.15±1.73 months, 363.52±3.52 days, 10,596±152 kg and 8647.0±58.0 kg, respectively. Based on the long FCA and CI averages of Austrian-origin SIM cattle, although it can be interpreted that there are some problems in terms of reproductive efficiency in the farm, finding long HL and PL and high milk yield, it can be said that the farm contributes to the increase of milk yield per cow by turning the negativity caused by the reproductive efficiency into an advantage.

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Introduction

Milk yield per cow shows significant differences between the farms raising the same foreign breed in Türkiye, depending on the applied management and feeding conditions. This situation reveals that the genetic potential of foreign breeds reared in many farms, especially Holstein-Friesian (HF), are not sufficiently utilized. It could be possible for the breeders to benefit from the full genetic potentials of these animals by providing appropriate management-feeding and health protection conditions at the farm level.

Simmental (SIM) is a breed that is preferred worldwide both in pure and crossbreeding due to its characteristics such as high growth rate, milk yield, milk fat and protein ratio, docility and adaptability (Koç, 2016a). Although it is difficult to determine the universal standard of the breed due to the development of various SIM-origin genotypes around the world, it is reported that there is no risk of narrowing the gene pool of this breed (Koç, 2016a). By using the SIM breed in intensive crossbreeding in many European countries such as Germany, Austria, France, Italy, Switzerland and in the former Soviet Union, different genotypes were developed. Breeders in the USA, on the other hand, brought the breed's meatiness to the fore and obtained genotypes called Black SIM and Red SIM, whose body color differs from the classical SIM colors, completely black and completely red (Koç, 2016a).

In Türkiye, for a long time, raw milk: concentrate feed parity has decreased below 1.5, close to 1.0, and occasionally below 1.0 (https://ulusalsutkonseyi.org.tr/cig-sut-yem-paritesi-627/). In recent years, the high prices of red meat due to the shortage of red meat in our country, the fertility problems experienced in HF cattle, and the relatively low resistance to diseases, especially mastitis, have led producers to prefer alternative cattle breeds, thus some producers have replaced HF breeds. Alternative breeds such as Red-Holstein (RH), Montbeliarde (MB), especially the Austrian and German origin SIM (Fleckvieh) breed with increased milk yield, have begun to be preferred more by cattle breeders. For this reason, a significant number of pregnant SIM heifers have been brought to Türkiye in recent years and this trend has made the SIM breed the second most grown foreign breed after HF, surpassing the Brown-Swiss breed, today.

Fertility, accepted as the basis of all yields in livestock, is also important in terms of transferring genetic variation to the next generation. There are some studies on the fertility of SIM cattle in Türkiye (Akbulut, 1998; Şekerden et al., 1999; Çilek and Tekin, 2006; Özkan and Güneş, 2011a; Erdem et al., 2015; Koç, 2016b; Koç, 2017; Okuyucu et al., 2018; Koç and Arı, 2020).

As a factor affecting profitability in dairy cattle, herd life (HL) is defined as the time elapsed between the date an animal was born and joined the herd and the date it was removed from the herd or left the herd for various reasons. Another measure of the HL is Productive life (PL) that is defined as a cow entering the herd, typically first-born heifers, and leaving the herd, typically because they are culled from the herd as voluntarily or compulsorily. Oltenacu (2009) stated that HL is expressed by the proportion of those still alive at the age of 48 months in the north of the USA, and that the ratio of those still alive at this age in HF cows decreased from 80% to 60% between 1957 and 2002, indicating that HL has decreased. There are some studies on HL in cattle in Türkiye and abroad (Savaş et al., 1999; Yaylak, 2003; Işık, 2006; Mundan and Karabulut, 2008; Sawa and Bogucki, 2010; Kara et al., 2010; Boğokşayan and Bakır, 2013; Fouz et al., 2014; Weller and Ezra, 2015; Gavrilă et al., 2015; Olechnowicz et al., 2016; Koç, 2017; Tutka, 2019).

There are also some previous studies on the milk yield of foreign cattle breeds raised in Türkiye, including the SIM breed (Şekerden and Erdem, 1997; Akbulut, 1998; Şekerden et al., 1999; Koç, 2001; 2006; 2009; 2016b; Çerçi, 2006; Çilek and Tekin, 2006; Yılmaz, 2010; Özkan and Güneş, 2011b; Erdem et al., 2015; Okuyucu et al., 2018; Koç and Arı, 2020; Koç and Gürses, 2020).

Although it has been brought to Türkiye to a large extent in recent years, the number of studies on fertility, herd life and milk yield of high yielding Austrian and German origin SIM (Fleckvieh) cattle is almost non-existent. That is why in this study, it was aimed to determine the fertility, HL and milk yield characteristics and some environmental factors affecting these from the records of Austrian origin SIM cattle raised in a private farm in the Menemen District of İzmir, Türkiye.

Material and Methods

The study was carried out in a farm located in Menemen District of İzmir Province, Türkiye, which raises SIM breed brought from Austria. From the herd management program used in the farm, the first calving age (FCA) and calving interval (CI) characteristics were calculated from the birth and insemination records of the existing animals and the animals culled from the herd. While 370 data were evaluated for the FCA, with a duration ranging from 310 days to 650 days, 619 data were evaluated for the CI. Two data (273 days and 299 days) with CI less than 310 days and 38 data with CI more than 650 days (663 days and 1294 days) were not included in the statistical analysis.

Lactation length (LL), lactation milk yield (LMY) and 305-day milk yield (305-dMY) from the milk yield records of the existing and culled cows were emphasized. The data for the 305-dMY varies between 5437 kg and 11768 kg, while the data for the LMY varies between 5930 kg and 22,569 kg. While LL and LMY data with a lactation period between 240 days and 550 days were taken into account, data with LL over 240 days for 305- dMY trait were evaluated, and 3 cows' 305-dMY values shorter than 240 days were excluded from the statistical analysis in addition to evaluate the first 305-d milk yield of the cows whose LL was longer than 305-d. Data with a LL longer than 550 days were excluded from statistical analysis, so 50 LL and 16 LMY data were excluded from evaluation according to this criterion.

Statistical analysis

Statistical analysis of the data was made in the SAS (2004) package program, and the comparison of subgroups was made according to Tukey ($P<0.05$). The following statistical model was used in the analysis of FCA:

$$y_{ijk} = \mu + a_i + b_j + e_{ijk} \quad (1)$$

Here, y_{ijk} is the observation value of the trait, μ; the mean of the trait, a_i; birth year effect (i= 2008, 2009, 2011, …..2019), b_j; birth month effect (j=1, 2, 3, …..12) and e_{ijk}; error term.

The following statistical model was used in the analysis of CI:

$$y_{ijklm} = \mu + a_i + b_j + c_k + d_l + e_{ijklm} \quad (2)$$

Here y_{ijklm}; observation value of the trait, μ; the mean of the trait, a_i; origin effect (i=born in Austria, born in Türkiye), b_j; calving year effect (j= 2011, 2012, ….. 2020), c_k; calving month effect (k=1, 2, …., 12), d_l: parity effect (l=1, 2, 3, 4 and 5+) and e_{ijklm}; error term.

The following statistical model was used in the analysis of HL and PL traits:

$$y_{ijklm} = \mu + a_i + b_j + c_k + d_l + e_{ijklm} \quad (3)$$

Here y_{ijklm}; observation value of the trait, μ; the mean of the trait, a_i; culling year (i=2014, 2015, ….2020), b_j; culling season (j=winter, spring, summer, autumn), c_k; number of calving (k=1, 2, 3, 4 and 5+), d_l: reason for removing the herd (l= slaughtering, death, sale for slaughtering or breeding) and e_{ijklm}; error term.

The statistical model used in the analysis of LL, LMY and 305-dMY is as follows

$$y_{ijkl} = \mu + a_i + b_j + c_k + e_{ijkl} \quad (4)$$

Here y_{ijkl}; observation value of the trait, μ; the mean of the trait, a_i; calving year effect (j= 2011, 2012, ….. 2020), b_j; calving month effect (j=1, 2, …., 12), c_k; parity effect (k=1, 2, 3, 4 and 5+) and e_{ijkl}; stands for the error term.

Results

Fertility traits

The mean FCA of SIM cattle was 842.35±5.30 days (28.08 months), and the effects of birth year ($P<0.01$) and month ($P<0.05$) on FCA were significant (Table 1). The longest mean of FCA was obtained for 2008 year (1019.04±30.56 days; 34.0 months), and the shortest for

2016 year (796.04±18.28 days; 25.5 months), and in all years the mean was calculated below 900 days (30 months), except 2008. In terms of birth month, the mean FCA ranged between 818.31±17.16 days (August) and 899.95±18.89 days (March).

The mean CI of SIM cattle was calculated as 422.98±3.18 days (14.1 months). The effects of calving year (P<0.01), calving month (P<0.01) and parity (P<0.05) on CI were significant (Table 1). In the statistical analysis of the CI data of SIM cattle, the effect of origin included in the model was also determined to be significant (P<0.05) and, the mean CI in cattle born in Austria (442.01±6.72 days) was 26.71 days longer than in cattle born in Türkiye (415.30±9.22 days).

The effect of calving year on CI was significant (P<0.01). The mean CI was calculated over 400 days for the years 2011-2020, the shortest year was 2012 (404.75±16.58 days), and the longest year was 2015 (469.16±10.09 days). While the month with the shortest CI mean was determined for cows that calved in December with 396.08±9.55 days, the longest month was determined for cows that calved in March with 476.42±13.97 days (Table 1). The difference of 37.32 days between the mean CI of cattle in the first lactation and the cattle in the third lactation was significant (P<0.05).

Longevity traits

Detailed records on the reasons for the culled cows from the herd could not be reached. The factors, averages and standard errors affecting the HL and PL traits of SIM cattle are given in Table 2. The overall means of HL and PL were calculated as 75.48±1.72 months and 47.15±1.73 months, respectively. The effects of the culling year and number of calving on HL and PL were significant (P<0.01), while the effects of the culling season and reason for culling were insignificant (P>0.05).

Table 1. First calving age (FCA, days) and calving interval (CI, days) of SIM cattle

Factor	FCA		CI	
	n	$\overline{X} + S_{\overline{X}}$	n	$\overline{X} + S_{\overline{X}}$
Birth/Calving year#		**		**
2008	11	1019.04±30.56^{a}	-	-
2009	83	843.88±11.44^{bc}	-	-
2011	27	812.31± 19.04^{bc}	89	406.20±17.33^{ab}
2012	25	804.91± 20.04^{bc}	59	404.75± 16.58^{a}
2013	16	896.29± 24.20^{b}	75	422.49± 11.58^{ab}
2014	31	872.81± 17.90^{bc}	74	412.19± 10.31^{a}
2015	33	852.81± 16.79^{bc}	60	469.16± 10.09^{b}
2016	29	796.04± 18.28^{c}	43	427.63± 12.07^{ab}
2017	35	823.50± 16.50^{bc}	51	462.97± 11.66^{ab}
2018	25	888.86± 19.08^{b}	58	424.87± 10.92^{ab}
2019	55	823.06± 13.54^{bc}	62	429.07± 10.68^{ab}
2020	-	-	48	427.22± 11.98^{ab}
Birth/Calving month		*		**
1	27	872.02± 18.92^{ab}	49	405.93±11.73^{ac}
2	27	881.90± 19.38^{ab}	42	432.51± 12.43^{abc}
3	29	899.95± 18.89^{a}	33	476.42± 13.97^{b}
4	24	864.03± 20.65^{ab}	27	468.15± 15.65^{b}
5	23	853.95± 21.63^{ab}	16	472.67± 19.08^{ab}
6	25	848.49± 20.35^{ab}	37	426.98± 12.83^{abc}
7	50	841.22± 14.38^{ab}	61	422.96± 9.96^{abc}
8	35	818.31± 17.16^{b}	66	399.48± 9.65^{c}
9	30	853.52± 17.61^{ab}	59	428.44± 10.03^{abc}
10	42	862.16± 14.97^{ab}	102	406.42± 8.04^{c}
11	29	877.23± 18.17^{ab}	60	407.82± 9.94^{c}
12	29	818.33± 18.46^{ab}	67	396.08± 9.55^{c}
Parity	-	-		*
1	-	-	240	446.88±7.06^{a}
2	-	-	162	436.44±7.32^{ab}
3	-	-	112	414.51± 8.18^{b}
4	-	-	61	428.62± 11.47^{ab}
5+		-	44	416.83± 14.31^{ab}
Origin				*
Austria	-	-	303	442.01±6.72^{a}
Türkiye	-	-	316	415.30±9.22^{b}
Overall	370	842.35±5.30	619	422.98±3.18

*: P<0.05, **:<0.01, a,b,c: the difference between groups with the same letter is insignificant according to P<0.05. #: Since the animals were brought to the farm as pregnant heifers from Austria and the animals born in the farm gave their first birth at the age of about two, there is no FCA data for 2010 in the farm records.

Table 2. Herd life (HL) and productive life (PL) of SIM cattle

Factor	n	HL, mo	PL, mo
		$\bar{X} + S_{\bar{X}}$	$\bar{X} + S_{\bar{X}}$
Culling year #		**	**
2014	22	68.47±2.74[a]	40.82±2.51[a]
2015	10	77.85±4.61[abc]	49.20± 4.23[ab]
2016	13	83.81±4.35[bc]	52.59± 3.99[ab]
2017	46	78.88±3.13[abc]	48.77± 2.87[ab]
2018	52	73.83± 2.77[ab]	43.82± 2.54[a]
2019	37	78.70± 3.57[abc]	50.05± 3.27[ab]
2021	32	84.81±3.37[c]	54.65± 3.09[b]
Culling season		NS	NS
1	96	77.46±2.79	48.58±2.56
2	19	78.24±3.47	47.56±3.18
3	43	77.53±2.59	48.90±2.37
4	54	78.98±3.20	49.18±2.93
No. of birth		**	**
1	41	48.62± 2.66[a]	18.36± 2.43[a]
2	47	66.77± 2.82[b]	36.38± 2.58[b]
3	32	73.99± 2.64[b]	45.37± 2.42[c]
4	41	89.81± 2.85[c]	60.38± 2.61[d]
5+	51	111.07± 2.91[d]	82.29± 2.67[e]
Culling reason		NS	NS
Slaughtering	170	74.05±1.43	45.95±1.31
Death	4	83.95±6.15	53.53±5.64
Selling	38	76.15±2.56	46.19±2.35
Overall	212	75.48±1.72	47.15±1.73

NS: non-significant, **:P<0.01, a,b,c,d: The difference between groups with the same letter is insignificant according to P<0.05. #: No record of the 2020 for culling.

It is understood that 212 animals were culled from the herd in the farm between 2014-2021 and, 17.9% (38 heads) of these animals were voluntarily culled from the herd and sold for breeding or slaughtering, and 82.1% (174 heads) were culled compulsorily. 1.9% (4 heads) of the forcibly culled animals were removed from the herd by death and 80.2% by being sent to slaughter.

According to the year of culling, the longest mean HL and PL were calculated as 84.81±3.37 months and 54.65±3.09 months, respectively, for 2021, while the shortest was 68.47±2.74 months and 40.82±2.51 months for 2014, respectively. Of the 212 herds, 19.3% (41 heads) once, 22.2% (47 heads) twice, 15.1% (32 heads) thrice, 19.3% (41 heads) four times and 24.1% (51 heads) were culled after five or more calving.

Milk yield traits

LL, LMY and 305-dMY were emphasized as milk yield traits of SIM cattle, and the averages and standard errors of the factors affecting these traits are given in Table 3. The mean of LL, LMY and 305-dMY were calculated as 363.52±3.52 days, 10,596±152 kg and 8647.0±58.0 kg, respectively. The effects of calving month on LL (P<0.01), calving year (P<0.01), calving month (P<0.01) and parity (P<0.01) on LMY (P<0.01), while only calving year effect on 305-dMY was significant (P<0.01).

The LL of SIM cattle raised in the farm was generally long, with 352.47±11.09 days the shortest in 2020 and the longest in 2015 with 388.53±10.44 days. When the situation is evaluated in terms of calving month, the LL average was over 400 days in March, April and May, and May was the month with the longest LL with 430.22±22.85 days.

While LMY average was above 10 tons in all other years except 2014, it exceeded 12 tons in 2019 and 2020 (Table 3). In terms of calving month, LMY average was over 10 tons in all other months except January, and it was around 12 tons in March, April and May, when the LL was longer in these months. In terms of the parity, while the third parity had a LMY average below 10 tons, it was around 11.5 tons on average due to the long LL during the first lactation.

The average of 305-dMY of SIM cattle was the lowest (6895.50±107.40 kg) in 2011 due to the fact that all of the cows were in the first parity, this year was followed by 2012 with 7719.70±114.32 kg and the averages, which were between 8-9 tons between 2013 and 2018, increased to over 10 tons in 2019 and 2020.

While the average of 305-dMY in terms of calving month was between 8-9 tons, the average of 305-dMY in terms of parity was over 8.5 tons or over for all parities (Table 3).

Table 3. Means and standard errors of lactation length (LL), lactation milk yield (LMY) and 305-day milk yield (305-dMY) of SIM cattle

Factor	n	LL, day		LMY, kg		305-dMY, kg
		$\bar{X} + S_{\bar{X}}$		$\bar{X} + S_{\bar{X}}$		$\bar{X} + S_{\bar{X}}$
Calving year		NS		**		**
2011	80	382.28±13.02	17	10277.08±670.84^{ab}	85	6895.50±107.40^{a}
2012	40	381.40±14.24	39	10036.59±480.09^{a}	49	7719.70±114.32^{b}
2013	45	379.70±13.77	39	10622.43±488.93^{ab}	50	8113.36±113.93^{bd}
2014	16	352.72±19.39	11	9625.98±728.16^{ab}	17	8786.37±162.46^{ce}
2015	52	388.53±10.44	28	10909.97±442.05^{ab}	59	8699.75±84.57^{ce}
2016	15	370.09±19.27	9	10178.81±754.51^{abc}	17	8339.84±156.61^{cd}
2017	36	387.24±12.05	19	11071.07±510.44^{abc}	44	8652.71±94.97^{cde}
2018	58	360.48±10.20	38	10507.78± 381.39^{a}	62	8922.44± 85.46^{e}
2019	60	360.23±10.02	37	12124.92±382.65^{bc}	67	10165.44± 81.97^{f}
2020	45	352.47±11.09	34	12765.94±395.16^{c}	47	10726.97±93.18^{g}
Calving month		**		**		NS
1	36	346.40±12.72^{ac}	22	9790.35 ±507.50^{a}	41	8551.71±102.48
2	26	376.90±14.76^{abc}	22	10419.27±494.86^{ab}	31	8562.45±116.81
3	23	413.96±15.52 ^{b}	16	12318.91±575.80^{b}	27	8700.27±125.00
4	20	420.11±17.35^{b}	11	11893.86±699.09^{ab}	21	8691.77±145.38
5	10	430.22±22.85^{ab}	8	12623.05±769.27^{bc}	11	8733.35±189.54
6	27	364.41±14.31^{abc}	17	10600.88±555.67^{ab}	30	8602.51±117.50
7	46	364.20±11.15^{abc}	32	10374.27±408.79^{ab}	49	8518.99±93.10
8	44	335.17±11.37^{c}	27	10479.21±433.66^{ab}	47	8795.46±95.07
9	39	361.32±12.05^{abc}	15	10840.55±571.39^{ab}	46	8830.90±96.90
10	80	354.57±9.13^{ac}	42	10203.36±366.83^{ab}	86	8864.64±75.69
11	40	347.46±11.60^{ac}	24	10200.84±456.24^{ab}	49	8784.34±91.85
12	56	343.46±9.96^{c}	35	10000.13±389.43^{a}	59	8790.11±83.30
Parity		NS		**		NS
1	172	383.80±8.09	81	11506.60±295.30^{a}	189	8720.43±65.72
2	111	365.65±8.74	89	10274.18± 322.38^{b}	123	8629.40±73.37
3	94	358.77±9.39	61	9989.93± 390.04^{b}	104	8639.46±77.75
4	32	373.81±13.43	21	11422.46± 498.86^{ab}	37	8745.20±107.86
5+	38	375.55±12.52	18	10867.12± 533.05^{ab}	44	8776.55±101.85
Overall	447	363.52±3.52	271	10596±152	497	8647.0±58.0

NS: non-significant, **:P<0.01, a,b,c,d,e,f,g: The difference between groups with the same letter is insignificant according to P<0.05.

Discussion

Fertility traits

The overall mean of FCA found for SIM breed (842.35±5.30 days or 28.08 months), which showed significant changes according to calving year and calving months, was found to be approximately 4 months longer than the 24 months accepted ideal for foreign cattle breeds. While the FCA overall mean had longer values ranging from 2.5 months (2016) to 10 months (2008) depending on the year of birth, it had longer values ranging from 4 (August and December) to 6 months (March) depending on the month of birth.

The mean FCA found for SIM cattle in this study was lower than all reported values (Akbulut, 1998; Şekerden et al., 1999; Özkan and Güneş, 2011a; Koç, 2016b, 2017) for the same breed. Akbulut (1998) and Koç (2016b) both of them compiled studies on SIM cattle raised in Türkiye, reported the average of FCA as 908±52.7 days, and 913±37.03 days, respectively. On the other hand, the averages of 955.2±13.62 days (Koç, 2016b) and 851.6±6.19 days (Koç et al., 2011) reported for MB and RH breeds reared in Aydın province conditions, respectively, are higher than the average obtained in this study.

As widely known, heifers are inseminated at the age of 14-16 months. If the heifers' birth is between January and April, it would clarify that insemination was applied on summer time, when the air temperature and humidity are high. Because of that FCA is prolonged in these months due to the increased rate of unclear estrus, decrease of the fertilization of sperm and egg, increase in embryonic deaths and failure of the embryo to attach to the uterus. In fact, it is observed that some animal breeders sometimes do not inseminate during this period because they know that the success of insemination is low in the summer months.

Similar to FCA, the overall CI mean for SIM cattle (422.98±3.18 days or 14.1 months) average is also determined as longer than 365 days, which is considered ideal, between 39.8 days (2012) and 104.2 days (2015) according to calving years, and between 34.1 days (December) and 111.4 days (March) according to calving months.

The mean CI (422.98±3.18 days) calculated for SIM cattle in this study was determined to be higher than the values reported by Akbulut (1998), Çilek and Tekin (2006), Özkan and Güneş (2011a), Erdem et al. (2015), Koç (2016b), Koç and Arı (2020) for the same breed and higher than the averages reported by Koç (2016b) for MB,

Koç and Arı (2020) for the RH, Koç et al. (2011) for RH. The mean of 446.88±7.06 days calculated for the SIM breed in this study in the first parity is higher than the mean (421.4±7.66 days) reported by Koç and Gürses (2020) for RH and HF breeds at the first parity.

Longevity traits

The 75.48-month HL average calculated for SIM cattle in this study can be considered a positive situation when Oltenacu (2009)'s rate of survival at 48 months of age is taken as a criterion. However, when both HL and PL are considered and considering that CI should be 12 months, cows with one birth stay in the herd for 18.36 months and cows with two births stay in the herd for 36.38 months and they culled at the age of 48.62±2.66 months and 66.77±2.82 months, respectively (Table 2), indicating that there is a fertility problem on the farm. It is understood that the farm prefers to keep these cows in the herd for a longer period of time, probably because their milk yield is satisfactory, instead of culling the animals that have fertility problems at an earlier age. On the other hand, it should be considered as a high rate that 19.3% of the cows after giving birth once, and 22.2% of the cows after giving birth twice removed from the herd.

The mean HL found for SIM cattle (75.48±1.72 months or 2264.4 days) in this study was longer than the values of Koç (2017) who found 1674.88±133.89 days (55.82 months), 1614.16±133.56 days (53.81 months) and 1634.93±110.54 days (54.5 months) for HF, RH and SIM breeds reared in the same farm, respectively, and the value (2073 days or 69.1 months) of Yaylak (2003) and the value (2229.07±18.53 days or 74.3 months) of Boğokşayan and Bakır (2013).

The mean PL (47.15±1.73 months or 1414.5 days) found for SIM cattle in this study was longer than the values of Koç (2017) who found 871.38±120.05 days (29.05 months), 773.84±120.65 days (25.8 months) and 740.49±99.11 days (24.7 months) for HF, RH and SIM breeds, respectively, and also the values reported for HF by Yaylak (2003), Kara et al. (2010) and Boğokşayan and Bakır (2013). Yaylak repoted 1060 days or 35.3 months, Kara et al. (2010) reported 36.8 ± 2.60 months and Boğokşayan and Bakır (2013) reported 1236.10±13.87 days (41.2 months) for HF breed.

Milk yield traits

In this study, although the LL of the SIM breed was found to be 58.5 days longer than the standard lactation period, the average LMY of the breed was above 10 tons in all years except 2014. In addition, since all cows on the farm are in 1st and 2nd lactation in the first 2 years, if these years are not taken into account, 305-dMY being over 8 tons should be considered as a very high milk production level for the SIM breed.

The mean LL obtained in this study of SIM cattle (363.52±3.52 days) was determined to be higher than the all means reported by Abkulut (1998), Çilek and Tekin (2006), Özkan and Güneş (2011b), Erdem et al. (2015), Koç (2016b), Koç and Arı (2020) for the same breed, and Çerçi (2006) for HF, Koç (2006) for HF and Brown-Swiss breeds, Yılmaz (2010) and Koç and Arı (2020) for RH breed and Koç (2016b) for MB breed.

The mean LMY obtained for the SIM breed (10,596±152 kg) in this study was higher than 3072±146 kg as reported by Akbulut (1998), 3368.11±38.49 kg as reported by Özkan and Güneş (2011b), 5746.5±65.47 kg reported by Erdem et al. (2015), 4756±59.41 kg and 5918.7±75.30 kg reported by Okuyucu et al. (2018) for the first and the second parity cows of SIM breed raised in intensive dairy cattle farms in the Konya region, and 7357.03±88.12 kg reported by Koç and Arı (2020) for SIM breed. Additionally, the LMY average calculated for the SIM breed in this study is higher than all of the LMY values reported by Çerçi (2006) for the HF breed, Koç (2016b) for the MB breed, Yılmaz (2010) and Koç and Arı (2020) for the RH breeds.

Similar to Koç (2009) who conducted a study on HF and MB breeds and Yılmaz (2010) for RH breed, the effect of year on 305-dMY was found to be significant in this study, but unlike Koç (2009) and Yılmaz (2010), the parity effect on 305-dMY was detected to be insignificant in this study ($P<0.05$).

In terms of the 305-dMY (8647.0±58.0 kg) mean of the SIM breed, a similar situation with LL and LMY was seen when compared with the mean values obtained in other studies. In this study, the 305-dMY average obtained for the SIM breed was determined to be higher than all the means reported by Şekerden and Erdem (1997), Akbulut (1998), Şekerden et al. (1999), Çilek and Tekin (2006), Özkan and Güneş (2011b), Erdem et al. (2015), Koç (2016b), Koç and Arı (2020) for the same breed, Koç (2001) and Çerçi (2006) for HF, Koç (2006) for HF and Brown-Swiss, Koç (2009) for HF and MB breeds, Yılmaz (2010) and Koç and Arı (2020) for RH, Koç (2016b) for MB and Koç and Gürses (2020) for the RH and HF.

In this study, LL, LMY and 305-dMY averages obtained for the SIM breed were found to be higher than all the averages reported for the same breed and other breeds in previous studies in Türkiye. The high LMY and 305-dMY averages of SIM breed reveals the reason why breeders have increased interest in breeding this breed of Austrian and German origin in recent years, in addition to its other characteristics.

In addition to the fact that the SIM breed has a high LMY and 305-dMY average in this farm, the management and feeding practices applied in the farm where it is grown are appropriate, as can be understood from the CI average of this breed (422.98±3.18 days) that the high milk yield potential of the breed by keeping the days open period long, it is understood that the LL was extended (363.52±3.52 days) to benefit from it.

Conclusion

Some important information was obtained about fertility, longevity and milk yield of Austrian origin SIM (Fleckvieh) cattle, known as dual purpose cattle but with increased milk yield, for which the interest of dairy cattle breeders in Türkiye has increased in recent years.

The FCA (842.35±5.30 days or 28.1 months) and CI (422.98±3.18 days or 14.1 months) means of SIM cattle found in this study were about four and two months longer than expected, respectively. The mean HL and PL of this breed were calculated as 74.48±1.72 months and 47.15±1.73 months. When the PL, FCA and CI averages of

SIM cattle are taken into account, it is understood that 3.34 births per cow participating in the herd are obtained. On the other hand, although the mean LL of SIM cattle (363.52±3.52 days) was about one year, the mean LMY (10,596±152 kg) was over 10 tones, and the mean of 305-dMY (8647.0±58.0 kg) was over 8.5 tones. The fact that FCA and CI averages are longer in these farm conditions where Austrian origin SIM cattle raised can be interpreted as a number of problems in terms of reproductive efficiency of SIM cattle, however, considering the high milk yield of this genotype and the dry period from the LL (363.52±3.52 days) and CI (422.98±3.18 days) averages, it is understood that the share of the dry period, which is an unproductive period, was tried to be reduced. Thus, it can be said that the milk yield per cow and accordingly the profitability of the farm increase.

In conclusion, it has been determined that the milk yield of Austrian origin SIM cattle is as high as the milk yield of HF breed, which is widely grown in Türkiye and the world, and even higher in some farms. While this genotype provides significant advantages in terms of HL, carcass yield and price are generally higher than the HF breed when culled animals are sent to slaughter, and considering the instability in milk prices and the increase in red meat prices, it is understood why breeders preferred to breed this genotype in recent years.

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Effect of Seed Rates and Sowing Dates on Productivity of Wheat (*Triticum aestivum* L.)

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ABSTRACT

A field experiment was conducted at the Agronomy farm of Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur to evaluate the effect of sowing dates and seed rates on yield and yield attributes of wheat. The experiment was laid in a split-plot design with three replications treated with 4 sowing dates as the main plot factor (12th Nov, 27th Nov, 12th Dec, and 27th Dec) and 4 seed rates as subplot factor (100 kg ha^{-1}, 120 kg ha^{-1}, 140 kg ha^{-1} and 160 kg ha^{-1}). Results revealed that the leaf area index was significantly affected by sowing dates and was comparatively superior in 2nd sowing date (27th Nov) wheat. Similarly, in the case of seed rates, the leaf area index was influenced significantly and was recorded to be increasing with an increase in seed rates. Phenological parameters like days to 50% heading, flowering, and maturity were observed maximum (116, 123, and 179 days, respectively) in early sown wheat and reduced with the subsequent delay in sowing. Maximum values of yield and yield attributes like effective tillers per meter square (635.6), spike length (9.56 cm), grains per spike (41.49), grain yield (7.59 Mt ha^{-1}), and straw yield (9.58 Mt ha^{-1}) were observed in the wheat sown in 2nd date (on 27th Nov) which differed significantly to wheat sown on other dates. Seed rates had no significant influence on grain yield and yield attributes. Thousand-grain weight was found maximum (46.26 g) in early sown wheat (on 12th Nov sown wheat, reduced with the subsequent delay in sowing, and the harvest index was observed as maximum (0.51) under December 27 sown wheat. Though the yield and its attributes were not influenced significantly by seed rate, the maximum yield (6.18 Mt ha^{-1}) was observed in wheat sown at the rate of 120 kg ha^{-1}. Considering seed yield and its parameters, 2nd date of sowing wheat (27th Nov) and seed rate of 100 kg ha^{-1} could be the best option to uplift the productivity of wheat in rainfed lowland conditions of Lalitpur, Nepal.

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Introduction

Wheat (*Triticum aestivum* L., 2n=42), also known as the king of cereals is an important source of food across the world. It belongs to the *Poaceae* family and globally, after maize, is the most widely cultivated cereal (Mourad et al., 2019; Ghahremaninejad, 2021). The extent of its success hinges on its adaptability and high yield potential, as well as the gluten protein fraction (Shewry, 2009; Sousa et al., 2021). Wheat cultivation has been a longstanding practice in Nepal, in various regions such as Terai, river basins, mid-hills, and high hills, occurring in the winter season from October to July. Though it is cultivated as a winter crop, differences in geography and topography makes the sowing period of wheat differ in different portions of the country (Poudel, 2013). It is produced on 0.70 million hectares of land in the country yielding about 2.2 million metric tons with a productivity of 3.08 Mt ha^{-1} (MoALD, 2020). There can be several factors which are responsible for low wheat productivity in the country like irrigation, fertilizer, climate, insects, pests, etc. but with proper management of the crop wheat production and productivity can be improved from the present scenario (Pokherel et al., 2007).

Sowing date and seed rate have always been a problem for the cultivation of wheat. Deviation from the optimal range of seed rate has an adverse effect on plant growth and yield. In the case of suboptimal seed rate, the available resources for plant growth are not efficiently utilized or even wastage of the resources occurs. Similarly, the use of more than optimal seed rate causes a dense population which makes the plant compete for radiant energy and other nutrients (Jan et al., 2000). Reducing the seed rate in

the vegetative growth stage leads to decreased competition between plants. However, during the grain-filling phase, increasing the number of tillers intensifies competition within each plant (Shazma et al., 2015).

Early planting ensures optimum emergence through sufficient tiller number per unit area. However, each weak delay of sowing reduces the vegetative and reproductive phases and affects the yield-attributing characteristics leading to yield reduction (Akmal et al., 2011; Malik et al., 2009). Similarly, the sowing date had a greater influence on grain yield quantity and quality compared to seed rate. However, consistently lower yields were obtained with reduced seed rates (McKenzie et al., 2011).

Proper sowing date with optimal seed rate is the need of farmers growing wheat crops. So this experiment focuses on the relationship between sowing date and seed rate in wheat crops in the hilly region of the country. Effects of sowing date and varied seed rate under study may result in the finding of appropriate sowing time and optimal seed rate for better productivity in the regions of similar climatic conditions which in return can improve the overall production trend of wheat. The findings might be fruitful directly to the regions having similar climatic conditions of research site only which seems to be a limiting factor for considering the overall wheat production trend in the country.

Materials and Methods

Location

The experiment was conducted in the research field of the Agronomy Division of Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur, Nepal from November 2020 to June 2021. The station is located at an elevation of 1360 meters above sea level.

Physio-chemical Characteristics of Experimental Soil

The soil of the experimental plot was tested by Nepal Agricultural Research Council (NARC) and the final soil status was provided. As per the available data, the soil was acidic in pH (5.98) containing low organic matter (2.01%), medium total Nitrogen (0.139%), high available P_2O_5 (478.6 kg ha^{-1}), and medium available K_2O (160.5 kg ha^{-1}). The soil texture was silty clay loam, and the average bulk density was 1.39 gm cm^{-3}(Table 1).

Climatic Conditions during Field Experimentation

Daily maximum and minimum temperatures for each month were recorded using maximum and minimum thermometer during the entire research period from November 2020 to June 2021. The maximum monthly average temperature (T_{max}= 27.8˚C and T_{min}=20 ˚C) was recorded in June and the minimum monthly average temperature was recorded in January (T_{max}=19.6˚C and T_{min}=3.5˚C). Daily rainfall for each month was recorded using rain guage and the total monthly rainfall was found higher in May (127.6 mm) and June (204 mm). Average relative humidity of 75±5 % was recorded (using hygrometer) for the entire research period (Figure 1).

Experimental Design and Treatments

The experiment was conducted in a split-plot design with two treatments and three replications. The sowing date was assigned as the main plot and the seed rate was assigned as a subplot in the experiment (Table 2).

Varietal Description

The variety under experimentation was WK3026 (zinc-fortified breeder seed). It was a pipeline variety that was under research by NARC and yet to be released at the period of the experiment. It was the variety that was foreseen as a promising one and of high potential from the previous trials done by the Division of Agronomy.

Field layout and crop geometry

A total of 48 plots with a dimension of 4m×3m was made with each plot consisting of 15 rows of wheat sown continuously at the spacing of 20 cm between the rows. A spacing of 0.75m between replication and 0.5m between plots was maintained. Ten rows were treated as net plot rows for harvesting, two for destructive sampling, and the other as guard rows.

Table 1. Physio-chemical characteristics of the soil in the experimental plot during 2020.

Soil Characters	Description
Soil texture	Silty clay loam
Soil pH	5.98
Bulk density	1.39 g cm^{-3}
Organic matter content	2.01%
Total nitrogen	0.139%
Available P_2O_5	478.6 kg ha^{-1}
Available K_2O	160.5 kg ha^{-1}

Source: NARC, 2021

Table 2. List of treatments used in the experiment.

Main plot factor A	Subplot factor B
1st date of sowing (D_1) = 12 Nov. 2020	Seed rate (S_1) = 100 kg ha^{-1}
2nd date of sowing (D_2) = 27 Nov. 2020	Seed rate (S_2) =120 kg ha^{-1}
3rd date of sowing (D_3) = 12 Dec. 2020	Seed rate (S_3) = 140 kg ha^{-1}
4th date of sowing (D_4) = 27 Dec. 2020	Seed rate (S_4) = 160 kg ha^{-1}

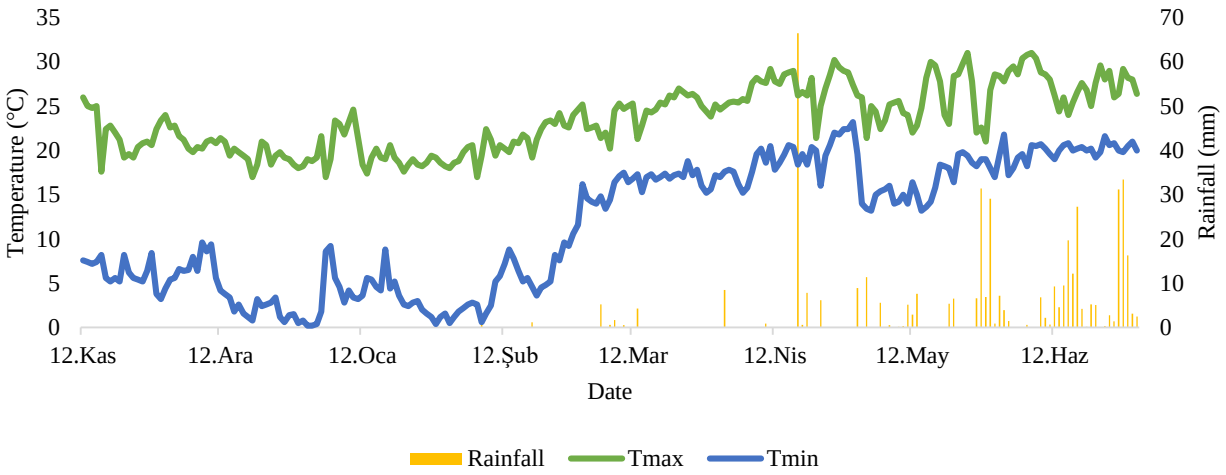

Figure 1 Daily temperature and rainfall during the experimental period.

Field Preparation

The experimental field was first cleared and then ploughed twice. Secondary and tertiary tillage operations were carried out to obtain a fine tilth of soil before sowing.

Fertilizer Application

The blanket recommended dose of the fertilizer for wheat cultivation in the hill region i.e., 6 mt ha^{-1} FYM and 100:40:40 N: P_2O_5:K_2O kg ha^{-1} was applied to meet the crop nutrient demand. Urea, DAP, and MoP were the fertilizer sources of the primary nutrients supplied to the crops. A full dose of P_2O_5, K_2O, and half dose of nitrogen was applied as basal dose at the time of final land preparation before sowing. The remaining half dose of nitrogen was applied in two splits, first at the active tillering stage and second at the booting stage (50% plant population) of the crop.

Seeds and Sowing

Pure breeder seeds of the WK3026 variety of wheat were sown continuously at the spacing of 20 cm between the rows and with seed rate as per treatment. The sowing of seeds was done manually on four different dates at an interval of fifteen days starting from the 12th of November, 2020.

Weeding and Irrigation

The crop-weed competition in the experimental field was checked by controlling the weed growth through manual or hand weeding. Weeding was done as per the need to make the field weed free. No chemical weedicides were used for checking the weed infestation. Irrigation was provided during the crown root initiation stage (CRI). The crop water requirement was fulfilled by rainwater on other critical stages and when short, external irrigation was provided.

Harvesting and Threshing

After the crop reached the harvest maturity stage showing different maturity indices (brittle/distorted awn, yellowing of stem, dry flag leaf, etc.), manual harvesting was done with the help of sickles. The harvested plants were left in the field to sundry. A pedal thresher was used for threshing and winnowing was done for cleaning the seeds.

Observations Taken

Leaf area index (LAI)

The leaf area measurement was done manually by measuring the average length and breadth of leaves of five randomly selected plants from the destructive sampling row. The length was measured directly with scale and for breadth, the leaf was folded thrice to divide the leaf into four equal halves, and the three breadths measured from folded points were worked out to find an average breadth of the leaf and then used for leaf area calculation. The leaf area obtained was then used to calculate the LAI using the following formula.

$$\text{Leaf area index (LAI)} = \frac{\text{Leaf area}}{\text{Ground area}}$$

Where, both the leaf area and ground area were worked out in cm^2.

Days to heading

The number of days taken from the day of sowing to the day to reach the heading stage (full exertion of the spike) in 50% plant population within a plot was noted.

Days to flowering

The number of days taken from the day of sowing to the day 50% plant population within a plot reached the flowering stage was noted.

Days to physiological maturity

The days to 50% maturity from the days of sowing within a plot were noted. It is the number of days taken by 50% plant population within a plot to reach the physiological maturity stage which is indicated by the maturity indices like yellowing and drying of flag leaf, yellowing of stem, yellowing of the spikes, etc.

Effective number of tillers

The effective number of tillers was determined by counting the number of tillers in an entire row in each plot before harvesting. This number was later worked to calculate the number of effective tillers per square meter.

Spike length

Ten spikes were randomly selected from the net plot and the length (cm) was measured from the base to the tip of the spike excluding the awn with the help of a measuring scale.

Number of grains per spike

The ten spikes that were randomly selected for measuring the spike length were used for the determination of the number of grains per spike. The grains were first detached/separated from the spike and then counted for ease of work.

Thousand-grain weight

A thousand grains were counted from randomly separated grain yield from the yield of the net plot and weighed (in grams) with the help of electronic digital balance.

Grain yield and straw yield

Grain yield and straw yield were taken at harvesting from the net plot. The crop was dried, threshed, sun-dried; cleaned, and the final weight of both straw and grain (before drying and after drying) was taken. The grain yield per hectare was calculated for each treatment. An automatic moisture recording meter was used for accessing the moisture percentage of grains.

$$\text{GYM} = \frac{(100 - \text{MC}) \times \text{plot yield (kg)} \times 10000}{(100 - 14) \times \text{net plot area}}$$

Where GYM is Grain yield (kg per ha) at 14% moisture and MC is the moisture content of grains in percentage. The straw yield was also recorded from the net plot and then later worked into a hectare.

Harvest Index

The Harvest index (HI) was calculated by dividing the grain yield by the biological yield.

$$\text{HI} = \frac{\text{Grain yield}}{(\text{Grain yield} + \text{Straw yield})}$$

Statistical analysis

The complete recorded data were entered in Microsoft Excel and further subjected to analysis of variance. The statistical package GENSTAT was used for the analysis of the data. Duncan's Multiple Range Test (DMRT) was used for mean comparison at a 0.05 level of significance.

Results

Leaf Area Index

The mean leaf area index was found to increase from 45 days after sowing (DAS) to 105 DAS and decrease thereafter (Table 3). The leaf area index was found significantly different among different sowing dates at different stages of crop growth except at 90 DAS. Within sowing dates, the LAI of wheat that was sown on Nov 12 and Nov 27 were statistically the same but were statistically different from the LAI of the wheat plants sown on Dec 12 and Dec 27. LAI of the wheat plants that were sown on Dec 27 was found lowest throughout the crop growth period. Similarly, among seed rates, there was no significant difference in LAI in the initial stages of crop growth. However, in the later stages, LAI was significantly affected by seed rate. The LAI of wheat crop sown at a seed rate of 160 kg ha^{-1} was found highest and that of wheat sown at a seed rate of 100 kg ha^{-1} was found lowest in the later stages. No interaction was found among seed rates and sowing dates for LAI.

Days to 50% Heading

The mean days to 50% heading was found to be 108 days (Table 4). The date of sowing had a significant effect on days to 50 % heading but no significant influence was observed on days to 50 % heading due to seed rate. The experiment showed that days to 50 % heading in wheat was shortened due to delays in the sowing of the crop. Wheat that was sown on November 12 took the highest (116 days) number of days to reach the 50% heading stage and the number of days gradually decreased with the subsequent delay in sowing. Wheat that was sown on December 27 reached 50 % maturity in the shortest (97 days) period. There was no significant impact of seed rates on days to the heading of wheat i.e., the effect of varied seed rates on days to heading was statistically the same. No interaction was observed among the treatments.

Table 3. Leaf area index as influenced by date of sowing and seed rates at different stages of wheat growth.

Treatments	Leaf Area Index (mean ± SE)						
	45DAS	60DAS	75DAS	90DAS	105DAS	120DAS	135DAS
Sowing Date (A)							
Nov 12	1.3±0.2^{a}	2.8±0.3^{a}	3.2±0.3^{a}	4.3±0.3	4.5±0.2^{ab}	5.5±0.6^{a}	4.9±0.4^{a}
Nov 27	0.8±0.1^{ab}	1.9±0.2^{ab}	3.3±0.3^{a}	5.0±0.5	5.2±0.3^{a}	6.6±0.7^{a}	4.8±0.5^{a}
Dec 12	0.4±0.0^{b}	1.2±0.2^{b}	1.9±0.3^{b}	3.7±0.5	4.0±0.3^{b}	2.4±0.2^{b}	1.8±0.2^{b}
Dec 27	0.5±0.1^{b}	1.1±0.1^{b}	1.7±0.3^{b}	3.3±0.6	2.7±0.2^{c}	1.5±0.2^{b}	0.5±0.1^{b}
SEM (±)	0.1448	0.3506	0.342	0.726	0.151	0.494	0.6523
LSD (<0.05)	0.5012	1.2133	1.182	NS	0.524	1.710	2.2574
Seed Rate (B)							
100 kg ha^{-1}	0.7±0.2	1.6±0.3	1.8±0.3^{b}	3.6±0.5	3.4±0.3^{b}	2.9±0.4^{b}	2.6±0.6^{b}
120 kg ha^{-1}	0.8±0.2	1.6±0.2	2.3±0.4^{ab}	3.6±0.5	4.1±0.4^{ab}	4.2±0.8^{a}	3.0±0.6^{ab}
140 kg ha^{-1}	0.8±0.2	1.9±0.2	2.9±0.3^{a}	4.8±0.6	4.2±0.3^{ab}	4.7±1.0^{a}	3.0±0.6^{ab}
160 kg ha^{-1}	0.8±0.1	1.9±0.4	2.9±0.4^{a}	4.3±0.4	4.8±0.4^{a}	4.2±0.8^{a}	3.4±0.8^{a}
SEM (±)	0.1157	0.1787	0.267	0.404	0.201	0.381	0.1995
LSD (<0.05)	NS	NS	0.781	NS	0.588	1.113	0.5823
A*B	NS	NS	NS	NS	NS	NS	NS
CV%	52.7	35.9	36.8	34.4	17.0	33.0	22.9
Grand Mean	0.76	1.72	2.52	4.07	4.10	4.00	3.01

Means followed by a common letter (s) are not significantly different from each other based on DMRT at a 5% level of significance. SEM= standard error of means, LSD= least significant difference, CV= coefficient of variance, and NS= non-significant.

Table 4. Phenological observations as influenced by date of sowing and seed rate in growth of wheat.

Treatments	Days to 50 % heading (mean ± SE)	Days to 50% flowering (mean ± SE)	Days to 50% maturity (mean ± SE)
Sowing Date (A)			
Nov 12	116±0.4[a]	123±0.5[a]	179±0.2[a]
Nov 27	113±0.2[b]	116±0.2[b]	168±0.2[b]
Dec 12	105±0.4[c]	109±0.3[c]	157±0.2[c]
Dec 27	97±0.4[d]	101±0.4[d]	145±0.1[d]
SEM (±)	0.27	0.24	0.21
LSD (<0.05)	0.95	0.84	0.72
Sowing Rate (B)			
100 kg ha^{-1}	108±2.0	112±2.3	162±3.9
120 kg ha^{-1}	108±2.3	113±2.7	162±3.8
140 kg ha^{-1}	107±2.1	112±2.5	162±4.0
160 kg ha^{-1}	108±2.3	112±2.5	162±3.8
SEM	0.37	0.34	0.15
LSD (<0.05)	NS	NS	NS
A*B	NS	NS	NS
CV%	1.2	1.1	0.3
Grand mean	108	112	162

Means followed by a common letter (s) are not significantly different from each other based on DMRT at a 5% level of significance. SEM= standard error of means, LSD= least significant difference, CV= coefficient of variance, and NS= non-significant.

Table 5. Yield and yield attributes as influenced by seed rates and sowing dates on wheat.

Treatments	Effective Tillers/m^2	Spike Length (cm)	Grains/Spike	Grain yield (Mt ha^{-1})	SDM (Mt ha^{-1})	TGW (g)
Sowing Date (A)						
Nov 12	477.6±16.1[b]	9.0±0.0[b]	39.5±0.9[ab]	6.5±0.2[b]	8.9±0.3[a]	46.3±0.5[a]
Nov 27	635.6±40.8[a]	9.6±0.1[a]	41.5±0.5[a]	7.6±0.2[a]	9.6±0.3[a]	45.8±0.3[a]
Dec 12	498.5±15.7[b]	9.0±0.0[b]	36.8±0.8[bc]	5.3±0.1[c]	7.4±0.2[b]	43.8±0.8[b]
Dec 27	382.3±18.8[c]	8.8±0.1[b]	35.2±0.4[c]	5.2±0.1[c]	4.9±0.2[c]	41.2±0.8[c]
SEM (±)	27.3	0.14	0.98	0.29	0.35	0.42
LSD (<0.05)	94.6	0.49	3.40	1.01	1.21	1.45
Seed Rate kg ha^{-1} (B)						
100 kg ha^{-1}	457.6±36.6	9.1±0.1	37.2±0.9	6.1±0.4	7.6±0.7	45.6±0.9
120 kg ha^{-1}	500.4±35.3	9.1±0.1	38.7±1.1	6.2±0.4	7.8±0.5	44.1±0.7
140 kg ha^{-1}	526.0±31.7	9.1±0.1	38.5±1.0	6.2±0.3	7.4±0.6	43.7±0.7
160 kg ha^{-1}	510.0±40.7	9.1±0.1	38.6±1.0	6.1±0.3	7.9±0.6	43.7±1.0
SEM (±)	28.3	0.03	0.50	0.16	0.25	0.64
LSD (<0.05)	NS	NS	NS	NS	NS	NS
A*B	NS	NS	NS	NS	NS	NS
CV%	19.6	1.2	4.5	9.0	11.3	5.0
Grand mean	499	9.1	38.25	6.13	7.69	44.27

Means followed by a common letter (s) are not significantly different from each other based on DMRT at a 5% level of significance. SDM= Straw dry matter, TGW= Thousand grain weight, SEM= standard error of means, LSD= least significant difference, CV= coefficient of variance, and NS= non-significant.

Days to 50% Flowering

In the experiment, the mean days to 50 % flowering was found to be 112 days (Table 4). The date of sowing had a significant effect on days to 50 % flowering but no significant influence was observed on days to 50 % flowering due to seed rate. The days to reach 50 % flowering was observed highest (123 days) in wheat sown on November 12 and this period decreased with the subsequent delay in sowing. Wheat sown on December 27 reached 50 % flowering stage in the shortest period (101 days). The effect of varied seed rates on days to reach 50 % flowering stage was statistically found to be the same. No any sort of statistical interaction was observed among the treatments.

Days to 50% Maturity

In the experiment, the mean days to reach 50 % maturity was found to be 162 days (Table 4). A significant influence on days to 50 % maturity was observed due to variation in sowing dates but there was no significant effect of varied seed rate on it. The number of days to reach 50 % maturity was found highest (179 days) in the wheat that was sown on November 12 and this period subsequently decreased over delay in sowing dates. Wheat that was shown on December 27 reached 50 % maturity in the shortest period (145 days). The effect of varied seed rates on days to reach the 50 % maturity stage was statistically found to be the same. No any sort of statistical interaction was observed among the treatments.

Effective Tillers/m^2

In the experiment, the mean effective number of tillers per square meter was found to be 499 (Table 5). It was observed that the effective number of tillers was significantly influenced by the date of sowing but there was no significant influence on the effective number of tillers due to varying seed rates. No interaction was observed between the two treatments. As per Table 5, the highest number of effective tillers per square meter (635.6) was observed in wheat sown on November 27. The effective number of tillers per square meter of wheat grown on November 12 and December 12 was statistically the same. Wheat that was sown on December 27 had the least number of effective tillers per square meter (382.3).

Spike Length

The mean spike length of wheat in the experiment was found to be 9.1 cm (Table 5). It was observed that the spike length of wheat was influenced significantly by the date of sowing but there was no significant influence of varied seed rate on the length of the spike. Wheat sown on November 27 had the highest length of the spike (9.56 cm) which was significantly different from the length of the spike of wheat sown on other dates. Wheat sown on December 27 had the least (8.77 cm) length of the spike. It showed that both early and late planting reduced the length of the spike. No interaction between the treatments was observed.

Number of grains per spike

In the experiment, it was found that the mean grains per spike were 38.25 (Table 5). The grains per spike were also significantly influenced by the date of sowing but not by the varying seed rates. Wheat sown on November 27 had the highest (41.49) grains per spike which was statistically similar to wheat sown on November 12 (39.49) but significantly differed from the values of wheat sown late in the season. The grains per spike in the wheat that was sown on December 27 had the minimum value as per the results of the experiment. Interaction among the treatments for grains per spike was not observed in the experiment.

Grain Yield (t ha^{-1})

The mean grain yield of wheat in the experiment was found to be 6.13 t ha^{-1} (Table 5). It was observed that the grain yield of wheat was significantly influenced by the date of sowing but no influence was observed due to a change in seed rate statistically. No interaction between the treatments was observed for the grain yield of wheat. As per the results, wheat sown on November 27 had a maximum grain yield (7.59 t ha^{-1}) that was statistically different from the yield of wheat sown on other dates. This value was followed by the wheat sown on November 12 i.e., 6.45 t ha^{-1} and the wheat that was sown on December 27 had the least grain yield of 5.17 t ha^{-1}. Although the grain yield was not significantly influenced by varied seed rates, it was observed that grain yield was maximum under wheat sown at a seed rate of 120 kg ha^{-1} and minimum under wheat sown at a seed rate of 160 kg ha^{-1}.

Straw Yield (t ha^{-1})

The mean straw yield in the experiment was 7.69 t ha^{-1} (Table 5). It was observed that the date of sowing had a significant influence on the straw yield but no significant difference was observed in the yield of straw due to changing seed rates. The straw yield of wheat that was sown on November 27 had a maximum value (9.58 t ha^{-1}) which was statistically similar to the straw yield of wheat sown on November 12 (8.88 t ha^{-1}) but significantly differed in the yield of straw sown later in the season. The straw yield of wheat sown on December 27 was found minimum i.e., 4.95 tons ha^{-1}. Although the straw yield was not significantly influenced by seed rates, the maximum yield of straw was observed under the seed rate of 160 kg ha^{-1}. The interaction between the treatments was not observed for the yield of straw in the experiment.

Thousand Grain Weight (g)

The mean thousand grains weight in the experiment was found to be 44.27 g (Table 5). A thousand grains' weight was also influenced significantly by the date of sowing but there was no significant influence due to seed rate on it. The thousand-grain weight was observed maximum in the wheat that was sown on November 12 (46.26 g) which was statistically similar to the thousand grains weight of the wheat that was sown on November 27 (45.84 g) but significantly differed in values in the wheat sown later on the season. Thousand-grain weight of wheat sown on December 27 was observed as a minimum (41.19 g). Although seed rate had no significant influence on thousand-grain weight, the highest value (45.58 g) was observed in the wheat sown at the rate of 100 kg ha^{-1}.

Harvest Index

In the experiment, the mean harvest index of the wheat crop was observed to be 0.45 (Table 6). It was found that the harvest index was significantly influenced by the date of sowing but no significant difference was found due to the seed rates. The Harvest index was statistically the same for varied seed rates. However, as per the results harvest index was found maximum in the wheat that was sown on December 27 (0.51) which significantly differed from the values obtained from the wheat sown on other dates. Wheat sown on December 12 and November 12 had the lowest harvest index (0.42). No interaction was observed between the treatments for the harvest index of the crop.

Discussion

Leaf Area Index

Duary & Yaduraju, (2006) revealed similar outcomes in their experiment except for 60 DAS in the second year, where it was not substantially different, the mid-November sown crop had a higher leaf area index than the mid-December sown crop and also at all phases, the leaf area index at 150 kg ha^{-1} seed rate was at its highest and greatly outperformed the 100 kg ha^{-1} seed rate. Tahir et al. (2019) also reported that more LAI was recorded with an increase in seed rates and in the case of sowing dates LAI decreased with late planting. The higher LAI of wheat sown in November was due to favorable weather conditions that increased the vegetative period and the lower LAI of December sown wheat could be due to lower temperatures during December and the late sown crop received less solar radiation at the critical growth stages (Tahir et al., 2019; Hussain et al., 2012a). Higher LAI in plants sown on high seed rate could be due to higher plant density per unit area that increased the percentage of light intercepted.

Table 6. Harvest index of wheat as influenced by date of sowing and seed rates.

Treatments	Harvest index (mean ± SE)
Sowing date (A)	
Nov 12	0.42±0.01[b]
Nov 27	0.44±0.01[b]
Dec 12	0.42±0.01[b]
Dec 27	0.51±0.01[a]
SEM (±)	0.02
LSD (<0.05)	0.06
Seed rate (B)	
100 kg ha^{-1}	0.45±0.02
120 kg ha^{-1}	0.44±0.01
140 kg ha^{-1}	0.46±0.01
160 kg ha^{-1}	0.44±0.01
SEM (±)	0.01
LSD (<0.05)	NS
A*B	NS
CV%	6.8
Grand mean	0.45

Means followed by a common letter (s) are not significantly different from each other based on DMRT at a 5% level of significance. SEM= standard error of means, LSD= least significant difference, CV= coefficient of variance, and NS= non-significant.

Days to 50 % Heading

Our results of days to 50% heading are in line with the findings of Hamid & Muhammad (2000) which showed that with a delay in sowing from 1st November to 20th December there was a corresponding decrease in the number of days to 50% heading and maturity. Also, both days to 50% heading and maturity were not significantly affected by seed rates. Khokhar et al. (2010) also reported that wheat sown in November took around 76-78 days for heading, providing more time compared to mid-December sowing which resulted in a shorter period of 70-69 days.

Days to 50% Flowering

Our findings regarding the time taken for 50% flowering are in line with Shazma et al. (2015) research. They observed that wheat planted on October 29th took more days (146) to reach anthesis, while the plot sown on December 10th exhibited a shorter period (109) to anthesis. The impact of seed rate on anthesis days, however, demonstrated inconsistency. Similar results were reported by Tahir et al. (2019), who noted that delayed sowing led to a reduction in days to anthesis. Notably, wheat sown on November 10th displayed the highest number of days to anthesis, in contrast to December 25th sowing which showed the shortest duration to anthesis.

Days to 50 % Maturity

The results of days to 50% maturity are in line with the findings of Tahir et al. (2019) who reported that the days to maturity were found higher in the wheat sown on November 10 and it gradually decreased with delay in sowing and was found least (121 days) on wheat sown on 25th of December. When wheat planting was delayed from 16 October to 15 March, there was a gradual shortening of the lifetime of the crop as higher temperatures enhanced crop processes over those at normal temperatures (Hakim et al., 2012).

Effective Tillers

The effective number of tillers was significantly affected due to the change in the date of sowing but had no significant impact on it due to varying seed rates. Also, no interaction was observed among the treatments for the effective number of tillers (Table 5). Akhtar et al. (2006) & Shah et al. (2006) found that the number of effective tillers was reduced by delayed sowing. Jan, Hamid & Muhammad (2000) also reported that productive tillers per square meter decreased with delay in sowing and the effect of seed rates on effective tillers per square meter was not significant. High tillers in early sowing may be due to a prolonged period for crop growth and temperature changes with delay in sowing cause low productive tillers in late-sown wheat. The temperature of the shoot meristem is linearly related to the rate of leaf initiation and appearance on the main column (Ong & Baker, 1985).

Spike Length

The length of the spike in wheat has a direct impact on its yield, considering its relationship with the grains per spike (Shahzad et al., 2007). In a study by Baloch et al. (2010), it was observed that delayed sowing led to a reduction in spike length. This emphasizes the significance of appropriate sowing timing for optimizing wheat yield potential. Spike length was not significantly affected by seed rate or its interaction with sowing time. According to Waraich et al. (1981), early planting led to a stronger spike in growth because of a longer growing season. Spike length was not significantly affected by seed rate or its interaction with sowing time, although on October 25th, with 100 and 175 kg of seed ha^{-1}, a larger spike length of 10.2 cm was observed. Farooq et al. (2016) reported that in early planting, spike length increased due to the longer time available for a spike to develop.

Number of grains per spike

Our results of grains/spike are in line with Tahir et al. (2019) who reported that higher grains per spike were observed at the 25th November sowing date, which was statistically similar to the 10th November sowing date. Fewer grains per spike were found on the last sowing date i.e., the 25th of December. In the study by Shazma et al. (2015), it was observed that there was a consistent decrease in the number of grains per spike with the delay in sowing dates, and the impact of seed rate on grains per spike was unpredictable. Similarly, Jan, Hamid & Muhammad (2000) found that the highest number of grains per spike was recorded in plots sown on November 20th, with lower counts in plots sown both earlier and later than this date. This corresponds with the findings of Yajam et al. (2013), where early planting was associated with a higher number of grains per spike.

Grain Yield

Shahzad et al. (2007) also obtained lower grain yield with delay in sowing due to a shorter duration of growth and development. Ebrahimi & Dastan, (2016) revealed that grain yield was significantly affected by different sowing dates while seed rate and interaction were non-significant. Baloch et al. (2010) also reported significant effects (P<0.05) of different sowing times, whereas, seed rate differed non-significantly for the grain yield. The higher

grain yield was obtained from October 25 and November 10 planting dates while December 25 planting date produced the lowest grain yield. Akmal et al. (2011); Baloch et al. (2012) & Poudel et al. (2020) reported significantly higher yields in early sown wheat compared to late sown wheat because of maximum partitioning of photosynthate to grain. According to Ahmad et al. (2018), an increase in temperature reduces yield since it accelerates growth and shortens the growing season.

Straw Yield

Although the impact of seed rate on straw yield was non-significant, the straw yield was observed maximum with wheat sown at maximum seed rate. The straw yield is reflected by growth parameters like the total number of tillers, leaf area, and plant height. Shazma et al. (2015) reported a biological yield maximum in early sown plots. Marasini et al. (2016) reported that the straw yield was significantly higher in wheat sown on November 29. Tahir et al. (2009) also reported similar results in straw yield in their experiment where the straw yield decreased with late planting of the crop. This might also be due to the shortening of the vegetative period of the late sown crop.

Thousand Grains Weight

Our results of thousand grains weight are in line with the findings of Shahzad et al. (2007) who also observed that earlier sowing resulted in better development of the grain due to a longer growing period. This is also supported by the findings of Baloch et al. (2010) where the results are similar to this experiment. Although seed rates had no significant impact on thousand-grain weight, increasing seed rates did not show a positive effect on grain weight which may be due to bulk plant density on account of high seed rates that eventually decreased seed weight. Due to a shorter grain filling phase caused by higher temperature during the reproductive period of late-sown wheat, grain weight was reduced which ultimately decreased the production (Hussain et al., 2012b; Ahmad et al., 2018).

Harvest Index

The results in Table 6 show that the harvest index in wheat was significantly influenced by the date of sowing but no significant influence of seed rate on it was observed. The harvest index of wheat sown on December 27 was observed as maximum and that of wheat sown on other early dates was statistically the same. The results are contrary to the findings of Thapa et al. (2020) where the harvest index was low for late-planted conditions (14th December) than for timely sown (14th November). But the results in this experiment were supported by the findings of Madhu et al. (2018) where the highest harvest index (31.66%) was recorded on 30 December sowing and the lowest (27.70%) was found on 15 November sowing. Farooq et al. (2016) reported similar results where the harvest index was maximum on wheat sown in December and a lower harvest index was observed on earlier sown wheat with the lowest in wheat sown on 25th October. Early planting may have had the lowest H.I. because of taller plants' larger biomass, more tillers per square meter, and dense population, all of which reduced the ratio of economic output.

Conclusion

The impact of the varied sowing date was clearly observed on yield and yield attributing characters of wheat where the best results on these were observed in wheat sown on November 27 as compared to other dates. While, seed rate had no significant impact on yield and attributing characters, though, maximum grain yield was observed on wheat sown under the rate of 120 kg ha^{-1}. Thus, early planting of wheat on November 27 at a seed rate of 100 kg ha^{-1} is better for wheat cultivators in mid hills having the conditions of Lalitpur, Nepal.

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Influence of Foliar Application of Boron on the Growth, Yield and Quality of Sesame (cv. BARI Til-4)

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ABSTRACT

Foliar supplementation may be useful for boron to a crop when its demands are higher compared to the supply from soil. Boron is an important micronutrient which had a substantial impact on oil content, seed yield, and the components of the sesame yield. A field investigation was implemented to determine the impact of foliar application of boron on seed yield, growth and oil content of sesame cv. BARI Til-4. The investigation included four concentrations of boron viz. 0, 25, 50, and 75 ppm and three frequencies of boron application viz. one time at 30 days after sowing (DAS), two times at 30 and 50 DAS, and three times at 30, 50, and 70 DAS. The experiment was conducted following randomized complete block design, which was replicated thrice. At the vegetative stage, the highest plant height (107.3 cm), branches/plant (5.0) and shoot dry weight (45.20 g/plant) resulted in 75 ppm boron spray at 30, 50 and 70 DAS. However, the highest root dry weight (5.80 g/plant) was recorded in 75 ppm with one application of boron at 30 DAS. The plants with the highest plant height (112.1 cm), branches/plant (5.13), pods/plant (44.13), seeds/pod (54.33), seed yield (609.0 kg/ha), harvest index (30.65%), and oil content (42.33%) were also observed with the combination of 75 ppm boron spray with thrice application at 30, 50 and 70 DAS. The lowest seed yield (368.7 kg/ha) resulted in without boron application. Therefore, it can be inferred that the most efficient method for increasing the sesame seed yield and oil content is thrice (30, 50, and 70 DAS) foliar spraying of 75 ppm boron.

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Introduction

One of the oldest oil seed crops is sesame (*Sesamum indicum* L.) under the Pedaliaceae family. It has been farmed for generations and is a valuable ancient oilseed crop cultivated in the tropics and subtropics, particularly in Asia and Africa (FAO, 2013). In terms of global oil crops, it comes in fourth place. Its primary producing regions are in Asia, East, and Central America's tropics and subtropics (Nayar et al., 1970). Sesame production drastically declined over time as a result of intense competition among various high-value crops (Paul et al., 2019). In addition to carbs, protein, and lipids, sesame is a versatile crop that produces excellent edible oil that synthesizes sulphur-containing amino acids and vitamins (Mohsana, 2009). Sesame oil contains 42 to 50 with 25% protein the oil contains 42% essential linoleic acid and 16-18% carbohydrates (Miah et al., 2015). It has lower levels of acetic acid but higher unsaturated fatty acids (more than 80%), particularly oleic and linoleic acids, which are resistant to rancidity. Sesame has noticeable antioxidant properties. It is sometimes referred to as the "seed of immortality" since it has a lengthy shelf life (Sanga, 2013). Sesame plants tolerate drought and can be cultivated in rain-fed highland settings. In Bangladesh, two-thirds of the total sesame is cultivated during the *kharif* season.

The population growth of Bangladesh is 1.22% and there is a severe shortage of edible oil (BBS, 2022). Sesame can also be used to extract high-quality oil for cooking, as well as medicinal and cosmetic uses, all of which have extended shelf lives. The yield of sesame in Bangladesh is lower than other sesame-producing countries. Lower yield might be caused by improper cultivar selection and inadequate management practices. Previous studies have shown that varietal potentiality influences on seed output (Mohsana, 2009; Raja et al., 2007).

One of the elements affecting sesame yield and oil content is boron. During the development of plants, boron is engaged in a number of physiological and biochemical processes. In Bangladesh, some soils and crops have shown evidence of B (Islam et al., 1997) and B, Zn, and Mo deficiencies (Khanam et al., 2001). On some soils and

crops, boron shortage is documented in particular (Islam et al., 1997). During the development of plants, boron is engaged in several physiological and biochemical processes. Low yield might be caused by its absence, among other things. According to Ahmed and Hossain (1999), a million hectares of land in Bangladesh has boron deficiencies. A lack of boron leads to a significant decline in grain set and a severe yield drop (Jamjod et al., 2004). This boron shortage is typically found in soils with a light texture and a high pH. Higher production potential and resistance to pests and diseases in improved sesame varieties are indicated to boost yield (Ssekabembe et al., 2002). The most recent high yielding sesame variety developed by Bangladesh Agricultural Research Institute (BARI) is BARI Til-4. This study was conducted to determine the impact of foliar application of boron on sesame growth, seed yield, and oil content.

Materials and Methods

Experimental Site, Soil and Weather

The Agronomy Field Laboratory of Bangladesh Agricultural University hosted the experiment between March and June of 2019. The study area is 18 meters above the nearest sea level and is located at 90° 50' East longitude and 24° 75' North latitude. A piece of land with a silty loam soil texture makes up the experimental plot, which is a little bit elevated and has a pH of 6.10, an electrical conductivity of 233 (µS/cm), organic carbon of 1.00%, nitrogen of 0.117 %, phosphorus of 3.19 ppm, potassium of 0.092 %, calcium of 8.30 g, magnesium of 3.29 g, sulfur of 9.52 ppm, and boron of 0.23 g. The experimental area experiences subtropical weather. The experimental site saw lowest and highest temperatures of 12.16°C and 31.69°C, relative humidity levels of 67.76% and 83%, and total rainfall amounts of 0.00 mm and 66.80 mm, respectively (Source: Department of Irrigation and Water Management, Bangladesh Agricultural University).

Experimentation

The study consisted of four concentrations of boron viz. 0 ppm (B_0), 25 ppm (B_1), 50 ppm (B_2) and 75 ppm (B_3) and three frequencies of boron application viz. one time at 30 DAS (T_1), two times at 30 and 50 DAS (T_2), three times at 30, 50 and 70 DAS (T_3). To set up the experiment, a Randomized Complete Block Design was utilized which comprised three replications. Each block included 12 plots. There were 36 such plots in all. The plot measured 2.5 m × 2.0 m. Blocks were separated from one another by 0.5 cm between each, while plots were separated by 0.25 cm. BARI Til-4 cultivar of sesame was utilized in this investigation.

Crop Husbandry

Using a power tiller, the experimental site was prepared. Laddering was done after using the country plough for ploughing and cross-ploughing to obtain the desirable tilt. There were 5 types of fertilizer used. The rate of urea 120 kg/ha, triple super phosphate (TSP) 145 kg/ha, muriate of potash (MoP) 50 kg/ha was applied to all plots as per recommended by Bangladesh Agricultural Research Institute. There were four concentrations of boron application *viz.* 0 ppm, 25 ppm, 50 ppm and 75 ppm with frequencies of boron application *viz.* once at 30 DAS, twice at 30 and 50 DAS, and thrice at 30, 50, and 70 DAS. Boron was obtained from boronic acid at 0, 147, 294 and 441 mg Boric acid per litre for 0 ppm, 25 ppm, 50 ppm and 75 ppm respectively. TSP, MoP, Gypsum, and 50% of Urea were all fully integrated during the last stage of soil preparation. Sesame seeds (BARI Til-4) were sown in 2-3 cm profound ridges created by manual raking, keeping rows 25 cm apart. After the seeds were planted in the furrow, dirt was added, and then the seeds were lightly pressed by hand. Seed rate was 7 kg/ha. Within three days of seeding, seedling emergence began, and it was finished seven days later. After the establishment of seedlings, healthy seedlings were kept within a distance of 5 cm between two seedlings in a row and the additional seedlings were carefully uprooted by hand pulling.

