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Exploration of Two *Cucurbitaceae* Fruit (Muskmelon and Watermelon) Seeds for Presence of Phytochemicals, and Antioxidant and Antimicrobial Activities

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ABSTRACT

Cucurbitaceae family fruits, especially melons, offers significant quantities of minerals carotenoids and phenolic compounds, contributing to their antioxidant activity. However, seeds of these fruits are usually discarded as waste by products. In current study, seeds of watermelon (*Citrullus lanatus*) and muskmelon (*Cucumis melo*) were separated, dried, grounded and extracted, with 70% ethanol, to investigate total phenolic content (TPC), flavonoid content (TFC), carotenoid content (TC) content, and total antioxidant activity (TAA). Further, antimicrobial activities of these extracts were tested against selected bacterial and fungus strains. Results showed that extracts of both *cucurbits* presented significant amounts of phytochemicals, with higher quantities presented by watermelon seeds. In watermelon seeds, TPC were found 156.50 mg/GAE 100 g, TFC 56.78 mg CE/100 g, TC 36.65 mg/100 g, and TAA 71%, and these amounts were significantly higher than those found in muskmelon seeds. Antimicrobial study results showed that extracts of both seeds exhibited significant zone of inhibitions against three bacterial and three fungal species, and these values were very comparable to the reference antimicrobial drug used, Ciprofloxacin. Findings of current research work provided significant grounds for presence of phytochemical bioactives in two melon fruits seeds, providing the basis for extraction and utilization of these bioactives, through processing and fortification different pharma foods.

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Introduction

Numerous natural substances that are found in plants are used to treat a variety of diseases. The usage of synthetic pharmaceuticals has become more prevalent in modern society, but they always have negative effects on humans. Fruits and vegetables in particular play a significant impact in promoting health and lowering the risk of disease (Mala and Kurian, 2016). By preventing cell oxidation and scavenging the free radicals generated in the body, antioxidants play a critical role in the body of life during various types of chronic diseases. In a live organism, antioxidants prevent oxidizing chain reactions. Antioxidants can be either synthetic or natural. Due to their carcinogenic consequences, the use of synthetic antioxidants is restricted (Skandrani et al., 2010). Human diseases are brought on by bacteria, viruses, fungi, and other parasites; as a result, millions of people have died, been disabled, and experienced social and economic difficulties. Despite the fact that many diseases may be treated with safe and effective medications, many people do not have affordable, safe, or healthy access to the resources they need to prevent or treat these illnesses. Seeds of the *cucurbit* fruits are a good source of antibacterial compounds (Hammer et al., 1999).

Melons are quite popular internationally, because of their delicate and delicious flavor, but seeds of these fruits, loaded with phytochemicals and sustainable ingredients for novel food formulations, go away as waste streams (Hussain et al., 2022b). Food waste is a major concern for the entire planet. Several fruits, including watermelon, muskmelon, and others, are accessible in the summer.

Fruits like muskmelons are eaten all around the world. Approximately 32% of muskmelon, which comprises 5% of the seeds and 27% of the peel by weight, is wasted. Muskmelons' seeds offer excellent nutritional value. They contain a huge variety of bioactive substances, including phenolics, flavonoids, and carotenoids. Muskmelon seeds can be used to cure or prevent a wide range of illnesses since they include antibacterial, antioxidant, and antidiabetic qualities. Thus, the seeds can be used as a meal that serves a purpose (Kumar et al., 2022). Watermelon seeds, a by-product of watermelon juice extraction, has high potential for recovery of value-added bioactive compounds (Rico et al., 2020).

Among *Cucurbitaceae* fruit crops that are commonly grown throughout the world, are the melons. In actuality, melons play a significant role in global fruit and vegetable production. The sweetness, pleasant flavour, and high nutritious content of melon are only a few of the qualities that characterize a melon's quality (Zhao et al., 2023). *Cucurbitaceae* fruits have significant quantities of carotenoids and phenolic compounds as well as excellent antioxidant activity, making them a rich source of phytochemicals. It has also shown that the various fruit components from this family responded differently in terms of antioxidant activity. At the immature stage, the majority of the antioxidant activity was discovered (Kubola and Siriamornpun, 2011). Due to presence of biochemical constituents in *cucurbits* and their role as pharma foods, these fruits have been widely consumed around the globe, as each part of these fruits have loads of bioactives (Hussain et al., 2023).

By utilizing the most recent methods, bioactive chemicals found in *Cucurbitaceae* seeds can be extracted to their fullest potential (Massa et al., 2019). According to Jukic et al. (2019), flour made from *cucurbit* seed oil press cake can be utilized to make functional and nutritious food ingredients. Functional food products could be developed by incorporation of seeds and peels of *cucurbit* fruits (Hussain et al., 2022c).

Although several research investigations have been done on different members of *Cucurbitaceae* family, but limited data could be found upon phytochemistry of the seeds of these fruits. Keeping in view the problems created as a result of waste generated due to consumption and processing of different melon fruits and vegetables, the objectives of current investigations were to explore seeds of two melons for presence of phytochemicals, and antioxidant and antimicrobial activities.

Materials and methods

Procurement of Fruits, Reagents, Chemicals and Microbial Strains for Study

Watermelon and muskmelon, for investigations of their seeds, were manually harvested from the fields, when they were fully developed and mature, and were brought to the Department of Food Science and Nutrition, University of Sargodha, where botanical identification of the fruits was carried out with the help of experts from the botany department. During the harvesting, uniformity of size, color, shape and weight was considered, and the harvested samples were precooled to remove field heat, and analyzed after being kept in a dark place at temperature between 20 and 25°C. All the reagents and chemicals used for this study were purchased from Tech Chemicals Ltd. Located in the Liberty market of Lahore, Pakistan. All these chemicals were of Sigma Aldrich, Germany. However, strains of bacteria and fungus were acquired from the Biochemistry Department, University of engineering and Technology, Lahore. Reference antimicrobial drug was purchased from Clinix pharmacy, Lahore, Pakistan.

Preparation of Muskmelon and Watermelon Seeds Powders

Muskmelon and watermelon seeds were rinsed with distilled water after being cleaned with tap water. To preserve the sample, the fruit seeds were immersed in 0.2% sodium metabisulphite for a period of fifteen minutes. Then, these seeds were dried in a hot air oven (model 400/D200°C, New Ethics®, São Paulo, Brazil) for 72 hours at 150°C, by following the procedure earlier adopted by Hussain et al. (2021a). The dried seeds were then ground into powder using a stainless-steel grinder, sieved, and stored in an airtight container in the laboratory shelf.

Development of Ethanolic Extracts From Muskmelon and Watermelon Seeds

For development of ethanolic extracts of the seeds, procedure from the studies of Hussain et al. (2021a) was followed with required adjustments. Briefly explaining, 200 g of each powder was well mixed with solvent having 70% ethanol and 30% water, and this solution was placed in a shaker for 48 hours after that filtration was carried out using muslin cloth. Then the extracts were further concentrated at 40°C in a rotary evaporator to find out the final crude extracts. Yield of extracts was found out by below given formula;

$$\text{Yield (\%)} = \frac{\text{Solvent free extract weight (g)}}{\text{Weight of dried extract (g)}} \times 100$$

Determination of Total Phenolic Contents (TPC) In Ethanolic Extracts of Muskmelon and Watermelon Seeds

The Folin-Ciocalteu colorimetric method, as described by Hussain et al. (2021a), was used to determine the TPC of the fruits seeds samples. In a nutshell, 20 mL of sample were combined with 1.57 mL of water, 100 mL of Folin-Ciocalteu reagent, and 300 mL of 7% Na_2CO_3 were added to the mixture, after 6 to 8 minutes. For two hours, the mixes were maintained at room temperature in the dark. A spectrophotometer (Spectronic, model 4001/4, ThermoFisher Scientific, Waltham, MA, USA) was then used to measure the absorbance at 765 nm. The results are presented as mg GAE/100 g of the dry weight of seed.

Determination of Total Flavonoid Contents (TFC) In Ethanolic Extracts of Muskmelon and Watermelon Seeds

For TFC determination in two melon seed powders, the protocols were followed from the procedure already adopted by Hussain et al. (2022d), with some modifications. Explaining briefly, 0.5 mL diluted solution was taken and 2 mL distilled water was added and then 5% $NaNO_2$, 0.15 mL was also added. Then, 1 mL of 1 M NaOH was added after 5 minutes. This final solution was thoroughly mixed for some time and using a spectrophotometer the absorbance at 510 nm was measured, in triplicate and results were presented as mg of quercetin equivalent per 100 g of dried weight.

Determination of Total Carotenoid Content (TC) In Ethanolic Extracts of Muskmelon and Watermelon Seeds

For total carotenoid determination, the spectrophotometric technique was modified per the one introduced by De Carvalho et al. (2009), with necessary changes. The melon seeds extracts were analyzed for total carotenoid content using a spectrophotometer (10S UV/visible spectrophotometer, TSG, Australia). The samples were examined by the spectrophotometer in the visible spectrum between 190 and 1100 nm. Then 3 mL of the diluted samples was utilized for analysis at wavelengths of 450, 470, and 502 nm after the samples were diluted with ethanol. The blank was comprised of ethanol. The spectrophotometer was constructed with a quartz cuvette. The absorbance coefficient for ethanol was determined using the extinction coefficient of 1% and the coefficient of A1%, respectively.

Determination of Total Antioxidant Activity (TAA) In Ethanolic Extracts of Muskmelon and Watermelon Seeds

According to the methodology adopted by of Aryal et al. (2019) with certain adjustments, the total antioxidant activity (TAA) of melon seeds powders was assessed using the DPPH method. Based on an estimation of the stable 1,1-diphenyl-2-picrylhydrazyl radicals' ability to scavenge free radicals, the antioxidant capacity of the samples was assessed. By detecting the drop in absorbance at 517 nm, the DPPH scavenging ability was assessed spectrophotometrically using spectrophotometer (Biochrom, Libra S22, and England). Each reaction was performed in triplicate to find out the means values of TAA, which were presented as mg trolox/100 g dry weight.

Determination of Antimicrobial Activities of Muskmelon and Watermelon Seeds Extracts

Antibacterial assay

Antibacterial activity of ethanolic extracts of two melon seeds was tested against three different microbial pathogens (*Bacillus cereus, Escherichia Coli* and *Streptococcus aureus*). Nutrient agar media was used for standardization of bacterial pathogens. Seeds ethanolic extract prepared were tested for the antimicrobial activity through well diffusion method. In each well 100 micro liters of ethanolic seed extract was added. Using sterile swabs, bacterial strain cultures were plated on Mueller Hinton agar and adjusted to a final concentration of 108 CFU/mL using a 0.5 McFarland standard. 40 L were pipetted into the different wells for each 500 mg/mL extract. As a standard, Ampicillin was utilized at a final concentration of 30 g/mL. Afterward, the plates were incubated for 24 hours at 37°C by following the guidelines of Neglo et al. (2021). Mean values of zone of growth inhibitions (mm) were calculated.

Antifungal assay

Pure cultures of three fungal species (*Candida albicans*, *Mucor meihi* and *Aspergillus niger*) were collected in same way as bacterial. Nutrient agar was used to provide the growth medium for the antifungal test, as was reported by Barbero-Lopez, (2020). Following an autoclave (120°C, 15 min), 15 mL of each growth medium was cast in a petri dish and cooled under sterile circumstances. As a control, a growth medium containing 4% malt, 2% agar, and milli-Q water was created. Using a plug measuring 0.28 cm^2, the fungus was inoculated under sterile circumstances. Following that, the petri dishes were subsequently maintained at 25°C and 65% relative humidity in a growth chamber. Depending on the fungal strain and replicates, it took between 8 and 14 days for the fungus to spread across the entire petri dish when they were growing in the control samples. Zone of inhibition in mm was calculated for the antifungal activity of the muskmelon and watermelon seed extracts.

Statistical Analyses of The Obtained Results

The findings of each analysis were produced in triplicate, and they were presented as means and standard deviations. The statistical analysis was conducted using the one-way ANOVA approach. Using the Duncan's multiple-range test, the mean values were separated. For statistical analysis, guidelines from Steel et al. (1980) procedures were used.

Results and Discussion

Extracts Yield of Muskmelon and Watermelon Seeds

Yield of ethanolic extracts of both watermelon and muskmelon seeds has been given in Table 1, from where it was evident that among the seeds of the two melons, watermelon seeds had the highest percentage of extract yield and muskmelon seeds had the lowest percentage of extract yield. Waste by products of melon and watermelon are cheap and sustainable sources of valuable bioactive compounds, utilization of their extracts in food industry could be proved useful in development of pharma foods, capable of lowering economic potential on populations (Rico et al., 2020).

Singh et al. (2016) extracted various *cucurbit* fruit components using various solvents, and their findings were generally consistent with those of the present, leading them to the conclusion that the seeds of these fruits contain valuable bioactives in their extracts. In a related investigation, Xanthopoulou et al. (2009) extracted four different types of *cucurbit* seeds groups using four different types of solvents and calculated the yield as g of extract per 100 g of seed. The acquired results were sufficient to support the findings of the current study. Similar findings were also reported by Hussain et al. (2021a), during extraction of *cucurbit s*eeds using 70% ethanol.

Since all volatile and non-volatile components are extracted by the mixing of two types of solvents, extracts of two melon seeds were made using the most environmentally friendly and productive solvents, ethanol and water in a 70:30 ratio. Because seeds are sites of higher metabolism, a higher output of extracts from them may reflect the presence of more active ingredients (Asif et al., 2017).

Table 1. Extracts yield of muskmelon and watermelon seeds

Melon seeds	Extracts yield (%)
Muskmelon seeds	9.13 ± 0.15^{b}
Watermelon seeds	11.05 ± 0.1^{a}

Values are presented as means of triplicate analysis along with standard deviation. Whereas, different alphabetical letters in a column represent significant results (P>0.05).

Table 2. TPC, TFC, TC and TAA of watermelon and muskmelon seeds extracts

Treatments	Total Phenolic Contents (mg GAE/100 g)	Total Flavonoid Contents (mg CE/100 g)	Total Carotenoids (mg/100 g)	Total Antioxidant Activity (%)
Muskmelon seeds extracts	105.25±0.25[b]	34.60±0.10[b]	22.90±0.20[b]	48.56±0.15b
Watermelon seeds extracts	156.50±0.30[a]	56.78±0.25[a]	36.65±0.15a	71.0±0.10[a]

Different alphabetical letters in a column represent significant results (P>0.05). (P< 0.05), TPC; total phenolic contents, TFC; total flavonoid contents, TC; total carotenoid content, TAA; total antioxidant activity, GAE; gallic acid equivalent, CE, catechin equivalent

TPC, TFC, TC and TAA of Watermelon And Muskmelon Seeds Extracts

Results presented in Table 2 showed that extracts of both *cucurbits* presented significant amounts of phytochemicals, with higher quantities presented by watermelon seeds. In watermelon seeds, TPC were found 156.50 mg/GAE 100 g, TFC 56.78 mg CE/100 g, TC 36.65 mg/100 g and TAA 71%, and these amounts were significantly higher than those found in muskmelon seeds. These results provided strong evidences of presence of phytochemicals in seeds of these two melon varieties, due to which these seeds have huge potential to be used as medicinal ingredients and to develop pharma foods that could promote health.

Among the significant phytochemical classes, carotenoids, flavonoids, and phenols are recognized for their ability to promote health. Bioactive chemicals are crucial for human nutrition and health since they prevent the start of numerous diseases (Algarni, 2020). For the purpose of evaluating the antibacterial and antioxidant activity of meat products, Boeira et al. (2018) isolated bioactive components from herbal plants and added them. They claimed that the main bioactives responsible for these therapeutic activities are phenolics and flavonoids. Lemon grass extracts may be used with chemotherapeutics to decrease the risk of drug-related toxicity and boost the efficacy of the therapy.

The values of antioxidant activity for seeds were consistent with the current findings when Singh et al. (2016) extracted different portions of *cucurbit* fruits with various solvents to assess their antioxidant activity by DPPH free radical scavenging technique. Alkaloids, saponins, flavonoids, and steroids are the phenolic compounds that have been discovered to be effective antioxidants and are abundant in pumpkin fruit sections, particularly the seeds and pulp (Mala and Kurian, 2016). According to Dissanayake et al.'s (2018) analysis of the antioxidant properties of the skin, seeds, and leaves of *cucurbit* fruits, seeds exhibit substantial antioxidant properties because they contain significant amounts of phenolic and flavonoid chemicals.

In their earlier investigations Hussain et al. (2022a) observed that seeds of *cucurbit* fruits have great potential to be used as antioxidants sources. Findings of Amin et al. (2018) were also in line, reporting antioxidant activities of muskmelon seed extracts. Similarly, presence of bioactives in watermelon seeds, leading towards antioxidant properties was reported by Lopusiewicz, (2018). Presence of significant amounts of carotenoids in melon seeds was reported by Fundo et al. (2018), supporting the current results regarding carotenoid contents in muskmelon and watermelon seeds extracts.

Antimicrobial Activities Of Melon Seeds Ethanolic Extracts

Table 3 presents the results of the antimicrobial activities of ethanolic extracts of two melon seeds extracts against bacterial and fungal strains, compared to the standard antibiotic ampicillin. The standard antibiotic ampicillin exhibited the highest zone of inhibition against all tested bacterial and fungal strains, indicating its potent antimicrobial activity, whereas antimicrobial activities of two melon seed extracts were also very comparable to the reference drug. Melons seeds extracts have the potential to be used as natural sources of antibacterial chemicals, as evidenced by the antimicrobial properties reported in this study. Incorporating these extracts into food products or developing topical applications could offer alternative strategies for microbial control and preservation.

Cucurbitaceae fruit sections contain phytochemicals such saponins, tannins, flavonoids, alkaloids, and steroids that may have been acting as antibacterial agents (Chonoko and Rufai 2011). In a related study, Dissanayake et al. (2018) used three bacterial and fungal strains to test the antibacterial efficacy of extracts from *cucurbit* skin, seeds, and leaves using three different types of solvents. They discovered that the seeds showed a prominent zone of inhibition. Melon seed extracts are effective antifungal agents, according to a subsequent investigation by Pandey et al. (2010). Three different fungi strains were used as test subjects, and volatile components from the essential oils of *cucurbit* seeds were discovered to be important in regulating the fungus' growth. Findings of Amin et al. (2018) were also in line, reporting high antimicrobial activities of muskmelon seed extracts. Studies of Lopusiewicz, (2018), also provided scientific evidences for strong antimicrobial potential of watermelon seeds.

Extracts of different plant-based household wastes, including those of melon, pumpkin, squash and watermelon, have potential to counter the growth of a range microbes, possibly due to presence of antimicrobial substances in their wastes (Barbero-Lopez, 2020). Experiment by Neglo et al. (2021) provided scientific results regarding peel, skin, rind and seeds of watermelon extracts for antibacterial activities, and zone of inhibitions against all bacterial species were prominent from seed extracts as compared to peel and pulp extracts. Further phytochemical analysis of watermelon seed extracts revealed the presence of phenolics, flavonoids, alkaloids, saponins, and tannins. Sovljanski et al. (2022) investigated the nutritional, antimicrobial, and phytochemical properties of five samples of peel, pulp, and seed extracts using spectrophotometric methods of *in vitro* antioxidant potential as well as in vitro antimicrobial testing methods.

Table 3. Antimicrobial activities of ethanolic extracts of muskmelon and watermelon seeds

Melon seeds extracts	Antimicrobial activities (Zone of inhibition mm)					
	Bacterial strains			Fungal strains		
	Bacillus cereus	*Escherichia coli*	*Streptococcus aureus*	*Candida albicans*	*Aspergillus niger*	*Mucor meihi*
Watermelon seed extract	15.20±0.1[b]	14.30±0.1[b]	17.40±0.2[b]	12.10±0.1[b]	14.20±0.2b	12.30±0.3[b]
Muskmelon seed extract	11.20±0.3[c]	12.10±0.2[c]	15.10±0.3[c]	10.10±0.2[c]	13.70±0.3c	11.20±0.2[c]
Ampicillin (Reference)	23.50±0.5[a]	21.30±0.4[a]	20.60±0.3[a]	16.20±0.10[a]	15.30±0.3a	17.50±0.2[a]

Values in a row or column with similar alphabetic letter are statistically non-significant, whereas with different alphabetic letters are significant (P< 0.05)

They came to the conclusion that the phytochemicals present in seed extracts, which affect antioxidant and antibacterial activity, are the most promising.

The researchers have received a variety of broad spectrum anti-microbial components from *Cucurbitaceae* seeds. Numerous bacterial and fungal species are inhibited by the oil from *Cucurbitaceae* seeds (Hammer et al., 1999). According to Caili et al. (2006), the antimicrobial qualities of melon seed extracts are correlated with antibacterial proteins, phenolic chemicals, and organic acids.

Conclusion

Muskmelon and watermelon are both valuable fruits contributing towards the healthy food with adequate nutritional contents. However, seeds of these fruits, which are considered as waste, have also strong potential of utilization as bioactive source. Current study provided scientific evidence of presence of useful bioactive components in muskmelon and watermelon seeds, and watermelon seeds ethanolic extracts presented significantly high amounts of TPC, TFC and TC, due to which antioxidant and antimicrobial activities of watermelon seed extracts were also significantly higher than those of watermelon seed extracts. Therefore, the use of these seeds for various food and pharmaceutical purpose could prove the useful valorization of these seeds.

Recommendations

It should be emphasized that the seeds of various melon fruits can be taken into consideration as a possible source of diverse antioxidant components, which are not currently utilized but may find use in many industrial fields. Fruit seeds are frequently waste products, thus using them again as antimicrobial and antioxidant source could result in quantifiable economic gains and help reduce environmental pollution caused by the fruit and vegetable sectors. Further *in vivo* and *in vitro* trials could be conducted to investigate the biological activities of extracts of melon seeds to correlate them with the possible medicinal activities.

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Investigation of the Effect of Pumpkin (*Cucurbita pepo* L.) Seed Oil on Pentylenetetrazole-induced Neuronal Damage in HT-22 Cell Line

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ABSTRACT

Recent studies have shown the positive effects of *Cucurbita pepo* L. (pumpkin) seed oil (PSO) in different disease models. However, the effect of PSO on neurological diseases has not been clarified yet. Therefore, this study aims to elucidate the effects of BBS on pentylenetetrazole (PTZ)-induced neuronal damage and the possible roles of oxidative and nitrosative stress in this effect *in vitro*. The HT-22 hippocampal neuronal cell line was used in the study. Cell survival after PTZ-induced neuronal damage was evaluated with the XTT test in the groups. While the effects of BBS on total antioxidant status (TAS) and total oxidant status (TOS) after PTZ were measured with colorimetric commercial kits, its effects on neuronal nitric oxide synthase (nNOS) and nitric oxide (NO) levels were also determined by ELISA kits. In light of the data obtained, it was found that pre-treatment with PSO prevented the decrease in cell survival after exposure to PTZ. In addition, it has been found that PSO normalizes the increase in TOS, nNOS, and NO in neuronal cells after PTZ. As a result, it was determined that the treatment of neuronal cells with PSO prevented neuronal damage caused by PTZ and showed neuroprotective properties. It is thought that PSO may achieve these effects through oxidative and nitrosative systems. Enrichment of a daily diet with PSO might be beneficial in reducing the risks of neurological diseases.

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HT-22 Hücrelerinde Pentilentatrazol ile Oluşturulan Nöronal Hasarlanma Üzerine Kabak (*Cucurbita pepo* L.) Çekirdeği Yağının Etkisinin Araştırılması

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HT-22 hücreleri

ÖZ

Güncel çalışmalar kabak (*Cucurbita pepo* L.) çekirdeği yağının (KÇY) farklı hastalık modellerinde olumlu etkilerini ortaya koymuştur. Ancak KÇY'nın nörolojik hastalıklar üzerine etkisi henüz aydınlatılamamıştır. Bu nedenle bu çalışmanın amacı KÇY'nın *in vitro* olarak pentilentetrazol (PTZ) ile oluşturulan nöronal hasar üzerine etkilerini ve bu etkide oksidatif ve nitrozatif stresin olası rollerini ortaya koymaktır. Çalışmada HT-22 hipokampal nöronal hücre hattı kullanılmıştır. Gruplar arasında hücre hücre sağkalımına etkisi XTT testi ile değerlendirilmiştir. KÇY'nın PTZ sonrası oluşan total antioksidan durum (TAS) ve total oksidan durum (TOS) üzerine etkileri kolorometrik ticari kitler ile ölçülürken nöronal nitrik oksit sentaz (nNOS) ve nitrik oksit (NO) seviyeleri üzerine etkileri ise ELISA kitleri ile belirlenmiştir. Elde edilen veriler ışığında, PTZ'e maruziyet sonrası ortaya çıkan hücre sağ kalımındaki azalmayı KÇY ile ön muamelenin engellediği bulunmuştur. Buna ek olarak KÇY'nın PTZ sonrası nöronal hücrelerde meydana gelen TOS, nNOS ve NO artışını normale çevirdiği tespit edilmiştir. Sonuç olarak nöronal hücrelerin KÇY ile muamelenin PTZ ile oluşturulan nöronal hasarlanmayı engellediği ve nöroprotektif özellik gösterdiği belirlenmiştir. Bu etkileri KÇY'nın oksidatif ve nitrozatif sistemler üzerinden gerçekleştirebileceği düşünülmektedir. KÇY ile günlük beslenmenin zenginleştirilmesi nörolojik hastalık risklerini azaltmada faydalı olabilir.

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Giriş

Pentilentetrazol (PTZ) deneysel nöbet ve epilepsi modeli oluşturmak için kullanılan farmakolojik bir ajandır (Taskiran ve ark., 2021). $GABA_A$ reseptörünü bloklayıp klor akışını baskılayarak bu etkinin ortaya çıkmasına neden olmaktadır. Bu özelliğinden dolayı nöbet ve nöbet sonrası nöronal hasarlanma oluşturmak için deneysel çalışmalarda sıklıkla kullanılmaktadır (Taskiran ve Tastemur, 2021; Ahlatci ver ark., 2022; Yildizhan ve ark., 2023).

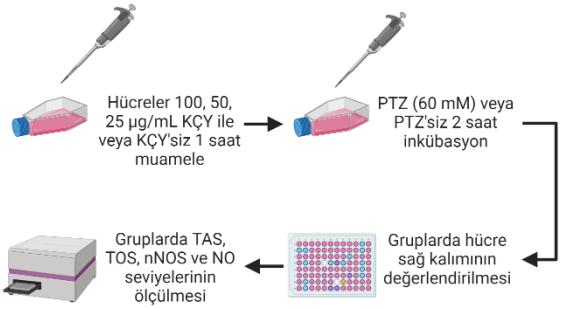
Oksidatif stres (OS) ve nitrozatif stres (NS) oksidan-antioksidan sistem arasındaki dengenin oksidan sistem lehine kayması sonucu oluşmaktadır. Denge halinde organizmada enerji üretimi başta olmak üzere hücresel düzeyde önemli fizyolojik rolleri bulunmaktadır (Yildizhan ve Naziroglu, 2019). Dengenin bozulması sonucu OS ve NS'in ortaya çıkması protein, lipit ve DNA gibi temel hücresel yapı taşlarının hasarlanmasına neden olmaktadır. Bu hasarlanma tamir edilemeyecek düzeye geldiğinde ise hücresel ölüm meydana gelmektedir. Bu durum kanser, diyabet ve birçok nörolojik hastalıklarla yakından ilişkilidir (Pizzino ve ark., 2017; Yildizhan, 2020). Nöbet sonrası artan elektriksel deşarjlar OS ve NS'in tetiklenmesine neden olarak nöronal hasarlanmaya neden olmaktadır (Salim, 2017).

İlk olarak 1980'li yıllarda Japonya'da ortaya çıkan "fonksiyonel gıda" kavramı, fizyolojik fonksiyonlar üzerinde olumlu etkileri olan bileşiklerle zenginleştirilmiş gıda ürünleri için kullanılmaktadır (Siró ve ark., 2008). Günümüzde fonksiyonel gıdalar, yalnızca besin ve enerji sağlamakla kalmayan, aynı zamanda belirli bir fizyolojik yanıta neden olup hastalık riskini azaltarak vücuttaki bir veya daha fazla hedeflenen işlevi olumlu yönde etkileyen gıdalar olarak tanımlanmaktadır (Nicoletti, 2012). Gıdaların sağlıkla ilgili özelliklerine ve fonksiyonel bileşenlerine karşı artan araştırmalarla birlikte, sadece geleneksel mutfağın bir parçası olarak kullanılan gıdalar, dünya çapında tıbbi açıdan dikkat çekmeye başlamıştır. Bu bağlamda son zamanlarda farklı özellikleri ortaya çıkmaya başlayan fonksiyonel gıdalardan biri de kabak çekirdeği yağıdır (KÇY).

KÇY doymamış yağ asitlerinden ve lif açısından zengin bir besin kaynağıdır (Makni ve ark., 2008). KÇY yapısında linoleik asit, oleik asit, palmitik asit gibi doymamış yağ asitlerini barındırmaktadır. Bununla birlikte, KÇY yüksek miktarda E vitamini içermektedir (Fruhwirth ve Hermetter, 2007). Ayrıca KÇY diğer bitkisel orijinli yağlara kıyasla β-karotenin seviyesi daha yüksektir ve bu sayede güçlü antioksidan özelliği göstermektedir (Šamec ve ark., 2022). Yapılan bir çalışma, KÇY'nın bening prostat hiperplazisini engellediğini bildirmiştir (Gossell-Williams ve ark., 2006). Buna ek olarak KÇY'nın hipertansiyon ve artritin ilerlemesini engellediği, meme, mide ve akciğer kanserine yakalanma oranını düşürdüğü tespit edilmiştir (Stevenson ve ark., 2007) Bütün bu faydalarının yanı sıra, çalışmalar KÇY'nın antiviral, antibakteriyel, antifungal, antihelmintik ve antikarsinojenik özelliklere sahip olduğunu ortaya koymuştur (Shaban ve Sahu, 2017). Fakat KÇY'nin sinir sistemi üzerine etkileri ve olası etki mekanizmaları henüz aydınlatılamamıştır. Bu nedenle bu çalışmada KÇY'nın PTZ ile oluşturulan *in vitro* nöronal hasar üzerine etkilerini ve bu etkide OS'in ve NS'in rollerini ortaya koymak amaçlanmıştır.

Materyal ve Yöntem

Deneye ait uygulanan yöntemsel basamaklar Şekil 1'de özetlenmiştir.



Şekil 1. Deney diyagramı (Biorender programı kullanılarak oluşturulmuştur).
Figure 1. Experimental Diagram (Created by Biorender)

Hücre Hattı ve Kimyasallar

Çalışmada HT-22 (SCC129) fare hipokampal nöronal hücre hattı kullanılmıştır. Hücre hattı Sigma Aldrich (Missouri, Amerika Birleşik Devletleri)'den temin edilmiştir. Hücre büyütme ve çoğaltmada kullanılan yüksek glikoz içeren dulbecco′s modified eagle′s medium (DMEM), fetal sığır serumu (FBS), L-glutamin, penisilin/streptomisin (10,000U/mL), tripsin-EDTA çözeltisi ve PTZ Sigma Aldrich (Missouri, Amerika Birleşik Devletleri) firmasından alınmıştır. KÇY Zade Vital (Konya, Türkiye)'den alınmıştır.

Hücre Kültürü Protokolü

Steril koşullar altında, 37 °C ve %5 CO_2'li ortamda HT-22 nöronal hücreleri %10 FBS, %5 L-glutamin ve %1 penisilin-streptomisin içeren DMEM hücre kültür besi yerinde çoğaltılmıştır (Yildizhan ve Ozturk, 2022). Hücreler %80 yoğunluğa ulaştıklarında pasajları yapılmış ve üçüncü pasajın ardından çalışmalara başlanmıştır. Hücreler temelde dört farklı gruba ayrılmıştır. Bunlar:

Kontrol grubu: Bu gruptaki hücrelere herhangi bir işlem uygulanmamıştır.

PTZ grubu: Bu gruptaki hücreler 2 saat 60 mM PTZ ile inkübe edilmiştir. PTZ doz seçimi için 240 mM, 120 mM, 60 mM, 30mM ve 15 mM konsantrasyonlar 2 saat olarak hücrelere uygulanmış, 60 mM konsantrasyon %50 öldürücü olarak bulunmuştur.

KÇY + PTZ grubu: Bu gruptaki hücrelere 1 saat 100, 50 ve 25 µg/mL KÇY ile muamele edildikten sonra 2 saat 60 mM PTZ ile inkübe edilmiştir.

KÇY grubu: Bu gruptaki hücrelere sonrasında PTZ uygulanmaksızın 1 saat 100, 50 ve 25 µg/mL KÇY ile muamele edilmiştir.

Hücre Sağ Kalımının Değerlendirilmesi

PTZ ile oluşturulan nöronal hasar sonrası hücre sağ kalımını değerlendirmek için mitokondriyal enzimler aracılığıyla renk veren XTT (Biological Industries, Kibbutz Beit-Haemek, İsrail) testi kullanılmıştır. Sitotoksisite için 96 kuyucuklu mikroplakaya Thoma lamında ekim öncesi sayım yapılarak her bir kuyucukta 15000 hücre olacak şekilde hücre ekilmiş ve hücrelerin yapışması için 24 saat bekletilmiştir. Hücrelerin ertesi gün

üzerindeki besi yeri uzaklaştırılmış, fosfat buffer solüsyonunda (PBS, pH: 7.4) ile yıkanmış, deney gruplarında yukarıda belirtildiği şekilde PTZ (60 mM olacak şekilde) uygulanmış ve 2 saat inkübasyon gerçekleştirilmiştir. 2 saatin sonunda besi yeri uzaklaştırılmış ve hücreler PBS ile yıkanmıştır. Her bir kuyucuğa 100 µl renksiz DMEM eklenmiş ve üzerine 50 µl XTT solüsyonu eklenerek 4 saat inkübe edilmiştir. Hücre canlılığına bağlı meydana gelen renk değişikliği mikroplaka okuyucuda (Spectrostar Nano, Allmendgrün, Almanya) 450 nm'de okunarak, kontrol grubunun hücre sağ kalım oranı %100 olarak kabul edilip % Hücre sağ kalım = (Konsantrasyon O.D. / Kontrol O.D.) × 100 formülünden yararlanarak hesaplanmıştır.

Hücre Lizatlarının Elde Edilmesi

Deney gruplarına belirtilen işlemler uygulandıktan sonra biyokimyasal analizler için hücreler tripsin ile kaldırılmış ve çöktürülmüştür. Hücreler ml'de 10^6 olacak şekilde PBS'da süspanse edilmiştir. Ardından -80 °C dondurucu yardımı ile üç kez dondurma-çözme işlemi yapılarak hücrelerin patlaması sağlanmıştır. Bu sayede hücre sitozolünde yer alan protein ve metabolitlerin PBS'ye geçmesi sağlanmıştır. Proteinlerin denatüre olmasını engellemek amacı ile dondurma-çözme işlemi üç tekrarla sınırlandırılmıştır. Sonrasında hücre süspansiyonları 10.000 rpm'de 20 dakika 4 °C'da santrifüj edilmiş ve elde edilen süpernatantlar biyokimyasal işlemler için kullanılmıştır.

Total Antioksidan (TAS) ve Total Oksidan (TOS) Seviyelerinin Ölçümü

Hücre lizatlarında PTZ sonrası oksidatif stres üzerine etkilerini değerlendirmek için kolorometrik TAS, TOS (Rel Assay Diagnostics, Antep, Türkiye) ölçümü ticari kitleri kullanılmıştır. TAS ölçümü hidrojen peroksit varlığında ABTS (2,2'-Azino-di-[3aehylbenzthiazolinesulphonate) molekülünün $ABTS^+$ molekülüne okside olmasına dayanmaktadır. ABTS radikali, antioksidan varlığına göre mavi ve yeşil rengini kaybetmektedir. Renk değişikliği, 660 nm dalga boyunda ölçülerek değerlendirilmektedir. Örneklerde bulunan antioksidan konsantrasyonları ile orantılı olarak renkteki açılma hızlanmaktadır. Sonuçlar µmol Trolox Equiv/mg protein başına ifade edilmektedir. TOS ölçümü örneklerde bulunan oksidanların, Fe^{+2}-o-dianisidine kompleksini Fe^{+3} iyonuna okside etmesine dayanmaktadır. Fe^{+3} iyonu asidik ortamda ksilenol oranı ile renkli bir kompleks yapar ve renk değişimi, örnekte bulunan oksidan moleküllerinin konsantrasyonu ile orantılı olup spekrofotometrik olarak ölçülebilmektedir. Renk değişikliği 530 nm dalga boyunda ölçülerek değerlendirilmektedir. Ölçüm hidrojen peroksit ile kalibre edilmekte ve sonuçlar µmol H_2O_2 Equiv/mg protein başına ifade edilmektedir.

nNOS ve NO Seviyelerinin Ölçümü

Elde edilen hücre lizatlarında nNOS ve NO seviyeleri, spesifik fare ELISA ticari kitleri (Sunlong, Zhejiang, Çin) kullanılarak ölçülmüştür. Üreticinin talimatlarına göre, ilk olarak kit içerisinde yer alan standartlar ve hücre lizatları kuyucuklara yüklenerek 37 °C derecede 60 dakika inkübe edilmiştir. Ardından kuyucuklar yıkama solüsyonuyla yıkanmıştır. Yıkama işleminden sonra kuyucuklara boyama solüsyonları eklenerek 37 °C derecede 15 dakika tekrar inkübasyona bırakılmıştır. Son aşama olarak kuyucuklara durdurma solüsyonu eklenmesini takiben, mikroplaka okuyucuda (Spectrostar Nano, Allmendgrün, Almanya) 450 nm dalga boyunda okutulmuştur. Standartların absorbanslarına göre doğrusal bir grafik oluşturulmuştur. Bu doğrusal grafikle elde edilen denklem yardımıyla örneklerin değerleri hesaplanmıştır. Bradford yöntemi kullanılarak, örneklerde total protein tayini gerçekleştirilmiştir (Kruger, 2009).

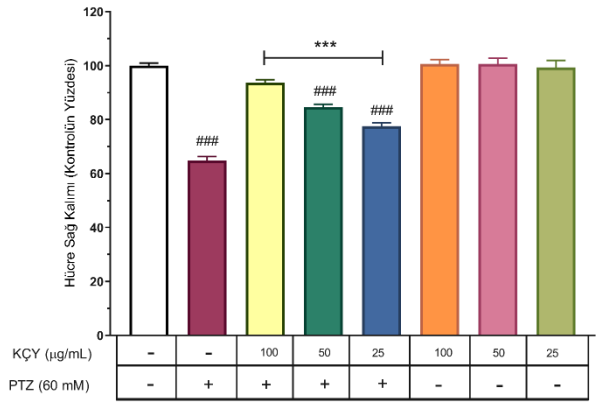
İstatistiksel analiz

İstatistiksel değerlendirme için SPSS 22.0 programı kullanılmıştır. Tüm gruplarda ölçülen XTT, TAS, TOS, nNOS ve NO verilerinin ortalama ± standart hatası (Ort. ± SH) alınarak değerlendirilmiştir. Verilerin normal dağılıma uygunluk göstermesinden dolayı tek yönlü varyans analizi (ANOVA), post-hoc olarak Tukey testi kullanılmıştır. İstatistiksel anlamlılık düzeyi $P < 0,05$ olarak kabul edilmiştir.

Bulgular ve Tartışma

KÇY'nın PTZ ile Oluşturulan Nöronal Hasar Sonrası Hücre Sağkalımı Üzerine Etkisi

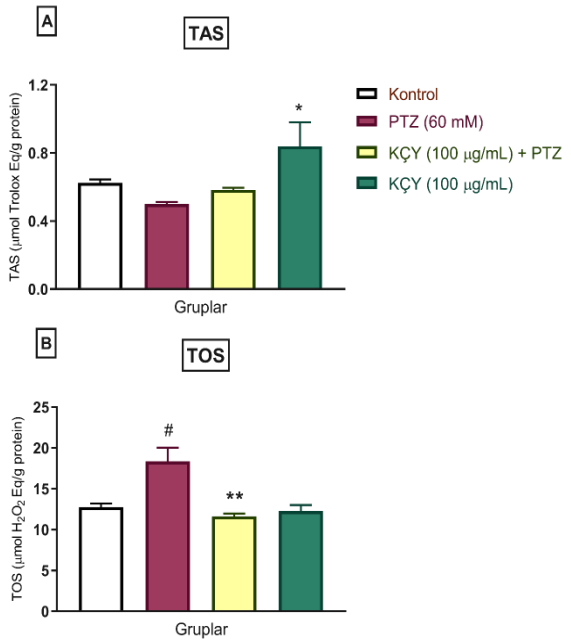
Çalışmanın bu aşamasında, artan KÇY dozlarının (25–100 µg/mL) HT-22 hipokampal nöronal hücrelerde PTZ ile oluşturulan hasar sonrası hücre sağkalımı üzerine etkisi XTT testi ile değerlendirilmiştir. Hücreler başlangıçta 1 saat boyunca artan dozlarda (25, 50 ve 100 µg/mL) KÇY ile muamele edilmiş ve ardından sonraki 2 saat boyunca 60 mM PTZ ile veya PTZ'siz inkübe edilmiştir. Şekil 2'de gösterildiği gibi, HT-22 hücrelerini 2 saat boyunca PTZ ile inkübasyonu, kontrole göre kıyasla hücre sağkalımını önemli ölçüde azaltmıştır ($P < 0,001$; Şekil 2). Bununla birlikte, tüm dozlarda KÇY'nın, sadece PTZ ile muamele edilen grupla karşılaştırıldığında hücre sağkalımını arttırdığı belirlenmiştir ($P < 0,001$; Şekil 2). Ayrıca tek başına KÇY'nın, uygulama dozlarında, kontrole kıyasla HT-22 hücrelerinin sağkalımı üzerine toksik bir etkisi belirlenmemiştir ($P > 0,05$; Şekil 2).



Şekil 2. KÇY'nın PTZ ile oluşturulan nöronal hasarlanma sonrası hücre sağ kalımı üzerine etkisi. Veriler Ort. ± SH olarak sunulmuştur. ### P< 0,001 diğer gruplar ile karşılaştırıldığında ve ***P < 0,001 PTZ grubu ile karşılaştırıldığında.

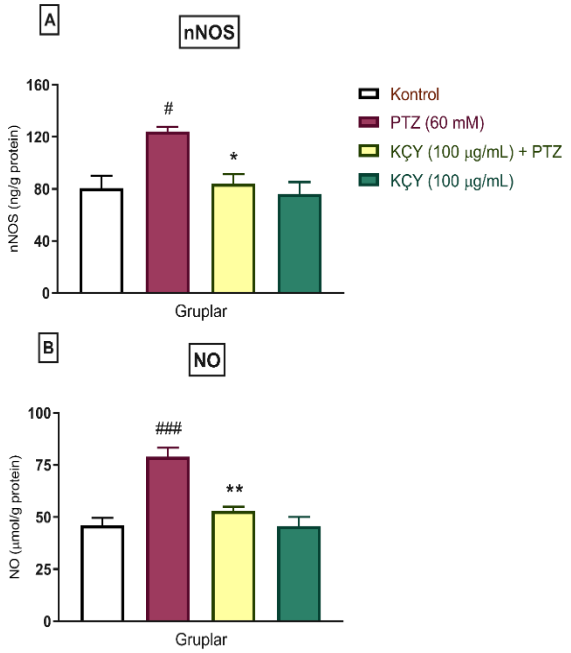
Figure 2. Effect of PSO on cell survival after PTZ-induced neuronal damage.