Data Collection, Harvesting and Post-Harvest Operations

At 60 DAS, to measure plant height, four plants were chosen at random from the core 1.0 m × 1.0 m and border rows, branches/plant, shoot and root dry matter production/plant Plant was uprooted average height and number of branches/plant was measured then shoots and roots were separated and a consistent weight was attained after oven drying. When around 80% of the plant leaves turned straw-yellow in colour then crops were harvested. In order to collect information on plant height, branches/plant, pods/plant, seeds/pod, 1000-seed weight, and oil (%) content of sesame seeds, five randomly chosen plants from each unit plot were uprooted. Border plants and the middle 1.0 m × 1.0 m area were not included in this process. After collection of the sample harvesting of 1.0 m^2 area was done. The harvested crops were tied into bundled, tagged separately, sun dried and threshed by bamboo rods. After separating the seeds from the pods, the yields of both the seeds and the stover were documented. The biological yield was determined using the following formula on a dry weight basis:

Biological yield = Seed yield + Stover yield.

The formula below was used to construct the harvest index (%), which determines the percentage difference between economic yield and biological yield (Gardner et al., 1985).

$$\text{Harvest index (\%)} = \frac{\text{Grain yield}}{\text{Biological yield}} \times 100.$$

Seed Oil Content Measurement

By using the Soxhlet apparatus method in accordance with the steps in the oil content was calculated (Singh et al., 1960). Oil content and seed yield were multiplied to determine the oil yield.

Statistical Analysis

The computer program MSTATC-C developed by (Russel, 1986) was employed to conduct the analysis of variance (ANOVA). The mean differences between the treatments were adjusted by DMRT at a 5% level of significance (Gomez and Gomez, 1984).

Results

Vegetative Traits at 60 DAS

Plant Height

The height of the plant was sown a substantial impact by various boron doses concentrations. However, the frequencies of boron application and the interaction between concentration and frequencies did not show any significant effects. Among the different concentrations, 75 ppm resulted in the tallest plants (104.70 cm), which were not significantly different from plants treated with 50 ppm and 25 ppm. The shortest plants (101.10 cm) were observed in the control group (0 ppm) (Table 1). Regarding the frequencies of boron application, plants treated three times at 30, 50, and 70 DAS exhibited the highest height of the plant (104.3 cm), while the lowest height of the plant (101.9 cm) was found when boron was applied only once at 30 DAS (Table 1). Table 2 shows that the combination of 75 ppm boron applied three times at 30, 50, and 70 DAS ($B_3 \times T_3$) resulted in the tallest plants (107.3 cm), whereas the shortest plants (100.7 cm) were observed in the group treated with 0 ppm boron two times at 30 and 50 DAS ($B_0 \times T_2$).

Number of Branches/Plant

Variable boron concentrations had a substantial impact on the quantity of branches/plant, but the frequencies of boron treatment and the relationship between the two did not. In the experiment, the highest number of branches/plant (4.56) was observed at a boron concentration of 75 ppm. Conversely, the lowest number of branches/plant (3.44) resulted with control group (0 ppm), which was identical to the results for 25 ppm and 50 ppm boron concentrations (Table 1). Regarding the frequencies of boron application, the highest number of branches/plant (3.91) was achieved when boron was applied thrice at 30, 50, and 70 DAS. Conversely, the lowest plant height (3.66 cm) was observed when boron was applied only once at 30 DAS, which was comparable to the results of twice applications at 30 and 50 DAS (Table 1). However, numerically, the highest number of branches/plant was recorded at 75 ppm with three boron applications at 30, 50, and 70 DAS ($B_3 \times T_3$) (Table 2). Conversely, the lowest number of branches /plant was found in the control group (0 ppm) with a single boron application at 30 DAS ($B_0 \times T_1$) (Table 2).

Shoot Dry Weight

The dry weight of the shoot of the plant was positively affected by varying the doses, frequencies, and combination of boron treatments. The highest shoot dry weight (43.47 g/plant) was observed at a dose of 75 ppm, comparable to 50 ppm and 25 ppm. In contrast, the lowest shoot dry weight (24.26 g/plant) was recorded in the control group with 0 ppm of boron (Table 1). When boron was applied thrice at 30, 50, and 70 DAS, the highest shoot dry weight (33.86 g/plant) was achieved while the lowest shoot dry weight (31.86 g/plant) was recorded when boron applied once at 30 DAS, which was at par with twice applications at 30 DAS and 50 DAS (Table 1). Furthermore, the highest shoot dry weight (45.20 g/plant) was recorded with 75 ppm boron applied thrice at 30, 50, and 70 DAS ($B_3 \times T_3$). Contrarily, the minimum shoot dry weight (23.66 g/plant) was found without boron application ($B_0 \times T_2$) (Table 2).

Root Dry Weight

Different concentrations, frequencies and their interactions of boron had a considerable effect on the plant's root dry weight. The highest root dry weight (5.66 g/plant) was observed at 75 ppm boron concentration and the lowest weight (4.86 g/plant) in without boron application (Table 1). Furthermore, when boron was applied at 30 DAS, the root dry weight varied depending on the frequencies of application. The lowest root dry weight (5.163 g/plant) was recorded when boron was applied once at 30 DAS, whereas the maximum root dry weight (5.321 g/plant) was observed when boron was applied twice at 30 and 50 DAS (Table 1).

Table 1. Effect of concentration and frequencies of boron on plant height, number of branches/ plant shoot and root dry weight of sesame plant at 60 DAS

Treatments	Plant height (cm)	Branches/plant (no.)	Shoot dry weight/plant (g)	Root dry weight/plant (g)
Concentration of Boron				
B_0	101.10b	3.44b	24.26d	4.86c
B_1	103.00ab	3.44b	28.46c	5.17b
B_2	103.90a	3.56b	34.30b	5.17b
B_3	104.70a	4.56 a	43.47a	5.66a
Sig. level	*	**	**	**
CV (%)	2.35	15.40	2.50	1.80
Frequencies of Boron				
T_1	101.9	3.66	32.15b	5.163b
T_2	103.3	3.66	31.86b	5.321a
T_3	104.3	3.91	33.86a	5.173b
Sig. level	NS	NS	**	**
CV (%)	2.35	15.40	2.50	1.80

According to the DMRT, figures in a column for each factor of treatment with the same letter or without a letter do not significantly differ from each other, however figures with dissimilar letter(s) do. ** = 1 % level of significant, * = 5 % level of significant, NS = Not significant; B_0 = 0 ppm, B_1 = 25 ppm, B_2 = 50 ppm, B_3 = 75 ppm, T_1 = Once at 30 DAS, T_2 = Twice at 30 and 50 DAS, T_3 = Thrice at 30, 50 and 70 DAS

Table 2. Interaction effects of concentrations and frequencies of boron application on plant height, number of branches/plant, shoot, root and total dry weight of plant at 60 DAS

Interaction (Concentration × Frequencies)	Plant height (cm)	Branches/plant (no.)	Shoot Dry weight/plant (g)	Root dry weight/ plant (g)
$B_0 \times T_1$	101.7	3.33	25.15g	4.70h
$B_1 \times T_1$	101.3	3.33	27.88f	4.95g
$B_2 \times T_1$	101.7	3.66	32.82d	5.20e
$B_3 \times T_1$	103.0	4.33	42.77b	5.80a
$B_0 \times T_2$	100.7	3.33	23.66h	4.90g
$B_1 \times T_2$	104.7	3.66	28.02f	5.40cd
$B_2 \times T_2$	104.0	3.33	33.32d	5.31de
$B_3 \times T_2$	103.7	4.33	42.43b	5.66ab
$B_0 \times T_3$	101.0	3.66	23.97gh	5.00fg
$B_1 \times T_3$	103.0	3.33	29.50e	5.16ef
$B_2 \times T_3$	106.0	3.66	36.76c	5.01fg
$B_3 \times T_3$	107.3	5.00	45.20a	5.51bc
Sig. level	NS	NS	**	**
CV (%)	2.35	15.40	2.50	1.80

According to the DMRT, figures in a column for each factor of treatment with the same letter or without a letter do not significantly differ from each other, however figures with dissimilar letter(s) do. ** = 1 % level of significant, NS = Not significant; B_0 = 0 ppm, B_1 = 25 ppm, B_2 = 50 ppm, B_3 = 75 ppm, T_1 = Once at 30 DAS, T_2 = Twice at 30 and 50 DAS, T_3 = Thrice at 30, 50 and 70 DAS

Table 3. Effect of concentrations and frequencies of boron on yield and yield contributing characters of sesame (BARI Til-4)

Treatments	Plant height (cm)	Branches/ plant (no.)	Pods/ plant (no.)	Seeds / pod (no.)	Biological yield (kg/ha)	Harvest index (%)
Concentration of Boron						
B_0	105.2c	3.75c	38.56c	43.10d	2174.0a	18.38d
B_1	106.9b	4.15b	36.62d	45.7c	2047.0b	21.19c
B_2	108.5a	4.26b	40.87b	46.50b	2078.0b	23.42b
B_3	109.3a	4.84a	43.09a	52.44a	2061.0b	27.82a
Sig. level	**	**	**	**	**	**
CV(%)	1.52	4.83	2.57	1.03	2.85	3.64
Frequencies of Boron						
T_1	106.2b	4.45a	40.02a	46.45b	2138.0a	21.64c
T_2	107.7a	3.88 b	40.23a	46.82b	2093.0a	22.78b
T_3	108.5a	4.43a	39.10 b	47.55a	2039.0b	23.69a
Sig. level	**	**	*	**	**	**
CV (%)	1.52	4.83	2.57	1.03	2.85	3.64

According to the DMRT, figures in a column for each factor of treatment with the same letter or without a letter do not significantly differ from each other, however figures with dissimilar letter(s) do. ** = 1 % level of significant, * = 5 % level of significant, B_0 = 0 ppm, B_1 = 25 ppm, B_2 = 50 ppm, B_3 = 75 ppm, T_1 = Once at 30 DAS, T_2 = Twice at 30 and 50 DAS, T_3 = Thrice at 30, 50 and 70 DAS

In Table 2, the most significant root dry weight was recorded (5.80 g/plant) at 75 ppm boron concentration with a single application at 30 DAS ($B_3 \times T_1$). This result was statistically similar to the root dry weight obtained from 75 ppm boron concentration with two applications at 30 and 50 DAS ($B_3 \times T_2$). Conversely, minimum root dry weight (4.70 g/plant) was recorded in without boron application ($B_0 \times T_1$).

Crop Characteristics, Yield Components and Oil Content at Harvest

Plant Height

Different concentrations, frequencies, and interactions of boron doses significantly affected the height of the BARI Til-4 plant. The tallest plant height (109.3 cm) was found with 75 ppm boron, which was comparable to the plant height at 50 ppm. Conversely, the shortest plant (105.2 cm) has resulted in 0 ppm boron (Table 3). Another observation was that applying boron three times at 30, 50, and 70 DAS resulted in the highest plant (108.5 cm), which was on par with applying boron twice at 30 and 50 DAS. However, the shortest plant height (106.2 cm) was found when boron was applied only once at 30 DAS (Table 3). Table 4 presents additional findings, showing that the tallest plant (112.1 cm) occurred with thrice application of 75 ppm boron at 30, 50, and 70 DAS ($B_3 \times T_3$). Conversely, the shortest plant (104.6 cm) was found in without boron application (B0 × T3).

Number of Branches/Plant

The number of branches/plant in BARI Til-4 was significantly influenced by various concentrations, frequencies and their interactions. The maximum branches/plant (4.84) was observed at 75 ppm boron, followed by 50 ppm and 25 ppm. The lowest number of branches /plant (3.75) was recorded without boron (0 ppm) (Table 3). When examining the timing of boron application, the highest quantity of branches/plant (4.45) resulted from a single application of boron at 30 DAS, which was similar to three applications of boron at 30, 50,

and 70 DAS. The lowest number of branches/plant (3.88) observed with boron application at 30 and 50 DAS (Table 3). Regarding the interaction of boron concentration and application timing, the highest quantity of branches/plant (5.13) was resulted in the combination of 75 ppm boron with three applications at 30, 50, and 70 DAS ($B_3 \times T_3$). Conversely, the lowest number of branches/plant (3.40) occurred in the interaction of 0 ppm boron with two applications at 30 and 50 DAS ($B_0 \times T_2$) (Table 4).

Number of Pods/Plant

Different doses of boron at different concentration, frequencies and their interactions all had a substantial impact on the quantity of pods/plant. The highest quantity of pods/plant (43.09) was observed when using 75 ppm boron. Conversely, the lowest number of pods/plant (36.62) was recorded with 25 ppm boron (Table 3). When analyzing the frequencies of boron application, having the most pods/plant (40.23) resulted from applying boron twice at 30 and 50 DAS, which was similar to a single application of boron at 30 DAS. The lowest number of pods/plant (39.10) occurred with three applications of boron at 30, 50, and 70 DAS (Table 3). Furthermore, in the interactions between boron concentrations and application frequencies, the most significant number of pods/plant (44.13) was found with 75 ppm boron and three applications of boron at 30, 50, and 70 DAS ($B_3 \times T_3$). Conversely, with the fewest pods/plant (36.67) resulted in without boron application ($B_0 \times T_1$) (Table 4).

Number of Seeds/Pod

The quantity of seeds/pod of BARI Til-4 was significantly affected by variations in boron concentration, frequencies, and their combinations. The highest seeds/pod (52.44) were observed when applying 75 ppm of boron and the lowest seeds/pod (43.10) were found in 0 ppm boron (Table 3). When examining the effect of boron application frequencies, the highest seeds/pod (47.55) occurred with thrice applications of boron at 30, 50, and 75 DAS. The lowest seeds/pod (46.45) was recorded when boron was applied only once at 30 DAS, which showed similar results to the twice application of boron at 30 and 50 DAS (Table 3). Regarding the interaction of boron concentrations and application frequencies, the highest quantity of seeds/pod (54.33) was found when using 75 ppm at 30, 50, and 70 DAS ($B_3 \times T_3$). Conversely, the lowest seeds per pod (42.13) were observed in the interaction of 0 ppm boron with a single application at 30 DAS ($B_0 \times T_1$) (Table 4).

Seed Yield

Variable boron concentrations, application frequencies, and their interactions had a significant impact on the seed production of sesame. The highest yield of seed (571.9 kg/ha) was observed with 75 ppm boron, while the lowest seed yield (399.6 kg/ha) was recorded in the control group with 0 ppm boron (Figure 1). When examining the effect of boron application frequencies, the highest yield of seed (481.9 kg/ha) was achieved with thrice applications of boron at 30, 50, and 70 DAS. This result was comparable to the yield (460.5 kg/ha) obtained from the twice application of boron at 30 and 50 DAS. The lowest seed yield was found in boron was applied only once at 30 DAS (Figure 1). In relation to how the frequencies and concentration of boron interact, the highest yield of seed (609.0 kg/ha) was observed when using 75 ppm boron in combination with three applications at 30, 50, and 70 DAS ($B_3 \times T_3$). Conversely, the lowest seed yield (368.7 kg/ha) was found in the interaction of 0 ppm boron with three applications at 30, 50, and 70 DAS ($B_0 \times T_3$) (Figure 1).

Stover Yield

The stover yield of BARI Til-4 was significantly affected by variations in boron concentration, frequencies, and their interaction. The highest stover yield (1774.0 kg/ha) was measured in the control group with 0 ppm boron, while the lowest stover yield (1489.0 kg/ha) was observed when using 75 ppm boron (Figure 2). Examining the effect of boron application frequencies alone, the highest stover yield (1678.0 kg/ha) occurred when boron was applied once at 30 DAS. Conversely, the lowest stover yield (1557.0 kg/ha) was found when boron was applied three times at 30, 50, and 70 DAS (Figure 2).

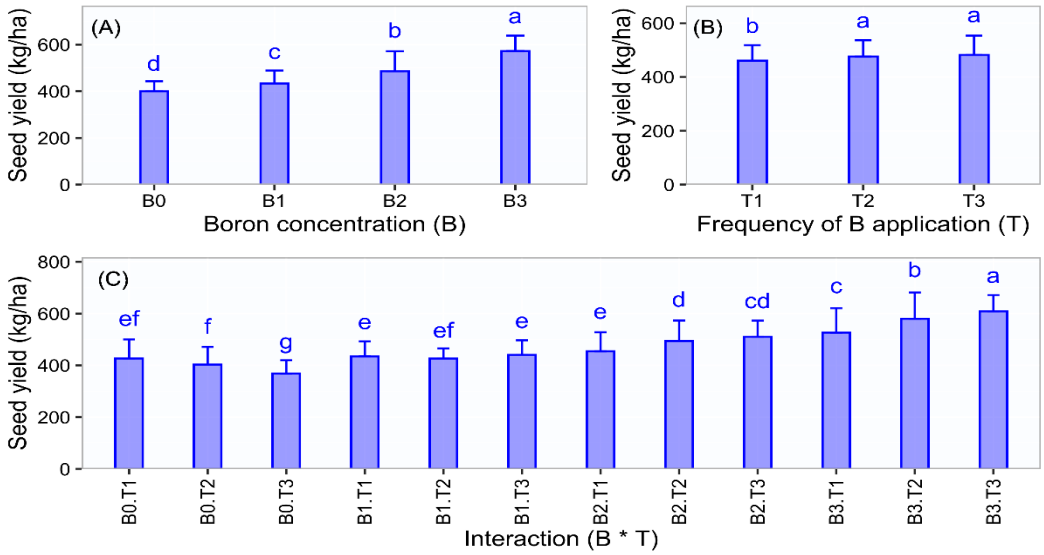

Figure 1. Effect of boron concentrations and application frequencies on sesame seed yield.

B_0 = 0 ppm, B_1 = 25 ppm, B_2 = 50 ppm, B_3 = 75 ppm, T_1 = Once at 30 DAS, T_2 = Twice at 30 and 50 DAS, T_3 = Thrice at 30, 50 and 70 DAS

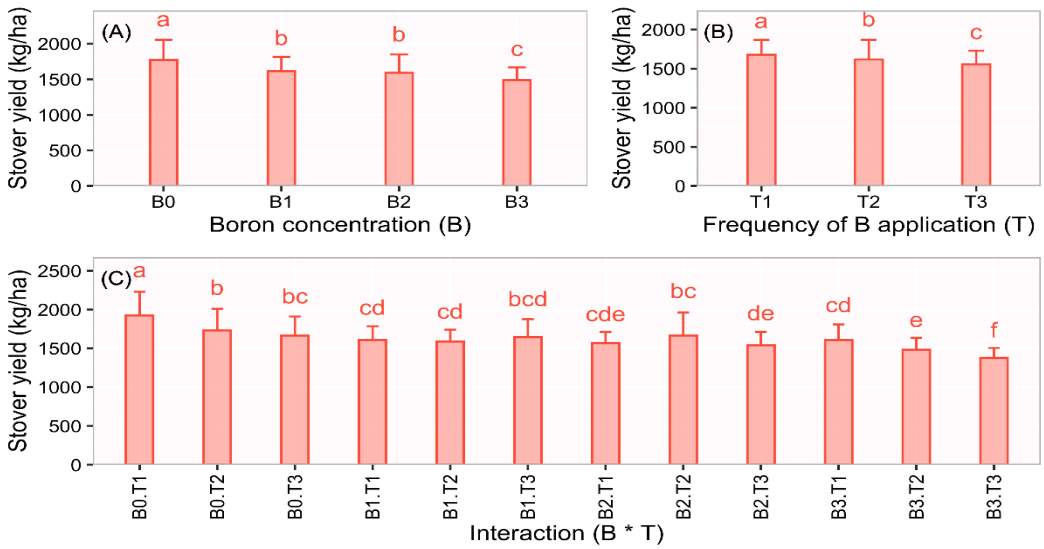

Figure 2. Effect of boron concentrations and application frequencies on sesame stover yield.
B_0 = 0 ppm, B_1 = 25 ppm, B_2 = 50 ppm, B_3 = 75 ppm, T_1 = Once at 30 DAS, T_2 = Twice at 30 and 50 DAS, T_3 = Thrice at 30, 50 and 70 DAS

Table 4. Interaction effects of concentrations and frequencies of boron application on yield contributing characters of sesame

Interaction (Conc. × Frequencies)	Plant height (cm)	Branches /plant (no.)	Pods/plant (no.)	Seeds/ pod (no.)	Biological yield (kg ha^{-1})	Harvest index (%)
B_0×T_1	104.9ef	3.86cd	36.67f	42.13 f	2354.0a	18.12f
B_1×T_1	105.1def	4.46b	41.27c	45.65 d	2044.0cd	21.28e
B_2×T_1	106.8c-f	4.66b	38.73de	46.88 c	2022.0cd	22.46de
B_3×T_1	107.9b-e	4.80ab	43.40ab	51.13 b	2133.0bc	24.70c
B_0×T_2	106.1c-f	3.40e	37.20ef	42.79 f	2133.0bc	18.90f
B_1×T_2	108.5bc	3.93cd	40.33cd	45.59 d	2014.0d	21.17e
B_2×T_2	108.2bcd	3.60de	41.67bc	47.04 c	2161.0b	22.90d
B_3×T_2	107.9b-e	4.60b	41.73bc	51.87 b	2063.0bcd	28.13b
B_0×T_3	104.6f	4.00c	41.80bc	44.37 e	2035.0cd	18.11f
B_1×T_3	106.9c-f	4.06c	28.27g	45.89 d	2084.0bcd	21.13e
B_2×T_3	110.5ab	4.53b	42.20bc	45.59 d	2050.0bcd	24.89c
B_3×T_3	112.1a	5.13a	44.13a	54.33 a	1987.0d	30.65a
Sig. level	*	**	**	**	**	**
CV (%)	1.52	4.83	2.57	1.03	2.85	3.64

According to the DMRT, figures in a column for each factor of treatment with the same letter or without a letter do not significantly differ from each other, however figures with dissimilar letter(s) do. ** = 1 % level of significant, * = 5 % level of significant, B_0 = 0 ppm, B_1 = 25 ppm, B_2 = 50 ppm, B_3 = 75 ppm, T_1 = Once at 30 DAS, T_2 = Twice at 30 and 50 DAS, T_3 = Thrice at 30, 50 and 70 DAS

Regarding the interaction of boron concentration and application frequencies, the highest stover yield (1927.0 kg/ha) was found in the interaction of 0 ppm boron with a single application at 30 DAS (B_0 × T_1). Conversely, the lowest stover yield (1378.0 kg/ha) was observed in the interaction of 75 ppm boron with thrice applications at 30, 50, and 70 DAS (B_3 × T_3) (Figure 2).

Biological Yield

The biological yield of BARI Til-4 was significantly affected by the variations in boron concentration, application frequencies, and their interactions. The highest biological yield (2174 kg/ha) was observed in the control group with 0 ppm boron. The lightest biological yield (2047 kg/ha) was found with 25 ppm boron, which was statistically similar to the yields obtained with 50 ppm and 75 ppm boron (Table 3). A single application of boron at 30 DAS resulted in the highest biological yield (2138.0 kg/ha), which did not differ considerably from the yields achieved with twice boron applications at 30 and 50 DAS. The lowest biological yield (2039.0 kg/ha) was recorded with three times applications of boron at 30, 50, and 70 DAS (Table 3). In the interaction of 0 ppm boron with a single application at 30 DAS (B_0 × T_1), the highest biological yield (2354.0 kg/ha) was found, while the lightest biological yield (1987.0 kg/ha) was recorded in the interaction of 75 ppm boron with thrice applications at 30, 50, and 70 DAS (B_3 × T_3) (Table 4).

Harvest Index

The harvest index of BARI Til-4 had a considerable impact on boron concentration, application frequencies, and their interaction. The greater harvest index (27.82%) was found with 75 ppm boron, the least harvest index (18.38%) was observed in the control group with 0 ppm boron (Table 3). With thrice applications of boron at 30, 50, and 70 DAS, the greater harvest index (23.69%) was achieved, and the lowest harvest index (21.64%) was found with a single application at 30 DAS (Table 3).

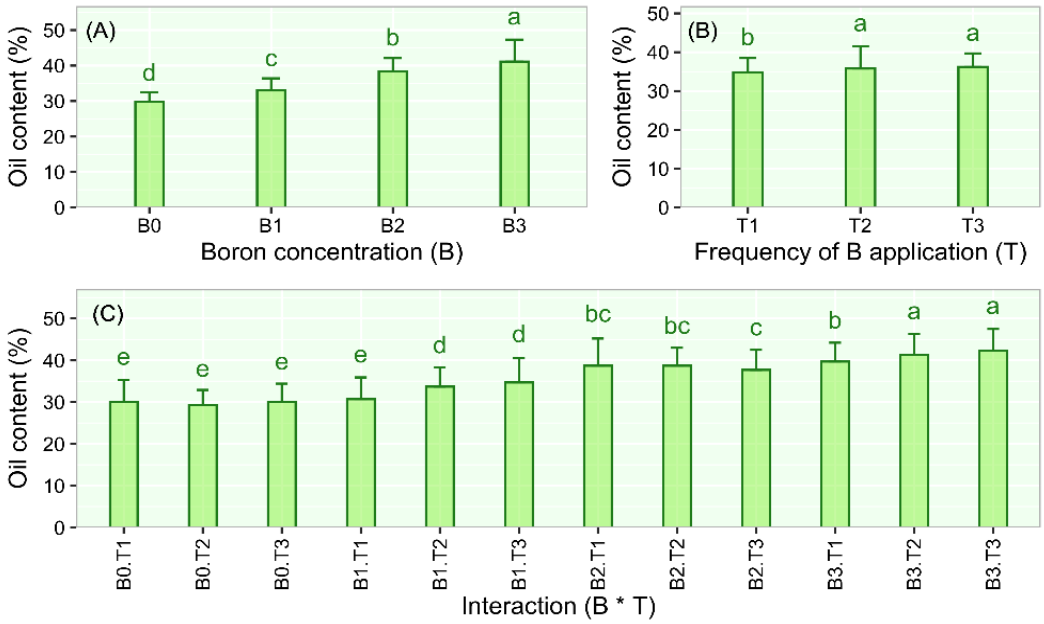

Figure 3. Effect of boron concentrations and application frequencies on sesame oil content.
B_0 = 0 ppm, B_1 = 25 ppm, B_2 = 50 ppm, B_3 = 75 ppm, T_1 = Once at 30 DAS, T_2 = Twice at 30 and 50 DAS, T_3 = Thrice at 30, 50 and 70 DAS

In the interaction of 75 ppm boron with thrice applications at 30, 50, and 70 DAS ($B_3 \times T_3$), the greater harvest index (30.65%) was observed, while the least harvest index (18.12%) was recorded in the interaction of 0 ppm boron with a single application at 30 DAS ($B_0 \times T_1$) (Table 4).

Oil Content

The oil content of BARI Til-4 was significantly affected by boron concentration, application frequencies, and their interaction. The highest oil content (41.11%) was obtained with 75 ppm boron, while the lowest oil content (29.78%) was recorded in the control group with 0 ppm boron (Figure 3). With three frequencies of boron application at 30, 50, and 70 DAS, the highest oil content (36.17%) was observed, followed by twice applications at 30 and 50 DAS. The lowest oil content (35.75%) was found with a single application of boron at 30 DAS (Figure 3). In the interaction of 75 ppm boron with three times applications at 30, 50, and 70 DAS ($B_3 \times T_3$), the highest oil content (42.33%) was observed, which was statistically similar to the interaction of 75 ppm boron with twice application at 30 and 50 DAS ($B_3 \times T_2$). The lowest oil content was found without boron application ($B_0 \times T_1$, $B_0 \times T_2$, and $B_0 \times T_3$) which was at par with the interaction of 25 ppm applied once at 30 DAS ($B_1 \times T_1$) (Figure 3).

Discussion

The growth characteristics of BARI Til-4 plants had a profound impact on the administration of various boron doses. The control group with 0 ppm boron had the lowest height, whereas the treatment with 75 ppm boron had the highest plant height and the most branches/plant (Table 1). The highest shoot and root dry weight was produced by the 75 ppm boron treatment as well (Table 1). When exposed to varying frequencies of boron treatment, how tall the plant is and the number of branches/plant did not show any appreciable differences. From the three times application frequencies, the tallest plants were found with three time applications at 30, 50 and 75 DAS whereas one application of boron at 30 DAS produced the shortest plants (Table 1). Three boron treatments at 30, 50, and 70 DAS resulted in the highest number of branches/plant in terms of numbers (Table 1). Furthermore, boron was treated three times at 30, 50, and 70 DAS to achieve the highest shoot dry weight, while two treatments at 30 and 50 DAS to get the highest root dry weight were recorded (Table 1). Several physiological processes, including cell division and elongation, which eventually lead to increased plant height, depend on the mineral boron. Similar observations showed that boron fertilization increases production potential through more branching (Schon and Blevins, 1990). According to Miller and Donahue (1997) boron is a crucial ingredient for the synthesis of new cells, which results in a considerable rise in leaf dry weight when boron supplies are adequate. Ramirez and Linares (1995) also reported that boron is applied optimally, sesame leaves produce more dry matter but this production is drastically reduced when boron levels in the leaf tissue are lower. The results of this study demonstrated that the highest growth criteria, including plant height, the number of branches/plant, and shoot dry weight, were recorded at concentrations of 75 ppm with three applications at 30, 50, and 70 DAS, while the highest root dry weight was recorded at concentrations of 75 ppm with a single application of boron at 30 DAS, which was statistically comparable to 75 ppm at twice applications at 30 and 50 DAS (Table 2). The height of the plant was greater at the optimal boron fertilization rate than at higher doses, according to Hemantaranjan et al. (2000). This might be because boron is crucial for physiological processes like cell division and elongation, which eventually helped the plant grow taller. Sarkar (2008) demonstrated that boron fertilizer promoted branching. The amount and frequencies of boron had a considerable influence on the BARI Til-4 crop's characteristics at

harvest time. The application of 75 ppm boron had the biggest effect on plant height, whereas 0 ppm boron had the lowest effect (Table 3). When boron levels were 75 ppm, the highest branches/plant was observed (Table 3). The highest values for the number of pods/plant and number of seeds/pod were achieved with either 50 ppm or 75 ppm boron, and these two dosages were statistically comparable. The lowest values were discovered in the 0 ppm boron treatment (Table 3). Except biological yield and stover yield on a dry weight basis, the 0 ppm boron treatment showed the least favorable outcomes in terms of seed yield, harvest index, and oil content (Table 3, Figures 1 & 3). Concerning the frequencies of boron treatment, the yield and oil content of BARI Til-4 fluctuated dramatically. When boron was treated three times, at 30, 50, and 70 days after sowing (DAS), the tallest plants were seen, whereas when boron was applied just once, at 30 DAS, the shortest plants were seen. Boron sprays at 30, 50, and 70 DAS, the highest number of branches/plant was observed (Table 3). Aside from that, three treatments of boron at 30, 50, and 70 DAS were found to produce the most pods/plant and seeds/pod, whereas only one application at 30 DAS produced the lowest values (Table 3). The three boron treatments at 30, 50, and 70 DAS resulted in the highest seed production, harvest index, and oil content values (Table 3, Figures 1 & 3).

The features of the BARI Til-4 crop were profoundly impacted by the connection between boron content and dose frequencies. The crop characteristics examined in this study plant height, number of branches/plant, number of pods/plant, number of seeds/pod, seed yield, harvest index, and oil content were shown to be best when 75 ppm of boron was administered three times at 30, 50, and 70 DAS (Table 4, Figure 1 & 3). The findings of the 75 ppm boron administered with a single treatment at 30 DAS are statistically equal to the results of the number of branches/plant and number of pods/plant (Table 4). The interaction between 0 ppm of boron and a single application of boron at 30 DAS produced the highest stover production and biological yield, while the interaction between 75 ppm of boron and three applications of boron at 30, 50, and 70 DAS produced the least favorable results (Table 4 and Figure 2).

Boron is one of the most important micronutrients for the proper growth and development of sesame. Boron deficiency results in fewer and shorter-lasting flowers, slower growth of the pollen tube, and fewer fruits as a result. A sufficient intake of boron increases the dry weight of the petiole and leaves. Capsule dry weight and yield are both greatly improved (Sindoni et al., 1994) and found that boron supplementation reduction only significantly reduced root and shoot dry weight at the 30 day mark, as opposed to boron removal at all ages. The reduction in boron concentration affected the development of seeds as well as the amounts of boron in leaves, sterns, and pods. The amount of boron in the pods and seed weight showed a substantial and linear association.

When the amount of boron in the leaf tissue fell below the necessary level, a significant reduction in the amount of dry matter generated in the shape of the leaves, stems, and roots occurred. However, when the amount of boron in the leaf tissue rose, seed oil content and dry weight declined (Sarkar, 2008; Huq, 2012) and found that the interaction between boron content and frequencies dramatically boosted the number of pods/plant. Pod production/plant could vary depending on how much boron is applied. According to a previous article (Sarkar, 2008), which claimed that boron deficiency resulted in the growth of less fruits, the control plot's lowest seeds/pod yield may be the cause of this. The mineral composition, capsule development, seed production, and stover output of sesame were all affected by the use of boron fertilizer (Bennetti, 1993). Mathew and George (2011) observed that stover yield affected for boron deficiency. Oil content significantly differs in seeds due to boron application (Haque, 2008; Liu et al., 2003).

Conclusion

It might be inferred from the investigation that among the boron treatments 75 ppm boron was found to be the most effective. Thrice (30, 50 and 70 DAS) application of 75 ppm boron had significant impact for improving yield components and increasing the yield of sesame**.** However further research at different Agro-ecological zones and in the *kharif* season is necessary to draw a definite conclusion and for recommendation with respect to application boron fertilizers for sesame cultivation.

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Conflicts of Interest

The writers attest to having no financial or other competing interests to disclose with relation to the current work.

Data Availability Statement

The study's data may be requested from the corresponding author. Since the statistics are being disclosed for the first time, they are not yet available to the general public. The writers are more than happy to provide them upon request.

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The Functional and Rheological Properties the Mesocarp Layer of the Oleaster (*Elaeagnus angustifolia* L.) grown in Karaman

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ABSTRACT

The oleaster (*Elaeagnus angustifolia* L.), also known as wild olive, is a small fruit with three parts: the outer peel or exocarp layer, the edible part or mesocarp layer, and the inner seed or endocarp layer. The mesocarp layer is rich in essential vitamins and has great potential for use in various food products. The flour made from the mesocarp layer has a moisture content of 8.99%, an ash content of 2.66%, a fat content of 0.55%, a protein content of 5.99%, a crude fiber content of 3.32%, and a total dietary fiber (TDF) content of 26.36%. The TDF content is divided into insoluble dietary fiber (IDF) and soluble dietary fiber (SDF), which are 21.35% and 5.01%, respectively. The flour has color values of L*: 75.14, a*: 2.86, b*: 23.87, and a water activity value of 0.314. The water solubility, water absorption, and oil absorption are 67.33%, 4.91 g water/g sample, and 2.26 g oil/g sample, respectively. Additionally, the mesocarp layer contains minerals such as Mg, P, K, Ca, Fe, and Na. The mesocarp layer significantly affected the thermomechanical properties of wheat flour. As the substitution level of the mesocarp layer increased from 10 to 30%, the water absorption capacity, dough development time and stability time of the wheat dough significantly decreased. Specifically, the water absorption capacity dropped from 53.5% to 47%, dough development time reduced from 1.10 to 0.75 min, and stability time decreased from 8.90 to 2.25 min. Substituting a mesocarp layer in wheat flour can significantly improve product shelf-life due to slower retrogradation. The mesocarp layer is an functional ingredient in the food industry.

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Introduction

Oleaster fruit, also known as wild olive, is a small-sized fruit that belongs to the Elaeagnaceae family. Originating from Turkey, this fruit is composed of three distinct parts, namely, the peel (exocarp), the edible portion (mesocarp), and the seed (endocarp). In the field of alternative medicine, all parts of the oleaster fruit are utilized for their various health benefits. The fruit is commonly consumed in the form of tea or powder, and is known to aid in alleviating several health conditions, such as nausea, vomiting, flatulence, gastric disorders, asthma, jaundice, and muscle relaxation. Moreover, oleaster fruit is loaded with bioactive compounds that possess antibacterial, anti-inflammatory, and antinociceptive properties. These properties make it an excellent natural remedy to fight infections and relieve pain. The mesocarp layer, in particular, has caught the attention of researchers in recent years, and has been studied for its potential use in various food products. The mesocarp of oleaster fruit is rich in essential vitamins, minerals, and dietary fiber, making it a valuable ingredient in several food products. Researchers have incorporated the fruit into various products such as biscuits, breakfast cereals, yogurt, gluten-free cakes, and bread (Şahan et al., 2013; Öztürk et al., 2018; Zangeneh et al., 2021; Tatari et al., 2022; Yavuz et al., 2022). This is primarily due to its ability to enhance the nutritional, functional, and sensory attributes of the food. With its numerous health benefits, oleaster fruit is fast becoming recognized as a superfood, and is a great addition to one's diet.

Composite flour has recently emerged as a promising alternative to traditional wheat flour for food production. Its use is primarily driven by the desire to enhance the functional and technological properties of food products while also generating economic benefits. Composite flour is created by blending multiple sources, including mango peel powder, apple peel powder, and apricot flour. To summarize, a few of them are as follows. Ajila et al. (2008) studied mango peel powder, a rich source of dietary fiber with health-promoting and functional properties to increase the nutritional properties of biscuits. Adding different levels of mango peel powder (5.0, 7.5, 10.0, 15.0

and 20.0%) enhanced the dietary fiber and antioxidant capacity properties of the biscuits. Rupasinghe et al. (2008) reported that apple peel powder, a very high dietary fiber content, increased phenolic substance and antioxidant activity of the muffins. Adding up to 24% of apple peel powder preserved most physical properties except the color properties. Ozbas et al. (2014) used apple and apricot kernel flours by replacing 10-40% wheat flour in biscuit formulation to investigate the effects of fruit powders on low-fat biscuit quality. In both additive types, the total dietary fiber amount increased due to increased concentration. It was also determined that adding 30% apricot powder was a better fat substitute than apple powder. It has also been reported that apricot flour can produce low-fat bakery products. The versatility and numerous benefits of composite flour have led to its growing popularity in functional foods and nutraceuticals. As such, it has become an increasingly important area of research for the food industry, with many companies and researchers exploring new ways to develop and utilize composite flour in the production of healthier, more nutritious, and more sustainable food products.

This research delves into the characteristics of the mesocarp portion of oleaster cultivated in Karaman, focusing on its physical, technological, functional, and dough rheological properties. The study offers valuable insights into the possible applications of oleaster in various industries. The results indicate that oleaster boasts significant properties that can be utilized for a wide range of purposes. This investigation is an important step in understanding the qualities and benefits of oleaster.

Material and Methods

Materials

The oleasters (*Elaeagnus angustifolia* L.) were collected from three different locations in Karaman, Turkey, in 2021. To analyze the samples, they were mixed and dried in an oven at 22-24 °C for 48 hours (Nüve, FN055, Ankara) and blended for 2 seconds in a Waring blender. The oleaster samples were then separated into three layers using sieving, which included the exocarp (upper 1000 microns), mesocarp (under 300 microns), and endocarp layers (upper 300 microns and under 1000 microns). For this study, the mesocarp layer was used, while wheat flour was used as a control.

Methods

The proximate composition of mesocarp layer

The mesocarp layer of oleaster underwent chemical analyses to determine its proximate composition. The AACC methods 44-15A, 08-01, 46-12, 30-25, and 32-10 were followed according to the standard procedures of AACC (2002) to measure moisture, ash, protein, fat, and crude fiber, respectively. Additionally, the dietary fiber contents of the mesocarp layer were analyzed based on AOAC 960.43 using the Megazyme Dietary Fiber Determination Kit (K-TDFR-100A, Ireland). The insoluble (IDF), soluble (SDF), and total (TDF) dietary fiber contents were determined, and the amount of SDF was calculated by subtracting the IDF from the TDF (AOAC, 2005).

Color analysis

The color analyses of the mesocarp layer (L*:lightness or darkness, a*:redness or greenness, and b*: yellowness or blueness) were conducted with the colorimeter (HunterLab Color Flex, USA). The results were given as means of triplicate measurements.

Water activity analysis

The water activity of the mesocarp was determined utilizing a Novasina (Labmaster device aw, Switzerland).

Water absorption, oil absorption and water solubility indices of the mesocarp layer

The experiment was conducted to ascertain the water and oil absorption indices (WAI & OAI) of the mesocarp layer by the Sharma et al. (2014) method. A slight modification was implemented in the method. Specifically, 0.5 grams of mesocarp layer were dispersed in 10 ml of water. The mixture was then allowed to stand for 10 min at 25°C, then centrifuged at 9000 rpm for 15 min. The WAI was expressed as the amount of water retained by the mesocarp layer (g/g). The oil absorption of oleaster flour was estimated by mixing 0.5 g sample with 10 mL corn oil, allowing it to settle for 10 min at room temperature, and then centrifuging at 9000 rpm for 30 min. The oil retained by the solids was expressed as g/g. WSI was expressed as the weight of dry solids in the supernatant per dry weight of the mesocarp layer.

Extraction of free and bound (hydrolyzable) phenolics and antioxidants

The extraction of free and bound (hydrolyzable) phenolics and antioxidants from the mesocarp layer was carried out using the method of Kaya et al. (2017). The total phenolic and antioxidant content values were calculated by summing up the free and bound phenolic and antioxidant contents. The free phenolics and antioxidants extraction procedures were followed as described. The 0.2 g sample was mixed with acetone: methanol (20 ml, 1:1, v/v) and concentrated HCl (13µL) and shaken in a water bath (200 rpm, 1 hour) at room temperature and then centrifuged (7800 rpm, 10 min) to extract the bioactive component. The supernatant was transferred to a rotary evaporator until the methanol and acetone were completely evaporated in the sample at 45°C. The sediment was dissolved with 1 mL of ethanol, 1 mL of acetone, and 4 ml of distilled water and filtered through a 0.45 µm membrane filter. It was stored at -18°C until analysis. The bound phenolics and antioxidants extraction procedures were followed as described. The process involved hydrolyzing the residue that remained in the free form, using NaOH (10 mL, 4N) in a water bath, maintained at 200 rpm for 3 hours at room temperature. The pH was then adjusted to 1.5-2.0 using concentrated HCl. The extracts were passed through coarse filter paper funnels and extracted using ethyl acetate (60 ml). The resulting extract was concentrated using a rotary evaporator at 45°C. The sediment portion of the sample was dissolved in ethanol, acetone, and distilled water and then passed through a 0.45 µm membrane filter before being stored at -18 °C for analysis.

The analysis of phenolic content

The phenolic content of the sample was analyzed using the Folin-Ciocalteau method, which involved measuring the free, bound, and total phenolic substances expressed as mg of gallic acid equivalents over the gr of dry weight (dw), described by Spanos and Wrolstad (1990).

The 100 µL sample extract, 900 µL distilled water, 5 mL Folin-Ciocalteau solution (0.2 N), and 4 mL sodium carbonate solution (75 g/L) were mixed and incubated for 2 hours in a dark room temperature, were measured in a spectrophotometer (Shimadzu, UV-1800, Japan) at a wavelength of 765 nm. The standard curve of the gallic acid curve was y=0.0011x+0.0135 (R^2=0.99). The results were based on the average of three replicates.

The analysis of Trolox Equivalent Antioxidant Capacity (TEAC)

The Trolox equivalent antioxidant capacity (TEAC) was determined using the Re et al. (1999) method with modifications. The ABTS radical solution was prepared by dissolving 0.0384 g of ABTS in distilled water and mixing it with 12.25 mM (2 mL) of potassium persulfate solution. The solution was then completed with distilled water to a final volume of 10 ml, wrapped in aluminum foil, and kept in the dark at room temperature for 12-16 hours before analysis. The ABTS+ solution was diluted with saline phosphate buffer (PBS, pH:7.4) to absorb 0.700±0.020 at a wavelength of 734 nm before analysis. Four different concentrations (5, 10, 15, and 20 µL) were added to 1 mL ABTS radical, and measurements were performed at a wavelength of 734 nm after 6 min. The calibration curve of Trolox absorbance versus concentration spanned from 5-20 µmol, and the results were expressed as µmol Trolox Equivalent per g sample.

The analysis of EC50 with DPPH assay

In a study conducted by Pellati et al. in 2004, various antioxidant activities were analyzed using the DPPH assay. The free, bound, and total antioxidant activity was assessed by Pellati et al. (2004) method by dissolving DPPH+ radical (0.03943 g) in methanol within a 100 ml flask. The sample extract was mixed with varying volumes (20-40-60-80-100 µl) of DPPH+ solution (600 µl) and brought to a total volume of 6 mL with methanol. The samples were then vortexed and incubated for 15 min at room temperature without light before being measured using a Shimadzu spectrophotometer (UV-1800, Japan) at a wavelength of 517 nm. The percentage inhibition was calculated using formula (1), and the results were expressed as the EC50 value. This value was determined as the concentration of the antioxidant substance required to inhibit 50% of the DPPH radical in the medium. The EC50 values were presented as the mean of duplicate analyses and reported as 'mg dw.'

$$\% \text{ inhibition} = [(A_{\text{DPPH}} - A_{\text{Extract}})/\text{A}_{\text{DPPH}}] \times 100 \quad (1)$$

Mineral analysis of mesocarp layer

The mineral content analysis of the mesocarp layer (Mg, P, K, Ca, Fe, Zn, Na and Cu) was conducted through the use of inductively-coupled plasma spectroscopy, specifically ICP-OES from the Vista series. Before analysis, the samples were treated using a closed vessel microwave digestion oven (MARS 5, CEM Corporation, USA) with concentrated nitric and sulfuric acid. The resulting mineral component concentrations were determined through ICP-OES, utilizing the methodology proposed by Skujins in 1998.

The dough reology analysis

The rheological properties of dough were measured using Mixolab® with Chopin protocol+. The methodology of Dubat (2010) was used to express the physical state of the dough during mixing and heating. Phase 1 involved initial mixing for water absorption, held at 30 °C for 8 min and achieved by 1.1 Nm (+/-0.05 Nm) torque values. Phase 2 represented the weakening of the protein at 30-60°C to indicate protein quality. Phase 3 measured the rate of starch gelatinization, with the value of C3 depending on the starch characteristics. Phase 4 represented the stability during baking to hold 90°C and define the starch gel stability. Finally, phase 5 expressed the retrogradation from 90 to 50°C. These measurements were taken for both wheat flour and composite flour containing various levels of mesocarp level.

Results and Discussion

The Functional Properties of Mesocarp Layer

Table 1 presents an extensive analysis of the mesocarp layer, covering its chemical, physical, and functional properties. The data reveals that the flour has a moisture content of 8.99%, an ash content of 2.66%, a fat content of 0.55%, a protein content of 5.99%, a crude fiber content of 3.32%, and a total dietary fiber (TDF) content of 26.36%. The TDF content is further broken down into insoluble dietary fiber (IDF) and soluble dietary fiber (SDF), which are 21.35% and 5.01%, respectively. The color values were L*: 75.14, a*: 2.86, and b*: 23.87, while the water activity value was measured as 0.314.

Tablo 1. The proximate composition of mesocarp layer

Chemical composition (%, dw)	
Moisture	8.99±0.00
Ash	2.66±0.05
Fat	0.55±0.17
Protein	5.99±0.56
Crude fiber	3.32±0.16
TDF	26.36±0.19
IDF	21.35±0.35
SDF	5.01±0.16
Physical and functional properties	
Color	L*: 75.14±0.13 a*: 2.86±0.03 b*: 23.87±0.05
Water activity (a_w)	0.314±0.004
Water solubility (%)	67.33±3.06
Water absorption (g water/g sample)	4.91±0.33
Oil absorption (g oil/ g sample)	2.26±0.07
Mineral content (mg/100 g, dw)	
Mg	30.21±0.27
P	97.11± 0.83
K	1100.58± 24.94
Ca	63.90±0.37
Fe	0.07±0.01
Zn	nd
Na	7.91± 0.57
Cu	nd

*Values represent mean±sd for triplicate measurements, nd: not detected

Table 2. The free, bound and total phenolic content and antioxidant capacity of mesocarp layer

Bioactive components of the mesocarp layer		
Phenolic content mg GAE/g, dw	Free	25.32±0.52
	Bound	4.83±0.37
	Total	30.15±0.15
Antioxidant capacity EC50 mg, dw sample	Free	0.78±0.03
	Bound	4.64±0.25
	Total	5.42±0.23
Antioxidant capacity µmol TE/g, dw	Free	263.15±3.61
	Bound	102.50±14.71
	Total	365.65±11.10

*Values represent mean±sd for triplicate measurements

Şimşek and Sufer (2020) reported that the crumb of oleaster fruits from İzmir, Aksaray, and Niğde were also analyzed, with the results showing a range of L*:78.91-82.59, a*:1.48-2.98, and b*:17.92-26.88. Additionally, the water solubility, water absorption, and oil absorption were calculated as 67.33%, 4.91 g water/g sample, and 2.26 g oil/g sample, respectively.

The mineral content of the mesocarp layer contained as ppm 30.21 Mg, 97.11 P, 1100 K, 63.90 Ca, 0.07 Fe, and 7.91 Na, while Zn and Cu were not detected. A study conducted in Bursa reported that the oleaster mesocarp flour with pericarp contained 73.5% dry matter, 0.5% crude fat, 2.22% ash, 4.69% protein, and 4.06% crude fiber. The water solubility and water absorption capacity of peeled and unpeeled oleaster were also analyzed, with the results ranging from 90.33-96.01% and 372.74-430.33%, respectively. The total fiber content of the peeled and unpeeled oleaster flour was 23.55-30.65 g/100 g. Furthermore, the study reported that the mineral content of the oleaster flour collected from Bursa was as follows: Fe 11.59 ppm, B 7.43 ppm, Zn 3.85 ppm, Mn 3.56 ppm, and Cu 3.45 ppm (Cansev et al., 2011). It is worth noting that the physical, chemical, and bioactive properties of the oleaster fruit may vary depending on the soil, climate, and ecological conditions in which it is grown. This was highlighted by a study by Saboonchian et al. (2014), which found differences in the characteristics of oleaster grown in different regions.

Table 2 provides information on the bioactive properties of the mesocarp layer, including the free, bound, and total phenolic content as well as the antioxidant activity. The free, bound, and total phenolic contents were found to be 25.32, 4.83, and 30.15 mg GAE/g, dw, respectively. The antioxidant activity was expressed as EC50, which is the concentration required to inhibit 50% of the DPPH radical in the medium, and Trolox Equivalent Antioxidant Capacity (TEAC). The mesocarp layer had 0.78 EC50 mg dw sample and 263.15 µmol TE/g, dw for the free, 4.64 EC50 mg dw sample and 102.2 µmol TE/g, dw for the bound, and 5.42 EC50 mg dw sample and 365.65 µmol TE/g, dw for the total. Recent scientific research has extensively explored phenolic contents and the antioxidant capacity of various components of oleaster fruits, including flour, shell, core, peel, pulp, crust, and crumb. According to Hassanzadeh and Hassanpour's research in 2018, the average value of total phenolic contents for both the peel and pulp of Iran-grown oleaster was found to be 518.07 and 480.16 mg GAE/100 g fresh weight, respectively. On the other hand, Karkar and Şahin's study in 2022 showed that the phenolic contents varied depending on the extraction method used. For instance, the phenolic contents ranged from 0.13 to 34.89 mg GAE/g of oleaster flour, from 0.37 to 36.16 mg GAE/g of oleaster shell, from 1.12 to 158.73 mg GAE/g of oleaster core, and from 2.36 to 205.26 mg GAE/g of oleaster flower. Comparing our findings to previous studies, our results align with Karkar and Şahin (2022) but are higher than those reported by Hassanzadeh and Hassanpour (2018). Karkar and Şahin (2022) conducted a study that revealed the diverse range of antioxidant capacity in oleaster flour, shell, and core, which was highly dependent on the method used for extraction. For instance, the values ranged from 11.60 to 39.71 mg TE/g for flour, 14.48 to 34.42 mg TE/g for shell, and 23.72 to 67.55 mg TE/g for core. On the other hand, Hassanzadeh and Hassanpour (2018) discovered that the mean antioxidant capacity of peel and pulp was 74.71% and 53.76%, respectively. Another study by Faramarz et al. (2015) reported that the antioxidant capacity measured by the DPPH method was 86.95% and 91.78%, respectively. Moreover, Şimşek and Sufer (2021) researched the antioxidant activity of crust and crumb extracts from oleaster fruits, which were found to be in the range of 6.28-14.05 µmol TE/g DM and 5.01-11.56 µmol TE/g DM, respectively. It is crucial to bear in mind that several factors can influence the phenolic contents and antioxidant capacities of oleaster fruits, including cultivars, genotypes, climate conditions, and geographical locations, as reported in other studies (Hassanzadeh & Hassanpour, 2018; Şimşek & Sufer, 2021).

The Rheologycal Properties of Mesocarp Layer

Table 3 and Figure 1 present the rheological properties of wheat flour (control) and composite flours that contain varying percentages (10%, 20%, and 30%) of mesocarp flour. Introducing the mesocarp layer into the composite flours has a noticeable impact on the thermomechanical properties of the control flour. One of the significant changes is the decreased hydration capacity of the composite flours in all ratios. The mesocarp layer incorporation (from 10 to 30) decreased the water absorption capacity of the wheat dough from 53.5 to 47.0%. This means that the composite flours with mesocarp layer are less capable of absorbing water than the control flour (Figure 1).

This decrease in water absorption capacity indicates that mesocarp layer incorporation hinders the hydration of granular starch and wheat protein.

Tablo 3. Rheological properties of the control and composite flours containing various levels of mesocarp layer as measured by Mixolab

S	R	WA	DDT	ST	C1**	C2**	C3**	C3-C2	C4**	C3-C4	C5**	C5-C4
CF	0	53.5	1.10	8.90	1.09	0.41	1.68	1.27	1.70	-0.02	2.53	0.83
MF	10	51.9	1.07	6.15	1.06	0.30	1.28	0.98	0.99	0.29	1.38	0.39
	20	49.3	0.95	2.15	1.12	0.27	1.10	0.83	0.77	0.33	1.13	0.36
	30	47.0	0.75	2.25	1.09	0.23	0.87	0.64	0.62	0.25	0.92	0.30

S: Samples; CF: Control flourl; R: Ratio (%); WA: Water absorption (%); DDT: Dough development time (min); ST: Stability time (min); *Values represent mean for duplicate measurements, MF: Mesocarp layer flour; ** Torque (Nm)

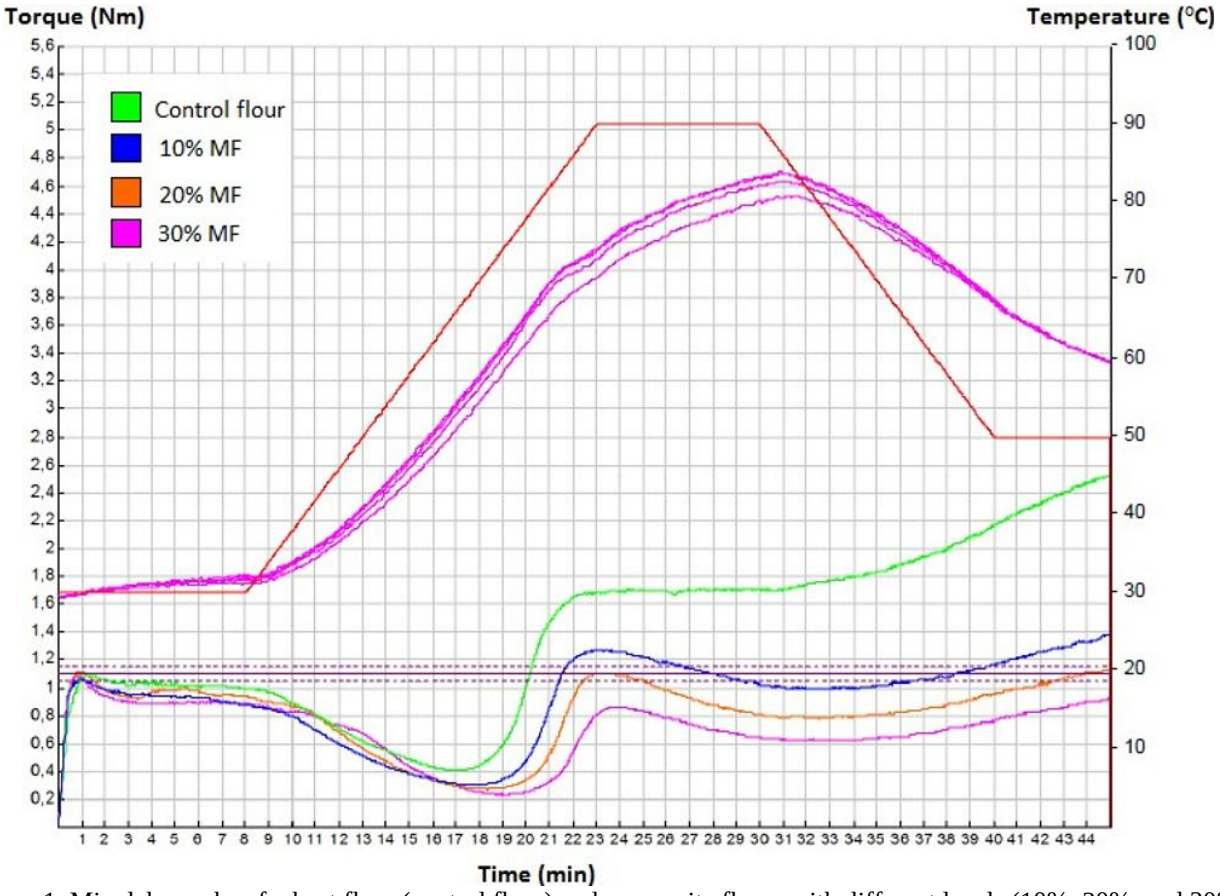

Figure 1. Mixolab graphs of wheat flour (control flour) and composite flours with different levels (10%, 20%, and 30%) of mesocarp layer

As the substitution level of the mesocarp layer increases from 0% to 30%, dough development and stability time significantly decrease from 1.10 to 0.75 and 8.90 to 2.25 min, respectively. The decrease in dough development time is likely due to the mesocarp layer being rich in total phenolics and antioxidants. These compounds may form protein-polyphenol interactions, incorporate phenolic compounds into the gluten network, and close them in the hydrophobic pockets (Sivam et al., 2010). A recent study by Welc et al. (2022) found that the presence of polyphenols in gluten can cause significant structural and functional changes in gluten proteins. These changes include aggregation and conformational alterations of disulfide bridges. Reducing these interactions is a desirable outcome, as highlighted by Pala (2012). One way to measure the strength of flour is to analyze dough stability time. A higher value indicates a stronger dough, critical for forming a three-dimensional viscoelastic structure that relies on wheat gluten (Rosell et al., 2007). However, adding the mesocarp layer, which has a protein content of 5.99%, can inhibit the development of gluten network structure due to the dilution of gluten concentration and competition for water between gluten, starch, and mesocarp layer. This results in lower dough stability time. Previous studies by Danno and Hoseney (1982) and Okada et al. (1987) have shown that free radical scavengers like phenolic acids can accelerate dough breakdown. On the other hand, Han and Koh (2011) reported that adding phenolic acids to dough can decrease kneading time, tolerance, elasticity, and bread volume. Composite flours containing mesocarp layer in all ratios are characterized by a lower C2 torque and a lower viscosity peak (C3 and C3-C2), as well as higher hot stability (C3-C4) and lower retrogradation (C5-C4). These qualities make the flour an excellent substitute for wheat flour in various food products. The changing hot viscosity (C3) of the composite flours may be attributed to a unique alteration in water distribution. Additionally, the mesocarp layer substitution in wheat flour can significantly enhance product shelf-life due to slower retrogradation (C5-C4), indicating a weaker starch gelling process.

Conclusion

In this study, the physical, technological, and dough rheological properties of the mesocarp layer of oleaster (*Elaeagnus angustifolia L.*) cultivated in Karaman were thoroughly examined, with a focus on its functional properties. The results showed that the mesocarp layer of oleasters grown in Karaman had high protein and ash content, low crude fiber, and a fat content comparable to those grown in other regions. The crumb color and dietary fiber amounts of the mesocarp layer were consistent with previous studies. However, the mineral properties of the oleaster were found to vary depending on the region, climate, and soil structure in which it is grown, leading to different characteristics. While the bioactive component properties were similar to previous studies, there were instances where they were lower, likely due to varying factors such as cultivars, genotypes, climate conditions, and geographical locations. Additionally, the inclusion of the mesocarp layer significantly affected dough rheology. The findings of this research highlight the numerous applications of oleaster in various industries, showcasing its significant properties that can be utilized for a broad range of purposes. This investigation provides valuable insights into the characteristics and potential advantages of the oleasters, contributing to our understanding of this plant's properties.

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Insecticidal Effect of a Natural Turkish Diatomaceous Earth Formulation on Greater Wax Moth

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ABSTRACT

In this study, the insecticidal effect of the Detech® (Turkish Diatomaceous earth) DE formulation against *Galleria mellonella* L. was determined. The study was conducted in a laboratory environment with materials taken from beehives produced at Muş Alparslan University in 2022. Diatomaceous earth (DE) was applied in two different forms (dust and slurry DE) and as positive control GüveSavar®, which is currently used against some pests in beekeeping. DE concentrations of 0, 3, 5, and 7 g/m^2 in different exposure times were tested for the control of *G. mellonella* larvae. As a result of all treatments, the highest mortality occurred at 7 g/m^2 dust DE concentration. Larvae (3rd stage) exposed to 7 g/m^2 concentration according to dust DE mortality rates reached 100% mortality after 40 hours. According to the results of the slurry DE, the larvae exposed to the slurry diatom at all concentrations achieved 100% mortality at the end of the 96 hours. When the dust and slurry DE results were examined, the direct use of dust formulations greatly accelerated the effectiveness against larvae. The study showed very promising results, suggesting that slurry DE and dust formulations could be a new alternative control method for Greater Wax Moth. In addition, for the first time, the insecticidal efficacy of DE against the honey bee pest, the greater wax moth, was determined.

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Introduction

Honey bees, *Apis mellifera* L. (Hymenoptera: Apidae) are one of the most ecologically and economically valuable bee species in the world. Thanks to beekeeping, 70% of the world's valuable bee products and the pollination of plant production take part in honey bees (Klein et al., 2007). For bees to perform these valuable activities, they must be protected against diseases and pests. Varroa mites, greater wax moth, and small hive beetle, *Aethina tumida* Murray, 1867 (Coleoptera: Nitidulidae) are known as the most important pests of bees. These pests cause great damage to bees and their products (Turker et al., 1993; Core et al., 2012; Dietemann et al., 2013; Neumann et al., 2016; Gunesdogdu et al., 2021). In addition, these pests carry bee disease agents between colonies and apiaries (Charriere & Imdorf, 1999; Kwadha et al., 2017). Great wax moth causes economic losses for beehives and beekeepers (Haewoon et al., 1995; Almadani & Hiware, 2020). Greater wax moth larvae cause extreme damage to beeswax by consuming nutrients stored in the bee comb, especially in the 4th and 5th stages (Kwadha et al., 2017; Almadani & Hiware, 2020). Wax moths feed on pollen stored in the honeybee comb (Milan, 1970; Gulati & Kaushik, 2004). Beeswax can be contaminated by moths in the colony, during the honey harvest, or in storage (Rajendran & Hajira Parveen, 2005; Zhu et al., 2016; Kwadha et al., 2017; Mansour, 2020). *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) is a serious pest of weak honeybee colonies and stored combs (Shimanuki, 1980; Williams, 1997; Ritter & Akratanakul, 2006). The larva is creamy white. It feeds on beeswax during the larval stage. The

insect develops in 7 larval stages (Desai et al., 2019). *Galeria mellonella* larvae were obtained from honeybee hives produced by the Animal Production and Technologies Department on the university campus. The larvae were collected from the honeycombs stored during the winter period when honey production was not produced and used in the experiments. Larvae (3rd instar) were tested under warehouse laboratory conditions. Insects and honeycombs were left in the containers as bait.

Various chemical (sulphur, aluminium phosphide, ethylene dibromide, methyl bromide, paradichlorobenzene (Napthalene), cold-hot applications, organic (vegetable oils and extracts) and biological insecticides (*Bacillus thuringiensis* Berliner, 1915) (Bacillales: Bacillaceae) control methods are applied in different ways (Burges, 1977; Ritter et al., 1992; Bisht et al., 2017; Telles et al., 2020; Beyene & Woldatsadik, 2019). These products negatively affect human health by making residues in beeswax and other bee products (Ritter & Akratanakul, 2006). Today, chemicals used to control this pest in particular cause residues in bee products. This situation negatively affects the quality and sales of bee products, which are very important for human health. Alternative control methods that do not leave chemical residues in bee products are the focus of many researchers. In particular, honey and bee products free from chemical products are important points to be considered. Therefore, the role of organic and mammalian non-toxic Diatomaceous earth in-pest control has been studied. Diatomaceous earth is one of the natural insecticides used as an alternative to chemical pesticides (Korunic, 1998).

There are different theories in the literature regarding the mechanism of action of DEs on insects. It is generally accepted that DE particles adhere to the cuticle of insects, causing death by absorption of fluids from the insect body by DE (Subramanyam & Roesli, 2000; Vayias & Vassiliki, 2009), but abrasion of the cuticle is also a complementary action through cuticular micro-wounds (Subramanyam & Roesli, 2000). Dose-dependent mortality results also show that a higher dose results in more DE particles in the insect's cuticle and faster death. Adsorbed DE particles immediately damage the protective waxy coating on the insect body, mostly through absorption and to a lesser extent, abrasion or both. It has been the common result of many studies that the main activity that causes the death of insects is the loss of water from the body of the insect through desiccation (Ebeling, 1971; Korunic et al, 1988).

Local diatomite preparation originating in Türkiye has been commercially formulated as named Detech® by Entoteam (Entoteam R&D Food Agriculture Co.). Previous studies have shown that the dust formulation of these two local diatomite preparations has high efficacy against various stored grain pests at different concentrations (Erturk et al., 2020; Saglam et al., 2020). Diatomaceous earth is non-toxic to mammals (oral LD50 value > 5000 mg/kg body weight in rats), does not leave toxic residues on crops, and is classified as GRAS (Generally Recognized as Safe) according to the US EPA because it is used as a food additive (FDA, 1995). Diatoms was formed because of the sedimentation of single-celled microscopic algae from the fossilized siliceous shells of the algae. The cell walls of diatomites are composed of amorphous silica. Diatomite dust is probably the most effective natural source that can be used as an insecticide (Korunic, 1998). Diatomite dusts are effective in the cuticle of insects, causing rapid drying of the insect and thus its death from water loss. It has been reported that Türkiye has very rich natural DE deposits and these large diatomite rock formations are in various parts of the country (Ozbey & Atamer, 1987; Mete, 1988; Sıvacı & Dere, 2006; Tas & Cetin, 2012).

In this study, the effect of dust and slurry formulations of Detech® formulation and GüveSavar® (essential oil-based) for the control of *G. mellonella* during stored honeycombs was investigated. The aim is to expand the use of natural products as alternatives to chemical pesticides against harmful insects in beekeeping. The insecticidal activity of organic DE against *Galeria mellonella* was investigated.

Materials and Methods

Turkish DE preparation (Detech®)

It has been determined that Türkiye has very rich natural DE resources and these great diatom resources are in different regions of the country. In recent years, because of scientific studies, Türkiye-origin diatom preparation Detech® has been enhanced for the physical control of pests. Detech® mainly consists of 80.6% (w/w) amorphous silicon dioxide and its median particle diameter (d (0.5)) is 14,061 µm (Bayram et al., 2019). Some properties of Detech® preparation are given in Table 1.

Commercial preparation used in beekeeping (GüveSavar®)

GüveSavar® consists of natural ingredients such as plant shells, nettle extract, walnut leaf extract, and molasses. It is reported that 250 ml of GüveSavar® protects approximately 220 Honeycombs between August and April. The concentrate is created by mixing 250 ml of GüveSavar® with 5 liters of water. The mixture is filled into clean spray bottles and sprayed homogeneously on the honeycombs and allowed to dry. The honeycombs applied with GüveSavar® are kept in suitable warehouse conditions. This commercial product is widely used in beekeeping with effective results in Türkiye, so it was used in trials as a positive control.

Table 1. Some physical and chemical properties of dust formulation of Detech® commercial local diatomite dust used in biological activity tests

DE preparation	SiO_2 (%) *	Median particle size (µm)**	pH value ± S.E.	Batch density ± S.E (g/l)	Color
Detech®	80.6	14.061	8.25±0.01	248.1±5.3	Yellowish white

*The physical and chemical of the diatomite sample was determined in the Analysis Laboratories of the General Directorate of Mineral Research and Exploration. ** Median value corresponding to 50% of the total particle volume in the volume of the cumulative particle size distributions (d(0.5)).

Table 2. Mortality (%) of greater wax moth larvae exposed to dust diatom formulations

AR	Mortality (%) ± S.E.				
	Exposure Time				
	8	24	32	40	
3	0 ± 0 Ab	46.6 ± 3.3 Aa	70 ± 10 Aa	73.3 ± 6.6 Ba	$F_{3, 8}$= 51.22 P<0.001
5	0 ± 0 Ab	50 ±15.2 Aa	80 ±10 Aa	90 ±5.7 ABa	$F_{3, 8}$= 16.97 P<0.001
7	0±0 Ac	43.3 ± 8.8 Ab	96.6±3.3 Aa	100 ±0 Aa	$F_{3, 8}$= 109.29 P<0.001
	$F_{2, 6}$= -P= -	$F_{2, 6}$= 0.12 P=0.893	$F_{2, 6}$= 2.71 P=0.145	$F_{2, 6}$= 8.93 P=0.016	

AR: Application rate of DE (g/m^2); *The data were subjected to one-way analysis of variance (ANOVA) and the differences between the means were determined at 5% significance level by applying TUKEY a test. Different lowercase letters in the same column and different uppercase letters in the same row indicate statistically different means.

Table 3. Mortality (%) of greater wax moth larvae exposed to slurry diatom formulations

AR	Mortality (%) ± S.E.				
	Exposure Time				
	24	48	72	96	
3	0 ± 0 Ad	13.3 ± 3.3 Ac	56.6 ± 3.3 Bb	100 ± 0 Aa	$F_{3.8}$= 547.48 P<0.001
5	0 ± 0 Ad	16.6 ± 6.6 Ac	60 ± 11.5 Bb	100 ± 0 Aa	$F_{3.8}$= 81.99 P<0.001
7	0±0 Ac	23.3 ± 3.3 Ab	93.3 ± 3.3 Aa	100 ± 0 Aa	$F_{3.8}$= 166.09 P<0.001
	$F_{2, 6}$= -P= -	$F_{2, 6}$= 1.27 P=0.347	$F_{2, 6}$= 8.55 P=0.018	$F_{2, 6}$= - P= -	

AR: Application rate of DE (g/m^2); *The data were subjected to one-way analysis of variance (ANOVA) and the differences between the means were determined at 5% significance level by applying TUKEY a test. Different lowercase letters in the same column and different uppercase letters in the same row indicate statistically different means.

Test procedures

The larvae were exposed to different dust and slurry concentrations (0, 3, 5 and 7 g/m2) for each application using a 1000 ml hand sprayer. Slurry DE formulations were prepared by mixing 0.12 g of DE/ml water. It was shaken by hand before the application so that DE and water had a homogeneous distribution. Dust and slurry diatom forms Detech® were used in biological test. The study was carried out in plastic containers (254*191*99 mm) containing bee bread (perga) for feeding the larvae. The diatomite dust form was homogeneously distributed on the plastic container surface with a small brush, the 3th instar larvae were transferred into the containers, and mortality were obtained at the exposure times (8, 24, 32, and 40 hours). Experiments were prepared in 3 replications and mortality rates were determined by leaving 10 Greater wax moth larvae in each replication. As a positive control, GüveSavar® (0, 3, 5 and 7 ml/100 ml) was applied as a spray in the form of a slurry in plastic containers and the larval mortality rates were determined at the exposure times (24, 48, 72, 96 hours). The larvae were collected from different wax frames. This study was carried out on the Muş Alparslan University campus, Faculty of Applied Sciences in 2022.

Statistical Analysis

Tables containing the obtained data, mean mortality rates, and standard errors for each application separately were created. Mortality rates were determined by recording the mortality results in the number of individuals in all treatments after certain periods. After applying the arcsin transform to individual mortality rates, they were subjected to analysis of variance using the statistical program SAS 9 (SAS Ins. 2009). One-way analysis of variance (ANOVA) was applied to the results and the differences between the averages were determined by using TUKEY test at 5% significance level.

Results

Mortality of Greater wax moth larvae was determined after 8, 24, 32, and 40 hours of exposure to dust D formulation (Table 2). According to the mortality, the larvae exposed to a concentration of 7 g/m^2 had a 100% at the end of the 40 hours. Mortality of 73.3% and 90% were obtained at an exposure time of 40 hours at concentrations of 3 and 5 g/m^2, respectively. It has been determined that dust diatom applications can be effective against larvae with increasing insecticidal efficiency results with increasing concentrations. Three different dust concentrations turned out to be ineffective on larvae after 8 hours of exposure.