*Data are presented as mean ± SEM. ###P<0.001 compared to other groups and ***P<0.001 compared to the PTZ group.*



Şekil 3. KÇY'nın PTZ ile oluşturulan nöronal hasarlanma sonrası hücresel TAS ve TOS seviyeleri üzerine etkisi.
Veriler Ort. ± SH olarak sunulmuştur. #P < 0,05 diğer gruplar ile ile karşılaştırıldığında. *P < 0,05 ve **P < 0,01 PTZ grubu ile karşılaştırıldığında.

Figure 3. Effect of PSO on cellular TAS and TOS levels after PTZ-induced neuronal damage.
*Data are presented as mean ± SEM. #P<0.05 compared to other groups. *P<0.05 and **P<0.01 compared to the PTZ group.*



Şekil 4. KÇY'nın PTZ ile oluşturulan nöronal hasarlanma sonrası hücresel nNOS ve NO seviyeleri üzerine etkisi.
Veriler Ort. ± SH olarak sunulmuştur. #P < 0,05 ve ###P < 0,001 diğer gruplar ile karşılaştırıldığında. *P < 0,05 ve **P<0,01 PTZ grubu ile karşılaştırıldığında.

Figure 4. Effect of PSO on cellular nNOS and NO levels after PTZ-induced neuronal damage.
*Data are presented as mean ± SEM. #P<0.05 and ###P<0.001 compared to other groups. *P<0.05 and **P<0.01 compared to the PTZ group.*

Bu çalışmada *in vitro* olarak oluşturulan nöronal hasarlanma üzerine KÇY'nın etkisi çalışılmıştır. Mevcut literatüre göre KÇY ile ilgili bu alanda gerçekleştirilen ilk çalışmadır. Farklı dokularda ve farklı modellere bakıldığında, *in vivo* bir çalışmada KÇY'nın sıçanlarda ince bağırsakta metotreksat kaynaklı oluşan hasara karşı koruyucu olduğu tespit edilmiştir (Yüncü ve ark., 2006). Başka bir *in vivo* çalışmada KÇY'nın kardiyoprotektif özellik gösterdiği bulunmuştur (El-Mosallamy ve ark., 2012). Bu çalışmalara ek olarak farelere diyet içerisinde KÇY verilmesinin karaciğer yağlanmasını ve ateroskleroz gelişimini önlediği belirlenmiştir (Morrison ve ark., 2015). Tüm bu çalışmalarla uyumlu olarak çalışmamızda KÇY'nın PTZ ile oluşturulan nöronal hasarlanmaya karşı da koruyucu olduğu ortaya konmuştur.

KÇY'nın PTZ ile Oluşturulan Nöronal Hasar Sonrası TAS ve TOS Seviyeleri Üzerine Etkisi

Hücreler 1 saat boyunca en etkili doz olarak belirlenen 100 µg/mL KÇY ile muamele edilmiş ve ardından 2 saat boyunca 60 mM PTZ ile veya PTZ'siz inkübe edilmiş ve kolorometrik ticari kitler ile hücresel TAS ve TOS seviyeleri belirlenmiştir.

Şekil 3'te gösterildiği gibi, tek başına PTZ alan grup, kontrol kıyaslandığında HT-22 hücrelerinde TAS seviyelerini azaltsa da bu istatiksel olarak anlamlı bulunamamıştır ($P > 0,05$; Şekil 3A). Öte yandan KÇY, tek başına PTZ alan grupla kıyaslandığında HT-22 hücrelerinde TAS'ı önemli ölçüde artırmıştır ($P < 0,05$; Şekil 3A).

HT-22 hücrelerini 2 saat boyunca tek başına PTZ'e maruz bırakmak, kontrole kıyasla TOS seviyelerini önemli ölçüde yükselttiği belirlenmiştir ($P < 0,05$; Şekil 3B). Fakat KÇY ile ön muamele görüp ardından PTZ'e maruz kalan hücrelerdeki TOS seviyesi, tek başına PTZ ile muamele gören hücrelerin TOS seviyesine kıyasla önemli ölçüde azaldığı tespit edilmiştir ($P < 0,01$; Şekil 3B).

Çalışmamızda nöronal hücrelere KÇY uygulanmasının nöronal hasarlanma sonrası TAS seviyesini arttırsa da bu anlamlı bulunamamıştır. Yapılan çalışmalarda KÇY'nın antioksidan özellik gösterdiği belirlenmiştir (Patel, 2013). Fakat bu özelliğin ekstraksiyon metodları ile değişebileceği vurgulanmıştır (Irnawati ve ark., 2022). Çalışmamızda KÇY'nın doğrudan TAS seviyesini arttırmaması endojen antioksidan sistem üzerinden değil vitamin C gibi eksojen olarak reaktif oksijen türlerini süpürücü etkisi ile açıklanabilir. Nitekim TAS seviyesini arttırmasa da nöronal hasarlanma sonrası artan TOS seviyesini düşürdüğü belirlenmiştir. Çalışmamıza benzer şekilde, KÇY'nın alfatoksin zehirlenmesi sonrası karaciğer, akciğer, böbrek ve beyin gibi dokularda azalan antioksidan sistem belirteçlerini arttırdığı ve artan oksidan sistem belirteçlerini ise azalttığı bulunmuştur (Eraslan ve ark., 2013). Buna ek olarak, KÇY'nın sodyum nitrat ile oluşturulan karaciğer hasarını oksidatif stresi azaltarak hafiflettiği rapor edilmiştir (Rouag ve ark., 2020).

KÇY'nın PTZ ile Oluşturulan Nöronal Hasar Sonrası nNOS ve NO Seviyeleri Üzerine Etkisi

Hücreler KÇY (100 µg/mL) ile 1 saat boyunca muamele edilmiş ve ardından 2 saat boyunca 60 mM PTZ ile veya PTZ'siz inkübe edilmiş ve ELISA ile hücrelerde nNOS ve NO seviyeleri belirlenmiştir.

Tek başına hücreleri 2 saat boyunca PTZ'ye maruz bırakmak, kontrole kıyasla nNOS seviyelerini ve NO seviyelerini önemli ölçüde arttırdığı bulunmuştur (nNOS için $P < 0,05$; Şekil 4A; NO için $P < 0,001$; Şekil 4B). Öte yandan hücrelerin KÇY ile muamele edilmesi tek başına PTZ'e maruz kalan hücrelere göre nNOS ve NO seviyelerini anlamlı olarak düşürmüştür (nNOS için $P < 0,05$; Şekil 4A; NO için $P < 0,01$; Şekil 4B).

Çalışmamızda KÇY ile yapılan ön tedavi PTZ ile oluşturulan nöronal hasar sonrası artan nNOS ve NO seviyelerini azaltmıştır. Literatürde yapılan çalışmalara bakıldığında, çalışmamıza benzer şekilde KÇY'nın sıçanlarda metotreksat kaynaklı oluşan hasar sonrası ince bağırsakta NO seviyelerini azalttığı belirlenmiştir (Yüncü ve ark., 2006). Benzer şekilde KÇY'nın deneysel olarak oluşturulan hipertansiyon modelinde serumda yükselen NO seviyesini düşürdüğü tespit edilmiştir (El-Mosallamy ve ark., 2012). Buna ek olarak KÇY'nın karaciğer dokusunda sodyum nitrat ile oluşturulan hasarlanma sonrası NO seviyelerini normale çevirdiği rapor edilmiştir (Rouag ve ark., 2020). Fakat çalışmamızda KÇY'nin nNOS seviyeleri üzerine de düzenleyici etkinlik gösterebileceği ilk olarak gösterilmiştir.

Sonuç

Bu çalışma mevcut ulaşılabilir literatür göz önünde bulundurulduğunda KÇY'nın nörolojik etkileşimi ve olası etkilerini inceleyen ilk *in vitro* çalışmadır. Çalışmamızda KÇY ile ön tedavinin nöronal hücrelerde PTZ ile oluşturulan hasarlanmayı engelleyerek hücre sağ kalımını arttırdığı belirlenmiştir. Bu nöroprotektif özelliği KÇY'nın oksidatif ve nitrozatif sistemler aracılıyla gerçekleştirebileceği bulunmuştur. KÇY ile beslenmenin zenginleştirilmesi nörolojik hastalık risklerini azaltmada faydalı olabileceği düşünülmektedir. KÇY'nın nörolojik sistemlerle ilgili farklı etkileşimlerini ve mekanizmalarını ortaya koymak için ileri çalışmalara ihtiyaç vardır.

Mevcut çalışmanın bazı kısıtlılıkları bulunmaktadır. Öncelikle araştırma *in vitro* olarak gerçekleştirilmiş olduğundan canlı sistemin bütününü yansıtamamaktadır. Bu amaçla *in vivo* modeller ile KÇY'nın epileptik nöbetler ve epileptogenez üzerine etkilerinin araştırılması hedeflenmektedir. Bununla birlikte çalışmamızda piyasada kullanılan bir ürün tercih edilmiştir fakat bu ürünün elde ediliş yöntemi ve besin özellikleri dışında kalan içerik detayları bilinmemektedir. Bu nedenle de ilerleyen çalışmalarda üretici firma ile iletişime geçilecek izin verilmesi halinde elde ediliş yöntem detayları paylaşılacak ve mevcut ürün özelliklerinin detaylı bileşenleri gaz kromatografik yöntemi ile ortaya konması sağlanacaktır.

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Investigation of the Protective Role of Quercetin on Oxidative Stress and Endoplasmic Stress Pathway in 4-aminopyridine-induced Neuronal Damage

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ABSTRACT

Quercetin (QU) is a flavonoid found in different fruits and vegetables. Studies report that QU may have positive effects on neurological diseases. However, the effect of QU on 4-aminopyridine (4-AP)-induced neurodegeneration in neuronal cells is still not fully elucidated. In this study, the effects of QU on 4-AP-induced hippocampal neuron damage *in vitro* and the possible role of oxidative stress and endoplasmic reticulum stress in this effect were investigated. The study was carried out using the HT-22 hippocampal neuronal cell line. The effect of pre-treatment with QU on cell viability after 4-AP-induced neuronal damage was determined by the XTT test. Cells were evaluated histopathologically for apoptotic nuclear change (ANC) using DAPI staining. The effects of QU on oxidative stress (total oxidant state (TOS) and total antioxidant status (TAS)) occurring after neuronal damage were evaluated with colorimetric commercial kits and endoplasmic reticulum stress markers (activating transcription factor 4 (ATF-4) and C/EBP homologous protein). (CHOP) was measured with the ELISA kits. While the cell viability rate decreased in the cells treated with 4-AP, it was determined that pre-treatment with QU reversed this situation. In terms of histopathology, treatment with 4-AP increased the number of ANC, while QU pre-treatment reduced it. In addition, in terms of biochemical evaluations, TOS, ATF-4, and CHOP increased in neuronal cells after 4-AP, and QU was determined to suppress this increase. In addition, QU normalized the decreased TAS levels following the 4-AP application. As a result, in the HT-22 cell line, it was found that QU treatment had a neuroprotective effect by suppressing oxidative stress and endoplasmic reticulum stress in 4-AP-induced neuronal damage.

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4-aminopridin ile Oluşturulan Nöronal Hasarda Oksidatif Stres ve Endoplazmik Stres Yolağı Üzerine Kuersetin'in Koruyucu Rolünün Araştırılması

MAKALE BİLGİSİ

Araştırma Makalesi

Geliş : 02.10.2023
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Anahtar Kelimeler:
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4-aminopridin
Nöronal Hasar
Oksidatif stres
Endoplazmik retikulum stresi

ÖZ

Kuersertin (KU) farklı meyve ve sebzede bulunan bir flavonoiddir. Yapılan çalışmalar KU'in nörolojik hastalıklar üzerine olumlu etkileri olabileceğini bildirmektedir. Ancak nöronal hücrelerde KU'in 4-aminopridin (4-AP) kaynaklı nörodejenerasyon üzerine etkisi hala tam olarak aydınlatılamamıştır. Bu çalışmada; KU'in 4-AP ile oluşturulan *in vitro* hipokampal nöron hasarı üzerine etkilerini ve bu etkide oksidatif stres ve endoplazmik retikulum stresinin olası rolü araştırılmıştır. Çalışma HT-22 hipokampal nöronal hücre hattı kullanılarak gerçekleştirilmiştir. KU ile ön tedavinin 4-AP ile oluşturulan nöronal hasar sonrası hücre canlılığına etkisi XTT testi ile belirlenmiştir. Hücreler DAPI boyası kullanılarak apoptotik çekirdek değişikliği (AÇD) açısından histopatolojik olarak değerlendirilmiştir. KU'in nöronal hasarlanma sonrası oluşan oksidatif stres (total oksidan durum (TOS) ve total antioksidan durum (TAS)) üzerine etkileri kolorometrik ticari kitler ile endoplazmik retikulum stres belirteçleri (aktifleyici transkripsiyon faktör 4 (ATF-4) ve C/EBP homolog proteini (CHOP) ELISA kitleri yardımıyla ölçülmüştür. 4-AP uygulanan hücrelerde hücre canlılık oranı azalırken, KU ile ön tedavinin bu durumu tersine çevirdiği belirlendi. Histopatolojik açıdan, 4-AP ile muamele AÇD sayısını arttırırken KU ön tedavisi bunu azalttı. Ayrıca biyokimyasal değerlendirmeler açısından, 4-AP sonrası nöronal hücrelerde TOS, ATF-4 ve CHOP artışı meydana geldi ve KU bu artışı baskıladığı belirlendi. Buna ek olarak KU, 4-AP uygulamasını takiben azalan TAS seviyelerini normale çevirdi. Sonuç olarak, HT-22 hücre hattında 4-AP ile indüklenen nöronal hasarda, KU tedavisinin oksidatif stres ve endoplazmik retikulum stresi baskılayarak nöroprotektif etki gösterdiği bulundu.

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Giriş

Kortikal nöronlardan aşırı ve anormal elektrik boşalması olan nöbetler, geçici beyin fonksiyon bozukluklarına neden olmaktadır (Taskiran ve Ergul, 2021). Epilepsi, genetik yatkınlık veya patolojik bozukluklara bağlı tekrarlayan nöbetlerle karakterize anormal bir merkezi sinir sistemi durumudur (Taskiran ve ark., 2020; Ahlatci ve ark., 2022). Dünya nüfusunun yaklaşık %1'i bu nörolojik bozukluktan muzdarip durumdadır (Taskiran ve ark., 2021). Nöbetler genellikle antiepileptik ilaçlarla (AEİ) tedavi edilir. Bununla birlikte, AEİ almalarına rağmen ilaca dirençli epileptik hastaların %20-30'u hala dirençli nöbetler yaşamaktadır (Das ve ark., 2011). Bu nedenle AEİ'ların terapötik etkilerinin yetersiz olması nedeniyle hala daha güçlü ve güvenli yeni ilaçlar üzerine araştırmalara ihtiyaç duyulmaktadır (Taskiran ve Tastemur, 2021; Yildizhan ve ark., 2023).

4-aminopridin (4-AP) beyin kesitlerinde *in vitro* olarak ve deney hayvanlarında *in vivo* olarak nöbet oluşturmak için kullanılan farmakolojik bir liganddır. Etkinliğini seçici olmayan potasyum kanallarını bloke ederek göstermektedir (Gean ve ark., 1990). Potasyum kanal blokajı hücre içerisinde fazla miktarda pozitif iyon birikmesine neden olarak aksiyon potansiyel oluşumunu kolaylaştırmaktadır (Pea ve Tapia, 2000). Bunun sonucu olarak nöbet aktiviteleri meydana gelmektedir. Bu özelliğinden dolayı 4-AP deneysel nöbet araştırmalarında sıklıkla kullanılmaktadır (Heuzeroth ve ark., 2019).

Oksidatif stres (OS) oksidan-antioksidan sistem arasındaki dengenin bozulması sonucu oluşmaktadır. OS'in ortaya çıkması temel hücresel yapı taşları olan protein, lipit ve DNA'nın hasarlanmasına neden olmaktadır (Pizzino ve ark., 2017). Bu hasarlanma hücresel tamir mekanizmaları tarafından tamir edilemeyecek düzeye geldiğinde hücresel ölüm meydana gelmektedir. Bu durum birçok nörolojik hastalıklarla da yakından ilişkilidir (Emerit ve ark., 2004; Yildizhan ve Naziroglu, 2019). Nöbet sonrası elektriksel uyarılmalardaki artış mitokondri disfonksiyonu ile OS'in tetiklenmesine neden olarak nöronal hasarlanmayı tetiklemektedir (Aguiar ve ark., 2012). Bununla birlikte OS'in artışı nöronal eksitasyonu arttırarak nöbetlerin kolaylaşmasını sağlamakta ve epileptogenez oluşum sürecini hızlandırmaktadır (Sun ve ark., 2022; Madireddy ve Madireddy, 2023).

Hücrelerde endoplazmik retikulum (ER) proteinlerinin sentezlenmesi, katlanması ve olgunlaşmasının sağlandığı temel organeldir. Hücresel kapasitenin aşılarak protein katlanma cevabının bozulması durumuna ER stresi denilmektedir. Yapılan çalışmalar ER stresinin birçok farklı hastalıkta rol aldığını göstermiştir (Almanza ve ark., 2019). Bununla birlikte ER stresinin nörodejeneratif hastalıklar ve epilepsi patofizyolojisi ile ilişkili olabileceği rapor edilmiştir (Lindholm ve ark., 2006).

Son zamanlarda, doğal olarak oluşan substratların, geniş yelpazedeki biyolojik aktivitelerden dolayı, özellikle de doğal fitokimyasalların tanımlanmasına büyük önem verilmiştir (Russo ve ark., 2012). Flavonoidler, bilim camiasının büyük ölçüde dikkatini çeken ve birçok hastalık için umut verici tedavi edici olabilecek doğal polifenolik bileşiklerdir (Russo ve ark., 2012). İnsan diyetindeki en yaygın bitki flavonoidlerinden ve önde gelen diyet antioksidanlarından biri olan kuersetin (3,3',4',5,7-pentahidroksiflavon; KU), çeşitli çaylarda, meyvelerde ve sebzeler bulunmaktadır ve özellikle Çin tıbbında klinik çalışmalarda kullanımı onaylanmıştır (Tang ve ark., 2020). KU'in antifibrotik, antiviral, antikanser, antiinflamatuar ve antioksidatif özelliklere sahip olduğu yapılan çalışmalarda gösterilmiştir (Wang ve ark., 2022; Manni ve ark., 2023; Mansour ve ark., 2023). Buna ek olarak, KU'in sinir sitemi üzerine olumlu etkileri ortaya konmuştur (Islam ve ark., 2021). Fakat 4-AP ile oluşturulan *in vitro* nöronal hasarlanma üzerine etkisi ve olası etki mekanizmaları henüz aydınlatılamamıştır. Bu çalışmada; KU'in 4-AP ile oluşturulan hipokampal nöronal hasarlanma üzerine etkilerini ve bu etkide OS'in ve ER stresinin rollerini ortaya koymak amaçlanmıştır.

Materyal ve Yöntem

Çalışmamızda gerçekleştirilen deneysel aşamalar basamaklar Şekil 1'de gösterilmiştir.

Hücre Hattı

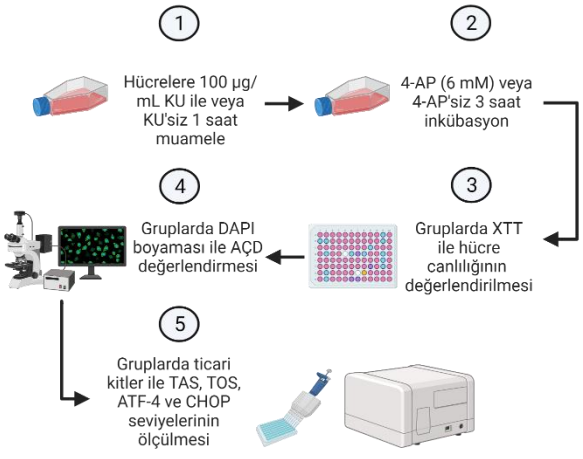
Çalışmada HT-22 (SCC129) fare orjinli immortalize edilerek çoğalma yeteneği kazandırılmış hipokampal nöronal hücre hattı kullanılmıştır. Hücre hattı Sigma Aldrich (Missouri, Amerika Birleşik Devletleri)'den temin edilmiştir.

Kimyasallar

Hücre büyütme ve çoğaltmada kullanılan yüksek glikoz içeren dulbecco′s modified eagle′s medium (DMEM), fetal sığır serumu (FBS), L-glutamin, penisilin/streptomisin (10,000U/mL), tripsin-EDTA çözeltisi ve 4-AP Sigma Aldrich (Missouri, Amerika Birleşik Devletleri) firmasından alınmıştır. KU Orzax (İstanbul, Türkiye)'den alınmıştır.

Hücre Kültürü Protokolü

HT-22 hipokampal nöronal hücreler steril koşullar altında 37 °C ve %5 CO_2'li ortamda, 25 cm^2'lik flasklarda, %10 FBS, %5 L-glutamin ve %1 penisilin-streptomisin içeren DMEM hücre kültür besi yerinde büyütülmüş ve çoğaltılmıştır (Yildizhan ve Ozturk, 2022).



Şekil 1. Deney aşamaları
(Biorender programı ile oluşturulmuştur)
Figure 1. Experimental Stages (Created by Biorender)

Hücreler %80 yoğunluğa ulaştıklarında pasajları yapılmış ve üçüncü pasajın ardından çalışmalara başlanmıştır. Hücreler dört farklı gruba ayrılarak işlemler gerçekleştirilmiştir. Bunlar: *Kontrol grubu*: Bu gruptaki hücrelere herhangi bir işlem uygulanmamıştır. *4-AP grubu*: Bu gruptaki hücrelere 3 saat boyunca %50 öldürücü olarak bulunmuş olan 6 mM 4-AP ile muamele edilmiştir. *KU + 4-AP grubu*: Bu gruptaki hücrelere 1 saat 10 µM/mL KU ile muamele edildikten sonra 3 saat 6 mM 4-AP içeren besi yeri inkübe edilmiştir. *KU grubu*: Bu gruptaki hücrelere 1 saat 10 µM/mL KU ile muamele edildikten sonra 3 saat 4-AP'siz besi yeri ile inkübe edilmiştir

Hücre Canlılık Testi

4-AP'in meydana getirdiği nöronal hasarlanma sonrası hücre canlılığını değerlendirmek için mitokondriyal enzimler aracılığıyla renk veren ve suda çözünen XTT (Biological Industries, Kibbutz Beit-Haemek, İsrail) testi kullanılmıştır. Sitotoksisite için öncelikle her kuyuda $15x10^3$ hücre olacak şekilde hücre alınıp steril 96 kuyucuklu mikroplakaya ekilmiş ve hücrelerin yapışması için bir gece bekletilmiştir. Tutunma sonrası yukarda belirtildiği şekilde 4-AP'li ve 4-AP'siz işlem uygulaması yapılmış, 3 saat sonra üreticinin talimatlarına göre gerekli ön işlemler gerçekleştirilmiş ve hücrelere XTT karışım solüsyonu eklenerek 4 saat inkübe edilmiştir. Sonrasında mikroplaka okuyucuda (Spectrostar Nano, Allmendgrün, Almanya) 450 nm'de okunarak, kontrol grubunun hücre canlılık oranı %100 olarak kabul edilip % Hücre canlılık = (Konsantrasyon O.D. / Kontrol O.D.) × 100 formülünden yararlanarak hesaplanmıştır.

Histopatolojik Değerlendirme

Gruplara KU ve 4-AP uygulanmasından 3saat sonra hücreler, oda sıcaklığında 10 dakika boyunca paraformaldehit (%4) ile fikse edilmiştir. Hücreler daha sonra üç kez PBS ile yıkanmış ve ardından 5 dakika DAPI (BioShop, Burlington, Kanada) ile boyanmıştır. Hücreler floresan mikroskob yardımıyla (Carl Zeiss, Jena, Almanya) görüntülenmiş mikroskobun kendi programı kullanılarak değerlendirilmiştir. Hücresel ve nükleer morfolojideki apoptozla ilişkili değişiklikler incelenmiş ve nükleer boyutta azalma, kromatin yoğunlaşması, nükleer parçalanma ve yoğun floresan görünümü apoptotik çekirdek değişiklikleri (AÇD) olarak kabul edilmiş ve toplam çekirdek sayısına göre yüzdesi belirlenmiştir.

Hücre Lizatlarının Elde Edilmesi

Biyokimyasal analizler için 25 cm^2'lik flasklarda gruplandırılan hücreler yukarda belirtilen ilaç uygulamalarını takiben tripsin ile kaldırılmış ve çöktürülmüştür. Ardından hücrelerin patlatılıp sitoplazmik içeriklerin ve metobolitlerin ortaya çıkmasını sağlamak amacıyla üç kez dondurma-çözme işlemi yapılmıştır. Proteinlerin denatüre olmasını engellemek amacı ile dondurma-çözme işlemi üç tekrarla sınırlandırılmıştır. Sonrasında hücre süspansiyonları 10.000 rpm'de 20 dakika 4°C'da santrifüj edilmiş ve elde edilen süpernatantlar biyokimyasal işlemler için kullanılmıştır.

Hücresel Oksidatif Stres Belirteçlerinin (Total Oksidan Durum (TOS) ve Total Antioksidan Durum (TAS) Ölçülmesi

Hücre lizatlarında 4-AP ile oluşturulan nöronal hasarlanma sonrası oksidatif stres belirteçleri üzerine etkilerini değerlendirmek için kolorometrik TOS ve TAS (Rel Assay Diagnostics, Antep, Türkiye) ticari kitleri kullanılmıştır. Üretici tarafından belirlenen aşamalar takip edilerek TOS ve TAS ölçümü gerçekleştirilmiştir.

TOS ölçümü örneklerde bulunan oksidanların, demir iyonunu okside etmesine dayanmaktadır. Bu oksidasyon ortamda bulunan özel renkli bir moleküle kompleks yapar ve turuncu renk değişimi spekrofotometrik olarak ölçülebilmektedir. Örnekte bulunan oksidan moleküllerinin konsantrasyonu ile orantılı olarak renk koyu turuncuya kaymaktadır. Renk değişikliği 530 nm dalga boyunda ölçülmektedir. Ölçüm hidrojen peroksit ile kalibre edilmekte ve sonuçlar µmol H_2O_2 Equiv/mg protein başına ifade edilmektedir. TAS ölçümü antioksidan varlığında yeşil rengini kaybeden özel bir molekül ile değerlendirilmektedir. Örneklerde bulunan antioksidan konsantrasyonları ile orantılı olarak renkteki açılma hızlanmaktadır. Renk değişikliği, 660 nm dalga boyunda ölçülmektedir. Sonuçlar µmol Trolox Equiv/mg protein başına ifade edilmektedir.

ER Stres Belirteçlerinin (ATF-4 ve CHOP) Seviyelerinin Ölçümü

İlaç uygulamasından sonra elde edilen hücre lizatlarından ER stres belirteçleri (ATF-4 ve CHOP) ELISA ticari kitleri (YL Biont, Shanghai, Çin) kullanılarak ölçülmüştür. Üreticinin talimatlarına göre standartlar ve örnekler kuyucuklara eklenip gerekli tüm aşamaların tamamlanmasını takiben mikroplaka okuyucuda (Spectrostar Nano, Allmendgrün, Almanya) 450 nm dalga boyunda okutulmuştur. Standartların absorbanslarına göre doğrusal bir grafik oluşturulmuştur. Bu doğrusal grafikle elde edilen denklem yardımıyla örneklerin değerleri hesaplanmıştır. Örneklerdeki seviyelerin normalizasyonu için total protein tayini Bradford yöntemi kullanılarak gerçekleştirilmiştir (Kielkopf ve ark., 2020).

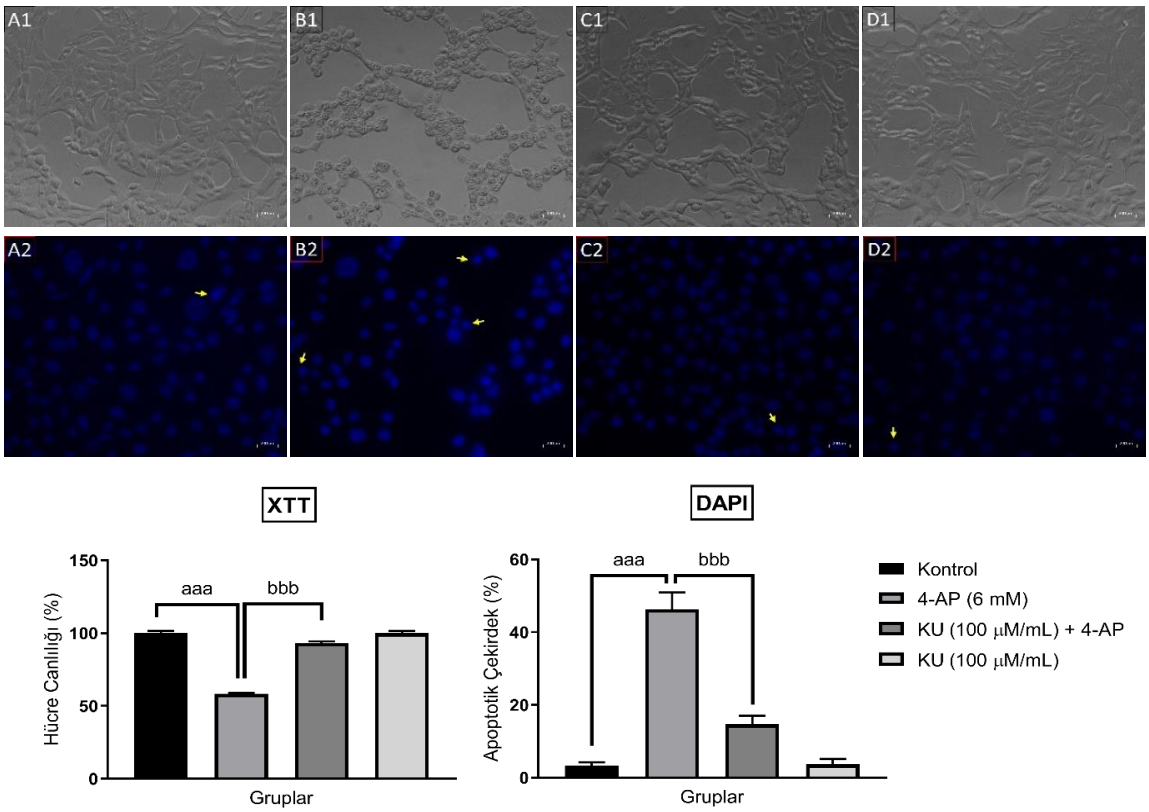
İstatistiksel analiz

İstatistiksel değerlendirme için SPSS 22.0 programı kullanılmıştır. Tüm gruplarda ölçülen XTT, AÇD, TOS, TAS, ATF-4 ve CHOP değerlerinin ortalama ± standart hatası (Ort. ± SH) alınarak analiz edilmiştir. Verilerin normal dağılıma uygunluk göstermesinden dolayı tek yönlü varyans analizi (ANOVA), post-hoc olarak Tukey testi kullanılmıştır. İstatistiksel anlamlılık düzeyi $P<0,05$ olarak kabul edilmiştir.

Bulgular ve Tartışma

KU'in 4-AP ile Oluşturulan Nöronal Hasar Sonrası Hücre Canlılığı ve AÇD Üzerine Etkisi

Mevcut çalışmada, hipokampal nöronal hücrelerde KU'in 4-AP ile oluşturulan hasar sonrası hücre canlılığı üzerine etkisi XTT testi kullanılarak belirlenmiştir.



Şekil 2. KU'in 4-AP ile oluşturulan hipokampal nöronal hasarlanma sonrası hücre canlılığı ve AÇD'ler üzerine etkisi.
A1, B1, C1 ve D1 farklı gruplarda nöronal hücrelerin 20X'lik büyütmede ışık mikroskobisi altında morfololojik görünümü. A2, B2, C2 ve D2 farklı gruplarda nöronal hücrelerin 40X'lik büyütmede DAPI çekirdek boyaması sonrası floresan mikroskobisi altında morfololojik görünümü. Sarı oklar AÇD lehine yorumlanan çekirdek değişikliklerini göstermektedir. Veriler Ort. ± SH olarak sunulmuştur. ^{aaa}P<0,001 kontrol grubu ile karşılaştırıldığında. ^{bbb}P<0,001 tek başına 4-AP uygulanan grupla karşılaştırıldığında.

Figure 2. Effect of QU on cell viability and apoptotic nuclear changes after 4-AP-induced hippocampal neuronal damage.
Morphological appearance of neuronal cells in different groups A1, B1, C1, and D1 under light microscopy at 20X magnification. Morphological appearance of the nucleus of neuronal cells in different groups A2, B2, C2, and D2 under fluorescence microscopy after DAPI nuclear staining at 40X magnification. Yellow arrows indicate apoptotic nuclear changes. Data are presented as mean ± SEM. ^{aaa}P<0.001 compared to the control group. ^{bbb}P<0.001 compared to the 4-AP group.

Hipokampal nöronlar öncelikle 3 saat boyunca (100 µM/mL) KU ile ön tedavi işleminden geçirilmiş ve ardından sonraki 3 saat boyunca 6 mM 4-AP ile veya 4-AP'siz inkübasyona bırakılmıştır. Nöronal hücrelerini 3 saat boyunca 4-AP ile inkübasyonu, kontrol'e göre kıyasla hücre canlılığını önemli ölçüde azaltmıştır (P < 0,001; Şekil 1). Bununla birlikte, KU'in tek başına 4-AP ile muamele edilen grupla karşılaştırıldığında hücre canlılığını normale çevirdiği bulunmuştur (P < 0,001; Şekil 1). Bunun yanı sıra tek başına KU'in, kontrole kıyasla nöronal hücre canlılığı üzerine toksik bir etkisi gözlemlenmemiştir (P > 0,05; Şekil 2).

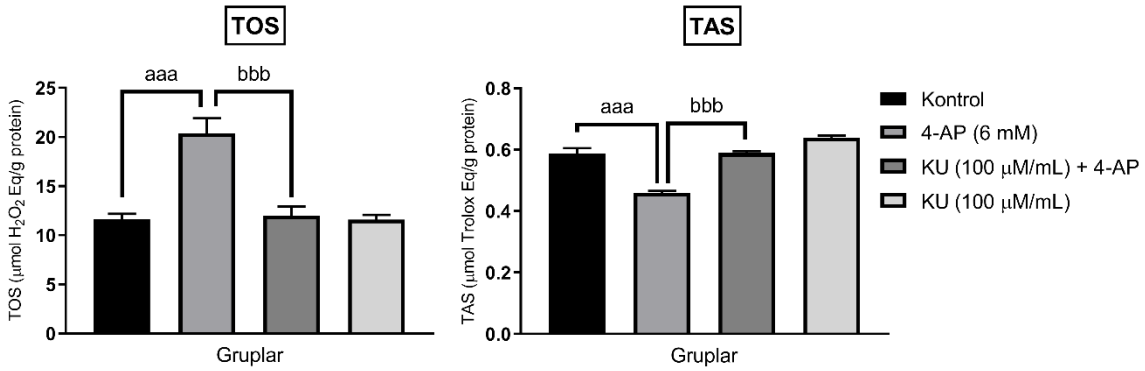
Işık mikroskobisinde elde edilen morfolojik görüntüler değerlendirildiğinde tek başına 4-AP ile muamele edilen hücrelerin hasarlanmaya bağlı hücresel uzantılarını kaybederek yuvarlak bir şekle girdiği belirlenmiştir (Şekil 2; A1-D1). Fakat KU ön tedavisi alan hücrelerin morfolojik yapılarını kontrole benzer şekilde sürdürdüğü tespit edilmiştir (Şekil 2; A1-D1).

DAPI boyaması açısından değerlendirildiğinde 4-AP ile muamele kontrole kıyasla AÇD'ni anlamlı olarak arttırdığı (P < 0,001; Şekil 2; A2-D2) bunun aksine KU ile ön tedavinin tek başına 4-AP uygulanan grupla kıyaslandığında AÇD sayısını azalttığı bulunmuştur (P < 0,001; Şekil 2; A2-D2). Tek başına KU uygulaması ise AÇD sayısını etkilememiştir (P > 0,05; Şekil 2; A2-D2).

Yapılan birçok *in vivo* ve *in vitro* çalışma KU'in nöroprotektif etkinliğini göstermiştir (Riche ve Lenard, 2022; Jiang ve ark., 2023; Jiao ve ark., 2023; AbdElrazek ve ark., 2023). Bununla birlikte KU'in pentilenterazolle oluşturulan nöbetleri azalttığı belirlenmiştir (Tavakoli ve ark., 2023). Buna ek olarak kainik asitle oluşturulan epilepsi modelinde KU'in nöbet oluşumunu engellediği ve nöbet sonrası nöronal hasarlanmayı azalttığı tespit edilmiştir (Xie ve ark., 2022). Bizim çalışmamızda ise *in vitro* olarak hipokampal hücrelerde 4-AP ile nöbet sonrası nöronal hasarlanma meydana getirildiğinde KU'in literatürdeki diğer çalışmalara benzer şekilde koruyucu etki ortaya koyduğu ve 4-AP sonrası meydana gelen AÇD'ni azalttığı bulunmuştur.

KU'in 4-AP ile Oluşturulan Nöronal Hasar Sonrası Oksidatif Stres Belirteçleri (TOS ve TAS) Üzerine Etkisi

Oksidatif stres belirteçleri olan TOS ve TAS kolorometrik ticari kitler ile belirlenmiştir. Nöronal hücreler 3 saat boyunca 100 µM/mL KU ile muamele edilmiş ve ardından 3 saat boyunca 6 mM 4-AP ile veya 4-AP'siz inkübe edilmiştir. Şekil 2'de ortaya konduğu gibi, tek başına 4-AP'ne maruziyet, kontrol kıyaslandığında nöronal hücrelerinde TOS seviyelerini anlamlı olarak arttırmıştır (P < 0,001; Şekil 3).

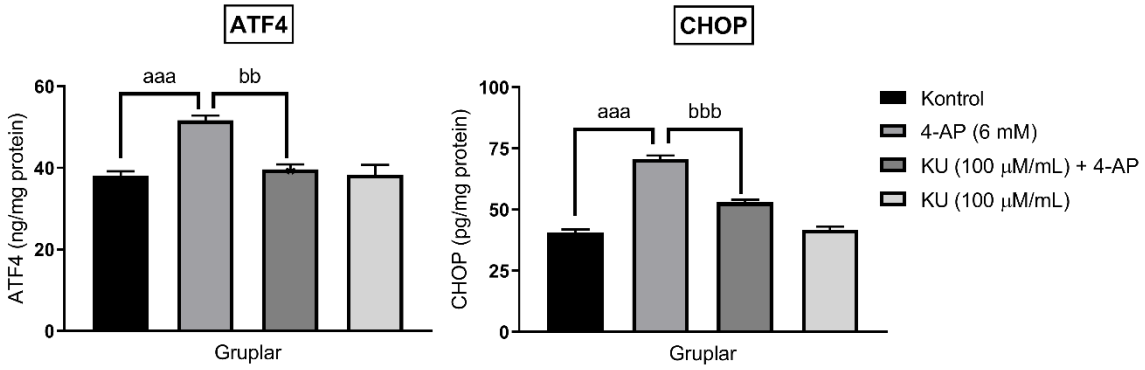


Şekil 3. KU'in 4-AP ile oluşturulan hipokampal nöronal hasarlanma sonrası oksidatif stres belirteçleri (TOS ve TAS) üzerine etkisi.

Veriler Ort. ± SH olarak sunulmuştur. ^{aaa}P<0,001 kontrol grubu ile karşılaştırıldığında. ^{bbb}P<0,001 tek başına 4-AP uygulanan grupla karşılaştırıldığında.

Figure 3. Effect of QU on oxidative stress markers (TOS and TAS) after 4-AP-induced hippocampal neuronal damage.

Data are presented as mean ± SEM. ^{aaa}P<0.001 compared to the control group. ^{bbb}P<0.001 compared to the 4-AP group.



Şekil 4. KU'in 4-AP ile oluşturulan hipokampal nöronal hasarlanma sonrası ER stresi belirteçleri (ATF-4 ve CHOP) üzerine etkisi.

Veriler Ort. ± SH olarak sunulmuştur. ^{aaa}P<0,001 kontrol grubu ile karşılaştırıldığında. ^{bb}P<0,01 ve ^{bbb}P<0,001 tek başına 4-AP uygulanan grupla karşılaştırıldığında.

Figure 4. Effect of QU on ER stress markers (ATF-4 and CHOP) after 4-AP-induced hippocampal neuronal damage.

Data are presented as mean ± SEM. ^{aaa}P<0.001 compared to the control group. ^{bb}P<0.01 and ^{bbb}P<0.001 compared to the 4-AP group.

Bunun aksine, KU ile ön tedavi tek başına 4-AP'ne maruz kalan kıyaslandığında TOS seviyelerini nöronal hücrelerde anlamlı düzeyde azaltmıştır ($P < 0,001$; Şekil 3). Nöronal hücrelerde TAS seviyeleri açısından bakıldığında, 3 saat boyunca tek başına 4-AP'ne maruz bırakmak, kontrole kıyasla hücresel TAS seviyelerini önemli ölçüde düşürmüştür ($P < 0,001$; Şekil 3). Fakat KU ile ön tedavi sonrası 4-AP'ne maruz kalan hücrelerdeki TAS seviyesi, tek başına 4-AP ile muamele gören hücrelerin TAS seviyesine kıyasla önemli ölçüde yükselmiştir ($P < 0,001$; Şekil 3).

OS'in nöbetlerin oluşumunda önemli rol oynadığı belirlenmiştir (Aguiar ve ark., 2012). Bu nedenle antioksidanlar ile epilepsi için koruyucu yöntemler ve yeni tedaviler geliştirmek hedeflenmiştir (Martinc ve ark., 2014). Bu açıdan güçlü bir antioksidan olan KU dikkat çekmektedir (Rarinca ve ark., 2023). Yapılan çalışmalar KU'in oksidan sistem üzerindeki etkinliği ortaya çıkarmıştır ve bu etki ile epilepsi için bir hedef molekül haline getirmiştir (Prakash ve ark., 2023). Çalışmamızda bu çalışmalara benzer şekilde fakat farklı bir model ve *in vitro* seviyede KU'in azalan antioksidan sistemi arttırdığı ve nöronal hasarlanma ile meydana gelen oksidan sistem artışını baskıladığı gösterilmiştir.

KU'in 4-AP ile Oluşturulan Nöronal Hasar Sonrası ER Stres Belirteçleri (ATF-4 ve CHOP) Üzerine Etkisi

Hipokampal nöronal hücreler 100 µM/mL KU ile 3 saat boyunca muamele edilmiş ve ardından 3 saat boyunca 6 mM 4-AP ile veya 4-AP'siz inkübe edilmiş ve hücrelerde bir protein ölçüm yöntemi olan ELISA ile ER stres belirteçleri (ATF-4 ve CHOP) ölçülmüştür. Şekil 4'te belirtildiği gibi, tek başına nöronal hücreleri 4-AP'ne maruz bırakmak, kontrole kıyasla hücresel ATF-4 ve CHP seviyelerini önemli ölçüde arttırmıştır ($P < 0,001$; Şekil 4). Fakat hücrelerin KU ile muamele edilmesi tek başına 4-AP'ne maruz kalan nöronal hücrelere göre ATF-4 ve CHOP seviyelerini anlamlı olarak azaltmıştır (ATF-4 için $P < 0,01$, CHOP için $P < 0,001$; Şekil 4).

ER stresi sonucu protein katlanma cevabının bozulması epilepsi patofizyolojisinde yer aldığı güncel çalışmalar ışığında ortaya konmuştur (Fu ve ark., 2020). ER stresinin

başlaması ATF4 protein seviyesinin yükselmesi ve bunun sonucu CHOP aktivasyonu ile hücresel apoptoz yolu tetiklenmektedir. Bu durum nöbet sonrası nöronal hasarlanma ile ilişkilendirilmiştir (Fu ve ark., 2020). Bu nedenle ER stresini azaltmak nöbet sonrası hasarlanmayı azaltmak açısından önem arz etmektedir (Liu ve ark., 2019). Çalışmamızda 4-AP ile oluşturulan hipokampal hasarlanma sonrası ER stres proteinleri ATF-4 ve CHOP'un arttığı belirlenmiştir. Bu artışın KU'in ön tedavisi ile azaldığı gösterilmiştir. Çalışmamıza benzer şekilde çalışmalar KU'in farklı dokularda ER stresi oluşumunu baskıladığını göstermiştir (Ileriturk ve ark., 2022; Boonyong ve ark., 2023).

Sonuç

Çalışmamızda KU ile ön tedavinin hipokampal hücrelerde 4-AP ile oluşturulan nöronal hasarlanmayı engelleyerek hücre canlılığını arttırdığı gösterilmiştir. Bu nöroprotektif özelliği KU'in oksidatif stres ve ER stresini baskılayarak gerçekleştirebileceği bulunmuştur. KU'in sağlıklı ve nörolojik hastalığa yatkın bireylerde takviye olarak kullanılması hastalık risklerini azaltmada faydalı olabileceği düşünülmektedir.

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Examination of Air Quality of Dr. Sami Yağız Street in Niğde

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ABSTRACT

Air pollution can be defined as the accumulation of gas, air, or particulate matter released as a result of fuel residues and chemical processes in the atmosphere in amounts that harm the lives of living things. Rapid urbanization, industrialization, increase in the number of motor vehicles, meteorological conditions, fuels used for heating, and the spread of industrial establishments and thermal power plants play an important role in the increase in air pollution. Air pollution is a major environmental problem affecting people in both developed and developing countries, and it is estimated that megacities in developing countries, and a quarter of the world's population are exposed to unhealthy concentrations of air pollutants. People living in cities with high outdoor air pollution are more likely to suffer from heart disease, respiratory problems, and lung cancer than those living in urban areas with clean air. In the industrialized western world, urban air pollution is in some respects in its final stages, with a dramatic decline in SO_2 and soot levels. The increase in the number of private vehicles is a newly emerging problem. Rapid urbanization in most developing countries has so far led to uncontrolled growth and environmental degradation. Air pollution levels are still rising in many cities. In this study, air quality was tried to be measured along Dr. Sami Yağız Street, which is one of the most heavily used areas of Niğde. H_2S, O_2 and CO measurements were made at 25 points at equal intervals on both sides of the street. Measurements were made at a total of 4 different time periods per day. The obtained values were transferred to ArcGIS 10.3 software and maps were produced. Measurement of gases other than H_2S is not distributed homogeneously along the street, and traffic density and the businesses on the sides of the street cause changes in the rates of the gases.

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Introduction

Damage to vegetation in urban ecosystems, change or decrease in its quantity and quality, causes air pollution and environmental problems that negatively affect public health and ecological functioning (Hutyra et al., 2011). The Right to Clean Air Platform (THHP) stated that air pollution, which is considered among the risk factors that cause the most diseases and deaths in our country, rose to the 6th place in 2017, while it was seventh in 2007 (THHP, 2020).

It is known that carbon monoxide (CO), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), and ozone (O_3) gases, which increase in the air, especially as a result of the use of fossil fuels, cause respiratory diseases, cardiovascular diseases and neurotoxic effects in humans (THHP, 2020). It has been determined that the air pollution seen in our cities during the winter months is caused by the fuels consumed for heating purposes, especially approximately 90% of SO_2 comes from this source, 10% comes from industry, traffic and wind erosion, and non-fuel sources have a 20% share in smoke.

In addition, it causes a wide variety of damages to plants, such as loss of vegetation and species, deterioration of chlorophyll structure, affecting the development of vegetative parts, fertilization biology, fruit set, yield, and quality (Turalıoğlu, 2011). In addition, carbon-based pollutants create a greenhouse effect, causing an increase in air temperature, the emergence of a heat island effect, acid rain, and climate change (Cui and de Foy, 2012; Tursun et al., 2018).

PM2.5 and PM10 particles are the most important air pollution components, which are expressed as a mixture of solid and liquid particles suspended in the air and are formed as a result of human activities such as heating, transportation, industry, and electricity generation.

These particles cause many diseases related to the respiratory, cardiovascular, and nervous systems,

especially cancer (Perez et al., 2015; Tonyaloğlu et al., 2021). On the other hand, the presence of trees in urban areas provides social, cultural and human health benefits as well as various ecosystem services on important issues such as carbon capture and storage, removal of atmospheric pollution, reduction of urban heat island effect and reduction of rainwater surface runoff (Nowak et al., 2013). In this context, mapping, measuring and evaluating ecosystem services are of great importance in terms of natural resource management, as well as the creation of planning decisions and policies and economic resource management (Burkhard and Maes, 2017; Kesgin Atak and Tonyaloğlu Ersoy, 2020; Tonyaloğlu Ersoy, 2020).

Green areas covered with vegetation improve air quality by filtering pollutants in the atmosphere, depending on the ecological characteristics of the plants, climate and environmental conditions. Trees improve the air quality of their environment by absorbing pollutants in the air through the stomata in their leaves during respiration or by trapping pollutants in the atmosphere with their leaves. These pollutants retained on the leaf surface remain on the plant until the tree loses its leaves or the leaves are washed away by rainfall. The air quality improvement functions of trees are high when they are close to pollutant sources. For this reason, in many cities, the focus of adaptation efforts to climate change is on reducing the amount of pollutants released into the atmosphere, afforesting roads that are sources of pollution to create green corridors, and calculating the ecosystem services provided by these corridors (Hepcan and Cangüzel, 2021).

Streets are exposed to intense air pollution due to traffic density and urbanization in the immediate vicinity. Although this pressure is tried to be reduced by afforestation and median works on the sides of the streets, the pavement width on the sides of the roads is not sufficient and the plant species selected in the medians are not among the types that reduce air pollution, causing these problems to continue.

Air pollution is generally grouped under three headings. These; air pollution caused by heating, transportation and industry. Air pollution caused by heating is a phenomenon that occurs especially in winter months. Fire has been one of the important needs for humanity since its discovery. It was used extensively, especially for heating purposes. Today, we use stoves and radiators in homes, schools and workplaces for heating purposes during the winter months. Wood, coal, fuel oil and natural gas are used as fuel in stoves and radiators. When these fuels are burned in stoves and radiators, carbon monoxide (CO), sulfur dioxide (SO_2), nitrogen oxides (NOx) and particulate matter coming out of the chimneys pollute the air.

In addition to the pollution caused by heating in cities, the harmful exhaust gases caused by motor vehicles, which increase in parallel with the increase in population and income level, also emerge as an important air pollution problem that needs to be taken precautions.Harmful substances in the exhaust gases emitted by gasoline and diesel vehicles cause much more damage to the environment, especially in large urban centers where population and traffic are dense.

It is a multifaceted and mutually influencing relationship with industry, which is one of the main sectors of development, and in addition to the positive results created by this interaction, if precautions are not taken in terms of environmental protection and appropriate technologies are not used, the problem of pollution arises, which has negative consequences on the environment, gradually leading to the destruction of resources and rapid pollution of the environment. and causes the benefits expected from the industrial sector to gradually decrease (Anonymous, 2023).

Main purpose of this study is to determine the main factor causing air pollution on the selected street. Changes in the measurement values of gases according to determined time periods and the reasons for this were determined. The most important feature of the study is that January was selected as the measurement time. The coldest month in Niğde is January. An attempt was made to measure the air quality of the street depending on heating and motor vehicle use.

Materials and Method

In the study, it was planned to produce maps of the gases that affect the air quality of Dr Sami Yağız Street in Niğde Province. Niğde is surrounded by Mersin to the south, Konya to the west, Nevşehir to the north, Aksaray to the northeast, and Kayseri to the east (Figure 1). Therefore, the main material of the study consists of data from Dr. Sami Yağız Street and its surroundings. Dr. Sami Yağız Street is one of the longest streets of Niğde Province and is approximately 1.5 km long and 15 meters wide. Dr. Sami Yağız Street is located at 37.966373 latitude and 34.672649 longitude (Figure 2).

Figure 1. Location of the study area

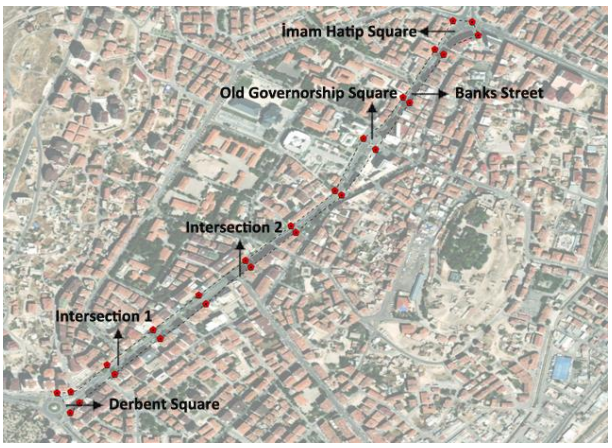

Figure 2. Dr. Sami Yağız Street and measurement points

O_2, CO and H_2S measurements were made at 25 points at equal intervals on both sides of the street. Measurements were made on weekdays. The reason for this is that the street is exposed to heavy traffic on weekdays, especially for reasons such as school, work, etc. In addition, measurements were made on weekdays to determine the effects of the restaurants on the sides of the street on air quality.

After determining the days to be measured, the time period in which the measurement would be made was discussed. As a result of the observations, the street is heavily used at 09:00, 12:00, 15:00 and 18:00. 09:00 and 18:00 are the time period for going home, work, and returning to school, work, etc. It is used intensively in the 12:00 and 15:00 time zones for various reasons (eating, public, banking, etc.). The obtained values were transferred to ArcGIS 10.3 software and maps were produced. Through the maps, it was determined in which time periods the gas density changed and in which parts of the street the gas density changed and the reasons.

Results and Discussion

The results of the measurements for CO are given in Figure 3-6. According to the CO gas measurement results along the street, it was determined that the gas rate changed according to the time periods measured.

In addition, it was determined that the CO gas rate was not distributed homogeneously throughout the street during the same time periods. The danger limit for the amount of CO in the air is given as 50 ppm or 55 mg/m^3. Symptoms of CO poisoning generally begin when the ambient level reaches 100 ppm.

According to the measurement results, it was determined that the amount of CO in the air along the street was below 50 ppm. However, in the measurements made at Imam Hatip Square, the westernmost end of the area, at 09:00 on Monday, a rate slightly above 50 ppm was detected. However, according to the measurement results, it was determined that the time period with the lowest CO amount was 09:00.

According to the measurement results, it was determined that the CO rate in the air was higher especially between 09:00 and 15:00 compared to other time periods. While the CO level is highest in the eastern part of the street in the measurements made at 09:00, the CO amount increases at 12:00 and 15:00, especially at intersections and areas where bus stops are located.

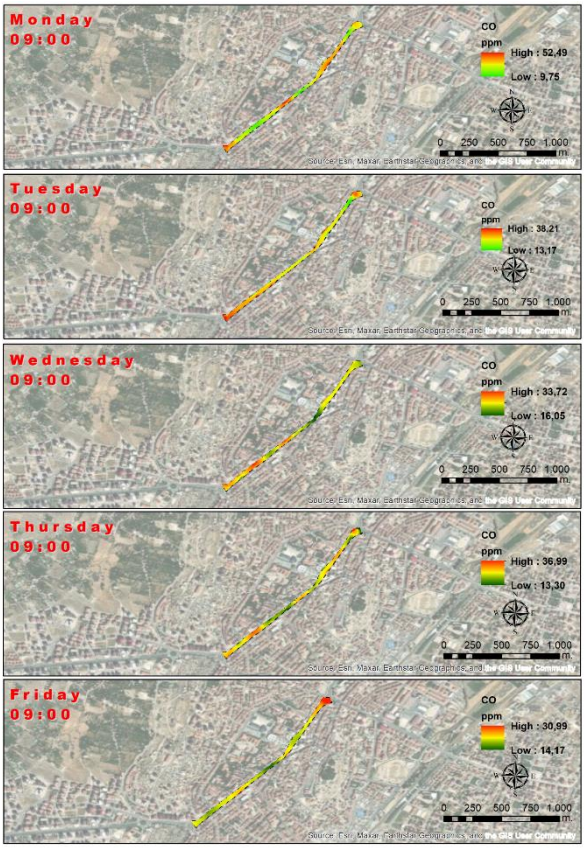

Figure 3. CO measurement – 09:00

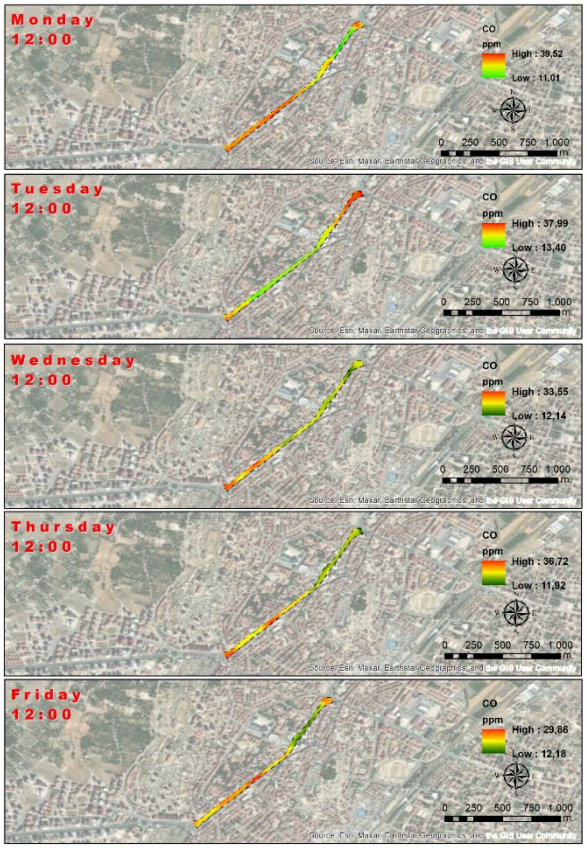

Figure 4. CO measurement – 12:00

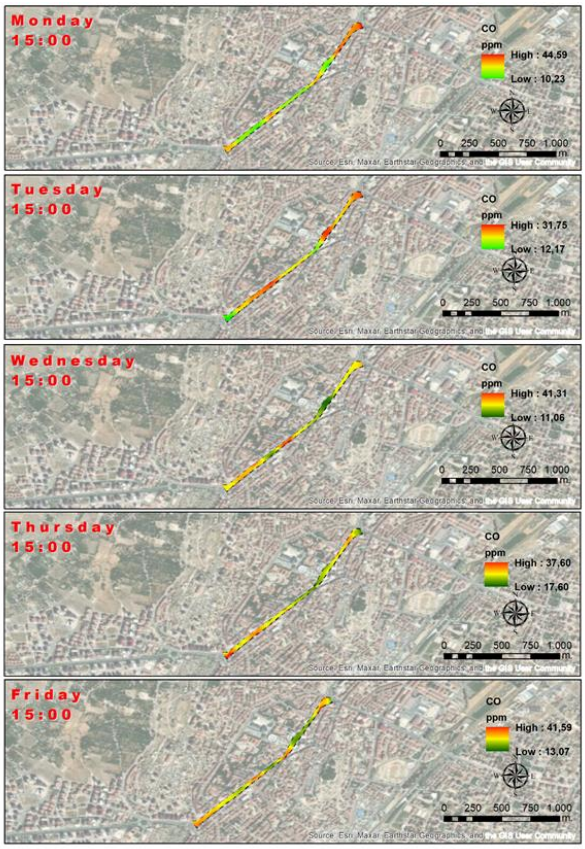

Figure 5. CO measurement – 15:00

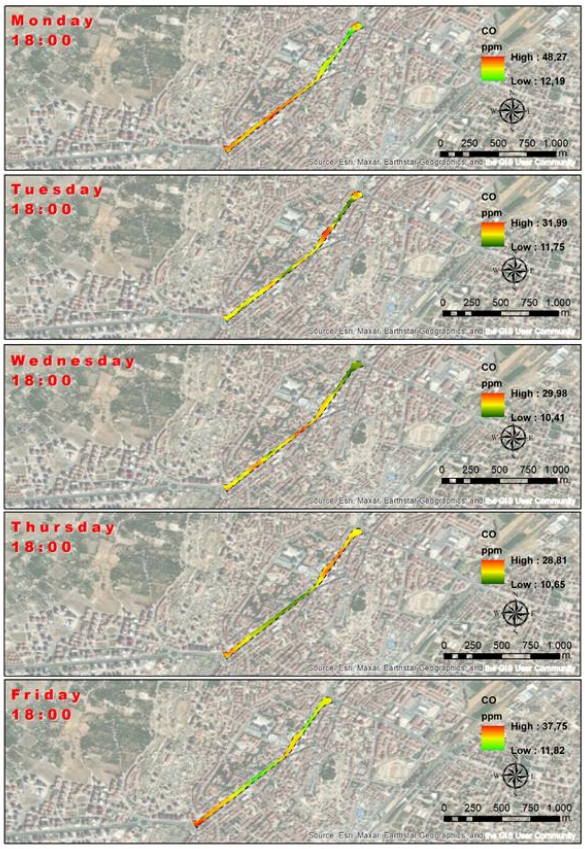

Figure 6. CO measurement – 18:00

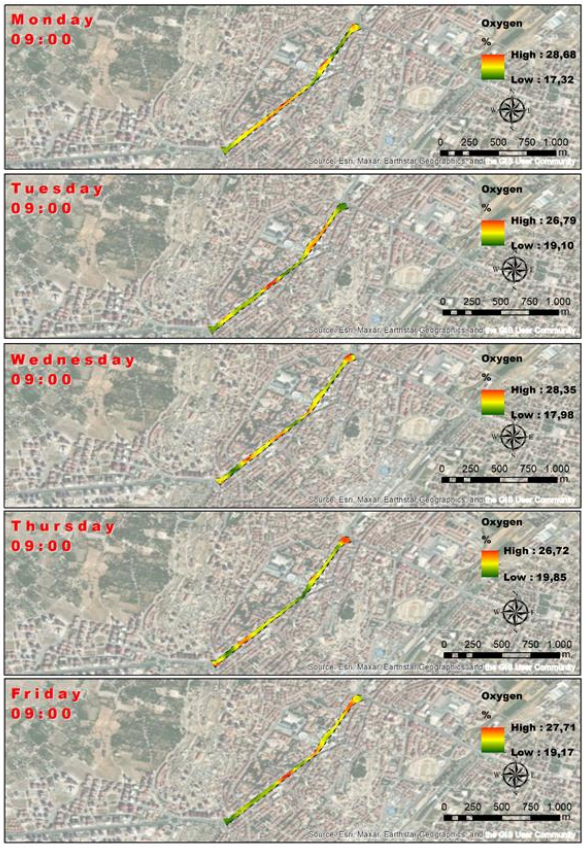

Figure 7. O_2 measurement – 09:00

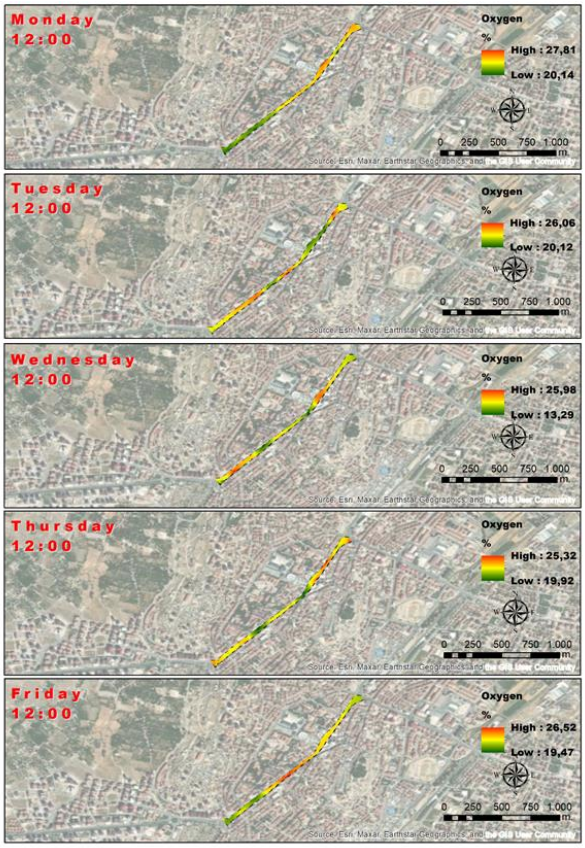

Figure 8. O_2 measurement – 12:00

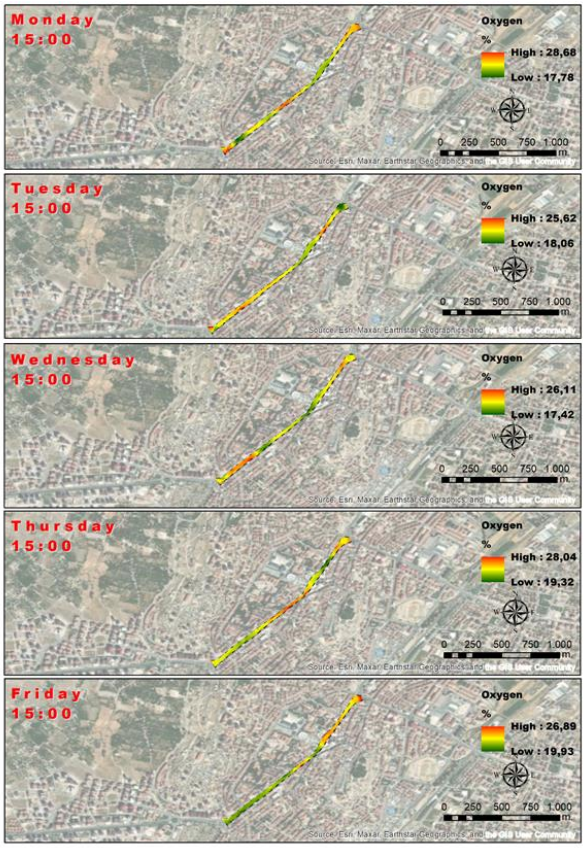

Figure 9. O_2 measurement – 15:00

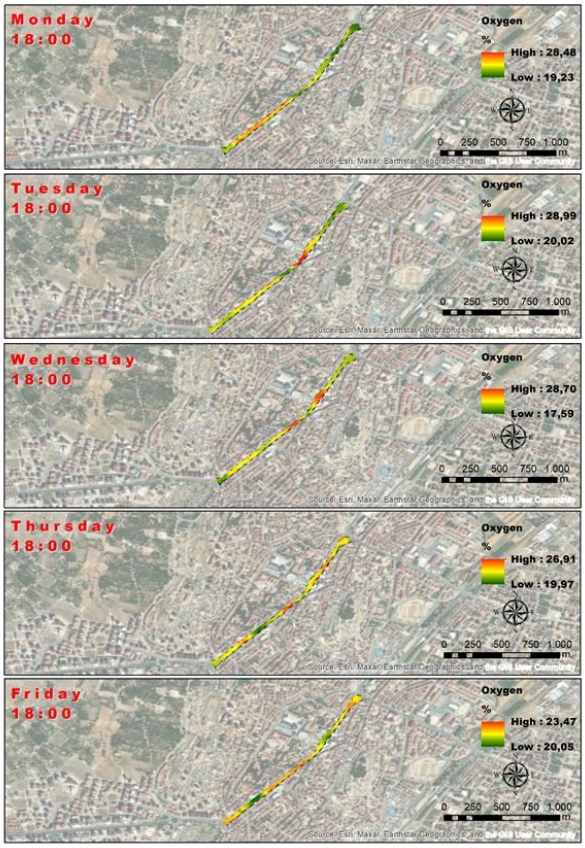

Figure 10. O_2 measurement – 18:00

Because the opening time of banks is 09.30, the amount of CO in Banks Street is low especially in the morning hours, but the CO gas rate in this street increases at 15:00 and 18:00. In measurements made at 12:00, 15:00 and 18:00, the CO level is at its highest levels especially at intersections and on Bankalar Street, where the street narrows. The results of the measurements for O_2 are given in Figure 7-10. There is normally around 21% oxygen in the air. In order to breathe healthily, the amount of oxygen in the air must not fall below this rate. According to the measurement results, it was determined that the oxygen rate in the air along the street was close to this value, was above this rate at certain times of the day, and was 1 - 2 points behind this rate in a very small part of the street. According to the measurement results, it was determined that the oxygen rate in the air was less than other time periods, especially between 12:00 and 15:00.

In the measurements made at 09:00, it was determined that the oxygen rate was lowest in the western part of the street, while the oxygen rate increased in this part of the street over time, and decreased towards the western part of the street. The concentration of Hydrogen Sulfide, which occurs naturally between 0.0001 - 0.0002 ppm in clean air, should not exceed 0.05 ppm according to standards and an hourly average of 0.125 ppm.

As a result of the measurements, it was determined that the H_2S rate in the air showed almost no change and had a value close to 0.0001 pm. H_2S gas generally changes instantaneously in areas located in heavy industrial facilities. There is no industrial facility that could cause environmental waste or pollution along the street examined within the scope of the study. For this reason, it has been determined that the rate of H_2S gas in the air remains within the determined limit values and there is no negative situation in terms of air quality.

Conclusion

According to the report published by the Ministry of Environment, Urbanization and Climate in 2020; In Niğde Province, there is felt and detected pollution caused by SO_2 during the winter months, late autumn and early spring.

This shows that the source of air pollution in Niğde (in terms of SO_2 pollution) is not the pollution caused by industry and motor vehicles, but the air pollution caused by heating and meteorological factors. owever, in this study, contrary to the report, it was determined that the amount of CO gas in the air increased especially at certain times of the day, especially due to motor vehicles.

The gases in which the measurements are made are close to the desired values and are slightly below or above the limit values determined at certain times of the day. The main reason why the rates of gases in the air vary in certain parts of the street according to the time periods when measurements are made is the traffic density on the street.

According to the measurement and observation results, the street has very intense usage at 15:00, when the oxygen rate is lowest and the C0 rate is highest. Likewise, the reason why the ratio changes like this at 09:00 or 18:00 is again related to the gases released into the environment by motor vehicles. One of the most important reasons why the oxygen rate is high in certain areas is the plant tissue on the edges of the street and median strips. Although the plant tissue is dense, especially in the area known as Bankalar Street, it has been determined that the O2 rate in these sections decreases due to the busy vehicles on the roadside.

Figure 11. a) İmam Hatip Square b) Banks Street

Figure 12. Restaurants on the street

It has been determined that another reason why the amount of oxygen in the street decreases and the amount of CO increases in certain periods of time is due to the restaurants located in certain areas of the street. The oxygen content in certain areas changes instantaneously, especially due to the harmful gases released from the chimneys of these areas**.** The industrial branches operating throughout Niğde vary. Air pollution caused by industry mainly occurs as a result of choosing the wrong location, using inappropriate fuel, and discharging waste gases into the receiving environment without taking adequate technical precautions.

Although the calcite quarries located around the city do not have a direct impact on residential areas, the dust created as a result of the activities of these facilities has negative effects on the air quality of the city. Facilities with a high risk of dust formation should be moved out of residential areas. In the layout planning of the city, construction patterns that would obstruct the flow of wind within the city should be prevented. Zoning regulations should be made to ensure a certain distance between industrial facilities and residential areas, and infrastructure works should be carried out to move industrial facilities and workshops within the city out of the urban settlement.

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Determination of Comfort Zones in Landscape Planning in Niğde

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ABSTRACT

Our cities, most of which do not show planned development, are turning into ecosystems that threaten the living life of the natural and cultural environment as a result of many environmental problems such as increasing human and building density and incorrect land use. This negative situation has the opportunity to be resolved with urban planning in which the physical structure of the city is balanced and with landscape plans that will create open and green areas in the city that contribute positively to human and environmental health. The urban heat island effect, which has emerged as a result of intense urbanization in recent years, creates problems for city residents. Unfortunately, most cities in the world face this negative climate phenomenon. It becomes difficult to combat the heat island effect, especially in cities where construction density increases unconsciously. Ecological planning appears as a factor that will prevent these problems from turning into important environmental problems in the future. The most important component to be considered in ecological planning is climate. In this study, thermal comfort areas were tried to be determined along Dr Sami Yağız Street, which is one of the most frequently used areas of Niğde. Temperature and humidity measurements were taken at 25 points at equal intervals on both sides of the street. Measurements were made on different days of the week and at 4 different times during the day. The obtained values were transferred to ArcGIS 10.3 software and maps were produced. A universal linear extension system was used in the evaluation of climate data, and climate factors and bioclimatically suitable areas on the street were determined and evaluated in terms of comfort level. It has been determined that the humidity rate in the study area varies according to measurement hours, and there is no significant change in temperature.

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Introduction

According to the United Nations World Urbanization Reports in recent years, population density in cities has increased significantly (United Nations, 2018). Accordingly, cities are growing day by day and the population density per unit area is increasing. This unplanned urban development negatively affects the quality standards of living spaces and brings with it many environmental problems, from air pollution to increased energy consumption and thermally uncomfortable spaces. At the same time, the residential areas needed to meet the needs of the increasing urban population lead to a decrease in open green areas. In addition to many known physical properties of open-green spaces (Payton et al., 2008; Nordh et al., 2011), they also provide important psychological contributions (Parsons and Daniel, 2002; Carlson 2010; Nordh et al., 2011). There are many studies aiming to determine thermal comfort values in residential areas with different qualities in cities (Unger, 1999; Yılmaz et al., 2007; Bulgan et al., 2014; Dikhan et al., 2018; Canan et al., 2019; Zengin et al., 2019). Many climate models, called simple and complex, are used to determine thermal comfort, and future scenarios can sometimes be produced with meteorological data from past years. In addition to the models used to determine thermal comfortable spaces, the use of thermal cameras has also gained momentum in this field in recent years. Thanks to these cameras, which can also be recorded by unmanned aerial vehicles, surface temperatures can be measured. In climate-focused studies, Thermal Cameras are used to determine the current status of urban living spaces and to work on forward-looking improvement scenarios in designs (Zengin et al., 2019).

Sustainable urban planning approaches aim to control urban heat island formation and develop strategies to reduce this effect. On a macro scale, structural densities, hard ground and green tissue density within the city are tried to be kept in a certain balance. When interventions are aimed at an existing urban fabric or when new urban areas are planned, it is important to predict the microclimatic conditions that may occur during the planning-design stages. Using simulation programs, preliminary information about the microclimatic conditions that are likely to occur can be obtained in a realistic manner in these preliminary planning/design stages.

In order to have information about the microclimate that will occur and to avoid gross mistakes, various scientific studies conducted in the same city or a city in a similar climate zone can be used. In some of these studies, the effects of various parameters that make up the structural environment on the urban climate are discussed and can provide various clues to urban planners, architects and landscape architects.

Within this scope, the study by Oke, in which the urban heat island effect was determined based on average sky openness values (SVF), is a good example to present (Oke, 1987). Again, in scientific studies on urban climate, the methods of obtaining information are obtained with the help of on-site short-term micro-meteorological measurements, long-term meteorological measurements, satellite data or simulation programs. An extensive literature has emerged on this subject in recent years (Canan and Geyikli, 2023).

The structure and characteristics of the urban climate are important issues that need to be addressed in the planning and design process. During the urbanization process, changes in the climate structure occur, albeit unintentionally, through planning and design. Landsberg (1981) emphasized the impact of changes in land use in urban areas on the urban area and climate; It states that in a city where vegetation has decreased by 35%, an area of 20 km^2 will be affected. In this affected area, urban heat island density increases, surface rainwater retention decreases, control of flood events becomes difficult, water management problems emerge, sudden and rapidly changing wind fields occur, differences occur in rain patterns, surface temperature increases, pollutant rate and their distribution is increasing (Balık and Yüksel, 2014).

From past to present, climate has been effective in directing human needs such as settlement, shelter, nutrition and health, which constitute the daily life cycle. The concept of climate, which affects daily life routine, has been tried to be better understood by people for centuries. As the relationship between humans and climate is established, it has been observed that people feel healthier and more vigorous physiologically and spiritually under certain conditions. Accordingly, the concept of bioclimatic comfort is defined as the conditions in which people adapt to their environment by consuming the least amount of energy. Temperature, humidity and wind parameters are evaluated to determine bioclimatic comfort status (Topay and Yılmaz, 2004; Erkek et al., 2020).

Due to the impact of bioclimatic comfort on humans and other living species, it is an important input especially for planning and design processes. Because, in physical planning and design processes, which are mostly carried out to create environmentally compatible and sustainable living spaces, the focus is on providing optimum living conditions for people while preserving environmental and cultural elements. In this context, it is important to evaluate climatic factors in a way that will enable people to live a more comfortable life and to turn them into planning input. In this study, it was planned to evaluate the detailed meteorological and climate analysis and results of Dr. Sami Yağız Street, and to create a design database for landscape planning. This database can be used for bioclimatic assessment of planning and design, climate balanced planning and design criteria as well as other criteria. The regional suitability of the street in terms of thermal comfort was discussed, and the effects of factors such as space use and traffic on heat exchange were determined.

Materials and Method

In the study, it was planned to produce maps of the gases that affect the air quality of Dr Sami Yağız Street in Niğde Province. Niğde is surrounded by Mersin to the south, Konya to the west, Nevşehir to the north, Aksaray to the northeast, and Kayseri to the east (Figure 1). Therefore, the main material of the study was prepared by Dr. Sami Yağız Street and its surroundings. Dr. Sami Yağız Street is one of the longest streets of Niğde Province and is approximately 1.5 km long and 15 meters wide. Dr. Sami Yağız Street is located at 37.966373 latitude and 34.672649 longitude (Figure 2).

Figure 1. Location of the study area

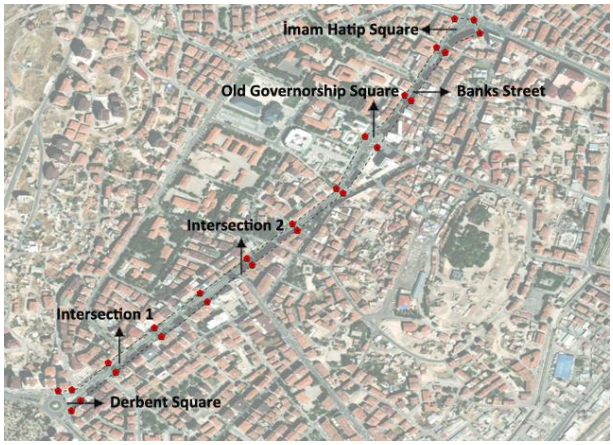

Figure 2. Dr. Sami Yağız Street and measurement points

Temperatur and humidity measurements were made at 25 points at equal intervals on both sides of the street. Measurements were made on weekdays. The reason for this is that the street is exposed to heavy traffic on weekdays, especially for reasons such as school, work, etc. In addition, measurements were made on weekdays to determine the effects of the restaurants on the sides of the street on air quality. After determining the days to be measured, the time period in which the measurement would be made was discussed. As a result of the observations, the street is heavily used at 09:00, 12:00, 15:00 and 18:00. 09:00 and 18:00 are the time period for going home, work, and returning to school, work, etc. It is used intensively in the 12:00 and 15:00 time zones for various reasons (eating, public, banking, etc.). The obtained values were transferred to ArcGIS 10.3 software and maps were produced. Through the maps, it was determined in which time periods the temperature and humidity values changed, in which parts of the street the temperature and humidity levels changed and the reasons why.

Results and Discussion

The warm season in Niğde is 3.5 months long and starts on June 13 and lasts until September 22, with the average daily high temperature being over 24°C. The cold season is 3.5 months long and starts on November 29 and lasts until March 10, with the average daily high temperature below 8°C. The coldest month in the Niğde is January; The average low temperature in this month is -5°C, while the high temperature is 3°C. The hottest month is July and the average temperature is around 30°C. For this reason, measurements were made in January. The obtained values were interpreted according to seasonal temperature norms. The results of the measurements for temperature are given in Figure 3-6. It was determined that the temperature values in the study area varied between 0°C and 8°C on average. According to the data received from the General Directorate of Meteorology, taking into account the years 1935 - 2022, the lowest average temperature in January, when the study was conducted, was determined as - 0.3°C, the lowest temperature was -4.6°C, and the highest temperature was 4.8°C.

This study was conducted at 09:00, 12:00, 15:00, 18:00 and it was determined that the temperature values were above the average values considering the regional conditions. It has been determined that the temperature value increases by approximately 1-2°C from east to west of the area. The highest temperature values on the street were reached between 12:00 and 15:00, and the lowest temperature values were reached in the measurements made at 18:00. The areas with high temperatures are Derbent Junction, Imam Hatip Square and the points where the Old Governorship Square is located. According to the temperature values of January 2023, it was determined that the average temperature was 8 degrees and above (Figure 7).

Compared to the average of previous years, there is warmer weather in 2023. According to the measurement results, the average temperature of the street is close to this value. The results of the measurements for humidity are given in Figure 8-11. It was determined that the humidity values in the study area varied between 36% and 90% on average.

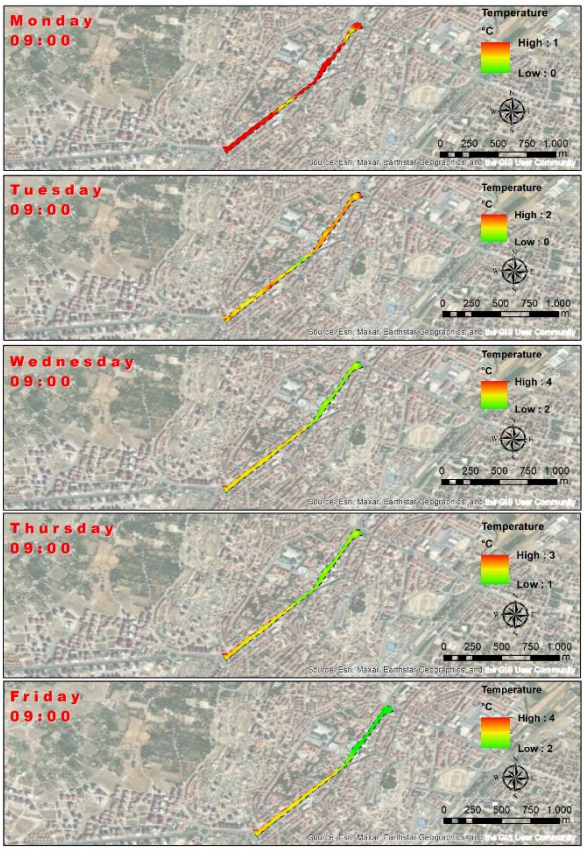

Figure 3. Temperature measurement – 09:00

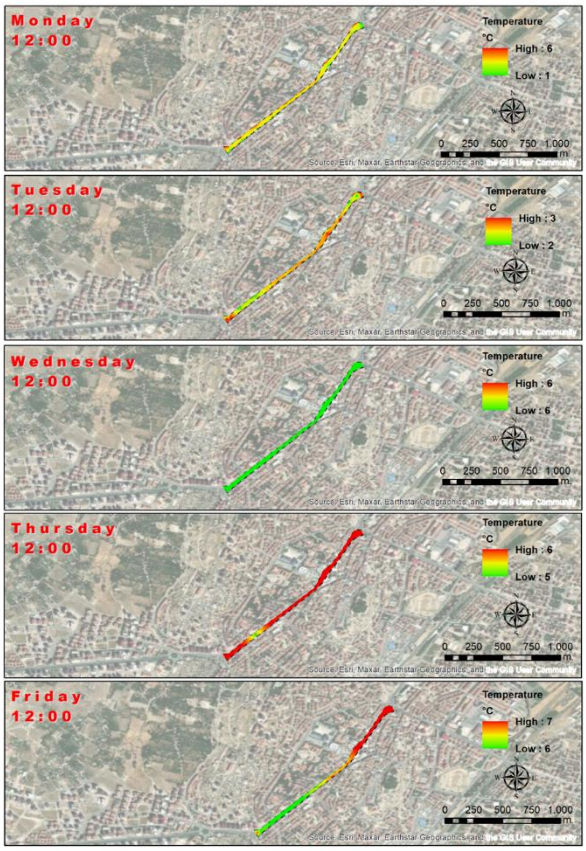

Figure 4. Temperature measurement – 12:00

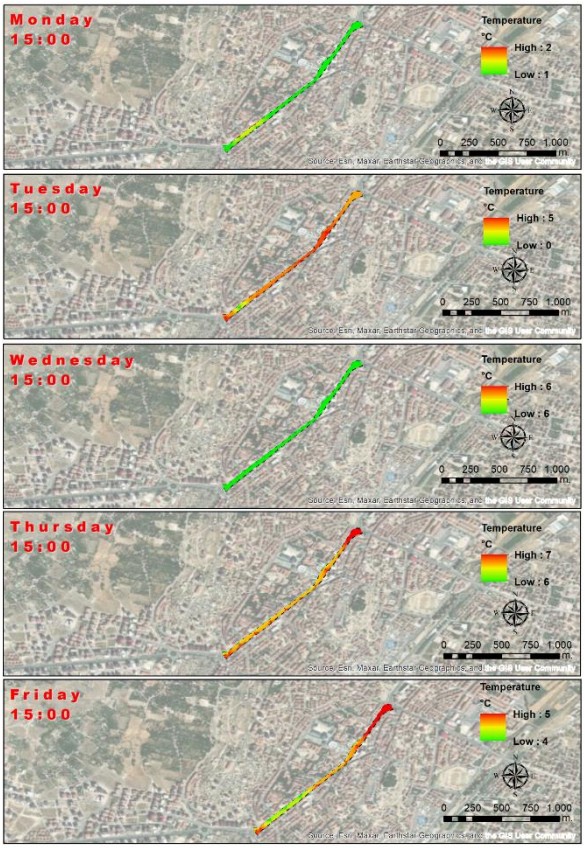

Figure 5. Temperature measurement – 15:00

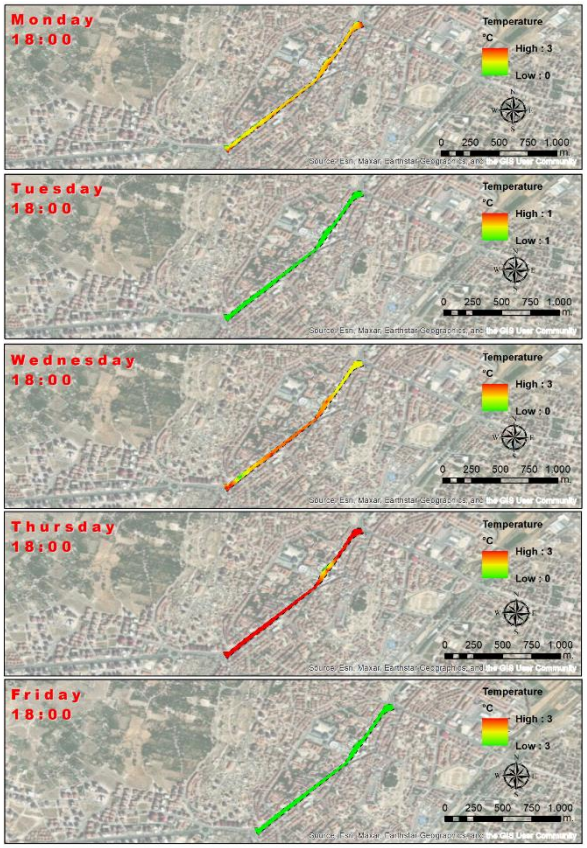

Figure 6. Temperature measurement – 15:00

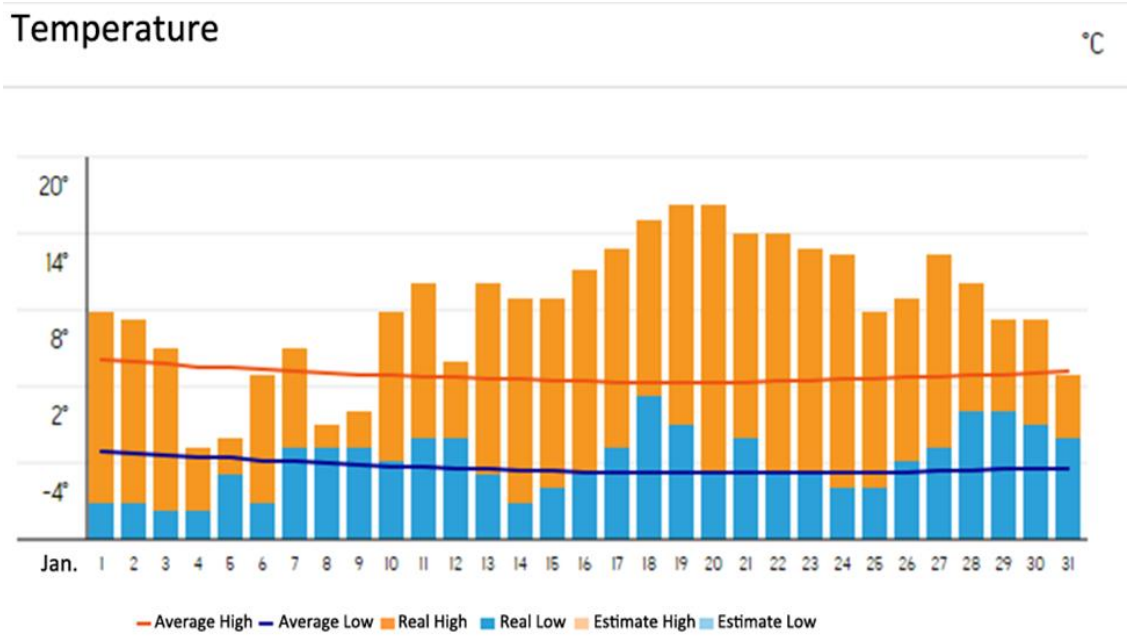

Figure 7. January temperature data (Anonymous, 2023)

According to the data received from the General Directorate of Meteorology, taking into account the years 1935 - 2022, it was determined that the lowest ambient humidity was 20%, the lowest humidity was 10% and the highest humidity was 75% in January, when the study was conducted.