Mortality of Greater wax moth larvae was determined after 24, 48, 7and 2, 96 hours of exposure to slurry DE form (Table 3). According to the mortality, the larvae exposed to three different slurry diatoms concentrations had 100% at the end the 96 hours. It has been determined that the insecticidal effect result from increasing with concentrations, slurry diatom applications could be effective against larvae. Three different concentrations of slurry diatoms appeared to be ineffective against larvae at 24 hours of exposure.

Mortality of Greater wax moth larvae were determined after exposure times of 4, 8, 12, and 16 hours to the GüveSavar® (Table 4). According to the mortality, the larvae exposed to a concentration of 7 g/m^2 had a 100% mortality rate at the end of the 12 hours. In the other concentrations (3 and 5 g/m^2), 100% mortality rates occurred after 16 hours. Insecticidal activity results increased with concentrations It was determined that moth-repellent preparation treatments conducted high-lethal activity against larvae.

Table 4. Mortality (%) of greater wax moth larvae exposed GüveSavar®

AR	Mortality (%) ± S.E.				
	Exposure Time				
	4	8	12	16	
3	6.6 ± 0 Ad	36.6 ± 3.3 Bc	70 ± 5.7 Bb	100 ± 0 Aa	$F_{3.8}$= 78.09 P<0.001
5	40 ± 5.7 Ad	76.6± 8.8 ABbc	93.3 ± 6.6 Aab	100 ± 0 Aa	$F_{3.8}$= 16.07 P<0.001
7	56 ± 8.8 Ac	90 ± 10 Aa	100 ± 0 Aa	100 ± 0 Aa	$F_{3.8}$= 10.10 P<0.004
	$F_{2, 6}$= 14.23 P= 0.005	$F_{2, 6}$= 8.08 P=0.020	$F_{2, 6}$= 9.54 P=0.014	$F_{2, 6}$= - P= -	

AR: Application rate of DE (g/m^2); *The data were subjected to one-way analysis of variance (ANOVA) and the differences between the means were determined at 5% significance level by applying TUKEY a test. Different lowercase letters in the same column and different uppercase letters in the same row indicate statistically different means.

Discussion

As a result, the potential of using the Detech® used in the study against moth pests during storage and storage of empty combs in beekeeping has emerged. In the study, it was revealed that slurry DE and dust formulations can be an important alternative as an organic insecticide to the control of Greater wax moth. During storage, when the combs are contaminated with moths, they become completely unusable. For this reason, dish combs are destroyed by burning. Therefore, the potential of DEs is promising in terms of protecting empty combs from pests during storage and reuse. In addition, diatoms are generally odorless powders, their moisture content varies between 2-6%, they are insoluble in water and do not burn, and there is no risk of flammability. DE is a highly stable organic substance that does not leave toxic chemical residues or react with other substances in its environment and is considered non-toxic to mammals (Quarles, 1992). Demirözer et al. (2022) reported that initial direct mortality to bees for DEs was kept below 25%, so DEs were classified as harmless to both honeybees and bumblebees. Also, mortality rates accelerated in parallel with increasing DE and GüveSavar concentrations. Prasantha et al. (2002), observed increased mortality linearly correlated with DE concentration. Mortality was reported to increase with increasing concentration in all treatments. Erturk et al. (2020) reported that the mortality rate of *Sitophilus oryaze* (L., 1763) (Coleoptera: Curculionidae) increased with Detech® WP dose and exposure time on both concrete and wooden surfaces. At the same time, the effectiveness of GüveSavar® swollen combs used in traditional beekeeping against the greater wax moth, which is a storage pest, was determined. Respectively, the most effective mortality percentages occurred with the GüveSavar® treatments, followed by dust DE. Almadani & Hiware (2020) found that thyme oil and homeopatic grug lodium treatment reduced the hatching of the great wax moth, Bisht et al. (2017) used neem oil, cedar oil, clove oil, pipermite oil, and karang oil in the treatment against Greater wax moth under storage conditions. Neem oil had the highest effect and Karang oil had the lowest effect. According to Ayman & Atef (2007), methyl salicylate, formic acid, clove oil, acetic acid and bacillus oil have high mortality. Fawzy et al. (2017), investigated the effects of propolis, cinnamon, clove, and peppermint ethanolic extracts on the 4th larval instar of the Greater wax moth and found that peppermint extract was the most effective and propolis extract had no effect. According to Swamy et al. (2006), mahua oil (63%), neem oil (62%), and pongamia oil (56%) cause a reduction in the Greater wax moth larvae populations. Paulraj et al. (2021) investigated the mortality of wax moth larvae using *Mentha piperita* (L., 1753) (Lamiales: Lamiaceae), eucalyptus oil and lemongrass oil. They reported mortality rates of 80.24%, 69.05%, and 50.48% respectively. Telles et al. (2020) investigated the effect of natural products of neem oil *Azadirachta indica* (A. Juss., 1830) (Sapindales: Meliaceae), eucalyptus oil (*Eucalyptus* spp.), tobacco extract *Nicotiana tabacum* L. (Solanales: Solanaceae) and malagueta pepper extract, *Capsicum frutescens* (Solanales: Solanaceae) on the control of greater wax moth. Neem oil and eucalyptus oil were reported to provide moth control at low doses and to be toxic to adult bees. They reported that tobacco and malagueta pepper extract-controlled moths and did not cause any adverse effects on bees. The untreated DE honeycombs (control group) quickly became contaminated with pests. In light of these observations, it has emerged that Diatom applications in empty honeycombs can be used as a preservative during the storage period. Ferreira et al. (2017), repellent and foraging of negramina oil *Siparuna guianensis* Aubl., 1775 (Laurales: Siparunaceae) against larvae and adults of *G. mellonella* and *Achroia grisella* F., 1794 (Lepidoptera: Pyralidae) wax moths reported that they were attracted to bees. Saglam et al. (2022) & Bayram et al. (2019) revealed that different concentrations (600 and 900 ppm) of Detech® local diatom preparation had high and moderate repellent effects against confused flour beetle *Tribolium confusum* Jacquelin du Val, 1863 (Coleoptera: Tenebrionidae) and rice weevil *Sitophilus oryzae* L., 1763 (Coleoptera: Curculionidae) adults, respectively, while no or low-level repellent-effects on *Rhyzopertha dominica* F., 1792 (Coleoptera: Bostrichidae) adults.

The use of Detech® as a natural insecticide to protect combs from insect pests offers a new alternative control and protection opportunity. As a result, it was determined that Diatomaceous earth could offer an alternative physical control method in beekeeping.

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Exploring the Efficacy of Essential Oils in Laboratory Conditions for Controlling Mediterranean Fruit Fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)

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ABSTRACT

The Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), poses a significant threat to agriculture worldwide. This study examines the potential insecticidal effects of essential oils from *Mentha arvensis* and *Cinnamomum zeylanicum* on controlling *C. capitata* under laboratory conditions. Even at low concentrations, toxicity assays indicated that both essential oils significantly increased the mortality of adult Medflies. The concentration-dependent effect of these oils on *C. capitata* mortality is demonstrated, with *Mentha arvensis* achieving 100% mortality within 48 hours at 1% concentration and *Cinnamomum zeylanicum* exhibiting rapid efficacy, reaching a low LC_{50} value after only 1 hour of application. The concentration and application time of essential oils were found to have a significant impact on their effectivness. This study highlights the potential of essential oils for controlling *C. capitata* populations. Essential oils offer a sustainable and eco-friendly alternative for managing *C. capitata* but further studies are necessary for their successful incorporation into integrated pest management programs.

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Introduction

The Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is a serious agricultural pest of global importance due to its invasive behavior and devastation of a broad variety of fruit crops. The medfly, which is native to sub-Saharan Africa, has extended its geographic range quickly, becoming a cosmopolitan species found on five continents (Gomulski et al., 2008; CABI/EPPO, 1998). Its adaptability to diverse climatic conditions and polyphagous nature, with a voracious hunger for more than 350 botanical species spanning 65 plant families, make it a tough agricultural challenge (Papadopoulos et al., 1996; Weems, 1981; Liquido et al., 1991).

In conventional farming, the management of *C. capitata* has mainly relied on the substantial use of synthetic insecticides and proteinic baits laced with toxic substances (Roessler, 1989). However, the continuous use of these compounds caused significant environmental and to health issues. Concerns include acute and chronic poisoning of pesticide applicators and consumers, damage to non-target organisms, disruption of pollination systems, pollution of groundwater, and causing insecticide resistance (Nerio et al., 2010; Picollo et al., 2005).

As a result of these concerns, there is an increasing interest in creating ecologically benign alternatives for the control of *C. capitata*. Due to their bioactive properties, which include insecticidal, repellent, and antifeedant effects, essential oils derived from numerous plant sources have emerged as promising candidates for this purpose. (Isman, 2006; Kilic et al., 2019).

The possible insecticidal effects of *Mentha arvensis* and *Cinnamomum zeylanicum* essential oils against *C. capitata* have been gaining considerable interest. These oils are renowned for their diverse chemical compositions, which include compounds with insecticidal effects, such as menthol and cinnamaldehyde. Previous studies have provided persuasive information that these essential oils have the ability to elicit toxicity in adult *C. capitata* (Passion et al., 1999; Miguel et al., 2010; Benelli et al., 2012; Benelli et al., 2013; Papanastasiou et al., 2020).

This study aims to determine the insecticidal effects of *M. arvensis* and *C. zeylanicum* essential oils on adult *C. capitata* under laboratory conditions. This research contributes to our understanding of the potential of these essential oils as viable alternatives for treating medfly infestations by determining mortality rates and concentration-dependent responses. In addition to this, this

study explores the concentration and timing factors that impact the efficiency of essential oils, therefore shedding light on their practical implementation in integrated pest management strategies.

Materıals and Methods

Ceratitis capitata Culture

Ceratitis capitata adults and larvae were collected from infested orchards.

The population has been consistently maintained under semi-mass rearing conditions, utilizing the rearing procedures outlined by Caceres (2002). The maintenance of the colony took place in controlled climatized chambers, with a consistent temperature of 25 ± 2°C, relative humidity of 75 ± 5%, and a photoperiod of 12:12 hours (L:D) throughout the entire life cycle. Additionally, total darkness was maintained during the pupal development stage. Adult flies were housed in "sandwich" cages (measuring 100 cm in length, 6 cm in width, and 75 cm in height). These cages were equipped with voile cloth on their sides. The diet provided to the adult flies consisted of a combination of refined sugar and lyophilized beer yeast extract in a ratio of 3:1, as described by Walder et al. (2014). Flies were provided unrestricted access to water. Oviposition cages were arranged in a randomized design, and egg deposition occurred on the screens of the cages. Eggs were collected from the cages after a 24-hours and subsequently, to obtain laboratory-reared larvae, the eggs collected from the maternal colony were subjected to 48 hours of bubbling in a water bath at a constant temperature of 24˚C before being introduced into the artificial larval diets.

Essential Oils

The essential oils tested in this study were *Cinnamomum zeylanicum* and *Mentha arvensis*. The essential oils were purchased from the Nature In Bottle company. The source of plants, plant parts and the obtaining methods are shown in Table 1.

Contact Activity Assays

Adult individuals were exposed to direct contact with essential oil residues from *Mentha arvensis* and *Cinnamomum zeylanicum*. This test was chosen to determine the insecticidal effect of essential oils on mortality of adults. This methodology closely emulates the practical scenario of flies coming into contact with treated vegetation, as outlined by Pavela in 2011.

Experimental units comprised of plastic cages 12 cm in diameter by 5 cm height, with a lid which had a cover which featured a hole 7.5 cm in diameter. Preliminary experiments showed similar mortality using ventilated Petri dishes (9 cm) diameter and ventilated lower cages (9 cm diameter), simply to make sure there is no fumigation impact of essential oils. The control was treated with pure acetone (EMSURE® ACS). Filter paper disks of 11 cm diameter were treated with 1 ml of acetonic solutions (0, 0.2; 0.4; 0.5; 0.6; 0.7; 0.8; 1%) of the oils and dried for at least 10 minutes, under a fume extractor. Later, each treated filter paper was fixed on the top of the cage with plasticine at two opposite points. A small plastic container (1 cm diameter) containing adult food as well as a drinking trough was supplied. Afterward, five couples of *C. capitata* adults previously sexed (<72 h old) were introduced in each cage and placed in a climatic chamber at 25°C, RH 75%, L:D 16:8. Mortality was scored after 1, 6, 24, 48, and 72 h. 5 replicates per concentration were carried out.

Statistical Analysis

The assessment of the toxicity was conducted by calculating the percentage of mortality observed on the 1st, 2nd, 24th, 48th and 72th hours following the application of essential oils. Data collected from the insecticide trials were subjected to statistical analysis, considering both the specific study conditions and the date of sampling. To enhance the suitability of percentage data for statistical analysis, an arc-sine square-root transformation was applied. The data were analyzed by the analysis of variance (ANOVA). Mean values were separated using Tukey's honestly significant difference (HSD) multiple comparisons test at a significance level of $P<0.05$. Dose-response results were subjected to probit analysis using Polo software (Polo Plus, version 1.0) to calculate the 50% lethal concentration (LC_{50}), 90% lethal concentration (LC_{90}).

Results and Discussion

Direct contact with essential oil residues from both essential oils resulted in increased mortality among adult Medflies.

The essential oils of *Mentha arvensis* and *Cinnamomum zeylanicum* caused a substantial increase in mortality over time, even at low concentrations (Table 2).

In the dose response tests of *M. arvensis*, *C. capitata* mortality reached 100% within 48 h at 1% concentration. For *C. zeylanicum* application within 72 hours, the mortality reached 100% at 0.8% concentration. The results show that the concentration of essential oils affected the mortality of *C. capitata* differently ($P<0.05$). These results for *M. arvensis* and *C. zeylanicum* revealed concentration-dependent mortality effects. This finding is consistent with the well-established principle that the efficacy of essential oils as insecticides is influenced by their concentration (Regnault-Roger, 1997). Higher concentrations often result in more rapid and complete insect mortality.

Mentha arvensis and *C. zeylanicum* were capable of controlling *C. capitata* based on the LC_{50} and LC_{90} values at 1, 6, 24,48, and 72 hours (Table 3). When examining the LC_{50} and LC_{90} values for *C. zeylanicum*, the LC_{50} value was determined to be 0.8455 even 1 hour after application. The LC_{90} values found 48 and 72 hours after application were 0.978 and 0.466, respectively. For *M. arvensis*, while the LC_{50} value was 0.724 at 1st hour of application, the LC_{90} value was 0.999 at 6th of application.

The determination of the LC_{50} and LC_{90} values is critical for assessing the insecticidal activity of essential oils. According to the results, *M. arvensis* was able to successfully kill the adults of *C. capitata*, as indicated by their LC_{50} and LC_{90} values. Similar findings have been reported in other studies involving the use of essential oils against fruit fly pests (Benelli et al., 2012).

Table 1. The source of plants, plant parts and the obtaining methods

Common name	Scientific name	Plant parts	Extraction method
Field mint	*Mentha arvensis*	leaf	Steam distillation
Cinnamon	*Cinnamomum zeylanicum*	leaf	Steam distillation

Table 2. The effect of varying concentrations of essential oils on the mortality rate of adult Mediterranean fruit flies at distinct time points of application.

EO	T	Concentration (%)						
		0.2	0.3	0.4	0.5	0.6	0.8	1
Mentha arvensis	1	10±7.07Bd*	16±15.17Cd	32±19.24Cc	40±12.47Cbc	46±5.48Db	48±13.04Cab	68±8.37Ba
	6	14±11.40Be	26±11.40BCd	52±13.04BCc	68±5.47Bb	68±8.37Cb	80±7.07BCab	90±7.07Aa
	24	26±5.47ABf	44±8.94ABe	60±14.14ABd	80±7.07ABc	88±8.37BCbc	92±4.47ABa	96±5.48Aa
	48	32±8.36Ad	46±11.40ABcd	68±10.95ABc	84±5.47Ab	88±8.37ABab	94±5.48ABa	100±0.00Aa
	72	34±8.94Ad	52±8.37Ac	84±8.94Ab	92±8.36Aab	94±8.94Aa	98±4.47Aa	100±0.00Aa
Cinnamomum zeylanicum	1	2±4.47Bd	2±4.47Bd	6±5.47Cd	14±5.47Ccd	22±4.47Dc	46±5.48Db	60±7.07Ba
	6	2±4.47Be	4±5.47ABe	10±7.07Cde	18±8.36Cd	34±5.48Cc	60±7.07Cb	80±14.14ABa
	24	4±5.47ABe	4±5.47ABe	12±4.47Cde	18±8.94Cd	42±4.47Cc	60±7.07Cb	80±14.14ABa
	48	4±5.47ABd	6±5.47ABd	60±7.07Bc	70±7.07Bb	74±8.37Bab	76±5.48Bab	80±14.14ABa
	72	10±7.07Ac	14±5.47Ac	78±4.47Ab	98±4.47Aa	98±4.47Aa	100±0.00Aa	100±0.00Aa

EO: Essential oil; T: Time (h); *Means followed in the same column by the same capital letter and in the same row by the same small letter are not significantly different (P≤0.05; Tukey's HSD test).

Table 3. Dose effects of essential oils on *Ceratitis capitata* adults

Essential oil	Time (h)	LC_{50}	LC_{90}	Slope± S.H	Chi square (χ^2)
Mentha arvensis	1	0.724	2.741	2.217±0.425	2.033
	6	0.416	0.999	3.373±0.365	2.718
	24	0.321	0.762	3.414±0.381	1.871
	48	0.290	0.629	3.811±0.419	2.995
	72	0.260	0.500	4.516±0.504	3.179
Cinnamomum zeylanicum	1	0.845	1.563	4.803±0.585	0.234
	6	0.712	1.248	5.252±0.555	1.028
	24	0.695	1.236	5.123±0.538	1.727
	48	0.469	0.973	4.049±0.403	36.552
	72	0.359	0.466	11.291±1.263	6.7178

Interestingly, the essential oil of *M. arvensis* displayed rapid efficacy, with a low LC_{50} value even at just 1 hour after application. This indicates that *M. arvensis* may also affect Medfly populations in the field, rapidly. This early effectiveness is useful for integrated pest management programs when rapid control is required to prevent additional crop damage.

This study showed that *Mentha arvensis* and *Cinnamomum zeylanicum* essential oils exhibit insecticidal properties that hold promise for controlling *C. capitata*. This potential efficacy can likely be attributed to the presence of bioactive compounds within these oils, such as menthol and cinnamaldehyde, both recognized for their insecticidal characteristics (Papanastasiou et al., 2020)

The results indicated that both *M. arvensis* and *C. zeylanicum* essential oils led to a substantial increase *in C. capitata* mortality over time, even at low doses and concentrations. This rapid mortality effect aligns with previous studies demonstrating the acute toxicity of essential oils to various insect pests (Isman, 2006; Papanastasiou et al., 2017). Essential oils can act quickly to disrupt vital physiological processes in insects, leading to their rapid demise.

It appears that the concentration and timing of essential oil treatments are crucial factors in determining their efficacy. This data underscores the necessity of accurate application methods and dose management to optimize the impact on *C. capitata* populations. For field application, studies on the appropriate timing of essential oil applications are essential (Sethi et al., 2014).

For our country, several researchers have evaluated the effectiveness of a variety of essential oils produced from both native and exotic plant species against *C. capitata*. These essential oils include those from citrus fruits like orange and lemon, as well as those from indigenous Turkish plants such as oregano and thyme. Kutca et al. (2021) and Tabanca et al. (2020) evaluated the insecticidal characteristics of these oils, as well as their effects on larval development, adult mortality, and oviposition inhibition.

The diverse flora of Türkiye provides a valuable resource for investigating the potential of indigenous plant essential oils for *C. capitata* management. In several studies the bioactive compounds in local plant species and their repellent or toxic effects against *C. capitata* have been examined (Kurtca et al., 2021; Benelli et al., 2012; Blythe et al., 2020). These studies have highlighted the significance of improving formulations and application strategies for essential oils. Researchers have investigated several different factors to improve the field stability and efficacy of essential oils (Yusufoğlu et al., 2021).

The agricultural industry in Türkiye has acknowledged the significance of integrated pest management (IPM) for sustainable agriculture. Essential oils are considered an important component of IPM techniques for *C. capitata* (Kurtca et al., 2021). This approach combines many control strategies to successfully manage insect populations. While essential oils provide promise, research has uncovered obstacles such as heterogeneity in efficacy across different environmental conditions and the requirement for consistent administration methods (Kilic et

al., 2019; Raff al., 2019). Future studies in Türkiye may concentrate on refining essential oil-based *C. capitata* management strategies, taking concentration, application time, and other variables into account.

Conclusion

The essential oils of *Mentha arvensis* and *Cinnamomum zeylanicum* show environmentally acceptable potential for the management of *Ceratitis capitata*. Their diverse insecticidal properties, such as larval development suppression, oviposition deterrence, and adult mortality, make them promising candidates for integrated pest management programs (Bempelou et al., 2018). However, more study is required to optimize the formulations, enhance their persistence in the field, and identify synergistic combinations with other pest control methods. In addition, it is crucial to comprehend the long-term effects of essential oil applications on the environment and the resistance development on *C. capitata* populations (Bempelou et al., 2018).

Essential oils have considerable potential as a sustainable and environmentally acceptable alternative for the management of *C. capitata*. They are effective against this destructive pest and pose fewer risks to the environment and human health than chemical pesticides. However, more research is required to overcome those challenges and assure successful implementation in integrated pest management strategies for *C. capitata*.

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Determination of Physical Properties, Color Properties, Mechanical Behavior and Germination Parameters of Three Different Forage Peas Cultivars

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ABSTRACT

The physical properties of seeds and grain is a wide knowledge that can be useful in the sowing, harvesting and storage or in processing such as drying, freezing and other. This knowledge is important in the designing of machinery to harvest and in preparation of processing chain from grain to food. In this study, the physical properties, color characteristics, mechanical behavior, and germination parameters of three different cultivars (Reis, Töre, and Özkaynak) of forage peas were examined and compared. The statistical differences were observed between the cultivars on the length, width, geometric mean diameter, sphericity, and surface area of the forage pea cultivars. Significant differences were observed between cultivars on mass, thousand mass, volume, and bulk density. The effects of cultivars on the true density, and porosity of forage pea seeds were not significant. Töre and Özkaynak cultivars constitute the highest statistical group in terms of mass and thousand mass (0.172 g, 0.174 g, 139.34 g, 138.54 g, respectively). The effects of cultivars on *L**, *a**, *b**, chroma, and hue angles of forage pea seeds were significant. Many features of the seeds should be considered in sowing, harvesting, and post-harvest processes and technological applications of forage pea seeds. In light of the data obtained in this study, it can be assumed that the operations to be carried out will contribute to the reduction of harvest losses, and the improvement of storage conditions at the pre-harvest and post-harvest engineering technologies and food production process applications.

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Introduction

The forage pea (*Pisum sativum* ssp. Arvense L.) is an leguminous crop having promising potential to be utilzed as a forage crop. It is considered as a source of nutritious feed for livestock breeders. The crude protein content is around 18.05% during the flowering period in hay (Tan et al., 2013) and 8.94% after the seeds are harvested (Mustafa et al. 2014, Cacan et al. 2018). Crude protein contains 20-30% in the seed, especially lysine (Acıkgöz, 1991). Forage pea is used in rations instead of soybean in Western European countries. Forage peas, which are used as both herbage and hay forage crops, are also used as pasture plants and green manure. (Dogan, 2013).

Forage peas, which are generally grown for herbage and grain, are not perennial but have a taproot. It provides nitrogen to the soil by fixing a high amount of nitrogen through Rhizobium bacteria. It improves the structure of the soil and provides the nutrient element it needs to the soil, and therefore, it has significance in the rotation (Aslan, 2017). It also prevents erosion at a high rate. The carbon/nitrogen (C:N) ratio of legume plants is 15-20 (Acıkgöz, 2001). Forage peas are also being utilized as silage for livestock feeding (Aslan, 2017).

Although Turkey's ecological conditions are suitable for forage pea cultivation, the quantum of forage production is not sufficient to fulfill the demnand owing to its high consumption. In terms of the sustainability and economy of the cultivation of a product, the quality must be as high as its yield (Karayel and Bozoglu, 2017).

The yield and quality, which are economic characteristics of plants, are related to genotype and environment. Genotype is the cultivar of plant species although the environment is climate, soil conditions, and cultivation techniques. The suitability of the cultivars to the climatic and soil conditions is necessary to increase the yield and improve the quality (Isler and Kılınç, 2016).

The properties of seeds are used in seed research, plant breeding studies, pre-harvest and post-harvest engineering technologies, and food production process applications. Also, fundamental characteristics of seeds are used in seed research, plant breeding studies and mechanization applications (Dumanoğlu, 2021). The reported literatüre corroborated that there are studies conducted by authors; Cacan et al. (2018), Ozyazici et al. (2019), Ozeroglu (2021), and to study about cultivar aspects of forage yield and quality. Dumanoglu et al. (2021) have determined the physical and physiological properties of forage pea seeds. Uslu et al. (2021) and Acikbas and Ozyazici (2021) have reported about the germination properties of forage pea seeds. However, a detailed evaluation of the physical attributes, color characteristics, mechanical behavior, and germination parameters of forage pea seeds is lacking in the literature. In this study, physical properties, color characteristics, mechanical behavior, and germination parameters of three different cultivars (Reis, Töre, and Özkaynak) of forage peas were examined and compared. This study focused on forage pea, which is an important member of forage crops. It is thought that the results obtained will be included in the physical plans and control mechanisms of authorized institutions and organizations and will be used in mechanization and breeding areas.

Matarials And Methods

Three different forage pea cultivars *cv*. Reis, Töre, Özkaynak were procured from private seed companies during December 2021. The pictorial presenteation of the selected cultivars is presented in the Figure 1. The seeds were cleaned of any foriegn matter including broken as well as damaged seeds and were stored at room temperature (20-24°C). The study was carried out in the laboratory of Tokat Gaziosmanpaşa University, Faculty of Agriculture, Department of Biosystem Engineering.

Physical Properties

The moisture content of the forage pea seeds was determined using the hot air oven (105°C for 24 h) method recommended by Braga et al. 1999. The length (*L*), width (*W*), and thickness (*T*) of randomly selected 100 seeds were measured using digital vernier caliper (Mitutoyo, Japan ± 0.01 mm) as shown in the Figure 2. To obtain the unit mass, each seed was weighted with an electronic balance to an accuracy of 0.001 g. The geometric mean diameter of the seeds (*Dg*, mm) was calculated using the following formula (Mahawar et al. 2019, Altuntas and Mahawar 2022):

$$D_g = (L \times W \times T)^{1/3} \quad (1)$$

The sphericity (Φ, %), surface area (S_a) and volume (*V*) of the seeds were calculated using the following formula (Mohsenin, 1980):

$$\Phi = (Dg/L) \times 100 \quad (2)$$

$$S_a = \pi \times (Dg)^2 \quad (3)$$

$$V = \pi/6\ (L \times W \times T) \quad (4)$$

(a)

(b)

(c)

Figure 1. The investigated forage pea cultivars (a) cv. Reis (b) Töre (c) Özkaynak

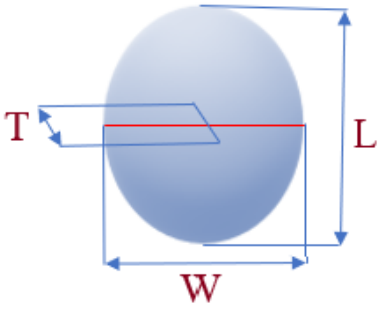


Figure 2. Three perpendicular axes: length (*L*), width (*W*) and thickness (*T*) of forage pea seed.

The seed volume and its kernel density were determined using the liquid displacement method. The hectoliter method was used to determine the bulk density (Mohsenin, 1980). The porosity (%Ɛ) was calculated according to Mohsenin (1980) by considering the bulk and kernel density values.

Color Properties

Color of the forage pea seeds was measured using a Minolta colorimeter (Konica Minolta, Model CR-400/410 Chroma Meters, Osaka, Japan). *L** indicates lightness: 100: white, 0: black, *a** indicates + red, - green, and *b** indicates + yellow, - blue (Itle and Kabelka, 2009). CIE *L**, *a**, and *b** color scales were determined for the color characteristics of forage pea seeds. The hue angle and chroma are effective parameters for characterizing visual

color appearance (Bernalte et al., 2003). The chroma value of the product is an indicator of the vivid or pastel tone of the seeds, and pastel tones are close to 0 and vivid tones are close to 100.

Chroma was calculated as follow:

$$C=(a^{*2}+b^{*2})^{1/2} \quad (5)$$

When $a^* > 0$ and $b^* > 0$; the hue angle (α) of the seeds and was calculated using the following formula (Lancaster et al., 1997),

$$\alpha = tan^{-1}(\frac{b^*}{a^*}) \quad (6)$$

Mechanical Properties

The coefficient of static friction is defined as the tangent value of the angle of slope between the sliding surface and vertical and horizontal planes. The coefficient of static friction was determined using a friction measurement device on different friction surfaces (PVC, galvanized steel, laminate, plywood, and rubber).

The angle of repose is the angle with the horizontal at which the seeds will stand when piled. It was determined using a topless and bottomless cylinder with a diameter of 300 mm and a height of 500 mm. The cylinder was filled to the top with seeds and slowly raised until a flat plate forms a cone on the surface. The angle of repose was calculated from the measurement of the height of the cone and the diameter of the cone (Kaleemullah and Gunasekar, 2002).

$$\text{Angle of repose} = \tan^{-1}(h / d) \quad (7)$$

when *h* the height of the cone (cm) and the diameter of cone (cm)

To determine the mechanical properties of forage peas, a biological material test device was used. This device has three main components, which are a moving platform, a driving unit, and a data acquisition (load cell, PC card, and software) system as shown in Figure 3. The rupture force in three different axes (L, W, T) and at three different speeds (20 mm min^{-1}, 40 mm min^{-1}, 60 mm min^{-1}) were taken Figureally on the test device. The *X*- axis (F_x force) is the longitudinal axis (L), the *Y*- axis (F_y force) is the transverse axis (W) at right angles to the *X*- axis in the plane of the suture, and the Z- axis (*Fz* force) is the transverse axis (T) at right angles to the plane of the suture (Figure 4). The values were read by keeping the speeds determined by the speed adjustment and fixation panel on the biological material test device. Samples were compressed along the *X*, *Y*, and *Z* axes to determine the rupture force (*F*), deformation (*D*), absorbed energy (E), hardness (*H*), and required power for cracking (*PC*) (Altuntas and Yildiz, 2007).

Absorbed energy, hardness and required power for cracking were obtained from the following equation:

$$E = (F \times D)/2 \quad (8)$$

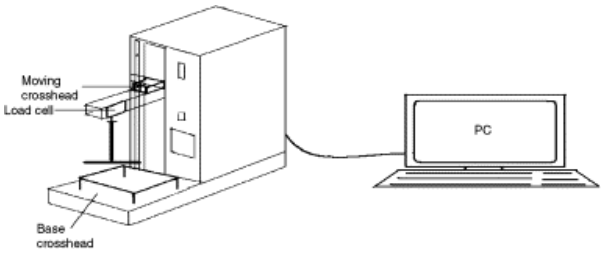
$$H = F/D \quad (9)$$

$$PC = \left[\frac{E*S}{60000*D}\right] \quad (10)$$

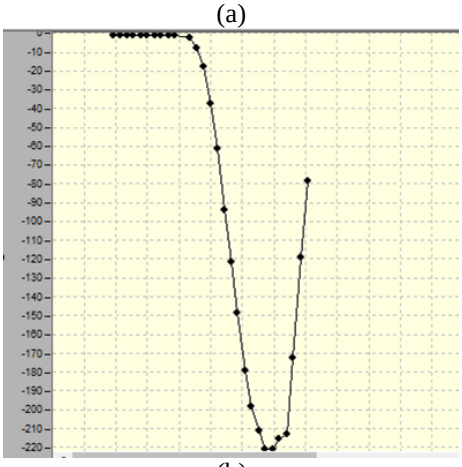
In equations; *F*: Rupture force (N), *D*: Deformation (mm), *E*: Absorbed energy (N mm), *H*: hardness, *PC*: Required power for cracking (W) and *S*: loading speed (mm min^{-1}).

Germination Properties

For germination test, the seeds were sterilized in 1% sodium hypochlorite solution for surface sterilization for 10 minutes and rinsed three times with distilled water before germination. Air-dried seeds were placed in 9 mm diameter petri dishes with filter paper (Ozkurt et al., 2018). Ten seeds were used in each petri dish and this process was repeated every two days to refresh the evaporated water. Petri dishes were kept at 21±2°C. All seeds were counted every day during the 14 days germination period, and seeds with a 2 mm radicle were considered germinated (Carpici and Erdel, 2015). In the study, germination percentage, germination rate, and radicle length Soltani et al. (2012), vigor index by Hamidi and Safarnejad (2010), and germination index by Torabi et al. (2011) were determined.



(a)



(b)

Figure 3. Biological material test device (Altuntaş & Yıldız, 2007) (a) and example of force-deformation curve of forage pea (b)

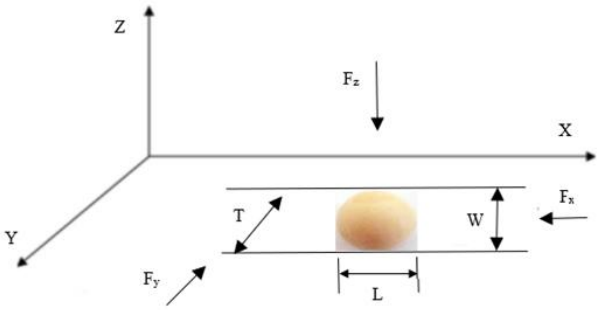


Figure 4. Representation of the three axial forces (*Fx*, *Fy*, and *Fz* axial forces) and three perpendicular dimensions of forage pea

Data Analysis

Statistical analysis of the obtained data was performed using SPSS statistical program. The data was analyzed using a randomized complete block design with a split block. In this design, the main factor was forage pea cultivars and the sub-factor was compression axis and speed. Before the analysis of variance for the data, a normality test was performed and its suitability for analysis was determined. Results were analyzed using analysis of variance (ANOVA) and the means were compared using the DUNCAN test.

Results and Discussion

The moisture contents of Reis, Töre, and Özkaynak cultivars were determined as 10.37%, 9.77%, and 9.10% d.b (dry basis), respectively. Detailed knowledge of physical properties is very important in the design of equipment for harvesting, cleaning, sorting, packaging, storage, and transportation during different agricultural operations. Size and shape are important in sizing, sieving, sorting, and other sorting processes.

The values of the L, W, T, Dg, sphericity, and surface area of the seeds of the forage pea cultivars are given in Table 1. Statistical differences were observed between the cultivars at the level of P<0.01 on the length, width, geometric mean diameter, sphericity, and surface area of the forage pea cultivars. Length, width, thickness, geometric mean diameter, and surface area values of Özkaynak cultivar were determined as 6.76 values may be added here 6.36 mm, 5.79 mm, 6.27 mm, 123.88 mm^2, respectively. It was determined that Reis cultivar was more spherical (94.17%) than other cultivars. A high sphericity value indicates that the forage peas are approaching a spherical shape.

Dumanoglu et al. (2021) reported the length value of forage pea seeds cultivars between 5.463 and 7.730 mm. It was reported that the highest width value was 7.022 mm in cv. Kristal and the lowest value was in cv. Taşkent (5.179 mm). Dumanoglu et al. (2022) reported that the seeds of cv. Vetch had a mean length of 4.876 mm, a width of 4.403 mm, and a surface area of 17.808 mm^2. Ganjloo et al. (2018) reported that the length, width, thickness, and geometric mean diameter values of green peas seeds at different moisture contents (15.21, 35.10, 55.18, and 75.15% w.b.) varied between 7.28 mm-10.48 mm, 5.80 mm-8.93 mm, 5.15 mm-8.61 mm and 5.90 mm-9.10 mm, respectively. It was seen that the data obtained were in harmony with the literature.

The values of seed mass, thousand mass, volume, bulk density, true density, and porosity of forage pea cultivars are given in Figure 5. Statistically differences (P<0.01) were observed between the cultivars on mass, thousand mass, volume, and bulk density. The effects of cultivars on the true density and porosity of forage pea seeds were not significant. Töre and Özkaynak cultivars constitute the highest statistical group in terms of mass and thousand mass (0.172 g, 0.174 g, 139.34 g, 138.54 g, respectively). The mass, thousand mass, volume, bulk density, true density, and porosity values of forage pea seeds were determined between 0.141g-0.174g, 85.93 g-139.34 g, 116.28 mm^3-130.67 mm^3, 844.79 kg m^{-3}- 872.59 kg m^{-3}, 1117.80 kg m^{-3}-1227.14 kg m^{-3} and 22.40%-27.80% respectively.

Thousand mass is an important quality factor and is related to yield. The higher the thousand mass of the seed, the higher the yield. In addition, the thousand mass is taken into account in determining the amount of seed to be planted per unit area (Moshatati and Gharineh, 2012). Dumanoglu et al. (2021) reported that the thousand mass of forage pea seeds varied between 112.57-266.40 g. The true density is one of the quality criteria, which is essential in separating the classes at world standards, as it gives information about the fullness of the grain (Moshatati and Gharineh, 2012). Ganjloo et al. (2018), reported that the bulk density and actual true density of green peas seeds are between 630.76-670.70 kg m^{-3} and 1088-1132 kg m^{-3}, respectively, at different moisture contents. Uzun et al. (2012) determined that the thousand seed mass of forage pea seeds were in the range of 167.1-193.6 g.

Table 1. Some geometric properties of seeds of forage pea cultivars.

Forage pea cultivars	L (mm)	W (mm)	T (mm)	S (mm)	Sp(%)	Sa(mm^2)
Reis	6,41±0,12b	6,10±0,09c	5,66±0,10	6,04±0,08c	94,17±0,89a	114,64±3,07c
Töre	6,69±0,11a	6,23±0,15b	5,69±0,14	6,17±0,12b	92,31±0,97b	119,97±4,59b
Özkaynak	6,76±0,13a	6,36±0,05a	5,79±0,12	6,27±0,08a	92,94±0,88b	123,88±3,12a
F value	23,21**	15,90**	$3,02^{ns}$	15,93**	10,78**	16,05**

L: Length, W: Width, T: Thickness, Dg: Geometric mean diameter, Sp: Sphericity, Sa: Surface area, power ±: standard deviation, **P<0.01, ^{ns}: Non significant. The difference between the same letters in the same column is insignificant.

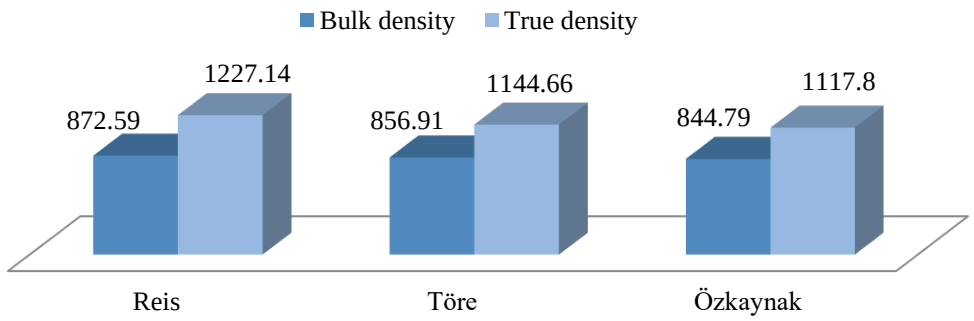


Figure 5. Bulk density and true density of forage pea cultivars.

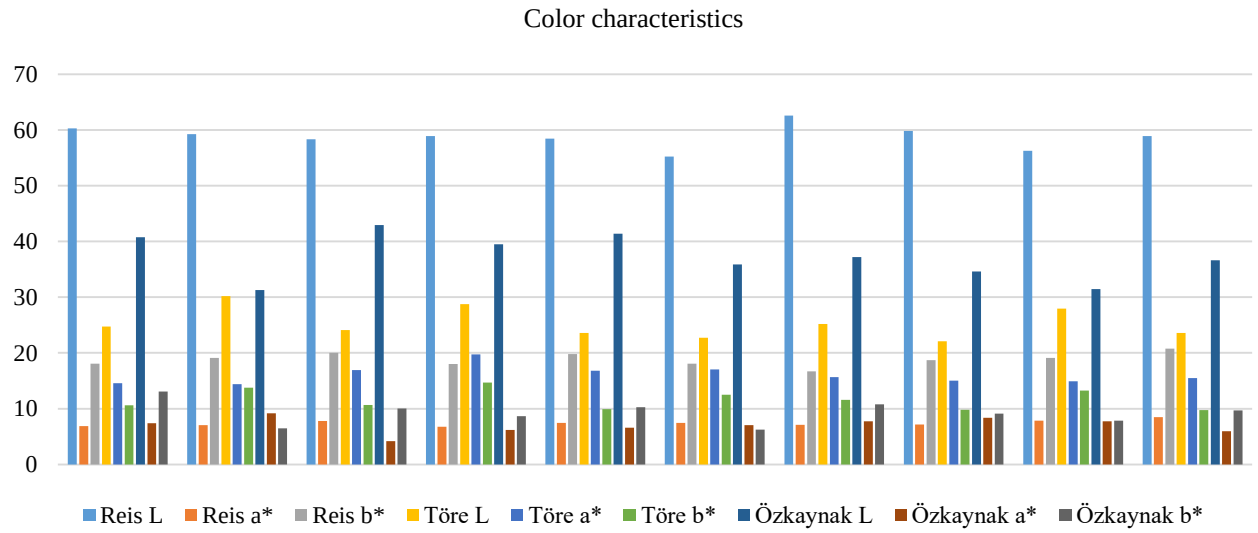


Figure 6. Color characteristics of the forage pea cultivars.

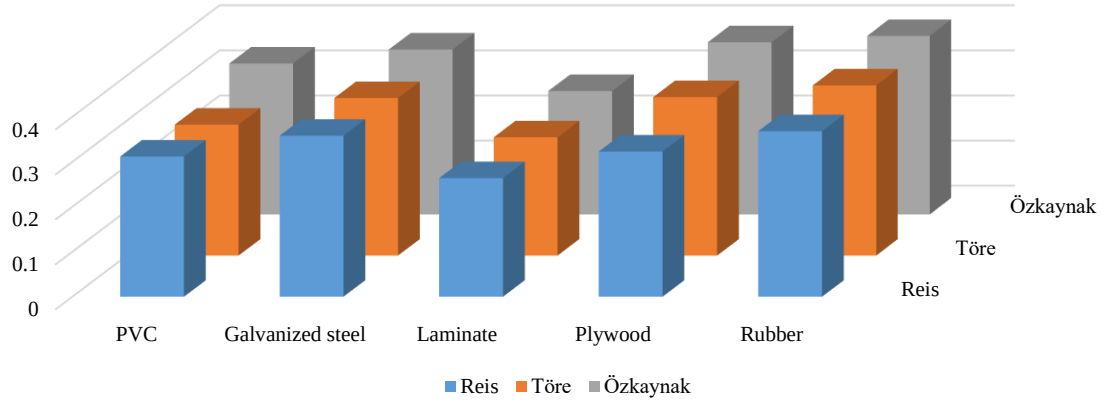


Figure 7. Static friction coefficient of seeds of forage pea cultivars.

The values of the color characteristics of the forage pea cultivars are given in Figure 6. The highest *L** and *b** values (mention the values) were measured in the Reis cultivar. In the Töre cultivar, the *a** value was measured the highest. Chroma and hue angle values were found to be high in Reis cultivar. The effect of cultivars on *L**, *a**, *b**, chroma, and hue angles of forage pea seeds was significant ($P<0.01$).

In seed production, quality is more important than the quantity of seed produced. This allows seed-producing companies to increase company credibility. It also means that producers get their money's worth when the seed grows (Mavi, 2010). One of the most important features in the grading and classification of seeds is color. Different seeds and cultivars are identified by their color characteristics (Anvarkhah et al., 2016). In addition, many studies have shown that color pigments in the seed coat affect germination, seedling emergence, seed quality, gas diffusion, seed dormancy, and water uptake and in some cultivars (Mavi, 2010).

For the mechanical behavior of the seeds of Reis, Töre, and Özkaynak cultivars, the static coefficient of friction, angle of repose, rupture force, deformation, rupture energy, hardness, and rupture power values were investigated. It was observed that the difference between the static coefficient of friction values on PVC and plywood surfaces was significant ($p<0.01$) level. The difference among the cultivar and static coefficient of friction (rubber) was statistically significant at $p<0.05$ level. No difference was observed on the laminate surface. The highest static coefficient of friction (values) was obtained on the surface with rubber material, while the lowest value (value) was obtained on the laminate material in all cultivars (Figure 7).

Different loading speeds and loading axes values of forage pea cultivars are given in Tables 2, 3 and 4. The rupture force along the width axis were higher than that of the length and thickness axis. The highest rupture force was observed for *cv*. Töre at 60 mm min^{-1} speed and width axis. The highest deformation value was observed in the Özkaynak cultivar at 60 mm min^{-1} speed and width axis. It is quite remarkable that there was a statistical difference in speed and axes in all cultivars.

Gul et al. (2021) suggested that the rupture force was higher at 30 mm min^{-1} loading speed as compared to the other loading speeds for grass pea cultivars. A decrease was observed in the rupture force values with the increase in loading speed. The reported literature iterates about the increase in the force due to the increase in speed. Mechanical damages cause more or less economic losses depending on the way the product was used.

Table 2. Textural attributes of various forage pea cultivars in different axes and 20 mm min^{-1} speed.

C		RF	D	RE	H	RP
Reis	L	158.51±26.61c	2.48±0.33b	200.08±57.44b	63.76±5.78b	0.026±0.004c
	W	317.94±36.29a	3.21±0.25a	512.63±86.02a	99.11±9.89a	0.053±0.006a
	T	204.97±55.44b	2.00±0.28c	207.75±77.82b	103.10±26.15a	0.034±0.009b
	F value	27.69**	31.20**	39.83**	12.08**	27.69**
Töre	L	166.86±8.71c	2.63±0.11b	219.33±18.85b	63.57±2.64c	0.028±0.001c
	W	334.80±12.49a	3.53±0.21a	591.69±49.53a	95.02±5.10b	0.056±0.002a
	T	275.13±55.55b	2.08±0.47c	290.00±109.66b	137.45±37.92a	0.046±0.009b
	F value	45.87**	40.13**	55.37**	19.63**	45.87**
Özkaynak	L	118.90±39.80c	2.59±0.32b	154.67±54.52c	46.26±16.44b	0.020±0.007c
	W	317.94±36.29a	3.24±0.26a	516.73±83.29a	98.33±10.80a	0.053±0.006a
	T	244.03±53.53b	2.14±0.26c	257.77±50.65b	117.05±35.68a	0.041±0.009b
	F value	36.87**	27.84**	58.57**	17.02**	36.87**

C:Cultivar, RF: Rupture force, D: Deformation, RE: Rupture energy, H: Hardness, RP: Rupture power ±: standard deviation, **P<0.01. The difference between the same letters in the same column is insignificant.

Table 3. Rupture force, deformation, rupture energy, hardness and rupture power of forage pea cultivars in different axes and 40 mm min^{-1} speed.

C		RF	D	RE	H	RP
Reis	L	174.44±13.05b	2.80±0.12b	244.60±23.32b	62.31±4.81b	0.058±0.004b
	W	322.81±32.50a	4.55±0.35a	734.14±95.13a	71.34±8.68b	0.108±0.011a
	T	221.94±64.37b	2.35±0.19c	260.37±78.23b	95.18±29.29a	0.074±0.021b
	F value	22.45**	162.27**	103.42**	6.34**	22.45**
Töre	L	177.14±3.91c	2.81±0.10b	248.89±13.37b	63.10±1.28c	0.059±0.001c
	W	358.21±10.81a	4.02±0.69a	721.43±135.94a	90.97±13.03b	0.119±0.004a
	T	279.47±26.12b	1.92±0.54c	271.75±88.80b	154.05±37.17a	0.093±0.009b
	F value	212.60**	29.91**	56.18**	29.35**	212.60**
Özkaynak	L	163.39±39.25c	4.45±0.46a	363.34±87.26b	37.01±9.72c	0.055±0.013c
	W	342.81±21.38a	4.55±0.35a	780.36±89.90a	75.69±6.06b	0.114±0.007a
	T	259.11±30.09b	2.37±0.29b	308.56±60.48b	110.13±14.28a	0.086±0.010b
	F value	58.31**	75.25**	72.24**	83.83**	58.31**

*C:Cultivar, RF: Rupture force, D: Deformation, RE: Rupture energy, H: Hardness, RP: Rupture power ±: standard deviation, **p<0.01. The difference between the same letters in the same column is insignificant.*

Table 4. Rupture force, deformation, rupture energy, hardness and rupture power of forage pea cultivars in different axes and 60 mm min^{-1} speed.

C		RF	D	RE	H	RP
Reis	L	181.63±18.33c	2.86±0.22b	261.40±44.13b	63.40±2.02b	0.091±0.009c
	W	338.00±26.38a	4.74±0.23a	800.32±73.51a	71.52±6.62b	0.169±0.013a
	T	261.46±89.79b	2.75±0.16b	363.54±137.42b	94.26±29.29a	0.131±0.045b
	F value	14.12**	211.76**	65.59**	5.93*	14.12**
Töre	L	190.54±6.81c	3.00±0.06b	286.25±15.88b	63.44±1.25c	0.095±0.003c
	W	370.63±10.89a	4.10±0.30a	759.40±64.49a	90.82±5.76b	0.185±0.005a
	T	287.76±25.95b	2.17±0.37c	311.85±60.14b	137.33±34.78a	0.144±0.013b
	F value	203.56**	83.75**	185.23**	23.54**	203.56**
Özkaynak	L	175.61±50.54c	4.54±0.49b	397.80±114.68b	39.14±12.67c	0.088±0.025c
	W	361.37±18.58a	4.99±0.33a	903.42±97.95a	72.54±4.03b	0.181±0.009a
	T	277.10±26.23b	2.56±0.23c	354.34±41.33b	109.09±15.61a	0.139±0.013b
	F value	50.65**	87.75**	80.01**	61.14**	50.65**

C:Cultivar, RF: Rupture force, D: Deformation, RE: Rupture energy, H: Hardness, RP: Rupture power ±: standard deviation, * P<0.05, **P<0.01. The difference between the same letters in the same column is insignificant.

As evident from Table 5, there was no statistical difference in the germination index, germination rate, final germination, and vigor index values of the cultivars. Although there was no statistical difference, the mean germination index of the cultivars was 21.55 and the lowest chinning index was obtained from Töre (20.0), while the germination index values of the other two cultivars were the same and gave the highest value. The mean germination rate of the cultivars was 4.39 and the Töre cultivar gave the lowest value (mention the value) and the Reis cultivar gave the highest value (mention the value). The germination rates of the cultivars were generally at a high level and the mean was as high as 97.77%. While the germination rate of the Töre cultivar was the lowest at 93.33%, the seeds of the other two cultivars were all germinated and a germination rate of 100% was obtained. İt has been reported in many studies that the mean germination rate of forage pea seeds is ≥95% (Okcu et al. 2005; Kücüközcü & Avci, 2020; Avci et al. 2020; Dumanoglu et al. 2021). The vigor index value, which is another examined parameter,

was realized as 16.82 on mean, and the lowest vigor index value was obtained from the Töre cultivar, while the highest was obtained from the Özkaynak cultivar. Although not statistically significant, Reis and Özkaynak cultivars were the most prominent cultivars for these parameters.

The examined characteristics of the cultivars used in the study are given in Table 6. Cultivars examined in the study did not make a statistical difference in terms of fresh root weights, dry root weights, and fresh root lengths, dry shoot lengths, and fresh shoot weights. However, the cultivars created a statistically significant difference ($P \leq 0.01$) between fresh shoot weights and root numbers. The mean fresh root weight of the cultivars examined in the study was 0.29 g, and this mean value did not make a statistical difference in the cultivars.

While the highest root weight was obtained from the Töre cultivar, the lowest fresh root weight was obtained from the Özkaynak cultivar. It is an advantage for the plant that the roots are dense and the roots are dense in the soil ensuring the resistance of plants against various stress factors. As a matter of fact, the amount of nitrogen that plants that are leguminous forage plants such as forage peas will add to the soil is closely related and directly proportional to the density of the plant roots.

The dry root weights obtained from the study were not statistically affected by the cultivars, as were the fresh root weights. The mean dry root weight of the cultivars examined in the study was 0.06 g, and the highest dry root weight (mention the value) was obtained from the Özkaynak cultivar, while the lowest (mention the value) was obtained from the Reis cultivar. Although the equity cultivar was determined as the lowest cultivar in terms of fresh root weight, it came to the forefront as the highest cultivar in terms of dry root weight.

The mean root length of the cultivars examined in the study was 17.59 mm and these means did not make a statistical difference in the cultivars. While the highest root length (mention the value) obtained from the research was obtained from the Reis cultivar, the lowest root length (mention the value) was obtained from the Özkaynak cultivar. It has been reported that there is quite a difference between cultivars in terms of root length. Okcu et al. (2005) have determined the root lengths of Bolero, Sprinter, and Utrillo cultivars as 13.99 cm, 11.69 cm and 3.19 cm, respectively. Avci et al. (2020) determined that the mean root lengths of 2020 are between 7.03-8.00 cm. Demirkol et al. (2019), investigated different salt doses in a forage pea genotype, which they considered promising, and they found the radicula length to be 6.24 cm in the control application.

It was expected by the authors about some differences in the studies in question with the results of this study. As a matter of fact, the different times and methods in which the observations were taken may have caused this difference. In addition, the length of the roots of the plants is also an important feature in terms of resistance to abiotic stress factors, especially to arid conditions. Knowing the characteristics of the plants selected for breeding is important for the new cultivars to be improved and resistant.

The area under arid regions will increase in the coming years with the climate change that has been experienced in recent years and is anticipated to increase in the coming future. This will augment the importance of drought-resistant plants in countries like Turkey where arid and semi-arid climates are experienced intensely. The mean fresh shoot weight obtained from the study was 0.27 g and the mean value created a statistically significant difference ($P \leq 0.01$) in the cultivars. The highest fresh shoot weight (0.31 g)obtained in the study was obtained from the Özkaynak cultivar, and the lowest fresh shoot weight (0.22 g)was obtained from the Reis cultivar. It has been determined in many studies that there is a difference in shoot age weights between cultivars. Okcu et al. 2005 used the same cultivars used in our study, Avci et al. 2020, the mean fresh shoot weights were between 199 to 243 mg/plant, and Küçüközcü and Avci 2020, in their research including similar cultivars, determined the mean fresh shoot weight as 132.06-161.16 mg/plant. Dumanoglu et al. 2021 In the light of the data obtained in their research, reported that species and cultivars with high shoot weight are an important criterion in the evaluation of forage plants used in animal nutrition, and it is important in terms of providing a good genotype preference to scientists, especially in breeding studies.

Table 5. Germination related attributesof the forage pea cultivars.

	Germination Index	Germination Rate	Final Germination	Vigor Index
Reis	22.33±2.08	4.68±0.33	100±0	17.27±4.26
Töre	20±2.65	3.89±0.38	93.33±5.77	16.46±1.29
Özkaynak	22.33±2.08	4.61±0.35	100±0	16.74±2.03
Average	21.55	4.39	97.77	16.82
F value	1.04 ^{ns}	4.53 ^{ns}	4.00 ^{ns}	0.06 ^{ns}

±: standard deviation, ^{ns}: non significant.. The difference between the same letters in the same column is insignificant.

Table 6. Fresh root weight, dry root weight, fresh root length, fresh shoot weight, dry shoot weight, fresh shoot length, and a number of roots of the forage pea cultivars.

	Wet Root Weight (g)	Dry Root Weight (g)	Wet Root Length (mm)	Wet Shoot Weight (g)	Dry Shoot Weight (g)	Wet Shoot Length (mm)	Number of roots (number)
Reis	0.29±0.11	0.05±0.01	18.38±2.89	0.22±0.03b	0.06±0.01	11.76±2.01	16±3.31b
Töre	0.34±0.09	0.06±0.00	17.66±3.83	0.28±0.06ab	0.07±0.00	10.98±2.88	23±7.18a
Özkaynak	0.26±0.07	0.07±0.02	16.74±3.24	0.31±0.09a	0.07±0.02	11.47±3.16	13±2.65b
Average	0.29	0.06	17.59	0.27	0.066	11.40	17.3
F value	2.02 ^{ns}	1.09 ^{ns}	0.54 ^{ns}	4.64**	0.88 ^{ns}	0.19 ^{ns}	10.59**

±: standard deviation, **P<0.01, ^{ns}: non significant. The difference between the same letters in the same column is insignificant.

Although fresh shoot weights have a statistically significant effect on cultivars, the same is not true for dry shoot weights. The mean dry shoot weight obtained from the study was 0.066 g. According to the results of the research, the highest dry shoot weight (value) was obtained from cv. Töre and Özkaynak, while the lowest (value) was obtained from cv. Reis. Avci et al. 2020, determined the mean dry shoot weight of 2020 between 18.8-20.1 mg/plant and determined that the effect of these values on cultivars was not statistically significant. The mean fresh shoot length of the cultivars examined in the study was 11.40 mm and this value did not make any statistical difference on the cultivars.

The highest fresh shoot length (value) was obtained from cv. Reis, and the lowest fresh shoot length (value) was obtained from cv. Töre. The fresh shoot length is also an important criterion in the selection of forage crops used for animal feding as the plants with higher shoot lengths will yield more green parts.

It would be a correct approach to evaluate the selection of these species and cultivars as an indicator of obtaining more abundant quality roughage. The number of rootsmade a statistically significant difference ($P \leq 0.01$) in the cultivars.

While the highest root number (value) was obtained from the Töre cultivar among the cultivars examined in the study, the lowest root number (value) was obtained from the Özkaynak cultivar, which constitutes the same statistical group as Reis cultivar. The resistance of plants to abiotic stress factors is closely related to the durability of their root systems. Thus, if more roots are there in the plant, the more the area in contact with the soil means an increase in the rhizosphere area, which plays an important role in the uptake of plant nutrients. This is an indication that the plant has the potential to absorb more water and plant nutrients through its roots.

Conclusion

Many features of the seeds should be considered in sowing, harvesting, and post-harvest processes and technological applications of forage pea seeds. In the light of the data obtained in this study, it is assumed that the operations to be carried out will contribute to the reduction of harvest losses and to the improvement of storage conditions. The physical properties of seeds is a vast amount of data that can be useful in harvesting and storage or drying and other processes. This data is involved in designing machines for harvesting and in every part of the food processing chain. The main purpose of post-harvest biotechnical properties technology is to increase agricultural production through the dissemination of quality seeds of high-yielding cultivars. This includes identifying cultivars with high seed quality, thereby improving seedling and plant growth, and assessing seed quality using different methods. In this study, physical properties, color characteristics, mechanical behavior, and germination parameters of three different cultivars (Reis, Töre, and Özkaynak) of forage peas were examined and compared. Moisture contents of Reis, Töre and Özkaynak cultivars were determined as 10.37%, 9.77%, and 9.10% d.b, respectively.

Length, width, thickness, geometric mean diameter, and surface area values of Özkaynak cultivar were determined as 6.76 mm, 6.36 mm, 5.79 mm, 6.27 mm, and 123.88 mm^2, respectively. It was determined that the Reis cultivar was more spherical (94.17%) than other cultivars. Statistically significant ($p<0.01$) differences were observed between cultivars on mass, thousand mass, volume and bulk density. Töre and Özkaynak cultivars constitute the highest statistical group in terms of mass and thousand mass (0.172 g, 0.174 g, 139.34 g, 138.54 g, respectively). Although there was no statistical difference, the mean germination index of the cultivars was 21.55 and the lowest chinning index was obtained from the Töre (20.0), while the germination index values of the other two cultivars were the same and gave the highest value. In next future, the physical parameters examined need to be made in different cultivars and species. Thus this will be a guide in future plant breeding and mechanization studies.

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Molecular Survey of *Toxoplasma gondii* Infection in Aborted Fetuses of Sheep in the Iğdır Province of Türkiye

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ABSTRACT

Toxoplasma gondii, an obligatory intracellular protozoan parasite, can infect a wide range of warm-blooded animals, including livestock species. *T. gondii* is a zoonotic protozoan parasite that affects both humans and other warm-blooded animals. The aim of this study was to detect *T. gondii* by using PCR in the brain tissues of 60 aborted sheep fetuses from the Iğdır Province in Türkiye. For this purpose, 60 brain tissue samples of sheep were collected within the lambing seasons of 2023 in Iğdır, Türkiye. The DNA extraction was performed using the PureLink™ Genomic DNA Mini Kit from brain samples. The PCR was performed with the appropriate primers from the obtained DNA samples. *T. gondii* was found in the brain (16.6%) samples of aborted sheep fetuses. According to the present study, *T. gondii* infection can be one of the causes of fetus abortion of sheep in Iğdır province, Türkiye. This result emphasizes the need for vigilance and preventive measures in managing this potential public and animal health concerns.

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Introduction

Toxoplasma gondii, an obligatory intracellular protozoan parasite, can infect a wide range of warm-blooded animals, including livestock species (Innes, 2010). The cats are the primary hosts for *T. gondii*. Sheep may become infected after consuming feed or pasture contaminated with sporulated oocysts (Elmore et al. 2010). *T. gondii* affects reproductive system organs resulting in reproductive failure such as abortion, stillbirths, and low viability of offspring in sheep. Therefore, it may cause important economic losses in the sheep industry (Anastasia et al. 2013; Gutiérrez-Expósito et al. 2021). *T. gondii* can also affect public health negatively. In pregnant women, the main way of transmission of *T. gondii* is through the consumption of raw or undercooked meat during pregnancy (Kapperud et al. 1996; Bilgili and Hanedan, 2019). The prevalence of *T. gondii* in pregnant women ranges between %13 and %55 (Bilgili and Hanedan, 2019). Therefore, it is an important risk factor for pregnant women worldwide. In addition, the serological prevalence of *T. gondii* is high among farm animal species such as pigs, sheep, and goats (Tenter et al. 2000).

Türkiye is one of the important countries in Europe for sheep breeding with 46.1 million head of sheep (TUIK, 2022). Therefore, sheep are an important source of meat, milk, and wool in Türkiye (Köseman and Şeker, 2015; Behrem and Gül, 2022). There are different studies showing the high seroprevalence of *T. gondii* in Türkiye (Tutuncu et al., 2003; Oncel and Vural, 2006; Acici et al. 2008; Çakmak and Karatepe; 2017). The high prevalence of *T. gondii* is an important problem for the sheep industry in Türkiye because it causes reproductive diseases. *T. gondii*-induced abortions are still reported in different countries (Edwards and Dubey, 2013; Chessa et al., 2014; Nayeri et al. 2021). However, there is limited information on *T. gondii*-induced abortion in sheep. The objective of the investigation was to detect *T. gondii* by using PCR in the brain tissues of 60 aborted sheep fetuses from the Iğdır Province in Türkiye.

Materials and Methods

Ethical Statement

This study was approved by Van Yuzuncu Yil University Animal Experiments Local Ethics Committee (Approval no: 2023/03-11).

Study Samples

Brain specimens were collected from 60 sheep fetuses that had undergone abortion at various stages of pregnancy, within the lambing seasons of 2023 in Iğdır, Türkiye.

The samples were exclusively sourced from Morkaraman breed sheep. Out of 60 aborted ovine fetuses, a total of 60 brain tissue samples were procured. To extract brain samples, each fetus was handled individually, with the calvarium opened and meninges dissected using a fresh disposable scalpel and forceps. Approximately 1 cm^3 of brain tissue from the right cerebral hemisphere was excised and subsequently frozen at -20 °C for DNA extraction.

DNA Extraction

The DNA extraction from the aborted fetus brain was carried out using the PureLink™ Genomic DNA Mini Kit (Invitrogen™, USA, K182002), and subsequently stored at -20°C.

PCR Amplification

The amplification of the 529-bp repetitive element region of *Toxoplasma gondii* was conducted using the TgTox4F (5'-CGCTGCAGGGAGGAAGACGAAAGTTG-3′) and TgTox4R (5'-CGCTGCAGACACAGTGCATCTGGATT-3′) primers (Sah et al. 2019). In a 20 µl master mix, the following components were used: 8 pmol of both forward and reverse primers, 4 µl of 5x FIREPol® Master Mix (containing 7.5 mM MgCl2, Solis BioDyne, Estonia), 1.6 µl of DNA, and 12.8 µl of Nuclease Free Water. The PCR protocol involved an initial denaturation step at 95°C for 5 minutes. This was followed by 35 cycles, each consisting of denaturation at 95°C for 60 seconds, annealing at 60°C for 60 seconds, elongation at 72°C for 1 minutes and final elongation at 72°C for 10 minutes. Subsequently, a 1.5% agarose gel was prepared and stained with RedSafe™ Nucleic Acid Staining Solution. The PCR products were then electrophoresed on the agarose gel, and images were captured using a gel imaging device (Syngene Bio imaging System).

Results

In this study, a total of 60 brain tissue samples were chosen from aborted fetuses for the isolation of the *Toxoplasma gondii* parasite through conventional PCR (Figure 1). Positivity for the presence of *T. gondii* was confirmed in 10 out of the 60 samples, accounting for 16.6% of the total.

Discussion

Toxoplasma gondii infection is a zoonotic protozoan parasite that affects both humans and other warm-blooded animals. Various molecular techniques, including serological methods, cell culture, laboratory animal vaccination, and PCR, are employed to detect toxoplasmosis (Fuente et al. 1996; Greg et al. 1996; Tavassoli et al. 2009). The increased sensitivity of PCR now enables to delve into alterations at the individual cell level, surpassing the typical requirements for parasite-related research. PCR has profoundly influenced advancements in fields such as parasite systematics and epidemiology, as well as in the domains of immunology and interactions between hosts and parasites (Ndao, 2009). PCR stands out for its high sensitivity and specificity, allowing for the detection of a specific segment of *T. gondii* DNA, making it the preferred choice over other techniques (Fuente et al. 1996; Greg et al. 1996; Tavassoli et al. 2009).

Indeed, numerous studies worldwide have employed aborted fetal tissues for diagnosing Toxoplasmosis infection. Molecular investigations have been carried out across different regions to assess the prevalence of *Toxoplasma gondii* in aborted fetuses. Prevalence of Toxoplasma gondii infection in aborted fetuses have been reported in various studies around the world. These include 10% in Germany (Steuber et al. 1995), 13% in Italy (Chessa et al. 2014), 14.3% in Brazil (de Moraes et al. 2011), 13.5% in Iran (Rassouli et al. 2011), 64% in Iran (Shahbazi et al. 2019), 11.8% in Romania (Paştiu et al. 2023), and 5.4% in Spain (Moreno et al. 2012). In our study, a positivity rate of 16.6% was observed.

Molecular studies have been carried out to investigate the prevalence of *Toxoplasma gondii* in aborted fetuses in Türkiye (Özkaraca et al. 2016; Irehan et al. 2022; Orunç Kılınç et al. 2023; Akpinar et al. 2023). Özkaraca et al. (2016) detected positivity in 1 out of the sheep abortion samples brought to Elazığ Veterinary Control Institute using the Duplex PCR method. Irehan et al. (2022) identified positivity in 7.27% of 55 aborted fetus samples from 13 different provinces in the Eastern and Southeastern Anatolia Regions of Türkiye using Real-time PCR. Orunç Kılınç et al. (2023) reported a 35.7% positivity rate in 42 aborted sheep fetuses in the Van region in 2023 using the conventional PCR method. Akpınar et al. (2023) found that 7.7% of 78 sheep fetuses from 9 provinces (Samsun, Sinop, Amasya, Giresun, Ordu, Rize, Tokat, Trabzon, and Sivas) between 2018 and 2020 were positive by the PCR method. In the present study, which focused on brain samples from aborted ovine fetuses, the prevalence of *T. gondii* infection was 16.6% (10 out of 60) based on the conventional PCR method.

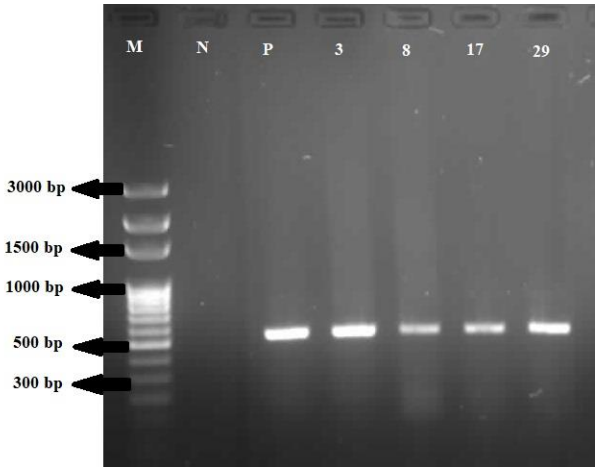

Figure 1. Agarose gel image of Toxoplasma gondii.
M - marker, P - positive control, N - negative control; lanes 3, 8, 17, and 29 are positive samples for Toxoplasma gondii (529 bp).

The clinical manifestation of toxoplasmosis in pregnant ewes can be influenced by the age and immune status of the fetuses. In the first trimester, when the fetal immune system is relatively immature, the likelihood of fetal demise due to infection is greater compared to later stages of pregnancy. Infections during mid-gestation typically lead to the birth of stillborn or frail lambs. In contrast, infections in the later stages of gestation may result in the birth of a live lamb that appears healthy but is infected (Salehi et al., 2020).

Conclusion

Sheep infection with *Toxoplasma gondii* carries significant implications for public health, underscoring the importance of ascertaining its prevalence for implementing requisite precautions. In the current study, which focused on brain samples from select aborted ovine fetuses, the prevalence of *T. gondii* infection was determined to be 16.6% (10 out of 60) using the conventional PCR method. This finding emphasizes the need for vigilance and preventive measures in managing this potential public health concern.

The findings of our study will play a crucial role in enhancing awareness among veterinarians, researchers, and farmers regarding the epidemiology and prevalence of *T. gondii* infection in the Iğdır region. However, further investigations are imperative to delve deeper into understanding the various genotypes of *T. gondii* and their potential connection to abortion and other reproductive complications within the sheep population. This will contribute significantly to a more comprehensive understanding of the infection's impact on the local livestock.

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Spatial Evaluation of Land and Soil Properties with Geography Information Systems (GIS): The Case Study from Meriç District of Thrace Region in Türkiye

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ABSTRACT

This research was carried out within the scope of spatial evaluation of the land and soil properties of Edirne-Meriç district, located in the Thrace region (Türkiye) by using GIS. Arc GIS 10.3.1 software was used in the classification of soil and land features. Digital soil maps (1/25.000 scale) were used to determine soil and land properties. Alos Palsar (12.5m) satellite images were used to determine land elevation and aspect distributions. As a result of the research, it was seen that the soil class with the largest area in terms of large soil groups in Meriç district is lime-free brown forest soils (261.2 km^2). III. It was determined that class lands (153.7 km^2) cover the largest area. In general, it was observed that the soil depth was greater than 150 cm (261.9 km^2). It was determined that 23.3 km^2 of the Meriç district lands were exposed to severe water erosion. It was determined that the study area consists of lands with a steep slope of 12-20% (126.7 km^2). It has been observed that the height distribution of Meriç district lands varies between 4.7-120.5 m. It was defined that the majority of the lands were in the southwestern direction group. It is thought that the soil and land information database created as a result of the research will make significant contributions to researchers and the public, institutions and organizations.

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Introduction

The population in the world and in Türkiye is in an increasing trend. This has led to an increase in consumption and the development of the industrial sector. The increase in population has affected the unconscious and incorrect use of natural resources (Tekinel, 2004). Soil is a living thing that is affected by the environmental situation and can show structural changes depending on the environmental situation they are in. The methods used in agricultural production are extremely important in terms of preserving a sustainable soil resource (Tunçay and Bayramin, 2010; Koca et al., 2017).

For the sustainable use of production areas, it is necessary to determine all the characteristics of the existing soils and review the management techniques applied from the beginning to the end of production in order to identify and solve the problems at this stage (Tunçay et al., 2010; Dingil et al., 2014). It is extremely important to protect and sustain soil and water resources. Soil survey and mapping studies come to the fore in studies on soil and water resources. (Anderson et al., 1976; Başayiğit et al., 1998).