It was determined that the humidity values, as well as the temperature data, were compatible with the previous climate data. Considering that the temperature values of January 2023 are above the average values of previous years, it is expected that the humidity value will also increase. The highest average humidity values on the street were reached in the 12:00 and 15:00 time periods, and the lowest average humidity values were reached in the measurements made in the 18:00 time period. The areas with high temperatures are Derbent Junction, Imam Hatip Square and the points where the Old Governorship building is located.

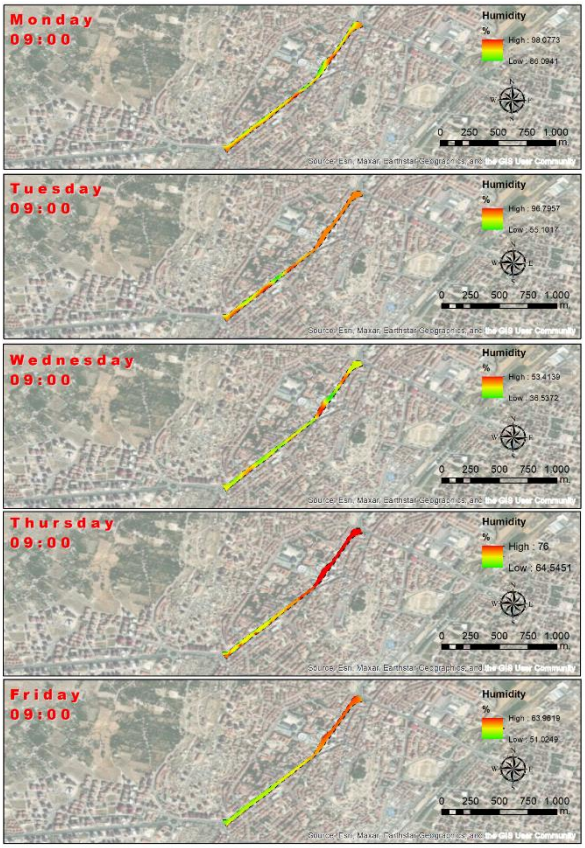

Figure 8. Humidity measurement – 09:00

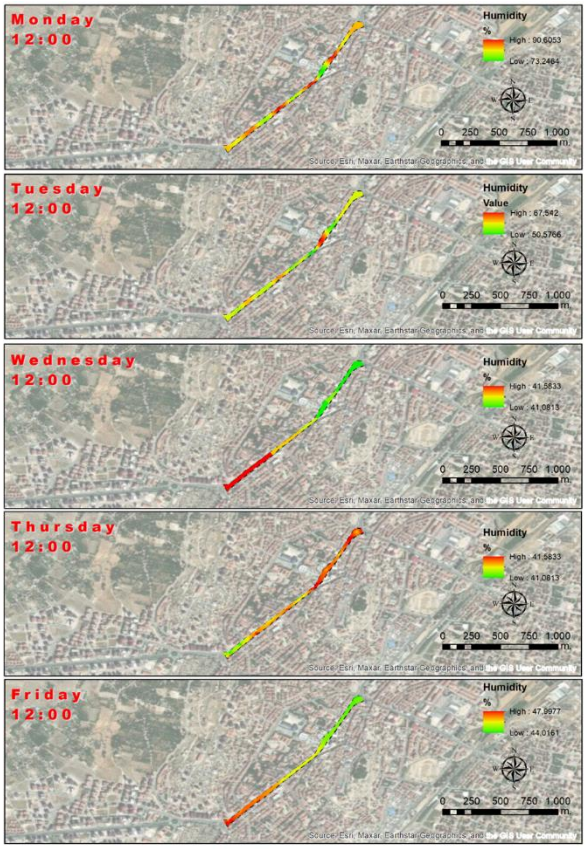

Figure 9. Humidity measurement – 12:00

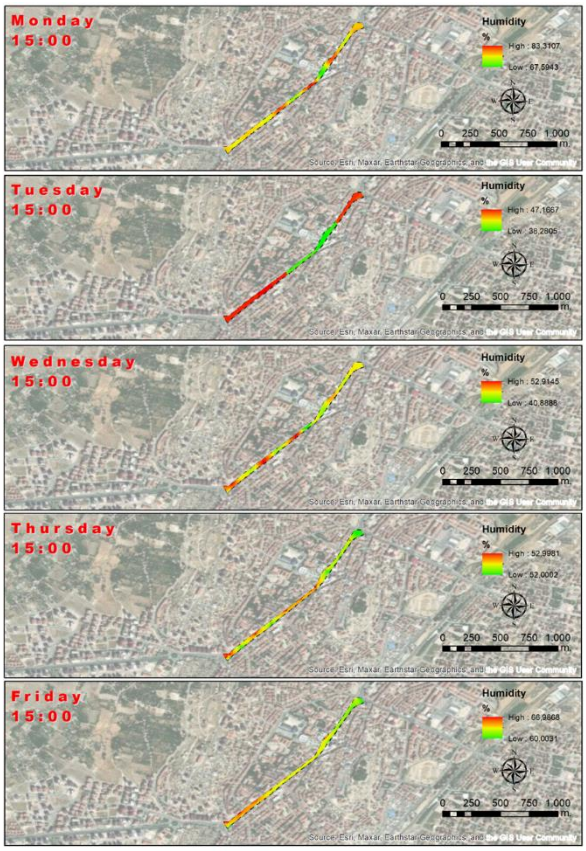

Figure 10. Humidity measurement – 15:00

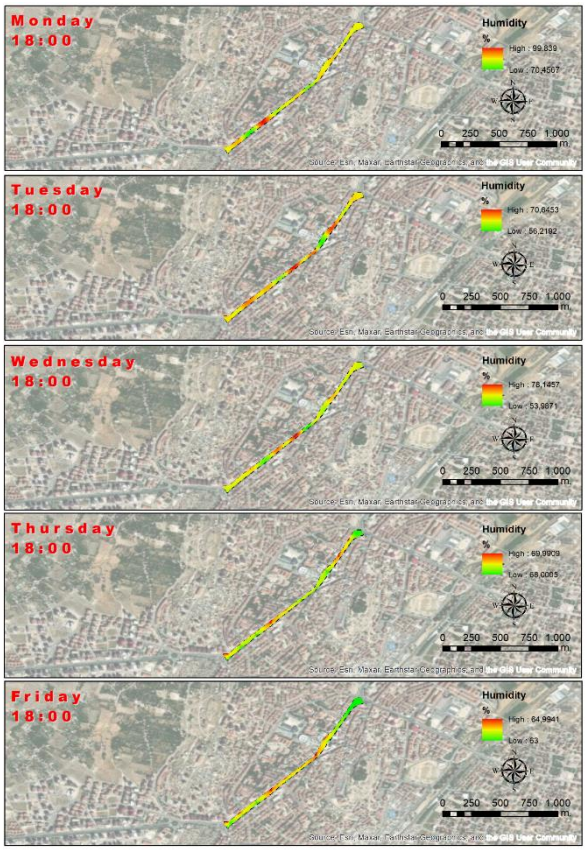

Figure 11. Humidity measurement – 18:00

Figure 12. Dr. Sami Yağız Street

Conclusion

In Turkey, studies on determining bioclimatic comfort areas are increasing day by day. Cetin et al. (2010) examined the bioclimatic comfort areas for Kütahya province in line with the landscape architecture profession and determined suitable areas for landscape activities in line with the landscape planning criteria. In another study, bioclimatic comfort areas suitable for landscaping activities in Kastamonu city center were determined (Çetin, 2015). Kestane and Ülgen (2013) determined bioclimatic comfort zones for İzmir province with the help of GIS. Çetin (2016) examined the determination of bioclimatic comfort areas in landscape planning using the Cide coastline example. This study is similar to other studies in terms of method. However, since the climatic characteristics and land use of the study areas will differ, it is not possible to compare the results. When all the data obtained were evaluated, it was determined that almost the entire region was suitable in terms of thermal comfort, considering the average data of the month in which the measurements were made. It has been determined that the buildings on the street further reduce the climatic comfort conditions. The climatic comfort value range for January in Dr. Sami Yağız Street is at an optimum level between 0-10°C with an average temperature of 8°C. Humidity comfort values vary. GIS map results show that Dr. Sami Yağız Street generally has suitable space for climatic comfort in large areas or square areas.

As a result of the study, it was determined that approximately 52.48% of the total area of Dr. Sami Yağız Street was suitable for climatic comfort. Climatic comfort areas with negative values are mostly located in the northeastern part of the street. It has been determined that this result is caused by the narrowing of the road width and the high building density in these parts of the street. High humidity values in this region reduce climatic comfort values. The southern part of the street has wider roads and more empty areas. Accordingly, with the influence of the aspect of the area, increasing sunshine duration causes the temperature to increase. High temperature and humidity levels in this region reduce bioclimatic comfort values. Landscape architecture and planning can be improved by applying bioclimatic comfort conditions principles and design criteria. Planning and bioclimatic comfort design in the wrong conditions can create extremely adverse conditions. In this study, Dr Sami Yağız street was examined in terms of bioclimatic comfort. The study results show that most of the street is suitable for bioclimatic comfort.

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Identification of *Alternaria, Aspergillus, Fusarium* and *Penicillium* spp. on Onion Plant (*Allium cepa* L.) Growing in Hatay, Amasya and Tokat Provinces Using MALDI-TOF Mass Spectrometry

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ABSTRACT

Plant fungal disease pathogens cause significant yield and quality losses in onion growing areas. In addition to yield losses, they cause negative effects that reduce the quality and export potential of the product, resulting in significant economic losses during harvest, post-harvest, processing and marketing stages. In recent years, Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) has emerged as a rapid, cost-effective, reproducible, and powerful technique for identifying microorganisms, and its impact on microbiological diagnosis has transformed workflow in equipped laboratories. In this study, proteomic analyzes were performed on *Alternaria, Aspergillus, Fusarium* and *Penicillium* species isolated from onion growing areas in Hatay, Amasya, and Tokat provinces. After extraction of mycelium from single spore cultures of the isolates with ethanol-formic acid, the spectra of the individual fungal isolates were determined using the Flex control software program. These spectra were compared with Maldi Biotyper Real-Time Classification (RTC) and identification was performed. Of 519 different fungal isolates, 435 representative fungal isolates (83.8%) were identified by MALDI TOF MS. Eighty-four fungal isolates could not be identified because they were not in a satisfactory range of purity and identification. Of the 435 isolates, 269 (61.8%) were identified as *Fusarium* spp., 80 isolates (18.4%) were identified as *Alternaria* spp., 60 isolates (13.8%) as *Aspergillus* spp., and 26 isolates (6.0%) as *Penicillium* spp. Among the fungal isolates, 72.5% of the *Fusarium* isolates, 78.8% of the *Alternaria* isolates, 90.0% of the *Aspergillus* isolates and 84.6% of the *Penicillium* isolates were identified as "highly probable" species with score values between 2.000-3.000 (green color). These species are *Alternaria alternata, Alternaria infectoria, Aspergillus flavus, Aspergillus niger, Fusarium culmorum, Fusarium moniliforme, Fusarium oxysporum, Fusarium proliferatum, Fusarium solani, Fusarium verticillioides, Penicillium commune* and *Penicillium glabrum*. The results clearly demonstrate that MALDI TOF MS biotyping may be used as a highly reliable and economical diagnostic method for routine diagnosis of diseases caused by *Alternaria, Aspergillus, Fusarium* and *Penicillium* species.

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Introduction

Onion (*Allium cepa* L.) is one of the vegetables with a great variety when examined considering its different characteristics. Many factors prevent production in the areas where onion cultivation is carried out. Among them, fungal, bacterial and viral disease pathogens cause significant yield and quality loss in different development periods of the onion plant (Schwartz and Mohan, 2008). These diseases and pests create negative effects that reduce the quality and export potential of the product, which cause significant economic losses during harvest, post-harvest, processing and marketing stages, as well as a decrease in yield. Among the fungal and fungal-like disease pathogens, *Alternaria porri*, *Alternaria alternata*, *Alternaria tenuissima*, *Alternaria palandui*, *Alternaria brassicola*, *Botrytis cinerea*, *Botrytis aclada*, *Botrytis allii*, *Botrytis byssoidea*, *Botyrtis squamosa*, *Botrytis porri*, *Cladosporium* sp., *Stemphylium* sp., *Urocystis cepulae* and *Peronospora destructor* cause significant product losses by causing disease in the green parts of the plant. Fungal disease pathogens such as *Aspergillus niger, Aspergillus oryzae, Penicillium georgiense, Penicillium polonicum, Penicillium glabrum* and *Penicillium expansum* cause bulb rot in onions before and after harvest. Soil-borne fungal disease pathogens such as *Pythium* spp., *Fusarium* spp.,

Rhizoctonia solani, *Sclerotium rolfsii* and *Sclerotium cepivorum* cause diseases such as wilt, root and root rot and damping-off in onions (Smith, 1988; Haq et al., 2003; Chilvers et al., 2007; Schwartz and Mohan, 2008; Dumbre et al., 2011; Bayraktar et al., 2014; Oh et al., 2015; Duduk et al., 2017; Chethana et al., 2018).

In the provinces where onion cultivation is intense in Turkey, the definitive diagnosis of fungal pathogens seen in onion plants using the latest technological diagnostic devices is of great importance for the development of correct control methods.

In recent years, Matrix-assisted lasser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) has emerged as a powerful technique for the identification of microorganisms and its impact in microbiological diagnostics has changed the workflow in well-established laboratories. Compared to traditional diagnostic methods that rely on biochemical testing and require long incubation procedures, MALDI-TOF MS has the advantage of identifying bacteria and fungi directly from colonies grown in culture media in a few minutes and with simple procedures (Kurt et al., 2020; Uysal et al., 2022). Many studies available in different systems have proven the reliability and accuracy of the method (Carolis et al., 2014, Uysal et al., 2019; Soylu et al., 2021; Kara et al., 2022). In this respect, it offers a powerful alternative to microscopic and molecular biology methods. Today, commercial MALDI systems are available for diagnostic applications in clinical medicine, biotechnology, and industrial as well as biological research studies. Although it is mostly used in bacterial biotyping, many experimental strategies have been developed for the analysis of fungi. Members of many fungal genera such as *Aspergillus*, *Fusarium*, *Penicillium* or *Trichoderma*, as well as various yeasts from clinical specimens (e.g. *Candida albicans*) have been successfully identified with MALDI-TOF MS (Chalupová et al., 2014).

In this study, *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium* species isolated from onion growing areas in Hatay, Amasya and Tokat provinces were identified by MALDI-TOF MS system.

Materials and Methods

Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) Analysis

All fungal isolates were preliminary diagnosed morphologically using dichotomic keys. Colony color, reverse colony color, shape and size of conidia and conidiophores, shape and size of macro and/or microconidia, presence of sexual structures, sclerotia production and formation of chlamidospores on selective and general nutrient media such as CDA (Czapek Dox Agar), CMA (Corn Meal Agar), MEA (Malt Extract Agar), YEA (Yeast Extract Agar), CLA (Carnation Leaf Agar) and PDA (Potato Dextrose Agar) media were used as morphological fungal parameters as suggested for each fungal genera (Nelson et al., 1983; Ellis, 1993, Barnett and Hunter, 2003; Frisvad and Samson, 2004; Chethana et al., 2018).

Fungal isolates, previously obtained from onion growing areas in Hatay, Amasya and Tokat provinces of Turkey, were grown in PDA medium for 5-7 days. Up to 3-5 mycelial discs (0.5 cm) were transferred to plastic tubes containing an average of 8 ml potato dextrose broth (PDB) broth. The cultures were allowed to grow on the rotator (13.000 rpm) in plastic tubes for 2-3 days at room temperature. Formic acid ethanol extraction processes were used for MALDI-TOF MS analyses. First of all, each isolates were taken into a 1.5 ml Eppendorf tube. It was centrifuged at 13000 rpm for 2 minutes. The liquid part was removed with the help of a Pasteur pipette so that the pellet remained at the bottom. Then, centrifugation was repeated by adding 1 ml of distilled water at HPLC value. The liquid part was removed so that the pellet remained at the bottom again. Then 300 µl of HPLC distilled water was added to the pellet and vortexed. Then, 900 µl of ethanol was added, vortexed again and centrifuged at 13000 rpm for 2 minutes. After centrifugation, the ethanol was completely removed and left to dry for 5-10 min at 37°C. Depending on the size of the pellet, between 10 and 80 µl of 70% formic acid was added to the dried pellet and vortexed. Acetonitrile was added as much as the amount of formic acid added and vortexed again. Finally, it was centrifuged at 13000 rpm for 2 minutes. After centrifugation, 1 µl of the upper liquid part was loaded into the wells of the MALDI-TOF MS target and left to dry. After drying, it was covered with HCCA (α-Cyano-4-hydroxycinnamic acid) matrix liquid. After the wells were completely dry, they were loaded onto the target device. Spectra were taken with the Flex control software program. Then, these spectra were compared with the MALDI Biotyper V9.0 software program and the species identification was completed (Biotyper 3.0; Microflex LT; Bruker Daltonics GmbH, Bremen, Germany).

As a result of the analysis; the scores between 2.300-3.000 (green color) probable species identification, 2.000-2.299 (green color) reliable genus level diagnosis and probable species level diagnosis, 1.700-1.999 (yellow color) probable genus level diagnosis, 0.000- a score of 1.699 (red color) was considered as an unreliable diagnosis (Uysal et al., 2019; Soylu et al., 2021; Kara et al., 2022; Uysal et al., 2022).

Results and Discussion

A total of 1691 fungal isolates were obtained from different fields and provinces in Tokat, Amasya and Hatay provinces where important onion cultivation was made. Pre-diagnosis of each isolates were made according to their morphological properties. Single spore cultures of 519 isolates pre-diagnosed as *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium* spp. were obtained for MALDI-TOF analysis. The spectra of the device were taken with the Flex control software program following the extraction of mycelium from single spore cultures of the isolates with ethanol-formic acid. These spectra were compared with the Maldi Biotyper Real-Time Classification (RTC) and diagnosis process was performed (Figure 1).

Among 519 different fungal isolates, 435 isolates were diagnosed (83.8%) using the MALDI TOF MS analysis. Eighty four fungal isolates could not be diagnosed because they were not in the satisfactory purity and identification spectrum. Of the 435 isolates identified, 269 (61.8%) were identified as *Fusarium* spp., 80 (18.4%) isolates were *Alternaria* spp., 60 isolates (13.8%) were *Aspergillus* spp., and 26 (6.0%) isolates were *Penicillium* spp.

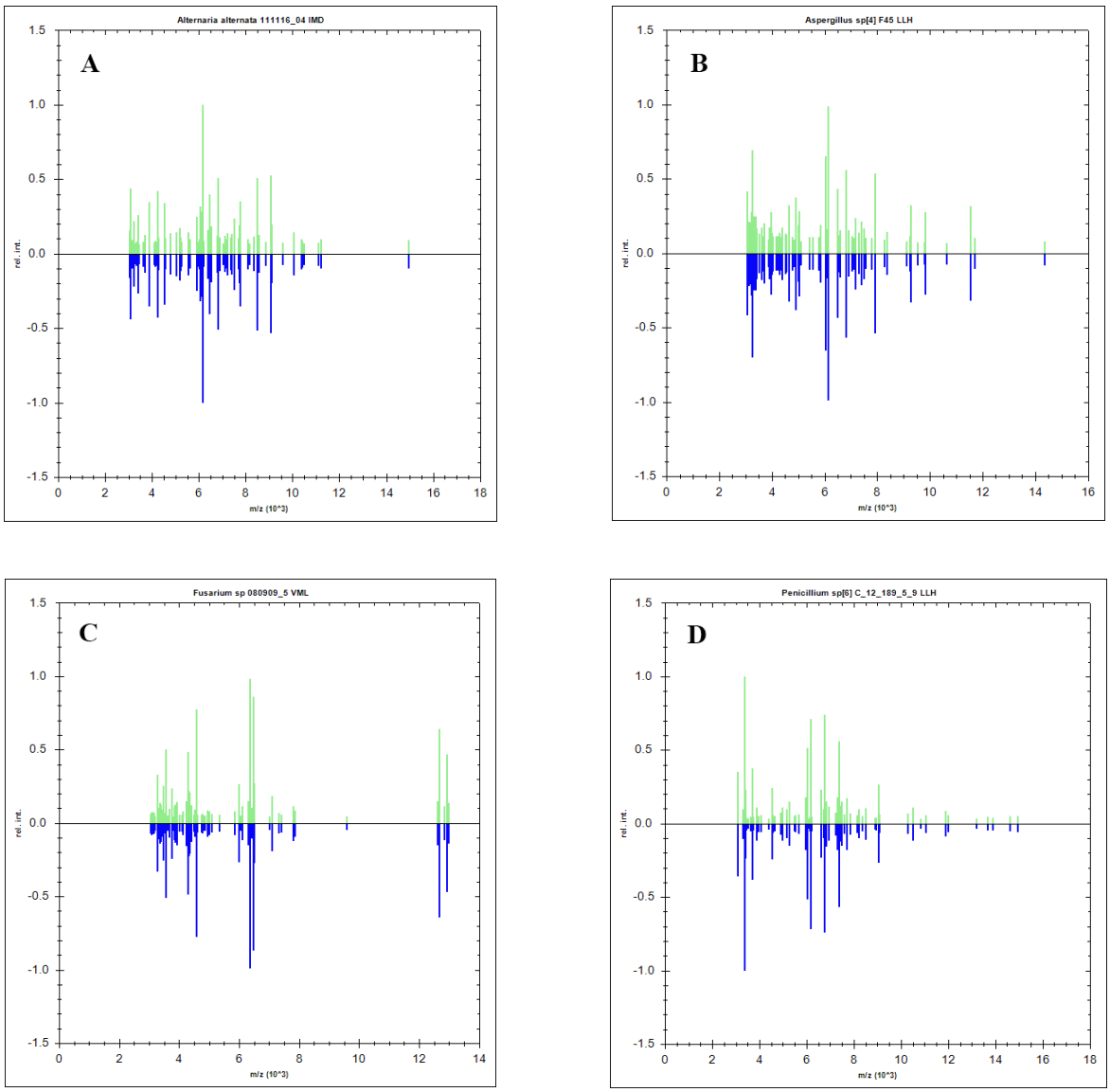

Figure 1. MALDI-TOF MS spectra of representative isolates belonging to *Alternaria* (A), *Aspergillus* (B), *Fusarium* (C) and *Penicillium* (D) genera identified using MALDI Biotyper 3.0 program

Amongst identified isolates, 72.5% of the isolates of the *Fusarium* genus, 78.8% of the *Alternaria* genus, 90.0% of the *Aspergillus* genus and 84.6% of the *Penicillium* isolates with score values between 2,000-3,000 (green color) were identified as "highly probable" species. These species are *Alternaria alternata, Alternaria infectoria, Aspergillus flavus, Aspergillus niger, Fusarium culmorum, Fusarium moniliforme, Fusarium oxysporum, Fusarium proliferatum, Fusarium solani, Fusarium verticillioides, Penicillium commune* and *Penicillium glabrum*.

The distribution of 435 isolates obtained from different provinces and identified with MALDI TOF MS according to the plant parts from which they were isolated on the basis of provinces and their % ratios are given in Tables 1 and 2. In this way, a preliminary diagnosis process was carried out according to protein analysis of species belonging to *Alternaria, Aspergillus, Fusarium* and *Penicillium*. This study is the first in Turkey to use the MALDI TOF MS technique for the identification of *Alternaria, Aspergillus, Fusarium* and *Penicillium* species in onions. When the MALDI TOF MS method is compared with molecular methods considering the economic cost aspect, it has clearly shown that MALDI TOF MS biotyping can be used as a highly reliable and economical diagnostic method for the routine diagnosis of diseases caused by *Alternaria, Aspergillus, Fusarium* and *Penicillium* species.

It has also been reported in many taxonomic identification studies that the MALDI-TOF MS technique is an effective device for the diagnosis of Ascomycetes phytopathogenic fungi belonging to the *Alternaria* genus, such as *Alternaria dauci* in carrots, *A. porri* in onions, *Alternaria solani* in potatoes, and *Alternaria tomatophila* in tomatoes (Brun et al., 2013; Chalupová et al., 2014). Hettick et al. (2008a) described 12 *A. flavus* species and 5 strains using the MALDI TOF MS technique. In another study, 12 *Penicillium* species were analyzed by MALDI TOF MS technique (Hettick et al., 2008b). The MALDI-TOF MS technique was also used to differentiate *P. expansum* and *Penicillium pinophilum* in apples and *P. citrinum, P. italicum* and *P. digitatum* in citrus fruits (Chen and Chen, 2005).

Table 1. The isolate numbers of fungal species obtained from Hatay, Amasya and Tokat provinces and identified by MALDI TOF MS analysis according to the samples from which they were isolated (piece)

Species Name	Hatay					Amasya					Tokat					T
	R	B	L	F	S	R	B	L	F	S	R	B	L	F	S	
Alternaria alternata	-	-	17	2	-	1	2	11	4	-	-	-	14	28	-	79
Alternaria infectoria	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
Aspergillus flavus	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
Aspergillus niger	3	21	-	-	-	2	3	3	1	1	2	7	8	8	1	59
Fusarium culmorum	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
Fusarium moniliforme	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
Fusarium oxysporum	11	9	5	-	5	51	27	19	-	-	16	11	4	6	3	167
Fusarium proliferatum	2	6	8	-	-	8	4	15	1	1	3	4	7	1	-	59
Fusarium solani	2	1	-	-	-	4	-	1	-	-	1	-	-	-	-	9
Fusarium verticillioides	2	1	2	-	-	10	5	6	-	-	2	-	3	1	-	32
Penicillium commune	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
Penicillium glabrum	5	4	-	-	-	3	5	1	-	-	2	3	-	2	-	25
Total	25	42	32	2	5	82	45	56	6	2	26	25	37	46	4	435

R: Root; B: Bulb; L: Leaf; F: Flower; S: Soil; T: Total

Table 2. Distribution of fungal species obtained from Hatay, Amasya and Tokat provinces of Turkey and identified according to MALDI TOF MS analysis according to the plant parts they isolated (%)

Species Name	Hatay					Amasya					Tokat				
	R	B	L	F	S	R	B	L	F	S	R	B	L	F	S
Alternaria alternata	-	-	89.5	10.5	-	5.6	11.1	61.1	22.2	-	-	-	33.3	66.7	-
Alternaria infectoria	-	-	-	-	-	-	-	-	-	-	-	-	100.0	-	-
Aspergillus flavus	-	-	-	-	-	100.0	-	-	-	-	-	-	-	-	-
Aspergillus niger	12.5	87.5	-	-	-	20.0	30.0	30.0	10.0	10.0	7.7	26.9	30.8	30.8	3.8
Fusarium culmorum	-	-	-	-	-	-	100.0	-	-	-	-	-	-	-	-
Fusarium moniliforme	-	-	-	-	-	100.0	-	-	-	-	-	-	-	-	-
Fusarium oxysporum	36.7	30.0	16.7	-	16.7	52.6	27.8	19.6	-	-	40.0	27.5	10.0	15.0	7.5
Fusarium proliferatum	12.5	37.5	50.0	-	-	27.5	13.7	51.7	3.4	3.4	20.0	26.7	46.7	6.7	-
Fusarium solani	66.7	33.3	-	-	-	80.0	-	20.0	-	-	100.0	-	-	-	-
Fusarium verticillioides	40.0	20.0	40.0	-	-	47.6	23.8	28.6	-	-	33.3	-	50.0	16.7	-
Penicillium commune	-	-	-	-	-	100.0	-	-	-	-	-	-	-	-	-
Penicillium glabrum	55.6	44.4	-	-	-	33.3	55.6	11.1	-	-	28.6	42.9	-	28.6	-

R: Root; B: Bulb; L: Leaf; F: Flower; S: Soil

Recently, two *F. solani* isolates (CFs4 and CFs8) that cause dry rot in citrus roots in the Eastern Mediterranean Region were identified with MALDI-TOF MS technique (Kurt et al., 2020). Similar to our study, Al-Hatmi et al. (2016) was successfully identified *Fusarium ficicrenscens* as a differential species in the *Fusarium fujikuroi* species complex by using the formic acid-ethanol extraction method. In our study, all obtained spectra were evaluated on the Bruker Mikroflex platform using MALDI Biotyper V2.0 software. As a result, the device made a reliable diagnosis of 3 isolates of *Fusarium* with 2.193, 2.200 and 2.226 score values. De Carolis et al. (2012) created their own library for Mucorales, *Fusarium* and *Aspergillus* species with the Biotyper system and identified 97% of 94 isolates at the species level. Santos et al. (2015) tested the MALDI-TOF MS technique to identify *Fusarium guttiforme* on pineapple side shoots in situ. The identification of a plant pathogen (*F. guttiforme*) and its antagonist (*Trichoderma asperellum*) using MALDI-TOF MS have been demonstrated. On the other hand, Masih et al. (2016) identified 95% of *Aspergillus* species using the Bruker system and the database they developed.

In conclusion, MALDI-TOF MS technique for the early detection of filamentous fungi infecting agricultural products has not been extensively studied and data is lacking in the literature.

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Phylogenetic Analysis and Lipoxygenase (LOX) Gene Family Variation in The Pistachio

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ABSTRACT

Lipoxygenases (LOX) gene family is a type of nonheme iron-containing dioxygenases, which has a very important aspect in plant development and fruit quality. LOX gene, which is responsible for lipid oxidation, the main role for the biosynthesis pathway of unsaturated fatty acids. Although some studies have investigated the LOX gene family in several species including arabidopsis, soybean, peanut and apple, there is no information from Pistachio; and the phylogeny of this gene family in the *Pistacia* genus is still not determined. In this study, *Arabidopsis thaliana* LOX1 gene (NCBI Reference Sequence: NM_104376.3) was selected and used as a query sequence for performing a BLASTN search. Among all sequence query which was found by NCBI platform, 9 sequences were selected for further analysis. Phylogenetic tree of full-length LOX gene sequences from the Pistacia genus was constructed using the Maximum Likelihood method with MEGA software. By using phylogenic analysis, we identified variations in gene structure and revealed the phylogenetic evolutionary relationship of this gene family. Additionally, this may serve as a reference value for assessing the genetic relationships among various LOX genes in *Pistacia* genus species. This variations provides us the possibility the design the primer to achieve us to find the exact LOX gene in *Pistacia* genus and future research on the evolutionary history and transgenic research on LOX genes.

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Introduction

Pistachio belongs to *Pistacia* genus, which is the most important genus of flowering plants from Anacardiaceae family. (Zohary, 1952; Motalebipour et al., 2018; Kafkas et al., 2023). Pistachios owe their economic significance to being highly favored nuts due to their rich content of unsaturated fatty acids and potassium. These elements possess both antioxidant and anti-inflammatory properties. Nevertheless, Pistachio (*Pistacia vera*) crops face substantial losses each year worldwide due to a range of factors, including drought, salt, herbivorous insects, and viruses. (Christensen et al., 2015).In plants, the production of oxylipins and related compounds, such as jasmonic acid (JA), green leaf volatiles, divinyl ethers, and traumatic acid, is facilitated by enzymes known as lipoxygenases (LOXs) within the LOX pathway (Christensen et al., 2015).

Lipoxygenases (LOXs), a type of non-heme iron-containing dioxygenase, are widely found in plants and contribute significantly to the development of fruit aroma, a crucial aspect of fruit quality (Needleman et al., 1986). These enzymes are also prevalent in the animal and plant kingdoms and play essential roles in resisting both biotic and abiotic stresses (Song et al., 2016). The biosynthesis of Lipoxygenases has been extensively studied in various plant species, including *Arabidopsis thaliana* (Melan et al., 1994), *Triticum aestivum* (Feng et al., 2012), *Glycine max* (González-Gordo et al., 2023), *Arachis hypogaea* (Mou et al., 2022) and *Malus* × *domestica* (Vogt et al., 2013). According to NCBI platform, 9 full length genes were recorded for Lipoxygenases (LOXs): 3 gene in *Arabidopsis thaliana*, 3 gene in *Homo sapiens*, 3 gene in *Rattus norvegicus* and one gene in *Triticum aestivum* . Among 3 gene in *Arabidopsis thaliana* (LOX1, LOX3, LOX5), LOX1 were used as a reference sequence in the current study. The aim of the study is to characterize the main Lipoxygenases gen in *Pistacia vera* and showed the distance between genes.

Material and Methods

Specific information on the LOX gene was obtained using the NCBI-Gene. A total of 9 species gene information was retrieved, including 9 known species information LOX gene of *Arabidopsis thaliana* with Reference Sequence: NM_104376.3 were selected for further analysis.

Phylogenetic trees of full-length sequences of different LOX genes of *Pistacia* genus were constructed by the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, 1993) using Molecular Evolutionary Genetics Analysis Version 11 (MEGA version 11 http://www.megasoftware.net, Tamura et al., 2021).

Results and Discussion

The present study provided basic information to understand the genetic diversity of LOX gene in the *Pistacia* genus. LOX gene has been studied in various plant species, such as *Arabidopsis thaliana* (Melan et al., 1994) and *Triticum aestivum* (Feng et al., 2012). Among 9 LOX genes, three genes in *A. thaliana*, were define in NCBI platform. NCBI's RefSeq of LOX1 gene of *A. thaliana* (NM_104376.3) was selected and used as a query sequence for performing a BLASTN search. BLASTN was done against all nucleotide database in *Pistacia* (taxid: 55512) organism. A Total of 11 putative non-redundant LOX gene were identified by performing BLASTN search against all database. Among 11 sequence query, 9 sequences with high coverage were selected for further analysis (Table 1). The sequence with zero E-value sequence represented the query sequence that matched the database sequence and was more significant. Nine sequence queries showed the predicted LOX family gene of *Pistacia* genus.

According to MEGA software, 9 different genes were analyzed and the genetic distance was calculated using the highest log likelihood method constructed a phylogenetic tree of the *Pistacia* genus (Figure 1).

Table 1. Information of all 9 gene sequence information of LOX in *Pistacia vera*.

No	Accession	Predicted name	Query Cover	E value	Accession length
1	XM_031404813.1	linoleate 9S-lipoxygenase 5	0.96%	0.0	2696
2	XM_031425379.1	linoleate 13S-lipoxygenase 3-1	0.96%	0.0	2976
3	XM_031406178.1	linoleate 13S-lipoxygenase 3-1	0.96%	0.0	2987
4	XM_031416247.1	linoleate 9S-lipoxygenase 5	0.96%	0.0	2992
5	XM_031426686.1	linoleate 13S-lipoxygenase 2-1	0.96%	0.0	2820
6	XM_031426696.1	linoleate 13S-lipoxygenase 2-1	0.95%	0.0	2864
7	XM_031398663.1	lipoxygenase 6	0.94%	0.0	2908
8	XM_031412135.1	lipoxygenase 6	0.94%	0.0	3062
9	XM_031392570.1	linoleate 9S-lipoxygenase 5	0.94%	0.0	2717

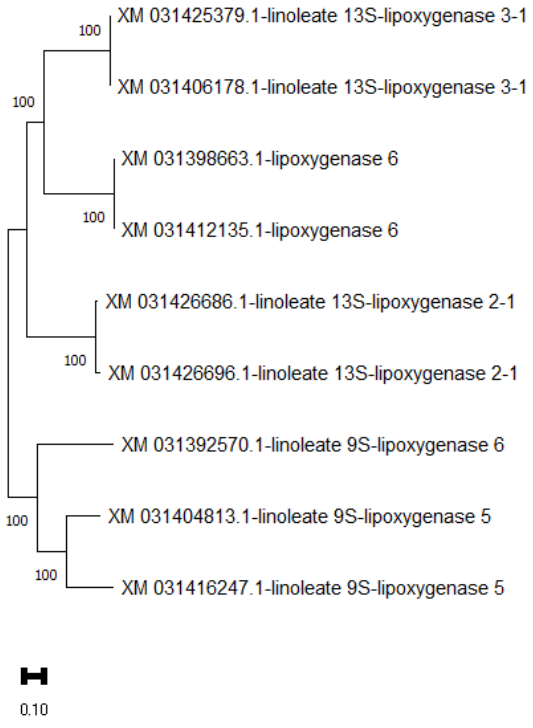

Figure 1, Phylogeny analysis for 9 predicted LOX gene sequences of *Pistacia vera* by using the Maximum Likelihood method and Tamura-Nei mode in MEGA software. The tree with the highest log likelihood (-20334.16) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches.

The results showed that the nine different LOX gene sequences from *Pistacia vera* were divided into two main groups. Each group was divided to 2 subgroups. The first group contained four sequences from two predicted gene (linoleate 13S-lipoxygenase 3-1, lipoxygenase 6), which separated in two branches. It means two predicted genes were very similar to each other. Furthermore, two gene of linoleate 13S-lipoxygenase 2-1genes were place in one branch and very close to the first group. Linoleate 9S-lipoxygenase 5 and linoleate 9S-lipoxygenase 6, were placed in different branches which means the differences of these genes. Two genes of linoleate 9S-lipoxygenase 5, and one gene of linoleate 9S-lipoxygenase 6 were placed very close and located in the same branch.

According to the results, the variation predicted by LOX genes in *Pistacia vera* showed the differences among the sequence and also protein followed by mechanism of a LOX gene. These variations are useful for primer design and finding the level of gene expression in other *Pistacia* species. The high level of gene expression can be concluded by the high amount of LOX gene in species for special purposes in breeding programs. Furthermore, some of the above species could be used directly as a breeding program.

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Effects of Leek Powder and Sunflower Oil in Guar Gum Edible Coating on the Preservation of Mushrooms (*Agaricus bisporus*)

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ABSTRACT

This study investigated the effects of various guar gum edible coating formulations, incorporating different proportions of waste leek powder and sunflower oil, on weight loss, color parameters (L*, a*, b* values, and ΔE), texture, and shrinkage of *Agaricus bisporus* mushrooms during a 7-day storage period. The goal was to assess the potential impact of these coatings on preserving the mushrooms' quality over time. The results showed that the coatings had a significant effect on reducing weight loss compared to uncoated samples. The lowest weight loss was observed in the 0.5% leek powder and without sunflower oil, while the highest was in 2.5% leek powder and 0.1% sunflower oil. Shrinkage was also positively affected by the coatings, with 1.5% leek powder and without sunflower oil showing the most promising results. The L* values of the coated samples declined slightly, indicating better color preservation, while the a* values exhibited stable redness/greenness. On the other hand, b* values increased, indicating an increase in yellowness during storage. The ΔE values were lower for the coated samples, suggesting less color deviation compared to uncoated ones. Overall, the study indicates that these edible coatings have the potential to maintain the quality of mushrooms during storage, leading to better preservation and extended shelf life.

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Introduction

In Türkiye, the cultivation of edible mushrooms, particularly the *Agaricus bisporus* species, has shown significant growth in recent years. According to estimates, the production of cultivated mushrooms was around 65,000 tons in 2018, and it is projected to reach 100,000 tons by the year 2025 (Eren and Pekşen, 2019). Among the various mushroom cultivation practices, *Agaricus bisporus* remains at the forefront due to its popularity and economic significance in the country. This upward trend reflects the increasing interest in mushroom production as a viable and profitable agricultural activity in Türkiye. However, the short shelf life of mushrooms constitutes a major problem in the field of mushroom production and preservation. The short shelf life of mushrooms can be attributed to the absence of a protective cuticle, which leaves them vulnerable to damage and water loss, consequently leading to microbial attacks (Zhang et al., 2019). To solve the problem, edible packaging options can be applied to mushrooms to prolong the freshness. Edible films or coatings create a protective barrier for mushrooms, offering benefits such as gas conditioning, delayed gas transfer, reduced moisture and flavor loss, slower color change, and improved appearance (Nasiri et al., 2018). While films are the structures that can be wrapped around food after molding, coatings involve directly applying liquid edible coating material to protect the food (Ali et al., 2010; Thakur et al., 2021). In one study, a blend of chitosan (1%) and guar gum (15%) coating helped maintain firmer tissue, slowed down the decline of soluble protein and ascorbic acid, and controlled changes in various components like total soluble solids and reducing sugar of the mushrooms (Huang et al., 2019).

Research has focused on preparing edible films and coating by environmentally degradable materials that are derived from plants, animals, and even their by-products or waste. Up to date, xanthan gum, alginate (Jiang 2013), chitosan (Nair et al., 2020), aloe vera (Kumar et al., 2023), and tragacanth gum (Nasiri et al., 2018) are commonly used in the production of edible coatings due to their biodegradability, recyclability, and sustainability. Although biopolymers have advantages, they possess poor mechanical and barrier properties. To address this, certain natural materials can be incorporated to enhance their flexibility, gas barrier, and mechanical properties. Notably, oils can be added to inhibit the growth of microorganisms and improve water-vapor barrier capacity.

The outer leaves of leeks (Allium porrum) can be considered as waste since these parts are typically removed and discarded during the preparation process before cooking or using leeks in recipes. Due to their high phenolic content (262.66 ± 18.05 mg GAE 100 g−1 dry weight) and significant antioxidant activity (48.58 ± 3.84%) coupled with antibacterial properties (Pellegrini and Ponce, 2020), leek leaves present a potential as an active ingredient in edible coating formulations. Sunflower oil in edible coatings may provide improved barrier properties, including enhanced water vapor resistance and flexibility, while also offering antioxidant benefits, antibacterial activity, and a mild flavor enhancement, making it a valuable addition for preserving and enhancing the quality of food products. Additionally, it serves as a source of essential fatty acids, contributing to the nutritional value of the coated food. In the literature, there have been no studies conducted on the application of edible coating using waste leek powder for the preservation of mushrooms. Thus, in this study, the impact of various guar gum edible coating formulations containing different proportions of waste leek powder and sunflower oil on weight loss, color parameters (L*, a*, b* values, and ΔE), texture and shrinkage of mushrooms (*Agaricus bisporus*) during storage was investigated.

Materials and Methods

Materials

Leek and sunflower oil were purchased from a local store. Guar gum and glycerol was purchased from Sigma-Aldrich (Merck İlaç Ecza ve Kimya Tic. A.Ş., an affiliate of Merck KGaA, Darmstadt, Germany). Mushrooms (*Agaricus bisporus*) with consistent sizes, appearance, color, and ripeness level, devoid of any defects, were carefully chosen for the study. Immediately after harvest, the mushrooms were brought to the laboratory and subjected to washing with 0.1% sodium hypochlorite. Following the cleaning process, they were allowed to air dry before the application of the coating.

Edible coating preparation

The outer two layers of leek were peeled. Subsequently, the peeled leek material was subjected to drying in an electric oven (Nuve NO55, Ankara, Türkiye) and then finely ground using a grinder (Kiwi Coffee and Spice Grinder, Türkiye). The ground leek particles were further sieved through a 100-mesh sieve to achieve a consistent and uniform particle size. The guar gum edible coating formulation (Table 1.) was based on method described by Saha et al. (2016) with minor changes. Guar gum, with its excellent water-binding properties that form a thick gel when mixed with water, enables the creation of a uniform and protective coating on the surface of food items, helping retain moisture and freshness. Leek powder was added to the coating formulation at concentrations of 0, 0.5, 1.5, and 2.5 g per 100 ml of coating solution. Ethanol was included in the formulation to dissolve alcohol-soluble ingredients with a powder to ethanol ratio of 0.67 (v/v). Glycerol is commonly used in edible coatings as a plasticizer, enhancing the flexibility and mechanical properties of the coating. Its hygroscopic nature helps retain moisture, improving the barrier properties and extending the shelf life of coated food products. Glycerol was incorporated into the coating solution at a concentration of 0.4 g per 100 ml. To assess the impact of sunflower oil in the coating, 0.1 ml of sunflower oil was added to the formulation of samples L0S0.1, L0.5S0.1, L1.5S0.1, L2.5S0.1.

Guar gum, glycerol, leek powder, sunflower oil and distilled water were stirred at room temperature for 60 min to dissolve adequately. The mushrooms were randomly divided into eight different sections, and immersed in their respective coating solutions for a duration of 2 minutes at a temperature of 20°C. In contrast, the control group was submerged in distilled water.

Following the treatment, the samples were placed on plastic trays and exposed to a fan for 30 minutes to facilitate the drying process. Subsequently, the treated samples were placed on polystyrene trays using toothpicks. The samples were then stored under controlled conditions at 4 ± 1°C and 85–90% relative humidity for a period of 7 days. Experimental assessments were performed on days 1, 3, and 7 to monitor the effects of the storage and coating on the samples. Each experiment was conducted in three replicates.

Weight loss (%) measurement

To assess weight loss, coated and uncoated mushrooms were initially weighed on day 1 and then weighed again at each storage period. Weight loss was calculated as the percentage of weight reduction relative to the initial mass. The experiment was performed in triplicate to ensure accuracy and reliability of the results. The mushrooms' weight was measured by an analytical scale (KERN PFB 1200-2A, Balingen, Germany). Weight loss was determined using the following equation where W_1 represents the initial weight of the mushrooms, and W_2 represents the weight after storage for 3 and 7 days.

$$\text{Weight loss (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad (1)$$

Table 1. Edible coating formulations

Coatings	Guar Gum (g)	Glycerol (g)	Leek powder (g)	Ethanol (ml)	Distilled water (ml)	Sunflower oil (ml)
L0S0*	0.9	0.4	0	0	100	0
L0.5S0	0.9	0.4	0.5	0.75	99.25	0
L1.5S0	0.9	0.4	1.5	2.25	97.75	0
L2.5S0	0.9	0.4	2.5	3.75	96.25	0
L0S0.1	0.9	0.4	0	0	99.9	0.1
L0.5S0.1	0.9	0.4	0.5	0.75	99.15	0.1
L1.5S0.1	0.9	0.4	1.5	2.25	97.65	0.1
L2.5S0.1	0.9	0.4	2.5	3.75	96.15	0.1

* the abbreviations L and S represent leek and sunflower oil, respectively. The subsequent numbers represent the percentage amount of each ingredient in the solution. For example, L0.5 indicates 0.5% (w/v) leek in the coating solution, and S0.1 indicates 0.1% (v/v) sunflower oil in the coating solution.

Table 2. Weight loss (%) and shrinkage ratio (%) in coated and uncoated mushroom samples between the 1st and 3rd days and the 1st and 7th days of storage

Coatings	Days	Weight loss (%)	Shrinkage ratio (%)
L0S0*	1-3	20.580±0.571^{ij}	11.468±0.0257^{bc}
	1-7	43.090±0.537^{def}	22.84±1.45^{abc}
L0.5S0	1-3	18.751±0.963^{jk}	13.329±0.288^{abc}
	1-7	41.287±0.460^{ef}	28.059±0.998^{a}
L1.5S0	1-3	14.860±0.567^{k}	8.873±1.082^{c}
	1-7	39.079±0.781^{f}	19.02±0.507^{abc}
L2.5S0	1-3	22.153±0.591^{hij}	8.739±0.732^{c}
	1-7	44.875±0.397^{de}	19.107±0.0108^{abc}
L0S0.1	1-3	23.120±0.460^{hij}	8.84±0.516^{c}
	1-7	46.670±1.498^{cd}	19.212±0.533^{abc}
L0.5S0.1	1-3	23.704±0.228^{hi}	9.25±1.94^{c}
	1-7	50.904±0.856^{bc}	21.76±3.06^{abc}
L1.5S0.1	1-3	26.127±0.964^{gh}	15.83±11.76^{abc}
	1-7	52.981±0.578^{b}	23.63±11.14^{abc}
L2.5S0.1	1-3	28.262±0.784^{g}	10.274±0.868^{bc}
	1-7	55.055±1.437^{b}	24.07±2.67^{abc}
Uncoated	1-3	29.125±0.902^{g}	15.95±1.58^{abc}
	1-7	63.110±5.200^{a}	25.838±0.434^{ab}

* the abbreviations L and S represent leek and sunflower oil, respectively. The subsequent numbers represent the percentage amount of each ingredient in the solution. For example, L0.5 indicates 0.5% (w/v) leek in the coating solution, and S0.1 indicates 0.1% (v/v) sunflower oil in the coating solution. Values are expressed as mean±standard deviation. Different superscripts show that values in the same column within each group are significantly different (P≤ 0.05)

Color measurement

Color of mushrooms on storage days 1, 3 and 7 were recorded using a handheld colorimeter (TES 135A Color Reader, TES, Taiwan). Color characteristics were evaluated and expressed using the CIE color coordinate system, which includes three components: L*, a*, and b*. The L* component represents lightness, ranging from 0 (black) to 100 (white). The a* component represents the position on the red-green axis, with positive values indicating redness and negative values indicating greenness. The b* component represents the position on the yellow-blue axis, with positive values indicating yellowness and negative values indicating blueness. Euclidean distance ΔE was obtained by Equation below by taking color data of fresh mushrooms as base (L* =99.6, a* = 3.5 and b* = 14.1)

$$\Delta E = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2} \quad (2)$$

Hardness measurement

A texture analyzer (Lloyd Ins. TA Plus, Hants, UK) was employed to conduct a penetration test on the mushrooms' caps. A cylindrical probe with a diameter of 5 mm was utilized to determine the hardness. The texture analyzer was set with a loading head speed of 2.0 mm s−1, a trigger force of 50 g, and a probe travel distance of 5 mm. The average value of each batch was determined as the hardness measurement taken from 6 different circular mushroom caps from each batch.

Shrinkage ratio (%)

The shrinkage ratio of mushrooms was calculated based on the measurement of cap diameter using a digital caliper, as described by Thakur et al. (2020). The formula for shrinkage ratio is as follows:

$$Shrinkage\ ratio\ (\%) = \frac{D_i - D_f}{D_i} \times 100 \quad (3)$$

where D_i represents the initial cap diameter of the fresh mushroom in millimeters (mm), and D_f represents the cap diameter of the mushroom on 3rd and 7th days of storage. The average value of six different measurements was calculated for each batch of mushrooms.

Statistical analysis

The collected data was subjected to analysis of variance (ANOVA), and subsequently, the means were compared using Tukey Post Hoc Analysis. The statistical analysis was conducted in MINITAB Release 17.1 (Minitab Inc. State College, PA, USA), and a significance level of $p < 0.05$ was employed to determine the statistical significance of the results.

Results and Discussion

Effect of coatings on weight loss of mushrooms

Weight loss is a key parameter in assessing the efficiency of edible coatings, as it directly impacts the preservation and quality of food products. Minimizing weight loss is crucial in extending the shelf life of perishable items, ensuring they remain fresh and marketable for a longer period. Edible coatings serve to protect food against moisture and gas permeability while simultaneously minimizing the respiration of the food, thereby extending its shelf life (Bozkurt et al., 2023). Edible coatings can help reduce the rate of weight loss in coated food products. Moreover, weight loss is often associated with moisture loss in fruits and vegetables, leading to undesirable changes in texture, appearance, and taste. Edible coatings may act as a barrier, preventing excessive moisture loss and maintaining the overall quality of the coated food.

The weight loss of coated mushrooms ranged from 39.079± 0.781% (coating L1.5S0) to 55.055± 1.437% (coating L2.5S0.1) between the first and seventh days of storage (Table 2). The lowest weight loss was observed in mushrooms coated with L1.5S0, while the highest weight loss was in mushrooms coated with L2.5S0.1. In contrast, uncoated mushrooms exhibited a weight loss of 63.110±5.200%. The coatings significantly reduced weight loss compared to the uncoated mushrooms, with the L1.5S0 formulation showing the most promising results. The addition of oil in the coatings was observed to lead to a significant increase in weight loss. The application of composite coatings consisting of carboxymethyl cellulose alone and in combination with garlic essential oil exhibited a significant delay effect on weight loss (Dong and Wang, 2017). Similarly, following a 12-day storage period, the uncoated bluberry exhibited a weight loss percentage 1.42 times greater than that observed in fruits coated with gum arabic and roselle extract (Yang et al., 2019). In the study, 1% and 2% garlic essential oil added coatings reduced weight loss, while 3% garlic essential oil increased weight loss. In comparison, garlic essential oil extracted from garlic cloves is more concentrated and known for its potential antimicrobial, antioxidant, and anti-inflammatory properties. However, the oil extracted from sunflower seeds is a mild, light oil rich in vitamin E and low in saturated fats. In our research, the use of 0.1% sunflower oil in the coatings may have led to a less pronounced effect of sunflower oil in reducing weight loss. Addition of oil in coating solution led to oil in water emulsion formation and this emulsion had negative effect on weight loss. The addition of oil increased the hydrophobicity of the solution; however, the leek powder decreased this effect due to the hydrophilic nature of the extract.

Effect of coatings on shrinkage ratio of mushrooms

Some edible coatings have been shown to slow down the ripening and senescence processes in fruits and vegetables, which can contribute to reduced shrinkage during storage. Moreover, edible coatings prevent excessive moisture loss from the food. This can lead to reduced shrinkage, as moisture is retained within the product, maintaining its overall size and shape. Regardless of the treatments applied, the shrinkage ratio steadily increased as the storage period extended (Kumar, 2020). The application of basil seed gum enriched with echinacea extract on fresh strawberries decreased gas permeability, inhibited microorganism growth, reduced respiration rates, and reduced shrinkage/cell wall degradation (Moradi et al., 2019). The minimum shrinkage ratio was observed in sample L1.5S0, with a value of 19.02±0.507%, while the maximum shrinkage ratio was found in sample L0.5S0, measuring 28.059±0.998%. In comparison, uncoated samples exhibited a shrinkage ratio of 25.838±0.434% (Table 2). The application of edible coatings, particularly L1.5S0, appears to have resulted in lower shrinkage ratios, indicating a potential positive effect in reducing the shrinkage of the coated mushrooms compared to the uncoated ones. So, there is approximately a 26.4% reduction in shrinkage ratio when using the coating L1.5S0 compared to the uncoated samples. 1.5% of waste leek powders showed promising results.

Effect of coatings on color values (L*, a*, b* and ΔE) of mushrooms

L*, a*, and b* values are color parameters used to characterize the color of a sample. L* corresponds to lightness, a* indicates the degree of redness (positive values) or greenness (negative values), and b* represents the extent of yellowness (positive values) or blueness (negative values). In many cases, edible coatings are formulated to preserve the natural color of the food, delay color changes, and maintain its visual appeal during storage (Medina-Jaramillo et al., 2020). As expected, L* values exhibited a declining pattern as the mushrooms were stored for longer period. Due to polyphenol oxidase activity, phenolic oxidation occurs, causing the L* value to decrease as darker pigments form, ultimately resulting in the browning of the fruit's surface. During the 7-day storage period, the lightness (L*) of the uncoated sample decreased from 93.44±4.5 to 82.69±2.67. On the other hand, the best coating based on both weight loss and shrinkage data only slightly decreased from 98.115±1.407 to 94.2±0.933, resulting in a relatively lighter color, thus preserving its original appearance better (Table 3). In a previous study, alginate-based coatings were reported to effectively preserve the lightness of mushrooms, resulting in an L* value of approximately 82.5 (Louis et al., 2021), which is darker compared to the L* value observed in our study. The difference in L* values between the two studies could be attributed to variations in the formulation and application of the coatings, as well as differences in the storage conditions in each study.

Among the color parameters, the a* value exhibited the least change, indicating relatively stable redness/ greenness, compared to other color components. Additionally, the coatings had a lesser effect on the a* value when compared to the uncoated samples, suggesting that the coatings had a minimal impact on the redness/greenness of the mushrooms during storage.

The b* values, representing yellowness/blueness, increased during the 7 days of storage (Table 3). This indicates that the mushrooms became more yellow over time. The increase in yellowness may be attributed to various factors, such as the natural ripening process, chemical reactions, or changes in the structure and composition of the mushrooms during storage. An increase in b* values is often associated with browning reactions, which can occur during the storage of fruits and vegetables. Browning reactions, such as enzymatic browning and Maillard reactions, can lead to the formation of pigments that contribute to a more yellow or brown color in the food. The study by Rokayya et al. (2021) observed a similar increase in b* values of mushrooms, providing further evidence of the occurrence of browning reactions during storage.

ΔE represents the total color difference between two color samples in the CIE Lab color space. It quantifies the perceptual difference between the colors and is used to evaluate how much one color deviates from another. ΔE was calculated with respect to fresh mushrooms CIE Lab color. The least color deviation was observed in samples coated with L0S0, L0.5S0, and L1.5S0, with ΔE values of 8.2202±0.0442, 8.769±0.217, and 9.812±0.945, respectively. These coated samples showed minimal color change and maintained their original color more effectively. In contrast, the uncoated samples exhibited a higher ΔE value of 18.64±2.3, indicating a more significant color deviation and changes during the storage period.

Table 3. Color values (L*, a*, b* and ΔE) of coated and uncoated mushroom samples on the 1st, 3rd, and 7th day of storage

Coatings	Days	L* value	a* value	b* value	ΔE
L0S0*	1	98.85±0.438^{a}	3.296±0.185^{abc}	14.345±0.276^{efgh}	0.853±0.421^{g}
	3	95.845±0.757^{abc}	3.303±0.258^{abc}	17.76±2.39^{cdefgh}	5.32±2.19^{defg}
	7	93.635±0.0495^{abc}	5.325±1.103^{abc}	19.39±0.368^{bcdefg}	8.2202±0.0442^{bcdefg}
L0.5S0	1	99.37±0.368^{a}	1.14±1.054^{c}	13.635±0.134^{gh}	2.453±0.954^{fg}
	3	98.065±1.138^{ab}	5.06±1.77^{abc}	14.1±1.47^{fgh}	2.56±1.77^{fg}
	7	94.295±0.742^{abc}	5.658±0.463^{abc}	20.705±0.46^{bcdef}	8.769±0.217^{bcdefg}
L1.5S0	1	98.115±1.407^{ab}	2.692±0.272^{bc}	11.865±0.615^{h}	2.906±1.117^{fg}
	3	95.89±2.29^{abc}	4.305±1.047^{abc}	19.2±3.18^{bcdefg}	6.37±4.01^{cdefg}
	7	94.2±0.933^{abc}	5.898±0.357^{abc}	21.925±0.431^{abc}	9.812±0.945^{bcdefg}
L2.5S0	1	97.545±0.7^{ab}	3.5875±0.0247^{abc}	14.83±1.174^{defgh}	2.278±1.009^{fg}
	3	92.56±4.21^{abc}	6.32±2.32^{ab}	21.92±4.84^{abc}	10.9±6.79^{abcdefg}
	7	91.03±2.61^{abcd}	6.73±2.08^{ab}	24.915±1.096^{ab}	14.24±2.87^{abcde}
L0S0.1	1	95.76±0.863^{abc}	3.708±0.711^{abc}	14.165±0.219^{fgh}	3.879±0.888^{efg}
	3	93±2.43^{abc}	6.703±1.015^{ab}	22.46±1.81^{abc}	11.14±3.09^{abcdefg}
	7	87.465±0.997^{cde}	6.252±0.449^{abc}	25.535±0.502^{ab}	16.907±0.982^{abc}
L0.5S0.1	1	93.545±0.247^{abc}	4.883±0.456^{abc}	16.88±1.69^{cdefgh}	6.885±0.993^{cdefg}
	3	89.565±0.672^{bcd}	7.15±0.771^{ab}	23.31±1.58^{abc}	14.13±1.305^{abcde}
	7	87.99±0.665^{cde}	7.38±0.658^{ab}	23.22±0.382^{abc}	15.282±0.11^{abcd}
L1.5S0.1	1	94.07±3.99^{abc}	6.12±2.36^{abc}	16.9±0.495^{cdefgh}	6.84±4.33^{cdefg}
	3	91.755±0.728^{abc}	7.25±0.714^{ab}	22.68±0.467^{abc}	12.239±0.359^{abcdef}
	7	88.16±0.424^{cde}	8.023±0.237^{a}	27.79±1.72^{a}	18.41±1.6^{ab}
L2.5S0.1	1	91.94±0.976^{abc}	6.697±0.916^{ab}	20.3±1.032^{bcdefg}	10.37±1.62^{bcdefg}
	3	95.74±4.34^{abc}	5.72±2.3^{abc}	19.09±3.41^{bcdefg}	6.8±5.71^{cdefg}
	7	92.73±3.24^{abc}	5.367±0.916^{abc}	22.81±1.52^{abc}	11.36±2.97^{abcdefg}
Uncoated	1	93.44±4.5^{abc}	5.1±1.63^{abc}	17.77±1.344^{cdefgh}	7.42±4.75^{cdefg}
	3	80.07±2.23^{e}	7.6±2.47^{ab}	21.47±2.08^{abcd}	21.42±1.8^{a}
	7	82.69±2.67^{de}	6.212±0.929^{abc}	21.345±0.643^{abcde}	18.64±2.3^{ab}

* the abbreviations L and S represent leek and sunflower oil, respectively. The subsequent numbers represent the percentage amount of each ingredient in the solution. For example, L0.5 indicates 0.5% (w/v) leek in the coating solution, and S0.1 indicates 0.1% (v/v) sunflower oil in the coating solution. Values are expressed as mean±standard deviation. Different letters show that values in the same column within each group are significantly different (P≤0.05)

Table 4. Hardness value of coated and uncoated mushroom samples on the 1st, 3rd, and 7th day of storage

Coatings	Days	Hardness value
L0S0*	1	2.05±0.0866^{bcdefg}
	3	1.9333±0.1528^{cdefg}
	7	1.56±0.0529^{hijk}
L0.5S0	1	2.1333±0.0289^{bcdef}
	3	2.243±0.277^{abcd}
	7	1.57±0.1127^{hijk}
L1.5S0	1	2.6±0.265^{a}
	3	2.3±0.1^{abc}
	7	1.8±0.05^{efghijk}
L2.5S0	1	2.417±0.176^{ab}
	3	1.9±0.1^{cdefghi}
	7	1.5333±0.0577^{hijk}
L0S0.1	1	2.25±0.05^{abcd}
	3	1.8±0.05^{efghijk}
	7	1.5±0.1323^{ijk}
L0.5S0.1	1	2.0967±0.0839^{bcdef}
	3	1.85±0.05^{defghij}
	7	1.38±0.0819^{k}
L1.5S0.1	1	2.2833±0.0289^{abc}
	3	1.587±0.201^{hijk}
	7	1.4667±0.0577^{jk}
L2.5S0.1	1	2.1567±0.0814^{bcde}
	3	1.717±0.362^{fghijk}
	7	1.3833±0.0289^{k}
Uncoated	1	1.9167±0.0764^{cdefghi}
	3	1.6333±0.0289^{ghijk}
	7	1.4033±0.138^{k}

* the abbreviations L and S represent leek and sunflower oil, respectively. The subsequent numbers represent the percentage amount of each ingredient in the solution. For example, L0.5 indicates 0.5% (w/v) leek in the coating solution, and S0.1 indicates 0.1% (v/v) sunflower oil in the coating solution. Values are expressed as mean±standard deviation. Different letters show that values in the same column within each group are significantly different (P≤0.05).

Effect of coatings on hardness of mushrooms

Monitoring the hardness of the coated mushrooms over the storage period is crucial to determine the effectiveness of the edible coating in preserving the desired texture. In general, edible coatings can help maintain the hardness or texture of the coated mushrooms during storage. The application of edible coatings forms a protective layer on the surface of the mushrooms, which can reduce moisture loss and prevent mechanical damage. This protective barrier helps retain the natural moisture content of the mushrooms, thereby minimizing softening or loss of firmness. The hardness values of both the coated and uncoated samples were observed to decrease significantly during storage. This reduction in hardness indicates that the mushrooms became softer over time, regardless of whether they were coated or uncoated. the short observation time of 7 days might have limited the ability to detect significant differences in hardness between the coated and uncoated samples. With longer storage durations, the positive effects of the coatings on hardness could become more pronounced. The findings from Mohebbi et al. (2012)'s study support the notion that the texture of mushrooms tends to deteriorate during storage, and this difference in texture becomes more pronounced over time. Therefore, the use of coatings is expected to help maintain the hardness and fresh texture of mushrooms. Initially, during the early days of storage, there might be minimal differences between the coated samples and the uncoated control in terms of preserving the initial texture quality. However, as the storage period extends, the positive effects of the coatings on hardness changes become more evident, particularly for mushrooms treated with gum tragacanth. This indicates that the coatings could provide protective benefits that effectively delay the degradation of hardness and texture in the mushrooms, especially when stored for longer durations.

Conclusions

The study investigated the effects of different guar gum edible coating formulations, which incorporated varying proportions of waste leek powder and sunflower oil, on weight loss, color parameters (L*, a*, b* values, and ΔE), texture, and shrinkage of *Agaricus bisporus* mushrooms during 7 days of storage. The research explored how these coating formulations could potentially impact the preservation and quality of the mushrooms over time. Overall, the study suggests that edible coatings have the potential to play a crucial role in preserving the moisture, shape and color quality of mushrooms during storage, leading to better quality and extended shelf life.

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Selçuk University Museum Interior Design and Application Example

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ABSTRACT

The aim of this study is to cover the design-implementation processes of the museum interiors of the museum building in Selçuk University campus, which consists of foyer, temporary and permanent exhibition areas that can realize the aims and functions of the museum in accordance with the culture of the institution to which it is affiliated. Two types of materials were used within the scope of the study. The first type of material is theoretical and visual information based publications, thesis studies, lecture notes, architectural and interior design printed publications and internet resources. The information scanned and analyzed was handled from general to specific. The second material is the analysis of the Selçuk University Museum building located in Selçuk University Alaeddin Keykubat Campus by the authors with the on-site identification stages, interviews with the relevant people, the requirements of the museum foyer, temporary, permanent exhibition areas and existing interior photographs. The method followed in the study is observation and detection. As a result of the researches and observations made by utilizing publications, thesis studies, course notes, architectural and interior architectural printed publications and internet resources on the subject, an identification study was carried out in the existing museum building. As a result of the research, it was stated that the museums within the university campus have an important place in terms of establishing a bond between the public and the university, apart from the educational areas affiliated to the institution. Apart from education and training structures, from a different perspective, it was mentioned that they are common multi-purpose areas and the positive aspects they add to the institution to which they are affiliated. The interior revisions made in the determined areas of the museum building, the project design process and the implementation phases were mentioned.

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Introduction

Museums can be classified according to their type, status, affiliation, etc. However, the most ideal classification can be made according to the collections museums contain. Museums are divided into various groups according to the contents or qualities of their collections, the institutions they are affiliated with, their service areas, the society they serve, the places where they exhibit their collections and their functions (Yücel, 1999).

In the classification of museums, university museums, which fall under the classification of the institutions they are affiliated with, begin to include the audience from pre-school onwards. Later, much attention is paid to school groups. The forms of activity with schoolchildren are varied - there are tours, lectures, workshops, competitions, scientific and educational events, and museum education programs. Initially, the museum collections of universities are created as academic museums - visual training bases for research and support of the educational process. Therefore, students play an important role in the structuring of museum education at the university.

For centuries, museums and their collections have been the basis for scientific research in which students participate. Based on the museum, students gain a lot of practical knowledge and experience with the help of various lectures, excursions, seminars, and training courses (Yurkin, 2019). Based on the collections of the museum, the student can start to engage in research activities. Also, students can be involved not only as students but also as volunteers who will actively participate in museum activities with guided tours. The involvement of students in museum activities makes it possible to form in young people a sense of spiritual attachment to the alma mater, and a sense of responsibility for future generations.

In foreign universities around the world, the tradition of museum education has been active for many years. Preserving the basic forms of museum activity for visitors (lectures, tours, talks, etc.), university museums develop educational and training practices. New ways of working with visitors, as well as the use of modern information technologies, are part of the interactive educational

process. Thanks to the efforts of private museum associations and their staff, today university museums abroad have become a large open educational platform designed for the public and meeting the needs of different social groups. Peter Vergo used the term "New Museology" for the first time, saying, "The old type of museology is too dependent on methods and is doomed to disappear if it is not renewed and radically changed" (Atasoy, 1997).

Nowadays, an audience-oriented understanding of museology, which ensures the existence in the social and cultural field and the cognitive, social, and emotional development of the individual, is gaining importance (Karadeniz, Özdemir, 2018).

Material and Method

Within the scope of the study, two types of materials were used. The first one is theoretical and visual information, including publications, thesis studies, and related internet data on museum and university museology. The scanned and analyzed information was sorted from general to specific through a filter. The second material is the on-site identification of the Selçuk University Museum building, interviews with the relevant people and analysis of the space with photographs.

The method followed within the scope of the study is observation, determination, and computer-based Autodesk programs used within the scope of interior architectural project design, interior architectural project design workflow chart, design phase, and interior space visualizations. As a result of the research and observations made by utilizing sources such as publications, thesis studies, etc. related to museums and university museums, an identification study was carried out. In the first part of the study, the concept of a museum, the brief history of the concept of museology in the world and Turkey, the formation of university museology, and the development process were mentioned. In the next section, the contributions of university museums to the institutions they are affiliated with and the city they are located in are emphasized. In the field study phase of the article, interior architectural project design and on-site application processes were transferred by the workflow. The exhibition methods in museums and the museum examples examined are the results of research, observation, and identification studies. The structure of the study is given schematically in Figure 1.

The Concept of Museum and the Formation of University Museums

Today, the word 'museum' in our language is derived from the Greek word 'Mouseion'. Erbay states that museums today are educational institutions that reflect the scientific and cultural past of society and combine the elements that shape the future with art and culture (Erbay, 2011).

There are many different definitions in the literature regarding museums, which play an important role in the collection, protection, preservation, and preservation of cultural heritage and come to the fore with their educational activities that shed light on scientific research as well as their exhibition function (Akçaova, Köse Doğan, 2020).

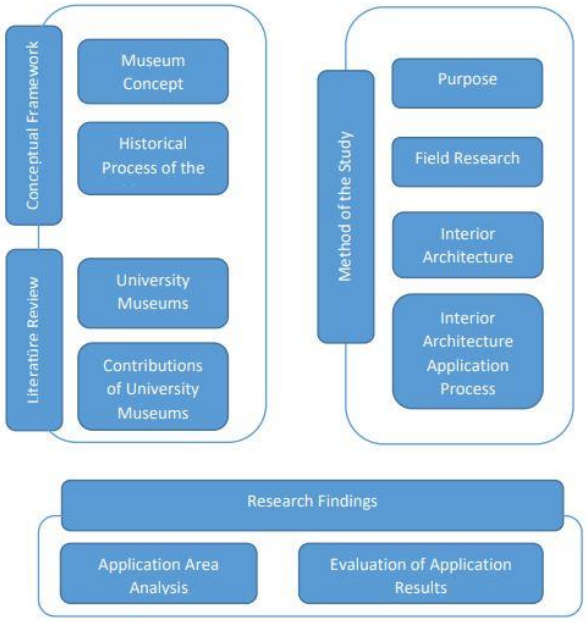


Figure 1. Structural Schematic of the Study (Akcaova and Noraslı Personal Archive).

Museums have emerged from the accumulation of people's interest and thoughts on art and artifacts for centuries. In the early ages, the most important artifacts were made and kept as gifts to gods and goddesses. The objects dedicated to the Muses mentioned in Ancient Greece are worthy of being an example of this situation (Atagök, 1999).

In the world, the concept of museology is seen in the Hellenistic era. The formation of collections and its relationship with educational planning dates back to the Renaissance period. The mid-19th century World Exhibitions pioneered the 20th-century museums with their structures and actions (Köse Doğan, 2018). In Turkey, we can categorize museology under two main historical headings. The first is the Ottoman period museology. It can be seen in many literature studies that the sultans of the period were good collectors (Cumhuriyet Dönemi Türkiye Ansiklopedisi, 1983). During the Ottoman period, the "Museum-i Hümayün" Imperial Museum was named as the first Ottoman museum (Türkiye Müzeleri, 1999). The second period is called the post-Republican understanding of museology.

The process of musealization of collections was exemplified by the establishment of the Ashmolean Museum in the 18th century to be open to teaching, research, and public service. This exemplary situation has expanded the usage areas of many universities by combining their collections with museum exhibition techniques (Özdemir, 2017). In the 20th-century postmodern approach, the relationship between the university museum and society has begun to be established.

The types and functions of museums vary according to the institutions they are affiliated with (Akçaova, Köse Doğan, 2020). University museums are museums that host the history, development process and cultural accumulation of the institution they are affiliated with in their collection. In a different definition, the concept of a university museum is a scientific and cultural institution whose collection is created by the university through

donation or purchase and contributes to the education and development of the students and academics at that university as well as the people of the region where the university is located (Onur, 2009).

In the research conducted by Pekgözlü Karakuş (2020), it was stated that there are 229 university museums in our country. When we look at different studies on the subject, a great increase in the number of university museums between 2010 and 2020 stands out.

The large and diverse collections of university museums allow them to engage people in the educational process, regardless of their age, social and national identity, activities and interests (Kretova, 2016).

The Relationship between Museum, Campus and Community

University museums establish a link between the campus and the community and play an important role in the field of public service, going beyond the missions of the university (King, 2001). For the city dweller, these museums provide access to the knowledge produced at the university.

In the last two decades, museums have strived to communicate and share with members of society through rich advisory programs and community projects. The proliferation of scholarly work and the policy of engaging the community in the museum from a variety of perspectives includes issues such as public culture and heritage (Were, 2010; Crooke 2007; Karp 1992; Kreps 2003).

As long as the university is engaged with its immediate surroundings, it becomes recognized and trusted. Recently, university-industry collaborations have provided consultancy services in various fields to contribute scientifically to the production of the university. In this context, boards and commissions have been established in many universities to improve the information sharing of university museums with the city (Özdemir, 2019).

University museums undertake missions such as conducting scientific studies, transferring scientific studies to the public, providing complementary environments to the educational activities of the university, creating programs that will appeal to all segments through curators, collaborating with other disciplines, and integrating the academic community and the public community (Özdemir, 2017).

University museums are a process of education, training and information sharing, which have an important place in their duties. Education and training within the scope of museums can be enriched with different processes as well as individualized. Falk and Dierking (2000) put forward the "Contextual Learning Model" by stating that learning is not only individual and is affected by all kinds of environmental environments. In this learning model, which is expressed under three main headings as personal, socio-cultural and physical context, the "lifelong learning" model emerges. Because each main heading is interrelated. In the personal context, the person's previous experiences, upbringing and interests are at the forefront. The socio-cultural context is the result of acting in groups, in harmony and collectively. The physical context includes the immediate environment in which the act of learning takes place, architectural elements, exhibition and display techniques, and other activities in closed, open or semi-open spaces.

In this context, this study aims to bring together museum, city and university culture while designing interiors that will enable university museums to fulfill their duties in the best way possible. By utilizing the values, historical process, mission and vision of the institution to which it is affiliated, creating interiors suitable for the university and the city also contributes to museum and community belonging.

Selçuk University Museum Building Application Area

The location of buildings that are built today and have a common use function by the society is important in terms of ease of transportation. It is seen that especially museum buildings are positioned as a transition area between the city and the campus according to the availability of space within the campus. The buildings are intended to be close to public transportation or main transportation arteries for visitors coming from the campus and the city. Indoor, open or semi-open spaces and the landscape areas around the building are designed to increase social interaction and allow for activities. The location of the museum building on the campus and its immediate surroundings are given in Figure 2.

Figure 2. Location of Selçuk University Museum Building on Campus

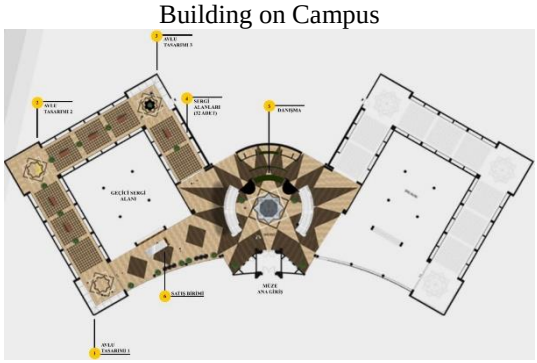


Figure 3. Selçuk University Museum Building Application Areas (Akcaova and Noraslı Personal Archive).

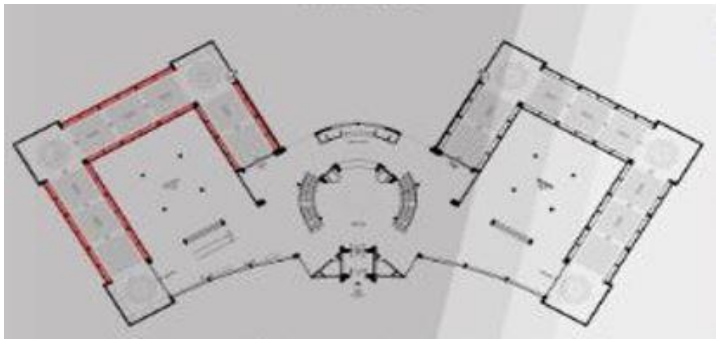
Today's museums offer new spatial experiences with multiple circulation areas, perceptual changes in exhibition spaces, regular and irregular spaces, uncertainty of boundaries, different colors, textures and materials (Canbakal, 2016).

The entrance, foyer area, temporary and permanent exhibition areas, foyer area, temporary and permanent exhibition areas of Selçuk University Alaeddin Keykubat Campus Museum were limited as the areas where the interior architectural application project will take place in terms of obtaining information, transportation and follow-up of the interior architectural project application. The areas to be applied in the museum building are given schematically in Figure 3 on the architectural plan of the building.

The symmetrical architectural structure of the Selçuk University Museum Building is among the design features that bring it to the forefront. With the glass facade cladding used on the front façade, the building is associated with the open space. This also provides natural lighting to the foyer, entrance and temporary exhibition areas within the museum. The museum building is designed as 2 separate sections symmetrical to each other in square forms. These sections are divided into corridors in the outer section and the inner sections are reserved as temporary exhibition areas, while the outer corridors forming the boundaries of the square are used as permanent exhibition areas.

In the entrance section of the museum, a water element was created based on the Seljuk star motif and a double-arm staircase was used as a horizontal circulation element. There is a multi-purpose hall and foyer area on the first floor of the museum. The Selçuklu star is part of the Turkish historical, cultural and artistic heritage and is used to express the Turks' attachment to their past.

Permanent Exhibition Area Location in Plan

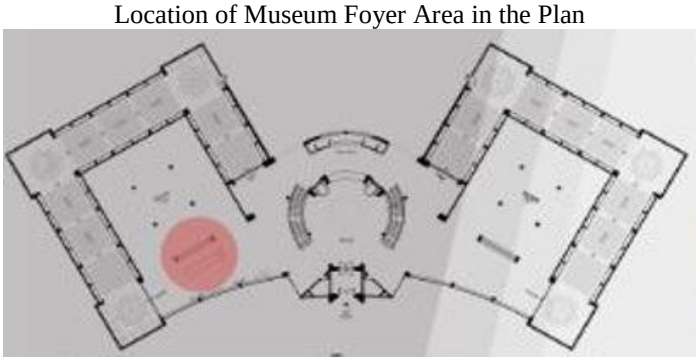


Pre-Application

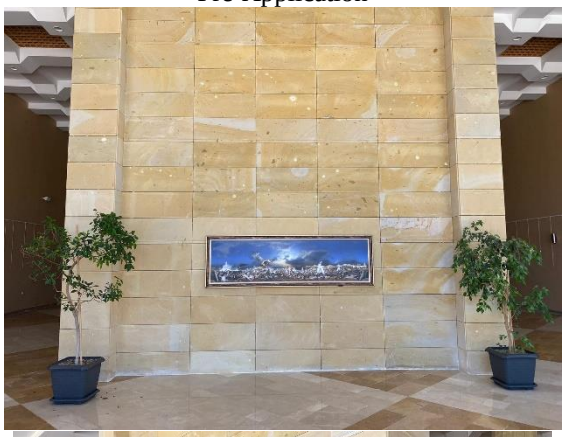
Post Application

Table 1. Permanent Exhibition Technical Plan - View / Before and After (Akcaova and Noraslı Personal Archive).

Location of Museum Foyer Area in the Plan



Pre-Application



Post Application

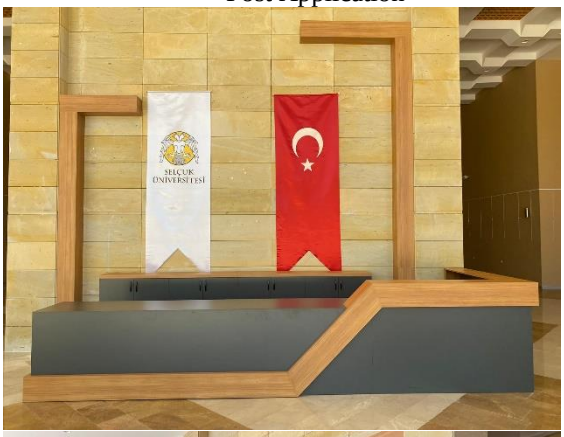


Table 2. Foyer Area Technical Plan - Appearance / Before and After (Akcaova and Noraslı Personal Archive).

Design and Implementation Integration

The integration of design and implementation started with the delimitation of the area covering the entrance, foyer, and exhibition areas of Selçuk University Museum Building, and assignments were made by defining the work from the relevant institutions. In the next stage, in order to decide on the spatial constructions, the exhibition, foyer and entrance areas of the museum building were experienced, observed, and the interior design preliminary project was shaped according to the requests and suggestions of the users. Table 1 shows the location of the permanent exhibition area on the plan, before and after the interior design application.

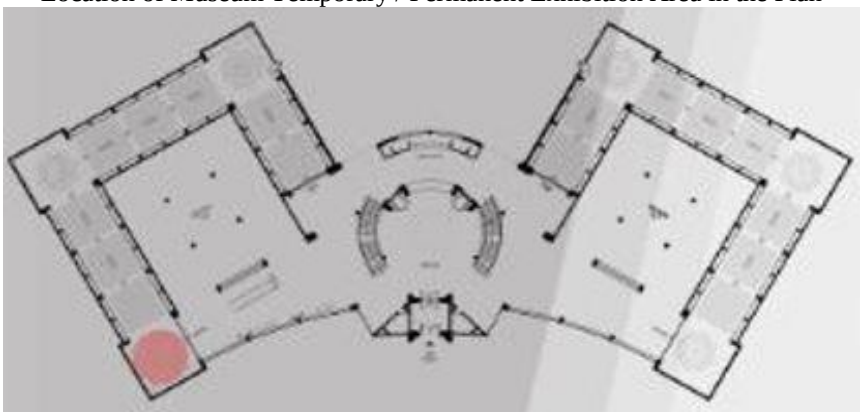
While implementing the permanent exhibition area of the museum building, the survey of the building was made in coordination with the Department of Building Works of Selçuk University. Plans, sections and front views were drawn with 2-dimensional Autodesk Autocad programming technique in accordance with the design. Material selections were made and the application process started. First, rough plaster process was applied within the permanent exhibition modules. In the second stage, satin plaster plaster was applied on rough plaster plaster. In order to highlight the products intended to be exhibited, dark interior paint and lighting were applied. The application was finalized by applying a Seljuk star application on the glass area with sanded cladding dc-fix material. Table 2 shows the location of the reception desk in the foyer area of the museum building in the plan, before and after the interior architectural application.