It is extremely important to present the data obtained in soil survey studies to users. Presenting soil data in a database makes significant contributions to environmental modeling studies, protection of natural resources and other engineering studies (Dengiz and Sarıoğlu, 2011). GIS has an important place in digitizing non-numeric data. GIS makes significant contributions to collecting different information in a database and presenting graphic and attribute data simultaneously (Başayiğit et al., 2008).

GIS technologies are a set of systems that perform the functions of storing, analyzing and presenting data obtained from different sources as a whole. In this environment, maps can be created by evaluating data spatially. These advantages provided by GIS make it easier for users to make quick and effective analyzes and make decisions (Kol and Küpçü; 2008).

GIS provides significant contributions to researchers in many subjects such as agricultural production planning, yield estimation, soil and water resources planning, plant monitoring, irrigation and drainage studies and rural settlement planning. (Delibaş et al., 2015; Öztekin et al., 2021; Öztekin and Dingil, 2022). It is necessary to evaluate the current situation in order to evaluate soil resources and make projects and investments for the future (Bağdatlı et al., 2014).

The rapid development of technology around the world has led to significant gains in engineering studies. The use of GIS technologies in planning studies and the ability to store and analyze the obtained data quickly and effectively have provided significant gains for decision makers. Effective evaluation of soil resources with the help of GIS technologies makes significant contributions to sustainable land management (Doğan and Aslan, 2013).

This study was carried out to determine some soil and land properties of Meriç district of Edirne province, located in the Thrace Region of Turkey. Digital soil maps prepared by the repealed Ministry of Agriculture and Rural Affairs were used in the research (Anonymous, 2000). In addition, for the numerical evaluation of some land features, land elevation and aspect features were revealed by using Alos Palsar (12.5 m) satellite images. GIS (Arc GIS) software was used to evaluate the data in the study. The distributions of major soil groups, land use capability classes, erosion degrees, soil depth classes, land elevation and aspect characteristics of the study area are revealed spatially (Anonymous, 2010). In this way, it is aimed to make significant contributions to investor organizations that will contribute to the development of the region and support agricultural production by sharing the research results.

Material and Method

This study was carried out to determine the soil and land properties in Edirne-Meriç district in the Thrace region of Türkiye. The location and location of Meriç district, which is the subject of the research, is shown in Figure 1.

The Thrace region is located in the northwest of Türkiye. There are 3 provinces, 26 districts and 678 villages in the Thrace region. Its surface area (excluding lakes) is 18,665 km^2. Edirne, the subject of the research, is one of the three provinces of the Thrace region. Edrine province has 9 districts in total, including the central district. One of these districts is Meriç district. Meril district is 89 km away from Edirne. The natural borders of the district are the Ergene River in the southeast and south, and the Meriç River in the west and north. The district lands consist of wavy plains and plains between these rivers. The people of the district make their living from agriculture. The main agricultural products grown in the research area are rice, wheat, sunflower, sugar beet and legumes. Animal husbandry is also practiced in the district, where small amounts of apples, grape apples, grapes, pears, sesame and barley are grown.

In the study, GIS software (Arc GS 10.3.1) was used in the analysis and classification of land and soil properties. (Anonymous, 2010). In the study, digital soil maps (1/25.000 scale) were used to determine soil and land properties. Digital elevation models with 12.5 m resolution provided from the Alos Palsar Satellite image were used to determine the aspect and elevation distribution from land features. The classification of soil and land properties was carried out by taking into account the values specified in the Ministry of Agriculture and Forestry in Türkiye, Soil and Land Classification Standards Technical Instruction and given in Tables 1-6 (Anonymous, 2005).

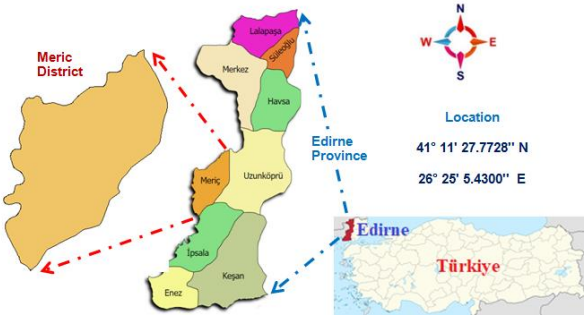

Figure 1. Location of Research Area

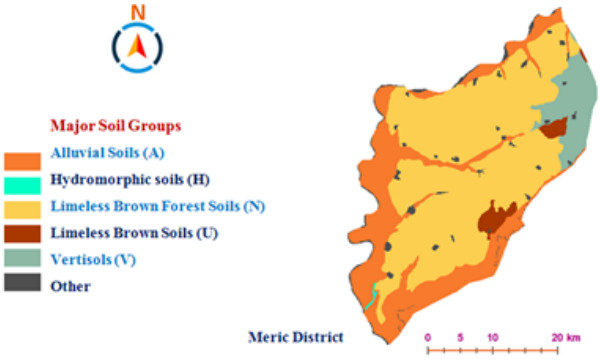

Figure 2. Spatial distributions of major soil groups

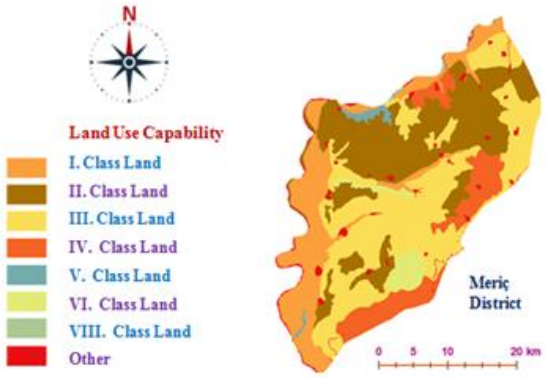

Figure 3. Spatial distributions of land use capability classes

Result and Discussion

In the research, some soil and land characteristics of Meriç district were examined spatially. In the study, the spatial distributions of the large soil groups of Meriç district are presented in Figure 2, and their areal amounts are calculated and presented in Table 6.

When we look at the distribution of large soil groups in Meriç district, it is seen that non-limestone brown forest soils are mostly distributed in the area. Alluvial soils are especially prevalent in areas close to the borders of Meriç district. Vertisol soil group is partially seen in the northeastern part of the district. Alluvial soils cover an area of 116.7 km^2 and their ratio in the total area is 26.78%. Limeless brown forest soils are most distributed in the study area and cover an area of 261.2 km^2 and their ratio in the total area is 59.95%. The spatial distributions of land use capability classes of Meriç district are gived in Figure 3 and the accordingly calculated areal distributions and ratios are summarized in Table 7.

Table 1. Layers of major soil groups (Anonymous, 2005)

Major Soil Groups	
Alluvial soils	Hydromorphic soils
Brown Soils	Colluvial soils
Limeless Brown Soils	Regosols
Chestnut Color Soils	Brown forest land
Reddish Chestnut Color Soils	Nursery Brown Forest Soils
Reddish Brown Mediterranean Lands	Red Yellow Podzolic Soils
Reddish Brown Soils	Rendzinal Soils

Table 2. Land use capability classes (Anonymous, 2005).

Land use Capability Classes	Explanations
Class I Land	These lands have deep soil structure. They are flat and nearly flat. It has fertile and easily cultivated soil.
Class II Land	In this type of land, the soil can be easily cultivated by taking some precautions. These lands may be slightly sloping and subject to moderate floods and erosion.
Class III Land	Agricultural activities can be carried out on these lands using appropriate agricultural methods.
Class IV Land	This type of land can be used as meadow land. There is poor soil character and exposure to erosion. They have poor drainage.
Class V Land	It is not suitable to grow cultural plants on these lands. This type of land can be used as meadow and forest area. These lands are stony and wet areas and agricultural production cannot be done..
Class VI Land	This type of land is very sloping. They are subject to severe erosion. They are not suitable for soil cultivation.
Class VII Land	These lands include sloping lands. It has been exposed to a lot of erosion. These are stony and swampy areas.
Class VIII Land	Agricultural production is not possible on this type of land. These lands should be left more naturally. These lands are extremely stony, mountainous and swampy areas.

Table 3. Erosion degree (Anonymous, 2005).

Erosion Degree	
1	Light (less than 25% of top soil eroded)
2	Hydrangea (25-75% of topsoil eroded)
3	Severe (more than 75% of topsoil and less than 25% of subsoil eroded)
4	Very Severe topsoil and 35-75% of subsoil eroded)

Table 4. Soil depth layers (Anonymous, 2005).

Soil Depths (cm)		
A	>150	Deep
B	90 - 150	Medium Deep
C	50 - 90	Shallow
D	20 - 50	Too Shallow
E	0 - 20	lithosolic

Table 5. Land slope groups (Anonymous, 2005).

Land Slope	
0 - 2 %, Straight	20 - 30, Very Steep Slope
3 - 6 %, Slight Slope	30 - 45, Steep Slope
7 - 12 %, Medium Slope	45 + Cliff
13 - 20 %, Steep Slope	

Table 6. Area distributions of major soil groups

Major Soil Groups	Area (km^2)	Ratio to Total Area (%)
Alluvial Soils (A)	116.7	26.78
Hydromorphic Soils (H)	0.60	0.14
Limeless Brown Forest Soils (N)	261.2	59.95
Limeless Brown Soils (U)	10.8	2.48
Vertisols (V)	34.4	7.90
Other	12	2.75

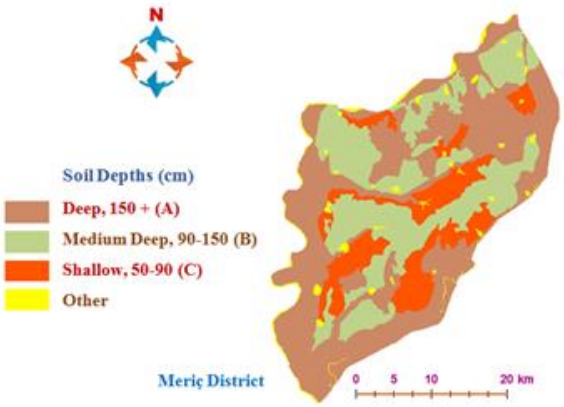

Figure 4. Spatial distributions of soil depth classes

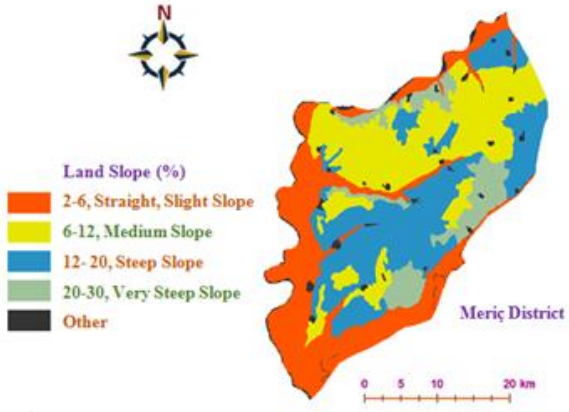

Figure 5. Spatial distributions of land slope classes

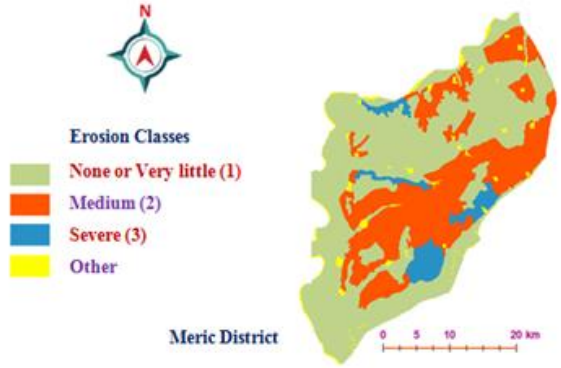

Figure 6. Spatial distributions of erosion classes

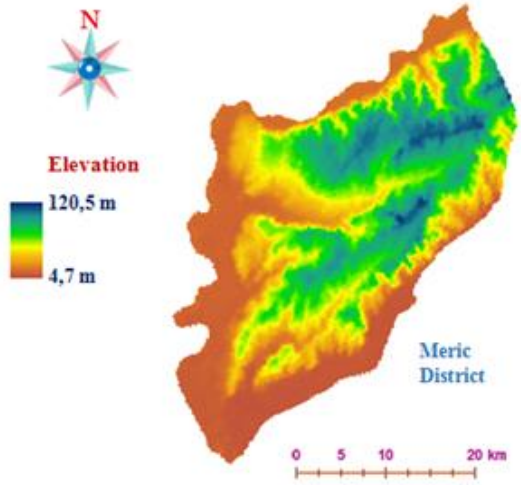

Figure 7. Spatial distribution of land elevations

In the study, land use capabilities of Meriç district were classified. In this context, the lands throughout the district are in III and VI. It is seen that it consists of lands that fall into this category. III. class lands correspond to 35.28% of the total area of the district. VI. class lands constitute 30.53% of the total area. In the research area, class I lands cover an area of 77.7 km^2 and cover 17.83% of the total area. Class I lands are distributed especially in the western and northern parts of Meriç district. Especially in the western part of the district, the Meri River, the 10th largest river of our country, passes. In this context, this region is a rich and favorable region in terms of both efficiency and agricultural production. In this respect, the lands in this section are areas where agricultural production is carried out effectively. II and III. Class lands are distributed in the slightly more central parts of Meriç district.

These lands are also suitable for agricultural production and agricultural production activities are carried out. The soil depth classes of Meriç district was analyzed as spatially and the results obtained are presented in Figure 4 and Table 8.

It is inevitable to have sufficient soil depth to carry out agricultural production effectively. Having different soil depths depending on the type and type of plant to be grown is extremely important in terms of plant root development and ensuring optimum productivity. An appropriate and sufficient soil depth provides a suitable development environment for seeds, seedlings and saplings. In this context, knowing the appropriate soil depth, which is extremely important for agricultural production, will make significant contributions to production.

In the examined area, it is seen that the soil depths are predominantly 90-150 cm and there are areas larger than 150 cm. Areas with soil depth greater than 150 cm correspond to 49,8% of the total area. Lands with a soil depth of 90-150 cm cover an area of 138.7 km^2 and constitute 31.8% of the surface area of Meriç district. In the research area, it was observed that the ratio of areas varying between 50-90 cm and described as shallow soil class in the total area was around 15.6%. It has been observed that the fields falling into the shallow soil class are distributed especially in the central parts of Meriç district. The land surface slopes of Meriç district, which is the subject of the research, were also evaluated spatially and the findings are given in Figure 5 and Table 9.

The land slope of Meriç district varies between 2-30%. Lands with a slope between 2-6% constitute 28% of the total area. These lands are mostly distributed on the western, northern and southern borders of Meriç district. Lands in the medium slope class are mostly spread in the northern regions and constitute 7.28% of the total area. Lands with very steep slopes (20-30%) constitute 21.18% of the total area. Areas with very steep slopes are partially distributed in the central part of Meriç district. The distribution map showing the erosion exposure of Meriç district is given in Figure 6 and the areal amounts are given in Table 10.

Water erosion is mainly observed in Meriç district. It is given that there are areas exposed to moderate erosion in the overall research area, which is 33.42% of the total area. Areas exposed to severe erosion correspond to 5.35% of the total area.

Table 7. Areal distributions of land use capability classes

Land Use Capability	Area (km^2)	Ratio to Total Area (%)
I. Class Land	77.7	17.83
II. Class Land	1.3	0.30
III. Class Land	153.7	35.28
IV. Class Land	45.2	10.37
V. Class Land	4.2	0.96
VI. Class Land	133	30.53
VIII. Class Land	1.7	0.39
Other	18.9	4.34

Table 8. Areal distributions of soil depth classes

Soil Depth Classes	Area (km^2)	Ratio to Total Area (%)
Deep (A), +150 cm	216.9	49.8
Medium Deep (B), 90-150 cm	138.7	31.8
Shallow (C), 50-90 cm	68.1	15.6
Other	12.0	2.8

Table 9. Areal distributions of land slope classes

Land Slope	Area (km^2)	Ratio to Total Area (%)
2-6 % Slight Slope	122	28.0
6-12 % Medium Slope	31.7	7.28
12-20 % Steep Slope	126.7	29.08
20-30 % Very Steep Slope	63	14.46
Other	92.3	21.18

Table 10. Areal distributions of erosion classes

Erosion Classes	Area (km^2)	Ratio to Total Area (%)
None or very little (1)	254.9	58.50
Medium (2)	145.6	33.42
Severe (3)	23.3	5.35
Other	11.9	2.73

Table 11. Areal distribution of land elevations

Elevation (m)	Area (km^2)	Ratio to Total Area (%)
4,7 – 27,9	155.3	35.64
27,9 – 51,1	81.7	18.75
51,1 – 74,2	83.5	19.16
74,2 – 97,4	86.6	19.88
97,4 – 120,5	28.6	6.56

Table 12. Areal distribution of land aspect (direction) groups

Aspect (direction)	Area (km^2)	Ratio to Total Area (%)
Straight	0.01	0.001
North	20.17	4.63
Northeast	34.07	7.82
East	40.06	9.19
Southeast	72.1	16.55
South	80.24	18.42
Southwest	53.18	12.21
West	53.28	12.23
Northwest	57.37	13.17
North	25.24	5.79

Areas exposed to no or very little erosion constitute 58.50% of the total area. The spatial distributions of land elevations in Meriç district are given in Figure 7 and the calculated areal amounts are given in Table 11.

Lands with an elevation range of 4.7-27.9 m correspond to 35.64% of the total area. Lands with land elevations ranging between 27.9-51.1 m cover 18.75% of the total area. Areas with land elevation between 97.4-120.5 m constitute 6.56% of the total area. Generally, the land elevations of Meriç district vary between 4.7-120.5 m. Especially in places where the district border is located, the land elevation is seen as the lowest compared to other areas. In the western part, where the land elevation is lowest and where the district borders pass, the Meriç River folowes from north to south. In the eastern part of the district, the Ergene River flowes from north to south. Therefore, these areas are seen as the areas with the lowest elevation of the land. The spatial distributions of the aspect

status of Meriç district lands are given in Figure 8, and the areal amounts of the aspect classes are calculated and presented in Table 12.

When we look at the land direction distribution, it is seen that there are no flat areas. Eastern oriented lands constitute 9.19% of the total area. Western oriented areas cover 12.23%. Generally speaking, it constitutes 4.63% of the total area.

It is possible to come across many researches in the literature on the evaluation of soil and land resources with GIS. A study was carried out within the scope of Mapping Some Soil Properties of the Lower Kelkit Basin with GIS and Remote Sensing. Within the scope of the research, soil samples were taken from the field to determine the physical and chemical properties of the soil. The data obtained as a result of the analysis were transferred to GIS software and spatial distribution maps of soil properties were produced (Doğan and Aslan, 2013).

In another research, it was determined some land and soil properties in Siirt province with GIS and to create a database of the results obtained. Digital soil maps were used in the study. Within the scope of the research, distribution maps of Siirt province's land slope and aspect distributions, erosion classes, distributions of large soil groups and current land use situations were produced (Özyazıcı et al., 2014).

Another research was carried out within the scope of determining and mapping the local changes in soil properties of the Dicle basin with geostatistical and geography information systems. In this context, soil samples were taken from the study area. Some physical and chemical analyzes were performed on the soil samples taken. The obtained analysis results were evaluated spatially in the GIS environment and distribution maps for the examined parameters were produced (Budak et al., 2018).

Many studies similar to this study have been carried out in Niğde, Nevşehir, Kırşehir, Kayseri, Malatya, Thrace Region and Kızılırmak Basin to evaluate soil and land properties. In these studies, some soil and land properties were evaluated in the GIS environment and distribution maps for the examined features were produced (Oztekin and Kosar, 2021; Bağdatlı and Can 2021b; Bağdatlı and Can, 2021a; Bağdatlı and Oztekin 2021; Bağdatlı and Arslan 2021; Bağdatlı and Ballı 2021; Bağdatlı and Arıkan 2021; Bağdatlı and Arslan, 2020; Bagdatlı and Nazari 2022a; Bağdatlı et al., 2022; Bağdatlı and Ballı 2022; Bağdatlı and Arıkan 2022; Bağdatlı and Nazari 2022b; Bağdatlı and Can 2023; Bağdatlı and Nazari 2023).

As can be seen from the literature, it is possible to come across many researches in the literature on the determination of soil and land resources by using GIS based mapping. The main feature of all these studies is that soil and land resources in the examined areas are analyzed digitally and distribution maps of the examined features are produced. It is thought that the results will make database to agricultural practices in the study areas.

In this study conducted in Meriç district, land and soil properties were evaluated numerically in the GIS environment and it was aimed that the results obtained would make significant contributions to investor organizations operating in the region.

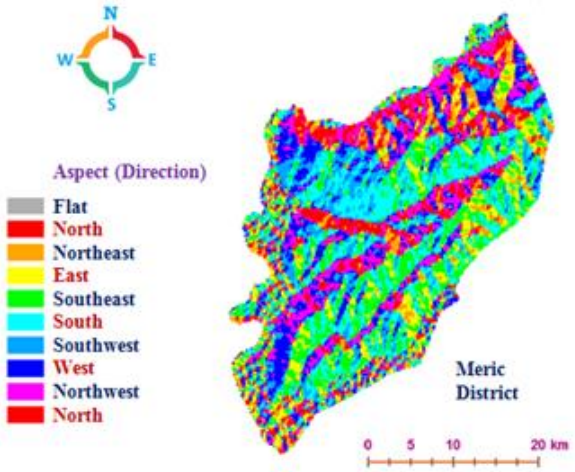

Figure 8. Spatial distribution of land aspect (direction) groups

Conclusion

GIS and Remote Sensing technologies are of vital importance in understanding and solving today's growing problems (Burrough, 1986; DeMers, 1997; Koçak, 1991). The main purpose of these systems is to manage data sets related to the environment or social structure with computer-aided studies and produce various information from these data for the benefit of society (Koçak, 1991).

GIS and its associated remote sensing are widely used in soil inventories, erosion control and pasture vegetation studies, as well as in many areas (Field, 1989).

These techniques, which are quite fast and sensitive compared to traditional methods, provide the opportunity to work in large areas and give very realistic results when combined with field studies (Chang et al., 1989, Mon Zan, 1989; Dogan and Dogan, 2006; Dogan, 2008). The main areas where GIS and Remote sensing are used in the world are issues related to soil structure, production systems and erosion (Hall-Bayer and Gwyn, 1997).

In this study, Meriç district of Edirne province, located in the Thrace region, was determined as the research area. In the study, some soil and land features of Meriç district were evaluated spatially with the help of GIS. In this context, distribution maps were created by classifying major soil groups, erosion degrees, land use capabilities, soil depths, land slope, elevation and aspect features.

The research area is a region where agricultural production is intense. Presenting the spatial distribution of data on some soil and land characteristics will provide significant contributions to investor organizations. The data obtained as a result of this study will provide infrastructure support, especially in the activation of areas that will be opened for agricultural production.

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Use of Probiotics for Safe Quail Meat Production

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ABSTRACT

Safe meat production is an important aspect to avoid human health hazards. The use of probiotics in poultry is an important tool to produce safe meat among several established biotechnological approaches. In this experiment, we studied the effects of probiotics for producing safe Japanese quail meat. 150 Japanese quail chicks were reared for a period of six weeks using various doses of probiotics (0, 0.5, 1, 1.5, and 2g per litre of water). The chicks were randomly distributed into five treatment groups with three replications each. The number of birds in each replication was 10. After rearing six weeks, significantly high body weight was found at probiotic concentrations of 1, 1.5, and 2g per litre of water. The feed intake in various treatments did not differ significantly, but comparatively better feed conversion ratios were observed at probiotic treatments. Water quality was not significantly differed as a result of addition of probiotics to the water. The reason for this better growth performance is probably due to the multiple benefits of probiotics in poultry. Probiotics could have maintained gut health with better nutrient utilization and availability that might have been led to higher body weight gain in the quail. In future experiments, challenging the birds with diseases or comparing probiotics with antibiotic growth promoters is required to ensure the efficiency of probiotics.

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Introduction

The poultry sector is playing a vital role in fulfilling protein requirements worldwide. The demand for poultry has increased over the past few years due to the diversified uses of poultry meat and eggs (Mottet and Tempio, 2017). The source of protein might be unsafe due to the transmission of infectious diseases and antimicrobial resistance, leading to human health hazards. One of the most commonly used techniques was to use antibiotic growth promoters to mitigate disease load and trigger the productive performance of poultry. For the safety of human health, the European Union banned the use of these growth promoters in 2006 (Huyghebaert et al., 2011). Almost all countries are now concerned about the use of antibiotics in the poultry industry. The governments of many countries have banned the use of these growth promoters in animals, but the research findings show that growth promoters are still being used in the poultry industry after being banned by the government (Islam et al., 2023). As the withdrawal of antibiotic growth promoters has led to poor poultry performance and increased production costs (Maria Cardinal et al., 2019), dishonest entrepreneurs and farmers are using growth promoters in spite of being banned by the government.

Production of antibiotic-free safe poultry, a nutritional biotechnological approach might be introduced in the poultry industry to replace antibiotic growth promoters and improve productivity (Abd El-Hack et al., 2022). Among the feed additives used in the poultry industry, probiotics could probably be a potential candidate to replace antibiotic growth promoters (Yaqoob et al., 2022). Probiotics might play an important role in improving overall productive performance through maintaining gut health, boosting immunity, decreasing disease load, and improving feed efficiency. Due to the beneficial effects of probiotics, several experiments have been conducted on several species. The researchers also reviewed the effects of probiotics in a multidimensional approach and explored the multi-benefits of probiotics on animal as well as poultry performance. In most cases, the effects in poultry were based on single probiotic species, like the effect of

Lactobacillus spp. (Fesseha et al., 2021), and *Bacillus* spp. (Biswas et al., 2022) on chicken performance. Even though there is research on the effects of compound probiotics on gut health and chicken performance (Chang et al., 2020), the research is not sufficient to fully understand their mode of action and health benefits. As a poultry species, the effects of probiotics on quail performance are limited, and the use of probiotics in quail production is yet to be elucidated. Among the poultry species Japanese quail is important and considered a potential species if managed scientifically and efficiently. Moreover, the doses of probiotics in quail have not been set up for optimum production. For this reason, the study has been designed to investigate the effects of probiotics in a dose-dependent manner to determine growth performance in Japanese quail. The research will help to determine the efficiency of probiotics in a dose-dependent manner on the production performance of quail.

Materials and Methods

Experimental birds and diets

The current study was conducted with 150 Japanese quails for six weeks at Poultry Farm, Department of Poultry Science, Patuakhali Science and Technology University, Babuganj, Barishal-8210. Before the arrival of chicks, the poultry shed was cleaned, washed, and disinfected properly. The day-old Japanese quail chicks were brooded and reared with the same management for two weeks. After two weeks of incubation, all chicks were weighed and distributed evenly into five treatments with three replications each. The number of birds in each replication was 10. A hand-mixed, balanced diet was prepared using available feed ingredients, and the prepared feed was analyzed at the laboratory of the Department of Livestock Services, Farmgate, Dhaka (Table 1). Feeds were supplied twice a day, and the birds were allowed to consume feed *ad libitum*.

Preparation of probiotics solutions

Probiotics were dissolved in water, and prepared different probiotic concentrations. The probiotics contained *Bacillus subtilis*, *Bacillus coagulans*, and *Saccharomyces boulardii* 4.5×10^9 CFU in each gram. Five treatments (T1: 0g probiotics per litre of water; T2: 0.5g probiotics per litre of water; T3: 1g probiotics per litre of water; T4: 1.5g probiotics per litre of water; and T5: 2g probiotics per litre of water) were considered in the experiment. Clean, safe, and sufficient drinking water was supplied to the birds throughout the experimental period.

Housing management

The chicks were reared in a cage with a space of 30 cm × 30 cm per 10 birds. The cages, feeders, drinkers, and surroundings of the shed were cleaned regularly. Bird droppings were collected in trays, and the trays were cleaned and disinfected twice a week. The poultry shed and cage were well ventilated. Strict biosecurity was maintained in the experimental area. The shed was open-sided, and the environmental temperature ranged from 26.1 to 34.5 °C with a relative humidity of 61 to 89% during the experimental period. Natural daylight was used as a light source; the average photoperiod was 12 hours of light and 12 hours of darkness.

Water quality measurement

We prepared various concentrations of probiotics in water and measured the water's quality with a multifunctional water quality tester (model: EZ-9909SP, China). At first, the water quality tester was calibrated with a standard pH solution at 6.86, 4.00, and 9.18. Then, placed the electrode in various concentrations of probiotics (0, 0.5, 1, 1.5, and 2g per litre of water) to determine water temperature (°C), pH, total dissolved solids (TDS) (ppm), salinity (%), and electrical conductivity (EC) (µS/cm).

Statistical analysis

Means ± standard deviations are used to represent data. The data were analyzed using IBM SPSS version 20. Differences among treatments were analyzed using Tukey's honestly significant difference test, and the significance level was declared based on $P< 0.05$.

Table 1. Ingredients and chemical composition of diet

Ingredients (%)	Basal diet
Yellow maize	56.3
Soybean meal (44%)	36.0
Protein concentrate	4.41
Limestone coarse	1.06
Dicalcium phosphate	0.811
Soyabean oil	0.531
Common salt	0.300
Vitamin premix	0.150
Mineral premix	0.150
Salmonella killer	0.100
L-lysine	0.050
DL-methionine	0.050
Toxin binder	0.050
Multi-enzyme	0.050
Choline chloride	0.040
Calculated values (% on DM basis)	
Moisture	12.57
Dry matter (DM)	87.43
Crude protein (CP)	25.93
ME (Kcal/kg)	3377
Total ash (TA)	6.06
Acid insoluble ash (AIA)	0.56
Crude fiber (CF)	3.53
Crude fat (EE)	3.86
Calcium (Ca)	1.11
Phosphorus (P)	0.63

Results and discussion

To produce safe poultry meat, the effects of probiotics were studied in a dose-dependent manner to observe the growth performance of Japanese quail. After 6 weeks of rearing, the average body weight of quail was 147.9, 154.6, 159.9, 157.9, and 154.9 g/bird when probiotics were added at 0, 0.5, 1, 1.5, and 2g per litre of water, respectively (Table 2).

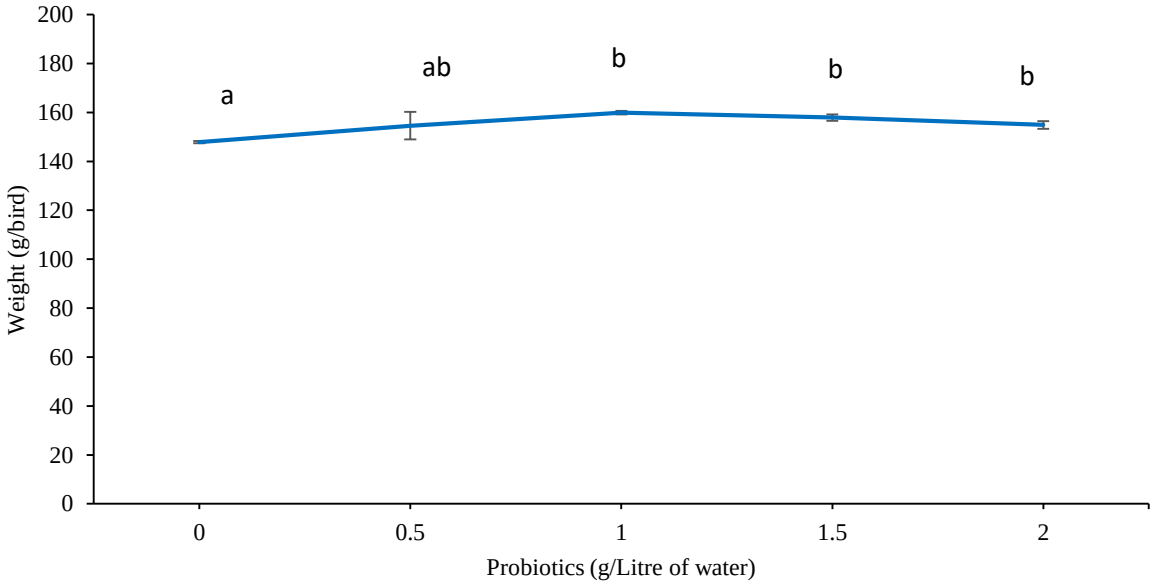

Figure 1. Use of probiotics on the growth performance of Japanese quail

Table 2. Growth performance of Japanese quail at 42 days of age

Parameters	probiotics g per litre of water					SEM
	0	0.5	1	1.5	2	
Final body weight (g/bird)	147.9^{a}	154.6^{ab}	159.9^{b}	157.9^{b}	154.9^{b}	1.50
Feed intake (g/bird)	607.65^{a}	602.10^{a}	595.05^{a}	605.75^{a}	606^{a}	1.80
Feed conversion ratio	4.11	3.89	3.72	3.84	3.91	0.063
Mortality (%)	0	0	0	0	0	-

Data are expressed as mean ± standard deviation; ^{a,b} meaning significant differences among treatment groups. P<0.05. SEM: standard error of the means

Table 3. Water quality after adding probiotics to water

Parameters	probiotics g per litre of water					SEM
	0	0.5	1	1.5	2	
Water temperature (°C)	29.47	29.3	29.03	29.17	28.93	0.071
pH	7.09	8.33	8.33	8.36	8.26	0.135
TDS (ppm)	993.33	980	983.33	980	976.67	2.06
Salinity (%)	0.1	0.09	0.09	0.09	0.09	0.054
EC (µS/cm)	2016.67	1956.67	1956.67	1953.33	1956.67	6.63

SEM: standard error of the means

It was found significantly higher body weight at probiotic concentrations of 1, 1.5, and 2g per litre of water, respectively (Figure 1). The reason for the weight gain might be due to probiotics act as a growth stimulator through multi-functional approach. In addition, several works and reviews have been performed that revealed the health benefits of probiotics. As viable microorganisms, probiotics can function in the intestines of poultry (Schrezenmeir and De Vrese, 2001). In the current study, we used *Bacillus subtilis*, *Bacillus coagulans*, and *Saccharomyces boulardii* 4.5×10^{9} CFU in each gram. *Bacillus subtilis* a gram-positive bacteria that can increase apparent metabolism of crude protein, crude fat and organic matters in poultry through better nutrient utilization of feed (Gao et al., 2017). It can also improve kidney functions and increase the digestive enzymes (Mohamed et al., 2022). The lactic acid forming bacterial species *Bacillus coagulans* may increase antioxidant capacity, boost immunoregulatory systems and decrease inflammation with increased microflora in poultry gut (Zhang et al., 2021). Yeast like *Saccharomyces boulardii* helps to available phosphate in the gut and increase calcium digestibility in poultry (Nari and Ghasemi, 2020). Therefore, the individual probiotic species have distinct benefits and probably not yet fully understood. Abd El-Hack et al., (2022) demonstrated the possible mechanisms of probiotic action, stating that probiotics exclude pathogenic microorganisms from the intestinal epithelium. Probiotics produce antimicrobial substances that inhibit the adhesion of toxins and pathogens in the mucus of epithelial cells, and pathogenic microorganisms cannot compete for intestinal nutrients. Thus, probiotics adhere to the intestinal epithelial layer, improve epithelial barrier functions, and enhance antibody production.

During the experimental period, feed consumption per bird was 607.65, 602.10, 595.05, 605.75, and 606g at probiotic concentrations of 0, 0.5, 1, 1.5, and 2g per litre of water, respectively. It was not found any differences in feed intake among the treatment groups. Several researchers studied the effects of probiotics on feed consumption in poultry and observed that the feed consumption was not affected by probiotic treatment in

chicken (dela Cruz et al., 2019) and Japanese quail (Abdel-Moneim et al., 2020). The research finding also reveals feed consumption increased significantly due to the application of dietary probiotics in Japanese quail (Taksande et al., 2009). Whatever the situation, the feed conversion ratio (FCR) was improved due to the application of probiotics in quail (Kazemi et al., 2019; Abou-Kassem et al., 2021). However, in the current study, the feed conversion ratios were 4.11, 3.89, 3.72, 3.84, and 3.91 when probiotics were added at 0, 0.5, 1, 1.5, and 2g per litre of water, respectively. There was no mortality of quail during the experimental period.

Probiotics in water improved growth performance, carcass quality, and antioxidant capacity in poultry (Zhang et al., 2021). The route of probiotics in poultry may be different, such as feed, water, oral gavage, and litter, but regardless of the route of probiotics in birds, probiotics reduce the load of harmful pathogens and increase the beneficial microflora in birds (Olnood et al., 2015). As we added probiotics to water and supplied to birds, we measured water temperature, pH, TDS, salinity, and EC to know the water quality at various concentrations of probiotics (Table 3). The water temperature for all the treatment groups ranged from 28.93 to 29.47 °C. The pH for the control group was 7.09, whereas it increased when probiotics were added to the water. The pH was 8.33, 8.33, 8.36, and 8.26 at probiotic concentrations of 0.5, 1, 1.5, and 2g per litre of water, respectively. The TDS values were 993.33, 980, 983.33, 980, and 976.67 for 0, 0.5, 1, 1.5, and 2g of probiotics per litre of water, respectively (Table 3). The EC was higher in the control group compared to others. The reason for this increase was probably due to the fact that the higher the TDS values, the higher the EC (Islam et al., 2017). The increase in this TDS might be safe for poultry because it can only affect growth adversely at higher doses of TDS. Higher TDS levels, like 3154 and 3448 ppm, affect growth negatively in poultry (Ahmed, 2013). The water quality should be checked regularly, and the salinity of the water should not exceed 1% NaCl (common salt) when balanced feed is provided to poultry. In our experiment, the salinity of the water ranged from 0.09 to 0.1%. The EC also seems to be the same in groups and ranges from 1953.33 to 2016.67.

Generally, probiotics increase the production of antimicrobial substances, exclude pathogenic microorganisms, reduce the pH of the lumen, increase villus length, and trigger the immune system in poultry (Anee et al., 2021). The performance of quail was also improved in various conditions when probiotics were added to the experimental conditions (Abdel-Moneim et al., 2020; Aydın et al., 2022). Currently, probiotics are used in humans and several animals to prevent diseases by controlling pathogenic bacteria and improving the gut microflora for a healthy life. It increases feed efficiency, growth, egg production, and profitability in poultry (Lokapirnasari et al., 2017). So, the findings show the multiple benefits of probiotics in poultry. In the current study, it is possible to maintain good gut health with better nutrient utilization, which likely leads to high body weight gain in the quail. In future studies, it might be useful to challenge disease birds or compare probiotics with antibiotic growth promoters to ensure the efficiency of probiotics.

Conclusions

The results show the higher body weight of quail at probiotic concentrations of 1, 1.5, and 2 g per litre of water. The feed intake did not differ significantly in various treatments, but comparatively better feed conversion ratios were seen at probiotic treatment. Water quality was not differed as a result of the addition of probiotics to the water. Probiotics might be used to produce safe meat and higher body weight in quail.

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Research Ethics Approval

We received departmental approval considering research ethics to use probiotics for safe quail meat production. The approval number was PSC/2023/272, dated March 1, 2023.

Authors' Contribution

Prodip Kumar Sarkar planned, designed, and conducted the experiment. He also prepared tables, graph and wrote the manuscript. Dip Majumder Ridoy and Mehedi Islam Moon conducted the experiment and collected the data. Swapon Kumar Fouzder reviewed the manuscript. All the authors confirmed the data and approved the final manuscript.

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Molecular Survey of the *Toxoplasma gondii* and *Neospora caninum* in brain tissue of aborted fetuses of Morkaraman sheep in Muş, Türkiye

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ABSTRACT

Toxoplasma gondii and *Neospora caninum* are obligate intracellular protozoan parasites that can affect different warm-blooded species worldwide. In this study, it was aimed to detect *T. gondii* and *N. caninum* using PCR method in brain tissues of aborted sheep fetuses. Brain specimens were collected from 50 Morkaraman sheep fetuses that had undergone abortion at various stages of pregnancy, within the lambing seasons of 2023 in Muş. Approximately 1 cm³ of brain tissue from the right cerebral hemisphere was excised and subsequently frozen at -20°C for DNA extraction. DNA extraction and PCR amplification were then performed. As a result of this study, 11 (22%) of 50 brain tissues were positive. All brain samples examined in this study were negative for *Neospora caninum.* Based on the results of this study, it is possible to say that *T. gondii* is an important abortion agent in sheep in this region. Although *N. caninum* was not detected in this study, larger scale studies are recommended. Moreover, this study provides important information to breeders and veterinarians in the evaluation and management of abortion in the field.

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Introduction

Toxoplasma gondii and *Neospora caninum* are obligate intracellular protozoan parasites that are distributed worldwide, have structural, genetic and immunological similarities, belong to the phylum Apicomplexa and can affect different warm-blooded species (Dubey, 2003; Basso et al., 2022).

Toxoplasma gondii causes toxoplasmosis, one of the most common parasitic diseases in humans and animals worldwide (Tenter et al., 2000; Wang et al., 2011). The definitive hosts are cats and felidae family, and the intermediate hosts are mammals, including humans, and birds (Dubey, 1994; Mor and Arslan, 2007). Infected cats can shed millions of oocysts. This causes widespread environmental contamination and is an important source of infection for herbivores (Innes et al., 2009; Can, 2010). The infective forms of the parasite are tachyzoites, bradyzoites and oocysts of the felidae family, especially in the brain and muscle tissue. (Muz et al., 2013). The disease causes significant economic losses especially in sheep due to pneumonia, enteritis, neurological disorders, encephalitis, premature birth, stillbirth, neonatal losses and abortions (Van der Puije et al., 2000; Bártová et al., 2009; Dubey, 2009; Anğ et al., 2011).

Neospora caninum is an obligate intracellular parasite first isolated in Norway in 1984 in puppies with congenital encephalomyelitis (Dubey et al., 2007; Uzêda et al., 2007). The definitive host of *N.caninum* is domestic and wild canids and the intermediate hosts are herbivores.

Herbivores become infected by ingesting infected oocysts scattered in the feces of the definitive hosts (Sharma et al., 2015; Gharekhani et al., 2016). This disease causes abortions and newborn deaths in cattle, sheep and goats (Dubey, 2003; Figliuolo et al., 2004; Uzêda et al., 2007). In many hosts, transmission is transplacental. Significant economic losses can occur due to abortions and neonatal mortality (Sharma et al., 2015). In experimental studies, congenital infection has been reported in sheep and goats (Gharekhani et al., 2016).

In this study, it was aimed to detect *T. gondii* and *N. caninum* using PCR method in brain tissues of 50 aborted sheep foetuses in the Muş, Türkiye.

Material and Methods

The Study Area and Samples Collection

Brain specimens were collected from 50 sheep fetuses that had undergone abortion at various stages of pregnancy, within the lambing seasons of 2023 in Muş, Türkiye. The samples were exclusively sourced from Morkaraman breed sheep. Out of 50 aborted ovine fetuses, a total of 50 brain tissue samples were procured. To extract brain samples, each fetus was handled individually, with the calvarium opened and meninges dissected using a fresh disposable scalpel and forceps. Approximately 1 cm³ of brain tissue from the right cerebral hemisphere was excised and subsequently frozen at -20°C for DNA extraction.

DNA Extraction

DNA extraction was performed in all aborted fetus brain using the PureLink™ Genomic DNA Mini Kit (Invitrogen™, USA, K182002), according to the manufacturer's protocol. The obtained DNAs were stored at -20°C.

PCR Amplification

The amplification of the 529-bp repetitive element region of *Toxoplasma gondii* was conducted using the TgTox4F (5'-CGCTGCAGGGAGGAAGACGAAAGTTG-3′) and TgTox4R (5'-CGCTGCAGACACAGTGCATCTGGATT-3′) primers (Sah et al., 2019). Protocol for reactions was performed according to Orunç Kılınç et al. (2023).

For amplification of the Nc5 gene region of *N. caninum*, nested PCR was performed using external (5'-CTGCTGACGTGTCGTTGTTG-3′) forward and (5' CATCTACCAGGCCGCTCTTC-3′) reverse primers inner (5'-GCGTCAGGGTGAGGACAGTG-3′) forward and (5'-CTCTCCGTTCGCCAGCAGTG-3′) reverse primers (Fish et al., 2007). Protocol for both reactions was performed according to Orunç Kılınç et al. (2023).

The reaction was performed in an automatic thermal cycler (Eppendorf Mastercycler® pro) device. Subsequently, 1.5% agarose gel was prepared and stained with RedSafe™ Nucleic Acid Staining Solution. The PCR products were run on an agarose gel afterward, and images were obtained on the gel imaging device (Syngene bioimaging system).

Ethical Approval

This study was approved by Van Yuzuncu Yil University Animal Experiments Local Ethics Committee (Approval no:2023/03-10).

Results

In this study, a total of 50 brain tissue samples were chosen from aborted fetuses for the isolation of the *Toxoplasma gondii* parasite through conventional PCR. Positivity for the presence of *T. gondii* was confirmed in 11 out of the 50 samples, accounting for 22% of the total. (Figure 1). All brain samples examined in this study were negative for *Neospora caninum.*

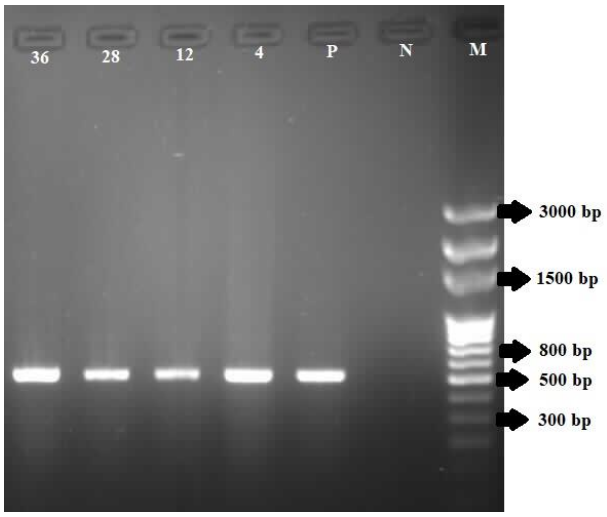

Figure 1. Amplification of *T.gondii* using conventional-PCR. Lanes M: Marker, N: Negative control, P: positive control; 36, 28, 12 and 4 represent *T.gondii*. (529 bp).

Discussion

Toxoplasmosis plays an important role in sheep abortions and causes heavy losses to the sheep industry worldwide (Dubey, 2009). Especially infectious abortions have important effects on public health not only with their economic dimension but also with their zoonotic importance (Har and Başbuğan, 2019). Although *N. caninum* has been reported to cause congenital infections, abortions and deaths in newborn lambs in sheep, it is not considered among the main causes of abortion in sheep (Innes et al., 2001; Koyama et al., 2001; Hässig et al., 2003).

Toxoplasma gondii infections have been recorded in sheep populations worldwide with highly variable seroprevalences (Basso et al., 2022). A prevalence of 51.76% was reported in Egypt (Ibrahim et al., 2017), 38.22% in Brazil (Ueno et al., 2009), 3.76% in India (Sharma et al., 2008) and 1.6% in Iran (Raeghi et al., 2011). In studies conducted in Turkey; 54.65% prevalence was reported in Afyonkarahisar (Çiçek et al., 2004), 66.66% in Yalova (Oncel et al., 2005), 10% in Nevşehir (Çakmak and Karatepe, 2017), 48.4% in Mersin (Öztürk et al., 2002), 95.7% in Kars (Mor and Arslan, 2007) and 13% in Konya (Aköz et al., 2009). It was reported that *T. gondii* DNA was detected in 3 of 20 aborted fetal brain tissues (Hässig et al., 2003), 4 of 74 sheep fetal brain tissues (Moreno et al., 2012), 48 of 75 sheep fetal brains (Shahbazi et al., 2019), 9 of 111 sheep fetal brains (Partoandazanpoor et al., 2020), and 9 of 53 sheep fetal brains (Hurtado et al., 2001). In this study, the brains of 50 aborted sheep fetuses were examined by conventional PCR method and *Toxoplasma*

gondii DNA was detected in 11 (22%) tissues. This result was lower than the findings of some researchers (Öztürk et al., 2002; Çiçek et al., 2004; Oncel et al., 2005; Ueno et al., 2009; Ibrahim et al., 2017) and higher than the results of some studies (Aköz et al., 2009; Çakmak and Karatepe, 2017).

In studies to determine the prevalence of *N.caninum* in sheep in the world; 10.1% in Spain (Panadero et al., 2010), 16.8% in Greece (Anastasia et al., 2013), 8.81% in Brazil (Ueno et al., 2009), 27.7% in Pakistan (Nasir et al., 2012) and 1.53% in Iran (Ezatpour et al., 2015) prevalence was reported. In Türkiye, studies investigating the prevalence of *N. caninum* in sheep are quite limited. Positive rates of 12.4% in Adana (Ekşi et al., 2018), 0% in Van (Har and Başbuğan, 2019), 0% in Elazığ (Özkaraca et al., 2016) and 2.1% in Kars (Gökçe et al., 2015).

In studies conducted on aborted fetal brain tissue, it was reported that *N. caninum* DNA was detected in 4 of 20 aborted fetal brain tissues (Hässig et al., 2003), 5 of 74 sheep fetal brain tissues (Moreno et al., 2012), 3 of 18 fetuses (Howe et al., 2008), 18.9% of 74 fetuses (Hughes et al., 2006). All tissue samples examined in this study were negative for *Neospora caninum*. This result is similar to the findings of researchers (Özkaraca et al., 2016; Har and Başbuğan, 2019). The reasons for the differences between the studies include geographical location, climate, sheep breed, end-host prevalence and the tests used.

Conclusion

Based on the results of this study, it is possible to say that *T. gondii* is an important abortion agent in sheep in this region. It was also concluded that PCR method is an important tool in the diagnosis of protozoal abortion agents. Although *N. caninum* was not detected in this study, larger scale studies are recommended. Furthermore, the results of this study are poised to have a significant impact on increasing awareness among veterinarians, researchers, and farmers regarding the epidemiology and prevalence of *T. gondii* and *N. caninum* in the Muş region. Nonetheless, additional investigations would be advantageous in elucidating the diverse genotypes of *T. gondii* and their potential correlation with abortion and other reproductive challenges in the sheep population. This will make a substantial contribution towards a more thorough comprehension of the influence of these factors on pertinent animals.

Conflicts of interest

Authors state no conflict of interest.

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Molecular Characterization of *Dermanyssus gallinae* in Türkiye Based on 16S and 18S rDNA

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ABSTRACT

The poultry red mite, *Dermanyssus gallinae* (De Geer, 1778), is widely regarded as the significant ectoparasite of egg-laying hens worldwide. Since many molecular studies on poultry red mites have focused on analyzing COI and ITS1-2 genes, the present study aimed to identify 16S rDNA and the relatively understudied nuclear 18S rDNA genes of Turkish *D. gallinae* populations. Twenty-eight different *D. gallinae* populations were collected from henhouses throughout Türkiye, and the target genes were amplified using conventional PCR after morphological analysis. Haplotype analyses of the 16S rDNA sequences revealed 14 different haplotypes, with Turkish *D. gallinae* grouped into two of these haplotypes. The intra-species genetic variation of the 18S rDNA and 16S rDNA sequences examined in the present study and the available sequences in public GeneBank were determined as 0.17% and 0.53%, respectively. The obtained sequences belonging to *D. gallinae* from Türkiye were submitted to GenBank for the first time. Given the importance of identifying genetic diversity within and between species across different geographical regions, the obtained data may contribute substantially to the genetic knowledge of the PRMs.

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Introduction

The poultry red mite, PRM, *Dermanyssus gallinae* (De Geer, 1778) is a vital ectoparasite threatening to the poultry industry worldwide (Sparagano et al., 2014). The PRM is an obligate blood-feeding parasite and leads to a considerable reduction in the production of eggs (Sleeckx et al., 2019). The PRM can also transmit disease-causing pathogens to hens (De Luna et al., 2008). As a result of these direct and indirect adverse effects, welfare problems and significant economic losses (estimated to be 231 million euros in Europe) often occur in the egg-laying hen industry (Sigognault Flochlay et al., 2017). According to a recent review, 83% of European poultry houses are infested by *D. gallinae* (George et al., 2015). This prevalence is even higher, reaching 100% in Türkiye's farms (Koç and Nalbantoglu, 2021; Konyalı and Savaş, 2021).

Over the last several decades, various molecular markers have been employed in several Acari to explore a variety of purposes, including the definition of taxonomy, the understanding of population structure, the detection of the geographical origin, and even their ecological adaptation as well as food preferences (Hebert et al., 2003; Dong et al., 2021). Several nuclear- and mitochondrial-encoded genes have preferably been amplified as molecular markers so far (Dabert, 2006). The mitochondrial DNA (mtDNA) genes, on the other hand, have been frequently employed in phylogenetic studies due to a large number of copies of mtDNA, their maternal inheritance, haploid state, and rapid mutation rates, as well as the simplicity of designing primers to amplify conserved mitochondrial genes (Avise, 1987; Behura, 2006). In the case of PRM, the genetic diversity studies have targeted several genes, including the *COI* (Karp-Tatham et al., 2020; Roy et al., 2021), 16S rDNA (Roy et al., 2009; Roy et al., 2010), nuclear internal transcribed spacer regions (Chu et al. 2015; Brännström et al., 2008; Oines and Brännström, 2011; Roy and Buronfosse, 2011) Tropomyosin, and elongation factor 1-alpha (EF-1α) (Roy et al., 2010; Roy et al., 2021).

The present study aims to determine and compare the genetic variation of molecular markers, including a nuclear (18S rDNA) and a mitochondrial (16S rDNA) gene in 28 *D. gallinae* populations collected from different locations in Türkiye. In addition, the comparisons were further expanded using available sequences in the public GenBank. The results will contribute to mite phylogeny and population-genetic studies on *D. gallinae*. The sequences of 18S rDNA and 16S rDNA genes of Turkish *D. gallinae* were submitted to GenBank for the first time.

Materials and Methods

Mite samples

Twenty-eight populations of *D. gallinae* were sampled using a fine brush from different integrated laying hen farms in Türkiye during 2022-2023 (Table 1). The collected mites were transported to the laboratory in 70% and 90% ethanol for further morphological and molecular processing, respectively. The sampled mites were identified at the species level based on their morphology using a stereomicroscope (Stemi 2000-C, Zeiss, Germany) (Naegele, 1963; Di Palma et al., 2012).

Genomic DNA extraction

All mites were grouped, including ten mites according to the poultry houses to extract the genomic DNA (gDNA). Pooled mites were rinsed with sterilized water and dried on a filter paper then extracted using the "Qiagen DNeasy Blood and Tissue Kit" following the manufacturer's instructions. Last, the quality of gDNA was evaluated using a spectrophotometer (NanoDrop™ 2000, Thermo Scientific). The extracted gDNAs were kept at -20°C until PCR was performed.

Amplification of target genes and phylogenetic analysis

The primers, Rh16S-1 GCTCAATGATTTTTTAAAT TGCTG and Rh16S-2 CCGGTCTGAACTCAGATCATG were employed to amplify the 442 bp of 16S ribosomal DNA at an annealing temperature of 55°C (De Rojas et al., 2001). The primers, 18S_F ATATTGGAGG GCAAGTCTGG and 18S_R1 TGGCATCGTTTATG GTTAG were used to amplify the 500 bp of 18S rDNA at an annealing temperature of 50°C (Otto and Wilson, 2001).

Each PCR reaction was conducted in a final volume of 30 µL, consisting of 2 µL of mite gDNA (61-74 ng/µL), one µL of each primer (forward and reverse), 11 µL of PCR-grade water, and 15 µL of EmeraldAmp Max PCR Master Mix (which includes buffer, MgCl2, and dNTPs) (Takara, Japan). The PCR products were purified using the "HighPrep PCR clean-up system" (MagBio Genomics Inc.) and followed by sequencing at Macrogen Inc. (Amsterdam, Netherlands).

Sequencing chromatographs were initially checked using 'BioEdit v7.0.9.0' (Hall, 1999). The obtained sequences were then examined in the GenBank database using the BLAST analysis. Multiple sequence alignment was carried out using 'MAFFT v.7' with the 'Auto' strategy (Katoh et al., 2019) and further refined with 'Bioedit v.7.0.5' software (Hall, 1999). Intra- and interspecific genetic distances were assessed using 'MEGA X' (Kumar et al., 2018) with the 'Kimura-2 parameter' (K2P) model with 1000 bootstrap supports. Due to the lack of available sequences in NCBI, the genetic distance assessments of 18S rDNA were performed using the obtained sequences from Türkiye and the sequences of the published draft genome of *D. gallinae* from Scotland (Burgess et al., 2018; accession number: QVRM00000000) and a sequence submitted from the USA (Dowling and Oconnor, 2010; accession number: FJ911836.1). In addition, haplotype determinations of PRMs were utilized with 'DnaSP v.6' (Rozas et al., 2017). A Maximum likelihood (ML) phylogenetic tree based on 16S rDNA sequences belonging to *Dermanyssus* spp. was constructed with 'MEGA X' using the 'HKY+G model' (identified to be the best-fit model in 'MEGA') (Tamura, 1992) with 1000 bootstraps.

Results

According to morphological analysis, the females of *D. gallinae* were typically measured 0.8-1.5 mm in length and around 0.4 mm in width, male mites were significantly smaller at about 0.6 mm in length and 0.3 mm in width. Additionally, the second cheliceral segment in females was notably elongated, extending well beyond the basal segment. *D. gallinae* possessed a genito-ventral shield that is narrowly rounded at the posterior, with the anus located at the posterior aspect. Furthermore, there were two setae on both the anterodorsal and posterodorsal sides of Tibia 1, and one seta each on the anterolateral sides of Tibia 2, 3, and 4.

The fifty-six sequences were subsequently obtained from *D. gallinae* populations belonging to partial fragments of 18S and 16S rDNA genes. All sequences showed the best BLAST hit (>99.1% identity) with the PRM sequences found in the NCBI database. Obtained sequences were submitted to Public GenBank, and accession numbers are presented in Table 2. Haplotype analyses of the 16S rDNA sequences of Turkish PRMs identified 14 haplotypes, presented in Table 3.

Alignments and intra- and interspecific genetic distances (Table 2) of 18S rDNA were mainly performed using the genome of *D. gallinae* and a sequence submitted from the USA (accession number: FJ911836.1) because of the absence of the sequences in the GenBank. According to this, the intra-species genetic variation among 18S rDNA sequences was calculated to be 0.11%. The 16S rDNA sequences showed an average intra- and inter-genetic distance of 0.22% (min-max, 0-1.07) and 11.84% (min-max, 7.96-16.75) among Turkish *D. gallinae* populations (Table 2). Additionally, results demonstrated that 16S rDNA sequences of Turkish PRMs were clustered in two haplotypes in the phylogenetic tree (Figure 1).

Discussion

Although there is a range of molecular data available for COI, 16S, and ITS sequences of *D. gallinae* from various countries (Çiloğlu et al., 2020; Karp-Tatham et al., 2020; Roy et al., 2021; Koç et al., 2022), no sequence data belonging to 18S and 16S rDNA of Turkish *D. gallinae*, considering the significance of the identification of genetic diversity within and between species from various geographical locations, there was a gap in the literature for the sequences of poultry red mites.

Poultry red mites collected from 28 poultry houses were initially identified based on morphological characteristics. This identification involved assessing factors such as size, setae localization, the structure of chelicerae, and the genito-ventral shield, as described in Naegele (1963) and Di Palma et al. (2012).

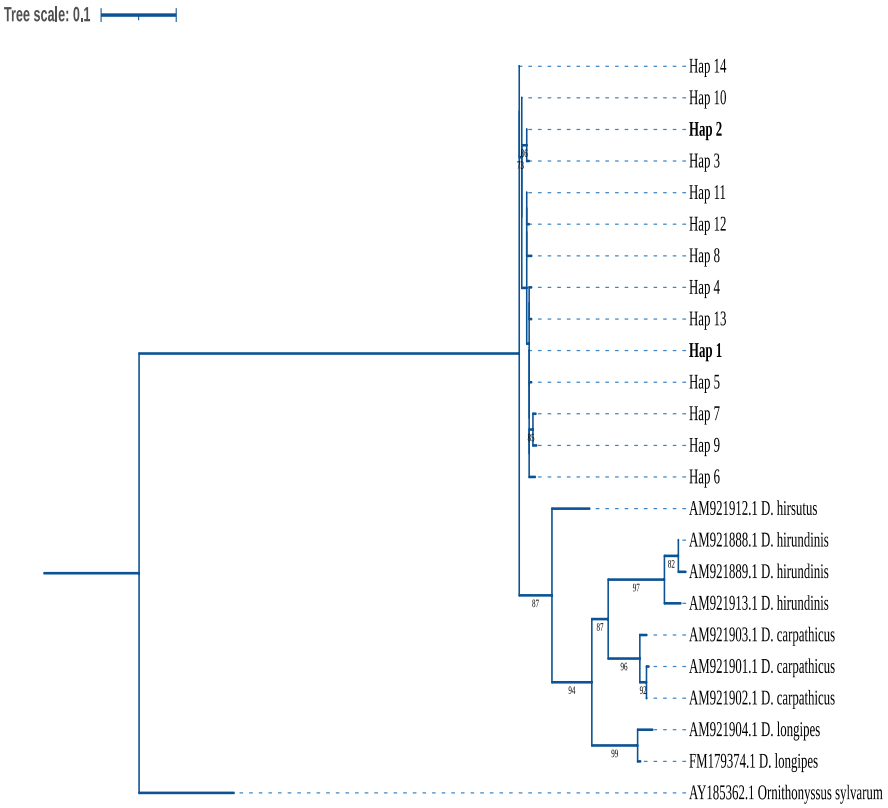

Figure 1. Phylogenetic tree of obtained *Dermanyssus gallinae* species based on 16S sequences belonging to the haplotypes available in GenBank. The sequences obtained in the present study are indicated in bold. Only the bootstrap values higher than 70% are shown. *Ornithonyssus sylviarum* sequence was used as an outgroup.

Table 1. Geographic origin, hen breeds, age of hens, and sampling dates of 28 *Dermanyssus gallinae* populations used in the present study

No	Location	Abbreviations	Hen breeds	Age of hens (weeks)	Collection date
1	Afyon	AFY1	Lohman brown	62	Sept 2022
2	Afyon	AFY2	Lohman brown	57	June 2023
3	Afyon	AFY3	Nick chick	55	June 2023
4	Ankara/Beypazarı	BYP1	Nick chick	73	August 2023
5	Ankara/Beypazarı	BYP2	Nick brown	82	August 2023
6	Ankara/Çubuk	CBK1	Nick chick	96	June 2023
7	Ankara/Çubuk	CBK2	Nick brown	89	August 2023
8	Ankara/Çubuk	CBK3	Lohman brown	64	Sept 2023
9	Ankara/Çubuk	CBK4	Nick brown	56	Sept 2023
10	Ankara/Elmadağ	ELM1	Nick brown	84	June 2023
11	Ankara/Elmadağ	ELM2	Lohman brown	98	June 2023
12	Ankara/Haymana	HYM	Nick chick	71	July 2023
13	Ankara/Gölbaşı	GLB	Lohman brown	70	July 2023
14	Ankara/Kalecik	KAL1	Lohman brown	64	Oct 2022
15	Ankara/Kalecik	KAL2	Lohman brown	65	May 2023
16	Ankara/Kalecik	KAL3	Lohman brown	68	Sept 2023
17	Ankara/Kalecik	KAL4	Nick brown	88	Sept 2023
18	Ankara/Kazan	KZN1	Nick brown	49	April 2023
19	Ankara/Kazan	KZN2	Nick brown	53	April 2023
20	Balıkesir	BAL1	Nick chick	68	August 2023
21	Balıkesir	BAL2	Nick brown	42	August 2023
22	Bayburt	BAY1	Lohman brown	56	May 2023
23	Bayburt	BAY2	Lohman brown	48	May 2023
24	Eskişehir	ESK1	Nick brown	45	Sept 2022
25	Eskişehir	ESK2	Lohman brown	56	Sept 2022
26	Konya	KNY1	Nick brown	71	June 2022
27	Konya	KNY2	Nick brown	96	June 2022
28	Uşak	USK	Lohman brown	62	May 2023

Table 2. Mean genetic distance (%) (mean, min-max) between *D. gallinae* from Türkiye and other countries, and other species in the same genus

Gene	Genetic distance				Nucleotide diversity within D. gallinae (π)	Accession numbers
	Between *D. gallinae* populations			Within the genus *Dermanyssus*		
	From Türkiye	From Türkiye and other countries	Other species within the genus *Dermanyssus*			
18S	0.11 (0-0.70)	0.17 (0-0.69)	0.87 (0.46-1.64)	0.21 (0-0.70)	0.00117	OR960601-OR960628
16S	0.22 (0-1.07)	0.53 (0-1.90)	11.84 (7.96-16.75)	1.77 (0-17.28)	0.00614	OR960571-OR960598

Table 3. Haplotypes of *Dermanyssus gallinae* populations based on 16S sequences

Haplotypes	n	Populations
Hap_1	43	AFY1, AFY2, AFY3, CBK1, CBK2, CBK3, CBK4, ELM1, ELM2, HYM, GLB, KAL1, KAL2, KAL3, KAL4, KZN1, KZN2, BAL1, BAL2, BAY1, BAY2, KNY1, KNY2, USK, FM207492, AM921890, AM921887, AM921884, LC029621, LC029622, LC029675, LC029677, LC029678, LC029679, LC029680, LC029681, LC029697, LC029698, LC029699, LC029706, LC029754, LC029755, LC029797
Hap_2	34	BYP1, BYP2, ESK1, ESK2, AM921914, AM921883, LC029566, LC029570, LC029592, LC029599, LC029625, LC029644, LC029687, LC029696, LC029711, LC029725, LC029730, LC029731, LC029732, LC029735, LC029737, LC029741, LC029749, LC029751, LC029752, LC029753, LC029759, LC029760, LC029766, LC029774, LC029776, LC029783, LC029792, LC029793
Hap_3	2	L34326, LC029798
Hap_4	1	FM207494
Hap_5	1	FM207493
Hap_6	1	AM921911
Hap_7	1	AM921910
Hap_8	1	AM921885
Hap_9	1	AM921886
Hap_10	43	LC029560, LC029565, LC029571, LC029575, LC029584, LC029585, LC029590, LC029591, LC029603, LC029606, LC029614, LC029617, LC029623, LC029626, LC029654, LC029661, LC029684, LC029688, LC029689, LC029693, LC029694, LC029707, LC029708, LC029713, LC029715, LC029716, LC029718, LC029719, LC029726, LC029728, LC029736, LC029740, LC029747, LC029748, LC029750, LC029757, LC029762, LC029768, LC029775, LC029779, LC029782, LC029789, LC029795
Hap_11	143	LC029561- LC029563, LC029567- LC029569, LC029572- LC029574, LC029576- LC029583, LC029586- LC029589, LC029593- LC029598, LC029600- LC029602, LC029604, LC029605, LC029607, LC029609- LC029613, LC029615, LC029616, LC029618- LC029620, LC029624, LC029627- LC029643, LC029645- LC029653- LC029660, LC029662- LC029667, LC029669- LC029674, LC029676, LC029682, LC029683, LC029686, LC029690- LC029692, LC029695, LC029700- LC029702, LC029704, LC029709, LC029710, LC029712, LC029714, LC029717, LC029720- LC029724, LC029727, LC029729, LC029733, LC029734, LC029738, LC029739, LC029742, LC029743, LC029745, LC029756, LC029758, LC029761, LC029763- LC029765, LC029769- LC029773, LC029777, LC029778, LC029780, LC029781, LC029784- LC029788, LC029790, LC029791, LC029794, LC029796
Hap_12	7	LC029564, LC029608, LC029668, LC029685, LC029703, LC029744, LC029746
Hap_13	1	LC029705
Hap_14	1	LC029767

Nuclear ribosomal DNA still provides one of the most complete tools for many molecular tasks. Among nuclear gene/gene regions, 18S rDNA, ITS1, and ITS2 have proved helpful in phylogenetic classification (Doolittle, 1999; Hebert et al., 2003). However, the evolutionary rates of the nuclear ribosomal genes are lower; therefore, they have used as molecular markers for phylogenies at higher taxonomic levels (Eickbush and Eickbush, 2007). In the current study, intra-species genetic variation among 18S rDNA sequences was calculated to be 0.11%. This distance indicates only a slight differentiation between populations in Türkiye from various geographical origins. The low difference is even revealed in the interspecific variance of 0.87% which is with the results of Dowling and Oconnor (2010) in superfamilies, Dermanyssoidea. Therefore, species-level identification should be avoided due to the low inter-specific distance of 18S rDNA sequences, or a combination of an additional marker should be favored.

The mitochondrial genome also contains two ribosomal RNA (rRNA) genes, including 12S and 16S rRNA. 16S rRNA is a small ribosomal RNA subunit responsible for the translation of genetic codes to functional cell components in all organisms (Woese and Fox, 1977). 16S rRNA region has been used for many years as a valuable tool to infer phylogenetic relationships for distantly related taxa (Dong et al., 2021). Due to its species-specific characteristics, it has been mainly employed in determining phylogenetic relationships between bacteria (Woese, 1987) and also popularly in ticks (Navajas and Fenton 2000). It was also included in several phylogenetic studies on PRM (Roy et al., 2010, Roy et al., 2009; Chu et al., 2015), and intraspecific variation was determined between 0-4% (Roy et al., 2010). Regarding the 16S rRNA results in the current study, the intra- and inter-genetic distances were determined consistent with Roy et al. (2010). Although low divergences were detected within species, the 16S rRNA gene could be particularly informative at the interspecific levels, as documented before. Supportingly, the phylogenetic tree shows a good clustering pattern at the species level (Figure 1).

In general, the molecular characterization of 18S rDNA and 16S rDNA of *D. gallinae* sampled from poultry houses in Türkiye were performed. The sequence data that was obtained was submitted to the NCBI database. This study may significantly contribute to the genetic data of poultry red mites regionally and globally. Still, additional sequences are required to elucidate the genetic diversity in PRMs fully.

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Ethical approval

Not applicable

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Comparison of Different Extraction Methods on the Recovery Efficiencies of Valuable Components from Orange Peels

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ABSTRACT

Supercritical-CO_2 extraction, Soxhlet extraction, and ultrasound-assisted extraction methods were conducted in this study to recover valuable components, specifically phenolic antioxidant compounds, from orange peels. Basic operating parameters such as temperature and pressure, which affect the extraction efficiency of phenolic substances in orange peel with supercritical-CO_2, were designed using the central composite design methodology. In the Soxhlet and ultrasound-assisted extraction methods, 2-hour extraction processes were carried out using ethanol at different concentrations (50%, 80% and 100%) as a solvent. Yield comparison was made by performing total phenolic content, antioxidant activity and total flavonoid content analyses in the extracts. The total phenolic content (TPC) in the extracts was determined to be 5034 mg GAE/L for supercritical-CO_2 extraction at 61.5°C and 20 MPa. In comparison, Soxhlet extraction yielded a TPC of 1728 mg GAE/L, while the ultrasound-assisted extraction method resulted in a TPC of 4056 mg GAE/L. It was determined that the optimum operating parameters of supercritical-CO_2 extraction were 60°C and 26.4 MPa in case all the responses were maximized. The best phenolic recovery was obtained at 100% ethanol in Soxhlet extraction and 80% ethanol in ultrasound-assisted extraction. Although supercritical-CO_2 extraction is an environmentally friendly application, the recovery rate of valuable components from raw materials is lower than in Soxhlet extraction and ultrasound-assisted extraction. However, since the volume of the extracts obtained from the supercritical-CO_2 extraction is small, the ratio of phenolic compounds is higher.

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Introduction

Citrus fruits are a natural source of many critical bioactive compounds for humans, such as ascorbic acid, flavonoids, phenolic compounds, pectins, and antioxidant substances (Fernández-López et al., 2005). Studies on flavonoids found in citrus fruits show that they minimize the risk of developing heart disease (Zayed et al., 2021). It has also been reported to produce antibodies that fight carcinogenic cells by strengthening the immune system due to its antioxidant properties (Elangovan et al., 1994; Javanmardi et al., 2003).

Valuable components are present in the edible parts of citrus fruits and the inedible peel, which make up almost half of the fruit mass. The peel is part of citrus fruits, containing nearly the highest flavonoid concentrations (Anagnostopoulou et al., 2006). These components have many biological effects such as antibacterial, antiviral, anti-inflammatory, anti-allergic and antithrombotic (Cook and Samman, 1996).