One of the main purposes of the counter applied in the museum foyer area is to inform visitors about the permanent or temporary exhibitions in the museum. Later, as a commercial action, it also functions as a sales counter. In addition to the corporate licensed products of Selçuk

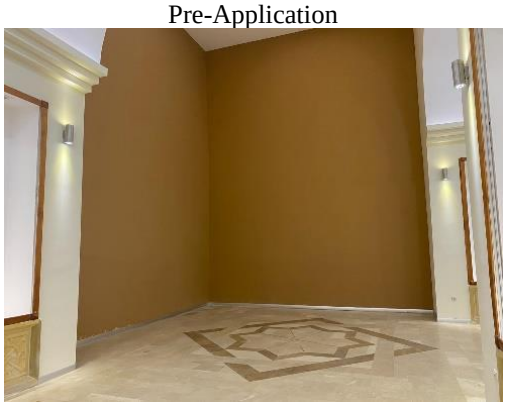
University, it is planned to sell different products of the existing exhibition or artist. While designing the form of the sales counter, the Seljuk star was stylized, reduced to simple lines and used as traces on both the counter furniture and the wall of the counter. Considering the cost, chipboard and MDF lam materials were preferred in the construction of the bank. The plan, section and front views of the counter furniture were drawn with 2-dimensional Autodesk Autocad programming technique in accordance with the design. Material selections were made and the application process was started. The counter furniture was produced by Selçuk University furniture workshop. In the next stage of the application project, wall exhibition panels for permanent or temporary exhibition areas were started to be produced and the photos before and after the application are given in Table 3.

Wall panels designed using recycled materials are generally designed for temporary exhibitions. They can also be used for informing about existing permanent artifacts in the areas where they are located. The 3mm galvanized sheet metal profile is designed to be supported on the wall with the help of dowels and screws. Galvanized sheets and intermediate gridal wires were obtained from scrapped bunk bed structural elements that were previously used in student dormitories. Oil paint was applied on them and painted in the specified color. Designed as two modules, the wall exhibition panels are also portable. In addition, different exhibition elements and lighting elements were designed for the temporary and permanent exhibition entrance sections. Technical plans were drawn and dimensioned in 2D with the support of Autodesk Autocad programs. Interior exhibition elements were prepared with Autodesk 3Dmax program and their visuals are given in Table 4. At another node of the exhibition areas, a multidimensional exhibition area was created. Table 5 shows the application of the multidimensional exhibition area.

Location of Museum Temporary / Permanent Exhibition Area in the Plan



Pre-Application



Post Application

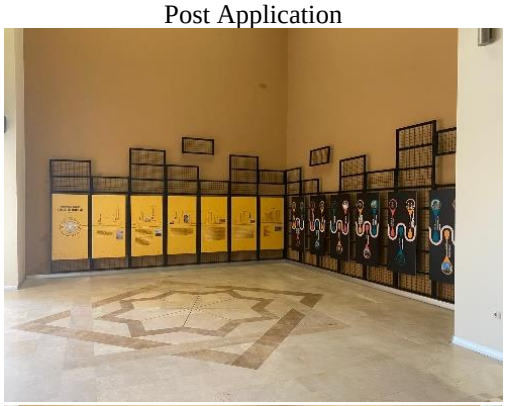


Table 3. Temporary / Permanent Exhibition Wall Panels Before and After (Akcaova and Noraslı Personal Archive).

Technical Drawings

3D Visuals

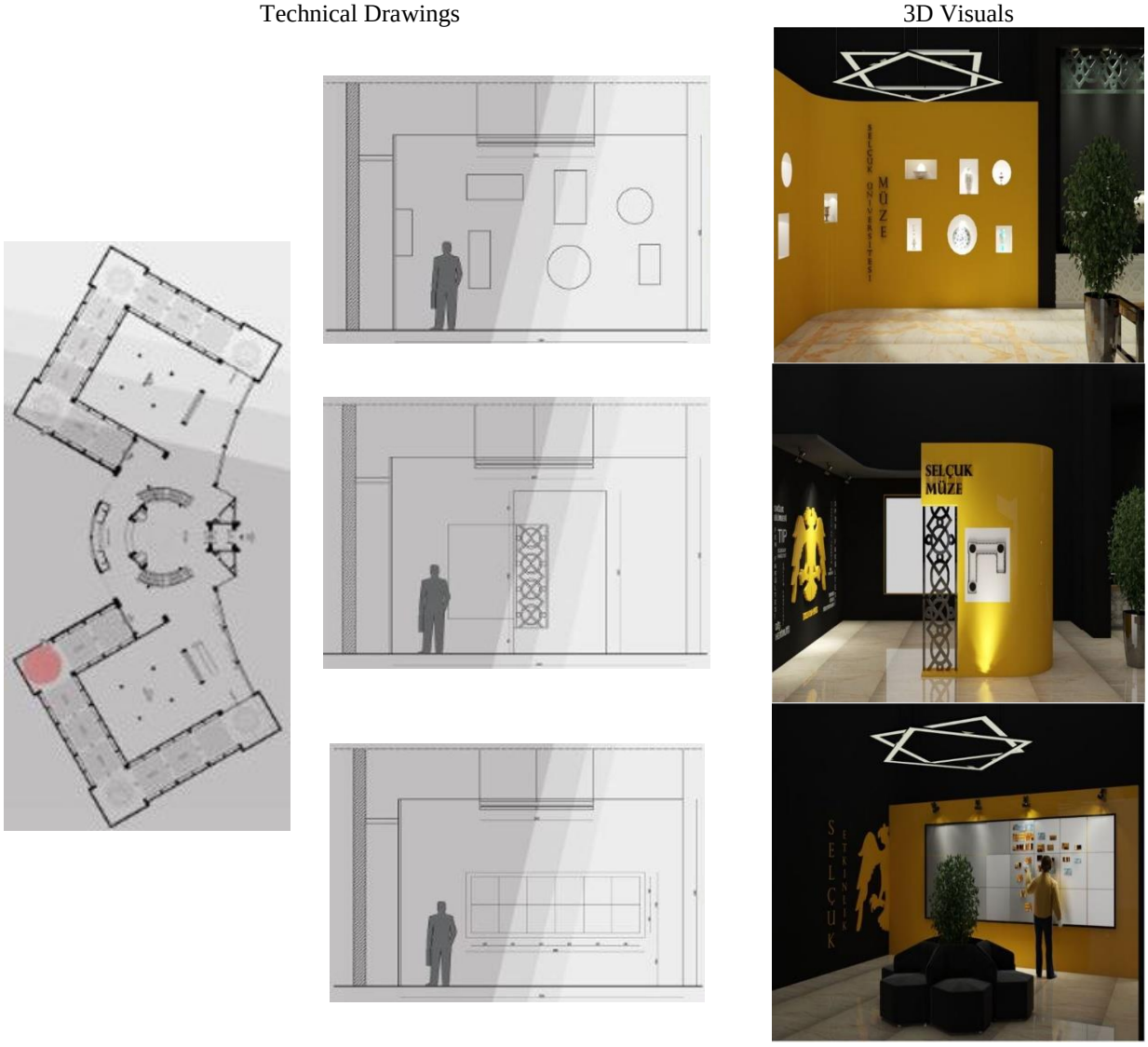


Table 4. Temporary / Permanent Display Wall Exhibition Panel Alternatives (Akcaova and Noraslı Personal Archive).

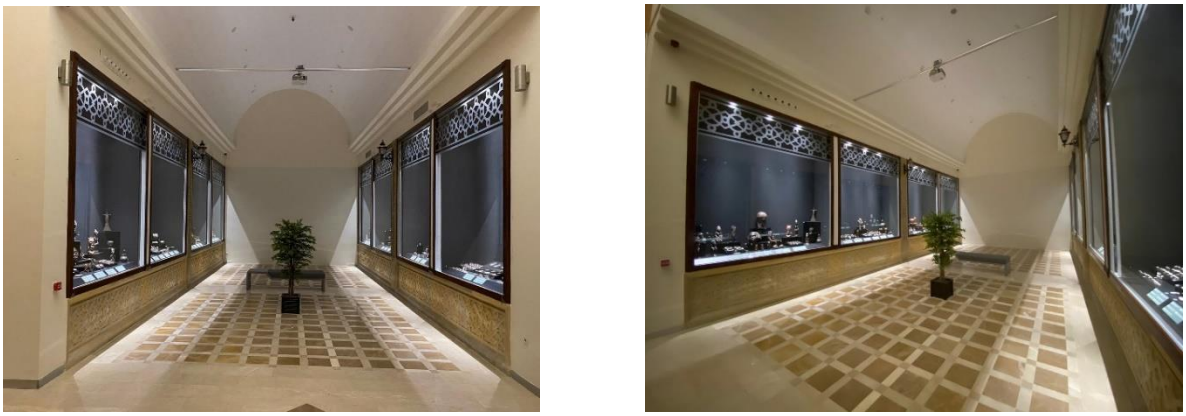


Table 5. Multidimensional Exhibition Area (Akcaova and Noraslı Personal Archive).

In the 21st century, with the developing and changing technology, interactive, digital exhibition techniques that establish a connection between the work and the visitor are widely seen in museums and exhibition areas. The multidimensional exhibition space is designed to serve many purposes such as the presentation of artifacts within the museum, special days and weeks, and exhibitions on the museum calendar. These and similar digital exhibition areas are available as exhibition and information sharing areas in many museums in our country such as Sakıp Sabancı Museum, Salt Galata, Salt Beyoğlu, Rahmi M Koç Museum. To give an example from the world, Athens Acropolis Museum, Caxia Form Museum are used as collective group information and digital multidimensional exhibition techniques in the entrance and foyer area.

Conclusion and Recommendations

Analysis and interpretation of the research material has shown that many university museums have become repositories of historical information about the history of universities, while fulfilling educational and scientific functions. In the context of this study, university museums are seen as an area of research that continues to develop.

In the study carried out within the scope of the Selçuk University Museum Building interior project application, it was inspired by Seljuk artifacts, which are an important part of the university's corporate identity. Stylization and linearization of the Seljuk star constituted the starting point of the design phase. The stylized Seljuk star was used in the flooring materials, wall covering materials, exhibition areas, various fittings and lighting designs of the museum building. In addition to reflecting the institutional identity, this situation is thought to help the active or graduated students of the university to form a sense of belonging and the people living in the city to adopt the museum.

In addition, among the advantages of the glass facade of the museum building included in the scope of the study, its relationship with the immediate environment enables the museum to be actively used in open or semi-open areas in relation to its immediate surroundings, not only limited to the interior.

The sheltered nature of most university campuses in Turkey may seem to be a barrier to communication between the university and the public, but university museums may have a contrary strategy.

This is an important aspect that museums need to explore in order to fulfill their mission of teaching, learning, research and information dissemination. Due to the recent worldwide pandemic and the recent earthquakes in our country, the educational process has been carried out through remote access. Therefore, the museum should initiate a digitization project that will enable collection information and images to be made available online.

Advances in communication technologies and the widespread use of the internet have made it easier for cultural institutions to reach their target audiences. Museums should be activated as institutions that attract visitors rather than expecting them. Museums should be equipped with services such as cafes, restaurants, cinemas and multi-purpose meeting halls, and should be able to serve people's active social lives just like shopping malls. In order to announce these services, billboards, announcement areas, social media networks, printed and publishing organizations and inter-institutional collaborations should be carried out to reach large masses.

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Chemical constituents in the essential oil of the endemic plant *Prangos platychlaena* from the Lakes Region (Türkiye)

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ABSTRACT

The fruits of *Prangos platychlaena* (Endemic), which grow wild in the Lakes Region of Türkiye, were collected at the seed maturing stage to study their essential oil composition. Fruit samples of the species were collected from two different locations, namely Isparta, Sütçüler/Çandır, and Burdur, Bucak/Kızılkaya, during the yellow ripening period. The essential oils of the fruits were extracted using a hydrodistillation apparatus, and the essential oil components were determined using GC-MS. The essential oil content in fruits grown in the Çandır location was 0.16±0.02%, while in the Kızılkaya location, it was 0.25±0.06%. Fruits from the Çandır location were found to contain 54 components, while those from the Kızılkaya location contained 44 components, resulting in a total of 73 different components identified in the species' fruits. Significant qualitative and quantitative variations in certain compounds were observed with respect to the collection locations. The major components were germacrene-D (17.08%-20.24%), β -bisabolene (7.53%-17.83%), β-copaene (0.92%-11.70%), caryophyllene oxide (%6.23-6.03%), β -farnesene (3.21%-5.66%), δ-cadinene (3.50%-3.87%) and ledane (3.35%-3.22%). It has been understood that the ecological factors of the region, especially the altitude, have a significant effect on the essential oil ratio and components.

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Göller Bölgesi'nde Doğal Olarak Yetişen Prangos platychlaena (Endemik) Uçucu Yağının Kimyasal Kompozisyonu

MAKALE BİLGİSİ

Araştırma Makalesi

Geliş : 06.10.2023
Kabul : 07.12.2023

Anahtar Kelimeler:
Prangos platychlaena
Uçucu yağ oranı
Uçucu yağ bileşenleri
Lokasyon
Endemik

ÖZ

Bu çalışma, Türkiye'nin Göller Bölgesi'nde farklı lokasyonlarda yabani olarak yetişen *Prangos platychlaena* (endemik) bitkisinin meyvelerinin uçucu yağ oranını ve bileşenlerini belirlemek amacıyla yürütülmüştür. Türe ait meyve örnekleri iki farklı lokasyondan (Isparta, Sütçüler/Çandır ve Burdur, Bucak/Kızılkaya) sarı olum döneminde toplanmış, meyvelerin uçucu yağları hidrodistilasyon cihazı ile elde edilirken, uçucu yağ bileşenleri ise GC-MS cihazı kullanılarak belirlenmiştir. Çandır lokasyonunda yetişen bitkilerde meyve uçucu yağ oranı%0,16±0,02, Kızılkaya lokasyonunda ise%0,25±0,06 olarak belirlenmiştir. Çandır lokasyonundan alınan meyvelerin 54 bileşenden, Kızılkaya lokasyonundan alınan meyvelerin ise 44 bileşenden oluştuğu belirlenmiş, türün meyvelerinde toplam 73 farklı bileşen tespit edilmiştir. Uçucu yağı oluşturan bileşenler ve oranları lokasyonlara göre kalitatif ve kantitatif varyasyonlar göstermiştir. Her iki lokasyonda da uçucu yağı oluşturan ana bileşenler germacrene-D (%17,08-%20,24), β-bisabolene (%7,53-%17,83), β-copaene (%0,92-%11,70), caryophyllene oxide (%6,23-%6,30), β-farnesene (%3,21-%5,66), δ-cadinene (%3,50-%3,87) ve ledane (%3,35-%3,22) olarak tespit edilmiştir. Çalışmada türün yetiştiği bölgenin ekolojik faktörlerinin, özellikle rakımın uçucu yağ oranı ve bileşenleri üzerinde önemli bir etkiye sahip olduğu anlaşılmıştır.

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Giriş

Göller Yöresi, Türkiye'nin en değerli tıbbi ve aromatik bitkilerin üretim alanlarından birisidir. Bu bölgedeki iller, bitki coğrafyası açısından Akdeniz ve İran-Turan bölgelerinin kesişim noktasında bulundukları için floristik açıdan oldukça zengindirler. Günümüzde Isparta yöresinde yaklaşık 600 endemik türün bulunduğu bilinmesine rağmen, bilim dünyasında sadece 40 türün tanımı yapılmıştır (Karadoğan ve ark., 2015). Apiaceae familyasına ait Prangos cinsinin dünya genelinde 28 türü bulunmaktadır. Türkiye'de toplam 19 taksona sahip olup, Prangos cinsine ait 12 tür bulunmaktadır ve bu türlerin 11'i ülkemiz için endemiktir. Bu türlere örnek olarak *Prangos platychlaena* Boiss, *Prangos ferulacea* L., *Prangos uechtritzii* Boiss. & Haussk., *Prangos peucedanifolia* Fenzl., *Prangos pumila* Boiss., *Prangos melicarpoides* Boiss., *Prangos denticulata* Fisch. & Mey., *Prangos acrisromanae* Boiss. & Huet., *Prangos uleptera* DC., *Prangos lophoptera* Boiss., *Prangos odontoptera* Boiss. ve *Prangos corymbosa* Boiss. gösterilebilir (Davis, 1972). *Prangos platychlaena* Boiss. Türkiye'de çağşır, çakşır, kirkor ve korkor olarak isimlendirilmektedir (Mottaghipisheh ve ark., 2020). Prangos cinsinin farklı türleri, kas gevşetici (Bouaoun ve ark., 2007), kanama durdurma, yara izlerini iyileştirilme (Ulubelen ve ark., 1995) gibi özellikleri nedeniyle modern ve geleneksel tıpta kullanılmaktadır. *P. platychlaena* Boiss. meyveleri farklı biyolojik aktivitelere sahip uçucu yağ ve diğer sekonder bileşikleri içermektedir (Uzel ve ark., 2006; Çelik ve ark., 2008). *P. platychlaena* bitkisinin sivrisineklere karşı repellent aktivitesi olduğu bildirilmiştir (Tabanca ve ark., 2018; Ulubelen ve ark., 1995). Ayrıca bitkinin köklerinin toz haline getirilip bal ile karıştırılarak afrodizyak olarak tüketildiği belirtilmektedir (Özek ve ark., 2018; Tabanca ve ark., 2018). Prangos türlerinden elde edilen ekstraktların, uçucu yağların ve diğer saf bileşiklerin önemli derecede antioksidan, antibakteriyel, antifungal ve antiviral etkiler gösterdiği bazı araştırmacılar tarafından bildirilmiştir (Sümer Ercan ve ark., 2013; Şimşek ve ark., 2016; Mottaghipisheh ve ark., 2020). *P. platychlaena* meyve uçucu yağının *Candida albicans*, *Candida krusei* ve *Candida tropicalis*, *Escherichia coli* ve *Bacillius subtilis*'e karşı yüksek antibakteriyel aktivite gösterdiği bildirilmiştir (Uzel ve ark., 2006). *P. platychlaena*'nın su ekstraktının yüksek antioksidan aktivite gösterdiği (Öke Altuntaş ve ark., 2011), meyve uçucu yağının orta derecede insektisidal ve repellent aktiviteye sahip olduğu bildirilmiştir (Tabanca ve ark., 2018). *P. platychlaena*'nın uçucu yağ bileşenleri hakkında çok az bilgi mevcuttur. Türkiye'nin farklı bölgelerinden toplanan *P. platychlaena* uçucu yağının önemli bileşenleri α-pinene (%69,75), β-phellandrene (%10,58), Δ-3-carene (%3,39) ve p-cimene (%3,38) olarak tanımlanmıştır (Uzel ve ark., .2006). Başka bir çalışmada Türkiye'nin doğusundan toplanan *P. platychlaena* uçucu yağ ana bileşenlerinin β-phellandrene (%22,4), α-phellandrene (%17,1) ve α-pinene (%12,8) olduğu rapor edilmiştir (Tabanca ve ark., 2018). Bu araştırmada, Göller Yöresi florasında doğal olarak yetişen *P. platychlaena* bitkisinin meyve uçucu yağ oranı ve bileşenlerinin lokasyonlara bağlı olarak değişimini belirlemeyi amaçlanmıştır.

Materyal ve Yöntem

Bitki materyali

Çalışmada, materyal olarak Göller Yöresi'nde 2 farklı lokasyonda (Burdur-Bucak, Kızılkaya; Isparta-Sütçüler, Çandır) doğal olarak yetişen *P. platychlaena* bitkisinin meyveleri kullanılmıştır. Bitki örnekleri 2016 yılında toplanmış, örnekleme tür teşhisi için bitkilerin tam çiçeklenme döneminde (Temmuz), uçucu yağ analizi için ise meyvelerin sarı olum döneminde (Eylül) yapılmıştır. Her iki lokasyondan da toplam 10'ar bitki örneği alınmıştır. Meyvelerin toplandığı lokasyonların konum bilgileri ile uçucu yağ oranları, Çizelge 1'de sunulmuştur. Bitki örneklerinin taksonomik sınıflandırması, SDÜ Fen Edebiyat Fakültesi Biyoloji Bölümü'nde Prof. Dr. Hasan ÖZÇELİK tarafından "Türkiye Florası 9. Cilt" (Davis ve ark., 1988) kriterlerine göre gerçekleştirilmiş ve herbaryum örnekleri SDÜ Fen Edebiyat Fakültesi GÜL Herbaryumu'nda (Herbaryum No: 63.18.5.1) muhafaza edilmiştir.

Uçucu yağ analizi

Bitkiye ait meyve örnekleri, her iki lokasyonda da meyvelerin sarı olgunluk dönemine göre toplanmış ve oda şartlarında gölgede kurutulduktan sonra 100 gram ağırlığındaki örnekler blender'da öğütülerek, Clevenger tipi hidro-distilasyon cihazında 3 saat boyunca damıtılmıştır. Distilasyon sonucu elde edilen uçucu yağların miktarı ml olarak ölçülerek, %oranları hesaplanmıştır (Council of Europe, 1980).

Gaz kromatografisi-kütle spektrometresi (GC-MS) analizi

Temin edilen uçucu yağların bileşenleri, GC/MS (QP Shimadzu 2010 Plus) cihazı kullanılarak belirlenmiştir (Stein, 1990). 10 µl uçucu yağ, 1 ml n-hekzan içinde çözündürüldükten sonra GC/MS cihazının CP-Wax 52 CB (50 m x 0,32 mm; film kalınlığı 0,25 µm) kolonuna enjekte edilmiştir. Kolon sıcaklığı, 60 °C'den başlayarak dakikada 10 °C artırılarak yükseltilmiş ve 220 °C'e ulaşıldığında 10 dakika boyunca bekletilmiştir. Enjeksiyon bloğu sıcaklığı 240 °C ve dedektör sıcaklığı 250 °C olarak sabitlenmiştir. Dedektör enerji akışı 70 eV olarak ayarlanmış, iyonlaştırma türü EI ve helyum akış hızı ise 20 ml/dak olarak kullanılmıştır. Bileşenler, kütle spektrumları standart maddelerin geliş zamanları ile karşılaştırılarak ve NIST ile Wiley kütüphanelerinde rapor edilen değerlere göre tanımlanmıştır (Rostad and Pereira, 1986; Adams, 2007).

Çizelge 1. *Prangos platychlaena* bitkilerinin lokasyon bilgileri ve meyve uçucu yağ oranları
Table 1. Locality information and fruit essential oil ratios of Prangos platychlaena plants

Lokasyonlar	Habitat	Rakım	Boylam	Enlem	Uçucu Yağ Oranı (%)
Burdur-Bucak, Kızılkaya	Kayalık yamaç alanlar	810 m	37˚29′97″	30˚44′34″	0.25±0.06
Isparta-Sütçüler, Çandır	Ormanlık alanlar	245 m	37˚37′90″	30˚88′02″	0.16±0.02

Bulgular ve Tartışma

P. platychlaena meyve uçucu yağ oranları Kızılkaya ve Çandır lokasyonlarında sırası ile %0,25±0,06 ile %0,16±0,02 olarak tespit edilmiştir. Lokasyonlara ait uçucu yağlarda sırası ile 44 ve 54, toplamda ise 73 farklı bileşen belirlenmiştir. Her iki lokasyonda da uçucu yağı oluşturan bileşenlerin önemli bir kısmı seskiterpen bileşenlerden oluşmuş ve yağların seskiterpen içerikleri Kızılkaya ve Çandır lokasyonlarında sırasıyla %85,82 ve %75,80 olarak tespit edilmiştir. Uçucu yağları oluşturan diğer önemli terpenoid gruplarından monoterpenler %7,02 ve %9,08, alkoller %0,20 ve %4,82, uzun karbon zincirli bileşenler ise %1,74 ve %0,13 oranında belirlenmiştir (Çizelge 2).

P. platychlaena meyve uçucu yağını oluşturan önemli bileşenler, her iki lokasyonda da benzerlik göstermiş olup, bileşen sayısı ve oranları arasında önemli varyasyonlar belirlenmiştir. Germacrene-D (%17,08-20,24), β -bisabolene (%7,53-17,83), β-copaene (%0,92-%11,70), caryophyllene oxide (%6,30-6,23), β -farnesene (%3,21-5,66), δ-cadinene (%3,50-3,87) ve ledane (%3,35-3,22) her iki lokasyonun da uçucu yağında bulunan önemli ortak bileşenler olarak saptanmıştır. Uçucu yağları oluşturan bileşenlerden 19 tanesi sadece Kızılkaya lokasyonunda, 29 tanesi ise sadece Çandır lokasyonunda tespit edilmiştir (Çizelge 2). β-copaene (%11,70), longifolene (%8,32), azulenol (%2,98), calarene (%2,88) ve cedrane (%1,56) sadece Kızılkaya, aromadendrene (%4,65), carotol (%3,49), 3-ısopropyl-6,7-dımethyl-trıcyclo (%3,46) ve pseudowiddrene (%2,31) ise sadece Çandır lokasyonunda belirlenen önemli bileşenlerdir (Çizelge 2). Diğer bileşenlerden cis-ocimene, n-undecene, n-undecanol, longifolene, β–funebrene, calarene, nerolidol, palustrol, β-citronellol, α-ethyl-o-methoxybenzyl alcohol, cycloheptane, azulenol, 9-octadecen-1-ol, cedrane, 3,9-dimethyl-tricyclo, cembrene, 2-phenanthrenecarboxaldehyde, cyclobuta, duvatriendiol bileşenleri sadece Kızılkaya lokasyonunda; β–myrcene, 2-ethylhexyl acetate, Z-citral, β –bourbonene, pseudowiddrene, myristcin, globulol, carotol, oplopenone, naphthalene, 7-tetradecene, torreyol, 3-isopropyl-6,7-dimethyl-tricyclo, 1,5-epoxysalvial-4(14)-ene, thujyl alcohol isomer, L-limonene, retro-ionone, cholesta-4,6-dien-3-ol, 4a-methyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3h)-one, 4-butan-2-ol, 2,5-furandione, dolichodial, limonene dioxide 4, isoaromadendrenepoxid, 5-isopropenyl-1,2-dimethyl-cyclohex-2-enol, platambin, 2-pentadecanone, 2-methyl-4-(2,6,6-trimethyl-cyclohex-1-enyl)-but-2-en-1-ol, lactaropallidin bileşenleri ise sadece Çandır lokasyonunda belirlenmiştir (Çizelge 2).

Farklı bölgelerden toplanan meyve örneklerinin uçucu yağ oranlarındaki tespit edilen farklılıkların, bitkilerin genetik yapıları ile olgunlaşma dönemlerindeki iklimsel farklılıklardan kaynaklanabileceği düşünülmektedir. Nitekim, bitkilerin yetiştiği lokasyonlar birbirinden oldukça farklı ekolojilere (Sütçüler lokasyonu ormanlık alan olup, zengin bitki örtüsüne sahip, nemli bir bölgedir, Kızılkaya lokasyonu ise kayalık ve kıraç bir alan olup, zayıf bitki örtüsüne sahip kurak-yarı kurak bir bölgedir) sahip oldukları için meyvelerin olgunluk dönemleri arasında önemli bir zaman farkı (15-20 gün arasında) gözlenmiştir. Meyvelerin olgunluk dönemlerindeki hava koşullarına bağlı olarak salgı kanalındaki uçucu yağ oranlarının değiştiği, bazı araştırmacılar tarafından da tespit edilmiştir (Özel, 2008; 2009). Kandil ve ark. (2002), meyvelerin olgunluk dönemlerinde gerçekleşen yağışlar veya yüksek sıcaklıkların, uçucu yağ oranının azalmasına neden olabileceğini bildirmişlerdir. Tıbbi ve aromatik bitkilerin bileşenlerini oluşturan aktif maddelerin sentezi, genellikle genetik faktörlerden (Palevitch, 1987) kaynaklansa da sıcaklık, yağış miktarı, hava nispi nemi, ışık durumu ve rakım gibi farklı etkenler, uçucu yağın kimyasal kompozisyonunda önemli değişikliklere neden olabilmektedir (Mammadov, 2014, Karık ve ark., 2017; Şanlı and Karadoğan, 2017; Sönmez ve ark., 2018). Kızılkaya lokasyonunda bitkilerin toplandığı alanlar yüksek rakımlı ve kayalık yamaç alanlar olup, bölge genel itibari ile kurak iklim ve bozkır bitki örtüsüne sahiptir. Bölgenin kötü iklim ve toprak koşullarına karşı adaptasyonunun sağlanması ve olumsuz şartlara karşı bitki direncinin arttırılması amacıyla Kızılkaya lokasyonunda yetişen bitkilerde seskiterpenoid bileşiklerin daha fazla sentezlendiği düşünülmektedir. Nitekim, oksidatif stres dahil farklı abiyotik stres koşullarına karşı, seskiterpenler grubuna ait bileşenlerin bitkilerde savunma sistemi güçlendirdiği bilinmektedir (Phillips and Croteau, 1999). Apiaceae familyasından kültüre alınan türlerle yapılan çalışmalarda, meyve uçucu yağ bileşenlerinin bitkilerin yetiştirildikleri vejetasyon periyotlarındaki ekolojik koşulların farklılığından kaynaklanan önemli değişimler gösterdiği tespit edilmiştir (Şanlı ve ark., 2012; 2019, 2020; Özel ve ark., 2014; Tosun ve ark., 2022). Uçucu yağ bileşenleri açısından belirlenen farklılıkların, özellikle bitkilerin genetik yapısı ile yetiştirildiği lokasyonlar arasındaki coğrafi (rakım, eğim, yön, toprak yapısı, vb.) ve ekolojik farklılıklardan kaynaklandığı düşünülmektedir. Aromatik bitkiler üzerinde yapılan farklı çalışmalarda, uçucu yağı oluşturan bileşenlerin ve bu bileşenlerin oranlarının rakımdan etkilendiği ve bu nedenle önemli değişiklikler gösterdiği, birden fazla araştırmacı tarafından da bildirilmiştir. (Mahzooni-Kachaip ve ark., 2014; Karadoğan ve ark., 2015; Sardrodi ve ark., 2017; Şanlı ve ark., 2019; 2020).

Türkiye'nin farklı bölgelerinden toplanan *P. platychlaena* uçucu yağı ile yapılan çalışmalarda uçucu yağın önemli bileşenleri α-pinene (%12,8-69,75), β-phellandrene (%10,58-22,4), α-phellandrene (%17,1), δ-3-carene (%3,39) ve p-cimene (%3,38) olarak tanımlanmıştır (Uzel ve ark., 2006; Tabanca ve ark., 2018). Rahman ve ark. (2020), *P. platychlaena* çiçek uçucu yağının önemli bileşenlerini (E)-β-Ocimene (%28,5), bornyle acetate (%24,18), γterpinene (%14,15), p-cymene (%6,48), α-pinene (%4,16), sylvestrene (%3,02) ve terpinolene (%2,41) olarak belirlemiştir. Azarkish ve ark. (2021), İran'da 13 farklı lokasyondan topladıkları *P. platychlaena* bitkisinin toprak üstü kısımlarında uçucu yağ oranının %0,04 ile %2,85 arasında değişim gösterdiği ve uçucu yağın önemli bileşenlerinin δ-3-carene (%9,25–43,17), α-pinene (%4,58–27,41), β-pinene (%3,72–25,55) ve β-phellandrene (%4,02–17,88) olduğunu bildirmişlerdir. Elde edilen bulgular ile karşılaştırıldığında, *P. Platychlaena* ile yapılan diğer çalışmalarda uçucu yağı oluşturan ana bileşenler ve oranları bakımından önemli farklılıklar görüldüğü anlaşılmaktadır.

Çizelge 2. *P. platychlaena* meyve uçucu yağının kimyasal kompozisyonu
Table 2. Chemical composition of P. platychlaena fruit essential oil

RI	Bileşenler	Kızılkaya	Çandır	RI	Bileşenler	Kızılkaya	Çandır
932	α-pinene	1,89	3,53	1849	Cholesta-4,6-dien-3-ol	-	0,64
974	β-pinene	0,89	1,13	1873	4a-methyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3h)-one	-	0,58
985	β-myrcene	-	0,21	1880	4-butan-2-ol	-	0,36
1032	Cis-ocimene	0,23	-	1885	9-octadecen-1-ol	0,79	-
1072	Octilin	0,43	0,08	1887	2,5-furandione	-	0,33
1249	Trans-anethole	1,48	0,61	1888	Cedrane	1,56	-
1270	n-undecene	0,27	-	1899	3,9-dımethyl-trıcyclo	0,28	-
1277	2-ethylhexyl acetate	-	0,41	1924	Kauran-18-al, 17-(acetyloxy)	0,41	0,63
1316	Z-citral	-	0,32	1937	Cembrene	0,47	-
1367	n-undecanol	0,68	-	1951	Dolichodial	-	0,40
1374	α –copaene	1,46	1,77	2015	Limonene dıoxıde 4	-	0,75
1387	β –bourbonene	-	1,06	2021	2-phenanthrenecarboxaldehyde	0,19	-
1392	β –elemene	2,28	1,7	2037	Isoaromadendrenepoxıd	-	0,53
1407	Longifolene	8,32	-	2048	1-3,3-dımethyl-2-(3-methyl-buta-1,3-dıenyl)	1,01	0,99
1408	Trans-caryophyllene	1,70	0,98	2058	Cyclobuta	0,45	-
1413	β-funebrene	0,46	-	2060	5-ısopropenyl-1,2-dımethyl-cyclohex-2-enol	-	0,27
1432	α-bergamotene	0,64	0,45	2073	Duvatrıendıol	0,67	-
1446	Aromadendrene	2,20	4,65	2075	Platambin	-	0,62
1454	β –farnesene	5,66	3,21	2079	2-pentadecanone	-	0,37
1479	Curcumene	0,98	0,54	2091	2-methyl-4-(2,6,6-trımethyl-cyclohex-1-enyl)-but-2-en-1-ol	-	0,50
1484	Germacrene D	17,08	20,24	2111	Lactaropallidin	-	0,26
1496	Valencene	0,57	0,33		Monoterpenler	7,02	9,08
1497	Ledane	3,35	3,22		Seskiterpenler	85,82	75,80
1498	Pseudowiddrene	-	2,31		Alkoller	0,20	4,82
1505	β –bisabolene	7,53	17,83		Uzun Karbon Zincirli Bileşenler	1,74	0,13
1513	γ –cadinene	0,26	1,26		Diğerleri	4,31	9,26
1517	Myristcin	-	0,35		Toplam (%)	99,09	99,09
1522	δ-cadinene	3,50	3,87		Bileşen sayısı	44	54
1537	Calarene	2,88	-	1899	3,9-dımethyl-trıcyclo	0,28	-
1561	Nerolidol	0,87	-	1924	Kauran-18-al, 17-(acetyloxy)	0,41	0,63
1567	Palustrol	0,65	-	1937	Cembrene	0,47	-
1582	Caryophyllene oxide	6,30	6,23	1951	Dolichodial	-	0,40
1590	β –copaene	11,70	0,92	2015	Limonene dıoxıde 4	-	0,75
1592	Globulol	-	0,27	2021	2-phenanthrenecarboxaldehyde	0,19	-
1594	Calvial-4(14)-en-1-one	0,29	0,29	2037	Isoaromadendrenepoxıd	-	0,53
1596	Carotol	-	3,49	2048	1-3,3-dımethyl-2-(3-methyl-buta-1,3-dıenyl)	1,01	0,99
1607	Oplopenone	-	0,41	2058	Cyclobuta	0,45	-
1608	Naphthalene	-	0,39	2060	5-ısopropenyl-1,2-dımethyl-cyclohex-2-enol	-	0,27
1618	Androstan-17-one, 3-ethyl-3-hydroxy	1,84	1,53	2073	Duvatrıendıol	0,67	-
1641	7-tetradecene	-	0,13	2075	Platambin	-	0,62
1642	β -citronellol	0,20	-	2079	2-pentadecanone	-	0,37
1644	Torreyol	-	0,39	2091	2-methyl-4-(2,6,6-trımethyl-cyclohex-1-enyl)-but-2-en-1-ol	-	0,50
1652	α -cadinol	0,87	0,35	2111	Lactaropallidin	-	0,26
1723	α -ethyl-o-methoxybenzyl alcohol	0,20	-		Monoterpenler	7,02	9,08
1754	3-ısopropyl-6,7-dımethyl-trıcyclo	-	3,46		Seskiterpenler	85,82	75,80
1761	1,5-epoxysalvial-4(14)-ene	-	0,3		Alkoller	0,20	4,82
1771	Spathulenol	2,22	1,51		Uzun Karbon Zincirli Bileşenler	1,74	0,13
1778	Thujyl alcohol ısomer	-	1,06		Diğerleri	4,31	9,26
1792	Cycloheptane	0,40	-		Toplam (%)	99,09	99,09
1793	L-limonene	-	0,37		Bileşen sayısı	44	54
1806	Azulenol	2,98	-				
1820	Retro-ıonone	-	0,70				

Aynı zamanda, diğer araştırmacıların bulguları arasında da belirgin farklılıklar bulunmaktadır. Bu farklılıkların muhtemelen doğal florada yetişen *P. Platychlaena* türünün popülasyon halinde yayılış göstermesi ve popülasyonlar içerisinde türe ait alt tür ya da farklı kemotiplerin bulunmasından kaynaklandığı düşünülmektedir.

Sonuç

Genel olarak yabani popülasyonların değerlendirilmesi, doğal popülasyon çeşitliliğini korumak amacıyla kemotiplerin tanımlanmasına yol açacak şekilde metabolik çeşitlilikleri hakkında bilgi sağlamaktadır. Bu çalışmada, *P. platychlaena*'nın yabani popülasyonlarının meyvelerindeki uçucu yağ değişimi araştırılmıştır. Çalışmada her iki lokasyonda yetişen bitkilere ait uçucu yağların kimyasal profilleri arasında önemli farklılıklar gözlenmiş, uçucu yağların önemli bileşenleri; germacrene-d, β-bisabolene, caryophyllene oxide, β–farnesene, δ-cadinene, β-copaene ve longifolene olarak tespit edilmiştir. Bu çalışmada, uçucu yağ oranı ile yağı oluşturan uçucu bileşenlerin sayı ve miktarlarının bitkilerin yetiştiği bölgenin coğrafi ve ekolojik koşullarından oldukça fazla etkilendiği ve buna bağlı olarak bazı yeni bileşenlerin sentezlenebildiği, dönüşebildiği veya miktarlarının faydalı veya zararlı olacak şekilde değişebildiği gözlemlenmiştir. Seskiterpenoidler, gıda, tarım ve farmakoloji sektörlerinde önemli kullanım alanlarına sahiptir. *P. platychlaena* uçucu yağı bu bileşenler açısından zengin olduğundan, bu yağın kullanım alanlarındaki etkinliklerine yönelik çalışmaların yürütülmesinin, türün ekonomik öneminin tespit edilmesine katkı sağlayabileceği düşünülmektedir. Sonuç olarak, türle ilgili çalışmaların artırılması ile hem kültüre alım ve ıslah çalışmalarında hem de farklı alanlarda kullanımında, türün yetiştiği lokasyonun dikkate alınmasının önemli olduğu sonucuna varılmıştır.

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The Effect of Corporate Identity on the Entrance of Educational Venues

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ABSTRACT

In the study, it was aimed to make the interior design qualified with the principle of corporate identity, and to make and apply the wall surface design in the education spaces, and thus to bring the corporate identity to the user in an inclusive way with the interior design of the determined sample area. Wall surfaces at the entrance of Selçuk University Social Sciences Institute and Selçuk University Faculty of Letters were used as material in the study. In the method of the study, literature research was conducted and focused on corporate identity and educational spaces through the discipline of interior architecture. Afterwards, observations and analyzes were made by taking the opinions and suggestions of the staff and students who constantly use the sampling areas. Accordingly, it has been observed that there should be wall surfaces where the guests and students can take photos, reinforced with focusing lighting, which represents the corporate identity with the logo at the entrances of the educational venues discussed. According to the data obtained, the wall surface design was made and applied according to the institutional identity of the educational spaces considered as material. After the implementation of the design, based on on-site observations and analyzes, it is seen that the sense of belonging of the staff and students to the institution develops, the guests and alumni increase the recognition of the institution by taking photographs, which are commonly carried out today, and the wall surface of the design becomes active by bringing it to the place it belongs to.

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Eğitim Mekânları Girişinde Kurumsal Kimliğin Etkisi

MAKALE BİLGİSİ

Araştırma Makalesi

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ÖZ

Yapılan çalışmada, iç mekân tasarımının kurumsal kimlik ilkesiyle nitelikli hale getirilmesi hedeflenerek eğitim mekânlarında, duvar yüzeyi tasarımının yapılması, uygulanması ve böylece belirlenen örneklem alanlarının iç mekân tasarımıyla kurumsal kimliği kapsayıcı şekilde kullanıcıya kazandırılması amaçlanmıştır. Çalışmada materyal olarak Selçuk Üniversitesi Sosyal Bilimler Enstitüsü ve Selçuk Üniversitesi Edebiyat Fakültesi'nin girişinde bulunan duvar yüzeyleri kullanılmıştır. Çalışmanın metodunda, literatür araştırması yapılarak iç mimarlık disiplini üzerinden kurumsal kimlik ve eğitim mekânlarına odaklanılmıştır. Sonrasında örneklem alanlarını sürekli kullanan personel ve öğrencilerin görüş ve önerileri alınarak gözlem ve analizlerde bulunulmuştur. Buna bağlı olarak ele alınan eğitim mekânlarının girişlerinde kurumsal kimliği logo ile temsil eden, odaklayıcı aydınlatmalarla pekiştirilmiş, gelen misafir ve öğrencilerin fotoğraf çekinebilecekleri duvar yüzeylerinin olması gerektiği gözlemlenmiştir. Edinilen verilere göre, materyal olarak ele alınan eğitim mekânlarının kurumsal kimliğine göre duvar yüzeyi tasarımı yapılmış ve uygulanmıştır. Tasarımın uygulanmasından sonra, yerinde gözlem ve analizlere dayanarak personel ve öğrencilerin kuruma karşı aidiyetlik duygusunun geliştiği, gelen misafirlerin ve mezunların günümüzde yaygın olarak gerçekleştirilen fotoğraf çekme eyleminde bulunarak kurumun tanınırlığını arttırdığı, tasarımı yapılan duvar yüzeyinin ait olduğu mekâna kazandırılarak aktif hale geldiği görülmektedir.

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Giriş

Kurumun iletişim biçimlerini, felsefesini, görsel unsurlarını oluşturan kurumsal kimlik; kuruluşların, işletmelerin, organizasyonların kimliğini ifade etmektedir. Ticari, kamusal ya da sosyal faaliyet gösteren birimlerin artış göstermesi, günümüzde birbirine benzer kuruluşların artmasına neden olmuştur. Bu duruma bağlı olarak kurumsal kimlik kavramını çözümleyemeyen kurumlar, benzerliğe dayalı olarak hizmet alan kişiler tarafından hatırlanamamaktadır (Süceddinov, 2008).

Uzun bir geçmişe sahip olan kurumsal kimlik kavramı, sıcak savaş dönemlerinde orduların askerlerine aidiyetlik katması ve karşı tarafın motivasyonunu düşürmesi amacıyla kullanılan araç gereçlerin üzerine simgeler ile yansıtılmıştır. Bu durum, ilerleyen zamanlarda ticari firmaların isim, logo, kart vizit gibi grafiksel alanda derinleşen görsel tasarımlar ile zenginleşmiştir. Günümüzde ise kurumsal kimlik kavramı, sadece grafiksel görsellerin temsiliyetinden öteye geçerek ticari, kamu, sosyal içerikli tüm kurumların iç mekânlarında da yaygın olarak kullanılmaya başlanmıştır. İç mekânda kurumsal kimlik çalışmaları, kurumların vizyon ve misyonlarını belirgin sınırlılıklar ile tanımlayarak nitelikli gelişimlere katkı sağlamaktadır (Noraslı, 2016).

İç mekânda kurumsal kimlik özgün tasarımlar ile çözümlenmelidir. Mekân içerisindeki nitelikli ve kurumu temsil eden tasarımlar, hizmet alan kişiler tarafından hatırlanabilirliği sağlamaktadır. Özgün mekânlar, mekânın çevresel faktörleriyle anlamlı bir bütün oluşturarak belli bir konsept üzerinde şekillenir. Bir mekânı tasarlarken renk, doku, ışık gibi çevresel faktörlerle mekânın içselleştirileceği ve buna bağlı olarak insanla mekânın etkileşim içerisine gireceği düşünüldüğünde mekân, kendisini deneyimleyen kişinin dışavurum şeklidir (Gündüzlü ve Erçevik Sönmez, 2021). Bu sebeple iç mimarlık disiplini, kurumsal kimlik kavramıyla kolayca özdeşleşmektedir.

Dünyanın küreselleşerek iletişim araçlarının artması ve her alanda başarılı bir sürecin yönetilme çabası, ticari işletmelerin yanı sıra kamu ve özel kurumlarında da kurumsal kimliği ön plana çıkarmaktadır. Ülkelerin kalkınmasında, belirleyici bir unsur olarak dışarıya prestij sağladığı için kurumsal kimliğin ön plana çıktığı bir alan da eğitim kurumlarıdır (Pürlüsoy ve Elibol, 2022). Bu doğrultuda eğitim kurumlarının iç mekânlarında kurumsal kimlik çalışmaları yaygın olarak kullanılmaktadır.

Yapılan çalışmada, kurumsal kimliğe bağlı olarak eğitim kurumlarında iç mekân tasarımının, örneklem alanı olarak belirlenen kurumlardaki personel ve öğrencilere etkilerinin; gözlem, görüş, öneri ve analizlere dayanarak araştırılması amaçlanmıştır. Çalışmanın materyali olarak Selçuk Üniversitesi Sosyal Bilimler Enstitüsü giriş holü ve Selçuk Üniversitesi Edebiyat Fakültesi giriş holü belirlenmiştir. Çalışmanın yönteminde öncelikle kurumsal kimlik ve iç mekân tasarımı sınırlılıklarıyla literatür taraması yapılıp örnek çalışmalar incelenmiştir. Sonrasında belirlenen alanlarda görev yapan personel ve öğrenim gören öğrencilerin görüş ve önerileri alınarak mekân içerisinde gözlem ve analizlerde bulunulmuştur. Edinilen verilere göre kurumların imajını yansıtan görsellerin mekân içerisinde bulunması, kullanıcılardaki aidiyetlik duygusunu arttırarak kurumu olumlu yönde etkilediği tespit edilmiştir. Bu doğrultuda, mekânlarda belirlenen duvar yüzeyleri tasarlanarak uygulaması yapılmıştır. Sonrasında ise tasarlanan alanların kullanımında artış görüldüğü, mekâna ve kurumu kazanım sağladığı tespit edilmiştir.

Kavramsal Çerçeve

Kurumsal kimlik kavramı ile ilgili edinilen bilgiler, kurumsal kimliğin ortaya çıkışının oldukça eskiye dayandığını göstermektedir. Kurumsal kimlik, bir ordunun karşısındaki orduya kim olduğunu bildirmek ve gövde gösterisinde bulunmak amacıyla ortaya çıkmıştır. Krallık dönemlerinde, yandaşları tarafından tanınmak ve karşı tarafa görsel mesaj göndermek için kullanılan simgeler; orduların, kimlik ve aidiyetlik duygusunu vurgulamıştır. Zamanla ülkeler, kendi kültürel ve toplumsal değerlerini yansıtan askeri üniformalar ile ordularını savaşa sürerek görsel anlamda algısal etki oluşturmuşlardır. Böylece kurumlar arası farklılıklar görselleşmeye başlamıştır. Başlangıçta orduların tanınması ve diğer ülkelerin ordularından ayırt edilebilinmesi amacıyla kullanılan simgeler, taşımacılığın gelişmesiyle değişik alanlarda kullanılmaya başlanmıştır. Tarihin başlangıcından beri belirleyici olan kurumsal kimlik kavramı zamanla gemilerde, yolcu vagonlarında, otobüslerde ve hava araçlarında uygulanmıştır (Balta Peltekoğlu, 2022).

Kurumsal kimlik kavramının tanımı, kimlik ve kolektif kimlik kavramlarının bilinmesiyle daha anlamlı hale gelmektedir. Kimlik, aslında insana özgü bir kavram olmakla birlikte insanı, diğer insanlardan ayırt eden bir özelliktir. Kolektif kimlik, toplumun kendine dönük bilinci ve duygularıyla ilişkili olarak özgün niteliklere sahip olan aidiyetlik duygusudur. Buna bağlı olarak kurumsal kimlik kavramı ise bireysel kimlikten farklı olarak ancak kolektif kimliğe benzer biçimde bir kurumun, işletmenin ya da organizasyonun kimliğini ifade etmektedir (Ülker Kaya, 2006).

İnsanları, birbirinden ayıran kimliklerin olduğu gibi kurumların da kendine özgü karakteristik özellikleriyle birbirinden ayrışıp farklılaşmasını sağlayan kimlikleri vardır. Kurumsal kimlik toplum ile iletişimi sağlayan en önemli araçlardan biridir (Uzoğlu, 2001). Bu bağlamda kurumsal kimlik; bir kurumun, işletmenin, organizasyonun vb. ortaya koymuş oldukları işe bağlı olarak görsel ve fikirsel anlamda akıllarda yer edinme şeklidir. Bir kurumun hem kurum içinde hem de kurum dışında duruş ve davranışlarını temsil etme biçimlerinin tümü, kurumsal kimliği oluşturur. Güçlü bir kurumsal kimlik, zamanla toplumların zihninde olumlu bir imaj çizerek güven duygusunu pekiştirir (Göktepe, 2013).

Kurumsal kimlik, kurumların rekabet içerisinde olduğu diğer kurumlardan ayrılmak için farkındalık oluşturma eylemleriyle sürdürülebilmektedir (Balmer ve Gray, 2000). Kurumların karakteristik özelliklerini dışavurumunun en önemli aracı olan iletişimi ve özgün taraflarını ifade edip, akıllarda yer edebilme biçimleri göz önünde bulundurulduğu sürece kurumsal kimlik temasını yerine getirmiş olmaktadır. Bu işleve bağlı olarak kurumsal kimlik, bir kurumun kendisinin nasıl tanındığına ve insanların kurumu nasıl tanımlaması, hatırlaması ve

anlatması gerektiğine imkân sağladığı anlamalar bütünü olarak görülebilir (Hepkon, 2023).

Kurumsal kimliğin iletişim, tanıtım ya da görsel ögeler çerçevesinde incelenen bir kavram olduğunu ifade etmek indirgeyici bir yaklaşımdır. Kurumu gelecekte ileriye taşıyacak ögelerin, belirlenen hedef kitleler tarafından fark edilerek hatırlanması için bir bütün olarak ele alınması ve kurumsal kimliğe uygun olarak tasarlanması gerekmektedir (Silsüpür ve Erdinç, 2021).

Şekil 1.'de görüldüğü gibi kurumsal kimliğin ögeleri; kurumsal felsefe, kurumsal iletişim, kurumsal davranış ve kurumsal tasarım olmak üzere dört ana başlık altında incelenmektedir. Kurumsal tasarım ise ürün, iletişim ve çevre tasarımı olmak üzere üç başlık altında incelenir. Kurumsal kimliğin kalkınmasında önemle üzerinde durulan iç mekân tasarımı, iletişim ve çevre tasarımı başlıkları altında ele alınan bir konudur (Yazıcıoğlu ve Meral, 2011).

Kurumsal tasarım, kurumları özgün konuma taşıyarak kurumlar arası rekabette başarı sağladığı için bir kurumun kimliğinin oluşumu ve gelişiminde en önemli parçalardan birisi haline gelmektedir (Melewar ve Saundres, 2000). Kavramsal bir boyut olan kurumsal kimliği, biçimsel bir görsel unsur haline dönüştüren eylem kurumsal tasarım aşamasıdır. Kurumsal tasarım, bir kurumun iç mekân tasarımı, logosu, personel kıyafeti, antetli evrakları, araç giydirmeleri, web sitesi, vb. tüm unsurların organize edilmesiyle anlamlı bir bütün haline gelmektedir. Böylelikle kurumsal kimlik, kurumlar için itibar yönetiminin önemli bir parçası haline gelerek kendisini, karşı tarafa ifade etmesinde etkin olarak kullanılmaktadır (van den Bosch vd, 2006; Birkigt ve Stadler, 1986).

İç mekân tasarımı, kurumsal tasarımdaki oluşumun en önemli parçalarından birisidir (Perry ve Wisnom, 2003). Kurumsal kimlik kapsamında iç mekân tasarımı ise özgün bir konsept çalışması ile mümkün hale gelmektedir. Konsept, tasarım sürecindeki şekillenmenin en önemli aşamalarından birisidir. Bir mekânın özgün niteliğe sahip olması, konseptin gelişimi ile oluşmaktadır. Bu bağlamda konsept, mekânda yansıtılmak istenen atmosferi niteleyen bir olgudur ve tasarım ilkelerinin somutlaştırılarak özgün yaklaşımlarla edinilen görsellerin bir sonucu olarak karşımıza çıkar (Gündüzlü ve Erçevik Sönmez, 2021).

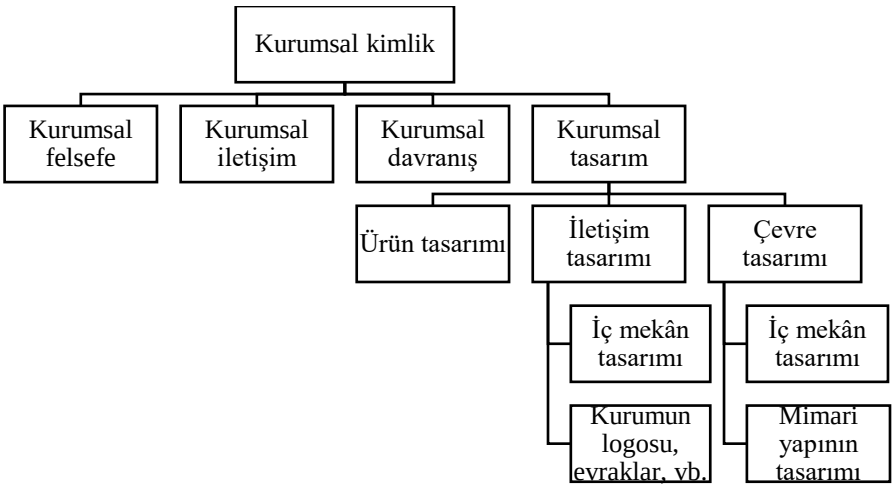
Bir iç mekân tasarımının kurumsal kimliğe uygun olduğunu anlayabilmek için o mekânı oluşturan çevresel faktörlerin taşıdığı algısal uyarılar, anlaşılabilir olmalıdır (Yazıcıoğlu ve Meral, 2011). Kurumsal kimliğe uygun iç mekân tasarımı, mekânı deneyimleyen kişinin; mekân içerisindeki renk, doku, aksesuar, mobilya, zemin ve duvar kaplaması gibi tüm bileşenlerin görsel etkilerinden etkilenerek her birine anlam yüklemesi ile oluşur (Akkaş ve Mutdoğan, 2021).

Kurumsal kimliğe bağlı olarak yapılan iç mekân tasarımlarında duvar yüzeyleri, en vurgulayıcı ögelerden biridir. Mekânlar, kavramsal olarak varlık gösterebildikleri gibi fiziksel olarak bir hacim ya da yüzey değerleriyle de varlık gösterebilir (Canbakal Ataoğlu, 2009). Duvar yüzeyleri, düşey yönelimi sebebiyle mekânın tanımlanmasında en etkili yüzeylerdir (Ching, 2002). Bu doğrultuda duvar yüzeylerinin algısal etkisi, iç mekânda bulunan diğer yüzeylere göre daha etkilidir. Tasarımcılar mekânın karakterini, kullandıkları tasarım ögeleriyle kullanıcılara görsel mesaj olarak iletir. Böylece kullanıcı, çevresel uyaranların kendisinde oluşturduğu çağrışımlarla birlikte mekânı düşünür, yorumlar ve davranışını sergiler. (Aydıntan, 2016).

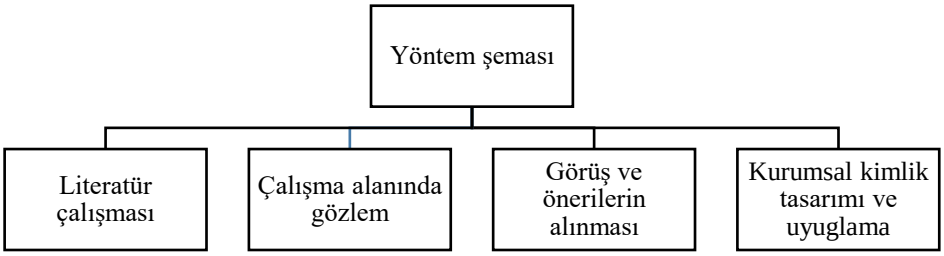
Kurumların kendini ifade edebilmesinde, kurumsal kimlik tasarımı etkin rol oynamaktadır. İç mekân tasarımı ise kurumsal kimlik tasarımının önemli parçalarından biridir ve yüzey tasarımları ile iç mekân tasarımında somut mesajlar verilebilir. Buna bağlı olarak yapılan çalışmada, eğitim mekânları üzerinden kurumsal kimlik tasarımının kullanıcılar üzerindeki etkisi araştırılmıştır.

Materyal ve Yöntem

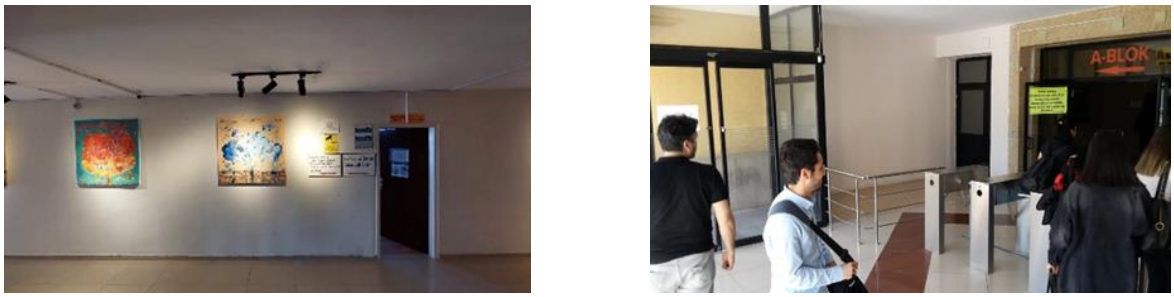
Çalışmanın materyalini, Selçuk Üniversitesi Sosyal Bilimler Enstitüsü ve Edebiyat Fakültesi girişlerinde bulunan duvar yüzeyleri oluşturmaktadır. Ele alınan iki mekânın duvar yüzeyleriyle kurumsal kimliğin kullanıcılar üzerindeki etkisi, yapılan uygulamalı tasarımla araştırılmıştır. Yapılan çalışmanın yöntemi Şekil 2.'de görüldüğü gibi dört aşamadan oluşmaktadır.



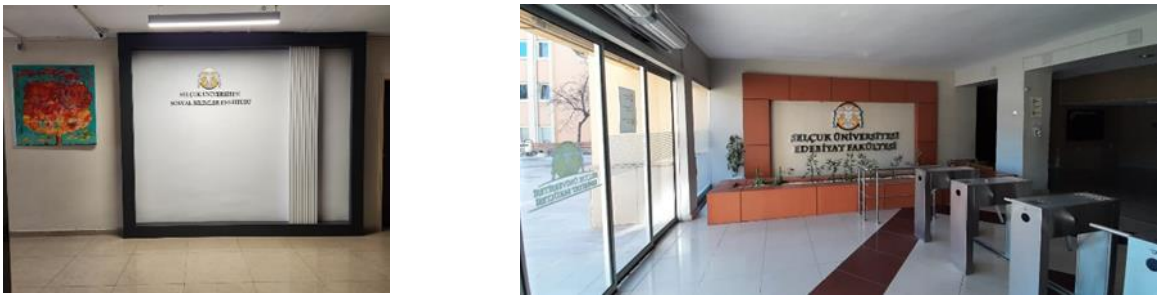
Şekil 1. Kurumsal kimlik ögeleri (Yazıcıoğlu ve Meral, 2011).
Figure 1. Corporate identity elements (Yazıcıoğlu and Meral, 2011).



Şekil 2. Çalışmanın yöntem şeması
Figure 2. Method scheme of the study.



Şekil 3. Enstitü ve fakültenin tasarım öncesi görselleri. (Noraslı ve Akçaova kişisel arşiv)
Figure 3. Pre-design visuals of the institute and faculty. (Noraslı and Akçaova personal archive)



Şekil 4. Enstitü ve fakültenin tasarım sonrası görselleri. (Noraslı ve Akçaova kişisel arşiv)
Figure 4. Post-design visuals of the institute and faculty. (Noraslı and Akçaova personal archive)

İlk olarak kurumsal kimlik tasarımı ile ilgili literatür çalışması yapılmıştır. Sonrasında materyal olarak ele alınan eğitim yapıları üzerinde gözleme dayalı araştırmalar yapılmıştır. Yapılan bir haftalık gözlem sürecinde, tasarlanacak olan mekânların rölövesi alınmıştır. Mekânların birincil kullanıcısı olan öğrenci ve personellerle röportaja dayalı görüş ve önerileri sorulmuştur. Tüm bu verilere dayanarak kurumsal kimlik kapsamında duvar yüzeyleri, tasarlanarak uygulaması yapılmıştır.

Yapılan literatür çalışmasına göre iç mekân tasarımı, kurumsal kimlik tasarımının en etkili ögelerinden biridir. Örneklem alanlarının tasarımı için mekânların, rölövesi alınmış ve bu alanlarda gözlem yapılmıştır. Yapılan gözlemlere göre Şekil 3.'te görüldüğü gibi, mekâna girişlerde nirengi noktası oluşturacak bir noktanın olmadığı görülmüştür. Bu rutin düzen, kullanıcıların mekânlardaki girişi kısmı hatırlamasını olumsuz yönde etkilemektedir. Yapılan görüşmelerde hem öğrencilerin hem de personellerin kullandıkları mekânlara karşı aidiyetlik hissetmedikleri öğrenilmiştir. Öğrenim gören ve mezun olan öğrencilerin kurumla bağlantılı fotoğraf çekinebilecekleri bir alanın olmadığı bilgisi edinilmiştir.

Yapılan gözlem, görüş ve önerilere göre Şekil 4.'te görüldüğü gibi örneklem alanı olarak belirlenen mekânların girişindeki duvar yüzeyine, kurumsal kimliği ön plana çıkaracak yüzey tasarımı yapılmıştır. Mekândaki aidiyetliğin hissedilmesi, fotoğraf çekimleri için kullanılabilmesi ve nirengi noktası oluşturarak mekânın hatırlanabilmesi için kurumsal kimliği görsel anlamda temsil eden kurumun logosu her iki mekânda da kullanılıştır. Ayrıca, mekândaki etkileşimi arttırmak için malzeme olarak ahşap panel ve profiller tercih edilmiştir. Tüm bu yapılan çalışmaları vurgulamak için ise bölgesel aydınlatmalar kullanılarak tasarımda kullanılan ögeler ön plana çıkarılmıştır.

Yapılan tasarımların uygulaması yazarlar tarafından yürütüldükten sonra, enstitü ve fakülte yetkilileriyle görüşülerek mekânın işleyişi ile ilgili veriler alınmıştır. Edinilen bilgilere göre; mekânlardaki girişlerin daha kucaklayıcı olduğu, öğrencilerin ve personellerin fotoğraf çekinerek ya da buluşma noktası belirleyerek mekânı daha verimli kullandıkları, kurumsal kimlik çalışmalarıyla kişilerdeki aidiyetlik duygusunun pekiştiği görülmüştür.

Sonuç ve Değerlendirme

Algı yönetimi için sıcak savaş dönemlerinde, araç ve teçhizatların üzerinde kullanılan semboller ile başlayan kurumsal kimlik kavramı, günümüzde kurumlar için itibar

yönetiminin en önemli parçalarından biri haline gelmiştir. Dünya'nın küreselleşmesiyle birlikte kurumsal kimlik, tüm alana yayılmış ve kurumların takip edenlere karşı kendisini ifade edebilmesinde etkin bir unsur olarak kullanılmaya başlanmıştır.

Bir kurumun nasıl tanındığına ve insanların kurumu nasıl tanımlaması, hatırlaması ve anlatması gerektiğine imkân sağlayan kurumsal kimlik; kurumu, rekabet içerisinde olduğu diğer kurumlardan ayırabilen en önemli faktörlerden biri haline gelmiştir. Günümüzde kurumsal kimliği, kurumların ifade gücü olarak tanımlamak yetersizdir. Kurumsal kimlik, farklı ögeleriyle ele alınan geniş kapsamlı bir konu haline gelmiştir.

İç mekân tasarımı, kurumsal kimliğin ögelerini oluşturan en önemli konulardan biridir. Kurumsal tasarım bağlamında iç mekân tasarımı, kurumu niteleyen özgün bir konsept çalışma ile mümkün hale gelmektedir. Bu doğrultuda iç mekân tasarımının çevresel faktörlerini oluşturan renk, ışık, doku gibi unsurlar; kurumsal tasarımda önemli yer tutmaktadır.

Kurumsal tasarım, her alanda olduğu gibi eğitim alanında da yoğun olarak kullanılmaktadır. Enstitü ve fakülte olmak üzere iki farklı eğitim mekânının girişinde kurumun kimliğini ön plana çıkaracak tasarımların yapılarak uygulanması, verilen görsel mesaj ile mekânı daha nitelikli hale getirerek aidiyetlik duygusunu arttırmıştır. Ayrıca kurumsal kimliğin algısal etkisi, mekânı ilk kez deneyimleyen kullanıcılar üzerinde de etkili olarak olumlu bir izlenim bırakmıştır. Yapılan çalışmada, ele alınan tasarımlara göre duvar yüzeylerinin kurumsal kimliği niteleyecek şekilde tasarlanması, kullanıcılar açısından ilgi uyandırmakta ve akılda kalıcılığı arttırmaktadır. Duvar yüzeylerinde farklı kaplama malzemelerini kullanarak bölgesel aydınlatmalar ile vurgulamalar yapmak, kurumun logosunu kullanmak ve uzayan koridor yüzeylerinde farklılaşmaya giderek nirengi noktaları oluşturmak mekânı hem özelleştirmekte hem de iç mekân tasarımıyla kurumsal kimliği ön plana çıkarmaktadır. İç mekân tasarımında kurumsal kimliği mekâna yansıtmak kullanıcılarda aidiyetliğin artmasını sağlamakta ve kurumun görsel anlamda ifade gücünü arttırmaktadır. Kurumların hedefleri, vizyon ve misyonları yazılı ve sözel olarak belirtilse de görsel hafızanın algıdaki etkisini kullanarak kurumsal kimliğe mekân içerisinde yer verilmesi önerilmektedir.

Bilgi

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A Workshop Example of Basic Design Education in Interior Architecture

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ABSTRACT

The "Basic Design" is one of the common introductory courses in design disciplines. It has great importance in interior architecture education as it forms the basis of design practice. Education that proceeds through abstract concepts creates difficulties for students to internalize this course. To avoid these difficulties, learning by doing is of great importance. The learning-by-doing approach was carried out in the form of a workshop within the scope of the "Basic Design 1" course of the Department of Interior Architecture at Konya Technical University in the fall semester of the 2022-2023 academic year. Students were asked to produce three-dimensional designs using basic design principles and elements. The class was divided into groups of eight people and studies were carried out with a workshop coordinator in each group. The duration of the workshop was planned as four weeks. During the workshop, students learned to use materials and colors, to design an original composition. At the end of the workshop, students learned to embody the abstract concepts they learned during the year by creating a composition that considers functionality and aesthetics. In order to evaluate the efficiency of the workshop, a survey study was carried out after the course period ended. As a result, it has been seen that the intelligibility of Basic Design 1, which is a course taught through abstract concepts, has increased thanks to the workshop.

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Introduction

Basic Design is one of the basic courses in all educational institutions related to the visual arts. Offered in the first year at all design schools, the aim of this course is to teach the general rules of design so as to develop students' design skills and empower them to make decisions in a limited amount of time (Ustaömeroğlu et al., 2015). Basic Design courses aim to enable students to produce designs from an original and aesthetic point of view that meet the needs of current social, cultural and technological developments. Interior Architecture Basic Design courses use disciplines such as music, literature, graphic design, cinema, performance arts, visual and digital arts to develop creative thinking (Aşkın, 2018).

Basic Design is a preparatory process in which art and design education is provided. With this education, students are expected to be able to learn the basic principles and elements of design and transfer this knowledge and skills to their designs. In this direction, they gained the ability to shape matter, use different materials together, color arrangement, convey basic design values such as integrity, rhythm, emphasis, and balance (Yıldırım, 2019).

The prominent studies in the literature in the last decade are as follows: Bağlı and Gelmez's (2013) study is a study that focuses on group work to reinforce the awareness of collective studying by moving away from individualism within the scope of the basic design course. Akbulut's (2014) study deals with the attempt to create a common basic design programme for students with a science background and students with an art background, studying in two different disciplines at Gazi University. Çetinkaya (2014) examined three universities from Europe and six universities from Türkiye in terms of basic design course methods. Neves and Duarte (2016) examined the advantages and methods of using Virtual Reality (VR) technology in basic design courses. Bostancı et al. (2016) in order to examine the method of using music in the basic design course, asked the students of the basic design course to listen to three separate pieces and transform these pieces into abstract three-dimensional designs. As a result of the study, students gained awareness about establishing interdisciplinary connections. Özdemir (2016) established a relationship between the academic performances of students taking a basic design course by classifying their learning styles. He suggested that students can increase

their success by being aware of their learning styles and internalizing their advantages and disadvantages. Düzenli et al. (2017) asked landscape architecture students to design a building environment in the last weeks of the basic design course, and as a result of the evaluations made at the end of the study, they observed that the basic design course increased creativity in design studies. Kasap and Türkmen (2018) evaluated form production in the transition from two-dimensional works to volumetric works in the basic design course, through student works. Felek (2020) compiled the scientific studies conducted between 2000 and 2019 in the field of basic design education in Türkiye. Kahraman (2020) measured students' experiences of the basic design course conducted via remote learning during the pandemic period with the help of a survey. Türkmen (2020) evaluated the effect of abstract and concrete concepts given to students for their study in the basic design course on the success of the work. Kılıç and Arabacıoğlu (2021) held a workshop with a four-day distance education model in five universities and supported the basic design course with a special parametric software in the computer environment. As a result of the study, it was seen that students showed more interest in the parametric workshop model than the typical distance education system. Coşkun and Çağdaş (2022) evaluated the design experience with the help of the computer game Minecraft as a part of the basic design course. Game-based learning has increased in-class communication and interaction. Yilmaz et al. (2023) theoretically examined the relationship between the basic design course and creative thinking.

Education that proceeds through abstract concepts creates difficulties for students to internalize this lesson. In order to avoid these difficulties, "learning-by-doing" is of great importance. In this context, learning-by-doing approach was carried out in the form of a workshop in the fall semester of the 2022-2023 academic year, within the scope of the Basic Design 1 course of the Department of Interior Architecture at Konya Technical University. This study was carried out on the basis of the hypothesis that the workshop, which supports the learning by doing process, can increase the intelligibility of the lesson. After the lecture period was over, a 10-question survey on the efficiency of the workshop was applied to the students. Thus, it was evaluated whether the workshop reached its goal or not. Ethics committee permission for the survey study was applied on 5.09.2023 and approved by the scientific research and ethics committee of Konya Technical University at the meeting numbered 2023/7.

Materials and Methods

Basic Design Courses

The English word "basic" is derived from the Latin root "base" and means belonging to the foundation. Epistemology, on the other hand, expands both the meaning and the usage area of the word and refers to providing support not only physically but also intellectually. It has a determining feature of the physical or intellectual structure that will be built on it. "Design" comes from the Latin word "signare", which means to show and point (Civcir, 2015). This corresponds to the words "Dessin" in French and "Designo" in Italian. Furthermore, it is explained as thinking or forming in the mind, formulating a purpose, finding a method, planning systematically, having a purpose, goal, or intention, to create, find, and invent on a subject that requires high skill. Design is the name of all these processes, as well as the name of a designed original example, visual presentation plans or draft doodles of a designed production process (Seylan, 2020).

The first studies on design education were made with the establishment of Bauhaus in 1919 by Walter Gropius. Aiming to combine arts and crafts, Bauhaus has adopted an educational curriculum centered on practice. Education at the Bauhaus includes all scientific and practical areas of creative work; students are trained in a craft, they learn techniques such as drawing and painting, and they are also developed scientifically and theoretically. In the Bauhaus curriculum, technology-art-science forms a three-floor structure. The biggest expansion Bauhaus brought to Basic Design Education through architectural education; The aim of this course was to start an introductory course on design, which is consciously organized, at the same time insightful and applied, and adopting a modern education program intertwined with art for this introductory course. The design education in this period allows the student to learn by doing, develop freely and get rid of stereotypes (Erkan, 2006). Figure 1 shows the basic design education formation process diagram. As can be seen in the figure, the development process took place under the influence of different artists and architects in different countries.

In general, the course curriculum includes topics such as:

- Elements of design: point, line, direction, size, shape, value, texture, color
- Visual perception: figure-ground relationship, organization principles, proximity relationship, similarity, shape properties,
- Principles of design: balance, concept, contrast, harmony, hegemony, repetition, unity
- Space, form and geometry: two and three dimensional concepts (Akbulut, 2010)

The course aims to develop the ability to use many concepts, techniques, and materials. In addition to the act of creation in the design process, manual dexterity is also expected from the student in the interpretation phase. Although the design process constitutes the essence of the studies, the student must be able to choose the materials used in the application phase and use them in the most appropriate way in the design. By providing this information, the educator acts as a guide for the student to implement the best practices using various techniques (Atmaca, 2014).

Basic design education at Konya Technical University is carried out in the form of a 8-hour training that covers the whole day. In the first two hours, basic design elements and principles are explained theoretically. Afterward, students are asked to create two-dimensional compositions related to the subject (Figure 2). These studies are collected at the end of the course and evaluated by the instructors. Students are given a two- or three-dimensional composition study on the subject as homework. Classes continue in this way throughout the semester.

	United States	France	Germany	Soviet Union
1890's	James Santayana		Munsterberg	
1900's	Munsterberg			
1910's+		Charles		Kandinsky
1920's+		Cubism Le Corbusier	Kandinsky	Ladovsky Ginsberg
1930's			Koffka Koehler Wertheimer Gropius Peterhaus Albers	
1940's +	Gropius Peterhaus Albers Kepes			
1950's+				
1960's+	Arnheim			

Figure 1. Basic design education formation process diagram (Erkan, 2006)

Figure 2. Some of the compositions which was designed within the scope of the course

At the end of the year, in the last four weeks, the class is divided into groups of 8 people and the workshop process is started. Each group is led by an instructor. During the workshop, each group produces a joint work and an individual work. Studies should be designed according to the basic design elements and principles learned in the first weeks. The workshop is based on the learning-by-doing approach.

Workshops in Design Education

Workshops, which mean short and intensive training studies, are frequently used in every field. Workshops are formations outside the functioning order; They enable people from different characters, different professions, different schools, jobs and countries to work together. At all stages of the workshop, facilitators and participants must be prepared to make changes and be flexible. Flexibility is one of the most important features of informal education (Yürekli and Yürekli, 2004). In order to define formal and informal learning environments; formal education means an education system based on a certain curriculum. The purpose of formal education is to teach people about the prevailing order and the values, norms and judgments associated with it. Informality, which aims at going beyond the ordinary, provides an environment excluding the current order and rules where hierarchy is taken down. A communicative environment where different ideas come together, expressing oneself individually, and gaining self-confidence are the main advantages of informality (Karslı and Özker, 2014). In this context, workshops are important in terms of providing informal learning environments.

No matter how qualified design education is offered, it is not possible for people to learn to design without having personal design experience and making their own effort. This effort is experienced more intensely with learner-driven desire in workshops that create independent environments for design. One of the important features of workshops is not to have a rigid curriculum, there is no single path to follow. It allows for more open-ended, more independent work. The reflection of the atmosphere created by sharing ideas on joint production ensures that the production, although personal, is now a product of the current atmosphere and common thoughts, in short, everyone participating in the process. Dialogue between students is strengthened in workshops. Communication skills increase. The studio managers' approach to the work with the curiosity of students, removes the boundaries between them and creates the opportunity to reinforce the knowledge learned in theoretical courses and understand the issues from different perspectives. A dialogue occurs between the instructor and the student. Being together all the time provides a more uninterrupted and fluid environment; as people get closer, tension decreases, and thus more information is shared (Ciravoğlu et al. 2009).

Method of the Study

The learning-by-doing approach was carried out in the form of a workshop in the fall semester of the 2022-2023 academic year, within the scope of the Basic Design 1 course of the Department of Interior Architecture at Konya Technical University. Students were asked to produce three-dimensional designs using the basic design principles and elements they learned during the year. The difference between this workshop from the two- and three-dimensional applications made throughout the year is that the product is larger in size and freely designed without being limited to a specific theme. In this context, students realized their designs by choosing one or more principles and elements. For the workshop, the class was divided into groups of 8 people and studies were carried out with a workshop coordinator in each group. The division of the class into small groups strengthened the one-to-one communication environment with each student. The duration of the workshop was planned as four weeks and the work was progressed in the company of the workshop coordinator during a total of four lessons. The studies were exhibited at an event held at the university at the end of the semester (Figure 3).

Figure 3. Workshop Exhibiton

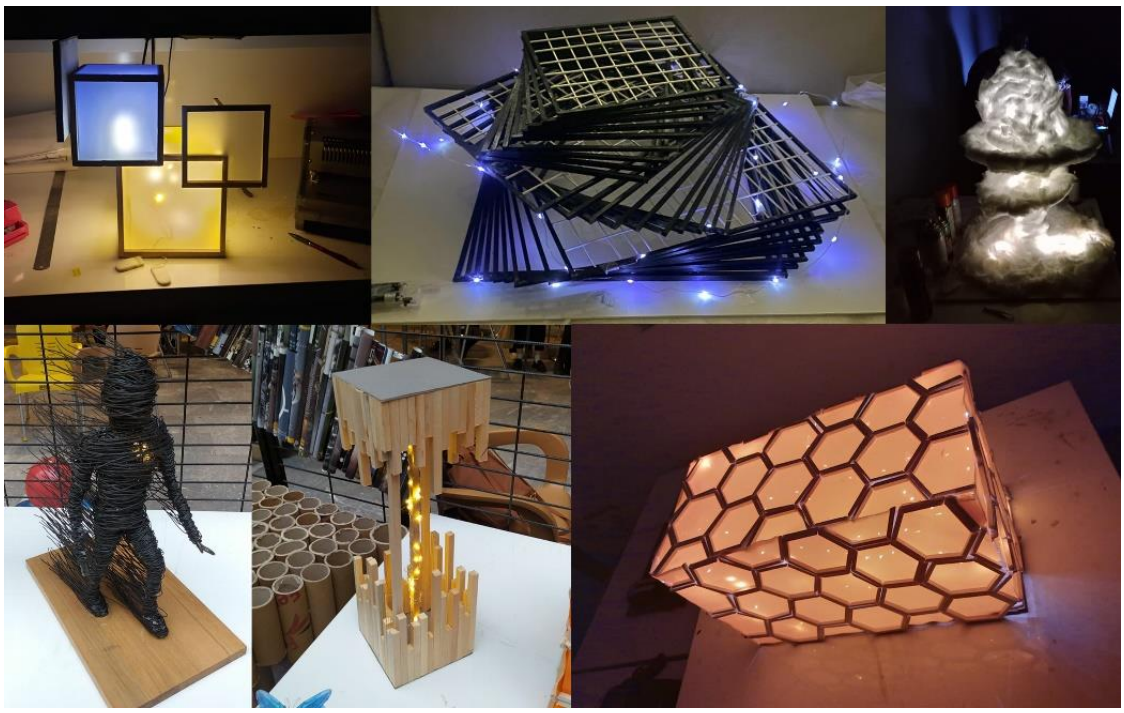

Figure 4. Some of the works produced within the scope of the workshop

Figure 4 shows the products of a group working within the scope of the workshop. In order to increase the functionality of the studies in the group, it was requested that the studies be designed as lighting elements. The type of lighting is released. With the addition of the lighting parameter to the design, the study has been transformed into a design problem by emphasizing the aesthetic features of the composition as well as its functionality. During the workshop, the students learned to use materials, to use color, to design an original composition and to integrate the lighting element into the design by experiencing it during practice. Concealing the lighting element in the design and standing still the design were some of the difficulties experienced by the students.

After the lesson period, a survey study was carried out with the workshop evaluation process. 30 volunteers from the 38-person class participated in the survey. The survey consists of 10 questions in total. The answers were created in the form of a 5-point Likert scale. In this context, it includes an answer template with 5 options: I strongly agree, I agree, Uncertain, I cannot agree, and I strongly disagree. The survey questions are as follows:

- In the workshop process, an approach was adopted in which theory and practice, design and construction are applied as a whole.
- With the workshop, a process-oriented learning environment was created within the scope of Basic Design 1 course.
- The creation of a student-centered design environment was provided during the workshop.
- During the workshop, studies were carried out for this purpose by emphasizing the culture of learning together.
- Workshops supported students' creative thinking skills.
- Workshop contributed to the development of students' design skills by transforming ideas into reality, empathizing with each other, reading, discussing, and experimenting with different production methods.
- During the workshop, the communication between the students and the instructors was strengthened.
- Learning in the workshop process is more fun when compared to theoretical learning.
- I used the elements and principles I learned in Basic Design 1 course in the product I designed within the scope of the workshop.
- With the workshop, the principles and elements I learned in Basic Design 1 were better understood.

Results and Discussion

The results in Table 1 were obtained in the questionnaire of 30 people after the workshop. 28 out of 30 people agreed with the idea that theory and practice were handled as a whole during the workshop. During the workshop process, 83.3% of the students agreed that a process-oriented learning environment was created. 73.4% agree with the idea that a workshop environment that puts the student in the center is created. While 16.6% remain undecided on this issue, 10% do not agree. 86.7% agree with the idea that the culture of learning together is applied in the workshop, which refers to joint group work. 80% of the participants agree with the idea that the workshop supports creative learning. 83.4% of the students agreed with the idea that the process improves students' design skills. 73.4% of the students agree that the relationship between students and instructors is strengthened during the workshop. 86.7% of the class agree that learning with a workshop is more fun when compared to theoretical learning. 83.4% of the class stated that they used the basic design elements and principles they learned in Basic Design 1 theoretical courses. Thanks to the workshop, 70% of the class agrees that the Basic Design 1 course is better understood.

Table 1. Workshop assessment survey results

	Strongly Agree	Agree	Uncertain	Disagree	Strongly Disagree
1. In the workshop process, an approach was adopted in which theory and practice, design and construction are applied as a whole.	%16.7 (23)	%76.7 (5)	%6.6 (2)	-	-
2. With the workshop, a process-oriented learning environment was created within the scope of Basic Design 1 course.	%13.3 (4)	%70.0 (21)	%16.7 (5)	-	-
3. The creation of a student-centered design environment was provided during the workshop.	%26.7 (8)	%46.7 (14)	%16.6 (5)	%10 (3)	-
4. During the workshop, studies were carried out for this purpose by emphasizing the culture of learning together.	%26.7 (8)	%60 (18)	%6.7 (2)	%6.6 (2)	-
5. Workshops supported students' creative thinking skills.	%26.7 (8)	%53.3 (16)	%10 (3)	%10 (3)	-
6. Workshop contributed to the development of students' design skills by transforming ideas into reality, empathizing with each other, reading, discussing, and experimenting with different production methods.	%23.4 (7)	%60 (18)	%10 (3)	%3.3 (1)	%3.3 (1)
7. During the workshop, the communication between the students and the instructors was strengthened.	%23.4 (7)	%50 (15)	%20 (6)	%3.3 (1)	%3.3 (1)
8. Learning in the workshop process is more fun when compared to theoretical learning.	%36.7 (11)	%50 (15)	%6.7 (2)	%3.3 (1)	%3.3 (1)
9. I used the elements and principles I learned in Basic Design 1 course in the product I designed within the scope of the workshop.	%30 (9)	%53.4 (16)	%13.3 (4)	%3.3 (1)	-
10. With the workshop, the principles and elements I learned in Basic Design 1 were better understood.	%6.7 (2)	%63.3 (19)	%26.7 (8)	%3.3 (1)	-

The results of the study revealed the positive contribution of workshops to education, in line with Ciravoğlu's thesis and Kılıç and Arabacıoğlu's (2021) studies. In addition, the positive results of the group work environment in the study of Coşkun and Çağdaş (2022), who integrated an informal education format into the basic design course, are compatible with the results evaluated in the 6th survey question of the study, which reveal the positive contribution of the exchange of ideas and trying different production methods to design skills. When the literature is examined, there are few examples where different learning styles are tried in basic design courses (Neves and Duarte, 2016; Bostancı et al. (2016); Coşkun and Çağdaş (2022)). Informal education given within the scope of the basic design course under the title of the workshop was examined in the study of Kılıç and Arabacıoğlu (2021). In this context, it can be seen that informal training organized under the title of workshops within the scope of the basic design course are few in the literature. For this reason, the positive results of the study encourage workshops that can be held within the scope of this course and contribute to the literature.

Conclusion

At the end of the workshop, the students learned to embody the abstract concepts they learned during the year by creating a composition that considers functionality and aesthetics at the same time. As a result, it has been seen that the intelligibility of Basic Design 1, which is a course taught through abstract concepts, has increased thanks to the workshop application.