Although citrus fruits are used in many sectors (such as fruit juice, puree, frozen pulp (fruit pulp), fermented beverages, gels, candies, and ice cream), the most industrial use is fruit juice processing. Compared to other uses, it is estimated that 50-60% is used in the fruit juice processing industry (Satari and Karimi, 2018; Zema et al., 2018). The fruit and vegetable processing industry generates significant by-product waste, accounting for approximately 25 to 30% of the commodity group (Sagar et al., 2018). These wastes can be used in the production of dietary fiber (Pathania and Kaur, 2022), enzymes, ethanol, biocolors (Sharma et al., 2016) and adsorbents (Shrivastava and Singh, 2022), or they can be utilized direct land spreading and composting (Zema et al., 2018). It is possible to use these wastes as animal feed (Panwar et al., 2021) and as raw material in biofuel production through biorefinery (Yadav et al., 2022). The fruit juice processing plant wastes are segment membranes, peels (albedo,

flavedo), pulp, and seeds (Zema et al., 2018; Suri et al., 2022). The pulp of orange fruit consists of 60-65% peel, 30-35% slices, 0-10% seeds, dice, juice sacs, and axis pieces on a dry basis. In the production of orange juice, waste is generated in the amount of 0.5 kg/kg of raw oranges. Citrus wastes have a high organic matter content and a low pH value, including valuable components that can be recycled (Alvarez et al., 2018; Zema et al., 2018; Bozkir et al., 2021).

Recycling methods of valuable compounds from plant wastes can be classified into two main categories: conventional and novel techniques. The conventional methods of steam distillation, hydro distillation, or Soxhlet extraction are often used to recover valuable components from waste. However, these techniques may not be suitable for sensitive compounds that may be lost or degraded at high temperatures (Sagar et al., 2018; Phong et al., 2022). Due to the increasing energy prices and the need to reduce environmental impacts, the infrastructure for the necessary experiments to recover valuable components from citrus wastes can be established using extraction techniques that require lower energy needs and minimize environmental problems. Examples of these extraction methods are supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), microwave accelerated distillation (MAD), microwave steam distillation (MSD), microwave hydro diffusion and gravity (MHY), and sonication-assisted extraction (SAE) (Negro et al., 2016). Supercritical-CO_2 (SC-CO_2) extraction is one of the most suitable methods for extracting valuable oils and some organic compounds from plants. Because considering the operating conditions of the process, the critical temperature and pressure of the solvent used must not affect the structure of the extract. CO_2 used as a solvent is non-toxic, inexpensive, non-flammable, and chemically stable. Thanks to its operating conditions, SC-CO_2 exhibits high diffusivity (similar to gases) and high solvent power (similar to liquids), allowing a higher mass transfer and extraction rate. In addition to these advantages, it can be said that low solvent consumption and no residue are left (Mira et al., 1999; Atti-Santos et al., 2005). Compared to other extraction methods, the Soxhlet extraction (SE) method has the most significant disadvantage: the amount of solvent used is high and causes health/environmental problems. The ultrasonic extraction method is based on increasing the interaction between solvent and solute through sound waves. Ultrasonic waves create compression and expansion cycles in the medium due to their movement. During the expansion cycle, the molecules separate and form bubbles that absorb energy and begin to increase in size, whereas during the compression cycle, the molecules come together due to the increase in pressure and temperature at the microscale collapse (Rao and Rathod, 2015). Compared to the Soxhlet extraction method, the ultrasound-assisted extraction (UAE) method can increase extraction efficiency using less solvent (Rathod et al., 2017).

In this study, SC-CO_2 extraction was optimized to recover valuable components from orange peel. Additionally, the efficiencies of SC-CO_2 extraction, SE and UAE methods were compared.

Material and Methods

Materials

Orange peel (albedo and flavedo) was obtained from Mersin/Türkiye and was used in extraction studies. SC-CO_2 extraction, 99.9% pure CO_2 was used as the supercritical fluid (Ar-Oksijen, Konya, Türkiye), and ethanol (Merc, Germany) was used as the solvent in the experiments carried out by Soxhlet extraction and ultrasonic extraction method.

Methods

Preparation of the samples

The citrus peels used in the experiments were dried in an oven at 75 °C for 12 hours. Argun et al. (2023) found that the decomposition of phenolics was not significantly affected at 70 °C unless exposed to sunlight. The dried samples were ground into powder in the GRT-10BL laboratory grinding mill (Akyol, Türkiye). The prepared samples were stored in the refrigerator at +4 °C until use.

Extraction methods

Supercritical CO_2 extraction

SC-CO_2 extractor has a 500 mL column in which temperature and pressure are controlled (Superex F-500; Figure 1). The pressure can be adjusted up to 35 MPa and the temperature up to 70 °C. The desired temperature and pressure values were adjusted, and then the sample (50 g) was placed in the extractor with the help of a cloth. After reaching the set operating conditions, the device was kept in a static state for 20 minutes, and then it was brought to a dynamic state with a carbon dioxide flow rate of 2 ± 0.3 mL/min for 100 minutes.

CO_2 was separated from the extracts spontaneously by reducing the pressure at the extractor outlet and the extracts were collected in a 50 mL falcon tube. The orange peel extracts were coded as PKE (Figure 2).

Figure 1. Photograph of the supercritical carbon dioxide extraction system

Figure 2. Photos of the orange peel extracts (PKE) obtained by SC-CO_2 extraction.

Table 1. Independent variables and working ranges used in SC-CO_2 extraction.

Variables	Working conditions		
	Minimum	Average	Maximum
Pressure (MPa)	8.5	20	31.5
Temperature (°C)	38.5	50	61.5

Table 2. Experimental conditions used in the extraction of orange peel samples with SC-CO_2.

Experiment Code	Working conditions			Extraction time and mode
	Temperature (°C)	Pressure (MPa)	CO_2 consumed (kg)	
PKE-1	38.5	20	0.5	20 min static, 100 min dynamic
PKE-2	40	10	0.4	
PKE-3	40	30	0.4	
PKE-4	50	20	0.5	
PKE-5	50	20	0.5	
PKE-6	50	31.5	0.6	
PKE-7	50	8.5	0.3	
PKE-8	50	20	0.5	
PKE-9	60	30	0.6	
PKE-10	60	10	0.4	
PKE-11	61.5	20	0.5	

Response surface methodology (RSM) was used in the experimental planning. RSM consists of a group of mathematical and statistical techniques that are based on the fit of empirical models to experimental data obtained in relation to experimental design. In applying RSM as an optimization technique, linear or square polynomial functions are used (Bezerra et al., 2008). The central composite design method was used to evaluate the effect of independent variables (pressure and temperature) on dependent variables: volumetric recovery, mass recovery, TPC, TPC recovery, DPPH, and ABTS. Experimental working ranges of the independent variables and specific conditions are summarized in Table 1 and Table 2.

Ultrasound-assisted extraction (UAE)

Ultrasound-assisted extraction was performed in a high-frequency ultrasonic bath (Kudos, China). In the study, 1 g of dried and ground orange peel samples was taken, and 10 mL of a solvent mixture with ethanol: water ratio of 50%, 80%, and 100%, respectively, was added. The extraction process was carried out at a frequency of 53 kHz, a power of 100 W, temperatures between 20-26 °C and for 120 minutes.

Soxhlet extraction (SE)

Ethanol was used as an organic solvent for Soxhlet extraction. 10 g of ground orange peel sample was placed in extraction cartridges. Ethanol/water ratios were determined as 50%, 80% and 100%. 100 ml of ethanol solution was taken into 200 ml flat-bottomed flasks, of which the empty weight was taken and placed in the apparatus. The Soxhlet extraction apparatus was operated on the heater for 2 hours under a fume hood. At the end of the extraction period, the solution collected in the balloon was evaporated at 65 °C under a fume hood using a Buchi R 100 brand rotary evaporator (Buchi, Germany). The amount of extract obtained at the end of this process was calculated gravimetrically.

Determination of total phenolic content (TPC)

TPC analysis of the extracts was performed using the Folin-Ciocalteu reagent method (Singleton et al., 1999). 20 µL of the sample or diluted sample was taken into 15 mL flasks, and 1580 µL of methanol/water mixture was added to it, and 1600 µL of methanol/water solution was taken for the blank. 100 µL of 2N Folin–Ciocalteu reagent (FCR) was placed on them, and they waited for 5 minutes. Then, 300 µL of sodium carbonate (20%, w/v) was added and mixed. The mixtures were waited for 30 minutes at 45 °C in darkness for color change. At the end of the incubation, the samples were transferred to 2 mL falcon tubes and were centrifuged at 4000 rpm for 5 minutes. Finally, absorbance values were read at 765 nm wavelength in a spectrophotometer (Hach Lange DR-5000). The calibration curve was prepared using standard gallic acid to calculate TPC ($y = 0.0441x$, R^2: 0.994). TPC was expressed as mg gallic acid equivalent (GAE)/L extract.

Determination of total flavonoid content (TFC)

TFC analysis of the extracts was performed according to Zhishen et al. (1999). 250 µL of the samples (250 µL of ethanol for the blank) was taken and placed in 15 mL falcon tubes. 1250 µL of pure water was added to it. 75 µL of 5% $NaNO_2$ solution was added to the mixture, mixed, and waited 6 minutes. At the end of the waiting period, 150 µL of 10% $AlCl_3$ solution was added, mixed, and waited 5 minutes. Finally, 500 µL of 1 M NaOH solution was added, and the total volume was completed to 2500 µL with 275 µL of pure water. A light orange color was observed in the prepared samples. The absorbance values of the prepared samples were read at 510 nm wavelength in a spectrophotometer (Hach Lange DR-5000). The calibration curve was prepared by using standard quercetin to TFC calculation ($y = 0.0009x$, R^2: 0.9868). TFC was expressed as mg quercetin equivalent (QE)/g extract.

Determination of DPPH$^{\bullet}$ scavenging capacity

The free radical scavenging capacity of the samples was measured according to Rai et al. (2006). 1900 µL of DPPH solution was taken into falcon tubes with a volume of 2000 µL, and 100 µL of the sample was added and shaken. Prepared samples were kept in the dark for 30 min. At the end of the time, the absorbance values of the samples were read at 517 nm wavelength. The calibration curve was prepared using the Trolox standard and expressed as µM TE.

Determination of ABTS$^{\bullet+}$ scavenging capacity

The radical cation scavenging capacity of the samples was determined according to Re et al. (1999). At room temperature, a seven mM ABTS$^{\bullet+}$ stock solution containing 2.45 mM potassium persulfate was prepared and kept in the dark for 12-16 hours. The ABTS$^{\bullet+}$ working solution was prepared by diluting the stock ABTS$^{\bullet+}$ solution with a 1:1 water:ethanol (v/v) mixture such that the absorbance of the total mixture was 0.70 ± 0.02 at 734 nm. 1000 µL of ABTS$^{\bullet+}$ solution was taken into 2000 µL falcon tubes, and 10 µL of the sample was added to it. The lids of the falcon tubes were closed, mixed with vortex, and waited for 6 minutes. At the end of the waiting period, the absorbance values of the samples were read by adjusting the spectrophotometer to a wavelength of 734 nm. The calibration curve was prepared using the Trolox standard and expressed as µM TE.

Calculation of recovery yields

The extraction yields were calculated concerning extract volume (%, v/w) and extracted mass (%, w/w) according to Equation (1):

$$Yield\ (\%) = \frac{X_{ext} \times 100}{M_{OP}} \quad (1)$$

where X is the volume (mL) or mass (g) of the extract and M_{OP} is the mass of the orange peel (OP) (g).

The TPC and TFC recoveries (%, w/w) were calculated considering the mass balance according to Equation (2):

$$\%R_{TPC,TFC} = \frac{C_{ext} \times M_{ext}}{C_{OP} \times M_{OP}} \times 100 \quad (2)$$

Where C is the concentration of the individual TPC and TFC in a particular matrix expressed in mg/g DW, and M is the mass of the extract and OP (g).

Statistical analysis

The significance of the statistical relationship between the independent variables and the results was evaluated according to the ANOVA results. The design of the experimental conditions and the ANOVA tests of the results were carried out with the Minitab 18 software. The obtained statistical model was analyzed at a 95% confidence interval (P <0.05).

Results and Discussion

Recovery studies with SC-CO_2 extraction

The appearance of the extracts obtained at higher pressures was dark orange, indicating more TFC in the extracts (Figure 2). These differences in color tones are also confirmed by TFC recovery efficiencies (Figure 3). It was observed that TFC is more enriched than TPC in the extracts. This richness indicates that SC-CO_2 dissolves more apolar flavonoids than other phenolics. The extraction yields for volumetric recovery and mass recovery were increased at lower temperatures and pressures, while TPC values were increased at higher pressures (Figure 3). It was determined that the volumetric recovery values of the extraction process varied between 4-8% and the mass recovery values between 2-5%. The highest recovery efficiencies in volume and mass were obtained for 38.5°C, 20 MPa, and 40°C, 10 MPa conditions.

It was determined that the TPC values of the extracts ranged between 1678-5034 mg GAE/L extract (R_{TPC}: 0.8-2.5%), and the TFC values ranged between 25-43 mg QE/g extract (R_{TFC}: 9-20%) (Figure 3 and Table 3). The ABTS$^{\bullet+}$ scavenging activity of the extracts was found to be 1708-16107 µM TE, and the DPPH$^{\bullet}$ scavenging activity was seen as 2423-7602 µM TE (Table 3).

ANOVA data showing the effects of experimental conditions on the responses are given in Table 4. It was observed that the effects of temperature on the extract yield and the effects of pressure on the TPC, TPC recovery, DPPH• scavenging activity, and ABTS•+ scavenging activity recovery values were significant (P<0.05). Argun et al. (2022) determined that increasing the temperature and pressure increased the extract yield and phenolic substance recovery from orange processing wastewater. Espinosa-Pardo et al. (2017) reported that the extract yield and TPC content increased in SC-CO_2 extraction of phenolic compounds from processed pulp from orange juice with increasing pressure.

Optimization of SC-CO_2 extraction according to the response surface methodology (RSM) is presented in Table 5. According to the central composite design, the optimum extraction conditions to maximize volumetric recovery, mass recovery, TPC, TPC recovery, DPPH$^{\bullet}$ scavenging activity, and ABTS$^{\bullet+}$ scavenging activity values of the extracts were determined as 22 MPa and 40°C.

Table 3. Valuable components variation at the different experimental conditions for SC-CO_2 extraction.

	TPC (mg GAE/L extract)	TPC recovery (%)	TFC (mg QE/g extract)	TFC recovery (%)	ABTS$^{\bullet+}$ scavenging activity (µM TE)	DPPH$^{\bullet}$ scavenging activity (µM TE)
PKE-1	4717	2.46	34.6	19.8	4791	5448
PKE-2	2880	1.72	29.9	18.6	1708	4926
PKE-3	3696	1.65	42.9	18.5	11245	6130
PKE-4	2827	1.78	38.2	19.6	16107	6550
PKE-5	2268	1.38	34.4	18.8	9066	7254
PKE-6	4263	1.27	36.1	13.1	13006	6947
PKE-7	1678	0.75	38.0	17.0	2947	4845
PKE-8	2222	1.16	33.6	17.7	9122	7602
PKE-9	4558	2.04	38.0	17.3	12168	6237
PKE-10	3356	1.00	25.3	9.1	1760	2423
PKE-11	5034	2.25	39.7	17.9	8144	6492

TPC: Total phenolic content; TFC: Total flavonoid content; GAE: Gallic acid equivalent; QE: Quercetin equivalent; TE: Trolox equivalents

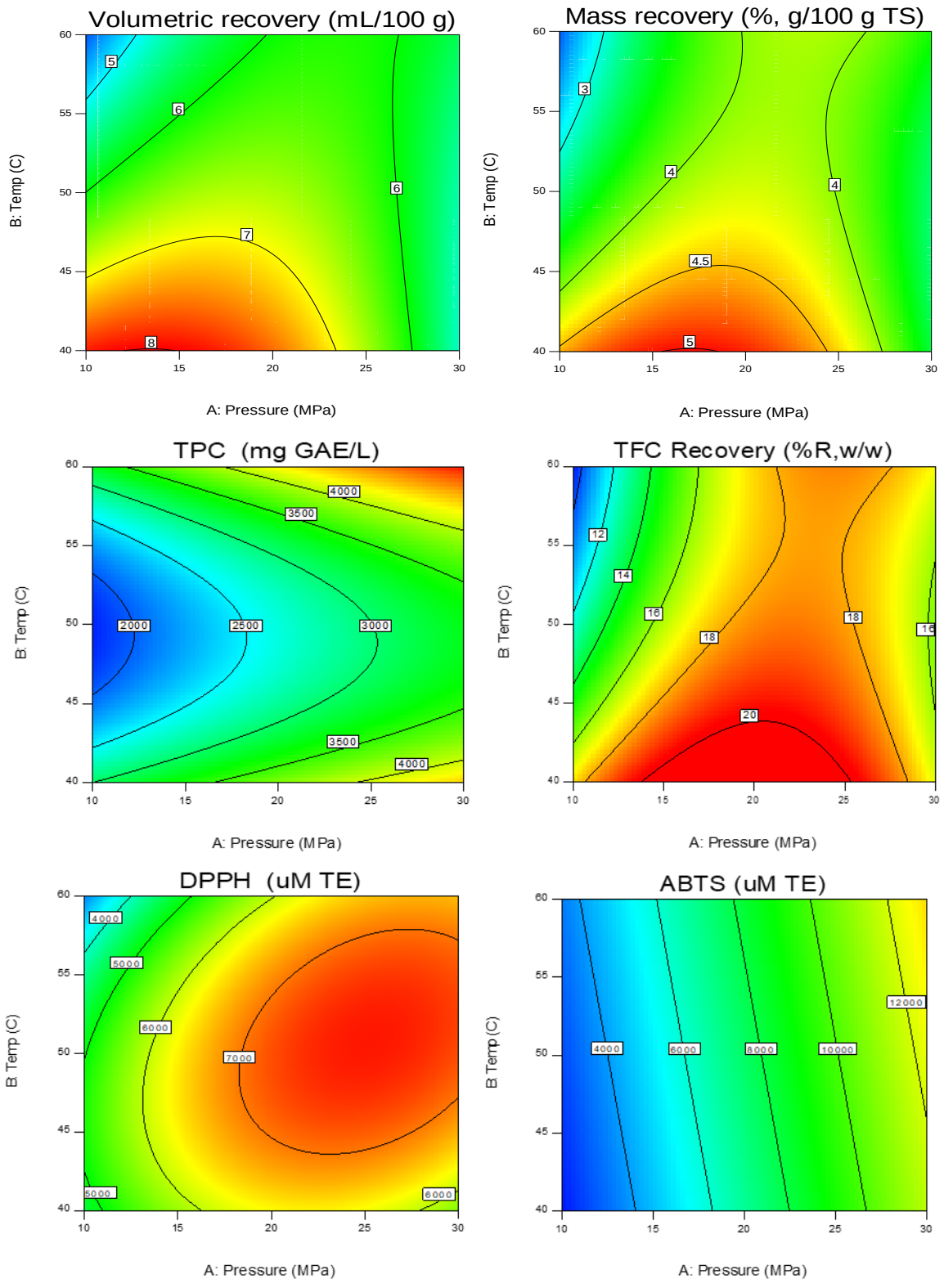

Figure 3. Dual effects of pressure and temperatures on the extraction yields and quality parameters of the extracts

However, it was determined that some values were higher at the 26 MPa and 60°C, and there was no significant difference between the two optimization solutions. Espinosa-Pardo et al. (2017) emphasized that the highest TPC content obtained using SC-CO_2 extraction from orange peel was at 40 °C and 350 bar. Still, there was no significant difference between the study performed at 60 °C and 250 bar. Based on these results, it can be concluded that temperature effects solvent density and selectivity in obtaining total phenolics. Argun et al. (2022) determined the optimum extraction conditions to maximize the mass recovery, TPC, TPC Recovery, and antioxidant capacity values of the extracts obtained from orange processing wastewater as 28.7 MPa and 60°C. Santos et al. (2019) obtained a TPC value of 23 mg GAE/g under 55°C and 30 MPa pressure (optimized parameters) in their extraction using SC-CO_2 extraction from feijoa peel.

Table 4. Statistical relevance between experimental conditions and obtained results for SC-CO_2 extraction.

Source	Volumetric recovery (mL/100 g)		Mass recovery (%, g/100 g)		TPC mg GAE/L		TPC recovery (%, w/w)	
	F	P	F	P	F	P	F	P
Model	Quadratic		Quadratic		Quadratic		Quadratic	
	4.8	0.06	4.1	0.07	4.9	0.05	8.4	0.018*
Pressure	1.5	0.27	5.10^{-3}	0.95	8.7	0.03*	5.9	0.059
Temperature	7.8	0.04*	4.7	0.08	1.0	0.36	0.8	0.42
Lack of Fit	9.7	0.09	31.9	0.03	5.7	0.15	0.2	0.91
Std. Dev.	0.72		0.55		656		0.25	
Mean	6.14		3.80		3409		1.57	
C.V. %	11.75		14.44		19.25		15.99	
R-Squared	0.83		0.81		0.83		0.89	
Adeq Precision	6.67		6.49		6.92		9.26	
Source	TFC mg QE/g		TFC recovery (%, w/w)		DPPH$^{\bullet}$ scavenging activity µM TE		$ABTS^{\bullet+}$ scavenging activity µM TE	
	F	P	F	P	F	P	F	P
Model	Linear		Quadratic		Quadratic		Linear	
	1.3	0.32	8.8	0.02*	6.9	0.03*	5.4	0.03*
Pressure	2.6	0.15	6.5	0.05	15.5	0.01*	10.6	0.01*
Temperature	0.1	0.81	8.2	0.04*	0.4	0.55	0.3	0.63
Lack of Fit	0.5	0.79	4.4	0.19	2.4	2.44	0.1	0.99
Std. Dev.	5.69		1.74		732		3755	
Mean	36.29		16.45		5896		7558	
C.V. %	15.69		10.56		12.41		49.68	
R-Squared	0.25		0.90		0.87		0.58	
Adeq Precision	2.75		9.89		7.64		5.58	

TPC: Total phenolic content; TFC: Total flavonoid content; GAE: Gallic acid equivalent; QE: Quercetin equivalent; TE: Trolox equivalents; *: P <0.05 level of significance.

Table 5. Optimum supercritical condition and results for orange peel extraction by using for pre-determined goals.

Variables		Goal	Range of experimental value		I	Solution 1 (Desirability: 0.74)	Solution 2 (Desirability: 0.73)
			Lower limit	Upper limit			
Experimental variables	A: Pressure (MPa)	in range	10	30	3	22.1	26.4
	B: Temperature (°C)	in range	40	60	3	40.0	60.0
Results	Volumetric recovery (mL/100 g)	maximize	4.00	8.00	3	7.25	6.03
	Mass recovery (%, g/100 g TS)	maximize	2.17	5.05	3	4.78	4.01
	TPC (mg GAE/L)	maximize	1678	5034	3	3876	4761
	TPC recovery (%, w/w)	maximize	0.75	2.50	3	2.12	2.14
	DPPH (µM TE)	maximize	2423	7603	3	6377	6643
	ABTS (µM TE)	maximize	1708	16107	3	7814	11300
	TFC recovery (%, w/w)	maximize	9.1	19.82	3	21.2	18.28

I: Importance; TS: Total solids; TPC: Total phenolic content; TFC: Total flavonoid content; GAE: Gallic acid equivalent; TE: Trolox equivalents.

Table 6. Comparison of the extraction yield, TPC and antioxidant activity values of the studied extraction methods.

Extraction type	Extraction conditions	Solvent /sample	Yield, %	TPC, mg GAE/g*	$\%R_{TPC}$	TFC, mg QE/g	DPPH, µmol TE/g	ABTS, µmol TE/g
Soxhlet extraction	50% ethanol	20	23	22.5^{abc}	28.5^{a}	0.6^{a}	12.9^{e}	33.8^{e}
	80% ethanol		12	33.9^{bc}	42.9^{ab}	5.8^{a}	15.1^{f}	41.5^{f}
	100% ethanol		49	34.6^{c}	45.7^{ab}	10.5^{ab}	13.3^{e}	33.9^{e}
Ultrasound-assisted extraction	50% ethanol	10	-	36.8^{c}	46.7^{ab}	1.2^{a}	6.6^{a}	20.8^{d}
	80% ethanol		-	42.3^{c}	98.2^{b}	13.0^{ab}	7.6^{b}	21.3^{d}
	100% ethanol		-	30.3^{abc}	55.2^{ab}	14.2^{abc}	7.7^{b}	21.5^{d}
SC-CO_2 extraction	38.5°C, 20 MPa	~10	4.6	7.1^{a}	2.5^{a}	34.6^{bcd}	8.2^{c}	7.2^{a}
	50°C, 20 MPa		4.4	4.7^{a}	1.4^{a}	38.2^{cd}	10.7^{d}	15.2^{c}
	61.5°C, 20 MPa		3.7	8.3^{ab}	2.3^{a}	39.7^{d}	10.6^{d}	13.4^{b}

TPC: Total phenolic content; TFC: Total flavonoid content; GAE: Gallic acid equivalent; QE: Quercetin equivalent; TE: Trolox equivalents; *: mg GAE/g extract for SC-CO_2 extraction, mg GAE/g dry peel for SE and UAE.; Mean values expressed with different letters in the same column are significantly different (P< 0.05).

Recovery studies with Soxhlet extraction (SE)

Extraction yield, TPC, DPPH$^{\bullet}$ scavenging activity, and ABTS$^{\bullet+}$ scavenging activity values of the extracts obtained by SE using 50% (v/v), 80% (v/v), and 100% (v/v) ethanol solutions from ground orange peel are given in Table 6. It was observed that the obtained extract yields changed significantly depending on the change in ethanol ratios, and the highest value was reached for 100% ethanol. Likewise, it can be said that TPC values get the best values in 100% ethanol. DPPH$^{\bullet}$ scavenging activity and ABTS$^{\bullet+}$ scavenging activity values were found to be higher at 80% ethanol. It is seen that the amount of antioxidant substance recovery from orange peels is higher than the antioxidant activity values of the extracts obtained by the SC-CO_2 method. The high extraction efficiency obtained by the Soxhlet method may be because ethanol dissolves the polar phenolic compounds better than SC-CO_2 (Azwanida, 2015).

Soxhlet extraction is a continuous process compared to percolation and maceration methods and is advantageous because it is easy, requires less time, and is less solvent (Azwanida, 2015; Alara et al., 2021). Alias and Abbas (2017) found the TPC values as 28.78 mg GAE/mg DW and 207.72 mg GAE/mg DW in the extracts they obtained from pineapple peels using SE and Microwave Assisted Extraction (MAE) methods, respectively.

Recovery studies with ultrasound-assisted extraction (UAE)

TPC and antioxidant activity values of extracts obtained from orange peels with UAE (53 kHz) at different alcohol ratios are summarized in Table 6. While the TPC value was higher at 80% ethanol, the antioxidant activity values were a little higher at 100% ethanol, which could be partly due to higher TFC concentration. The amount of flavonoids with higher antioxidant activity increases in the extracts because of high ethanol concentration. Rodrigues et al. (2015) observed the positive linear effect of ethanol concentration on the extraction of monomeric anthocyanin and cyanidin-3-O-glucoside from jabuticaba peel. It was concluded that UAE obtained maximum extraction efficiencies of TPC with the range of 47%-98%. Odabaş and Koca (2016) reported that higher extraction time (45 min) and medium ethanol concentration (approximately 67%) for UAE application resulted in increased extraction of total phenolic compounds. Some researchers reported that optimum TPC concentration could be obtained by using ethanol concentration near 70% in maceration, like our findings (Nepote et al., 2005; Vongsak et al., 2013). In their study on the extraction of bioactive components from lemon peels, Jagannath and Biradar (2019) found the TPC and TFC values to be 7.17 mg GAE/100 g and 4.52 mg CE/100 g, respectively, with the UAE method under optimum conditions. Their study stated that the UAE method was better than Soxhlet in the extraction of total phenolics and flavonoids, retention of vitamin C, and antioxidant activity.

Comparison of different extraction methods

TPC concentration of the SC-CO_2 extracts was a maximum of 8.3 mg GAE/g which is lower than Soxhlet and ultrasound-assisted extraction. However, the concentration value of the extracts (if calculated as mg GAE/L extract) obtained by SC-CO_2 extraction reached up to 5034 mg GAE/L while the TPC concentrations in the Soxhlet and UAE extracts were a maximum of 1728 mg GAE/L and 4056 mg GAE/L, respectively. This situation may be due to the dilution of phenolics in the increasing extract volume because of the higher yield of methods other than SC-CO_2 extraction. The DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ scavenging activity values of the extracts obtained by the SE method were the highest. The highest %R_{TPC} value (98.2%) was reached in UAE using 80% ethanol (Table 6). It is reported that UAE is a more economical extraction method than Soxhlet, provides higher extraction efficiency and requires less extraction time (Ciğeroğlu et al., 2018). By using a co-solvent, SFE can be made an effective technique for the extraction of essential oils and polar compounds, although the performance of UAE is better. Extraction performance can be improved by combining or integrating two different extraction techniques. Combining ultrasound with SFE increases extraction efficiency (Osorio-Tobón, 2020). Although SC-CO_2 extraction is an environmentally friendly and least damaging method to bioactive components, the extract yield and recovery rate of valuable components were found to be lower.

Conclusion

In this study, valuable components found in orange peels, generated as waste in various sectors, were tried to be recovered using SC-CO_2, SE, and UAE methods. In SC-CO_2 extraction, optimum extraction conditions were found to be 22 MPa and 40 °C (or 26.4 MPa and 60 °C) to maximize the volumetric recovery, mass recovery, TPC, TPC recovery, DPPH$^{\bullet}$ scavenging activity, and ABTS$^{\bullet+}$ scavenging activity values of the extracts according to the central composite design. While SE and UAE methods give higher values in terms of extract efficiency and recovery of valuable components, it is a fact that SC-CO_2 extraction is an environmentally and product-friendly method. The use of co-solvent can increase the efficiency of SC-CO_2 extraction. Increasing sensitivity to environmental protection and the spread of zero waste policies bring environmentally friendly applications such as SC-CO_2 extraction to the fore rather than applications that use chemicals such as SE and UAE methods. However, the effectiveness of this application needs to be increased. In addition, in SC-CO_2 extraction, since the extract is obtained in pure form without solvent removal, the products obtained may be more practical and economical.

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Determination of Probiotic Viability in Yoghurts Produced with Acid Adapted *Lactobacillus acidophilus* ATCC 4356 and *Bifidobacterium bifidum* ATCC 11863 During Refrigerated Storage

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ABSTRACT

Microorganisms have various stress response systems to maintain their viability when exposed to different stress conditions. In this study, *Lactobacillus acidophilus* ATCC 4356 and *Bifidobacterium bifidum* ATCC 11863 strains, used in probiotic yoghurt production, were subjected to acid (lactic and hydrochloric acid) stress to induce acid tolerance response (ATR). Yoghurt samples produced with both acid-adapted and non-adapted strains were stored at +4°C for 21 days. During the storage period, the pH and titratable acidity values of the yoghurts were measured, and the viability levels of the probiotic strains in the yoghurts were determined. In all yoghurt groups, a decrease in pH values and an increase in titratable acidity were observed during storage. The highest viability levels of the probiotic strains were detected on the first day of storage. Lactic acid-adapted *Lb. acidophilus* ATCC 4356 and *B. bifidum* ATCC 11863 in yoghurt showed growth at a level of 8.08 ± 0.12 and 8.08 ± 0.09 log10 Cfu/g at the first day of storage, respectively. Additionally, hydrochloric acid-adapted *Lb. acidophilus* ATCC 4356 and *B. bifidum* ATCC 11863 in yoghurt exhibited growth at levels of 7.90 ± 0.08 and 5.99 ± 0.03 log10 Cfu/g, respectively. The viability of acid-adapted *Lb. acidophilus* ATCC 4356 and *B. bifidum* ATCC 11863 showed a decrease similar way to that of the control group (non-acid adapted) during the storage period.

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Introduction

Starting from the late 20th century and encompassing the first quarter of the 21st century, there has been an increase in the quality of life for individuals due to various factors such as technological advancements that have impacted their lives and the rise in healthcare expenses. Therefore, the need for individuals to consume healthy food has arisen, and in this context, the demand for foods with beneficial properties such as "functional foods" has increased (Evren et al., 2017).

The term "functional food" was initially defined as "Foods for Specified Health Uses (FOSHU)" in Japan in the early 1980s (Granato et al, 2010). Functional foods are natural or processed foods that contain biologically active compounds with proven health benefits when consumed in adequate amounts (Martirosyan and Singh, 2015). Foods enriched with probiotics, on the other hand, are known as functional food products containing a sufficient number of live microorganisms capable of altering the microbiota in the host to create beneficial effects on health (Chávarri et al., 2010; Tufarelli and Laudadio, 2016).

The term "probiotic" is derived from the Latin word "bio-tikos", meaning "for life", and it was first used by researchers Lily and Stillwell in 1965 to refer to substances that promote the growth of other microorganisms (Parracho et al., 2007). Probiotic bacteria, which have been defined in various ways to date, are broadly described as living microbial food supplements that can survive in the host's intestinal microbiota and exert beneficial effects there to maintain the microflora (Saarela et al., 2000; Fuller, 2004). The first experimental study related to probiotic microorganisms was conducted by the Russian scientist Metchnikoff in 1907, who investigated the intestinal microflora and reported that fermented dairy products prevented the effects of toxic substances in the body (Vasiljevic and Shah, 2008; Galdeano et al., 2010).

In the first quarter of the 21st century, approximately 500 probiotic food products have been introduced to the global market, indicating a continuous expansion of the applications of probiotic bacteria (Dinkçi et al., 2019). Probiotic products can contain one or more types of microorganisms. Among the various types of microorganisms, species belonging to the *Lactobacillus* and *Bifidobacterium* genera are the most commonly used (Timmerman et al., 2004; Yaşar and Kurdaş, 2009). These bacteria are commonly used in the production of fermented foods, where they can remain highly viable. They exhibit their therapeutic effects only when consumed in specific quantities (10^6-10^7 Cfu/g or Cfu/ml) in the body. Therefore, while various foods are being researched as probiotic carriers, fermented foods are recommended as the best probiotic carriers. Yoghurt, fermented milk, and other fresh fermented products, or non-fermented products with an equivalent number of live probiotic bacteria added, are preferred food carriers for probiotic bacteria that have been used until today (Lourens-Hatting and Viljoen, 2001; Afzaal et al., 2019).

Milk and dairy products have become the primary product group in the probiotic market due to their buffering capacity, diverse product varieties, and the presence of nutrient elements that support the viability of probiotic microorganisms during fermentation and storage. These products are also known as functional dairy products and/or probiotic dairy products. Among them, yoghurt is considered the best carrier food (Gürsoy and Kınık, 2004; Meybodi et al., 2020; Gao et al., 2021). However, traditional yoghurt is not a probiotic product. The bacteria used in yoghurt production, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, are not resistant to stomach acid, bile salts, and digestive enzymes. Consequently, they lose their viability in the gastrointestinal system. Additionally, since they are not part of the intestinal microbiota, they lack the ability to adhere to and colonize the intestines (Çelikel et al., 2018).

Traditional yoghurt, while not carrying probiotic microorganisms, is still an important functional product with many properties, such as containing health-beneficial components like organic acids and providing lactase enzyme to the body through yoghurt starters. The use of probiotic cultures in yoghurt production has become a common practice with the aim of enhancing the positive effects and functional properties of yoghurt on health. This helps transform yoghurt, which holds a significant place in the Turkish diet, into a carrier for probiotics, contributing to the intake of these beneficial microorganisms. This application allows the production of a high-nutrient functional yoghurt, also known as "bio-yoghurt". The use of *Bifidobacterium* species and *Lb. acidophilus* in yoghurt production is increasingly popular, and the resulting product is sold under the name "probiotic yoghurt" (Lourens-Hatting and Viljoen, 2001; Güler-Akın et al., 2007).

The pH level of the environment is crucial for maintaining the viability of probiotic bacteria. Probiotic bacteria belonging to the *Lactobacillus* genus have higher acid tolerance (pH 3.70 - 4.30). However, for *Bifidobacterium* species, it becomes more challenging to maintain their viability below pH 5.00. The pH value of yoghurt, which is commonly used as a probiotic carrier, typically ranges from 4.00 to 4.50. As a result, the number of probiotic bacteria tends to decrease during storage, which can also reduce their viability during passage through the digestive system (Boylston et al., 2004; Tripathi and Giri, 2014).

Exposing microorganisms to adverse conditions for a short period can lead to these microorganisms' developing tolerance or adaptation to these adverse conditions (Hill et al., 1995; Uğuz and Andiç, 2016). A similar situation exists for probiotic microorganisms. Short-term adaptation of probiotic bacteria to an acidic environment can enhance their viability (Shah, 2000).

In this study, probiotic bacteria commonly used in yoghurt production were exposed to moderately high acidic conditions (pH 4.5) before fermentation to determine whether the bacteria developed an acid tolerance response. Therefore, two probiotic bacteria frequently used in probiotic yoghurt production, *Lb. acidophilus* ATCC 4356 and *B. bifidum* ATCC 11863, were adapted to both organic (lactic acid (LA)) and inorganic (hydrochloric acid (HCl)) acids, and the bacteria's acid tolerance responses were examined. Additionally, the viability of probiotic bacteria in yoghurt samples was monitored during storage.

Materials and Methods

Material

The UHT milk used in the research was obtained from Torku - Panagro Tarım Hayvancılık Gıda Sanayi ve Ticaret A.Ş. (Konya, Türkiye) company. The thermophilic yoghurt culture used for probiotic yoghurt production was obtained from Chr. Hansen/İstanbul company. The probiotic cultures (*Lb. acidophilus* ATCC 4356 and *B. bifidum* ATCC 11863) were provided from the distributor Oxoid/Türkiye.

Method

Activation of probiotic culture, preparation and preservation of stock culture

In the study, *Lb. acidophilus* ATCC 4356 was activated and prepared as a stock culture using MRS Broth (Merck, Germany). *B. bifidum* ATCC 11863 was activated and prepared as a stock culture using MRS Broth supplemented with 0.05% L-cysteine HCl (for anaerobic medium) (Dave and Shah, 1996; Tharmaraj and Shah, 2003). The probiotic cultures were inoculated into MRS Broth and incubated at 42°C for 24-48 hours. The activation of cultures was repeated for two passages. The active cultures obtained from the second passage were transferred to sterile tubes containing glycerol (1:1) and stored at -20°C (VELP Scientifica, Italy) for long-term preservation.

To determine the microbial loads of the activated cultures, the spread plate method was used for *Lb. acidophilus* ATCC 4356 on MRS-Sorbitol Agar (Merck, Germany), and the Petri dishes were incubated at 42°C for 72 hours in a carbon dioxide incubator containing 10% CO_2 (under microaerophilic condition) (Nüve EC 160, Türkiye). For *B. bifidum* ATCC 11863, MRS-NNLP Agar (Merck, Germany) was used, and the plates were placed in an anaerobic jar (containing Gas pack) and incubated at 42°C for 72 hours.

The solutions of L-cysteine HCl, sorbitol, and NNLP (nalidixic acid, neomycin sulfate, lithium chloride, and paromomycin sulfate from Sigma-Aldrich, Germany) used in the growth media were sterilized using a 0.45 µm pore-sized sterile syringe filter (Millipore, Ireland).

Adaptation of probiotic cultures to acid environment

In order to adapt the probiotic cultures to an acidic environment, sterilized MRS growth media were prepared by heating at 121°C for 15 minutes and then cooled to 45-50°C. Sterilized 1 N HCl and 1 N LA solutions were added to the MRS media using a 0.45 µm pore-sized sterile syringe filter to adjust the pH of the media to 4.5 (Shah and Lankaputhra, 1997; Matsumoto et al., 2004). For *B. bifidum* ATCC 11863, sterilized L-cysteine HCl solution was also added to the media at a concentration of 0.05% (Tharmaraj and Shah, 2003).

A 1 ml of inoculum was taken from active cultures and inoculated into centrifuge tubes containing 9 ml of acidic broth. The tubes were then incubated at 42°C for 3 hours. After incubation, the tubes were centrifuged using a cooling centrifuge (Hettich Universal 32R, Germany) at 4°C, 4500 rpm for 10 minutes due to cell pellets. The cell pellets were washed three times with sterile peptone water to remove the remaining acidic media.

Probiotic yoghurt production

For the production of probiotic yoghurt, UHT (Ultra-High Temperature) cow's milk was used. The milk was subjected to a heat treatment at approximately 85-90°C for about 10 minutes and rapidly cooled to 44-45°C. The yoghurt culture + non-adapted probiotic culture pellets and yoghurt culture + acid-adapted probiotic culture pellets were added into the milk at the same time for production of yoghurt groups. The yoghurt culture was inoculated at a 2% ratio and probiotic bacteria were added at a level of 10^7 Cfu/g (7.30 log Cfu/g, which is pellets's microbial load). The cultured milk was quickly distributed into sterile sample containers of 100 ml each. A total of three groups of yoghurt have been produced in this research. The yoghurt samples were codded *L0+B0* (non-acid adapted *Lb. acidophilus* ATCC 4356 (L0) + non-acid adapted *B. bifidum* ATCC 11863 (B0) – control group), *LL+LB* (lactic acid-adapted *Lb. acidophilus* ATCC 4356 (LL) + lactic acid-adapted *B. bifidum* ATCC 11863 (LB)), *HL+HB* (HCl-adapted *Lb. acidophilus* ATCC 4356 (HL) + HCl-adapted *B. bifidum* ATCC 11863 (HB)).

The containers were immediately sealed, and the yoghurt samples were placed in an incubator set at 44-45°C. Fermentation was stopped when the pH of the yoghurt reached approximately 4.6. The probiotic yoghurt samples were then stored at 4 ± 1°C for 21 days. During the storage period, pH measurements, titratable acidity analysis, and cultural count of probiotic bacteria were performed on the yoghurt samples on the 1st, 7th, 14th, and 21st days.

pH and titration acidity analysis

The pH values of yoghurt samples were determined using a benchtop pH meter (OrionTM Star A215, Thermo Fisher Scientific Inc, the USA). Before each analysis, the pH meter was calibrated using pH 4.0 and pH 7.0 buffer solutions at 20°C (Bradley et al., 1992).

For the titration acidity analysis, 10 g of the yoghurt sample was taken and mixed with 10 ml of distilled water. Then, 4-5 drops of phenolphthalein indicator were added to the mixture, and it was titrated with 0.1 N NaOH solution (Sigma, Germany) until a permanent light pink color was formed. The result was expressed as the acidity percentage in terms of lactic acid (AOAC, 1995).

Cultural counts of probiotic bacteria

MRS-Sorbitol Agar was used for *Lb. acidophilus* ATCC 4356, and MRS-NNLP Agar was used for *B. bifidum* ATCC 11863 in order to the count of probiotic bacteria (Dave and Shah, 1996; 1997). To prepare the samples, 1 gram of yoghurt was weighed and added to a test tube containing 9 ml of 0.1% sterile peptone water. Serial dilutions were then prepared from the initial dilution (10^{-1}) by taking appropriate dilution volumes. A 0.1 ml sample was taken from the suitable dilutions and spread onto Petri dishes using the spread plate method. For the count of *Lb. acidophilus* ATCC 4356, the Petri dishes were incubated in a carbon dioxide incubator containing 10% CO_2 at 42°C for 72 hours. For the count of *B. bifidum* ATCC 11863, the Petri dishes were placed in an anaerobic jar (containing a Gas pack) and incubated at 42°C for 72 hours. After incubation, the Petri dishes were examined, and those containing 30-300 colonies were selected for counting. The results were then calculated as colony-forming units per gram (Cfu/g). To present the results in a table, the obtained bacterial counts were subjected to logarithmic transformation (log10) for better representation and comparison.

Statistical analysis

In this study, the obtained results were analyzed using the SPSS software package (version 20.0 for Windows, SPSS Inc., Chicago, Illinois). One-Way ANOVA (Analysis of Variance) was used to determine whether there is a statistically significant difference between the group means of the yoghurt samples. To assess the significance of differences, the Duncan multiple comparison test was employed.

Results and Discussion

After activation of probiotic culture, the colonies were counted, and the microbial loads were determined to be $1.36x10^8$ Cfu/ml for *Lb. acidophilus* ATCC 4356 and $1.6x10^8$ Cfu/ml for *B. bifidum* ATCC 11863. In our study, we monitored both the viability of *Lb. acidophilus* ATCC 4356 and *B. bifidum* ATCC 11863 after 3 hours acid adaptation and the viability of these bacteria during storage in yoghurt.

Adaptation of probiotic cultures to acid environment

Acid stress exhibits lethal or sublethal effects on numerous microorganisms (Beales, 2004, Uğuz and Andiç, 2016). Although this effect primarily affects the viability of various microorganisms, short-term acid stress (acid adaptation) is one of the strategies to improve the survival of probiotic bacteria. Bifidobacteria are more sensitive to acids than lactobacilli (Upadrasta et al., 2011; Tripathi and Giri, 2014).

Following 3 hours exposure to an acidic environment at pH 4.5, the viability of *Lb. acidophilus* ATCC 4356 and *B. bifidum* ATCC 11863 was found to be 9 log10 Cfu/g. The microbial loads before and after acid adaptation of probiotic cultures are given in Table 1. Considering the bacterial counts after 3 hours incubation, it is seen that ATR was formed and acid adaptation was successful for both types of acids (De Angelis and Gobbetti, 2011; Guan and Liu, 2020).

Table 1. Microbial loads of probiotic bacteria before and after 3 hours acid adaptation

Acid type	Bacteria group	Adaptation Time (hours)	Counts (log 10 Cfu/g)
LA	LL	0	8.13 ± 0.02
		3	9.02 ± 0.03
	LB	0	8.20 ± 0.01
		3	9.16 ± 0.02
HCl	HL	0	8.13 ± 0.01
		3	9.22 ± 0.02
	HB	0	8.20 ± 0.01
		3	9.04 ± 0.02

LA: Lactic acid, HCl: Hydrochloric acid, LL: Lactic acid-adapted *Lb. acidophilus* ATCC 4356; LB: Lactic acid-adapted *B. bifidum* ATCC 11863, HL: Hydrochloric acid-adapted *Lb. acidophilus* ATCC 4356; HB: Hydrochloric acid-adapted *B. bifidum* ATCC 11863

Table 2. Changes in pH and acidity values of yoghurts produced with yoghurt culture + non-adapted and acid-adapted probiotic cultures

pH	Storage time (Days)	L0 + B0	LL + LB	HL + HB
pH	1	4.70 ± 0.04^{Aa}	4.32 ± 0.01^{Ab}	4.20 ± 0.01^{Ac}
	7	4.59 ± 0.01^{Ba}	4.23 ± 0.01^{Bb}	4.14 ± 0.02^{Bc}
	14	4.59 ± 0.02^{Ba}	4.19 ± 0.01^{Cb}	4.11 ± 0.00^{Cc}
	21	4.56 ± 0.02^{Ba}	4.01 ± 0.01^{Dc}	4.07 ± 0.01^{Db}
		L0 + B0	LL + LB	HL + HB
Acidity (%)	1	0.86 ± 0.02^{Bc}	0.97 ± 0.01^{Ba}	0.92 ± 0.01^{Cb}
	7	0.90 ± 0.01^{Ac}	0.97 ± 0.01^{Bb}	1.00 ± 0.01^{Ba}
	14	0.90 ± 0.02^{Ac}	0.99 ± 0.02^{Bb}	1.05 ± 0.01^{Aa}
	21	0.91 ± 0.02^{Ac}	1.01 ± 0.01^{Ab}	1.06 ± 0.01^{Aa}

a-c: Different letters on the same line indicate a statistically significant difference between samples (P<0.05); A-D: Different letters in the same column indicate a statistically significant difference between days (P<0.05); L0 + B0: Non-lactic acid-adapted *Lb. acidophilus* ATCC 4356 + non-lactic acid-adapted *B. bifidum* ATCC 11863; LL + LB: Lactic acid-adapted *Lb. acidophilus* ATCC 4356 + lactic acid-adapted *B. bifidum* ATCC 11863, HL + HB: Hydrochloric acid-adapted *Lb. acidophilus* ATCC 4356 + hydrochloric acid-adapted *B. bifidum* ATCC 11863

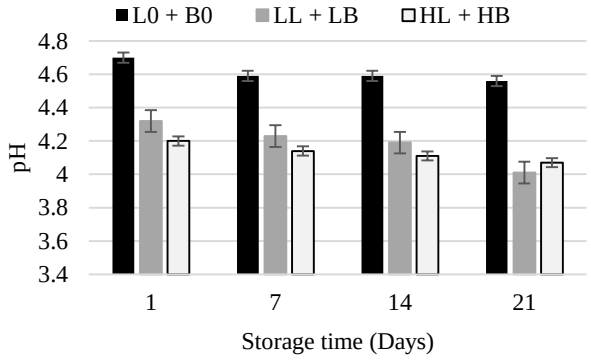

Figure 1. Changes of pH values during storage time

L0 + B0: Non-lactic acid-adapted *Lb. acidophilus* ATCC 4356 + non-lactic acid-adapted *B. bifidum* ATCC 11863; LL + LB: Lactic acid-adapted *Lb. acidophilus* ATCC 4356 + lactic acid-adapted *B. bifidum* ATCC 11863, HL + HB: Hydrochloric acid-adapted *Lb. acidophilus* ATCC 4356 + hydrochloric acid-adapted *B. bifidum* ATCC 11863

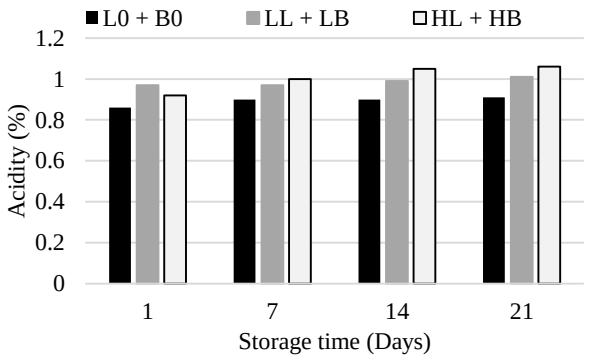

Figure 2. Changes of Acidity (%) during storage time

L0 + B0: Non-lactic acid-adapted *Lb. acidophilus* ATCC 4356 + non-lactic acid-adapted *B. bifidum* ATCC 11863; LL + LB: Lactic acid-adapted *Lb. acidophilus* ATCC 4356 + lactic acid-adapted *B. bifidum* ATCC 11863, HL + HB: Hydrochloric acid-adapted *Lb. acidophilus* ATCC 4356 + hydrochloric acid-adapted *B. bifidum* ATCC 11863

pH and titration acidity values of yoghurts

The pH changes of probiotic yoghurt samples during the 21-day storage period are presented in Table 2 and Figure 1. Throughout the storage period, it was observed that the pH values of probiotic yoghurts ranged from 4.20 - 4.70 on day 1, 4.14 - 4.59 on day 7, 4.11 - 4.59 on day 14, and 4.07 - 4.56 on day 21. In all probiotic yoghurts produced with the non-adapted culture (L0+B0) and with the acid-adapted culture (LL+LB and HL+HB), a decrease in pH values was noted during storage, and this decrease was statistically significant (P<0.05).

It was observed that yoghurt produced with acid-adapted cultures had significantly lower pH values compared to the control samples throughout all storage periods. Yoghurts produced with HCl-adapted cultures generally had lower pH values than those produced with LA-adapted cultures. The effect of acid adaptation and the type of acid on the pH of yoghurts was found to be statistically significant (P<0.05).

Shah et al. (1995) found that the pH of commercially produced probiotic yoghurts (containing *Lb. acidophilus* and *B. bifidum*) decreased during storage by 0.07 to 0.42 units, compared to the pH values observed 2-3 days after production. Yerlikaya et al. (2013) reported a regular decrease in pH values of probiotic fermented beverages produced using *Lb. acidophilus* (0.75%), *Bifidobacterium animalis* subsp. lactis (1.0%), and *Lactobacillus casei* (1.0%) cultures during the storage period.

Settachaimongkon et al. (2015) produced set-type probiotic yoghurts using *Lactobacillus rhamnosus* GG and *B. animalis* subsp. *lactis* BB12 cultures exposed to sublethal levels of NaCl and acid stress, along with yoghurt starter culture. During the storage period, the pH values of probiotic yoghurt samples showed a decrease; however, this decrease was not statistically significant (P>0.05). In the study conducted by Çomak-Göçer et al. (2016), probiotic yoghurts were produced using *Lb. acidophilus* ATCC 4356 in combination with yoghurt starter culture. The yoghurts were subjected to different incubation temperatures and terminated at different pH values. After storage, all yoghurt samples showed a decrease in pH values due to the storage period. In our study, it was determined that the decrease in pH values observed in yoghurt samples during storage is consistent with the findings of other studies on probiotic yoghurt.

The changes in titration acidity of probiotic yoghurt samples are shown in Table 2 and Figure 2. Throughout the storage period, the titration acidity values increased, while the pH decreased during storage. The titratable acidity of yoghurt samples varies between 0.86% and 1.06%. This increase was found to be statistically significant (P<0.05).

The lowest titratable acidity values were determined on the 1st day of storage. The yoghurt produced using HCl-adapted cultures showed the highest titration acidity values, whereas the yoghurts produced with non-adapted culture exhibited the lowest titratable acidity values during the end of storage period. According to the Turkish Food Codex Regulation on Fermented Dairy Products, the titration acidity in yoghurt should be between 0.6% and 1.5% (Anonymous, 2022). All of the titration acidity values determined in this research are within the limits specified in the regulation. Overall increase from first day to end of the storage period is compatible with the results reported by Shah et al. (1997), Çakmakçı et al. (2012), Shoji et al. (2013), Başyiğit-Kılıç and Akpınar Kankaya (2016), Demirci et al (2017), and Ghaderi-Ghahfarokhi et al. (2021).

Counts of probiotic bacteria in yoghurts

In the probiotic yoghurts produced using yoghurt starter and probiotic cultures (*Lb. acidophilus* ATCC 4356 and *B. bifidum* ATCC 11863), it was determined that the non-adapted cultures showed a decrease in counts during storage; however, their viability was maintained throughout all analysis periods. *Lb. acidophilus* ATCC 4356 and *B. bifidum* ATCC 11863 strains managed to maintain their viability during storage. The viable cell count of non-adapted and acid-adapted probiotics in yoghurt was given Table 3, and changes in count were shown Figure 3 and Figure 4.

On the first day of storage, *Lb. acidophilus* ATCC 4356 exhibited a viability of 7.71±0.04 log10 Cfu/g, while *B. bifidum* ATCC 11863 showed a viability of 7.12±0.04 log10 Cfu/g. Throughout storage, except for a slight fluctuation observed on the 14th day for *Lb. acidophilus* ATCC 4356, the counts of both bacteria decreased during the storage period. This reduction in probiotic bacteria counts was found to be statistically significant (P<0.05).

In the research conducted on 5 commercial probiotic yoghurts containing *Lb. acidophilus* and *B. bifidum*, it was observed that during the storage period, only 3 yoghurt samples were able to maintain *Lb. acidophilus* counts at approximately 7-8 log10 Cfu/g. However, for *B. bifidum* counts in the same yoghurt samples, only one sample was able to maintain a count of 6 log10 Cfu/g until the 9th day of storage, while none of the samples could sustain their viability by the end of the storage (Shah et al., 1995). Ng et al. (2011) investigated the relative viability rates of 5 different *Lb. acidophilus* strains (NCFM, ATCC 700396, PIM703, SBT2062, and LA-5) in combination with yoghurt culture. They found that the SBT2062 strain, which was used at levels of 7-8 log10 Cfu/g along with yoghurt culture, exhibited the highest viability rate. However, they observed that the relative viability rates of the other *Lb. acidophilus* strains rapidly decreased throughout the storage period, reaching levels between 4.11 to 5.04 log10 Cfu/g by the end of storage period. In the search by Çakmakçı et al. (2012), they observed a general decrease in the counts *of Lb. acidophilus* DSMZ 20079 and *B. bifidum* DSMZ 20456 in probiotic yoghurt produced using banana marmalade during the storage period. Due to a significant reduction in the counts of *Lb. acidophilus* and *B. bifidum* in these yoghurts, they reported that the products lost their probiotic properties after the 7th day of storage. The viability of *Lb. rhamnosus* GG and *B. animalis* subsp. *lactis* BB12 cultures used in yoghurt production without sublethal stress, it was found that after 28 days of storage, there was a decrease of 0.5 log10 Cfu/g in *Lb. rhamnosus* GG culture count and 1.2 log10 Cfu/g in *B. animalis* subsp. *lactis* BB12 culture count (Settachaimongkon et al., 2015). Similarly, the probiotic bacterial counts in our study also showed a decrease during storage. As the acidity of the yoghurt increased, the viability of the probiotic bacteria decreased. The possible reason for this decrease could be attributed to the increased acidity in the yoghurt. In yoghurt production, the number of organic acids such as lactic acid and acetic acid increases due to the metabolism of lactose by yoghurt cultures and probiotic cultures. Due to the organic acids, the increased ionized hydrogen in the environment interferes with microbial cell membrane integrity, disrupts the cell's internal pH balance, and fundamental biochemical processes. As a result, it can hinder the growth and survival of probiotic bacteria. On the other hand, the impact of metabolites produced by yoghurt cultures and probiotic cultures, along with antagonistic interactions between cultures, may have reduced the viability of probiotic bacteria (Shah, 2000; Tripathi and Giri, 2014; Sendra et al., 2016; Bisson et al., 2023).

In the yoghurt samples produced using *Lb. acidophilus* ATCC 4356 and *B. bifidum* ATCC 11863 adapted to lactic acid (LL and LB groups), both bacteria exhibited a growth level of 8.08 log10 Cfu/g on the first day of storage. It is known that bacteria can maintain their viability in acidic environments through certain mechanisms present in their structures. The activation of stress response systems and the development of acid tolerance response (ATR) in bacteria exposed to moderately low pH are common phenomena, inducing the synthesis of proteins that help bacteria survive in low pH conditions (Ventura et al., 2011; Uğuz and Andiç, 2016). Probiotic cultures can survive in the low pH environment resulting from milk fermentation by developing ATR during adaptation to the acidic conditions (De Angelis and Gobbetti, 2011).

Table 3. The viable cell counts of non-adapted and acid-adapted probiotics in yoghurt (Log CFU/g)

Probiotics	Storage time (Days)	L0	LL	HL
Lb. acidophilus ATCC 4356	1	7.71 ± 0.04^{Ac}	8.08 ± 0.12^{Aa}	7.90 ± 0.08^{Ab}
	7	6.96 ± 0.05^{Ca}	5.47 ± 0.06^{Bb}	7.00 ± 0.01^{Ba}
	14	7.06 ± 0.04^{Ba}	ND	5.63 ± 0.03^{Cb}
	21	5.94 ± 0.03^{Da}	ND	ND
		B0	LB	HB
B. bifidum ATCC 11863	1	7.12 ± 0.04^{Ab}	8.08 ± 0.09^{Aa}	5.99 ± 0.03^{Ac}
	7	7.07 ± 0.01^{Aa}	5.92 ± 0.07^{Bb}	5.61 ± 0.05^{Bc}
	14	6.00 ± 0.02^{Ba}	4.22 ± 0.02^{Cb}	ND
	21	5.61 ± 0.05^{Ca}	ND	ND

a-c: Different letters on the same line indicate a statistically significant difference between samples (P<0.05); A-D: Different letters in the same column indicate a statistically significant difference between days (P<0.05); L0: Non-lactic acid-adapted *Lb. acidophilus* ATCC 4356, LL: Lactic acid-adapted *Lb. acidophilus* ATCC 4356, HL: Hydrochloric acid-adapted *Lb. acidophilus* ATCC 4356, B0: Non-lactic acid-adapted *B. bifidum* ATCC 11863; LB: Lactic acid-adapted *B. bifidum* ATCC 11863, HB: Hydrochloric acid-adapted *B. bifidum* ATCC 11863, ND: not detected

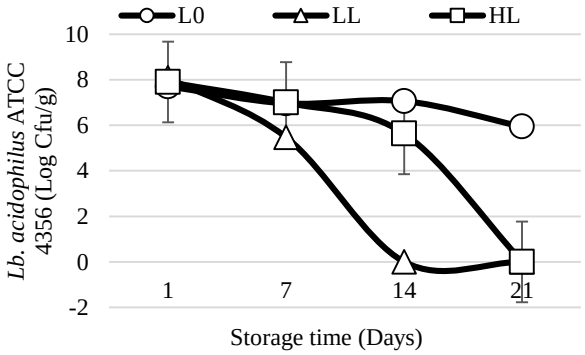

Figure 3. Changes in viable counts of *Lb. acidophilus* ATCC 4356 during storage time

L0: Non-lactic acid-adapted *Lb. acidophilus* ATCC 4356, LL: Lactic acid-adapted *Lb. acidophilus* ATCC 4356, HL: Hydrochloric acid-adapted *Lb. acidophilus* ATCC 4356

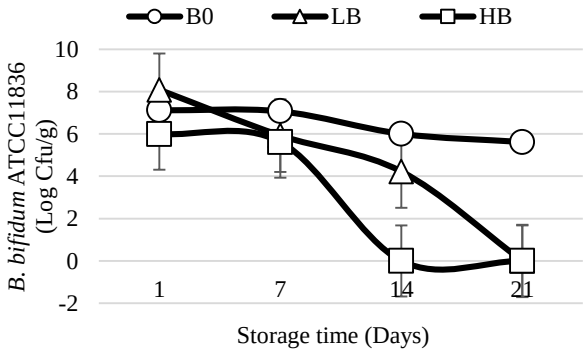

Figure 4. Changes in viable counts of *B. bifidum* ATCC 11863 during storage time

B0: Non-lactic acid-adapted *B. bifidum* ATCC 11863; LB: Lactic acid-adapted *B. bifidum* ATCC 11863, HB: Hydrochloric acid-adapted *B. bifidum* ATCC 11863

In our study, the probiotic bacterial counts on the first day of storage being above 7.30 log10 Cfu/g, as inoculated in yoghurt production, can be explained by this mechanism. According to this result, both probiotic bacteria have developed ATR in the acidic environment. In the yoghurt group containing *B. bifidum* ATCC 11863, viability was observed until the 14th day of storage, while in the yoghurt containing *Lb. acidophilus* ATCC 4356, viability was observed until the 7th day.

In yoghurt samples produced with hydrochloric acid-adapted probiotic bacteria (HL and HB groups), on the first day of storage, *Lb. acidophilus* ATCC 4356 showed growth at a level of 7.90 ± 0.08 log10 Cfu/g, while *B. bifidum* ATCC 11863 strain showed growth at a level of 5.99 ± 0.03 log10 Cfu/g. Considering the initial microbial load in the pellet inoculated into this yoghurt group (7.30 log10 Cfu/g), it can be said that *Lb. acidophilus* ATCC 4356 was positively affected by HCl adaptation, whereas *B. bifidum* ATCC 11863 was negatively affected. In this yoghurt group, *Lb. acidophilus* ATCC 4356 could maintain its viability until the 14th day of storage, while *B. bifidum* ATCC 11863 could only maintain its viability until the 7th day of storage. Probiotic cultures belonging to the *Bifidobacterium* genus have less tolerance to HCl in the stomach environment compared to species belonging to the *Lactobacillus* genus (Ventura et al., 2011, Tripathi and Giri, 2014; Soni et al., 2020). It is predicted that the decrease in the number of *B. bifidum* ATCC 11863 observed in this yoghurt group is due to this reason.

The counts of both acid-adapted and non-adapted *Lb. acidophilus* ATCC 4356 and *B. bifidum* ATCC 11863 exhibited a decrease in all yoghurt groups during the storage period. Bifidobacteria generally show optimal growth within a pH range of 6.0 to 7.0. When the pH drops below pH 5.0, the growth of bifidobacteria decrease significantly (Boylston et al., 2004; Dinkçi et al., 2019). The decrease in the counts of probiotic bacteria can be attributed to organic acids (lactic acid, acetic acid), bacteriocins (bifidin and bifidosin), and antibiotic-like substances produced by both yoghurt cultures and probiotic bacteria (Hill et al., 1995; Çelikyurt and Arıcı, 2008; Fraise et al., 2013, Güngör and Özçelik, 2014, Alipour and Mofarrah, 2022). Additionally, the induced acid tolerance responses in probiotic bacteria can vary based on the bacterial species, growth phase of the bacteria (log phase, stationary phase, etc.), and the type of acid used for adaptation (organic or inorganic acid) (Saarela et al., 2004).

In the study investigating the viability of *Bifidobacterium longum* biotype *longum* NCIMB 8809 and its mutant strain adapted to HCl (pH 4.0 - *B. longum* biotype *longum* 8809dpH), a significant decrease in viability was observed in the non-adapted strain, where it decreased by 5 log after a 90-minute incubation in the simulated gastric environment. However, there was no significant decrease in the viability of the mutant strain (Sánchez et al., 2007). Saarela et al. (2009) aimed to determine the stability of lyophilized *Lb. rhamnosus* cells, which they developed at pH 5.0 and pH 5.8, in their

research. For this purpose, they inoculated cultures into environments containing malic acid and HCl. They observed that the bacteria developed at pH 5.0 showed higher viability than those developed at pH 5.8. Additionally, in acidic environments, higher viability was achieved in the HCl medium. Jiang et al. (2016) investigated the impact of acid stress on *B. longum* BBMN68 (wild type) and found that *B. longum* BBMN68m (mutant strain - acid-adapted), incubated for 2 hours in a medium adjusted to pH 2.5 with HCl, exhibited 4.4 log10 Cfu/g higher viability compared to the wild-type strain. In the research evaluating the viability of different probiotic bacteria and their binary combinations at different pH levels (pH 1.0-4.0), the yoghurt group containing a combination of *Lb. acidophilus* and *B. bifidum* showed the highest probiotic viability in all pH environments, with a survival rate of 66.1% (Soni et al., 2020). In a similar manner, studies conducted by Çakmakçı et al. (2012), Söküt et al. (2021), and Akan (2022) in yoghurt research have also shown a decrease in probiotic counts during storage. Throughout these studies, some products maintained therapeutic levels of probiotic viability by the end of the storage, while others exhibited viability levels below the therapeutic range. In our own study, similar to these previous works, a reduction in probiotic counts was observed in both the control group and yoghurt produced with acid-adapted cultures. However, it was found that the *therapeutic level* was generally maintained within the first 7 days.

In some yoghurt studies produced with probiotic cultures, the detection of high viability during storage might be depend on the use of commercial lyophilized cultures (10^{11} to 10^{12} Cfu/g; e.g., *Lb. acidophilus* LA5® and *B. animalis* subsp. *lactis* BB-12®). However, in our study, despite the low initial levels of acid-adapted probiotic cultures (10^{7} Cfu/g), therapeutic level of viability was observed at the end of storage period.

Conclusion

In this research, it was observed that *Lb. acidophilus* ATCC 4356 and *B. bifidum* ATCC 11863 cultures adapted to both organic (lactic acid) and inorganic acid (hydrochloric acid) environments and they developed an acid tolerance response of bacteria. Following a 3-hours acid adaptation, both probiotic cultures exhibited a viability level of 9 logs, indicating the successful adaptation process. *Lb. acidophilus* ATCC 4356 showed better adaptation to the HCl (9.22 log 10 Cfu/g), whereas *B. bifidum* ATCC 11863 adapted more effectively to the lactic acid (9.16 log 10 Cfu/g).

In yoghurt samples, pH decreased and titratable acidity increased over the storage period. The viability of probiotic bacteria adapted to lactic acid and HCl environments decreased during storage, similar to the control group. HCl-adapted *Lb. acidophilus* ATCC 4356 and lactic acid-adapted *B. bifidum* ATCC 11863 could maintain their viability until the 14th day. However, by the end of the 21-day storage period, viability of probiotic culture was only observed in yoghurts produced with non-adapted strains. It has been observed that the majority of probiotic viability levels maintained the therapeutic dosage range that should be present in probiotic yoghurts.

To maintain the survival of these probiotic bacteria in the product, some measures can be taken, such as adding prebiotic substances to the product and selecting appropriate packaging material. Additionally, applications such as using components that reduce oxidation-reduction potential, incorporating antioxidant compounds, or using food products containing these substances can be used to preserve and enhance the viability of acid-adapted probiotic cultures in the product. Moreover, in potential products where probiotic cultures are used, such as cheese, ice cream, bakery products, and meat products, the cultures can be exposed to stress conditions like high salt, low temperature, high temperature, and acidic environments to induce the development of stress response systems. This approach would enable the use of probiotic cultures in products with high salt content or products subjected to low or high temperatures.

Conflict of Interest

No conflict of interest was declared by the authors.

Ethics Committee Approval

This research does not require ethics committee permission.

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Near- and Mid-Infrared Spectroscopy Combined with Machine Learning Algorithms to Determine Minerals and Antioxidant Activity in Commercial Cheese

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ABSTRACT

Erzincan Tulum Cheese (ETC) holds a significant place among the most popular cheeses in Türkiye. It has been awarded Protected Geographical Indication status, which restricts the allowable milk species, its production area, and specific sheep breed used in its production. Mineral content and antioxidant activity of ETC were aimed to be predicted using conventional FT-NIR and a portable FT-MIR spectrometer combined with partial least square regression (PLSR) and machine learning algorithms based on conditional entropy. Seventy ETC samples were analyzed for their mineral (Al, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, and P) content using ICP-MS. The samples' antioxidant activity was measured using the DPPH•+ scavenging activity method. PLSR combined with FT-NIR spectral data correlated with antioxidant activity (r=0.89) and minerals (as low as r=0.83) except for Cr and Fe. FT-MIR data provided a good correlation for minerals (as low as r=0.82) except for Cr and Mn and a moderate correlation with antioxidant activity (r=0.64). Information theory was applied to select wavenumbers used in machine learning algorithms, and better results were obtained compared to PLSR. Overall, FT-NIR and FT-MIR spectroscopy provided rapid (~ 1 min), non-destructive, sensitive, and reliable output for mineral and antioxidant activity predictions in commercial cheese samples.

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Introduction

Cheese consumption is of great value because it consists of numerous micronutrients and trace elements. Milk and dairy products are estimated to be the primary dietary sources of calcium (Ca) and phosphorus (P), contributing to 59% and 27% of the average human daily intake, respectively. They also provide about 7% of the daily intake of Na, 9% of K, and 11% of Mg (Lombardi-Boccia et al., 2003). Calcium and phosphor are essential for human health (Bonjour et al. 2009); however, Na has been correlated in hypertension and cardiovascular disease (Matthews and Strong 2005; Aburto et al. 2013). Therefore, the European Food Safety Authority and the World Health Organization have advised a daily sodium (Na) intake of no more than 2.4 grams. Furthermore, in accordance with mandatory labeling regulations outlined in Regulation (EU) No 1169/2011, the term "salt" (calculated as Na multiplied by 2.5) must be indicated on product labels. This aims to assist consumers in making informed purchasing choices.

Consequently, cheese producers may need to develop at-line tools to determine mineral content in cheese manufacturing, comply with labeling requirements, and add more detailed health claims to their products. The minerals are detected using standard analytical methods such as atomic absorption spectrometry (AAS), atomic emission spectrometry (AES), inductively coupled plasma–optical emission spectrometry (ICP-OES) (Prieto et al. 2002; Lucas et al. 2006; Mendil 2006). However, these analytical methods necessitate sample preparation techniques that involve the decomposition or destruction of the food sample. Instead of these methods in milk technology, new methods have been sought in analysis

measurements (Hürkan & Bulut 2023). This can include methods such as wet digestion, dry ashing, and microwave oven dissolution. (Ibanez et al., 2008). Furthermore, these methods require expensive instrumentation, high-cost maintenance, time, well-trained personnel to operate the instrument, and chemicals. As an alternative to those techniques, infrared spectroscopy may be used because functional groups interacting with transition minerals correspond to specific infrared light frequencies.

FT-NIR and FT-MIR spectroscopy provide rapid, sensitive, and unique information about food matrix. This information, however, is buried in the multi-dimensional data. While the machine learning algorithms are commonly applied on such data to reveal the information, the process begins with data cleaning and pre-processing steps, such as de-noising. Such intrusive ways theoretically cause losing some information valuable to the food matrix. Additionally, in case of having a data with excessive amount of dimensions, such as spectral data, the dimension reduction and feature selection methods are used to lessen the number of variables intended to be introduced to the machine learning algorithms. Otherwise, the products of these algorithms, either prediction or classification models, focus on the given data and fail to possess a general connection between the data and the information in it. (Rossi et al. 2006; Vergara and Estévez 2014; Wang et al. 2020; Rong et al. 2020; Jia et al. 2020; Liu et al. 2022). Therefore, the spectral features highly associated with the food matrix need to be identified in order to build generalized models. In this study, to measure the correspondence of wavenumbers as spectral features with the food matrix, the conditional entropy defined in the field of Information Theory is used (Cover & Thomas, 2005). The selected wavenumbers that are introduced to the machine learning algorithms, and results; prediction models with their accuracy and R-Square scores are presented.

Erzincan Tulum Cheese (ETC), a highly popular cheese in Türkiye, received Protected Geographical Indication (PGI) status from the Turkish Patent and Trademark Office (Turkish Patent and Trademark Office, 2001). By that PGI, Erzincan Tulum Cheese is described as follows; "A cheese variety produced from the milk of Karaman sheep breed grazed by 90-100 plants endemic to the hills of Erzincan mountains between the fifth and ninth months of the year." To the best of our knowledge, machine learning algorithms have not been employed in conjunction with FT-NIR and portable FT-MIR spectrometers to assess minerals and antioxidant activity in cheese.

Materials and Methods

Sample Collection

Seventy samples of Erzincan Tulum Cheese were procured from local grocery stores located in Erzincan, and Sivas, Türkiye. These cheese samples were acquired in vacuum-sealed packages, each containing no less than 250 grams, and were then stored at a temperature of 4°C until further examination. Afterwards, the cheeses were carefully placed into securely sealed plastic storage bags, manually crushed, thoroughly blended, and kept at 4°C until reference analyses and spectral data collection were carried out.

Reference Analysis

Mineral analysis

The mineral content (Al, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, P) of Erzincan Tulum Cheese was determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) with a slight modification of the method (Milani et al. 2015). First, 0.5 g of the sample was taken, and 2 mL HNO_3 and 1 mL H_2O_2 were added. Then, heating was performed at 200°C for 15 minutes, incineration for 15 minutes, and cooling for 10 minutes in a microwave oven for 40 minutes in total. Finally, the burned sample was placed in a 50 mL plastic flask and completed to 50 mL with ultrapure water. ICP-MS mineral analysis of the cheese was performed at the Gumushane University Central Research Laboratory Application and Research Center.

Antioxidant activity

Sample extracts were prepared with a slight modification of the method (Kuchroo & Fox, 1982). First, in a Stomacher, the cheese sample was homogenized with deionized water for 10 minutes at 20°C. The mixture, then, was kept at 40°C for an hour and centrifuged at 10,000 rpm for 20 minutes at 4°C. Next, the supernatant (oil phase) was removed, and the rest were passed through a 0.45 µm membrane filter. Then, an antioxidant activity analysis was performed.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assay was conducted in accordance with the method outlined by BLOIS (1958). First, 100 µl of the sample was transferred to test tubes, and the total volume was completed with ethanol to 2.0 mL. Then 0.5 mL of the stock DPPH• solution was added to each sample tube. After half an hour of incubation at room temperature, their absorbance at 517 nm against blank was measured. The reduced absorbance was determined as the remaining DPPH• scavenging activity. The % DPPH radical inhibition was calculated according to the formula below.

$$\%\text{DPPH radical inhibition} = [1-(\lambda_S / \lambda_C)] \times 100 \quad (1)$$

(λ_S: the sample absorbance measured at 517.; λ_C: the control absorbance measured at 517 nm)

Near- and Mid-Infrared spectroscopy

The cheese samples' NIR spectra were captured using a Nicolet IS50 Flex Gold infrared spectrometer (Thermo Fisher Scientific, Madison, WI, USA) equipped with FT-NIR diffuse reflectance. This device functions in both the near and medium IR ranges and is outfitted with a Ge-coated KBr (potassium bromide) beam splitter, covering the spectrum from 11,000 to 375 cm^{-1}. Before collecting the spectra, all samples were allowed to reach room temperature (25°C) for approximately 30 minutes. During the data acquisition, a spectral resolution of 4 cm^{-1} was employed, and 64 spectra were averaged for each measurement to improve the signal-to-noise ratio. The process initiated with the acquisition of a background spectrum, in which no sample was present, to compensate for environmental variations. Subsequently, the absorption spectrum was generated by dividing the sample's spectrum by the background spectrum. This procedure was repeated twice for each sample, and the resulting spectra were averaged to yield the final spectrum for each sample. Finally, all spectra were imported into software for subsequent chemometric analysis.

The cheese samples' MIR spectra were obtained using a portable Fourier transform mid-infrared spectrometer manufactured by Agilent Technologies Inc. (Santa Clara, CA, USA). This device was equipped with a zinc selenide (ZnSe) crystal and a deuterated triglycine sulfate (dTGS) detector. Spectra were collected at room temperature, covering a range from 4,000 to 700 cm^{-1}, with a resolution set at 4 cm^{-1}. To improve the signal quality, 64 co-scans were averaged. For each measurement, approximately 10 grams of the sample were directly placed onto the dial path accessory opening. Before each spectral acquisition, a background spectrum was recorded to compensate for any potential environmental effects. The spectral data were presented in absorbance and analyzed using Resolutions Pro Software provided by Agilent (Santa Clara, CA, USA). The FT-MIR analyses were performed in duplicate to ensure accuracy.

Chemometrics

Partial Least Square Regression

The spectral data obtained from the FT-NIR and FT-MIR spectrometers were processed using multivariate data analysis software (Pirouette® 4.5, Infometrix Inc., Bothell, WA, USA). Before analysis, the spectral data underwent preprocessing steps. For FT-MIR, this included mean-centering, normalization, and smoothing, while for FT-NIR, mean-centering, normalization, smoothing, and the application of the 2nd derivative (Savitzky–Golay 35-point window) were performed. These procedures were carried out to enhance the spectral features.

The spectral data were randomly divided into calibration (80% of the entire sample set) and validation (20%) sets. Partial Least Squares Regression (PLSR) analysis was employed on the calibration set to establish quantitative models correlating the reference mineral content (determined by ICP-MS) and antioxidant activity (measured using the DPPH scavenging activity method) with their corresponding spectral data. PLSR, originally developed by Herman Wold (Wold, 1975) is particularly useful for analyzing data with collinearity issues. It combines features of Principal Component Analysis (PCA) and Multiple Linear Regression (MLR) to construct prediction algorithms. The goal of PLSR is to predict the dependent variables (in this case, mineral content) based on the independent variables (wavenumber), extracting orthogonal factors with the highest predictive power from the independent variables (Abdi, 2010). The assessment of model performance involved the consideration of metrics including factor numbers, standard error of cross-validation (SECV), correlation coefficient of calibration (rcal), standard error of prediction (SEP), correlation coefficient of prediction (rval), and outlier diagnostics. Samples exhibiting large residuals indicate that their structure does not align well with the model, while those with high leverage suggest they have a significant impact on the calibration model, classifying them as outliers.

Feature selection algorithm based on conditional entropy

Wavenumbers of the spectral data are actually the dimensions of it and can be considered as its features. The absorption values at these wavenumbers differ based on the content of the specimen. Associating the wavenumbers and specimens' ingredients is the first step through modelling. In general, some, but not all, of the spectra conveys some information about the content of the sample. In other words, a set of wavenumbers will be functional for modelling (Liu et al. 2022; Ozturk et al., 2022; Aykas et al., 2022). Therefore, the information shared between such a wavenumber set and the specified ingredient must be determined, and the way is to use the entropy defined in Information Theory.

To be more specific in terms of modelling, there are predictors as inputs and responses as outputs of a model which are, in this study, the absorption values at the set of wavenumbers and the reference values of the samples, respectively. Saying that all mentioned are variables, their information amount can be calculated.

In the information theory, given its probability distribution, the amount of information that a random variable possesses is measured by the entropy $H(.)$ as follows;

$$\mathrm{H(X)} = \sum_{\mathrm{i}} \mathrm{p(x_i)} \log_2 \mathrm{p(x_i)} \tag{2}$$

Here, X, x_i, and $p(x_i)$ denote a random variable, the ith element of its sample space, and the probability of that element respectively. The unit of entropy is bits per event as the base of logarithm is 2. The entropy solely indicates the difficulty level of the prediction on the variable, however does not express a correspondence with any another variable. In order to figure out how another variable eases the prediction; the conditional entropy is required to be calculated as follows;

$$\mathrm{H(X|Y)} = \sum_{\mathrm{i,j}} \mathrm{p}\left(\mathrm{x_i}, \mathrm{y_j}\right) \log_2 \mathrm{p}\left(\mathrm{x_i}|\mathrm{y_j}\right) \tag{3}$$

The extension of this equation for a number of conditions is given by

$$\mathrm{H}(\mathrm{X}|\mathrm{Y_1}, \mathrm{Y_2}, \dots, \mathrm{Y_r}) = \sum_{\mathrm{i,j,k,l}} \mathrm{p}\left(\mathrm{x_i}, \mathrm{y_j}, \mathrm{y_k}, \dots, \mathrm{y_l}\right) \log_2 \mathrm{p}\left(\mathrm{x_i}|\mathrm{y_j}, \mathrm{y_k}, \dots, \mathrm{y_l}\right) \tag{4}$$

In order to create a set of wavenumbers, another version of Eq.(4) is used as follows;

$$\mathrm{H}(\mathrm{X}|\mathrm{Y_1}, \mathrm{Y_2}, \dots, \mathrm{Y_r}) = \mathrm{H}(\mathrm{X}, \mathrm{Y_1}, \mathrm{Y_2}, \dots, \mathrm{Y_r}) - \mathrm{H}(\mathrm{Y_1}, \mathrm{Y_2}, \dots, \mathrm{Y_r}) \tag{5}$$

The given expressions require the joint probability distributions. In order to obtain the distributions, the continuous variables are quantized, and then the relative frequencies are taken as probabilities. Saying that $n(y_i)$ is the number of occurrences of the event y_i, the distributions are derived as follows:

$$\mathrm{Y} = \begin{cases} \mathrm{y_1}, & \tilde{\mathrm{y}} < \mathrm{l_1} \\ \mathrm{y_2}, & \mathrm{l_1} \le \tilde{\mathrm{y}} < \mathrm{l_2} \\ \vdots & \vdots \\ \mathrm{y_n}, & \mathrm{l_{n-1}} \le \tilde{\mathrm{y}} \end{cases}, \ \mathrm{p(y_i)} = \frac{\mathrm{n(y_i)}}{\sum_{\mathrm{k}} \mathrm{n(y_k)}} \tag{6}$$

The next is to find the variables making the conditional entropy $\mathrm{H}(\mathrm{X}|\mathrm{Y_i}, \mathrm{Y_k}, \mathrm{Y_l}, \dots)$ zero or close to zero, which are accepted as the functional set of wavenumbers. This is

accomplished by a feasible search algorithm. It starting with calculating $H(X|Y_i)$ for each variable to find which variable provides the minimum conditional entropy, then this variable is taken as the first variable of the set. In order to pick the second variable, the first variable is kept in the condition, and $H(X|Y_{1st}, Y_i)$ is calculated with in the same way. The search potentially ends when $H(X|Y_1, Y_2, \dots, Y_r)$ hits to zero, which is evaluated as the information X carries is already buried on the set of Ys.

Results and Discussion

Minerals

In our study, the 70 samples' mineral composition was determined by ICP-MS. The mineral levels of the Erzincan Tulum Cheese (ETC) samples were determined as; sodium (Na) (5114-14210 mg/kg), phosphorus (P) (547-7537 mg/kg), calcium (Ca) (518-1772 mg/kg), potassium (K) (216-920 mg/kg) magnesium (Mg) (61-305 mg/kg). Erzincan Tulum Cheese samples were rich in Na, P, Ca, K, and Mg minerals. The intake of these minerals into the body is important in metabolism. Calcium (Ca) is essential for various biological functions in several tissues, including bones and teeth, as well as the musculoskeletal, nervous, and cardiac systems. Potassium (K) plays a vital role in maintaining the balance of the physical fluid system and aids in nerve functions by facilitating the transmission of nerve impulses. Magnesium (Mg) functions as a calcium antagonist, impacting vascular smooth muscle tone and insulin signaling after receptor activation. Sodium (Na) plays a pivotal role in human physiology by maintaining the equilibrium of physiological fluids, affecting blood pressure, kidney function, as well as the functionality of nerves and muscles. Phosphorus (P) is closely associated with calcium homeostasis and is also involved in the formation of bones and teeth (Martínez-Ballesta et al., 2010). These minerals, which have many tasks that we cannot list here and are high in Tulum cheese samples, must be consumed daily for essential metabolic activities. The high mineral content results suggest that the minerals are in need, which constitutes an adult's daily animal protein requirement that can be met by consuming ETC. In literature, the mineral content of Tulum cheese (not ETC) was determined. The mineral content of 60 samples sold in Elazığ province, Türkiye (30 Tulum and 30 fresh white cheese) were detected by ICP-OES (Oksuztepe et al., 2013). Mineral content of Tulum cheese was found as 8330-11025 mg/kg, 763- 1487 mg/kg, 4310-6620 mg/kg, 5432-12367 mg/kg, 557-620 mg/kg, for Ca, K, P, Na, and Mg, respectively. In a different study, 58 Tulum cheese collected from grocery stores in Izmir, Türkiye, were found as 4750-9500, 1000-1800, 3250-18000, 3100-5200 mg/kg, for Ca, K, Na, and Mg, respectively (Kilic et al., 2002). When compared with our study, it was observed that the Ca and Mg concentration in ETC were lower, K was higher, and Na and P levels were similar.

In our study, the metal concentrations of the ETC were determined as; aluminum (Al) (0.17-9.59 mg/kg), chromium (Cr) (0.00-3.28 mg/kg), copper (Cu) (0.10-1.65 mg/kg), iron (Fe) (0.48-24.18 mg/kg), and manganese (Mn) (0.07-1.22 mg/kg). In the study of Oksuztepe et al. (2013); the metal content of Tulum cheeses was found to be Al (0.10-0.59 mg/kg) Cr (0.03-0.60 mg/kg), Cu (0.10-0.58 mg/kg), Fe (6.12-18.90 mg/kg), and Mn (0.47-2.79 mg/kg). The iron content in studies is due to the use of iron-containing materials during the heating and processing of milk in dairy products (Prieto et al., 2002). Our results show similarities with reported studies.

Antioxidant activity

Among the antioxidant activity determination methods, the DPPH method is the simplest, fastest, and most common method used to determine the antioxidant activity of foods. In a study on determining some quality parameters and bioactivity of 15 herby cheese, % DPPH inhibition was found between 3.60-9.69 (Kara & Kose, 2020). In another study, the inhibition results of the DPPH radical indicated that the antioxidant activity of Tulum cheese samples increased as the ripening days progressed. Specifically, there was an average change of 20.34 in cow milk Tulum cheese and 25.97 in goat milk Tulum cheese over the course of the 120-day ripening period (ÖZTÜRK & AKIN, 2017). In our study, when the DPPH radical inhibition (%) values of 70 Erzincan Tulum Cheese samples were calculated, it was observed that inhibition values varied between 0.02 and 25.14. Our results complied with the reported studies.

Spectral information

The average FT-NIR and FT-MIR spectra, along with their major vibrations, are presented in Figure 1. Peak assignments were made based on the findings reported in the literature (Ayvaz et al., 2021). In the near-infrared (NIR) spectra, the peaks at 6880 and 5164 cm^{-1} were assigned to the first overtone stretching of the unbound O-H group and the combination bands of OH originating from water, respectively. The absorptions at 4331 and 4258 cm^{-1} were attributed to combination bands involving C-H and C-O stretching vibrations in fats. Additionally, the peaks at 5781 and 5669 cm^{-1} were linked to the first overtone of the C-H stretching vibration in fats. The absorptions around 8265 cm^{-1} were a result of the second overtone of the C–H stretching vibration in fats. Due to the elevated moisture content in the cheese samples, carbohydrate peaks around 5900, 4650, and 4380 cm^{-1} were prominently featured.

The peak assignments for FT-MIR spectra were based on the work of Rodriguez-Saona et al. (2006). The highest absorption peak at 3274 cm^{-1} is ascribed to the stretching vibration originating from the O-H bond in water. The 1625 cm^{-1} peak encompassed both the bending vibration of the O-H bond and the amide I vibration of the proteins. Absorption bands ranging from 3000 to 2800 cm^{-1} were attributed to both symmetrical and asymmetrical C-H stretching vibrations, predominantly originating from long-chain fatty acids. The absorption observed at 1740 cm^{-1} was a consequence of the C=O stretching vibration of fatty acid esters. The vibration at 1535 cm^{-1} was associated with the amide II structure in proteins. The absorption at 1448 cm^{-1} corresponded to the C-H bending vibrations of fats. Additionally, absorptions at 1237 and 1174 cm^{-1} were also indicative of fat content, specifically related to the C-O ester linkage and C-O stretching, respectively. The peak at 983 cm^{-1} was linked to the C-O and C-C stretching vibrations of carbohydrate.

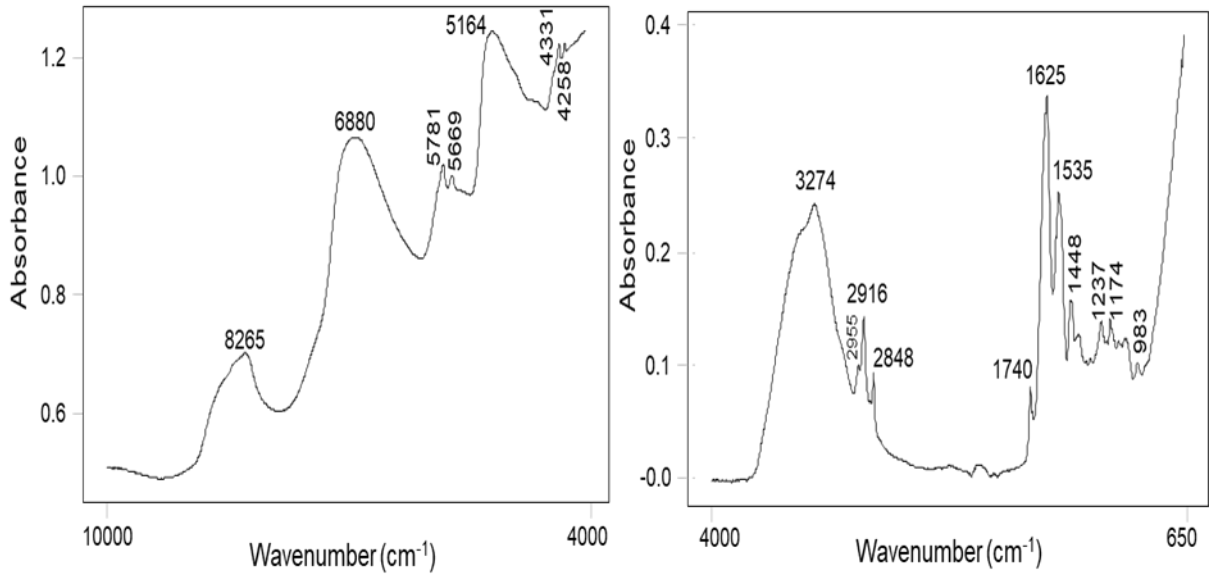

Figure 1. The average spectrum of FT-NIR (10000-4000 cm-1) and FT-MIR (4000-650 cm-1) spectra collected from 70 Erzincan Tulum Cheeses with major vibration bands

Prediction models by Partial Least Square Regression

Minerals and antioxidant activity of Erzincan Tulum Cheese (ETC) samples (*n*=70) were predicted using FT-NIR, and FT-MIR combined with Partial Least Square Regression (PLSR). Figure 2 shows the Ca, K, Mg, Na, and P models obtained from FT-NIR and FT-MIR spectral data. Mineral predictions obtained from FT-NIR spectroscopy are primarily reliant on the presence of minerals within functional groups of organic matter. In the case of cheese, the high predictability of mineral content may stem from its association with various organic components, including lipids (such as phospholipids), proteins, and carbohydrates. These organic compounds serve as carriers or binders for minerals, contributing to the accurate prediction of mineral content using FT-NIR spectroscopy. Calsium and phosphorus exist in cheese with different forms; soluble and bound. Bound P also is divided into two categories; phosphoserine residues called as organic P. Indeed, inorganic phosphorus (P) refers to the inorganic components that are held within the structural framework of casein (Schmidt, 1980). Additionally, these inorganic constituents within caseins engage with phosphoserine residues of casein, functioning as cross-linking agents within casein micelles. This interaction plays a significant role in the overall stability and structure of casein micelles in dairy products. (Aoki et al., 1987). Regression vectors for Ca models obtained from FT-MIR spectral data were 1608 cm^{-1}, 1079 cm^{-1}, and 983 cm^{-1} related to amide I band C-O stretching and C-C stretching, respectively. These results comply with the reported studies (Upreti and Metzger 2006). Regression vectors for the P model obtained from FT-NIR spectral data were 5835 cm^{-1} and 4354 cm^{-1} associated with the C–H stretching vibration and the second overtone of the C–H stretching vibration of fats, respectively. High predictions values were obtained from FT-NIR and FT-MIR units (r_{val}>0.88) (Table 2). Indirect predictions of minerals are possible due to their close association with the organic fraction in milk. Through the process of milk fermentation, the reduction in milk pH leads to the solubilization of a portion of minerals, which becomes evident during the cheese-making process. This phenomenon helps explain the accurate predictions of potassium content using this method. FT-MIR showed slightly better performance in predicting K. The prediction of magnesium (Mg) content in cheese samples may be linked to its crucial role in lipoprotein metabolism and its involvement in processes related to the synthesis of fatty acids and proteins. The presence and quantity of magnesium in the cheese could potentially reflect these underlying metabolic and biochemical processes. Mg serves as a co-factor for multiple enzymes and has demonstrated associations with high-density lipoproteins (Patel et al., 2020). The regression vector indicates that the presence of the bending vibration of the O-H bond at 1600 cm-1, which coexists with the amide I vibration of the proteins, plays a role in the prediction. Despite the absence of absorbance in the NIR region, sodium salts can alter the water spectrum in the infrared overtone region. Therefore, it is possible to indirectly estimate sodium salts using FT-NIR spectroscopy. Possibly, sodium chloride can induce a wavenumber shift in the water absorption band, moving it from 5570 to 5537 cm^{-1}. This shift likely contributes significantly to the accurate prediction of sodium chloride content using spectroscopic techniques. Overall, both FT-NIR and FT-MIR spectroscopy provided a very good correlation with the correlation of coefficient (r_{val}) at least 0.88 for Ca, K, Mg, Na, and K concentrations in ETC samples.

Metallic minerals (Al, Cr, Cu, Fe, and Mn) were predicted using FT-NIR and portable FT-MIR spectra, and regression models are shown in Figure 3. The aluminum content of ETC samples was predicted, and both FT-NIR and FT-MIR units provided a good correlation; r_{val} 0.83 and 0.82, respectively. This correlation could be due to Al salts such as aluminum sulfate, which absorption arises around 3620 and 1022 cm^{-1}. The chromium content of ETC did not correlate with both spectroscopic techniques. This could be due to very low concentrations of Cr (average Cr

level is 0.19 mg/kg). Copper concentrations of ETC were predicted, and both spectroscopic units provided a good correlation. FT-MIR showed a better performance since absorption bands at 1623 $cm^{-1,}$ and 1589 cm^{-1} correspond to COO-Cu, and $CuCl_2$, respectively. Both absorptions were well-correlated with Cu concentrations of ETC and provided r_{val}=0.93. Iron-binding proteins can explain the high Fe prediction by FT-MIR spectral data. MIR range absorption at 1522 cm^{-1}, corresponding to amide II in proteins, and at 1653 cm^{-1} corresponding to the amide I vibration of the proteins, are related to the prediction of iron content. Although FT-MIR provided a good correlation (r_{val}=0.86), FT-NIR showed a weak correlation (r_{val}=0.58). This may be related to weak intensities of protein bands in FT-NIR, resulting in poor correlation with the Fe content of cheese. Interestingly, FT-NIR showed a good performance in predicting Mn (r_{val}=0.87), whereas FT-MIR did not provide a good correlation (r_{val}=0.33). Similarly, FT-NIR showed very good performance on DPPH prediction (r_{val}=0.89), but FT-MIR spectral data correlated with a moderate performance providing r_{val}=0.64. Overall, both spectroscopic units can be used as an alternative to traditional methods to determine the mineral content of cheese.

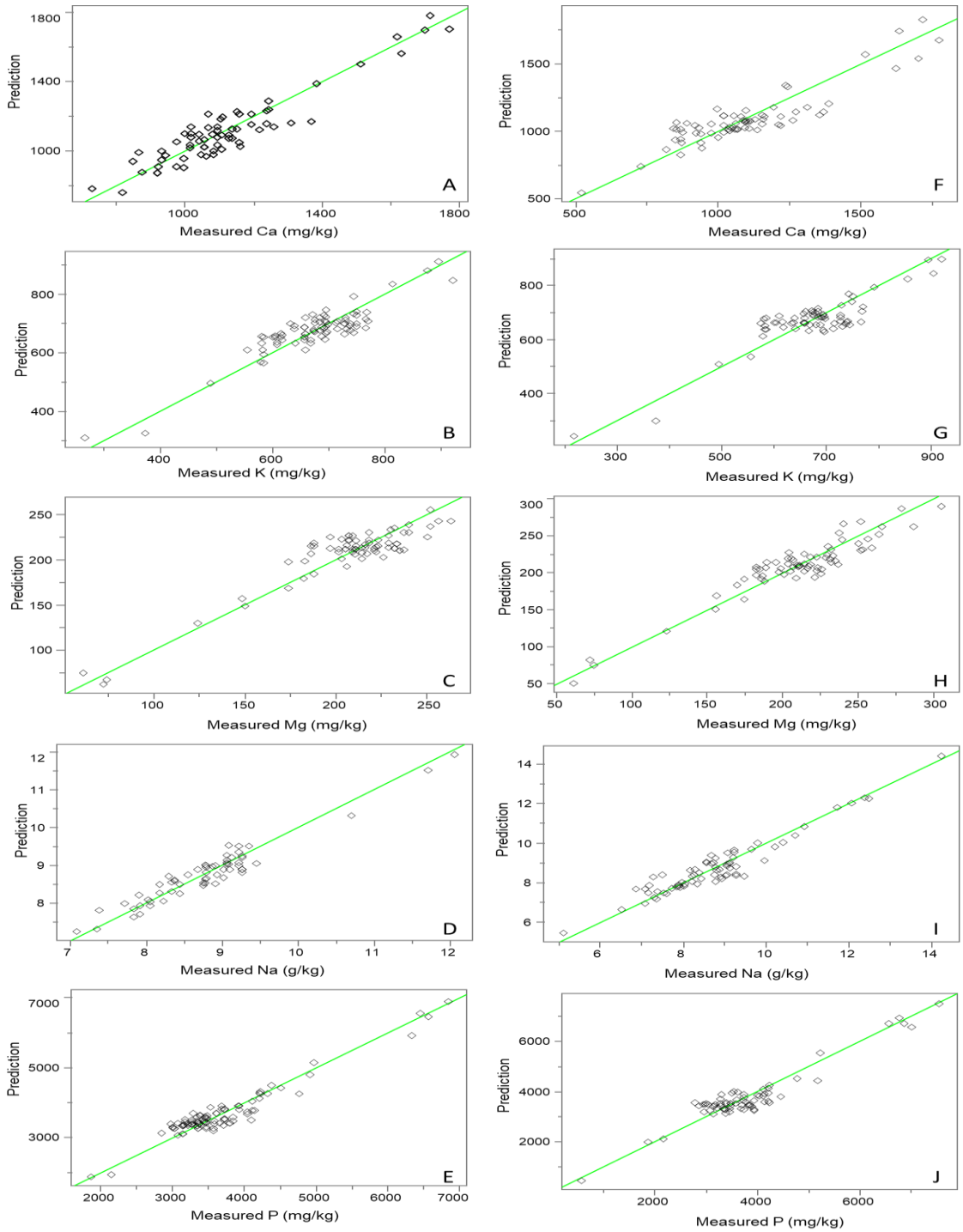

Figure 2. Partial Least Square Regression models for Ca, K, Mg, Na, and P were obtained from FT-NIR spectral data (A-E) and FT-MIR spectral data (F-J), respectively

Table 1 Statistical performance of the prediction models developed using FT-NIR and FT-MIR spectrometers for predicting minerals and antioxidant activity levels of Erzincan Tulum Cheese samples

Unit	Parameter	Calibration model					Validation model		
		Range[a]	N[b]	F[c]	SEC[d]	r_{cal}[e]	N	SEP[f]	r_{val}[g]
FT-NIR	Al	0.17-9.59	54	7	0.91	0.88	15	1.09	0.83
	Ca	518-1772	54	8	66.43	0.96	16	81.41	0.93
	Cr	0-3.28	52	5	0.07	0.49	14	0.08	0.32
	Cu	0.1-1.65	53	4	0.11	0.86	15	0.12	0.83
	Fe	0.48-24.18	52	7	2.69	0.74	15	3.29	0.58
	K	216-920	54	5	37.85	0.91	16	43.65	0.88
	Mg	61-305	54	6	14.15	0.94	16	15.80	0.92
	Mn	0.07-1.22	51	3	0.06	0.92	14	0.07	0.87
	Na*	5.11-14.21	54	6	174.64	0.98	15	271.83	0.95
	P	547-7537	54	4	232.94	0.94	15	239.69	0.93
	DPPH	0.02-25.14	53	7	1.74	0.93	16	2.06	0.89
FT-MIR	Al	0.17-9.59	52	7	1.06	0.88	15	1.19	0.82
	Ca	518-1772	53	6	101.08	0.91	16	108.97	0.88
	Cr	0-3.28	54	3	0.05	0.35	13	0.06	0.13
	Cu	0.1-1.65	53	6	0.12	0.95	15	0.14	0.93
	Fe	0.48-24.18	52	8	1.79	0.90	16	2.00	0.86
	K	216-920	54	5	44.85	0.93	15	47.06	0.91
	Mg	61-305	54	7	13.05	0.96	15	14.53	0.94
	Mn	0.07-1.22	51	5	0.21	0.42	15	0.29	0.33
	Na*	5.11-14.21	53	4	415.9	0.96	16	434.04	0.95
	P	547-7537	54	7	314.34	0.96	16	348.05	0.95
	DPPH	0.02-25.14	52	6	3.97	0.70	15	4.46	0.64

[a]The unit of the range is mg/kg. [b]Number of samples used in calibration models. [c]The number of latent variables. [d]Standard error of calibration. [e]Correlation coefficient of calibration. [f]Standard error of prediction. [g]Correlation coefficient of prediction for external validation. *g/kg

Table 2. Performance of FT-NIR and FT-MIR on the prediction of selected parameters using conditional entropy and machine learning algorithms

Unit	P	H(X)	Y_1	H1	Y_2	H2	Y_3	H3	Y_4	H4	RM	R^2	RMSE
FT-NIR	Al	2.76	7186	1.95	9939	1.09	4088	0.56	8439	0.28	GPR-E	0.85	0.66
	Ca	2.34	5917	1.71	8929	0.95	7054	0.45	4339	0.21	GPR-E	0.99	23.91
	Cr	0.90	7182	0.63	9812	0.29	9380	0.17	5246	0.09	Ensemble	0.06	0.41
	Cu	2.08	9889	1.37	5377	0.71	8416	0.43	4898	0.21	GPR-E	0.91	0.11
	Fe	2.27	7560	1.49	4343	1.00	9959	0.59	7132	0.29	GPR-E	0.34	3.35
	K	1.96	7232	1.18	5296	0.67	9847	0.30	5789	0.09	GPR-RQ	0.99	8.06
	Mg	2.24	7305	1.34	9862	0.76	5053	0.37	6017	0.17	GPR-E	0.97	7.41
	Mn	2.43	7243	1.65	8725	1.00	5789	0.49	5006	0.23	GPR-E	0.92	0.06
	Na	2.21	7386	1.46	5249	0.85	8917	0.42	8428	0.21	GPR-RQ	0.99	70.08
	P	1.88	7182	1.24	8983	0.53	5770	0.26	8763	0.09	GPR-E	0.99	66.41
	DPPH %	2.85	8401	1.97	5458	1.11	4343	0.64	8933	0.33	GPR-RQ	0.79	2.51
FT-MIR	Al	2.75	1616	1.65	654	0.68	3308	0.25	1124	0.00	SVM-FG	0.82	0.88
	Ca	2.32	1521	1.39	3314	0.54	2915	0.26	660	0.11	GPR-E	0.94	54.41
	Cr	0.90	1621	0.51	2915	0.13	678	0.00	-	-	GPR-E	0.14	0.39
	Cu	2.06	1618	1.30	650	0.55	3397	0.15	2188	0.00	GPR-E	0.97	0.05
	Fe	2.26	1623	1.42	2915	0.57	3056	0.24	652	0.09	GPR-E	0.89	1.29
	K	1.95	1618	0.96	652	0.37	2917	0.00	-	-	GPR-E	0.98	9.55
	Mg	2.25	1633	1.25	3258	0.61	658	0.23	1444	0.03	GPR-E	0.96	8.32
	Mn	2.43	1618	1.65	654	0.75	3308	0.28	2037	0.11	SVM-FG	0.08	0.23
	Na	2.19	3235	1.27	1511	0.49	2930	0.22	1942	0.07	GPR-E	0.94	346.88
	P	1.86	1623	0.87	2919	0.27	652	0.06	-	-	GPR-E	0.99	68.47
	DPPH %	2.85	1616	1.84	2913	0.86	652	0.31	2041	0.06	GPR-E	0.61	3.77

P: Para-meter; RM: Regression model; H1: (X|Y1); H2: (X|Y_1, Y_2); H3: (X|Y_1, Y_2, Y_3); H4: (X|Y_1, Y_2, Y_3, Y_4); H(X): Entropy; Y: selected wavenumber; R^2: correlation of determination; RMSE: root mean squared error; GPR: Gaussian Process Regression; E: Exponential; RQ: Rational Quadratic; SVM: Support Vector Machine; FG: Fine Gaussian

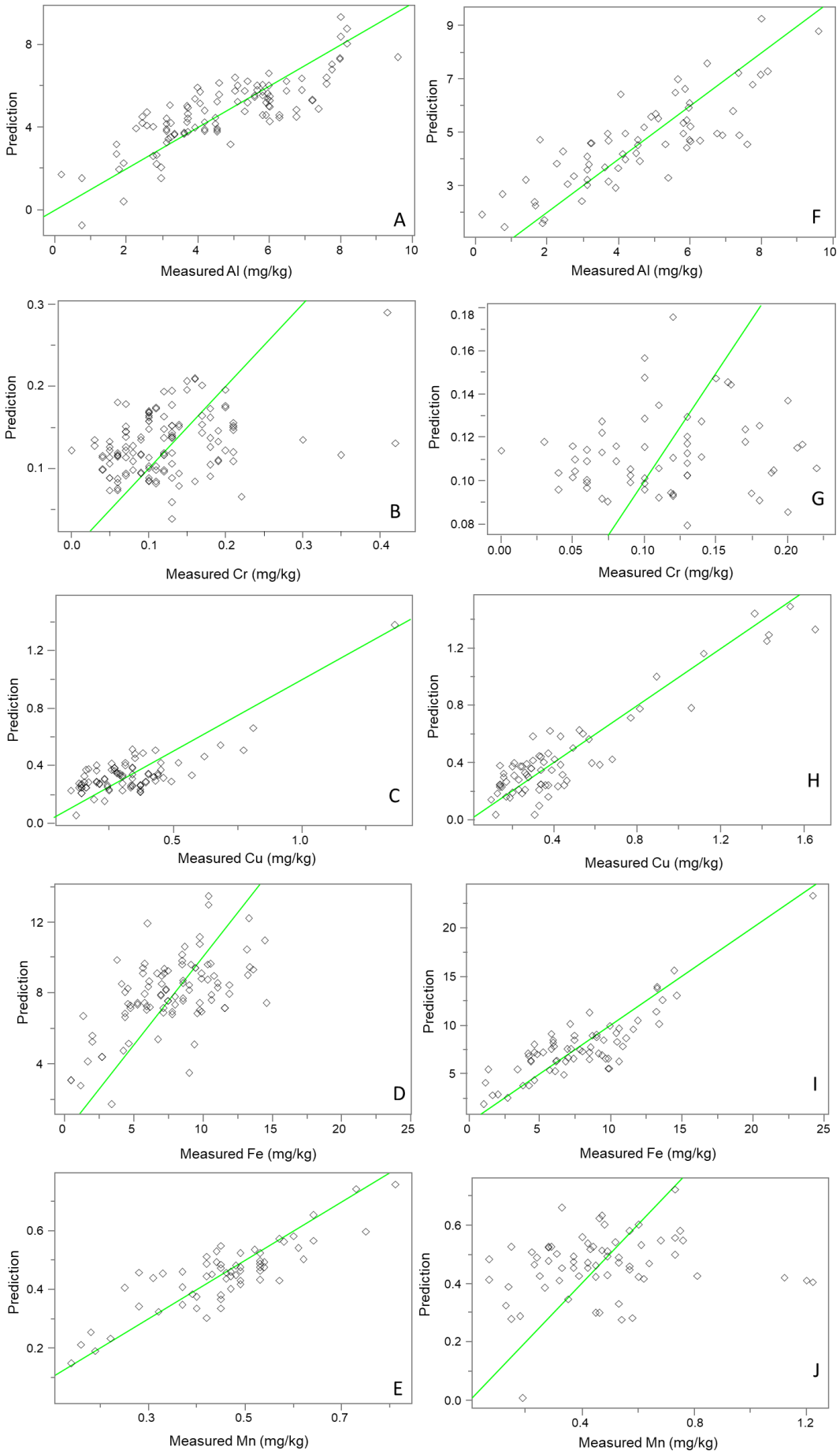

Figure 3. Partial Least Square Regression models for Al, Cr, Cu, Fe, and Mn were obtained from FT-NIR spectral data (A-E) and FT-MIR spectral data (F-J), respectively

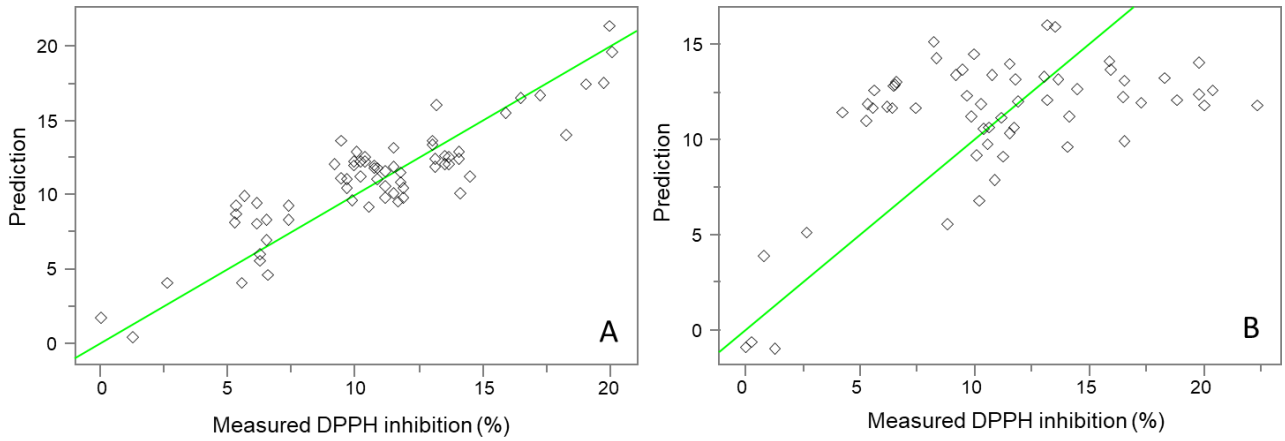

Figure 4. Partial Least Square Regression models for antioxidant activity (DPPH) were obtained from FT-NIR spectral data (A) and FT-MIR spectral data (B), respectively

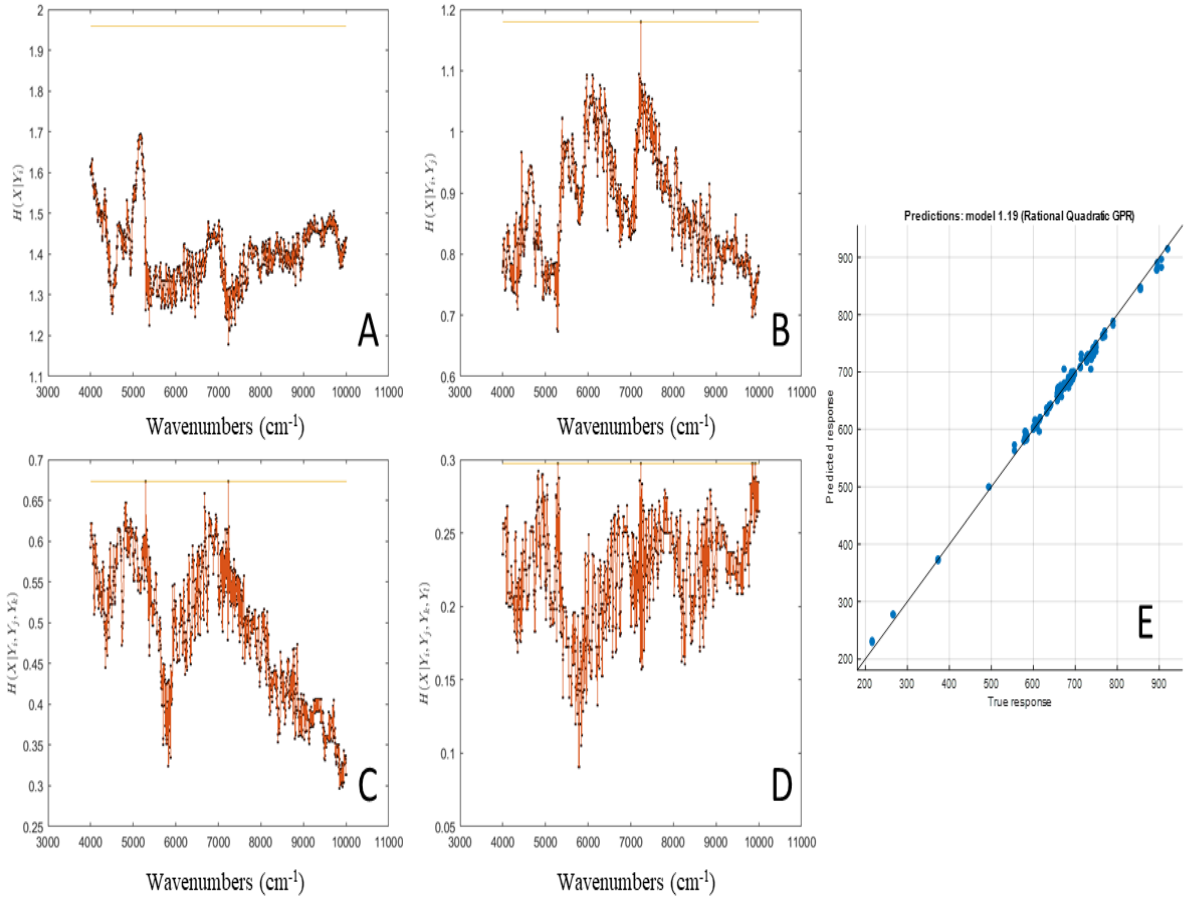

Figure 5. Feature selection based on the conditional entropy (A-B-C-D), and prediction model based on the selected features (E) for potassium prediction using FT-NIR

Prediction models by information theory and machine learning algorithms

Machine learning algorithms were used to predict minerals and the antioxidant activity of ETC. As explained in material and methods (2.3.2.), first, conditional entropies were calculated per parameters, and then features were selected based on the minimum conditional entropy. For instance, total entropy was 1.96 bits/sample in K predictions using FT-NIR spectral data (Figure 5). Then, the first feature was selected as 7232 $cm^{-1,}$ and it dropped the entropy to 1.18. Second, third, and fourth variables were selected as 5296, 9847, and 5789 cm^{-1}, respectively, and these variables dropped the entropy to 0.67, 0.30, and 0.09, respectively. After variables were selected for predictions of K, MATLAB regression learner was used to create a prediction model. FT-NIR showed a good correlation with potassium using Gaussian Process Regression (GPR) algorithms combined with Rational Quadratic (RQ) kernel providing R^2 as 0.99, and RMSE as 8.06 mg/kg. Table 2 shows the performance of FT-NIR and FT-MIR spectrometers on the prediction of minerals and antioxidant activity of ETC. Overall, both units well-correlated with the most minerals determined, except Cr and Mn.

Conclusions

Erzincan Tulum Cheese samples' minerals and antioxidant activity were aimed to be predicted using FT-NIR and portable FT-MIR spectrometers combined with PLSR and machine learning algorithms based on conditional entropy approach. Both units allowed for the accurate determination of the minerals. A basic FT-NIR and FT-MIR protocol minimized the sample heterogeneity of ETC that provided predictive models with a high correlation coefficient for minerals (r_{val}>0.83) except for Cr and Mn. ETC samples' antioxidant activity was correlated with FT-NIR spectral data; however, FT-MIR spectral data did not provide a good prediction model. Therefore, a machine learning approach was used to predict the minerals and antioxidant activity of ETC. Similar or slightly better performances were obtained using machine learning algorithms compared to PLSR. Overall, FT-NIR and FT-MIR spectrometers provided rapid (~1 min), non-invasive, and reliable determination of minerals in ETC.

CRediT authorship contribution statement

Ahmed Menevseoglu: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing - original draft; Writing - review & editing. ***Nurhan Gunes:*** Machine learning analysis; Software; Visualization; Writing - review & editing. ***Huseyin Ayvaz:*** Formal Analysis; Investigation. ***Sevim Beyza Ozturk Sarikaya:*** Formal analysis; Investigation; Writing - review & editing. ***Cuma Zehiroglu:*** Formal analysis.

Declaration of competing interest

The authors declared that there is no conflict of interest.

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Examination of the Relationships between Internal and External Egg Quality Traits: A Structural Equation Model

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ABSTRACT

This study aimed to determine the structural relationship between internal and external egg quality (IEEQ) traits. In this study, 114 eggs produced from 24 weeks-old laying hens reared at the Ondokuz Mayis University Research Farm were used. Egg weight (EW), egg width (EWi), egg length (EL) and shell weight (SW) measurements were examined as external quality traits. Also, albumen height (AH), albumen width (AW), yolk height (YH), yolk weight (YW) and yolk diameter (YD) parameters were used as internal quality traits. Structural Equation Model (SEM) was used to determine the relationships between IEEQ traits. Data analysis was performed with the LISREL package. It has been determined that the variables that are important in determining the external egg quality are SW, EWi and EL. When the variables explaining the internal traits were examined, it was determined that the YW, YD, AW, AH and YH were significant. It was determined that the relationship between external egg quality and internal quality was 0.96 and external quality explained the internal quality by 91%. It has been determined that the SEM used in this study is sufficient to explain the relationship between internal and external quality.

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Introduction

Poultry eggs are of great importance in a balanced and healthy diet. It is important to determine the quality characteristics of eggs, which have both nutritional properties and reproductive material (Aysondu and Ozyurel, 2021). For this reason, in recent years, importance has begun to be given to the quality of eggs and egg products at all stages of the egg industry, from producers to consumers (Gul et al. 2021). Egg quality is generally evaluated in two parts, internal and external quality (Mollazade et al. 2021) and it is one of the factors affecting both incubation efficiency (Boleli et al 2016) and consumer demands (Hisasaga et al. 2020). The quality factor plays a significant role in marketing, consumer satisfaction, and the shelf life of table eggs. Additionally, it has a notable impact on various criteria, including preserving egg quality during storage, hatching efficiency, hatchability, and producing healthy chicks in breeding eggs (Kamanli and Turkoglu, 2018).

It is not possible to increase egg quality after laying, but it is possible to maintain quality with minimum losses by providing necessary conditions (Erensayin 2000). In addition, improving shell quality is an important issue to minimize economic losses caused by egg cracking and breakage (Arango et al. 2016). The decrease in egg weight (EW) which is one of the egg quality criteria, is an effect of reducing components, or at least some components such as albumen, egg yolk and shell (Alves et al. 2007). For this reason, it is important to determine the characteristics that affect the internal and external quality of eggs and the relationships between them. Previous studies examined the effects of nutrition (Selim and Hussein 2020), egg processing conditions and long-term storage (Jones et al. 2018), and environmental temperature with advancing age on egg quality traits (Freitas et al. 2017). Particularly, some studies have been conducted to increase the yolk/albumen ratio (Jun et al. 2017; Sapkota et al. 2020). Sarica et al. (2012) conducted a study with principal components analysis to determine the most effective variables in internal and external egg quality (IEEQ) according to different breeds and their body weights. This study examined IEEQ traits bilateral relations between them with Pearson correlation, and the variables that most explain internal and external quality were determined under different factors with principal component analysis.

Pearson correlation (provided that the necessary assumptions are made) only determines the relationship between two variables, while canonical correlation determines the relationships between two sets of variables (Tahtali et al. 2012). However, these methods indicate the relationship between variables or the relationship between variable sets, respectively. In addition, principal component analysis can be used to determine the most effective one among the variables (Sarica et al. 2012).

As an alternative to these methods, the Structural Equation Model (SEM), which is created by combining factor analysis and multiple regression analysis, can be used to analyze the structural relationship between variables. This technique analyses the structural relationship between measured variables and latent constructs (internal and external). While the findings identify the variables with the most explanatory power among internal and external quality characteristics, they also determine their relationships with other variables. In this way, variables that are determined to be significant among the variables can be taken as breeding parameters or prioritized within the quality criteria. Researchers prefer to use this method to estimate multiple and interrelated dependencies in a single analysis (Statistics Solutions, 2021). This study aims to determine the relationship between IEEQ traits (latent variables) and determine the variable/variables (IEEQ traits) that explain these latent variables the most by the SEM.

Material and Method

Material

IEEQ traits were determined on 114 eggs obtained from Lohmann Brown laying hens at 24 weeks of age reared at the Ondokuz Mayıs University Research Farm. Egg weight (EW), egg length (EL), egg width (EWi), and shell weight (SW) were determined as external quality traits. EW was measured with a scale with an accuracy of 0.1 g (Shimadzu, Kyoto, Japan). EWi and EL were measured with a 0.01 mm precision digital calliper. Yolk diameter (YD), yolk weight (YW), yolk height (YH), albumen height (AH) and albumen width (AW) were determined as internal quality traits. These traits were measured on a mirrored glass table after the eggs were broken. AH and YH were measured using a digital tripod micrometer with 0.01 mm precision. AH was measured from the part closest to the yolk. The distance between the two widest edges of the albumen was assessed as the width of the albumen. YH was measured from the middle of the yolk (Erensoy et al. 2021).

Method

Descriptive statistics (mean, standard deviation, coefficient of variation (%)) of IEEQ traits examined in the study were calculated. Normality assumptions of the data were examined with the Kolmogorov-Smirnov test. It was found that they were normally distributed ($P>0.05$). Pearson correlation coefficient values were calculated to determine the relationships between the variables examined. Confirmatory Factor Analysis (CFA) to verify the structure of IEEQ traits and SEM was used to determine the relationships between IEEQ traits. The correlation matrix was used instead of the covariance matrix in the calculation of the structural relationship due to the unit difference in the data.

SEM is a multivariate statistical technique that estimates causal relationships between variables (Rahi et al., 2018). This method is widely used in many fields such as economics, marketing, biology, and medical research (Raykov and Marcoulides 2006) because direct and indirect effects between observable and unobservable variables can be tested in a single model. As such, SEM can also be considered as more than one regression analysis performed at the same time. SEM consists of two parts: the structural model and the measurement model (Yilmaz et al. 2016). The set of connections between the observed and latent variables constitutes the measurement model (Bollen 1989; Arbuckle 2007) and the connections between unmeasured (latent) variables are defined as the structural model (Bollen 1989). Following the purpose of the study, a general model with two factors is given in Figure 1, which is edited from Kenny's (2016) work.

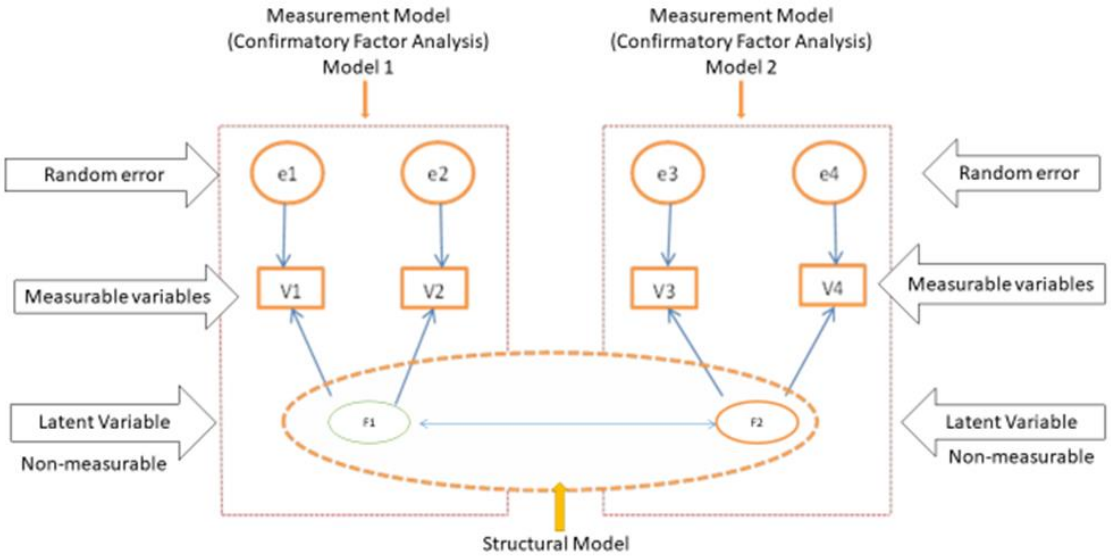

Figure 1. Two factor model

Table 1. Goodness of fit criteria

Compliance Measurement	Perfect Compliance	Acceptable Compliance
χ^2/df	$0 \leq \chi^2/df \leq 2$	$2 \leq \chi^2/df \leq 3$
Root Mean Square Error of Approximation	$0 \leq RMSEA \leq 0.05$	$0.05 \leq RMSEA \leq 0.08$
Normed Fit Index	$0.95 \leq NFI \leq 1.00$	$0.90 \leq NFI \leq 0.95$
Non-Normed Fit Index	$0.97 \leq NNFI \leq 1.00$	$0.95 \leq NNFI \leq 0.97$
Comparative Fit Index	$0.97 \leq CFI \leq 1.00$	$0.95 \leq CFI \leq 0.97$
Goodness of Fit Index	$0.95 \leq GFI \leq 1.00$	$0.90 \leq GFI \leq 0.95$
Adjusted Goodness of Fit Index	$0.90 \leq AGFI \leq 1.00$	$0.85 \leq AGFI \leq 0.90$

Table 2. Descriptive values for egg quality traits used in the study (n=114)

Traits	Mean	Std Deviation	CV (%)
EW	56.02	4.14	7.32
EL	54.02	1.51	2.78
EWi	42.48	1.16	2.82
SW	5.85	0.62	10.17
AW	64.93	4.95	7.70
AH	77.67	5.56	7.21
YD	37.09	1.41	3.77
YH	17.62	0.86	5.11
YW	12.66	0.91	7.09

CV (%): Coefficient of variation

Table 3. Correlation coefficients between egg quality traits

	EW	EL	EWi	SW	AW	AH	YD	YH
EL	0.692**							
EWi	0.906**	0.387**						
SW	0.721**	0.456**	0.624**					
AW	0.505**	0.276**	0.458**	0.425**				
AH	0.318**	0.336**	0.207*	0.366**	0.634**			
YD	0.359**	0.204*	0.362**	0.415**	0.202*	0.123		
YH	0.318**	0.239*	0.277**	0.240*	-0.103	-0.113	0.107	
YW	0.532**	0.394**	0.462**	0.585**	0.289**	0.270**	0.471**	0.390**

**P<0.01 *P<0.05

A typical model of the structural equation is as follows:

$$\eta = \beta\eta + \Gamma\xi + \zeta$$

In Equation, η : the m×1 dimensional endogenous variable vector, β: the coefficients matrix between the m×m dimensional endogenous latent variables whose elements are β_{ij}, Γ : It shows the coefficients matrix between exogenous latent variables and endogenous latent variables with m×n dimension and elements γ_{ij}, ξ :n×1 dimensional extrinsic variable vector, ζ : m×1 dimensional latent error terms vector, m is the number of endogenous latent variables, n is the number of extrinsic latent variables (Yilmaz et al. 2016).The suitability of the structural model created from the obtained data is tested with some criteria. These criteria are given in Table 1. For these criteria, there are two compliance criteria as perfect and acceptable (Schermelleh-Engel et al. 2003).

SPSS – 21 package program was used to obtain descriptive statistics, Kolmogorov-Smirnov test, and Pearson correlation analysis results, and LISREL 8.8 program was used to obtain the results of CFA and SEM.

Results

Descriptive statistics for egg quality traits are given in Table 2. The coefficient of variation (CV%) values for egg traits ranged from 2.78 to 10.17, and it can be said that the data are reliable (CV% < 30).

The correlation coefficients between the quality traits used in the study are given in Table 3. The highest significant correlation was found between EW and EWi, while the lowest significant correlation was found between YD and AW. There was no significant correlation between YH and AW, AH and YD (P>0.05).

CFA results are given in Table 4 and Figure 2. As a result of this analysis, EW was found insignificant in explaining the external egg quality along with other traits. While the most important factor in explaining external quality was SW (0.86), YW (0.72) was the most important in explaining internal quality. Also, the least important factor in explaining the external quality was EL (0.54), while it was YH (0.27) in the internal quality. According to Nunnally (1978) and Hair et al. (1998), when the reliability estimates of latent factors are examined to evaluate whether the observed variables defined under

latent variables describe the structures they are related to, the structural reliability of the internal quality is 75.96%, while the external quality characteristics are 61.43% in this study (Yilmaz and Celik 2010).

The findings of the fit criteria according to the CFA results are given in Table 5. It has been determined that the model obtained is perfect according to $\chi 2$/df criteria and acceptable according to other all criteria (RMSEA, NFI, NNFI, CFI, GFI and AGFI).

According to SEM results, it was determined that the variables that are important in determining the external egg quality were the SW (0.86), EWi (0.73) and EL (0.54), respectively. When the variables explaining the internal quality traits were examined, it was determined that the YW (0.72), YD (0.53), AW (0.51), AH (0.40) and YH (0.27) were significant. It was determined that the relationship between IEEQ traits was 0.96 and external quality explained the internal quality by 91%. The structural reliability of the internal quality was 75.96%, while structural reliability of the external quality was 61.43% (Table 6, Figure 3).

The findings of the fit criteria according to the structural equation model are given in Table 7. When the model fit criteria were examined, it was determined that the model obtained was perfect according to $\chi 2$/df criteria and acceptable according to other all criteria (RMSEA, NFI, NNFI, CFI, GFI, AGFI).

Table 4. Confirmatory Factor Analysis results

Factors/Traits	Standard Loadings	t-Values	Structure Reliability	R^2
External Traits (Ext)				
EL	0.54	5.70**		0.29
EWi	0.73	8.27**	75.96	0.53
SW	0.86	10.20**		0.73
Internal Traits (Int)				
AW	0.51	5.18**		0.26
AH	0.40	3.99**		0.16
YD	0.53	5.47**	61.43	0.28
YH	0.27	2.54*		0.072
YW	0.72	7.59**		0.51

*P<0.05; **P<0.01; R^2: Coefficient of determination

Table 5. Compliance measurement for Confirmatory Factor Analysis

Compliance Measurement	Values	Compliance
χ^2/df	38.16/23= 1.66	**
RMSEA	0.076	*
NFI	0.90	*
NNFI	0.95	*
CFI	0.95	*
GFI	0.92	*
AGFI	0.88	*

*Acceptable; **Perfect

Table 6. Structural Equation Model results

Factors/Traits	Standard Loadings	t-Values	Structure Reliability	R^2
External Traits (Ext)				
EL	0.54	6.96**		0.29
EWi	0.73	5.23**	75.96	0.53
SW	0.86	5.53**		0.73
Internal Traits (Int)				
AW	0.51	5.18**		0.26
AH	0.40	3.99**		0.16
YD	0.53	5.47**	61.43	0.28
YH	0.27	2.54*		0.072
YW	0.72	7.59**		0.51
Int ⟶ Ext	0.96	7.11**	-	0.91

*P<0.05; **P<0.01; R^2: Coefficient of determination

Table 7. Compliance measurement for Structural Equation Model

Compliance Measurement	Values	Compliance
χ^2/df	38.16/23= 1.66	**
RMSEA	0.076	*
NFI	0.90	*
NNFI	0.95	*
CFI	0.95	*
GFI	0.92	*
AGFI	0.88	*

*Acceptable; **Perfect

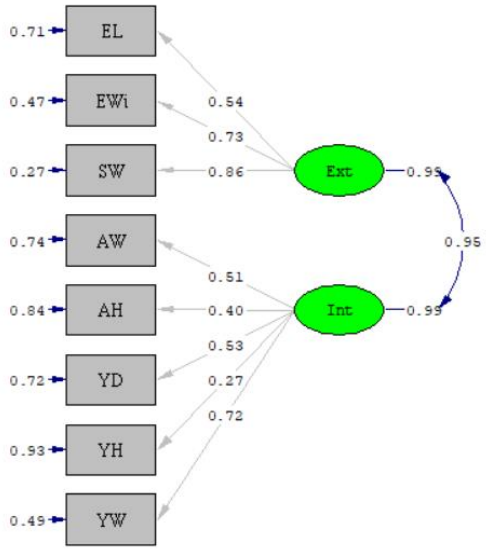

Figure 2. Confirmatory Factor Analysis results

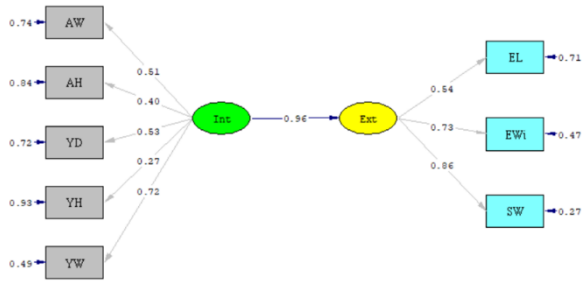

Figure 3. Structural Equation Model result

Discussion

Eggs are of the highest quality at the time of laying and impossible to increase afterward (Mauldin 2002). For this reason, studies have accelerated for the preservation and sustainability of egg quality since laying time. It is important to determine the most important criteria used in quality evaluation and the relationships between the criteria to ensure this quality protection. Some studies have examined the effect of egg storage on quality. Feddern et al. (2017) reported that higher egg quality (first week) was mainly associated with higher EW and specific gravity, Haugh Unit, and AH. Sarica et al. (2012) reported that the most effective parameters in genotypes were the breaking strength and shell thickness among the egg external quality traits, and the AH, albumen index and Haugh Unit among the internal quality traits.

Hrnčár et al. (2017) reported that egg weight, egg shape index, yolk percentage, egg shell (ES), albumen weight and YW increased, while ES and albumen percentage decreased with the advancing of the laying period. In addition, studies have been carried out on the determination of egg quality in different laying hen breeds (Freitas et al. 2017; Saribas and Yamak 2020).

In our study, SW and EWi were among the external qualities; YW, YD, and AW were determined as the most influential parameters among internal quality traits. Egg quality criteria can be determined by considering external quality traits, primarily among these variables. In addition, according to the result of the structural equation model, it was determined that a one-unit change in external quality variables would cause a 0.96-unit change in internal quality variables. It has been determined that the SEM used in this study is sufficient to explain the relationship between internal and external quality. The results of this study can contribute to increasing the internal and external quality of both hatching and table eggs.

Conclusion

Eggs have a large share in human nutrition, health and the commercial sector. For this reason, studies should be reliable and applicable first. In previous studies, the relationships between IEEQ traits were examined by correlation analysis and the results were interpreted. However, in this study, the relationship between the variables explaining the IEEQ were examined with the SEM, which is used especially in social sciences, and found to be applicable. As a result, it was determined that while the SW had the highest importance and explanatory power in the external egg quality, the YW had the highest importance and explanatory power for the internal quality. Also, a significant correlation (0.96) was found between IEEQ traits. It is thought that the use of different variables in addition to the variables used in this study and the determination of such relationships for eggs obtained from different poultry breeds and species will contribute to the literature.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Environmental Awareness Evaluation within the Scope of Noise Pollution: The Case of Adana-Çukurova District

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ABSTRACT

Today, environmental issues are rapidly increasing due to the growing population, rapid and unplanned urbanization, industrialization pressure, and advancing technology. Consequently, there is an accelerated search for solutions to environmental problems. As in the formation of these problems, humans will be a key factor in solving them. Therefore, individuals need to be developed and equipped in terms of environmental awareness, environmental consciousness, and environmental sensitivity. Many studies in the literature advocate the necessity of education to increase environmental awareness; however, first and foremost, individuals' environmental awareness must be identified and their levels must be revealed. In this study, noise pollution, which has been increasingly impactful in the last 30 years and is ranked as the second-highest burden of disease by the World Health Organization after air pollution, with less awareness compared to other environmental issues, is evaluated. In this context, the research area is selected as the Çukurova District of Adana Province, and the awareness of noise pollution among the residents in the region is assessed through survey forms and SPSS software. Additionally, using the survey results, the proportional values of noise pollution as the most significant environmental issue are evaluated as spatial analysis and mapped.

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Introduction

Informing individuals and increasing the level of environmental consciousness or enhancing environmental awareness play a crucial role in preventing environmental problems (Taycı, 2009). Environmental consciousness is fundamentally defined as understanding the importance of not causing harm to the environment and utilizing it at a sustainable level (Yücel et al., 2006; Mansuroğlu et al., 2010). In other definitions, environmental consciousness is described as raising sensitivity regarding the use and preservation of the natural environment (Başal, 2003), supporting living in a balanced and healthy environment, and serving as an indicator of changes in human attitudes and behaviors in the face of environmental problems (Çolakoğlu, 2010). According to Erten (2005), the aim of environmental consciousness includes environmental knowledge, positive attitudes towards the environment, and behaviors beneficial to the environment. However, it cannot be asserted that the level of internalizing environmental consciousness is the same in all individuals within society; there may be variations in the degree to which individuals internalize environmental consciousness (Karataş, 2013).

As understood from the definitions, the development of individual responses to prevent or reduce environmental problems and the formation of a consistent environmental attitude among all individuals are necessary. For this purpose, the development of environmental consciousness and, consequently, awareness is essential.

In today's world, people and their surroundings are confronted with numerous environmental issues due to factors such as intensive and unplanned industrialization, population growth, rapid and unplanned urbanization, technological advancements, various methods of energy production, new inputs in agriculture, transportation facilities, vehicles, and networks (Yücel, 2000). The increasing connection between environmental problems threatening natural life, humanity, and living environments and their significant impact on the quality of human life has led to a rise in societal environmental sensitivity, environmental conservation awareness, and awareness of environmental issues.

When considering environmental issues that are easier to analyze and visually perceive, such as soil, water, and air pollution, noise stands out as a environmental problem that is more challenging to perceive, dependent on the ongoing process, and relatively new in terms of awareness compared to other environmental issues. Noise, which arises due to factors such as unplanned urbanization, transportation, and industrialization in the process of urbanization, is defined as a type of technological residue (Kurra, 2009; Basner et al., 2014; Onay, 2021).

Environmental noise sources can be categorized as transportation, industry, construction, and entertainment and commercial noises resulting from human activities (Akça, 2009; Kurra, 2009). When evaluating noise sources, as seen in Table 1, the most impactful noise source on individuals in a residential area is traffic noise from highways (Fan et al., 2010; Paşaoğlu, 2013).

Table 1. Impact rates of human and the environment based on noise sources (MEB, 2011)

Noise Sources	Impact Rate (%)
Road traffic	50.0
Rail systems	18.0
Aircraft	13.0
Industry	6.0
Neighbors	3.5
Construction	3.0
Outdoor	2.5
Other sources	4.0

When considering all types of noise sources, there are three main approaches to combat noise, aiming to reduce or prevent it: controlling noise at the source, controlling noise in the area between the source and the receiver (environment), and controlling noise in the receiver, the individual exposed to the noise (user) (Beranek, 1983; Şahin, 2003). For these noise control methods to achieve their goals, it is essential for society to have awareness of noise pollution.

The success of efforts to minimize or even eliminate environmental problems depends not only on a global and political scale but also on fulfilling the necessary responsibilities at the societal level and fostering societal awareness (Erkal et al., 2011; Tunç et al., 2012). Effective planning for environmental protection can only succeed when the public is sensitive to environmental issues. Enhancing environmental sensitivity will contribute to people living in a healthier and safer environment (Özmen et al., 2005; Yeşil and Turan, 2020).

As evident in the resolution of environmental problems, the fundamental aspect in reducing and/or preventing noise pollution is the identification of individuals' awareness and consciousness of noise pollution, as well as fostering the development of this awareness. Various approaches, including surveys, assessments, and scale development, have been evaluated in the literature to assess environmental sensitivity and the public's awareness of environmental issues (Şama, 2003; Yücel et al., 2006; Oğuz et al., 2011; Yeşilyurt et al., 2013; Yeşil and Turan, 2020).

In this study, the survey assessment method is employed to evaluate individuals' knowledge about noise pollution, the level of impact, proposed solutions against noise, and consequently, their awareness. Considering environmental issues, the urban center of Çukurova District in Adana Province, identified as the area most affected by noise pollution, was selected as the research area. The social, demographic, and economic structure of the individuals living in the research area was determined, and the effects of noise pollution were evaluated in terms of perception, knowledge level, experience, opinions, and proposed solutions to mitigate noise pollution as an environmental problem. Additionally, individuals' assessments of noise pollution in the context of environmental issues were analyzed proportionally and spatially, and the distribution was mapped.

Material and Method

Material

According to the 'Turkey Environmental Issues and Priority Assessment Report' prepared by the former Ministry of Environment and Urbanization in 2019, the primary environmental issue in Adana Province is noise pollution. In recent years, Adana Province, particularly Çukurova District, has rapidly developed both vertically and horizontally, transforming into a densely populated urban area. Moreover, due to its possession of dual-directional, 3-4 lane boulevard-like urban roads and its proximity to the TAG highway, the region is highly exposed to traffic-related noise pollution (Bozkurt, 2013; Yücel et al., 2015; Kahveci, 2016; Çolakkadıoğlu and Yücel, 2017). Noise measurements were conducted at the points indicated in Figure 1 to assess the presence of noise pollution in the research area. The measurements were evaluated within the limits defined by the 'Environmental Noise Assessment and Management Regulation' dated June 4, 2010 (Table 2).

When evaluating Table 2, it is observed that the Leq values obtained from all measurement points exceed the limit values, indicating noise pollution originating from the highway.

For all these reasons, the main material of the study consists of the central urban area of Çukurova District in Adana Province. As depicted in Figure 2, nine neighborhoods with the highest residential and transportation density in the city center were included in the research. According to the data from TUIK (2020), the population of Çukurova District is 386,684, while the total population of the 9 neighborhoods comprising the research area is approximately 363,898, accounting for about 95% of Çukurova District's population.

The other materials of the study include a questionnaire consisting of 27 questions. The IBM-SPSS Statistics 26.0 software, which provides opportunities for statistical analysis, was used in the evaluation of the questionnaire.

Furthermore, due to the large scope of the study, the ongoing pandemic, and the need for more accurate and reliable results, professional support was sought during the implementation of the questionnaire. In this context, support was obtained from 'Ayna Public Relations and Research Center,' which provides services in areas such as policy, social, scientific, and consumer domains.

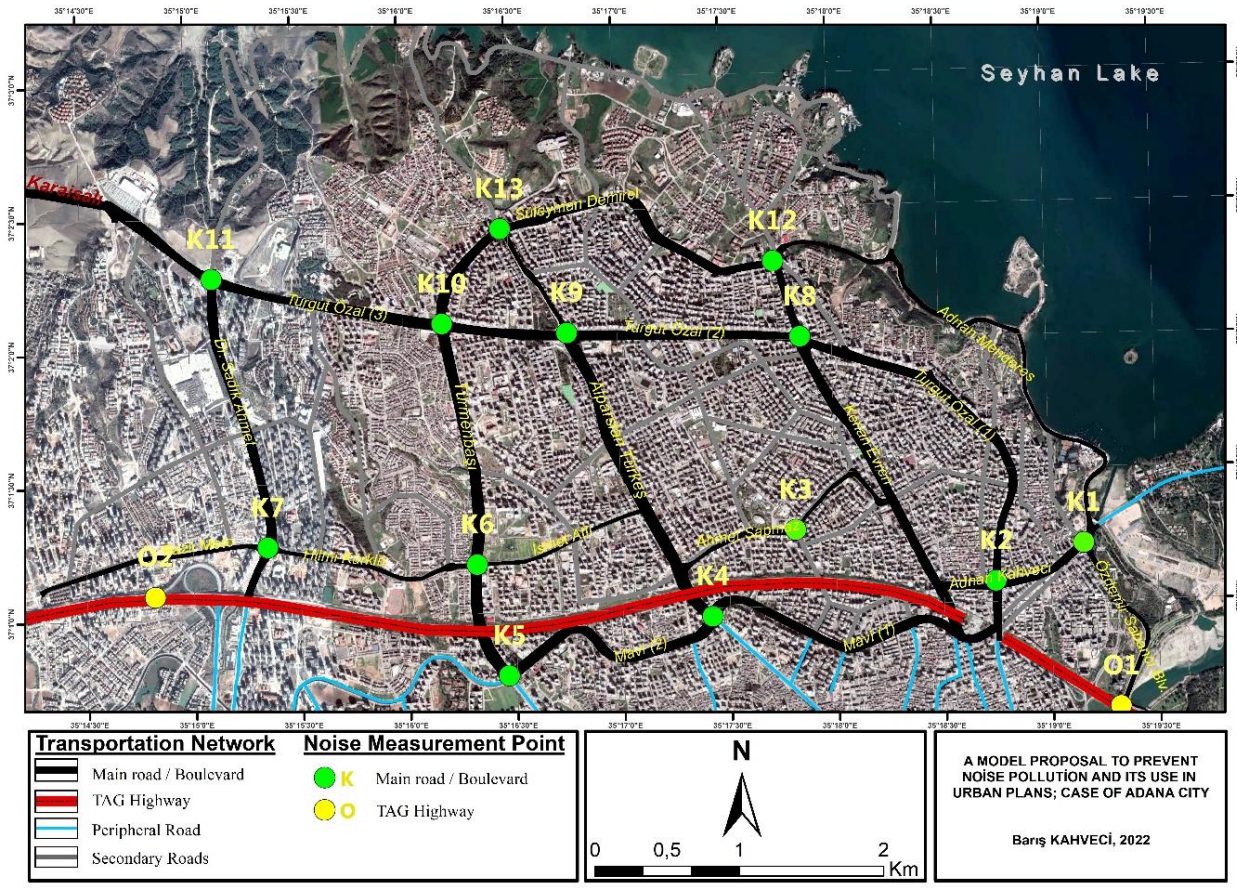

Figure 1. Noise measurement points in the research area

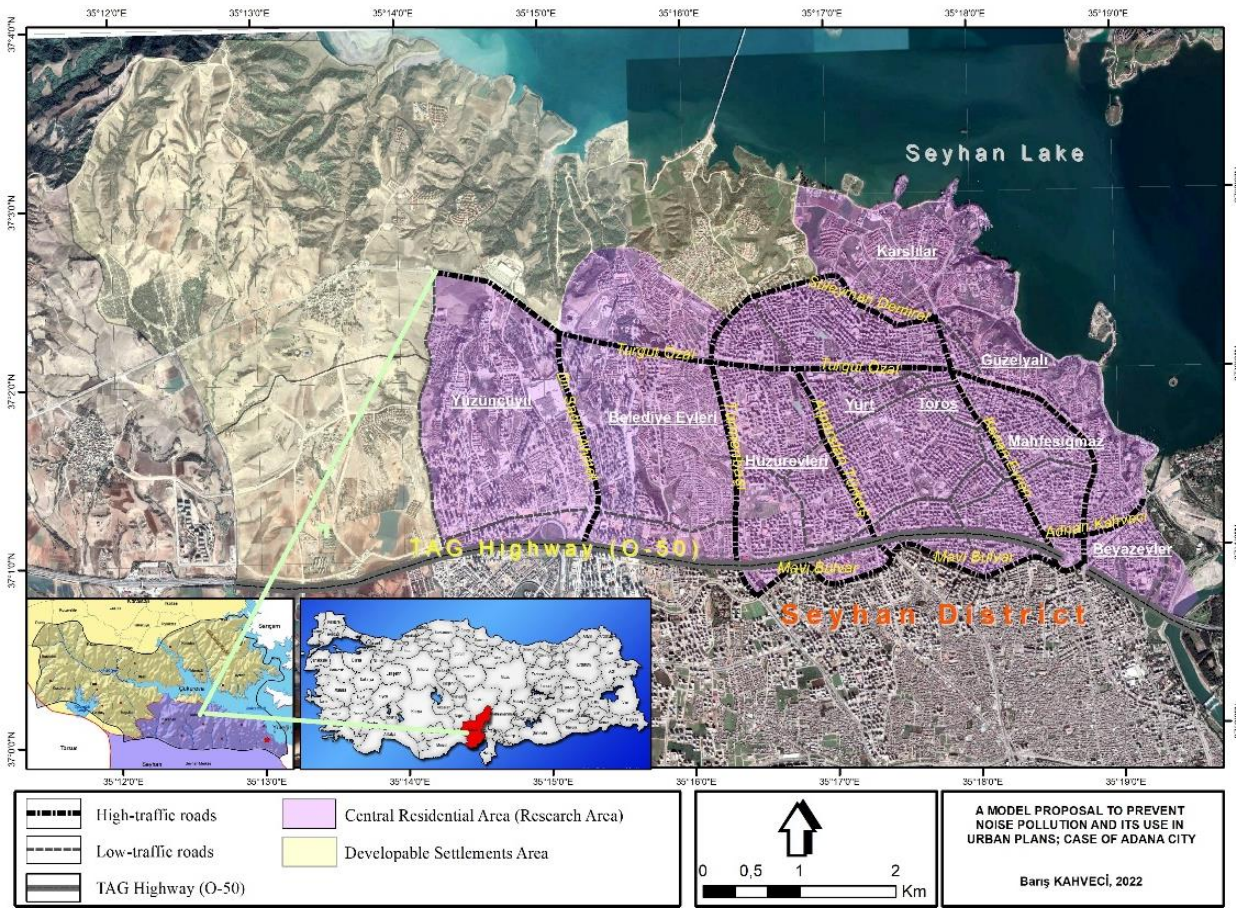

Figure 2. Location map of the research area

Table 2. Lmin, Lmax, and Leq values obtained from noise measurement points.

Measurement Point	Measurement Results								
	Daytime (L_day) (07:00-19:00) (dB(A))			Evening (L_evening) (19:00-23:00) (dB(A))			Night (L_night) (23:00-07:00) (dB(A))		
	Lmin	Lmax	Leq	Lmin	Lmax	Leq	Lmin	Lmax	Leq
K1	61.6	82.8	72.4	63.2	83.4	77.2	56.2	74.5	64.4
K2	58.9	79.6	70.5	62.4	80.5	75.4	55.4	75.2	65.4
K3	57.5	74.8	66.5	58.6	75.7	68.3	52.2	73,4	65.0
K4	56.7	76.3	69.5	57.4	75.6	68.5	50.3	70.6	64.4
K5	58.6	75.7	68.4	56.5	74.7	67.5	54.2	74.6	66.1
K6	57.5	74.8	66.5	58.6	75.7	68.3	53.6	72.4	64.8
K7	56.5	73.8	64.5	57.6	74.9	65.8	54,4	73.8	66.4
K8	62.4	76.6	69.8	60.2	78.8	70.2	59.7	73.4	63.7
K9	65.8	83.6	70.8	65.2	80.4	75.6	63.2	79.5	65.3
K10	61.1	77.4	68.5	62.8	79.4	71.4	55.8	72.6	61.5
K11	60.2	76.5	69.7	60.9	75.4	67.8	54.8	73.8	63.5
K12	59.7	75.6	68.8	61.5	77.1	69.2	55.6	72.8	62.8
K13	58.4	74.2	66.4	60.5	76.4	68.4	53.8	70.9	61.3
O1	71.9	88.0	79.4	69.4	88.6	79.8	68.6	86.2	76.4
O2	70.2	84.4	77.6	68.6	86.4	76.0	68,4	85.7	75.8

Areas	Existing Roads		
	Lday (dB(A))	Levening (dB(A))	Lnight (dB(A))
Areas predominantly characterized by noise-sensitive uses such as education, culture, and health facilities, as well as recreational and camping areas	65	60	55
Areas with a dense concentration of residences, where commercial structures coexist with noise-sensitive uses	68	63	58
Areas with a high density of businesses, where commercial structures coexist with noise-sensitive uses	70	65	60

Environmental Noise Limit Values for Road Traffic (ÇGDYY, 2010)

Method

In line with the aim of the study, the method of the research revolves around evaluating the survey. A survey was conducted to determine the social, demographic, and economic structure of individuals living in the study area and to identify perceptions, knowledge levels, experiences, opinions, and proposed solutions regarding the effects of noise pollution and its transformation into an environmental issue.

In the study area, it was determined that 363,898 people reside in the 9 neighborhoods, constituting the most densely populated region in terms of population and urban area. Based on this population, the sample size was determined as 625 with a 4% acceptable error rate at a 95% confidence interval and 400 individuals with a 5% acceptable error rate (Table 3). In this study, surveys were administered to 415 individuals.

Regarding the survey method, telephone interviews were chosen for the study, aiming for a safer and faster process during the pandemic and to ensure the participants do not have face-to-face contact with the interviewer, which is expected to yield more successful results. The survey aimed to evaluate four sections: determining the social situation of individuals living in the study area, identifying the individual's living area (neighborhood, proximity to the main road, floor of residence, etc.), determining information about noise pollution and its impact, and identifying suggestions to reduce and/or prevent the effects of noise pollution.

The questionnaire consists of 27 single-choice questions. The first five questions in the survey were designed to determine the participant's gender, age, education level, occupation, and monthly income, aiming to establish the social status and profile of the participant. Questions 6–12 investigated the participant's place of residence and their relationship with the research area. Questions 13–22 aimed to gather information about how participants are affected by noise pollution in terms of manner, time, and location. Questions 23–26 inquired about participants' knowledge and suggestions regarding the reduction and/or prevention of the effects of noise pollution. The preparation of the survey form involved reviewing studies on similar topics and evaluating them for the development of survey questions (Yücel et al., 2009; Kahveci, 2016; Yücel et al., 2015; Öner, 2018; Öner and Sesli, 2018).

Before conducting the survey, participants were first informed by the interviewer about the purpose and scope of the study. Then, a clear explanation of how the survey would be conducted was provided. Surveys were implemented through personal interviews, with interviewers reading the questions and recording participants' responses on a standard form. The surveys were evaluated using the IBM-SPSS 26.0 statistical software.

In the final stage of the study, a map was created to better understand the surveys and enable spatial analysis of the survey. For the mapping, the responses to the question about the most significant environmental problem were

evaluated based on the side of the neighborhood and buildings facing the road (main road or boulevard name and side road). The main road was considered with respect to boulevards and the blocks where the first building masses were located. Two sides (right and left) were created within a 100-meter distance from the main road. The other areas of the neighborhoods were considered on the side roads.

Additionally, in the final stage, the survey outputs were evaluated in terms of individuals' environmental awareness regarding noise pollution, mapped, and results and recommendations were developed.

Table 3. Characteristics of the sample, specified limits in percentages, and sample sizes for sensitivity (Yamane, 2001)

Population Size	Sample Size for Specific Sensitivities					
	%1	%2	%3	%4	%5	%10
500	b	b	b	b	222	83
1000	b	b	b	385	286	91
1500	b	b	638	441	316	94
2000	b	b	714	476	333	95
2500	b	1.250	769	500	345	96
3000	b	1.364	811	517	353	97
3500	b	1.458	843	530	359	97
4000	b	1.538	870	541	364	98
4500	b	1.607	891	549	367	98
5000	b	1.667	909	556	370	98
6000	b	1.765	938	566	375	98
7000	b	1.842	959	574	378	99
8000	b	1.905	976	580	381	99
9000	b	1.957	989	584	383	99
10 000	5.000	2.000	1.000	588	385	99
15 000	6.000	2.143	1.034	600	390	99
20 000	6.667	2.222	1.053	606	392	100
25 000	7.143	2.273	1.064	610	394	100
50 000	8.333	2.381	1.087	617	397	100
100 000	9.091	2.439	1.099	621	398	100
⟶	10.000	2500	1111	625	400	100

Table 4. Socio-economic Status of Participants in the Survey

Category	Number	%	Category	Number	%
Gender			Age		
Female	182	43.90	Under 18 Years Old	1	0.20
Male	233	56.10	18 - 24	12	2.90
Total	415	100.00	25 – 39	98	23.60
Educational Status			40 -59	8	1,90
Illiterate	5	1.20	60 Years and Older	104	25.10
Primary School	80	19.30	No Response	2	0.50
Secondary School	38	9.20	Total	415	100.00
High School	137	33.00	Income Level (Minimum Wage Net 2300 TL in 2020)		
Undergraduate Degree	137	33.00	0 – 1500	10	2.40
Postgraduate Degree	16	3.90	1501 - 2500	64	15.40
No Response	2	0.50	2501 - 4000	154	37.10
Total	415	100.00	4001 – 8000	134	32.30
Occupation			8001 ve Üstü	48	11,60
Worker	24	5.80	No Response	5	1.20
Civil Servant	57	13.70	Total	415	100.00
Retired	103	24.80	Residential Neighborhood		
Homemaker	105	25.30	Güzelyalı	43	10.40
Academician	11	2.70	Beyazevler	43	10.40
Trader/Artisan	13	3.10	Toros	51	12.30
Private Sector Employee	48	11.60	Mahfesığmaz	36	8.70
Student	7	1.70	Karslılar	37	8.90
Freelancer	40	9.60	Huzurevleri	45	10.80
Farmer	1	0.20	Yüzüncüyıl	62	14.90
Unemployed	4	1.00	Belediyeevleri	50	12.00
Other	2	0.50	Yurt	48	11.60
Total	415	100.00	Total	415	100.00

Findings and Discussion

In previous studies conducted to determine environmental sensitivity and awareness (Yücel et al., 2006; Oğuz et al., 2011; Yeşilyurt et al., 2013; Yeşil and Turan, 2020), a method based on scoring and weighting has been followed to identify surveys and scales. However, in this study, in line with the purpose and methodology of the research, an assessment was made specifically for a single environmental issue and awareness and/or consciousness of noise pollution. Through the prepared questionnaire, analysis and inferences were made using multiple-choice questions and directly provided responses.

The survey was conducted in August-September 2020 (during the pandemic) through telephone interviews, involving a total of 415 participants. IBM SPSS Statistics v26.0 was used for evaluating the results.

The characteristics of individuals participating in the survey in terms of gender, age, education level, occupation, and monthly income are presented in Table 4.

Table 4 shows that 56.10% of the participants in the survey are male, while 43.90% are female. When participants are evaluated in terms of age groups, it is determined that the majority, with 25.10%, is 60 years and older, and 23.60% are adults aged 25-39. Of the participants, 33.00% have a high school education, and the same percentage has a university degree, making a total of 66.00% with high school and university graduates combined.

The occupations of the participants were investigated with 10 options, including 9 choices and one open-ended. Considering the possible impact of noise in the research area, it is anticipated that housewives and retired participants who spend a significant portion of the day at home would be most affected. Therefore, 25.30% of the surveys were conducted with housewives, and 24.80% with retirees, who are mostly at home during the day. Of the participants, 13.70% are civil servants, 11.90% work in the private sector, 9.60% are self-employed, 5.80% are workers, and 2.70% are academics.

17.80% of the participants earn minimum wage or below, while the majority have an income above the minimum wage. In order to make the survey more understandable and analyzable, it was aimed to have similar numbers of participants in each neighborhood. Therefore, participants are distributed with very small differences according to neighborhoods, with the lowest being 8.70% in Mağfesığmaz and the highest being 14.60% in Yüzüncüyıl.

To assess the durations of participants' residence and the potential impact of noise in the research area, Table 5 and Table 6 evaluate the participants' neighborhoods, durations of residence, and the conditions of residential facades, considering that buildings close to main roads (boulevards) are most affected by traffic-related noise.

When Table 5 is evaluated by neighborhoods and in total, it is determined that the majority of participants have a residence duration of 10 years and above, constituting 62.41% of the total.

The preference for the location where participants live, regardless of whether they are tenants or homeowners, as seen in Table 6, is determined to be "central location" with a total of 26.75%, irrespective of facade and homeownership status. Other significant reasons for preference include proximity to family at 15.66% and transportation facilities at 12.77%.

Table 7 examines the impact of the facade condition of participants' residences (main roads (boulevards) and secondary roads (streets, avenues, and side streets)) and the floor they reside on in terms of being affected by traffic noise.

Table 5. Duration of Residence According to Participants' Neighborhoods

Neighborhood		Residence Period (Years)				
		0-2	3-5	6-10	10 and above	Total
Güzelyalı	Number	1	2	10	30	43
	%	0.24	0.48	2.41	7.23	10.36
Beyazevler	Number	0	2	5	36	43
	%	0.00	0.48	1.20	8.67	10.36
Toros	Number	1	8	13	29	51
	%	0.24	1.93	3.13	6.99	12.29
Mahfesığmaz	Number	0	2	3	31	36
	%	0.00	0.48	0.72	7.47	8.675
Karslılar	Number	5	3	6	23	37
	%	1.20	0.72	1.45	5.54	8.92
Huzurevleri	Number	2	7	11	25	45
	%	0.48	1.69	2.65	6.02	10.84
Yüzüncüyıl	Number	4	15	19	24	62
	%	0.96	3.61	4.58	5.78	14.94
Belediyeevleri	Number	5	3	8	34	50
	%	1.20	0.72	1.93	8.19	12.05
Yurt	Number	4	4	13	27	48
	%	0.96	0.96	3.13	6.51	11.57
Total	Number	22	46	88	259	415
	%	5.30	11.08	21.20	62.41	100.00

Table 6. Residential preferences of participants based on homeownership and building facade conditions.

Housing Status		Primary Reason for Choice											
		CF	EF	NL	NW	RC	CL	HF	CC	SF	TF	O	T
Main Road													
Homeowner	Number	31	10	11	10	1	56	2	6	12	21	5	165
	%	15.27	4.93	5.42	4.93	0.49	27.59	0.99	2.96	5.91	10.34	2.46	81.28
Tenant	Number	5	2	0	8	7	11	0	1	2	2	0	38
	%	2.46	0.99	0.00	3.94	3.45	5.42	0.00	0.49	0.99	0.99	0.00	18.72
Total	Number	36	12	11	18	8	67	2	7	14	23	5	203
	%	17.73	5.91	5.42	8.87	3.94	33.00	0.99	3.45	6.90	11.33	2.46	100.00
Secondary Road													
Homeowner	Number	25	7	17	15	8	37	3	12	11	25	13	173
	%	11.79	3.30	8.02	7.08	377	17.45	1.42	5.66	5.19	11.79	6.13	81.60
Tenant	Number	4	4	2	7	3	7	0	4	2	5	1	39
	%	1.89	1.89	0.94	3.30	1.42	3.30	0.00	1.89	0.94	2.36	0.47	18.40
Total	Number	29	11	19	22	11	44	3	16	13	30	14	212
	%	13.68	5.19	8.96	10.38	5.19	20.75	1.42	7.55	6.13	14.15	6.60	100.00
Total													
Homeowner	Number	56	17	28	25	9	93	5	18	23	46	18	338
	%	13.49	4.10	6.75	6.02	2.17	22.41	1.20	4.34	5.54	11.08	4.34	81.45
Tenant	Number	9	6	2	15	10	18	0	5	4	7	1	77
	%	2.17	1.45	0.48	3.61	2.41	4.34	0.00	1.20	0.96	1.69	0.24	18.55
Total	Number	65	23	30	40	19	111	5	23	27	53	19	415
	%	15.66	5.54	7.23	9.64	4.58	26.75	1.20	5.54	6.51	12.77	4.58	100.00

CF: Close to Family; EF: Educational Facilities; NL: Near the Lake; NW: Near my workplace; RC: Rent is Cheap; CL: Central Location; HF: Health Facilities; CC: Calm and Clean; SF: Social Facilities; TF: Transportation Facilities; O: Other; T: Total

Table 7. Participants' housing facade condition and the impact of traffic-related noise based on the floor of residence.

Noise Discomfort Level		Floor					
		1	2 – 4	5 – 7	8 – 10	11 +	Total
Main Road (Boulevard)							
Yes	Number	34	69	40	14	27	184
	Percentage%	91.89	93.24	90.91	87.50	95.83	92.61
	Floor Percentage%	16.75	33.99	19.70	6.90	13.30	92.61
No	Number	3	5	4	2	1	15
	Percentage%	8.11	6.76	9.09	12.50	4.17	7.39
	Floor Percentage%	1.48	2.46	1.97	0.99	0.49	7.39
Total	Number	37/13*	74/28*	44/28*	16/4*	28/10*	199
	Percentage%	100.00	100.00	100.00	100.00	100.00	100.00
	Floor Percentage%	18.23	36.45	21.67	7.88	13.80	100.00
Secondary Road (Street/Alley)							
Yes	Number	49	84	25	21	7	186
	Percentage%	85.96	89.36	86.21	91.30	66.67	87.74
	Floor Percentage%	23.11	39.62	11.79	9.91	3.29	87.74
No	Number	8	10	4	2	2	26.00
	Percentage%	14.04	10.64	13.79	8.70	33.33	12.26
	Floor Percentage%	3.77	4.72	1.89	0.94	0.94	12.26
Total	Number	57/17*	94/35*	29//13*	23/12*	9/5*	212
	Percentage%	100.00	100.00	100.00	100.00	100.00	100.00
	Floor Percentage%	26.89	44.34	13.68	10.85	4.25	100.00
Total							
Yes	Number	83	153	65	35	34	370
	Percentage%	88.30	91.07	89.04	89.74	90.00	90.12
	Floor Percentage%	20.00	36.87	15.66	8.43	8.20	90.12
No	Number	11	15	8	4	3	41
	Percentage%	11.70	8.93	10.96	10.26	10.00	9.88
	Floor Percentage%	2.65	3.61	1.93	0.96	0.72	9.88
Total	Number	94/30*	168/63*	73/41*	39/16*	37/15*	411
	Percentage%	100.00	100.00	100.00	100.00	100.00	100.00
	Floor Percentage%	22.65	40.48	17.59	9.40	8.92	100.00

In the number section, values separated by '/' and presented with '*' represent the numbers of buildings with sound insulation.

Table 8. Assessment of participants' discomfort with traffic-related noise based on the housing facade condition.

Noise Discomfort Level	Number	%
While Reading a Book/Studying	26	6.27
While Working	16	3.86
While Watching TV/Movies	39	9.40
While Resting/Sleeping	293	70.60
While Sitting in the Park	5	1.20
While Taking a Walk	11	2.65
While in a Vehicle	8	1.93
Other	0	0.00
All	13	3.13
Total	415	100.00

Table 9. Participants' opinions on the most significant environmental issue based on the facade of their residences.

Konut Cephesi		The Most Significant Environmental Issue/Pollution								
		Visual	Noise	Air	Water	Waste	All	None	Other	Total
Main Road	Number	10	140	13	1	8	6	2	1	181
	%	2.41	33.73	3.13	0.24	1.93	1.45	0.48	0.24	43.62
Secondary Road	Number	33	123	20	0	10	5	13	8	212
	%	7.95	29.64	4.82	0.00	2.41	1.20	3.13	1.93	51.08
Other	Number	1	19	1	0	0	0	0	1	22
	%	0.24	4.58	0.24	0.00	0.00	0.00	0.00	0.24	5.30
Total	Number	44	282	34	1	18	11	15	10	415
	%	10.60	67.95	8.19	0.24	4.34	2.65	3.61	2.41	100.00

Table 7 has been evaluated based on the information that four participants did not provide their floor details; therefore, out of 415 participants, 411 have been considered. When Table 6 is assessed, 92.61% of the 188 participants residing on the main road side mentioned being bothered by noise, while 87.74% of the 186 participants residing on the secondary road side expressed discomfort. The highest level of discomfort with noise is observed at 93.24% among those residing on floors 2-4 and along the main road, whereas the lowest level of discomfort is noted at 66.67% among those residing on 11 floors and above.

This finding indicates that the impact of noise pollution varies based on factors such as proximity to the noise source and barriers between the noise source and the receptor, as identified through participant perspectives.

In Table 8, participants' discomfort with traffic noise is assessed based on their housing facade condition, with a total of 9 options, including an open-ended one, for what they do most when bothered by traffic noise.

When Table 8 is evaluated, it is determined that the majority of participants, with a rate of 70.60%, are bothered by traffic-related noise "while resting/sleeping."

In Table 9, participants' opinions regarding the most significant environmental issue based on the facade of their residences are queried. The purpose of the survey is to reveal differences in the option of the most important environmental issue among participants residing on the side facing the secondary road, where traffic and thus potential noise pollution are less intense.

When Table 9 is evaluated, it is found that the majority of participants, with 67.95%, consider noise pollution as the most significant environmental issue. Among participants who identify noise pollution as the most important environmental issue, it is observed that 33.73% of them reside on the main road side. Those residing along the main road consider air pollution as a secondary issue, while those living on the secondary road consider visual pollution as one of the most important problems.

In Table 10, opinions on the most significant environmental issue are queried based on participants' education level, as an addition to the survey in Table 8.

When Table 10 is evaluated, it is determined that participants, regardless of their education level, identify noise pollution as the most significant environmental issue. For participants with a bachelor's or high school education level, visual pollution is mentioned as the second most important environmental issue.

Another point to consider is that the survey was conducted during a period when stubble burning, one of the causes of air pollution in Adana Province, was taking place. Despite this, the majority of participants expressed noise pollution as the most important environmental issue. In Table 11, daily results from the Adana-Governorate air quality measurement station covering the survey period (August 15 – September 30, 2020) are provided, showing minimum, maximum, and average values.

When evaluating Table 11, it is observed that the average values of PM10 (particulate matter) and CO (Carbon Monoxide) from the National Air Quality Monitoring Network (UHKİA) Adana data are 78.40 $\mu g/m^3$ and 292.92 $\mu g/m^3$, respectively, exceeding the limit values during the specified period.

Çukurova District is one of the new and rapidly developing residential areas where construction is ongoing, featuring boulevard-like roads, a light rail system, and numerous entertainment centers. The assumption that such noise sources also affect participants has prompted the need to inquire about which noise source causes the most discomfort. Table 12 queries participants about the noise source they are most bothered by based on the facade of their residential building.

Table 10. Participants' opinions on the most significant environmental issue based on their education level.

Education Level		The Most Significant Environmental Issue/Pollution								
		Visual	Noise	Air	Water	Waste	All	None	Other	Total
Illiterate Primary School	Number	0	4	0	0	0	0	1	0	5
	%	0.00	0.96	0.00	0.00	0.00	0.00	0.24	0.00	1.20
Secondary School High School	Number	9	52	5	0	7	3	3	1	80
	%	2.20	12.50	1.20	0.00	1.70	0.70	0.70	0.20	19.30
Undergraduate Degree	Number	6	27	2	0	0	2	1	0	38
	%	1.40	6.50	0.50	0.00	0.00	0.50	0.24	0.00	9.20
Illiterate Primary School	Number	14	92	11	1	7	3	5	4	137
	%	3.37	22.17	2.70	0.20	1.70	0.70	1.20	0.96	33.00
Secondary School High School	Number	14	94	12	0	4	3	5	5	137
	%	3.37	22.65	2.90	0.00	0.96	0.70	1.20	1.20	33.00
Undergraduate Degree	Number	1	13	2	0	0	0	0	0	16
	%	0.24	3.10	0.50	0.00	0.00	0.00	0.00	0.00	3.90
Other	Number	0	0	2	0	0	0	0	0	2
	%	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.50
Total	Number	44	282	34	1	18	11	15	10	415
	%	10.60	69.95	8.19	0.24	4.34	2.65	3.61	2.41	100.00

Table 11. Adana Province air quality results (August 15 – September 30, 2020 Adana-Governorate measurement station)*

Parameter	Minimum Value (µg/m³) - Date	Maximum Value (µg/m³) - Date	Average (µg/m3)	Limit Value (µg/m3)
PM10	34.24 - 22.08.2020	141.93 - 04.09.2020	78.40	50
CO	145.75 - 07.09.2020	412.02 - 29.09.2020	292.92	10

*(UHKİA, 2022)

Table 12. The Noise Source Participants Are Most Bothered by Based on the Facade of Their Residential Building

Residential Facade		Noise Source								
		RVT	LRS	OA	EV	CS	M	A	O	T
Main Road	Number	144	1	5	8	10	5	2	6	181
	Percentage%	34.70	0.24	1.20	1.93	2.40	1.20	0.48	1.44	43.62
Secondary Road	Sayı	98	1	27	36	16	19	2	13	212
	Percentage%	23.61	0.24	6.51	8.67	3.86	4.58	0.48	3.13	51.08
Other	Sayı	17	0	0	2	1	1	0	0	22
	Percentage%	4.10	0.00	0.00	0.48	0.24	0.24	0.00	0.00	5.30
Total	Sayı	259	2	32	46	27	25	4	20	415
	Percentage%	62.41	0.48	7.71	11.08	6.51	6.02	0.96	4.81	100.00

RVT: Road Vehicle Traffic; LRS: Light Rail System; OA: Other Apartment; EV: Entertainment Venue; CS: Construction/Site; M: Market; A: All; O: Other; T Total

When Table 12 is evaluated, it is determined that 62.41% of participants are most bothered by traffic-related noise. Among the 212 participants residing in buildings facing secondary roads, 36 of them stated that they are bothered by noise from entertainment venues.

In Table 13, participants were asked about the change in traffic-related noise pollution based on the facade of their residence compared to previous years, with options "increased, decreased, no change, and I don't know." The purpose of this survey is not only to gather information about noise pollution but also to measure participants' awareness and consciousness regarding whether there has been an increase or decrease in noise pollution over the years.

When Table 13 is evaluated, it is observed that 82.20% of participants expressed the opinion that noise pollution has increased compared to previous years, while 10.60% of participants stated that there was no change in noise pollution.

In Table 14, participants were asked about the times they are most bothered by traffic-related noise. Within this context, the season, weekdays – weekends, and the time of day when they are most bothered by noise were determined. When Table 14 is evaluated, it is found that a total of 41 participants did not specify the time they were bothered by noise. The majority, 36.96% of participants, reported being bothered by noise during the spring/summer season, on weekdays, and during the daytime interval (07:00-19:00). Considering the temporal period, it is expected that the period with the least barriers between the noise source and the receptor, when doors and windows are likely to be open due to warmer temperatures, would be identified as the time when noise is most bothersome.

Table 15 queries participants about their opinions on the effects of traffic-related noise pollution on their health. The aim of this survey is to determine not only the awareness of noise but also the awareness of its effects on health and whether participants are informed about these effects.

When Table 15 is evaluated, it is observed that 40.00% of participants stated irritability as the most significant effect of traffic-related noise on their health. Other significant effects include restlessness with 18.55% and insomnia with 10.60%. According to the World Health Organization (WHO), noise levels of 35 dB(A) and above have cognitive performance effects, those above 45 dB(A) can lead to sleep disturbances, and levels above 55 dB(A) may contribute to social behavior disorders such as distress, anger, and depression. Noise levels between 65-70 dB(A) are associated with cardiovascular and psychophysiological risks (WHO, 1999). In this context, it can be inferred that 40.00% of participants were exposed to noise levels of 55 dB(A) and above.

Table 13. Temporal Change in Noise Pollution Based on Participants' Residential Facade

Residential Facade		The Direction of Change in Noise Pollution Compared to Previous Years				
		Increased	Decreased	No Change	I Don't Know	Total
Main Road	Number	150	2	22	7	181
	Percentage%	36.15	0.48	5.30	1.69	43.62
Secondary Road	Sayı	168	13	19	12	212
	Percentage%	40.48	3.13	4.58	2.89	51.08
Other	Sayı	19	0	3	0	22
	Percentage%	4.58	0.00	0.72	0.00	5.30
Total	Sayı	337	15	44	19	415
	Percentage%	81.20	3.61	10.60	4.58	100.00

Table 14. Temporal Distribution of Participants' Discomfort with Traffic-Related Noise

Noise Discomfort Level	Weekday/Weekend		All Day Time Interval				
			07:00-19:00	19:00-23:00	23:00-07:00	Fikri yok	Total
No	Weekdays	Number	9	5	2	0	16
		Percentage%	21.95	12.20	4.88	0.00	39.02
	Weekend	Number	8	6	4	3	21
		Percentage%	19.51	14.63	9.76	7.32	51.22
	Not Disturbed	Number	0	0	0	4	4
		Percentage%	0.00	0.00	0.00	9.76	9.76
	Total	Number	17	11	6	7	41
		Percentage%	41.46	26.83	14.63	17.07	100.00
Yes	All the Time						
	Weekdays	Number	4	1	1	0	6
		Percentage%	33.33	8.33	8.33	0.00	50.00
	Weekend	Number	1	3	2	0	6
		Percentage%	8.33	25.00	16.67	0.00	50.00
	Total	Number	5	4	3	0	12
		Percentage%	41.67	33.33	25.00	0.00	100.00
	Spring/Summer						
	Weekdays	Number	119	75	43	2	239
		Percentage%	36.96	23.29	13.35	0.62	74.22
	Weekend	Number	26	30	25	2	83
		Percentage%	8.07	9.32	7.76	0.62	25.78
	Total	Number	145	105	68	4	322
		Percentage%	45.03	32.61	21.12	1.24	100.00
	Fall/Winter						
	Weekdays	Number	17	12	2	1	32.00
		Percentage%	42.50	30.00	5.00	2.50	80.00
	Weekend	Number	1	4	2	1	8
		Percentage%	2.50	10.00	5.00	2.50	20.00
	Total	Number	18	16	4	2	40
		Percentage%	45.00	40.00	10.00	5.00	100.00
Total	Weekdays	Number	149	93	48	3	293
		Percentage%	35.90	22.41	11.57	0.72	70.60
	Weekend	Number	36	43	33	6	118
		Percentage%	8.67	10.36	7.95	1.45	28.43
	Not Disturbed	Number	0	0	0	4	4
		Percentage%	0.00	0.00	0.00	0.96	0.96
	Total	Number	185	136	81	13	415
		Percentage%	44.58	32.77	19.52	3.13	100.00

Table 15. Participants' Opinions on the Most Significant Effect of Noise on Their Health

Noise's Effects on Health	Number	Percentage%
Headache	36	8.67
Restlessness	77	18.55
Hearing Loss	10	2.41
Performance Decline	5	1.20
Irritability	166	40.00
Insomnia	44	10.60
Mental and Physical Fatigue	36	8.67
All	19	4.58
Not Causing Health Issues	3	0.74
Other	19	4.58
Total	415	100.00

Table 16. Participants' Opinions on Methods Implemented in Turkey to Prevent Traffic-Related Noise Pollution

The method implemented to prevent noise pollution	Number	Percentage%
Noise Offenders Being Fined	261	62.89
Ban on Loud Music Broadcasts	185	44.57
Conducting Noise Measurements	67	16.14
Imposing Speed Limits on Vehicles Due to Noise	73	17.59
Restricting Vehicle Horn Usage in Some Specific Times and Areas	123	29.63
Using Natural (Landscaping) or Artificial (Noise Barrier) Noise Screening Along Roadsides	11	2.65
Constructing Buildings with Noise-Reducing Insulation Systems	40	9.63
Other	0	0.00
Total Number of Surveys Conducted	415	

Participants selected multiple options, but the evaluation was based on the total number of surveys conducted.

Table 17. Participants' Opinions on the Most Important Measure to Be Taken to Prevent Noise from Traffic

Measures to Prevent Traffic-Related Noise	Number	Percentage%
Imposing Financial Penalties	285	68.72
Monitoring Vehicle Horn Usage	195	46.98
Conducting Speed Controls for Vehicles	139	33.49
Mandatory Sound Insulation in Residences	62	14.93
Preserving Distances Between Residences and Main Roads/Boulevards	54	13.01
Implementing Landscaping or Noise Barrier Walls Along Main Roads/Boulevards	26	6.26
Other	0	0.00
Total Number of Surveys Conducted	415	

Participants selected multiple options, but the evaluation was based on the total number of surveys conducted.

In Table 16, participants were asked about their opinions on the methods implemented in Turkey to prevent traffic-related noise pollution. Since participants were allowed to choose multiple options, each participant expressed a different number of opinions. As there were no limitations, only participants' opinions were considered, and proportional analyses were provided based on the total number of survey participants. The same calculation was applied for Table 16.

When Table 16 is evaluated, it is observed that the method of imposing fines on noise offenders is the most supported practice, with the opinions of 261 participants. The ban on loud music broadcasts received 185 opinions, and the control of horn usage in vehicles received 123 participant opinions, identifying these options as other significant measures.

In Table 17, participants' opinions on the most important measure that can be taken to prevent noise from traffic were evaluated.

When Table 17 is evaluated, participant opinions indicate that the most supported measures to be taken at the source of noise are financial penalties, with 285 responses, monitoring vehicle horn usage with 195 responses, and conducting speed controls for vehicles with 139 responses

Results and Recommendations

UNDP (United Nations Development Programme) Turkey has presented the goals for 2030 for Sustainable Cities and Communities in the 11th Article of the Global Goals for Sustainable Development report. Accordingly, the statement includes, "strengthening capacity for inclusive and sustainable urban development and planning and managing participatory, integrated, and sustainable human settlements in all countries by 2030" (UNDP Turkey, 2023). In this context, noise control should also be considered, and cities should be made livable by creating peaceful areas, thus improving the quality of life.

Nature conservation and policies for addressing environmental issues should begin with increasing individual awareness, attitudes, and sensitivity on the subject. However, efforts to determine the environmental values of specific social groups and develop measures based on the findings are limited in Turkey, as in other countries.

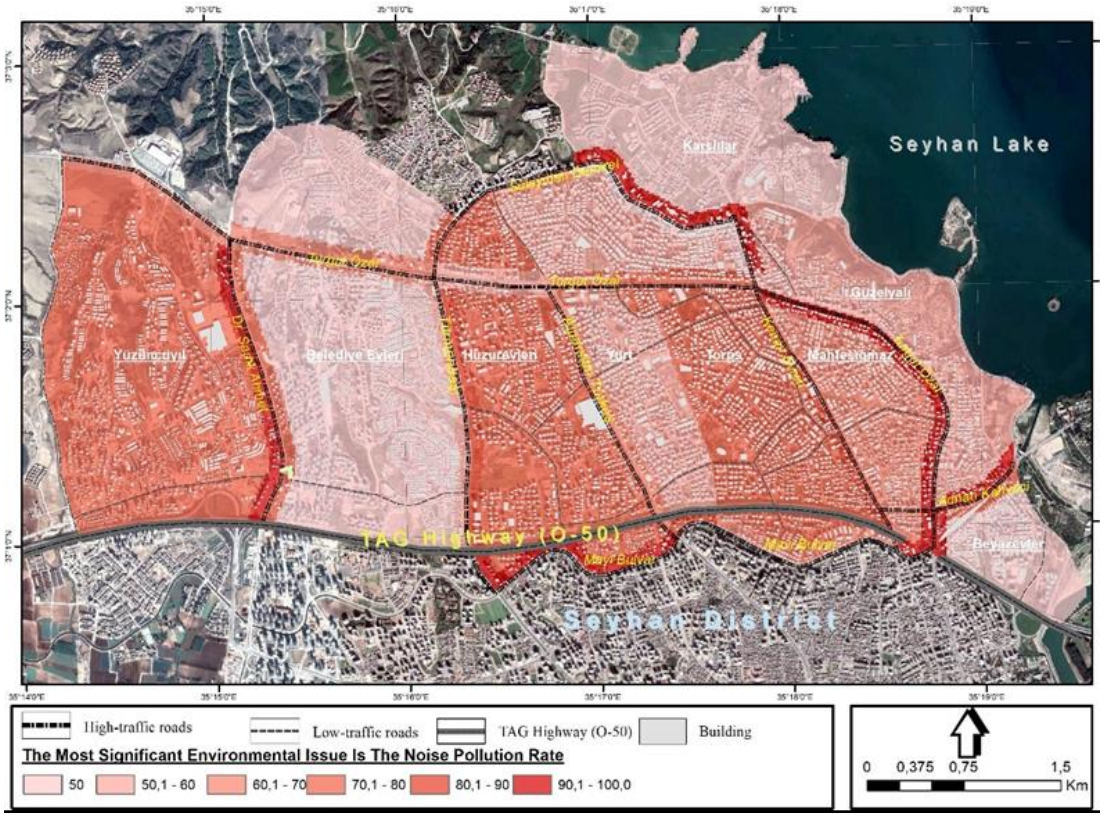

Figure 3. Spatial analysis of noise pollution awareness.

According to Yücel et al. (2006), determining the environmental awareness, attitudes, and sensitivity values of individuals in any region will provide a framework for taking measures to protect the environment and nature.

As with all environmental issues, the impact and damage caused by noise pollution, which is increasing every day, should be evaluated separately, as demonstrated in this study. According to the United States Environmental Protection Agency, the overall intensity of environmental noise doubles every decade parallel to social and industrial growth, and if unchecked, it will continue to increase uncontrollably, with the cost of reducing it in the future becoming insurmountable (Meyer, 1971, cited in Evans, 2017). In Western European countries, the Disability-Adjusted Life Years (DALY) index for traffic-related noise shows 61,000 years lost for heart disease, 45,000 years for cognitive impairment in children, 903,000 years for sleep disorders, 22,000 years for tinnitus, and 654,000 years for discomfort and anger-related disorders. This indicates that at least 1 million healthy life years are lost annually due to environmental noise related to traffic. The majority of this burden is mainly attributed to sleep disorders and discomfort caused by road traffic noise. Current assessments rank the disease burden caused by environmental noise as the second highest after air pollution (WHO European Regional Office & JRC, 2011; Hänninen et al., 2014; WHO, 2018).

Considering all these predictions and evidence, we find ourselves at a point where Dr. Robert Koch's prediction in 1910 is coming true: "One day, people will have to wage an relentless war against noise, just like cholera and plague." In this assessment, it is essential to first be aware of the encountered danger, raise awareness about preventing this danger, and act sensitively.

The existence of noise pollution from road traffic in the urban settlement area of Adana-Çukurova District, as determined by previous studies and measurements, has been proven to be known and recognized by the residents in this study (Table 9, Table 10, and Table 11). In Figure 3, a spatial analysis for noise pollution awareness has been obtained and mapped by evaluating the percentages of participants who assessed noise pollution as the most important environmental issue in the survey and considering neighborhood-road information.

When evaluating Figure 3, it is observed that individuals located near the main road (boulevard) in the research area have higher awareness of noise pollution compared to other regions. This indicates that people experiencing environmental issues have higher awareness. However, the effective solution to environmental problems lies in prevention before the environmental issue occurs. In this context, rather than increasing environmental awareness after the environmental problem arises, it is necessary to develop individuals and societies with enhanced environmental attitudes/sensitivities and use education as a means to achieve this.

In conclusion, this study is significant in terms of closely monitoring the impacts of environmental issues within the life cycle and the participation of those affected. It is important both for raising awareness in society and evaluating the participatory approach of the community in finding solutions to environmental problems. Additionally, in terms of converting the survey into a spatial analysis and usage, a unique approach has been developed in this study compared to previous works. The findings obtained will form a crucial foundation for reducing and/or preventing environmental issues such as noise pollution, and spatial analysis will play an effective role in planning measures.

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A Comprehensive Study on the Competitiveness of Governing Structures of Bulgarian Farming

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ABSTRACT

This article incorporates the interdisciplinary New Institutional Economics assumptions and principles and tries to give new insights on the real competitiveness of economic organizations in modern agriculture. It suggests a holistic framework for assessing farm' competitiveness taking into account economic, financial, and governance efficiency, and evaluates absolute and comparative competitiveness of governing structures of Bulgarian farming. The novel assessment system includes four pillars, four criteria, 17 particular, and 5 integral indicators. The first-in-kind evaluation, based on survey data, found that the competitiveness of Bulgarian farms is good. The competitiveness of cooperatives is highest, followed by corporations and associations, sole traders, and physical persons. Critical for competitive positions of farms are: low productivity, income, financial security, and adaptability to natural environment, where public support and farms' management strategies should be directed. Large shares of the country's farms have low competitiveness, and if measures are not taken to improve management, restructuring, state support, etc., many farms will cease to exist in the near future. In some cases, other characteristics of governing structures like size, specialization, market orientation, and ecological location, are critical for determining competitiveness level. The suggested and successfully tested framework for assessing the competitiveness of farms should be further improved and applied more widely and periodically in the country and internationally. The precision and representativeness of the information used should also be improved by increasing the number of surveyed farms and their important characteristics. The later requires close cooperation with producer organizations, national agricultural advisory service, and other interested parties as well as extending and improving the system for collecting agro-statistical information in the country and the EU.

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Introduction

The question of a proper understanding and evaluation of the levels and factors of competitiveness of governing structures of farming activities (farms of different type), have been among the most topical academic, ago-business, and policies issues (Falciola et al., 2020; Dresch et al., 2018; Westeren et al., 2020; Wisenthige and Guoping, 2016). There are numerous publications on the competitiveness of farms of different sizes (Alam, et al., 2020; Giaime et al., 2016; Котева, 2016; Latruffe, 2013; Mmari, 2015; Ngenoh et al., 2019; Orlowska, 2019), in major agrarian industries (Alam et al., 2020; Ngenoh et al., 2019; Benson, 2007; FAO, 2010; Иванов et al., 2020; Kleinhanss, 2020; Marques et al., 2015; Oktariani et al., 2016; Zietara and Adamski, 2018), diverse farming systems (Marques et al., 2015; Oktariani et al., 2016; Zietara and Adamski, 2018; Котева et al., 2021), specific geographical and ecological regions (Lundy et al., 2010; Ngenoh et al., 2019; Marques et al., 2015; Nowak, 2019), its key driving factors (Giaime and Mulligan, 2016; Mmari, 2015; Ngenoh et al., 2019; Oktariani et al., 2016; OECD, 2011), etc.

In the Economics theory the Agricultural Farm (or Firm) is an abstract category for describing the agent(s) managing and/or carrying out farming activity. However, the real governing structures in agriculture are the farms of different (juridical) types such as one person, family, cooperative, corporative, public, etc. farms (Bachev, 2023). The later classification is also broadly used by the international and governmental agencies, professional organizations, business community, counterparts in supply and agri-food chains, official (agro)statistics, and most researchers and experts in the area. Nevertheless, there are few profound evaluations on the absolute and comparative competitiveness of farms of different juridical types, mostly qualitative ones.

Another key starting point of the modern economic analyses is the "existence" of market and market competition ("invisible hand of market") - "in the beginning was market". In fact, that has not always been true: in not distant past during the Communist period in the Central and East European countries, market competition (governance) was not important. For a long period of time a different type of "competition" for centrally distributed targets, quotas, resources, etc. dominated the life of farming agents (structures) – namely central planning governance. Accordingly, a quite specific approach and set of indicators were used for assessing farms efficiency, viability, etc. The same was true for the first years of the post-communist transition when different governance structures coexisted for a long period of time – subsistence holdings, public farms under reorganization, privatization and/or liquidation, unsustainable farming structures based on provisional, not specified or badly specified and enforced private ownerships on lands and other resources, etc. Nevertheless, universal framework for assessing farm competitiveness is broadly recommended and practically used independent of the specific governance system of a particular country, subsector, region, ecosystem, historical period, etc.

In the modern market economy, the competitiveness of farming enterprises has been predominately assessed through traditional indicators of technical and accountancy efficiency, factors' productivity, profitability of activity, market shares, etc. However, the critical governance aspects of farm competitiveness, have been some-how ignored by most of the assessment frameworks. The later has impeded the adequate understanding and assessments of the "real" competitiveness, efficiency and sustainability of diverse governing structures that can be seen in contemporary agriculture. Consequently, many "strange" phenomena observed around the world are staying unexplained by the dominating economic orthodoxy like: why in certain periods, subsectors, regions, ecosystems, etc. often coexist diverse farm governance structures, while in others only some or a single one prevail; why there are significant variations in the competitiveness and (production) efficiency levels of different type of farming enterprises; why there are so many low efficient but highly sustainable farms in certain subsectors or regions; why some highly profitable, productive and "competitive" farms are unsustainable and constantly disappearing; why competition for re-sources and buyers do not bring to equal (Neoclassical Economics) efficiency in all farms; why there are various kinds of economic (governing) organizations in farming at all, etc.

Therefore, the first important issue tackled in this article is how to adequately assess the "real" competitiveness of major governing structures in modern Bulgarian farming – the farms of different type: unregistered individual, family or group farms, registered agro-firms, cooperatives, corporations, etc. It is logical to presume that "rational" agrarian agents will tend to select or design such a mode for governing their farming activities and relations which is most efficient (competitive) in their specific conditions (Bachev, 2011).

Incorporations of the interdisciplinary New Institutional Economics assumptions and principles give new insights on many phenomena related to the economic organizations in modern agriculture (Bachev et al., 2020; Sykuta and Cook, 2001). For instance, there has been a "successful" ex-planation of "high" efficiency and sustainability of dominating farming structures in the post-communist transition and the EU integration of Bulgarian agriculture (Bachev, 2010). However, it has been somehow "strange" that most framework evaluating the competitiveness of governing structures in farming stay blind to important governance efficiency of farms.

In recent years, a novel comprehensive approach for understanding and assessing the competitiveness of governance structures of farming activity was suggested, operationalized, experimented and gradually improved (Котева et al., 2021; Bachev and Koteva, 2021). In addition to the production and the financial efficiency, that new holistic framework takes into account the governance aspects of farms' ("competitive") potential to compete in a certain market, institutional and natural environment. Both current and long-term governance efficiency are included though assessment of farm' adaptability and sustainability. That new approach has been already applied for the assessment of competitiveness levels of Bulgarian farms in general and farms with different product specialization using macro (agro-statistical) and micro (survey) economic data (Bachev and Koteva, 2021; Котева et al., 2021).

However, there are no comprehensive assessments of the competitiveness of major governing structures of Bulgarian farming – the farming enterprises of different juridical types. Neither, there have been studies for revealing the specific relations of the competitiveness level of governing structures with other key features of farms such as operational size, market orientation, product specialization, ecological and geographical locations, etc. Therefore, the second issue delt with in this article is whether there are other critical factors, besides the governance mode, determining the competitive-ness of farming strictures in Bulgaria. If that is the case, there might be other (besides governance form) reasons for existence of certain farming structures, which are to be identified and studied.

The goal of this paper is to incorporate a holistic multi-pillars framework taking into account the Economic efficiency, Financial endowment, Adaptability and Sustainability of farms, and assess the absolute and comparative competitiveness of major governing structures of Bulgarian farming. Implementation of that new approach helps to solve the economic "puzzle" of the content and critical factors of farm's competitiveness, reveal the relations between farm's competitiveness, efficiency and sustainability, gives a new insight on the competitiveness level and prospects of evolution of diverse farming structures, and specify the importance of legal, operational, product, and territorial dimensions of farms at current stage of development in Bulgaria.

Materials and Methods

In this study a comprehensive and holistic framework for assessing the competitiveness of Bulgarian farms is incorporated including their production, financial and governance ability to compete in the specific market, institutional and ecological conditions. Detail presentation and justification of applied framework was done in

previous publications (Котева et al., 2021; OECD, 2011). According to the suggested more adequate understanding, the competitiveness of a farm means the capability (production, financial and governance potential) of an agricultural holding to maintain sustainable competitive positions on (certain) market(s), leading to high economic performance through continuous improvement and adaptation to changing market, natural and institutional environment (OECD, 2011).

The main "pillars" (aspects) of farm competitiveness are Economic efficiency (Production Pillar), Financial endowment (Financial Pillar), Adaptability (Governance Pillar for current governance efficiency) and Sustainability (Governance Pillar for long-term governance efficiency) (Figure 1). Subsequently, Good competitiveness refers to the state in which a farm (1) produces and sells its products and services efficiently on the market, (2) manages its financing efficiently, (3) is adaptable to the constantly evolving market, institutional and natural environment, and (4) is sustainable in time. On the other hand, a low or lack of competitiveness means that the farm has serious problems in efficient financing, production and sale of products and services due to high production and/or transaction costs, inability to adapt to evolving environmental conditions and/or insufficient sustainability over time.

For evaluation of the level of competitiveness of Bulgarian farms, a network system of 4 criteria for each Pillar and 17 particular indicators are selected (Figure 1). For instance, assessment criteria "Sufficient Economic efficiency", "Sufficient Financial endowment", "Sufficient Adaptability", and "Sufficient Sustainability" are used for each of the pillars of farm's competitiveness. Accordingly, appropriate Indicators for each Criterion are selected to measure the level of compliance with a particular Criterion. For the Economic efficiency and Financial endowment aspects of the competitiveness widely used traditional Indicators are used such as: Labor productivity, Land productivity, Profitability, and Income levels, and Profitability of own capital, Liquidity, and Financial autonomy.

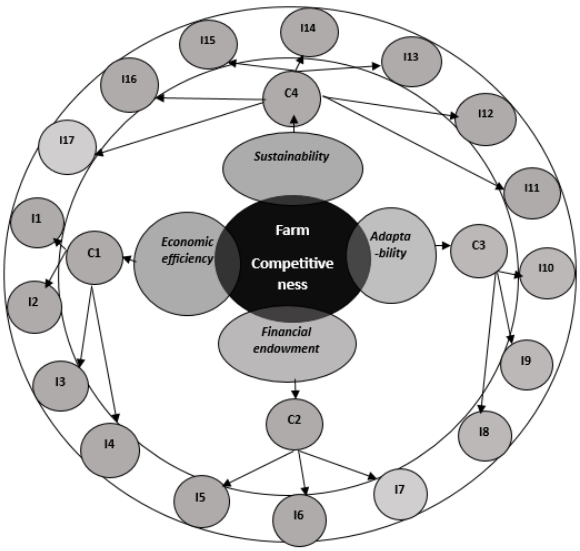

Figure 1. Framework for Assessing Completeness of Bulgarian Farms

For the other two aspects of farm's competitiveness, related to the governance efficiency, "new" Indicators are suggested. For assessing the governance structure's potential for adaptation (current governance efficiency) three measurements are select-ed: Adaptability to the market environment (market demands, prices, preferences, norms, etc.), Adaptability to the institutional environment (formal and informal rules, regulations, standards, etc.), and Adaptability to the natural environment (agro-environmental conditions, climate change, etc.). The governance structure's Sustainability (long-term governance efficiency) is determined by assessing the "level of problems and costs" for the effective supply of the necessary for the farm factors of production (land and natural resources, labor, inputs, services, innovations, and finance), and for the effective utilization and marketing of farm's products and services. Detailed justification of that novel approach for assessing farms sustainability is done by Bachev (2010).

Evaluation of farm competitiveness is made at three interconnected levels – individual competitiveness Indicators, individual competitiveness Pillars, and overall competitiveness. For the later two levels five integral indicators are suggested - Integral Aspect index (for each of the four Pillars) and the Overall Competitiveness index. That approach allows both to assess the absolute and comparative (to other governing structures) competitiveness level, to specify the competitive potential of a farm for each pillar, and identify critical factors giving competitive advantages and disadvantages (individual Indicators) of a farm. Individual competitiveness indicators often indicate quite different (unconvincing or even controversial) competitiveness of a farm which necessitates "co-measurement" and integration of indicators. In order to integrate individual indicators value with specific measurement units they are to be transferred into unitless indices which make co-measurement, comparison and integration practically possible.

The aggregate competitiveness index of farms of a particular juridical type is calculated as an arithmetic average of the competitiveness of the farms in the corresponding group. In addition, for each district legal type of farming, the aggregate competitiveness indices are calculated for the relevant farms with different operation-al size, market orientation, product specialization, and ecological and geographical locations. The later, demonstrate whether there are other important characteristics of farms (like size, market orientation, product, ecology, location) which are critical for differentiation of competitiveness level. The specific size, market orientation, specialization, and location categorization of each farm is done (self-selected or self-determined) by the farm manager according to the official classification of agri-cultural farms in Bulgaria and European Union.

The distinct and available alternative governance structures of contemporary farming activity in Bulgaria (supported by different Laws and Regulations such as Trade Law, Cooperative Law, Regulation for Registration of Agricultural Producers, etc.) are: Physical persons, Sole traders, Cooperatives, Corporations, and Associations (In Bulgaria, there are no any legal restrictions for setting up farms and carrying out farming activity by agents through

any of the legal entities in the country.) which in 2020 account for accordingly 91.4%, 1.3%, 0.54%, 6.5% and 0.21% of the total number of farming enterprises in the country (Котева et al., 2021). There are no available statistical, accountancy, report, etc. data for comprehensive assessment of the absolute and comparative competitiveness of farming enterprises in Bulgaria. Therefore, the competitiveness levels estimate in this study are based on the first-hand (survey) micro data collected from the managers of 319 "typical" farms of different juridical types in Autumn of 2020. The primary information was collected by the National Agricultural Advisory Service and major Agricultural Producers Organizations, and the structure of surveyed farms approximately corresponds to the real farm structure in the country.

During the survey, the farm managers provided relevant information for calculating competitiveness indicators of their own holdings. For the Economic efficiency and the Financial endowment pillars the indicators were calculated in the specific units such as Income level in Euro per Utilized Agricultural Area or per Labor unit, etc. For Adaptability and Sustainability Pillars the qualitative assessments of managers were used – e.g. serious, normal or no problems and costs associated with the effective supply of the necessary for the farm lands, labor, inputs etc. Besides, the managers were given possibilities to select one of the three levels (Low, Good, or High), which most closely corresponds to the condition of their own enterprise, for all indicators. Previous and parallel assessments using specific and qualitative assessments of the managers have shown similar results for the competitiveness level (Котева et al., 2021; Bachev and Koeta, 2021). Therefore, in this study, only qualitative assessment of the managers was used for calculating all competitiveness indicators to avoid problems (difficulties, controversies) for adequate ranging and co-measurement (integration) of the specific indicators' levels. Qualitative assessments have another big advantage since they give insights on farm's status and potential overcoming misleading caused by the "normal" (for Bulgaria) but considerable fluctuations of economic and financial indicators values over time.

There is no other agent but the Farm manager who knows the best and can judge precisely the (absolute and comparable) status of their holdings for each of competitiveness indicator. Thus, besides being the only feasible option that approach for primary data collection has been also most precise one for the practical experimentation of the new framework for assessing competitiveness of Bulgarian farms. Moreover, previous experimentation of the new framework using micro (farms survey) and macro (statistical) data for assessing farms' competitiveness in general and with different product specialization in Bulgaria gave similar results which proved that using farm survey data is reliable (Bachev and Koeta, 2021; Котева et al., 2021).

The qualitative evaluations of the farm managers were transformed into quantitative values, as the High levels were valued 1, the Intermediate ones 0.5, and the Lows ones 0. Following that, for each of the surveyed farms, an Integral Competitiveness Index is calculated for individual pillars and as a whole, as arithmetic averages. The competitiveness indices of the farms with different types (legal status, size, region, product specialization, etc.) were calculated as an arithmetic average from the individual indices of the constituent farms in the particular group. An equal weight is given for individual indicators and pillars as well as for each of the surveyed farm during integration of all indices. Differentiation of importance (weight) of competitiveness pillars is by definition unacceptable while differentiation of importance (weight) of individual indicators has proven to be difficult, controversial, arithmetically unsignificant (many indicators), and not recommended by the experts panel (Bachev and Koeta, 2021).

Any evaluation system is to include specific "reference values" for each particular and integral indicator to judge about the level of farm' competitiveness. For assessing the specific (indicator and aspect) and the overall levels of competitiveness of governing structures in Bulgarian farming, the following benchmarks, suggested by a panel of leading experts in the area, are applied: High competitiveness level 0.51-1, Good competitiveness level 0.34-0.5, and Low competitiveness level 0-0.32.

Results

Competitiveness Levels of Governing Structures

There is considerable variation in the level of competitiveness of agricultural farms of different legal types (Figure 2). With the highest competitiveness are cooperatives, and corporations and associations. The level of competitiveness of sole traders is good and above the industry average. The lowest is the competitiveness of physical persons, which is at a good level, but below the industry average.

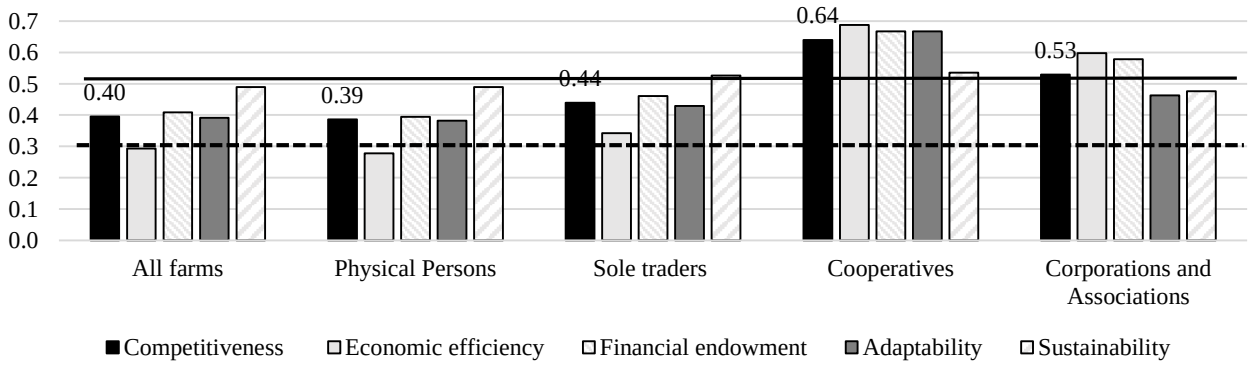

Figure 2. Competitiveness of governing structures in Bulgarian farming in general and for main pillars

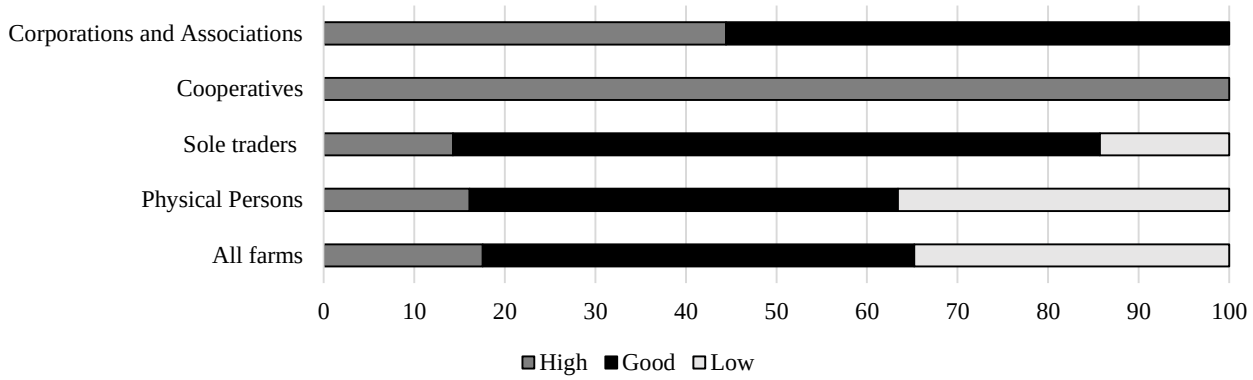

Figure 3. Share of agricultural holdings with different levels of competitiveness in Bulgaria (%)

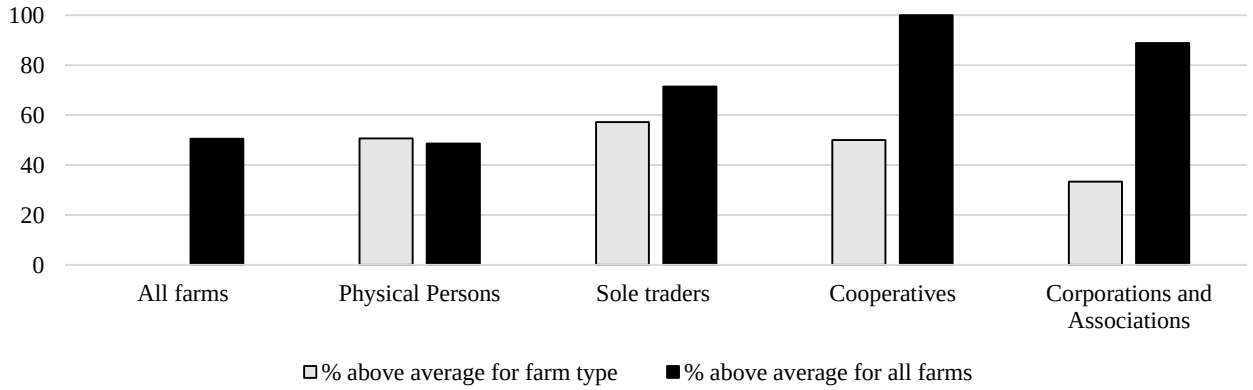

Figure 4. Share of governing structures in Bulgaria farming with a level of competitiveness above the average for all farms and the respective group (%)

All of the surveyed cooperatives, corporations and associations have a good or high level of competitiveness, including every cooperative farm (Figure 3). The share of sole trader with good and high competitiveness is also significant. At the same time, almost 37% of all physical persons have low competitiveness. Moreover, only 48.7% of physical persons have a level of competitiveness above the national average, and almost one in two is with competitiveness below the average for the group of physical persons (Figure 4). Along with this, the share of cooperatives, corporations and associations, and sole traders with competitiveness above the industry average is significant.

Integral levels for each pillar of the farms' competitiveness demonstrate that (relatively) low economic efficiency to the greatest extent contributes to the deterioration of the competitiveness of physical persons and sole traders, the low financial security of physical persons, the low sustainability of cooperatives, and the low adaptability of corporations and associations (Figure 2). At the same time, high economic efficiency conditions the strong competitive positions of cooperatives, corporations and associations, and the high sustainability of sole traders. Cooperative and corporate farms have the highest financial security and potential for adaptation to changes in the market, institutional and natural environment, and cooperatives and sole traders have the highest sustainability. Good sustainability also contributes to the greatest extent to maintaining the competitiveness of physical persons in the country.

Most competitiveness indicators of the farms of physical persons have values lower than the average for the country (Figure 5). Only in terms of inputs supply, these farms have competitive advantages compared to other governing structures.

The competitiveness of sole traders is supported by (better) good liquidity, profitability, and financial security, adaptability to the market and institutional environment, and advantages in terms of supply of services and innovations, and in the realization of production and services. Moreover, in terms of the supply of workforce and inputs, these holdings are superior to other legal types. The main factors for lowering the competitiveness of sole traders are relatively low productivity, productivity, financial autonomy, potential for adaptation to the natural environment, and weaker positions in supply of land and natural resources, and finance.

Cooperative farms have comparative competitive advantages over other legal types in terms of levels of productivity, profitability, liquidity, financial autonomy, adaptability to the market, institutional and natural environment, in the supply of labor and finance, and in the realization of production and services. Another significant part of the cooperatives' competitiveness indicators surpasses the average for the country. To the greatest extent, greater problems in supplying the necessary land and natural resources and services contribute to lowering the competitiveness of cooperative farms.

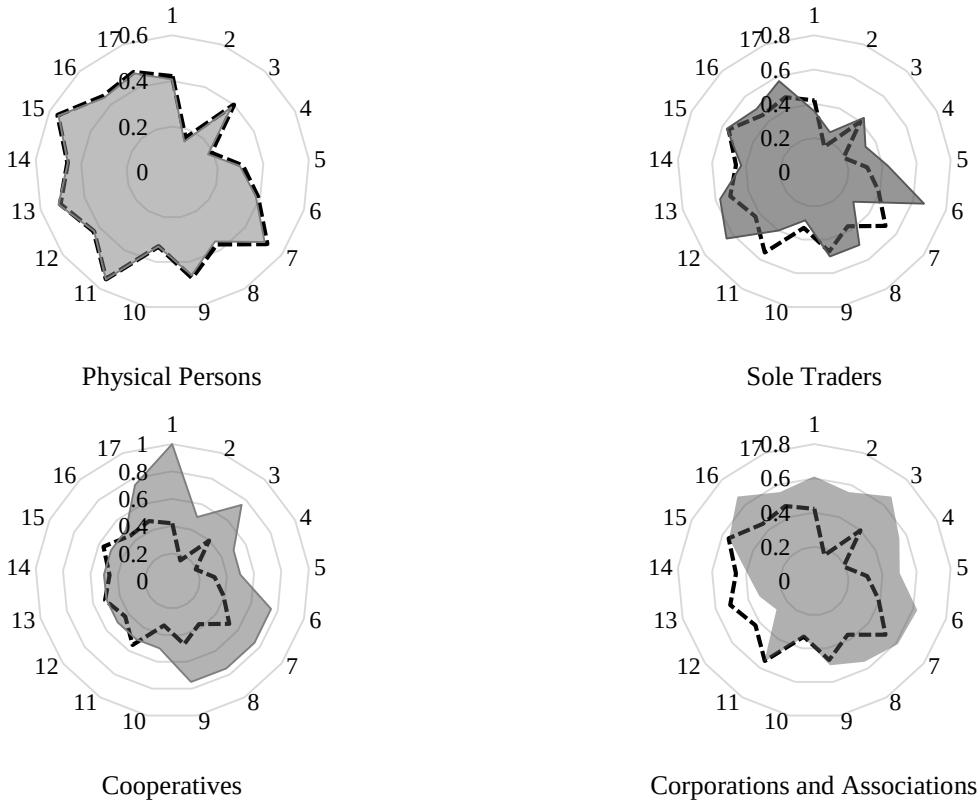

Figure 5. Competitiveness indicators* of different governing structures in Bulgarian farming (dashed line – average for agriculture)

* 1 – Labor Productivity; 2 -Land Productivity; 3 - Profitability; 4 - Income; 5 - Profitability of own capital; 6 – Liquidity; 7 - Financial autonomy; 8 - Adaptability to the market environment; 9 - Adaptability of the institutional environment; 10 - Adaptability of the natural environment; 11 - Supply of land and natural resources; 12 - Labor supply; 13 – Inputs supply; 14 – Finance supply; 15 – Services supply; 16 – Innovations supply; 17 – Utilization and marketing of produce and services

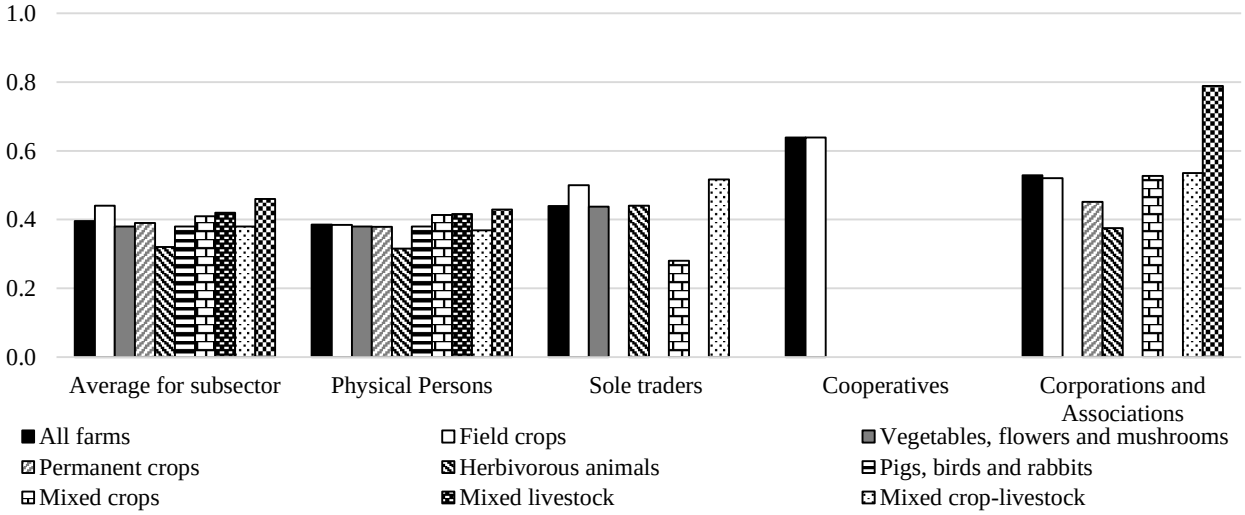

Figure 6. Competitiveness of governing structures of different type and specialization in Bulgarian farming

Corporations and associations outperform other legal types with high levels of labor and land productivity, and advantages in terms of supply of land and natural resource, and innovations. In addition, most of the remaining indicators of competitiveness of these farms are above the average for the country. Critical to maintaining the competitiveness of corporative farms are problems in supplying the necessary labor, inputs and finance, as well as average levels of adaptability to changes in the natural environment and efficiency in supplying the necessary services.

Importance of operational size, product specialisation, and location

There is considerable variation in the competitiveness of farms depending on their product specialization (Figure 6). Deviations from the average for the legal type are largest for physical persons specialized in herbivores (-0.07), sole traders specializing in mixed crop production (-0.16), and corporations and associations specialized in herbivores (-0.15) and bees (+ 0.26). These deviations are towards the average level for the sub-sector for physical persons, and corporations and associations specializing in herbivores.

On the other hand, for sole traders specialized in mixed crop production, and for corporations and associations specializing in bees, the deviations are in opposite directions from the average levels for the sub-sector.

Farms of physical persons dominate in the major types of production such as vegetables, flowers and mushrooms, herbivores, pigs, poultry and rabbits, mixed crop production and mixed livestock production. In these sub-sectors, the levels of competitiveness of physical persons predetermine the sub-sector level, while at the same time matching or being close to the average for this legal type of holdings.

In the case of farms of physical persons, and corporations and associations, there is a positive correlation between the level of competitiveness and the increase in the size of the activity (Figure 7). All of the surveyed sole traders are in the group of small farms and have a competitiveness level exceeding the average for this size group and the industry as a whole. The same applies to cooperatives, all of which are in the medium-sized group.

The situation is similar with corporations and associations, which are divided into only two groups - small and medium in size.

All governing structures in Bulgarian farming are market oriented, with exception of portion of physical persons which are mainly for subsistence farming. The competitiveness of market-oriented farms of all types is much higher than the subsistence holdings.

In the plain regions, farms with any legal status have a higher competitiveness than the rest of ecological regions, while preserving the differences reviled for the individual legal types (Figure 7). Only physical persons, and corporations and associations operating in the protected zones and territories have the lowest competitiveness.

The detailed analysis of the relationships of the level of competitiveness of governing structures in the different agrarian (administrative and geographical) regions of the country did not establish specifics different from those already established and described.

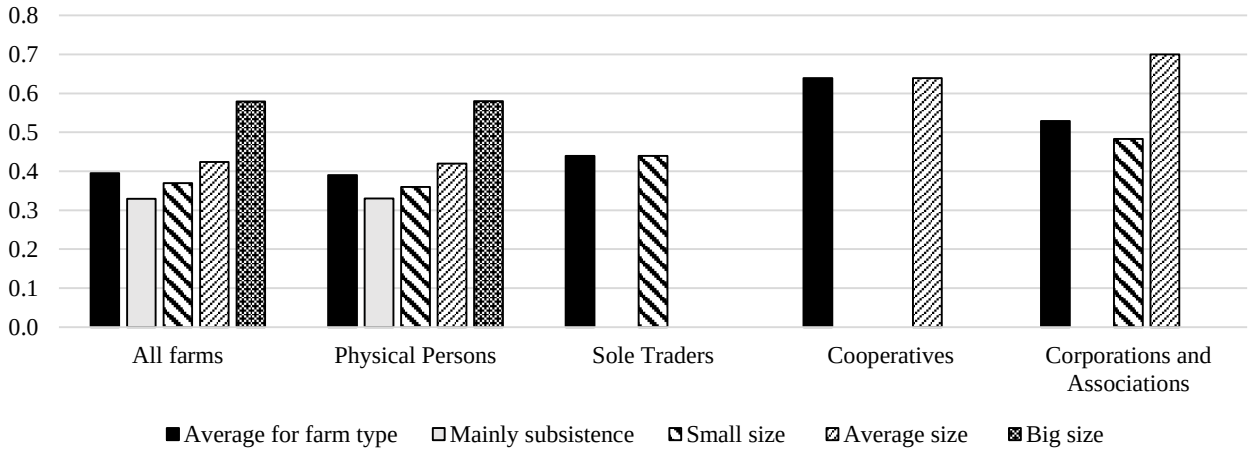

Figure 7. Competitiveness of governing structures of different sizes in Bulgarian farming

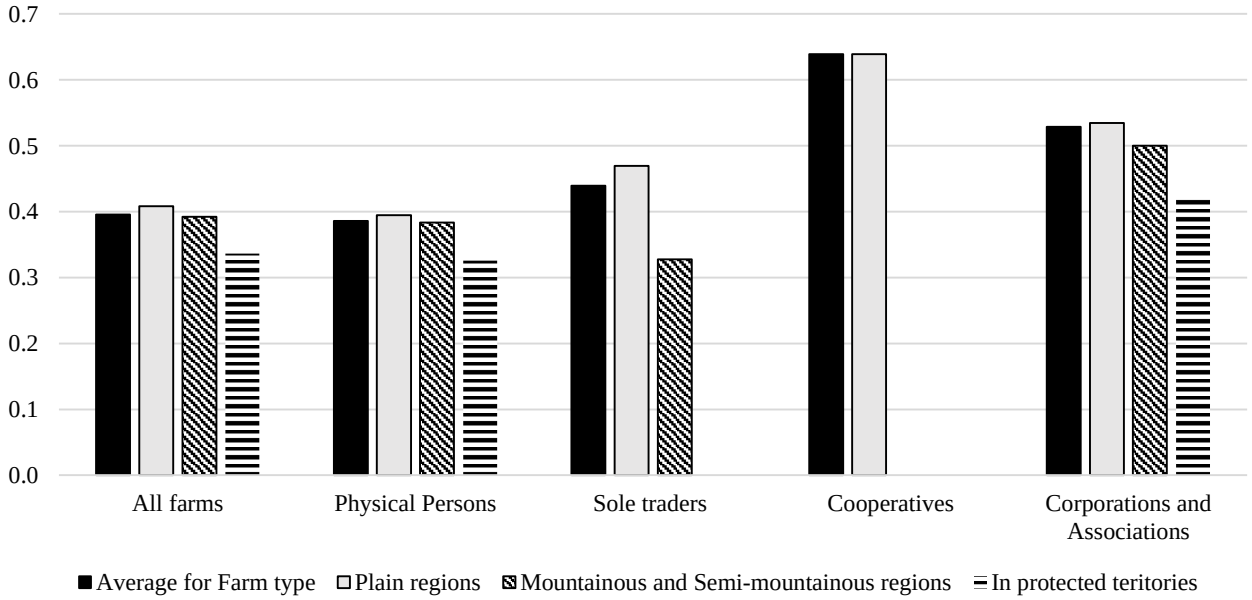

Figure 8. Competitiveness of governing structures in main ecological regions of Bulgarian farming

Discussion

This first in-kind holistic assessment of competitiveness of governing structures of Bulgarian farming found out a quite unlike levels in farms of different juridical types. The competitiveness of unregistered individual, family and group holdings (Physical Persons) is the lowest while other type of farms are with much higher competitiveness. This means that historical and current trend of transfer of agrarian resources and activity from the less competitive governing structures of the physical persons to cooperative, corporate and firm management with higher competitive advantages will continue.

Significant share of all physical persons in the country are with low competitive-ness. This means that a good part of the farms of physical persons will cease to exist in the near future, if measures are not taken in a due time to increase competitiveness by improving the management and restructuring of farms, adequate state support, etc. as a result of weak competitive positions, bankruptcies, transformation into companies and partnerships, acquisition by more efficient structures, etc. Two-thirds of corporations and associations also have below-average levels of competitiveness for this group, indicating a need for modernization to "align" with corporate governance and competition standards.

Low economic efficiency to the greatest extent contributes to the deterioration of the competitiveness of physical persons and sole traders, the low financial security of physical persons, the low sustainability of cooperatives, and the low adaptability of corporations and associations. At the same time, high economic efficiency is reason for the strong competitive positions of cooperatives, corporations and associations, and the high sustainability of sole traders.

Cooperative and corporate farms are with the highest financial security and potential for adaptation to changes in the market, institutional and natural environment, and cooperatives and sole traders have the highest sustainability. Good sustainability also contributes to the greatest extent to maintaining the competitiveness of physical persons in the country.

Individual competitiveness indicators vary considerable depending of type of farm governance. For physical persons most of their values are lower than the average for the country. Only in terms of inputs supply, farms of physical persons have competitive advantages compared to other governing structures.

The competitiveness of sole traders is supported by good liquidity, profitability, and financial security, adaptability to the market and institutional environment, and advantages in supply of services and innovations, and in the realization of production and services. Moreover, in terms of the supply of workforce and inputs, these holdings are superior to other legal types. The main factors for lowering the competitiveness of sole traders are relatively low productivity, productivity, financial autonomy, potential for adaptation to the natural environment, and weaker positions in supply of land and natural resources, and finance.

Cooperative farms have comparative competitive advantages over other legal types in terms of productivity, profitability, liquidity, financial autonomy, adaptability to the market, institutional and natural environment, in the supply of labor and finance, and in the realization of production and services. Another significant part of the cooperatives' competitiveness indicators surpasses the average for the country. To the greatest extent, problems in supplying the necessary land and natural resources and services contribute to lowering the competitiveness of cooperative governance of farming.

Corporations and associations outperform other legal types with high levels of labor and land productivity, and advantages in terms of supply of land and natural resource, and innovations. Most competitiveness indicators of these farms are above the average for the country. Critical to maintaining the competitiveness of corporative farms are problems in supplying the necessary labor, inputs and finance, as well as average levels of adaptability to changes in the natural environment and efficiency in supplying the necessary services.

There is considerable variation in the competitiveness of farms depending on their product specialization. Deviations from the average for the legal type are largest for physical persons specialized in herbivores, sole traders specializing in mixed crop production, and corporations and associations specialized in herbivores and bees. These deviations are towards the sub-sector's average for physical persons, and corporations and associations specializing in herbivores which shows that the product specialization of this group of farms is a more important factor for their competitive-ness than the legal status.

For sole traders specialized in mixed crop production and for corporations and associations specializing in bees, the deviations are in opposite directions from the sub-sector' average. This shows the additional comparative competitive advantages of corporations and associations and comparative competitive disadvantages of sole traders in certain sub-sectors of agriculture – beekeeping and mixed crop production, respectively.

Farms of physical persons dominate in major productions (vegetables, flowers and mushrooms, herbivores, pigs, poultry and rabbits, mixed crop production and mixed livestock production) and predetermine the sub-sector's competitiveness level which is close to the average for this type of holdings. This means that there is an "optimal" (competitive) specialization for the physical persons and there is practically no com-petition with other legal types in these subsectors.

Therefore, it is to be expected that the restructuring of holdings of different legal types will continue, through the concentration of resources in the most efficient groups, diversification and/or change of specialization, transformation of the legal type of the farms, etc.

There is a positive correlation between the level of competitiveness and the size of activity for physical persons, and corporations and associations. All sole traders are in the group of small farms having competitiveness exceeding the average for this group and the sector. The same applies to cooperatives, all of which are in the medium-sized group. Thus, an optimal size has been reached for realizing the maximum competitive positions of sole traders and cooperatives. Corporations and associations are only in the small and medium in size

groups. This means that competitive advantages of corporations and associations are fully realized in small and/or medium sizes depending on production (specialization, etc.), management (need to coalition of resources, etc.), or other reasons.

All governing structures in Bulgarian farming are market oriented, with exception of portion of physical persons which are mainly for subsistence farming. The competitiveness of market-oriented farms of all types is much higher than the subsistence holdings. Therefore, their future "efficiency" and sustainability would depend on other factors such as lack of income alternatives due to age of farmers, lack of skills, and remoteness of region, or as source to supplement household income, preference for independent operations or as a free time occupation, desire to preserve farm for next generation, etc.

All governing structures of farming have a higher competitiveness in the plain regions compared to other ecological regions of the country, while preserving the differences related to the legal status. Physical persons, and corporations and associations operating in the protected zones and territories are with lowest competitiveness. This shows that the specific ecological location is an additional critical factor that benefits or impairs the competitiveness of Bulgarian farms. At the same time, there are no differences in competitiveness of governing structures related to the administrative and geographical region they are located. The later demonstrates that legal, size, product and ecological characteristics of farms is more important for the competitiveness then the agrarian region they are operating.

Conclusions

This study has demonstrated the needs and proved the possibilities for (more) adequate assessment of the competitiveness of diverse governance structures in farming taking into account farm's economic, financial and governance potential. It also reviled the "complicated" relations between farm's competitiveness, efficiency and sustainability, the last being critical pillars of governance structures competitiveness. In that way, it can be explained why some type of farms maintain satisfactory production and financial efficiency indicators but are quite successful for competing in certain markets of resources and/or agrarian products.

The multi-criteria assessment of the competitiveness of farming structures in Bulgaria found that it is at a good level, but there is significant differentiation in the level of competitiveness of holdings with different juridical types. Furthermore, the study has found out that besides the juridical type, other dimensions of governance structures like economic size, market orientation, product specialization, ecological location, are critical (and sometimes more important) for determining their absolute and comparative competitiveness. This study proved results of previous assessments on competitiveness of efficiency of governing structures in Bulgarian farming based on pure qualitative (Discrete structural) analysis (Bachev, 2010; Bachev and Koeta, 2021; Котева et al., 2021; M3X, 2021; Bachev and Tsuji, 2001; Bachev et al., 2023).

The low adaptive potential and economic efficiency to the greatest extent contribute to lowering the competitiveness of Bulgarian agricultural producers. Especially critical for maintaining the competitive positions of farms are the low productivity, income, financial security and adaptability to changes in the natural environment, in which directions the public support of farms and their management strategies should be directed. A large share of farms of different types has a low level of competitiveness, and if measures are not taken in a due time to increase competitiveness by improving the management, restructuring of farms, adequate state support, etc., a large part of Bulgarian farms will cease to exist in the near future.

Nevertheless, transformation of farming governance to more competitive structures often take (a long) time because of the high transaction costs associated with initiation, transfer, development and maintenance of different governing forms in the specific market, institutional and natural environment. What is more, in addition to market competition, there are other mechanisms for governing farming activities ("visible hand of manager", "collective decision making", public intervention, etc.), and diverse contractual, informal etc. modes for governing horizontal and vertical integration of activity of agrarian and related agents. All these governing structures, be-yond the legal form and boundaries of the farm, have to be identified, studied, and their factors, importance and complementarities assessed. In that way the competitiveness of diverse governance structures in agriculture can be properly understood, evaluated, and factors and prospects of development correctly identidied.

The suggested and successfully tested framework for assessing the competitive-ness of farms should be further improved and applied more widely and periodically in the country and internationally. The precision and representativeness of the information used should also be improved by increasing the number of surveyed farms and their important characteristics (e.g. farmers age, gender, education level, agrarian experience, etc.). The later requires close cooperation with producer organizations, national agricultural advisory service, and other interested parties as well as extending and improving the system for collecting agro-statistical information in the country and the EU.

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Ecological Advancements and Developments of Agroforestry

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ABSTRACT

Agroforestry is a conventional method of land use that could help to address agricultural environmental issues. In order to take advantage of the ensuing ecological and economic interactions, agroforestry is the technique of consciously integrating woody vegetation (trees or shrubs) with crop and/or animal systems. According to recent studies, the global agri-food industry may reach more sustainable methods of producing food and fiber by adopting agroforestry techniques and principles more widely. This would benefit farmers economically and would benefit society as a whole in terms of the environment. Agroforestry promotes eco-intensification based on resource efficiency and offers a wide range of provisioning, regulating, cultural, supporting ecosystem services, and environmental advantages. In this review, we discussed agroforestry with its advantages and developments.

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Introduction

Agroforestry is a land use pattern which includes intentional growing of woody perennials along with agricultural crops or animals on the same piece of land in spatial or temporal arrangement for economical or ecological benefits ensuring an interaction between woody and non-woody components (Torquebiau, 2000). The definition figures out some key points such as i) agroforestry involves more than one species of plant, ii) the cycle of this system is more than one year, iii) the system is complex in nature, iv) agroforestry ensures multiple outputs. Although it's not the perfect definition for the term agroforestry but it's the most widely accepted. All agroforestry system must possess three vital attributes. These are i) productivity: all agroforestry system should increase productivity of the land such as increased yield or increased soil fertility etc., ii) Sustainability: the system should be able to sustain the improvement of soil fertility and the environment, iii) adoptability: the system must be accepted by farmers (Nair,1993). Poplar-wheat intercropping was observed by (Kanzler et al. 2019) to considerably lower wind speed in the system, raise nighttime temperature, and decrease daytime temperature. Compared to sole-cropping, agroforestry systems can withstand more harsh climate events like temperature changes but significantly reduce light and throughfall (Niether et al., 2018). According to (Yang et al. 2021), PAR and temperature were lower in poplar with alfalfa and jujube with wheat intercropping compared to monoculture. Farmers' desire to plant agroforestry systems is typically poor due to a lack of income information and suitable species-matching suggestions. However, the benefits of agroforestry systems in improving field microclimate have been well shown (Yang et al., 2021).

In this review, we discuss all aspects of agroforestry in brief. People can take an overall knowledge of its advantage and disadvantage from this review. We discussed how to spread agroforestry to make a sound environment.

History

The history of agroforestry is ancient. The practice started a few thousand years ago with human burning a piece of land followed by cultivating it. It is now known by slash and burn cultivation technique. However, the term 'Agroforestry' was first coined by John benny and his team in 1977 as a part of research of International Development Research Centre (IDRC) of Canada. This study paved the way of establishment of ICRAF (International Council for Research in Agroforestry). ICRAF played a vital role in conducting research on agroforestry worldwide and funding them. In 1991 the Council changed its name to International Centre for Research in Agroforestry. From 2002 ICRAF obtained the tag of "World Agroforestry"

indicating its leadership in the field of agroforestry research and development worldwide. Now a days ICRAF works for invention of modern technologies and disseminate them among farmers (King,1987; Nair 1993).

Classifications

Agroforestry system can be classified into five major categories according to dominance of its components. These are i) Agrosilviculture: in this system agricultural products are main component while woody plants are secondary component, ii) Silvoagriculture: Taungya or Shifting cultivation is the typical example of this system where woody components play major role and agricultural components are secondarily integrated, iii) Silvopasture: in this system trees are vital component and pastures are accessory component such as grazing forests, iv) Pastoral silviculture: grazing lands are suitable example for this system in which pastures are main element and woody plants are accompanying element, v) Agrosilvopasture: it is the summation of agricultural crops, woody plants and pastures where crops along with trees play authoritative role on pastures, vi) Silvoagropasture: in this system woody plants, agricultural component and pastures are pooled together where woody plants are prevalent component over other components (Atangana et al., 2014). According to the arrangement of components agroforestry system can be classified into two major categories i) spatial arrangement: It is the deliberate growing of trees with crops on same piece of land such as alley cropping, ii) Temporal arrangement: Shifting cultivation is a typical example of this. The land is cultivated for 2 to 4 years and then its left fallow for natural vegetation to regenerate.

Advantages of agroforestry

Agroforestry provide considerable advantage to environment as well as economy (Jose, 2019). As agroforestry includes trees and crops where leaves of trees fall to the ground and adds nutrients (Dossa et al.,2008). Many trees including agroforestry produce food for humans as well as animals (Table 1). The trees are deep rooted while the crops are usually shallow rooted. So, crops can utilize nutrients only from the top or upper portion of the soil column. But trees roots reach to the deep portion of soil column and uptake nutrients. These nutrients are recycled by litter fall and brought back to the surface soil (Nair, 2011). Agroforestry system including leguminous trees can add nitrogen to soil as leguminous plants can fix atmospheric nitrogen by symbiotic association with *Rhizobium* bacteria. In a study it was found that integration of poplar trees in agroforestry system has doubled the availability of nitrogen in soil by 7 kg N ha^{-1} $year^{-1}$ (Thevathasan & Gordon,2004). It has been scientifically proved that agroforestry can increase soil organic carbon (SOM). Extensive root system of woody plants serves as source for soil organic carbon in deeper layers of soil (Kell, 2012). Agroforestry system in cropland adds 3 to 1.5 times higher Carbon in soil through roots than shoots (Johnson et al.,2006).

A study conducted on soil plough depth (0-23 cm) in the presence of trees as an alley crop shows that SOM increased by 5300 kg ha^{-1} on an average (Pardon et al.,2017). Agroforestry also manifests to higher productivity. On the basis of the land equivalent ratio (LER) a few studies provide evidence to this phenomenon. A study conducted in Denmark shows that agroforestry systems requires 14-34% less input (light, water, nutrients etc.) than monoculture and the LER of the agroforestry system is 1.24-1.34 (XU et al.,2019). An experiment of silvoarable agroforestry system LER ranges from 1.3-1.6 (Lovell et al.,2018). Soil biota is very diverse including bacteria fungi, algae, protozoa, nematode etc. the abundance of these organisms depends on quality and quantity of litter, enzymatic activity, availability of nutrients (Lacombe et al.,2009). About 70% studies states positive relation between agroforestry system and soil microbial population while only 2 studies imply to negative association (Sollen et al.,2020). This establishes a positive role of agroforestry in increasing soil biodiversity. Microbes are known as decomposers. All kind of organic matter in soil is decomposed by microbial population of soil. Along with decomposition nutrients also releases and aids in nutrient cycling (Nair et al.,1999).

Table 1. Trees produce food for agroforestry systems.

Common Name	Species	Edibility	Principle Uses in Agroforestry
Cashew	*Anacardium occidentaIe*	Flowers, seeds	Garden, fence, pasture
Soursop	*Annona muricata*	Flowers	Garden, fence, pasture
Borassus-	*Borassus aethiopicum*	Multiple food uses	Garden, pasture
Pigeon Pea	*Cqjanis cajan*	Seed, leaves	Hills, nitrogen fixation, fuel, hedgerow
Papaya	*Carica papaya*	Flowers	Garden, quick shade
Chaya	*Cnidoscolus chayamansa*	Leaves	Rapid hedge
Coconut	*Cocos nucifera*	Multiple food uses	Pasture, roadside, construction
Coffee	*Coffea arabica*	Seeds (bean)	Hedges, hills, fuel
Mother of Cacao	*Gliricidia sepium*	Flowers	Living fence, feed, fuel
Leucaena	*Leucaena leucocephala*	Leaves	Hills, alley cropping, nitrogen fixation
Cassava	*Manihot esculenta*	Roots, leaves	Rapid hedge
Drumstick	*Moringa pterygosperma*	Leaves, flowers, pods	Fence, garden
Cocoa	*Theobrama cacao*	Pulp, seeds	Understory tree, pasture
Guava	*Psidium guajava*	Flowers	Pasture, fuel
Katuk	*Sauropus androgynus*	Leaves	Hedge, alley cropping
Izota	*Yucca elephantipes*	Flowers	Hedge
Jujube	*Ziszyphus mauritiana*	Flowers	Erosion control, fuel

Earthworms are known as natural plough as it stirs the soil and increases porosity. In agroforestry systems of France, the abundance of earthworms is considerably high than lands under monoculture (Barea et al., 2005). Agroforestry reduces soil erosion and increase soil water holding capacity by litter fall. The leaves of trees serve as mulching material and reduce evapotranspiration (Figure 1). Also, the leaves act as a physical barrier to run off water. Increased soil organic matters help to hold soil moisture (Sepúlveda & Carrillo,2015). Trees of agroforestry system also provide shading effect which increase humidity and reduce soil temperature as well as evapotranspiration (Schwendenmann et al.,2010). Today's one of burning topic is climate change and global warming. Agroforestry is a possible way of combating this global issue. Trees can be planted like border plants and this will help in reducing the amount of greenhouse gas like carbon dioxide (Toppo & Raj,2018).

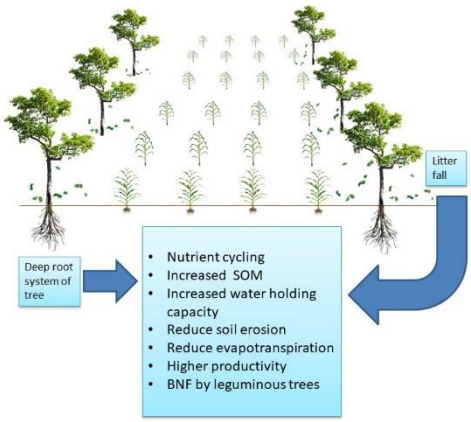

Figure 1. Role of agroforestry in agriculture and soil.

Disadvantages of agroforestry

There are some disadvantages of agroforestry too. The component of agroforestry like tree and crop affects growth of each other by interspecific competition. Tree and crops compete for light, nutrient, water etc. Through shading effect trees can reduce number of grains per spike up to 35% and grain weight up to 16% in durum wheat (Dufour et al.,2013). For nutrients, both components compete if the root system explores same depth of soil. Maize, red oak or black walnut plant's root extends up to 30 cm of soil in a dense manner. So, the yield of maize is reduced by 35% and yield of red oak or black walnut reduced by 33% as a result of nutrient competition between them (Jose et al.,2004). Allelopathic relation of many plants with crops can impose disadvantage. For example, black walnut, pecan, eucalyptus etc. secrete some kind of allelochemicals which inhibit the growth of any other plants near them (Devi, 2017).

Major challenges for today's Agroforestry are the adoption of agroforestry system by farmers. There are several reasons behind the low adoption rate of agroforestry over monoculture. Farmers do not want to pay higher amount of money for agroforestry purpose with a view to environmental benefits. In Germany 65.1% of the taxpayers are willing to invest in agroforestry while 82.1% believe it's the duty of government to take care of the environment (Otter & Langenberg,2020). So, it's easily understandable that there's a lack in public awareness in environmental conservation. Lack of capital, marketing facilities, technical knowledge among farmers are also vital reason for lower adaptability (Nouman et al.,2008). Implementation of new farming technologies comes with higher cost of production. Agroforestry system needs laborers which either cuts down manpower for crops or adds more money to production cost. In addition, Agroforestry products' profitability is still a big concern for growers (Graves et al.,2017). Research conducted on agroforestry is too limited and often there's a scarcity of data to prove hypotheses regarding the benefits of agroforestry.

Future works

The future work of agroforestry involves further research and practical implementation to solve the challenges. A study showed that community-level agroforestry adoption could be successful by conducting farmers' needs assessments and restoring farmers' confidence. It is necessary to focus on strengthening the capacity of farmers which greatly affect the adoption of agroforestry system. This may include involving the farmers in extension projects on farm-level plantation awareness campaigns and building their capacity through community engagement (Ullah et al., 2022).

The olive tree (Olea europaea L.), most widely-planted tree crop in Italy, which is covering an area of 1.16 million ha. For preventing soil erosion and soil degradation and to increase biodiversity green mulching in olive orchards has been increasingly recommended and adopted in recent decades. Preservation and the maintenance of attractive olive landscapes can play great roles in tourism. Growing alfaalfa in wide-spaced (i.e. 5 m x 10 m) olive orchards in Tuscany, it was observed that the nutritive value of the alfalfa was unaffected by the trees despite lower yields than in open field conditions.

Asparagus, a well-known perennial crop, can help reduce soil erosion by agroforestry system. By minimizing the administrative barriers associated with tree management on farm land adoption and maintenance of agroforestry can be promoted (Paris et al., 2019).

The naturally regenerating woodlots could be converted into high-value agroforestry systems by using conventional silvicultural practices. It gives supportive policies for selling farm-grown timber. Currently, the regulatory regime around the sale of farm-grown timber is highly expensive and difficult to navigate.

Tree selection is the most important factor in the agroforestry system. Such as high-density hedgerows of nitrogen-fixing trees have many benefits. Addressing terrace-based agroforestry can maximize the production of tree products on small landholdings. Research is needed to find the best way to arrange new species into existing terrace-based agroforestry.

To examine optimal spatial and temporal arrangement of trees, crops and livestock more research is needed including silviculture trials. It is necessary to pay attention on policy and regulatory barriers to the sale of farm-grown timber to remove disincentives in farm-tree growing (Cedamon et al., 2018).

The timely adoption of agroforestry is an important matter, that can improve the effectiveness of the agroforestry program. So, it is essential to motivate farmers for early adoption. Forestry extension agents can engage the less educated farmers in informal educational campaigns about the benefits of agroforestry in crop productivity. Village farmers can be motivated by establishing functional community-based organizations such as VDCs (Village Development Committees). The government could take necessary steps for the tenant farmers and establish a policy for them so tenure insecurity may not affect their timely adoption of agroforestry. Finally, the household head's age-related factors positively impact farmers' timely adoption of agroforestry. Thus, the government could ask the help of old farmers in the diffusion of agroforestry (Ullah et al., 2023).

The mulberry-dykes and fishponds agroforestry system (MFS), developed through the centuries as a result of a flood control system to protect Huzhou city from recurring floods. MFS is not only related to the cultural heritage or as an example of adaptation and mitigation, but also to it has great effect in reducing flood risk. To protect this unique agroforestry system development of adequate and specific planning instruments is necessary (Santoro et al., 2022).

Government of developing countries should provide incentives to farmers in order to encourage them for adopting agroforestry system. Extension services should introduce modern technologies among farmers. Research and studies of agroforestry should be encouraged by providing funds.

Conclusion

Agroforestry is the road to sustainable agriculture. Agroforestry provides a lot of benefits including nutrient recycling, carbon sequestration, soil conservation, better productivity any many more. Agroforestry encouragement is a relatively low-cost choice when it comes to enhancing rural living and mitigating the effects of climate change. By varying diets, agroforestry may improve food security in addition to its benefits for the environment. In this paper, we highlighted the overall of agroforestry with its advantages and disadvantages. Students can easily understand the purpose of agroforestry. We also talk about future initiatives in this area, which points to upcoming agroforestry research. It is high time for challenges in the path of agroforestry to be addressed.

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Potential Renal Effects of Cigarette Smoking in the Diabetic State-A review

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ABSTRACT

Diabetes is an alarming global systemic metabolic disorder that can pose a major threat to patients. The serious consequences of cigarette smoking on the diabetic kidney are not well known among people in different countries. According to different studies, smoking enhances albuminuria in diabetic patients. On the other hand, urinary albumin is a sensitive indicator of glomerular injury. The abnormal trans-glomerular passage of albumin may be seen due to increased permeability of the glomerular capillary wall and their subsequent impaired reabsorption by the epithelial cells of the proximal tubule. Smoking with hyperglycemia increases lipid accumulation and oxidative stress, which mainly up-regulates TGF-β, accumulates AGEs, reduces nitric oxide production, and eventually causes glomerular basement membrane thickening and mesangial expansion that results in the development of glomerulosclerosis and nephropathy. The complex interaction between cigarette smoking and diabetic mellitus poses multiple challenges for researchers, physicians, and patients. Therefore, the present review article aims to find out the feasible consequences for the kidney of a diabetic patient due to the habit of cigarette smoking which may be useful for academicians and researchers in the future.

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Introduction

Diabetic nephropathy (DN) is a microvascular complication with a high level of albuminuria that is mostly recorded in the Asia Pacific region in diabetic patients (Schena and Gesualdo, 2005). According to the International Diabetes Federation (IDF) survey in 2022, one in ten adults worldwide is currently living with diabetes, and the total number is estimated at 537 million people (WDD, 2022). Diabetic nephropathy has been also reported in about 20-25% of type I or type II diabetes patients which may increase by 6% of people per year (Kim et al., 2001). The pathophysiological mechanisms of cigarette smoking-induced changes in renal architecture in diabetic conditions have already been noted to be complex. Podocytes are specialized epithelial cells that surround the glomerular capillaries. Podocytes act as a filtration barrier attaching with endothelial cells of the glomerular capillary loop and the glomerular basement membrane and also give support to the structure and function of the glomerulus. Reduction of podocytes causes scarring, persistent proteinuria, and finally chronic kidney disease (CKD) progression. Smoking plays an important role in the progression of proteinuria and cellular alteration in the diabetic kidney, where early podocyte loss is reported as the major cause of promoting DN progression (Jaimes et al., 2021). On the other hand cigarette smoking causes vascular pathology and its long-term association can lead to hypertension (Salvatore et al., 2015). Smoking increases blood volume and blood pressure which finally enhances the glomerular filtration rate (GFR) though there are many controversial statements regarding this (Yoon et al., 2009). Pathological confirmation like glomerular basement membrane (GBM) thickening, tubular basement membrane (TBM) parallel thickening, and major mesangial proliferation, etc. ensure the actual state of diabetic nephropathy (Fioretto and Mauer, 2007). However, the present study aimed to focus on the renal complications due to cigarette smoking in diabetic conditions.

Diabetes and its Mechanism

Diabetes is characterized by chronic hyperglycemia, and impaired carbohydrate, protein, and lipid metabolism due to insufficient insulin secretion which has become an alarming public health issue. There are two types of diabetes mellitus (DM) such as insulin-dependent diabetes mellitus (type 1 diabetes mellitus T1DM) and non-insulin-dependent diabetes mellitus (type 2 diabetes mellitus T2DM). T2DM is the most common form of diabetes (Yacoub et al., 2010). In Diabetes mellitus (DM), the body

cannot regulate glucose in the blood. People eat foods that are converted into glucose by the liver. This glucose provides energy to the body. Especially blood glucose is regulated by hormones secreted from β-cells of the pancreas. Pancreas also secrete some enzyme that helps to digest food and insulin allows the glucose to move from the blood into cell throughout the body. By which the blood glucose is maintained. The pancreas is unable to produce enough insulin in type 1 diabetes, and cannot transport glucose into the cells to produce energy. For this reason, animals or people may go to unconsciousness or comma situations even this condition may turn to death. On the other hand, T2DM is a complex endocrine or metabolic disorder. Obesity is the main cause of producing insulin resistance and glucose intolerance. Finally, hyperglycemia and hyperlipidemia appear and are converted into a severe threat to the body (Sugiyama, 2011).

Associated risk factors of diabetes

Insulin is the key hormone produced by the β-cells of the pancreas that helps to transport glucose from the bloodstream into the body's cells. Several associating factors for the formation of diabetes mellitus (DM) are stated below:

- Cow milk consumption may increase the risk of type 1 diabetes.
- Hypertension, polycystic ovarian syndrome, hyperlipidemia, asthma, and sleeping disorders are associated with type 2 diabetes.
- Various medications including diuretics, immunosuppressants, some antidepressants, and chemotherapy drugs can increase the risk of developing secondary diabetes.
- Radiation therapy and pancreatectomy are also risk factors for diabetes.
- Using certain agricultural pesticides during pregnancy can lead to gestational diabetes.
- Free radicals, air pollution, and cold weather can destroy insulin-producing cells and contribute to the development of various types of diabetes.
- Cigarette smoking and alcohol consumption may also destroy the insulin-producing cells of the pancreas (Moussa, 2008 and Salvatore, 2005)

Risk factors for developing diabetic renal complications

- Long-term diabetes
- Cigarette smoking in diabetic condition
- Pre-existing hypertension
- Family history of diabetic nephropathy
- Presence of other microvascular complications
- Family history of hypertension (Ayodele et al., 2004)

How does smoking affect kidneys?

Smoking has some baleful effects on kidney functions. Blood flow disruption in the kidneys is the most common problem created by smoking. The possible way to deteriorate kidney function is given below:

Kidney structure is surrounded by many significant vascular systems. In diabetes, the small blood vessels become stiffened and gradually increase the blood pressure. In this stage, continuous smoking also increases blood pressure (BP) and may weaken or narrow the blood vessels, as a result, damaged renal arteries reduce the ability to filter blood. In turn, a damaged kidney cannot regulate blood pressure which is generally called ischemic nephropathy (Virdis et al., 2010). This high BP occurs due to peripheral vascular resistance and it gradually increases the cardiac output. This is the normal phenomenon of increasing blood pressure due to smoking in diabetes by damaging renal arteries. On the other hand, from an in vitro study, the nicotine present in the cigarette increases the level of mesangial cell proliferation and fibronectin production which plays a vital role in CKD. Endothelial cell dysfunction, and activation of growth factors (angiotensin II, endothelin-I, and *TGF*-β 1), can also be seen due to smoking which enhances oxidative stress (Dasgupta and Chellappan, 2006). Mesangial cell proliferation mainly increases the production of *TGF*-β 1, the major player in the genesis of renal fibrosis (Mur et al., 2004). According to studies from Egypt, cigarette smoking enormously accumulates cadmium (Cd) and lead (Pb) in the kidney tissue which may also affect the tubular cells. Whereas dietary exposure plus cigarette smoking are associated with tubular and glomerular dysfunction (EL et al., 2003).

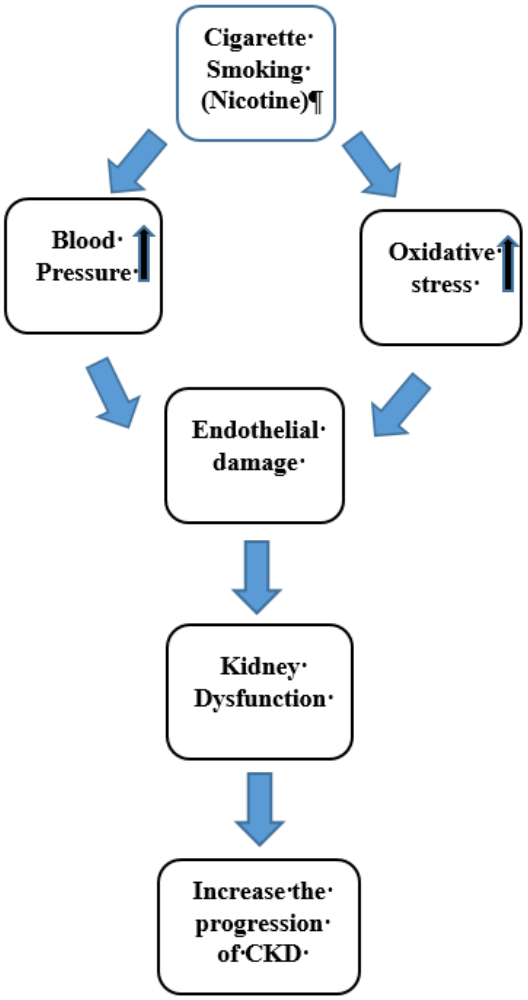

Figure 1. Possible mechanism of kidney disease by cigarette smoking (Schena and Gesualdo, 2005)

Association between cigarette smoking and diabetic nephropathy

People affected with diabetes and high blood pressure are more at risk of having heart attack or stroke. Several studies revealed that diabetic people with high blood pressure may have a chance of getting chronic kidney disease (CKD). Mainly, patients with diabetes mellitus have increased peripheral arterial resistance due to excess body fluid volume (Van Laecke and Van Biesen, 2017). According to the Multiple Risk Factor Intervention Trial (MRFIT), there is a cyclic relationship between CKD and hypertension (HTN). The elevated blood pressure gradually constricts and narrows the arteries, which finally damages and weakens throughout the body including in the kidney, and thus hampers to delivery of enough blood in the renal tissue. Long-term, uncontrolled, high blood pressure (BP) leads to high intraglomerular pressure, impairing glomerular filtration (Buffet and Ricchetti, 2012). Damage to the glomeruli leads to increased protein filtration, resulting in an abnormally high amount of protein in the urine (albuminuria or proteinuria). Albuminuria is the indicative first sign of CKD (Buffet and Ricchetti, 2012). According to the American Diabetes Association, the nicotine present in smoking suppresses appetite and increases the resting metabolic rate by which subsequent weight loss may occur. On the other hand, tobacco smoking triggers a free radical process that interferes with the functioning of vascular endothelium, increases oxidative stress, and directly damages the β-cell function (Gordon and Flanagan, 2016). Neuronal nicotinic acetylcholine receptors (nAChRs) are found in β-cell of pancreatic islets. According to several studies, there is a toxic influence of nicotine on insulin-secreting β cells. Because nicotine affects the development of pancreatic cells and increases the apoptosis of islet of β cells and finally contribute to the progression of diabetes (Bruin et al., 2010). That's why diabetes-affected people who smoke often need larger doses of insulin to keep their blood sugar close to the target levels. Furthermore, nicotine is an active compound in cigarettes that promotes excessive oxidative stress and leads to vascular endothelial cell dysfunction (VED). Eventually, nicotine also up-regulates the expression of transforming growth factor-β (TGF-β) which is the main risk factor involved in causing diabetic nephropathy (Forbes et al., 2008).

Role of macrophages (M1/M2) in the progression of diabetic kidney disease

Diabetic nephropathy is a condition of end-stage renal disease. Resident macrophages may activate by the accumulation of glucose and metabolites which have a role in the development of renal disease (Collins et al., 2015). The M1 macrophages involved in the inflammatory response secrete pro-inflammatory cytokines like CD68+/iNOS+, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-12, and IL-23 and M2 macrophages involved in anti-inflammatory response by secreting anti-inflammatory cytokine-like CD68+/Arg-1+ and transforming growth factor-β (TGF-β) (Ma et al., 2022). Though M1 and M2 play the opposite role in renal inflammation. At the early stage of renal injury, macrophages are activated by pathogen-associated molecular patterns (PAMPs), danger-associated molecular patterns (DAMPs), interferon-gamma (IFN-γ) and M1 macrophages have inflammatory effects with high expression of pro-inflammatory mediators like inducible nitric oxide synthase (iNOS) and promote tissue inflammation and damage. M2 macrophages have immunomodulatory, pro-fibrotic, and repairing effects with high expression of CD206, CD163, and arginase-1 (Arg-1). Alternatively, M2 macrophages secrete anti-inflammatory (IL-10) and pro-fibrotic cytokines (TGF-β) that promote tissue repair and fibrosis. High expression of TGF-β activates mesangial cells to produce extracellular matrix deposition via TGF-β1/Smad3 signaling pathway (Zhu et al., 2019). On the other hand, podocytes are the important parenchymal cells of the kidney, and M1 macrophages promote podocyte apoptosis by secreting the TNF-α (Lee et al., 2011).

Histopathological alteration of kidney in diabetic nephropathy condition

Glomerular changes are the most common characteristics in DN conditions. Diabetic patients with the habit of cigarette smoking, develop elevated serum creatinine levels as well as occur glomerulonephritis. Smoking can seriously affect the renal tissue, damage the vascular system as well as effects cardiovascular function. The most common pathological changes of diabetic nephropathy are the glomerular basement membrane thickening (Pourghasem et al., 2015) nodular sclerosis, (Markowitz et al., 2015) mesangial expansion, glomerular sclerosis, arteriosclerosis, (Pourghasem et al., 2015) hyalinosis of kidney blood vessels, tubular interstitial fibrosis (Pourghasem et al., 2015 and Vujičić et al., 2012). Stimulation of resident renal cells that produce the transforming growth factor TGF-β1 and upregulate GLUT-1 induce intracellular glucose transport, and TGF-β1 initiates extracellular matrix protein deposition (collagen types I, IV, V, and VI; fibronectin, and lamin) at the glomerular layer, thus causing mesangial expansion and thickening of the glomerular basement membrane. Mesangial cell expansion collapses the lumen of the capillaries that increases the glomerular volume (Salvatore et al., 2015; Schena and Gesualdo, 2015) and Lipofuscin storage (Pourghasem et al., 2015) in diabetic nephropathy due to changes in plasma lipoproteins, as it cannot be digested by tubular lysosomal enzymes and is eventually seen as storage as a residual body. Several studies described that protein glycosylation is the main reason for diabetic nephropathy. On the other hand, tubular hypertrophy and interstitial inflammation with mononuclear cell infiltration are indicative of histological alterations in the diabetic kidney. Such Progression of tubulointerstitium abnormalities finally leads to tubulointerstitial fibrosis and tubular atrophy (An et al., 2015). Moreover, nicotine in cigarettes possesses a toxic effect on podocytes that play a vital role in the kidney filtering function. Podocytes filter plasma proteins from leaking into the urine and are crucial for the healthy functioning of the kidneys. The loss of too many podocytes is the major risk factor for diabetic nephropathy and renal failure. According to the findings of different researchers

from both human podocytes and diabetic nephropathy in mouse models, large expression of the inflammatory enzyme COX2 and signs of oxidative stress are contributing to cellular injury. Nicotine also increases cell death and decreases the levels of synaptopodin, a protein that helps to prevent podocyte damage or death (Shafi et al., 2022).

Conclusion

Diabetes is now a well-known condition that is generally found in every family member who is not careful about their daily lifestyle. Several complications can arise as life-threatening problems. According to the literature, smoking undoubtedly causes various complications, especially kidney disease. From the present study, it is believed that cellular changes specifically albuminuria and macrophages (M1/M2) are the hallmarks of the progression of diabetic renal complications. Therefore, several clinical studies in model diabetic mice are needed to illustrate the pathophysiological mechanisms of cigarette smoking in producing kidney complications.

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The Relationship Between Vitamin B12 and Telomere Length: A Systematic Review

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ABSTRACT

Telomeres are natural nucleoprotein structures that cover the ends of chromosomes. The phenomenon of telomere shortening, which plays a crucial role in maintaining the stability of the genome, occurs gradually over time when cells undergo division due to the end replication issue. Multiple studies have demonstrated a correlation between telomere shortening and a range of illnesses, including diabetes, dyslipidemia, cardiovascular disease, cancer, and mortality. Diet and lifestyle can affect telomere length. There exists a beneficial association between telomere length and the Mediterranean diet, particularly with regards to the consumption of dietary fiber derived from whole grains and vegetables. Micronutrients such as vitamins and trace elements also play a role in cell metabolism. Some micronutrients, such as vitamin D, folate, and vitamin B12, are associated with telomere biology and cellular aging. Vitamin B12 is essential for DNA synthesis and epigenetic methylation processes. The present systematic review examines the results from clinical trials conducted in humans evaluating the role of vitamin B12 on telomere length. Cellular senescence is a state characterized by inflammation, altered cellular metabolism, genomic instability, and telomere dysfunction, which can be induced by changes in methylation patterns and oxidative stress. Vitamin B12 maintains antioxidative defense. Through these pathways, sufficient amounts of vitamin B12 may potentially play a role in the restoration of DNA damage. Most of the evidence is based on very few randomized clinical trials. Therefore, more extensive prospective cohort studies and better-designed randomized clinical trials are required to validate the correlations outlined in this review.

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Introduction

Telomeres are repetitive nucleotide sequences that protect the ends of chromosomes and ensure genomic stability and are considered a biomarker of biological aging (Pusceddu et al., 2015). Telomere shortening is a phenomenon that takes place with every occurrence of cell division, functioning as a reliable marker of the cell's chronological age and its near to senescence (Nomura et al., 2017). The reduction in telomere length has been found to be correlated with an increased susceptibility to various illnesses and mortality (Zarei et al., 2021). Certain micronutrients, namely vitamin D, folate, and vitamin B12, have a role in telomere biology and the process of cellular aging (Pusceddu et al., 2015). Vitamin B12, which has an antioxidant function that reduces oxidative stress, is involved in methylation reactions and increases homocysteine, the deficiency of which can compromise telomere length through increased oxidative stress (Praveen et al., 2020). The presence of sufficient vitamin B12 in the human body has the potential to facilitate the process of DNA damage repair. Additionally, it is reasonable to anticipate that vitamin B12 might play a role in safeguarding the integrity of telomeric DNA (Nomura et al., 2017).

This review aims to assess the correlation between vitamin B12 and telomere length.

Methods

Search strategy

The studies included in this systematic review were identified by a literature search conducted in both PubMed and Cochrane Library databases. The databases were searched from their inception to November 2023 using the following term: “vitamin b12 and telomere length”. The search included cross-sectional studies and Randomized Controlled Trials (RCT) as well as a manual search. All studies included in the current systematic review are summarized in Table 1.

Table 1. Studies related to vitamin B12 and telomere length

Reference	Design	Population	Method	Results
Pusceddu et al., 2019	cross-sectional study	2970 participants of the LURIC study.	qPCR	Vitamin B12 was associated with all-cause-mortality, telomere length and high-sensitive CRP in a non-linear fashion.
Praveen et al., 2020	cross-sectional study	428 apparently healthy subjects: 219 men and 209 women aged 21–88 years	qPCR and radioimmunoassay	Elderly people (≥60 years) have shorter telomeres and lower mtCN than the younger ones (<60 years). Vitamin B12 status may delay aging by preventing the reduction in length and mitochondrial DNA copy number
Shin and Baik, 2016	cross-sectional study	798 men and women aged 55-79 years	qPCR	A weak inverse relationship was found between serum homocysteine levels and leukocyte telomere length in those with elevated serum high-sensitive CRP levels. A weak inverse relationship was observed between serum vitamin B12 levels and leukocyte telomere length.
Nomura et al., 2017	cross-sectional study	7458 US adults (≥20 years of age) of the 1999–2000 and 2001–2002 cycles of the NHANES	qPCR	Serum vitamin B12 and α-tocopherol were not associated with LTL in all 4 years combined.
Ulak et al., 2023	RCT	600 Nepalese infants (aged 6 -11 mounth)	qPCR	Providing daily vitamin B12 for 1 year during infancy in a population at risk of vitamin B12 deficiency does not affect LTL.
Tucker, 2019	cross-sectional study	5581 adults of the NHANES study.	qPCR	Serum vitamin B12 and telomere length had a nonsignificant, inverse relationship in women, but no relation in men. Dietary vitamin B12 was linearly related to telomere length in women, after adjusting for age and race, but not in men.
Pusceddu et al., 2017	RCT	65 subjects (>54 years)	qPCR	After 1 year of supplementation with B and D vitamins, the relative telomere length correlated negatively with methylmalonic acid. Subjects with a change in relative telomere length above the group median also had a greater change in choline when compared to subjects below the median RTL. Changes in relative telomere length correlated positively with 5,10-methenyl-THF.
Dhillon et al., 2017	RCT	56 subclinically vitamin B12 deficient participants (27 males and 29 females)	qPCR	Whey protein isolate improves vitamin B12 and folate status in adults with subclinical vitamin B12 deficiency. The intervention provided suggestive evidence that whey protein isolate may exert significant effects on the maintenance of genome integrity.Whey protein isolate is more beneficial than soy protein isolate in people with subclinical vitamin B12 deficiency.
Pusceddu et al., 2016	RCT	60 elderly subjects	qPCR	tHcy was significantly reduced in the group taking B vitamins supplements. 5-methylTHF has been shown to be significant determinants of LINE-1 methylation in the group taking B vitamins supplements.
Chen et al., 2022	cross-sectional study	1247 pregnant women of the GUSTO study.	qPCR	Lower vitamin B12 levels are associated a higher risk of giving birth to offspring with shorter TL.

Abbreviations: qPCR, quantitative Real-Time Polymerase Chain Reaction; CRP, C-reactive protein; mtCN, mitochondrial DNA copy number; RCT, randomized clinical trial; LTL, leukocyte telomere length; RTL, relative telomere length; THF, tetra hydro folate; GUSTO, Growing Up in Singapore Towards healthy Outcomes

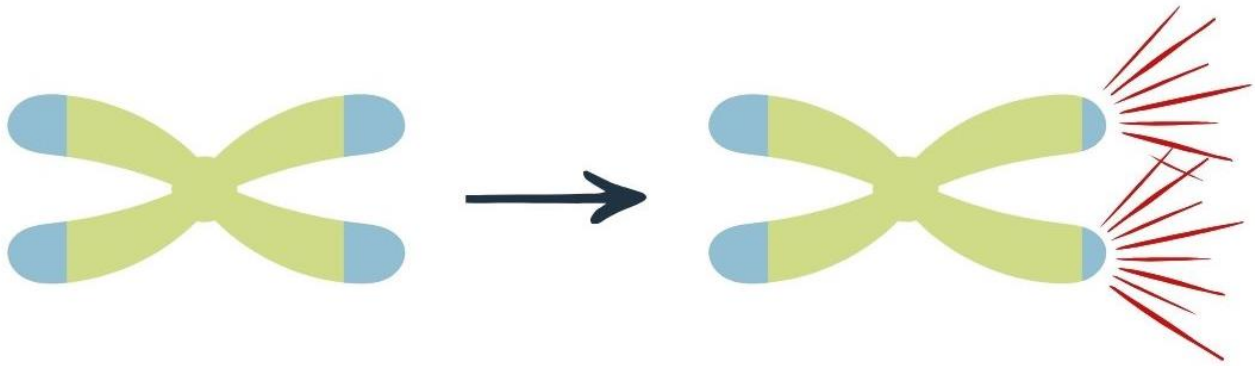

Figure 1. DNA Damage Occurring After Cell Divisions.
(Shay and Wright, 2019).

Telomere Structure and Properties

Telomeres in humans are the natural ends of chromosomes. Telomeres are composed of hexameric tandem repeat DNA sequences, namely the highly conserved "TTAGGG" motif, along with related proteins. These ends, which close and protect the eukaryotic chromosome ends, are processed as DNA double-strand breaks (Raftopoulou et al., 2022; Shin and Baik, 2016).

Telomeres are of paramount importance in preserving the integrity of the genome and controlling the process of cellular senescence. Telomere length control is influenced by several factors, including telomere binding proteins, telomere covering proteins, telomerase, and DNA replication enzymes (Zarei et al., 2021). The length of telomeres undergoes a gradual reduction over time due to the end replication issue, and this phenomenon is closely linked to the development of age-related ailments (Taub et al., 2022).

Telomeres are specialized nucleotide sequences situated at the terminal ends of chromosomes, serving as protective structures that safeguard the chromosome against destruction and maintain its structural integrity. Having long telomeres is an advantage. Telomere regions serve to mitigate the destruction of genes located in proximity to the terminal sections of chromosomes by facilitating the inevitable shortening of chromosome ends that transpires during the process of chromosomal replication. Telomere lengths undergo a gradual reduction as a consequence of repeated cellular divisions occurring over the course of an individual's lifespan (Zarei et al., 2021).

The length of telomeres is greater in females than to males (Paul, 2011). A study involved the examination of blood samples obtained from a sample of 43 young individuals (aged 18-32 years) and 47 older adults (aged 65-83 years). The analysis revealed that the telomere length in the younger cohort was observed to be 11.52% greater compared to the older cohort. In the older age group, it has been observed that females exhibit a telomere length that is 12.5% more than that of males (Bull et al., 2009).

Telomeres in humans refer to the repeated DNA sequences located in the terminal regions of linear chromosomes. In the context of normal cellular division, it is seen that telomeres undergo a progressive shortening process. When some chromosomal termini experience a reduction in length, an uncapped telomere has the potential to induce a signal of DNA damage, leading to the cessation of cellular growth (Figure 1) (Taub et al., 2022).

The regulation of telomere length is governed by epigenetic mechanisms including DNA and histone methylation. Deficiency in the DNA methyltransferases DNMT1 or both DNMT3a and DNMT3b or the histone methyltransferases Suv39 or Suv4-20h results in longer-than-normal telomeres without epigenetic markers. The deficit of methyltransferase does not result in any changes to telomerase expression, either through DNA loss or histone methylation. The regulation of telomere length is influenced by the methylation status of telomeric and subtelomeric regions, which governs the accessibility of telomere extender proteins to these areas (Paul, 2011).

Genomic DNA damage triggers a transient DNA damage response (DDR) that is not sufficient for the occurrence of senescence. The occurrence of irreversible DNA damage at telomeres leads to an extended DDR and the activation of senescence-associated secretory phenotype (SASP)-mediated inflammation, ultimately resulting in cellular senescence. Senescent cells undergo chromatin rearrangement, resulting in the creation of heterochromatin. This process leads to significant alterations in gene expression, an increase in cell size and protein content, as well as modifications in the structure of both the cell and its organelles (Tchkonia et al., 2013). These events in the stem cell context disrupt the properties of the stem cell and alter differentiation. In tissues that undergo rapid cell division, the length of telomeres decreases and elicits a DDR when they reach a dangerously short threshold. Telomere malfunction in non-proliferating, post-mitotic tissues can be attributed to irreversible DNA damage occurring inside the telomeres. Persistent DDR activation maintains an senescent phenotype characterized by aborted proliferation and activation of the SASP (Rossiello et al., 2022). Senescent cells have the potential to induce chronic inflammation throughout the aging process, hence contributing to the development of several age-related illnesses. SASP factors are the main mediators of this effect (Di Micco et al., 2021).

The absence of telomeres during cell divisions results in the loss of chromosomal ends and the vital genetic material they carry. Telomeres serve as protective caps located at the termini of chromosomes, effectively safeguarding their integrity. Over the course of cellular division, telomeres undergo attrition, although they are subsequently restored by the action of the enzyme telomerase. Telomerase deficiency has been shown in association with a range of chronic illnesses and pathological states, including diabetes, dyslipidemia, bacterial infections, cancer, and psychological stress. Risk of early death may increase with telomere shortening (Schellnegger et al., 2022; Zarei et al., 2021).

Aplastic anemia, Alzheimer's disease, chronic kidney disease, chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, age-related macular degeneration, ischemia-reperfusion injury, myelodysplastic syndrome, non-alcoholic fatty liver disease, alcoholic liver disease, primary biliary cirrhosis, Parkinson's disease, and Type 2 diabetes are examples of age-related diseases that are linked to cellular aging and telomere dysfunction (Rossiello et al., 2022).

Nutrition and Telomere Length

The process of biological aging involves the depletion of telomeres and changes in mitochondrial DNA, whereas dietary variables can impact genomic stability (Praveen et al., 2020). Telomere length gradually decreases with each cell division, and emerging research indicates that lifestyle factors may contribute to the process of telomere shortening (Welendorf et al., 2019). The length of telomeres can be influenced by diet and lifestyle factors, since these factors have the potential to impact inflammation, oxidative stress, and psychological stress, all of which contribute to the erosion of telomeres (Paul, 2011).

The consumption of whole grains and other plant-based diets has been found to have a mitigating effect on inflammation. Hence, it may be inferred that the consumption of dietary fiber, particularly derived from whole grains, exhibits a favorable correlation with telomere length (Paul, 2011).

Micronutrients, including vitamins and trace elements, exert a significant influence on cellular metabolism, hence directly affecting telomere biology and the process of cellular aging (Zarei et al., 2021).

The Mediterranean diet is widely acknowledged as a prominent dietary regimen for the purpose of disease prevention and promoting healthy aging. This reputation is mostly attributed to its well-documented anti-inflammatory and antioxidant characteristics, which have been seen to have an impact on the length of telomeres. The findings of a comprehensive examination and statistical analysis of cross-sectional research indicate a positive correlation between increased adherence to the Mediterranean Diet and extended telomere length (Canudas et al., 2020). In a study examining the effects of dietary patterns and inflammation indicators on telomere length, a negative relationship was observed between processed red meat intake and telomere length (Nettleton et al., 2008).

The findings derived from a sample of American adults that accurately represents the nation's population indicate a positive correlation between the oxidative balance score and the length of leukocyte telomeres in women. The utilization of the oxidative balance score serves as a means to assess the impact of one's food and lifestyle on the exposure to oxidative stress. There exists a positive correlation between a higher oxidative balance score, which signifies a greater prevalence of antioxidant exposure relative to prooxidant exposure in one's food and lifestyle, and an extended leukocyte telomere length. The present discovery implies that the adherence to an antioxidant-based diet and lifestyle has a safeguarding influence on the length of telomeres (Zhang et al., 2022).

A probable association exists between the length of telomeres and lifestyle factors, such as levels of physical activity and dietary patterns. The implementation of regular physical activity and a healthy diet have been postulated to potentially mitigate the process of telomere shortening, hence exhibiting potential anti-aging properties (Güneşliol et al., 2023).

Vitamin B12

Vitamin B12 is found in foods of animal origin and is necessary for genomic stability and cellular metabolism. Vitamin B12 is known to have a significant effect on the development of the nervous system and blood cells, especially in cases where cells change rapidly (Owen et al., 2021).

B vitamins (Folate, vitamin B6, and vitamin B12) in the one-carbon metabolism pathway have been associated with DNA methylation (An et al., 2019). Vitamin B12 plays an important role in DNA methylation metabolism due to its participation in homocysteine metabolism. (Boughanem et al., 2020). Vitamin B12 acts as a cofactor for the methionine synthase enzyme, ensuring the conversion of homocysteine to methionine (Ankar and Kumar, 2022). This enzyme catalyzes the formation of methionine from homocysteine using 5-methyltetrahydrofolate, which is then converted to tetrahydrofolate. Methionine undergoes a conversion process leading to the formation of 5-adenosylmethionine, which serves as a crucial element in several biological methylation events, such as DNA and histone methylation (Boughanem et al., 2020).

In the context of vitamin B12 insufficiency, the conversion of homocysteine to methionine and methyl tetrahydrofolate (THF) to THF is impaired. Consequently, the accumulation of homocysteine levels occurs, leading to the impairment of pyrimidine base formation, so impeding the process of DNA synthesis and resulting in the development of megaloblastic anemia. Anemia subsequently gives rise to symptoms such as weariness and paleness, which are frequently observed in individuals with a shortage in vitamin B12 (Ankar and Kumar, 2022). Furthermore, increased homocysteine levels can cause cognitive decline through oxidative damage (An et al., 2019).

Vitamin B12 serves as a cofactor for the enzyme methylmalonyl-CoA mutase, facilitating the conversion of methylmalonyl-CoA to succinyl-CoA. In individuals suffering from vitamin B12 deficiency, the concentration of methylmalonic acid (MMA) will increase due to the inability of its conversion to succinyl-CoA.

There exists a hypothesis suggesting that the coexistence of elevated levels of MMA and homocysteine contributes to the occurrence of myelin destruction, hence elucidating the underlying mechanisms behind the observed neurological impairments, such as neuropathy and ataxia, in affected individuals (Ankar and Kumar, 2022; Green and Miller, 2022). The presence of a deficiency in Vitamin B12 has been linked to several health conditions, including pernicious anemia, abnormal neurological development, neural tube defects, insulin resistance and, paradoxically, an increased risk of gestational diabetes mellitus. (Owen et al., 2021).

Absorption of vitamin B12 in the distal ileum requires intrinsic factor (Silverstein et al., 2022). The production of intrinsic factor, a glycoprotein, occurs inside the parietal cells located in the stomach. The glycoprotein in question serves a pivotal function in promoting the absorption of vitamin B12, particularly inside the terminal ileum. Once absorbed, vitamin B12 is used as a cofactor for enzymes that participate in the biosynthesis of DNA, fatty acids, and myelin (Ankar and Kumar, 2022).

Due to the higher prevalence of malabsorption in the elderly, vitamin B12 deficiency appears to be more common (Green and Miller, 2022). Those who have had a stomach or small bowel resection, those with inflammatory bowel disease, those who have used metformin for more than four months, those who have used proton pump inhibitors for more than 12 months, those who use histamine H2 blockers, vegans or strict vegetarians, and adults over the age of 75 are at risk for B12 deficiency (Langan and Goodbred, 2017).

The Role of Vitamin B12 in Telomere Length

The regulation of telomere length and mitochondrial DNA structure involves epigenetic mechanisms, namely methylation and histone modifications. These epigenetic processes can be influenced by dietary micronutrients, including vitamin B12 (Ma et al., 2019).

Vitamin B12 is an essential cofactor required for two enzymatic reactions in the human body. One important aspect is that vitamin B12 serves as a cofactor in the remethylation process of homocysteine to methionine, which is facilitated by the enzyme methionine synthase. On the other hand, vitamin B12 is the cofactor for the isomerization of methylmalonyl CoA to succinyl CoA by the enzyme methylmalonyl CoA mutase (Pusceddu et al., 2019). Figure 2 shows the reactions related to the recruitment of vitamin B12 as a cofactor.

Homocysteine due to its role in one-carbon metabolism; folate is considered a functional marker of vitamin B6 and B12 availability. The inadequate presence of any of these vitamins might impede the process of homocysteine detoxification, resulting in hyperhomocysteinemia. This condition can lead to an imbalance in oxidative reactions and an elevated production of reactive oxygen species (ROS), including peroxides and free radicals. ROS has the capability to induce detrimental effects on DNA, such as causing damage to DNA bases, breaking DNA strands, and hastening the process of telomere shortening (Herrmann and Herrmann, 2022).

Vitamin B12 plays a crucial role in the maintenance of the body's anti-inflammatory defensive mechanisms (Herrmann ve Herrmann, 2022). Cellular senescence is a state that is brought about by changes in methylation and oxidative stress, resulting in many manifestations such as inflammation, disturbances in cellular metabolism, instability in the genome, and failure of telomeres (Pusceddu et al., 2019; Shin and Baik, 2016). DNA damage and altered DNA methylation are important risk factors for cancer, cardiovascular diseases, developmental and neurological abnormalities (Fenech, 2012).

Vitamin B12 is essential for the biosynthesis of methionine and S-adenosyl methionine (SAM), which serves as a crucial methyl donor involved in the maintenance of DNA methylation patterns that regulate gene expression and chromosomal conformation (Fenech, 2012; Liu et al., 2013). The process of converting homocysteine into methionine, which serves as the precursor for SAM, is catalyzed by a reaction that relies on the presence of vitamin B12 (Paul, 2011). Adequate concentrations of vitamin B12 may contribute to the repair of DNA damage or would be expected to promote the maintenance of telomeric DNA (Nomura et al., 2017). DNA damage is significantly higher in individuals with inadequate folate, vitamin B12, and vitamin B6 intake (Jiang-Hua et al., 2014; Rossiello et al., 2022). Figure 3 shows the relationship between DNA methylation and vitamins.

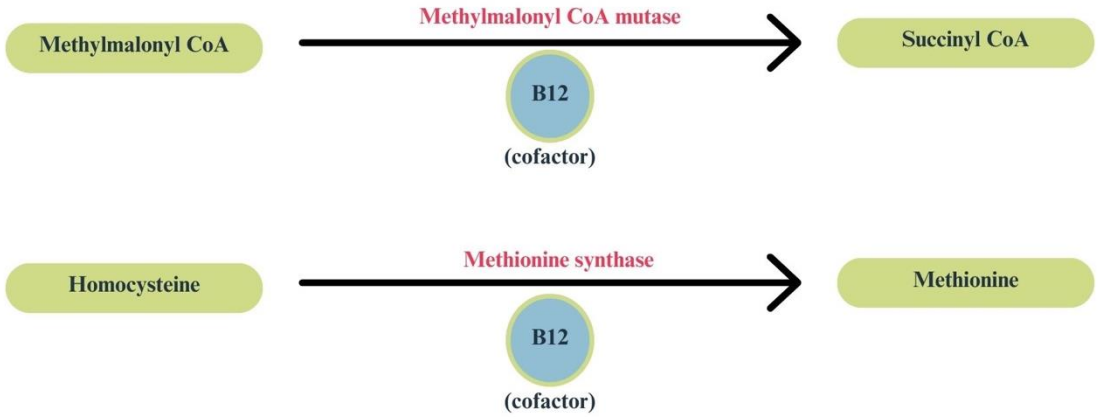

Figure 2. Reactions in which Vitamin B12 Serves as a Cofactor (Boachie et al., 2020).

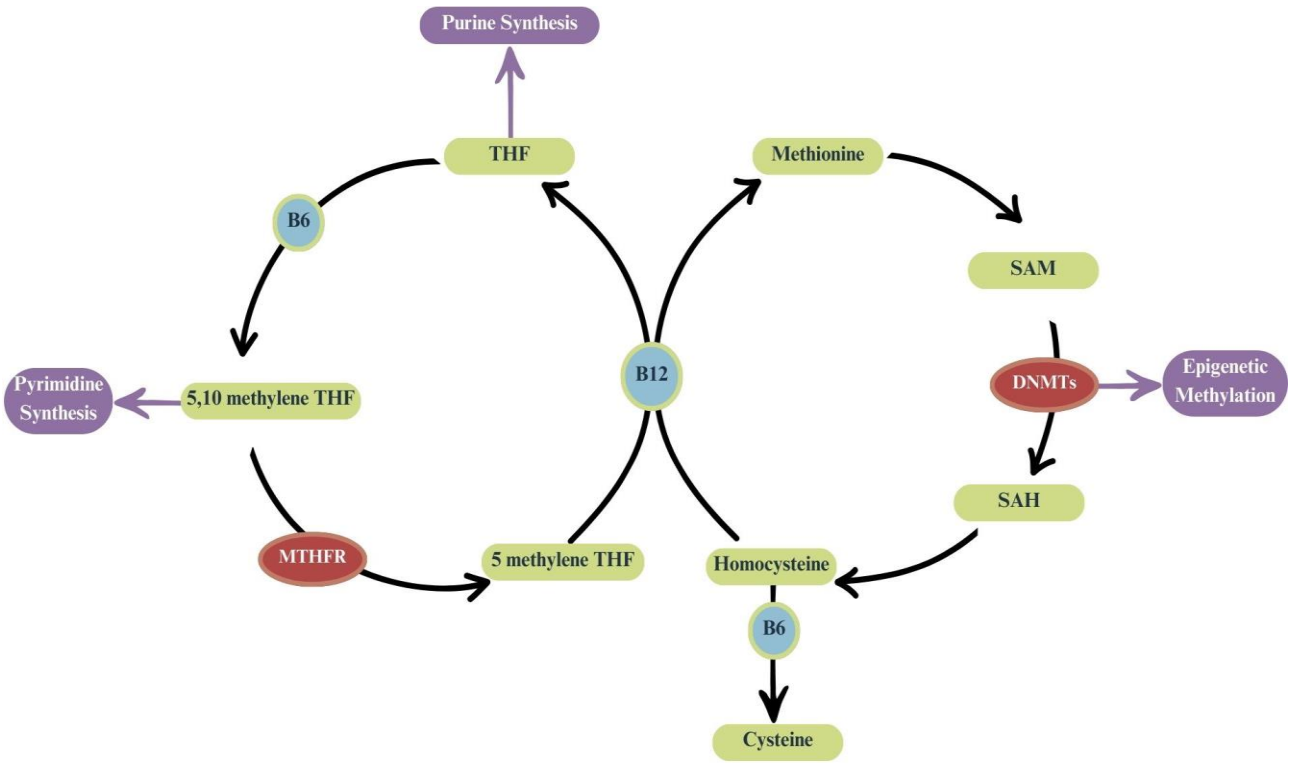

Figure 3. DNA Methylation and Vitamin Relationships (Froese et al., 2019).
Abbreviations: THF, tetra hydro folate; MTHFR, methyl tetra hydro folate reductase; DNMTs, DNA methyltransferases; SAM, S-adenosyl methionine; SAH, S-adenosyl homocysteine.

In a cross-sectional study evaluating the relationships between telomere length and serum folate, vitamin B12 and homocysteine; Serum homocysteine levels due to low-grade systemic inflammation and the presence of adequate folate and vitamin B12 were found to be inversely related to leukocyte telomere length. The research further proposed the maintenance of circulating homocysteine and C-reactive-protein (CRP) levels within the range of normal values as a potential strategy for postponing biological aging in individuals who are in good health (Herrmann and Herrmann, 2022).

It has been shown that plasma folate and vitamin B12 levels in elderly individuals may affect the integrity of the mitochondrial genome, telomere length, and epigenetic regulation of telomere length through DNA methylation (Chou et al., 2007). Plasma folate and vitamin B12 levels may influence aging by stabilizing telomere length and mitochondrial DNA copy number. (Praveen et al., 2020).

According to the findings of Pusceddu et al. (2019), individuals exhibiting either a deficiency or an excess of vitamin B12 display certain features that are linked to increased death rates when compared to those individuals with intermediate levels of vitamin B12. Insufficient vitamin B12 levels and hyperhomocysteinemia appear to be associated with overall DNA methylation and telomere length.

In a different study, telomere length shortened as serum folate and vitamin B12 levels decreased in women. Folate and vitamin B12 have been identified as significant micronutrients in the biological aging process of women (Tucker, 2019).

The length of telomeres in women is positively correlated with the consumption of vitamin B12 supplements. The administration of excessive amounts of vitamin B12 through supplements has the potential to impede the activity of nitric oxide synthase, leading to a potential decrease in inflammation. The potential mechanism underlying the observed elongation of telomeres in individuals who use high doses of vitamin B12 supplements might be attributed to the mitigated levels of oxidative stress and inflammation resulting from the supplementation (Paul, 2011).

The potential impact of folate, vitamins B6, and B12 on telomere biology has been seen in blood cells. Additionally, insufficient levels of vitamin B and elevated levels of homocysteine in the blood have been linked to changes in DNA methylation and telomere length (Pusceddu et al., 2016). Vitamin B12 (cofactor for methionine synthase) plays a role in the conversion of homocysteine to methionine in one-carbon metabolism and is important for the development of the placenta and fetus in sufficient concentrations during pregnancy (Wilson et al., 2016). Given the crucial impact of maternal nutrition on fetal development, a recent study examined the potential influence of prenatal plasma fatty acids and vitamin levels on the length of telomeres in newborns. The findings of this study revealed a noteworthy positive correlation between the level of vitamin B12 during pregnancy and the length of telomeres in newborns (Chen et al., 2022).

Vitamin B12, which is involved in the production of methyl groups and nucleotides necessary for DNA and histone methylation, is also linked to genome stability. Research has demonstrated a correlation between plasma levels of vitamin B12 and the length and functionality of telomeres (Herrmann and Herrmann, 2022; Praveen et al., 2020; Pusceddu et al., 2019).

All studies included in the current systematic review are summarized in table 1.

Conclusion

The present review aims to examine the impact of vitamin B12 on telomere length. Telomeres are natural regions that envelop and safeguard the termini of chromosomes. The process of biological aging is influenced by telomere attrition, which may be modulated by variables such as genomic stability, dietary elements, stress, and elevated levels of ROS. Vitamin B12, recognized for its involvement in methylation processes, furthermore exhibits antioxidant properties that mitigate oxidative damage. B12 deficiency increases homocysteine, which can compromise telomere length through increased oxidative stress. The length of telomeres has the potential to be influenced by dietary micronutrients, including vitamin B12. Further research is required to explore the correlation between vitamin B12 and telomere length.

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