In this context, workshops that create more democratic and independent production environments and where mutual information exchange comes to the fore, instead of only one-way information transfer, should be included in the education process in Basic Design courses. In workshops, students should be encouraged to share their ideas and produce new collective ideas.

In future studies, in order to further expand the design visions of students, coordinators from other branches of art can be included in the workshop to create a multi-disciplinary environment and test its efficiency.

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Examination of Kreuzberg Protective Urban Renewal Principles Specific to Tepebağ-Kayalıbağ

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ABSTRACT

Renewing and reuniting the regions of cities that have become collapse areas with the city; urban renewal, which enables the city to be restored, is a term frequently used today. These collapsed areas need to be physically and socially renewed and revitalized. The main purpose of urban renewal studies is to reintegrate the isolated living spaces with the city. This integration should be realized not only physically and economically, but also socially and culturally. Kreuzberg Region in Germany suffered great damage in the World War II and turned into a depression zone over time. The "Protective City Renewal" method, which has been applied in the region since 1961, has an important place in urban renewal studies with its 12 basic principles. Urban renewal works in Tepebağ-Kayalıbağ neighborhoods, which constitute the historical city center in Adana province, are still at the very beginning of the process. Several projects have been carried out within the scope of street rehabilitation studies in the area, but these are thought to be insufficient. The aim of this study is to evaluate the "Protective City Renewal" method and to conduct an experiment on how the 12 principles of this method can be applied to the Tepebağ-Kayalıbağ section, which is an important historical texture to be preserved in Adana.

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Introduction

The concept of urban renewal, which started with the Industrial Revolution, is to reconsider the physical aging caused by factors such as rapid urbanization, change in urban structure, earthquakes, destruction due to war, and population growth in the urban environment. The concept of urban renewal is an important factor in the change of cities. The concept of urban covers the process of reconsidering and improving the physical, social and ecenomic deterioration in cities (Yiğit, 2011, p.3,4). In otheer words, this concept is the replacement and renewal of places that have become dilapiated as a result of the deterioration of economic conditions in a certain area over time. The main purpose of the renewal process is to make urban enviroments more attractive for financial investments and economic developments, as well as physical conditions (Çiçek, 2014, p.13).

It is an exemplary project for urban renewal studies with its 12 basic principles determined by the "Kreuzberg region project" located near the Berlin Wall, which is successful in the world and in Turkey. The region, which was previously dominated by the destroy-build policy, has achieved to managed the process positively over time. Some studies are carried out within the scope of street rehabilitation projects in Tepebağ-Kayalıbağ Urban Renewal area (Adana historical city center)in Adana province, which is determined as the study area. However, the area has not yet got rid of the ruined appearance and does not include activities that will allow you to spend time in the area.

It is thought that discussing and evaluating these two projects together will be a guide for the study in the Adana Tepebağ-Kayalıbağ region. Based on this, the 12 basic principles created for the Kreuzberg urban renewal project will be tested on the historical city center of Adana, which covers the Tepebağ-Kayalıbağ neighborhoods determined as the study sample area and turns into a collapse area.

Conceptual Framework

In this section, the concept of urban renewal and the emergence and development of the phenomenon of urban renewal are examined.

Concept of Urban Renewal

The concept of urban renewal, which emerged in urban studies as a concept for the regulation of collapsed areas, is a phenomenon that and aims to improve the economic, social and environmental conditions of cities (Erden, 2003).

Urban renewal is a concept that aims to improve the socio-economic, cultural and physical development of the project areas. In a comprehensive sense, it is a study that ensures the participation of the public in urban renewal studies, tries to improve the collapse areas, and aims to advance the economy of the region (Kubat, Özden, 2003).

The main purpose of urban renewal studies is to create healty cities, and in this context, it covers the transformation of illegally built areas, the improvement of areas that have been or will be affected by natural disasters, the transformation of unqualified and unhealty areas within the city into livable areas, and the transformation of historical places and conservation areas that have lost their functions.

Urban renewal examines each region by taking into account the socio-economic conditions within its own physical fabric. Because each region has its own physical and socio-economic structure. Decisions taken for one region will not be appropriate in another region (Erden, 2003).

Emergence and Development of Urban Renewal Phenomenon

The concept of urban renewal is a concept that emerged at the end of the 19th century, at the beginning of the 20th century. The emergence of the concept is parallel to the period of socio-cultural, economic and physical transformations. With the population growth in the cities, renovation works have begun and the population living in the city centers has been replaced by new social layers. As a result of the loss of importance of historical city centers, the housing function available in these areas has been replaced by small commercial units. The area has lost its main user and a social layer brought by new functions has settled in the center. This physical and functional change has negatively affected historical urban centers (Dıefendorf, 1989)

According to Couch (1997), the concept of urban renewal first emerged in European countries and America. The concept gained importance in the 1950s and gained momentum after the 1970s and 1980s. In 1981, the Council of Europe launched a campaign to renew many European cities. The Bellagio Conference held in 1987 brought reconstruction to the agenda in post-war cities. With the contribution of the "Green Paper" prepared by the European Community Commission in 1990, Western European governments set their own goals for urban renewal. One of these goals is to revitalize the urban region (Özden, 2001, p.259).

In 1994, "Living and Livable City Centers: Struggle Meeting" was held. With the Aalburg Convention signed in the same year, criteria were determined to create sustainable cities. In the "American Society Renewal Law" dated 1997, it was decided to transfer the empty spaces and unqualified buildings owned by the American Department of Housing and Urban Development (HUD) to the local governments authorized in the region (Olson, 1997, as cited in Özden, 2001, p.260).

The concept of urban renewal, which European countries and the United States have been working on for a long time, advocates the protection and survival of urban areas together with their immediate surroundings, while at the same time reclaiming and revitalizing the collapse areas of cities (Özden, 2001, pp. 260-261).

In Türkiye, it developed later than Europen countries and America. Discourses related to this concept emerged in Türkiye in parallel with ecenomic growth in the 1950s, and began to take on the agenda of cities after 1980, but the real development and applications of the concept of urban renewal correspond to the years after 2000. The laws and regulations that emerged in Türkiye in 2004 and after focused on this concept. In the phenomenon of urbanization, new zoning actives were organized and implementation studies were carried out in cooperation with local goverments. In this context, it has been used in the phenomenon of unplanned urbanization, in the rehabilitation of historical city centers, in many dilapidated areas that are considered unhealty, and continues tı be used intensively today (Çağla, İnam, 2009, p.1).

Materials and Methods

The hypothesis of the study; the process of reintegrating urban renewal areas with the city needs to be addressed not only physically and economically, but also socially and culturally. In this context, the 12 basic principles of the Kreuzberg Project, which is a successful example among the Urban Renewal Projects in the World, were examined, and the Tepebağ-Kayalıbağ Urban Renewal area in the province of Adana, which is still at the very beginning of the renewal process. In this context, the method in the study was carried out in three stages. First, the Kreuzberg Urban Renewal area and its basic principles, then the Tepebağ Kayalıbağ Urban Renewal area were examined in detail. In the final stage of the method, 12 basic principles of Kreuzberg Protective City Renewal were tested and evaluated specifically in Tepebağ-Kayalıbağ. In the study, literature data were also examined and on-site detection, observation and photography techniques were used.

Kreuzberg Urban Renewal Project

Kreuzberg District in Germany is a district of Berlin. Kreuzberg region damaged in World War II, over time the city turned into a depression zone (Figure 1). At the end of the 19th century, the Kreuzberg region was used by the working class. In 1960-70, the destroy-build policy continued to exist in the area. In this process, the concept of Urban Renewal meant demolishing the damaged structure, opening large gaps in the area and building new structures. The buildings damaged after the war were abandoned by the main user. Some of the artists, foreigners, the unemployed and some of the lower class of the city continued to use the area. 1980-82 was a period of transition to urban renewal in Kreuzberg. In the studies conducted after these years, priority was given to the protection of existing areas (Tunçer, 2006).

Figure 1. Kreuzberg Region (Tunçer, 2006).


Figure 2. Tepebağ and Kayalıbağ Urban Protected Area (Seyhan Municipality, 2017).


Figure 3. Urban Renewal Area (Büyüköztürk, 2020).

The urban renewal area includes the renovation of 12 buildings on Islands 103 and 104 in the Luisenstadt district of Kreuzberg and the 5036 neighbourhoods of Kreuzberg, which survived World War II without much damage and were envisaged to be rehabilitated in 1983. The contract for the rehabilitation works in the region was signed in 1983 and the works started in 1986. Repairs were completed in 1991 (Yaygel, 2007).

The social structure of Kreuzberg in that time period consists of 25% of the user group newly moved to the area after the destroy-build policy, 25%of the low-income group, and the rest of the foreign Turkish group coming from abroad with immigration (Ünlü, 2009).

Awareness about conservation increased with the International Housing Exhibition (IBA) held in the region. At this stage, 12 basic principles of Urban Renewal have been developed (Tunçer, 2006).

These include:

- Renovation works should be organized according to the needs of the inhabitants of the region and planned together with them.
- Renovation works should be created with harmony between users and application coordinators.
- Damage threatening the essence of the structures must be repaired quickly.
- Renovation works should be able to be carried out progressively over time.
- Public facilities should be renovated and expanded and completed to meet the requirements.
- Renovation work requires agreement on the principles of social planning as a prerequisite.
- In order to guide urban renewal; it is necessary to have a clear form of decision-making, on the other hand, to strengthen the representation of renewal addressees and to establish decision-making rules that hold their meetings in the relevant region.
- Renovation works need definitive financing guarantees.
- It is necessary to use all chances to develop new forms of contracting organizations.
- All measures should ensure that the renovation works are carried out over time after 1984 (Ünlü, 2009).

Kreuzberg Urban renewal project has been prepared in line with the needs of the field user. Thus, people living in the region were included in the project process (Yaygel, 2007). Public areas found inadequate in the project have been increased. It is aimed to create new social spaces such as schools, kindergartens, youth centers, elderly clubs, district women's centers, neighborhood centers. In addition to social areas, plans have been made to find jobs for the unemployed. (Ünlü, 2009).

The Kreuzberg Renovation project is a study carried out with wide participation. It was carried out with the support of the Berlin Municipality and IBA organizations. In addition, tenants, business owners, owners, architects and urban planners, artists, redevelopment contractors, Berlin public institutions, politicians, mass media officials participated in the project (Ünlü, 2009).

The project has an important place in urban renewal works with its 12 basic principles. Especially the inclusion of the user's in the project and making plans in line with the user needs are the most important reasons for the success of the project.

Tepebağ-Kayalıbağ Urban Renewal Works

Tepebağ-Kayalıbağ Urban Renewal Area constitutes the whole of the urban protected area of Adana province. The study area constitutes the historical texture, which is the first settlement of the city (Figure 2).

After the proclamation of the Republic, there was an increase in the need for housing with the improvement of the economy of the city of Adana and the increase in immigration to Adana. Changes have been made in the traditional houses in the area that constitutes the historical city center of Adana in line with the needs. Partitions were added to the buildings, floors were built on top of them, and unqualified structures were started to be built in the historical area and the texture was damaged. Thus, the historical texture was damaged (Umar, 2010).

The historical city center, which is preferred by people with high income levels, has turned into a region where people from rural areas settle over time. New users who settled in the region could not take care of the area due to financial impossibilities, which caused the houses to wear out (Seyhan Municipality, 2023).

Adana Conservation Zoning Plan was prepared between 1996-1998. The conservation zoning plan prepared for Tepebağ Mound and its surroundings could not be implemented due to the 1998 Adana earthquake (Reel, 2006). In the examinations carried out in the area after the earthquake, it was determined that there was damage and destruction in 241 registered buildings (Gürçınar, 2000). Improvement works in the area after the earthquake have not been carried out for a long time. A new Conservation Zoning Plan was adopted in 2016. In this plan, partial improvements have been proposed in the area within the borders of Seyhan Municipality, which is determined as OPA. There are a total of 597 buildings in the study area. 63 of these structures are registered and 6 of them are monumental buildings (Büyüköztürk, 2020) (Figure 3).

Studies Conducted to Date in Adana Tepebağ-Kayalıbağ Region

In the Urban Renewal Area within the borders of Tepebağ-Kayalıbağ neighborhood of Adana province, restoration projects and street rehabilitation projects were carried out on the basis of individual buildings until 2023.

Restoration Works in the Area

Single projects restored within the boundaries of Tepebağ-Kayalıbağ neighborhoods; Atatürk House project, Gazipaşa Primary School Project, Şeyhoğlu Mosque Project, Green Masjid and Tomb Project, Şefika Hatun Mosque Project, Tepebağ Secondary School, Inventory Project No. 63, Inventory Project No. 64, Adana Cultural Heritage Preservation Regional Board project, Adana Cinema Museum project, Bosnian Hotel project, Inventory Project No. 109, Salvation School (Kadem Building) project, Inventory Project No. 175 (Turkish Pharmacists Association) project, Tepebağ Kuran Course Project (Figure 4-17).

Figure 4. Atatürk House (2023)

Figure 5. Şeyhoğlu Mosque (2023)

Figure 6. Şefika Hatun Mosque (2023)

Figure 7. Cinema Museum (2023)

Figure 8. Inventory No. 99 (2023)

Figure 9. Bosnian Hotel (2023)

Figure 10. Gazi Pasha Primary School (2023)

Figure 11. Inventory Projects No. 63-64

Figure 12. Green Masjid (2023)

Figure 13. Tepebağ Secondary School (2023).

Figure 14. Adana Regional Board for the Protection of Cultural Heritage (2023)

Figure 15. Tepebağ Kuran Course (2023)

Figure 16. Liberation School (Kadem Building) (2023)

Figure 17. Inventory No. 175 (Turkish Pharmacists Association) (2023)

Figure 18. Atty. Turhan Arın Street (Seyhan Municipality, 2019).

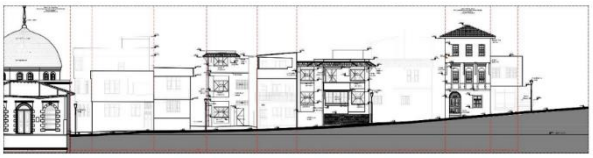

Figure 19. 26012 Street Silhouette (Büyüköztürk, 2023).

Figure 19. 26005 and 2012 Street Rehabilitation Project (Seyhan Municipality, 2023).

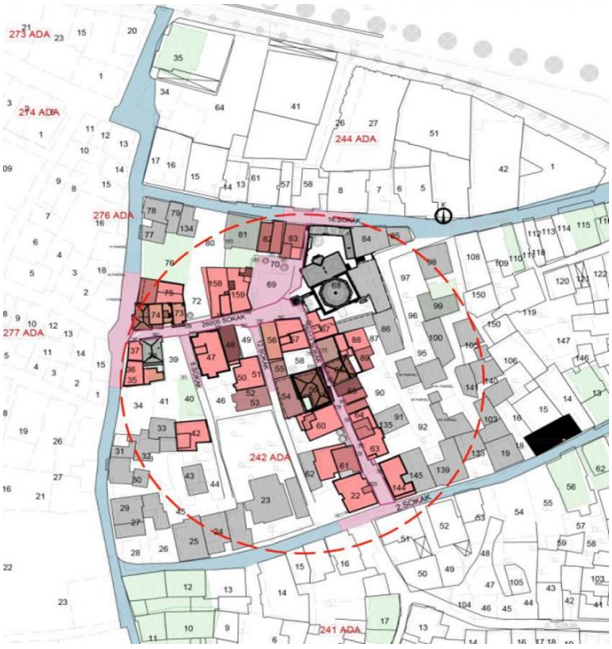

Figure 20. 26005 and 26012 Street Rehabilitation Project (Büyüköztürk, 2023).

Atty. Turhan Arın Street Rehabilitation Project

Tepebağ is a region between Kayalıbağ neighborhoods. There are Green Masjid, Tepebağ Kuran Course, school, dormitory building, registered buildings and today's buildings in the area (Büyüköztürk, 2020) (Figure 18).

Within the scope of Av.Turhan Arın Street Rehabilitation Project; infrastructure works, facade and roof renovation of buildings, lighting and renovation of urban furniture (benches) have been carried out (Seyhan Municipality, 2019).

26005 and 26012 Street Rehabilitation Project

Within the scope of the street rehabilitation project numbered 2012, the restoration projects of the building named Inventory No. 95 in the Conservation Zoning Plan have been completed and the restoration of the building called café library and inventory No. 103 is ongoing and it is planned to be used as a playhouse (Seyhan Municipality, 2023) (Figure 19-23). Within the scope of the street rehabilitation project No. 2005, the restoration of the ground floors of Inventory No. 92 and Inventory No. 93 was carried out (Figure 3.24).

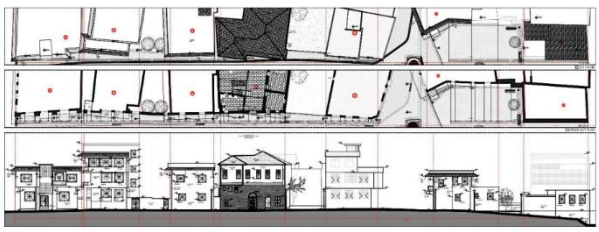

Figure 21. 26005 and 26012 Eastern Front Street Silhouette (Büyüköztürk, 2023).

Figure 23. 26012 Post-Street Rehabilitation (2023)

Figure 24. 26005 Post-Street Rehabilitation (2023)

Evaluation Of Kreuzberg Protective City Renewal Principles On Adana Tepebağ-Kayalıbağ Urban Renewal Area

In this study, an evaluation is made on how the "Protective City Renewal" principles applied in the transformation in the "Kreuzberg" neighborhood in Berlin and found successful all over the world will be handled in the historical city center within the borders of Tepebağ-Kayalıbağ neighborhoods in Adana province.

Principle 1. Renovation should be planned together with the local people, taking into account the needs of the local people.

Ensuring public participation in the projects increases the success level of the project. Since the deficiencies in the area are determined correctly in the projects designed with the user and the wishes of the local people are taken into consideration, urban renewal works are longer-lasting.

Problem: Tepebağ-Kayalıbağ region lost its main user due to the shift of the city to the north and the 1998 earthquake. Over time, the region has turned into an area preferred by narrow families, especially by those who migrated from the eastern provinces to Adana to work.

Experience: New users welcome any improvement to the region, which has become a depression zone due to financial impossibilities.

Evaluation: In the studies carried out in the Adana-Tepebağ Kayalıbağ Urban Renewal Area, interventions were made by obtaining a letter of appreciation from the users during the project and repair phase. In the studies, expropriation studies were carried out because the area lost its first user (Seyhan Municipality, 2023).

Problem: Decisions about the area are taken by the administrative administration (Adana Metropolitan Municipality, Seyhan Municipality and Seyhan Municipality KUDEB unit). Public participation could not be ensured in the studies in the field.

Principle 2. Urban Renewal should emerge with the harmony between users and project implementers.

In the works carried out, city councils should be established and areas should be created to allow the public to make decisions together.

Problem 2. In the studies, city councils were not established to fully cover the subject. They stated that they were open to all kinds of work carried out in face-to-face interviews with the people of the region, but there were deficiencies at some points.

Experience 2. The security problem continues in the region, but solutions are insufficient in this regard (The lighting elements have been replaced.) Another problem is that the vacant lands are used as parking lots. The location of the houses destroyed in the earthquake is still used as a parking lot. These parking lots are unlicensed. Since the area is in the middle of 4 heavily used commercial axles, the need for parking is very high. However, this should be resolved by the project executives where it is licensed and deemed appropriate. Parking and security problems in the region are the topics that area users are uncomfortable with from the beginning of the process. In the face-to-face interviews with the users, it was learned that they had difficulty in using the area at night. If the public had been included in the studies from the beginning of the urban renewal process, these headings would not have been ignored, and the security and parking problem could have been handled more meticulously (Figure 25-26).

Figure 25. Parking problem in the area (2023)

Figure 26. Parking problem in the area (2023)

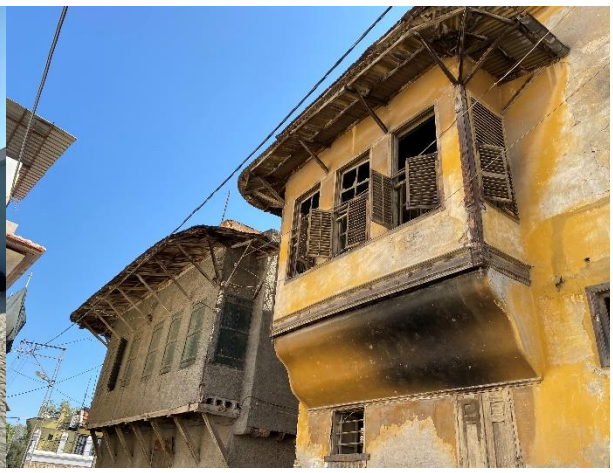

Figure 27. Tepebağ-Kayalıbağ Region

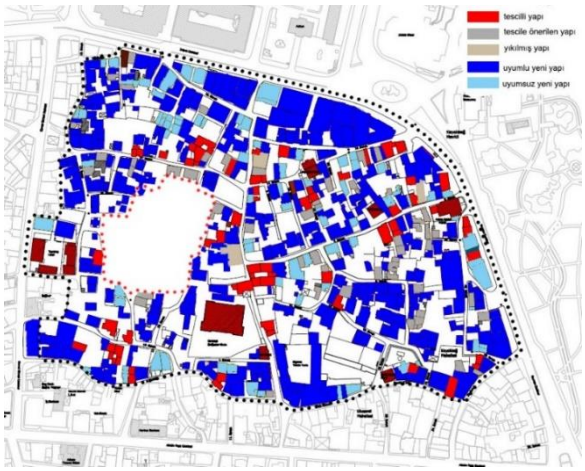

Figure 28. General Characteristics of Tepebağ-Kayalıbağ Region (Büyüköztürk, 2020).

Figure 29. Atty. Turhan Arın Street (2023)

Figure 30.26005 Street(2023)

Figure 31. Atty. Turhan Arın Street (2023)

Figure 32. 26005 Street(2023)

Figure 33. Yeşil Masjid (2023)

Figure 34. Şeyhoğlu Mosque (2023)

Evaluation 2. Users have a positive view of the work done. Because the area is very neglected and idle. However, there are still deficiencies in the region. These deficiencies should be resolved by user satisfaction analysis. Positive results can only be achieved with the cooperation of users and project managers.

Principle 3. Damages that occur on historic buildings must be repaired quickly.

Problem 3. Tepebağ-Kayalıbağ urban renewal works were delayed. Since the damages in the buildings were not repaired quickly, the area turned into a collapse zone. The lack of applications for repairing damages in the area other than street rehabilitation projects still poses a major problem (Figure 27.).

Experience 3. Little investment was made in the area after the earthquake, but these were limited to a few private entrepreneurs. Financial impossibilities have delayed the recovery in the field.

Evaluation 3. The region should have been intervened quickly after the 1998 earthquake, but decisions about the region were taken in 2016 (in the Conservation Zoning Plan) (Figure 28).

Facade and roof repairs, sewerage, infrastructure, lighting elements and flooring were renovated within the scope of street rehabilitation projects in the area (Figure 29-30).

Principle 4.5.6. Projects should be progressively realizable. In the studies conducted, the destruction should be kept to a minimum. Studies in which green areas are increased and walls are improved should be envisaged.

Problem 4.5.6. There is very little green space in the area (Figure 31-32).

Experience 4.5.6. During the work carried out in the new regions, additions are made to the previous period's work. However, this situation is insufficient.

Evaluation 4.5.6. The studies carried out in the region are carried out gradually. First, Atty. Turhan Arın Street Rehabilitation Project (1st Stage and 2nd Stage), then implementation projects of 26005 and 26012 Street Rehabilitation Projects were carried out. Afterwards, individual project repairs were made and rehabilitation works were continued. During each stage project, the deficiencies of the previous stage are also intervened.

Principle 7. Public structures and spaces should be renewed in line with the needs.

Problem 7. Renovation of public buildings takes a very long time. This is especially the case in projects carried out by the General Directorate of Foundations. Another problem is that public spaces are not created. Areas such as playgrounds, parks, etc. have been given very little space in the project and there is no such application in the area yet.

Evaluation 7. Within the scope of renovation and rehabilitation projects in the area; Gazipaşa Primary School, Yeşil Masjid, Şefika Hatun Mosque, Bekir Sapmaz Girls' Dormitory, Şeyhoğlu Mosque, Tepebağ Secondary School, Tepebağ Kuran Course structures have been improved and repaired.

Principle 8. Urban Renewal projects primarily require agreement on the project work.

Problem 8. At the social and economic level, the decisions taken about the area at the project stage aimed to transform this place into a tourism region. However, the studies conducted are insufficient in this regard. The functions given to the buildings during the project phase have not yet been implemented.

Experience 8. There is no study that will allow the visitors coming to the area to stay here for a long time.

Evaluation 8. In order to determine the Social Planning Principles in the example of Tepebağ-Kayalıbağ, it is necessary to determine the social, economic, cultural and conservation levels of the building owners.

Principle 9. In order to increase the level of success in the implementation of urban renewal, decisions should be taken clearly and decision-making boards should be established.

Problem 9. Decisions were taken by the authorities. The people of the region did not participate in these decisions.

Evaluation 9. In the studies carried out in Tepebağ-Kayalıbağ region, the decisions were taken by Adana Metropolitan Municipality, Adana Seyhan Municipality, Seyhan Municipality KUDEB, Adana Governorship, General Directorate of Foundations.

Principle 10. Financing guarantee is very important in renovation works.

Problem 10. The main reason for the inability to work for a long time in the region is the inability to provide financing guarantees.

Evaluation 10. The financing guarantee for the region was provided by Çukurova Development Agency, Adana Governorship YIKOB (It has given a certain rate of real estate tax as guarantee.), Cultural Heritage Protection Board (Contribution).

Principle 11. We should take our chances to the full to develop new forms of contracting organizations.

Problem 11. In the studies carried out in the field, contracting organizations are limited to public companies.

Evaluation 11. Renovation and expropriation works carried out in the region are widely used.

Principle 12. Decisions taken in the field should also be open to studies to be carried out over time.

Evaluation 12. The gradual realization of projects in the Tepebağ-Kayalıbağ area and the ability to intervene in the previous stage are important in terms of ensuring sustainability. However, it is necessary to ensure the continuity of this situation.

When the Adana Tepebağ-Kayalıbağ region is examined within the principles; it has been determined that the user of the area has not been included in the project and implementation process, all the damages have not yet been eliminated, functions that will contribute economically to the area have not been proposed, problems such as parking, green space and security have not been solved in the area, and indoor and outdoor areas have not been created to spend time in the area (food and beverage unit, park, cafeteria, buffet, children's playground, etc.).

The main factor in the success of the project implemented in the Kreuzberg region is the inclusion of the field user in the project and application. In the projects designed together with the user; the main needs and requests in the region are determined. Thus, studies with increased permanence emerge. Another factor in the success of the projects in the Kreuzberg region is the rapid repair of the damages in the region. Removal of the ruined image in the area with an urgent program positively affects both the people of the region and the visitors to the area. Units that offer new jobs in the field and studies that aim to find jobs for the unemployed are another success criterion of the project. The urban renewal area of the Kreuzberg region is a multi-actor study in which high financial support is also provided.

In Tepebağ-Kayalıbağ region; a disciplined study should be carried out by considering the 12 basic principles adopted in the Kreuzberg Urban Renewal area in full. In addition to public participation in decisions related to the field, the number of actors involved in the process should be increased and financing support should be provided.

Results and Discussion

It was emphasized in the study that urban renewal projects have become a great need for our cities. Especially historical city centers have been exposed to aging over time and turned into depression areas. The Kreuzberg region, which was damaged after World War II, was previously a depressed area of the working class, and it correctly

managed urban renewal projects with the studies carried out over time. The Kreuzberg urban renewal project envisages the development of projects that take into account the needs of the people of the region with the decisions taken, enable economic recovery, ensure public-private cooperation in studies, enable sustainability that can be carried out gradually, and ensure the rapid elimination of damages in buildings. Adana Tepebağ-Kayalıbağ region is at the very beginning of the urban renewal process. Studies by local governments to save the area from the collapse area are insufficient and need to be handled systematically, such as the Kreuzberg project.

In this study, the principles of the Kreuzberg Urban Renewal project were examined in the Tepebağ-Kayalıbağ area in Adana province. When these 12 principles are examined in the area, it is observed that the users living in the historical city center of Adana province are not included in the process, the problems experienced in the area have not yet been solved (security, green area problem), the functions that will provide economic input to the region are not recommended, and the damages are eliminated in certain parts of the region.

Addressing these two projects together will be a guide for the Adana Tepebağ-Kayalıbağ project. The study applied in Kreuzberg region is a guide to get positive results from the studies in Tepebağ-Kayalıbağ region.

Conflict of interest

The authors devlare that they do not have any conflict of interest.

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Determination of Yield and Quality Characteristics of Lavandula Cultivars in the Kahramanmaras Region

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ABSTRACT

In recent years, there has been a growing interest in herbal remedies for therapeutic purposes. Aromatic plants have become a significant source of raw materials for the fragrance, food, and cosmetics industries. Additionally, the emergence of new applications and the trend towards natural nutrition and alternative healing methods, often referred to as "going natural," have increased the interest in medicinal and aromatic plants in our country, as well as in other countries around the world. In this study were investigated to determine yield and quality characteristics of seven different lavender (*Lavandula intermedia Emeric* ex Loisel. = L. *hybrida* L.) (Grasso, Süper-A, Seguret, Dutch, Abrial, Akmeşe, English) cultivars under dry and irrigated cultivation in Kahramanmaraş conditions between 2019 and 2020. The research was carried out in a randomized block design with 4 replications. Fresh stem flower yield was the highest variety Grasso(672.81kg/da). The lowest fresh stem flower yield was found in Seguret variety with 611.33kg/da The highest dry stemless flower yield was determined in Grasso variety with 59,66 kg/da. There were significant differences determined between cultivars the end of 2 years. The highest average fresh stem flower yield (693.067 kg/da) and the highest average dry branched flower yield (252.588 kg/ha) were measured in GRASSO variety.

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Introduction

Lavandula (Lavandula spp.) is one of the most important essential oil plants within the Lamiaceae family (Guenther, 1952). Lavender (Lavandula *angustifolia* Mill.), lavandin (Lavandula intermedia Emeric ex Loisel.), and spike lavender (Lavandula spica) are three varieties of lavender that hold high commercial value worldwide. The majority of lavender (L. angustifolia Mill.) and lavandin (L.x intermedia Emeric ex Loisel.) cultivars are grown globally (Baydar, 2010; Karık et al., 2017). In recent years, medicinal and aromatic plants have found applications in various industries, including paint production, landscaping, ornamental plants, insecticide manufacturing, and even the food industry. Their utilization continues to grow annually, making medicinal and aromatic plants increasingly significant. As interest in these plants rises, both their usage and trade have experienced notable growth in recent times (Hui et al., 2010; Kara and Baydar, 2013: Baydar, 2013). It is challenging to compile precise production and consumption data on medicinal and aromatic plants globally due to the absence of a specific classification system for these plants. Consequently, it remains difficult to consistently assess and explain these figures. While Turkey boasts a conducive climate and ecological conditions for cultivating numerous medicinal and aromatic plants, some, such as laurel, mahaleb, linden flower, sage, rosemary, licorice root, and juniper bark, are primarily collected from natural sources. In contrast, cumin, anise, thyme, fenugreek, fennel, mint, and coriander are cultivated through field agriculture (Kırmızıpekmez et al., 2009; Gül and Çelik, 2016; Karık et al., 2017).

The demand for medicinal and aromatic plants, which were previously primarily sourced from nature, has led to increased efforts to cultivate these plants through field agriculture. Today, many countries, including our own, have undertaken cultivation projects for medicinal and aromatic plants, resulting in the development of various varieties within these plant species (Baydar, 2007; Atalay,

2008; Sönmez et al., 2018; Sekeroglu et al., 2022). In our country, as in other parts of the world, medicinal and aromatic plants, especially those that are not cultivated, are still collected from their natural habitats and utilized in various applications. Consequently, it is challenging to maintain accurate and comprehensive statistics, as there is no consensus on which plants should be classified as medicinal and aromatic.

This study aims to identify the morphological characteristics of seven different commercial cultivars (Seguret, Abrial, Dutch, Akmeşe, Grasso, Süper-A, and British) under Kahramanmaras ecological conditions. The goal is to provide valuable information to producers about the cultivation of the appropriate species and cultivars for their specific needs.

Materials and Methods

Material

This study was conducted in the research greenhouses of the Eastern Mediterranean Transition Zone Agricultural Research Institute (DAGTEM) between 2019 and 2020. We utilized seven commercial lavender cultivars (Seguret, Abrial, Dutch, White Oak, Grasso, Super-A, and English) with one-year-old roots, all belonging to *Lavandula intermedia* Emeric ex Loisel. = L. hybrida L..

Method

We employed a randomized blocks split-plot design with four replications to investigate the impact of seven irrigated conditions (rain + irrigation) and non-water conditions (rain only) on the lavender cultivars. Each plot measured 6 meters in length and had four rows, with 12 plants in each row, resulting in a total of 48 plants per plot. The experiment was organized into four blocks, totaling 192 plants for each cultivar. The overall trial area spanned 1800 square meters. Drip irrigation was consistently applied during June, July, and August at 15-day intervals.

Morphological Characteristics Examined in the Study

Plant Height (cm): Before harvest, the height from the soil surface to the tip of ten plants within each plot was measured in centimeters, and the average height per plot was calculated.

Fresh Stem Flower Yield (kg/da): Flower stalks representing 50 percent flowering of the plants in each plot were cut and weighed fresh without moisture loss. These measurements were then converted to yield per decare (da), yielding the fresh branched flower yield per decare.

Dry Stem Flower Yield (kg/da): Fresh branched flowers from each plot were combined and air-dried in the shade for approximately seven days. Afterward, the dry herb yields were calculated in kilograms per decare.

Dry Stemless Flower Yield (kg/da): The dry flower yield per decare was determined by separating the stems from the flowers in the dry, air-dried branched flowers (dried in room temperature and shade for seven days) and weighing the flowers using a precision scale.

Statistical Evaluation

We performed variance analyses according to the experimental design in the randomized blocks using the collected data. Subsequently, we grouped the means based on their significance using the Least Significant Difference (LSD) at a 5% level of significance. We employed an appropriate software package for the statistical analysis of the examined features.

Results and Discussion

Plant Height

The results of the variance analysis regarding the plant height (BB) values of the lavender cultivars we used in the research are given in Table 1. Upon examining the factors influencing plant height values, we observed significant differences at the 1% significance level between treatments and annual applications.

Table 1. Average Plant height values of Lavandula spp for 2019-2020 for varieties and applications in the study.

Cultivars	No Irrigated		Cultivars × Application Average	Irrigated		Cultivars × Application Average.	Application Average		
							Year × Cultivars		Cultivar
	2019	2020		2019	2020		2019	2020	
Abrial	52.75	50.75	51.75	62.24	63.93	63.09	57.50	57.34	57.42abc
Akmeşe	47.70	45.60	46.65	66.55	62.94	64.75	57.13	54.27	55.70bc
Dutch	55.94	55.65	55.80	67.95	66.20	67.08	61.95	60.93	61.44a
Grasso	47.60	45.70	46.65	64.73	67.37	66.05	56.17	56.54	56.35bc
Ingiliz	54.27	52.28	53.28	61.85	59.95	60.90	58.06	56.12	57.09bc
Seguret	49.39	47.39	48.39	60.60	61.83	61.22	55.00	54.61	54.80c
Super A	53.60	51.60	52.60	63.57	65.75	64.66	58.59	58.68	58.63ab
YAA	51.61b	49.85b		63.93a	64.00a				
AA	50.73b			63.96a					
Year Average.							57.77	56.92	
LSD year	2.04n.i								
LSD Application	2.89**								
LSD Çeşit	3.82*								
LAY	2.87**								
LSD cultivar x year	7.40n.i								
LCA	8.92n.i								
LCAY	9.64n.i								
CV(%)	9.45								

YAA: Year × Application Average; AA: Application Average; LAY: LSD Application × year; LCA: LSD cultivar × application; LCAY: LSD cultivar × application × year; n.i: no important; *: 0.05 important; **: 0.01 important

Table 2. Average fresh stem flower yield of Lavandula spp for 2019-2020 for varieties and applications in the study.

Applications	Cultivars (kg/da)							Application
	Ingılız	Seguret	Akmeşe	Grasso	Süper-A	Abrial	Dutch	Average
No Irrigated (Dry)	633.39	625.44	629.14	662.79	629.13	623.17	627.40	632.92
Irrigated	663.77	597.18	664.64	682.823	599.11	652.82	657.90	645.46
Average	648.58	611.31	646.88	672.81	614.36	637.99	642.65	639.23
F Application	8.93*							
F cultivars.	0.53 n.i							
F application x cultivars	0.0064 n.i							
CV %	12.82							

n.i: no important; *: 0.05 important; **: 0.01 important

Additionally, the average values of two-year-old cultivars showed significance at the 5% level and are grouped in Table 1. However, we did not find any significant relationships between year, variety-year, variety-application, or variety-application-year. Among the treatments, plant height under wet conditions was 63.96 in Group A, while under dry conditions, it recorded 50.73 in Group B. Notably, in 2020, under wet conditions, plant height reached 64 cm in Group A, but in dry conditions, it decreased to 49.85 cm in Group B. An examination of climate data revealed that the total precipitation amount in 2019 was 840 mm, but it decreased to 744 mm in 2020. In dry conditions, plant height was 51.61 cm in 2019, dropping to 49.85 cm in 2020, with an average of 56.92 cm. This result underscores a linear relationship between precipitation levels and plant height. Among the varieties, the Dutch variety exhibited the tallest plants, with an average height of 61.44 cm, while the Seguret variety had the lowest average plant height at 54.80 cm. Previous studies have reported varying plant height values for lavender (Table 1).

For instance, Ceylan et al. (1996) found lavender plant height to be 41.3 cm, while Arabacı and Bayram (2005) reported a range of 43.7 to 69.5 cm for lavender types. Kara and Baydar (2011) observed that lavandin-type cultivars had a higher average plant height (86.2 cm) compared to the lavender group (63.2 cm) in the Isparta region. Balyemez and Özel (2014), in ecological conditions similar to Harran Plain, reported plant height values between 29.30 and 31.15 for seven different lavender cultivars, including L. angustifolia Mill. (Grosso Tina, English, Little Lady) and L. x intermedia Emerice x Loisel. (Grosso, Super A, Dutch, Abriel). While plant height is influenced by various environmental factors, it is widely recognized that genetic potential plays a pivotal role in determining this trait. In a study by Aslancan (2016) featuring five different varieties (Seguret, Abrial, Grasso, Dutch, Super A) and one ecotype (White oak) of Lavandula intermedia, the Super A cultivar exhibited the highest plant height (86.1 cm) and spike stem length (58.67 cm). Our findings regarding lavender plant heights at the harvest period align with plant height values reported in prior studies (Ceylan, 1988; Hassiotis,et al.,2014; Karık et al.,2017; Ozyazıcıoglu ve Kevseroglu, 2019; Sonmez et al., 2019).

Fresh Stem Flower Yield

The analysis results of the fresh branched flower yield (FBFY) values of the lavender variety we used in the research are given in Table 2. The observed no significant differences in fresh branched flower yield among the cultivars at the 5% significance level under both irrigated and non-irrigated conditions. The highest fresh branched flower yield was achieved by the Grasso variety, totaling 672.81 kg/da, while the Seguret variety exhibited the lowest yield at 611.33 kg/da. The differences in fresh branched flower yield between cultivars were deemed statistically insignificant. In a study conducted by Arabacı and Bayram (2005) in Aydın, fresh flower yield in lavender ranged from 201.90 to 1499 kg/da. Karık et al. (2017) reported the highest fresh branched flower yields of 937.64 kg/da and 913.25 kg/da in the lavandin type Provence variety among eight different commercial lavender cultivars over two yield years. Among the lavandin group varieties, Seguret had the lowest yield value over two years, with an average yield of 251.00 kg/da. Lavander-type cultivars consistently exhibited the lowest fresh branched flower yield in both years (Table 2). Kara (2011), in a study conducted in Isparta ecological conditions, observed fresh branched flower yields ranging from 290.5 to 820.4 kg/da. In our study, fresh branched flower yield ranged between 183.0 and 937.64 kg/da, and these results align with the findings of previous studies. Our study results partially overlap with those from other studies conducted on lavender species and varieties in diverse ecological settings. Researchers have noted that fresh-branched flower yield in lavender can vary based on cultivar characteristics, harvest timing, environmental factors, cultivation conditions, planting frequency, and maintenance practices (Arabacı and Bayram, 2005; Salinas et al., 2007; Atalay, 2008; Karık et al., 2017).

Dry Stem Flower Yield (%)

The results of the variance analysis for the dry branched flower yield (KDÇV) values of the lavender variety used in our research are presented in Table 2. In a study conducted by Kara (2011) in Isparta, it was reported that the dry branched flower yield of lavender varied between 145.10 kg/da and 460.40 kg/da. In our study, there was no significant difference in dry branched flower yield among the varieties under both irrigated and non-irrigated conditions. The highest dry branched flower yield was recorded in the Grasso variety under irrigated conditions, reaching 252.59 kg/da, followed by the English variety at 248.80 kg/da. Subsequently, the Dutch (248.12 kg/da), Akmeşe (246.05 kg/da), Abrial (239.58 kg/da), and SüperA (239.57 kg/da) varieties followed in yield, while the Seguret variety had the lowest dry branched flower yield at 233.23 kg/da. The yield differences among the varieties were found to be statistically insignificant.. Karık et al. (2017), in a two-year study with eight different commercial varieties, reported that the lavandin type

Provence variety achieved the highest dry branched flower yield in both yield years, with 539.11 kg/da and 451.25 kg/da. Researchers noted that among the lavandin group varieties, Seguret had the lowest yield value over two years, with an average yield of 114.00 kg/da.

Lavander type varieties had the lowest dry branched flower yield in both years, with an average of 101.37 kg/da from the Munstead variety and 85.62 kg/da from the Hidcote variety.

Dry Stemless Flower Yield ((kg/da)

The results of the variance analysis for the dry branched flower yield values of the lavender variety used in our research are presented in Table 4. In the study, there was no significant difference in dry flower yield among the varieties under both irrigated and non-irrigated conditions at a 5% significance level. The highest dry flower yield was recorded in the Grasso variety at 59.66 kg/da, followed by the English variety at 58.51 kg/da. Subsequently, the Dutch (56.83 kg/da), Akmeşe (56.25 kg/da), SüperA (55.81 kg/da), and Abrial (55.00 kg/da) varieties followed in yield, while the lowest dry flower yield was observed in the Seguret variety at 54.35 kg/da. The yield differences among the varieties were found to be statistically insignificant. Researchers Arabacı and Ceylan (1990), Salinas et al. (2007), Atalay (2008)] have also noted that the dry stemless flower yield of lavender varies according to factors such as variety characteristics, harvest timing, environmental conditions, cultivation practices, planting frequency in rows and rows. In a study by Çimen (2016), the effect of mulch application on flower yield and essential oil components of Lavender (*Lavandula officinallis* L.) was investigated.

Lavender was grown both traditionally in open fields and on plastic black nylon covers. The results showed that the fresh stem flower yield per decare ranged from 33.95 to 168.22 kg, dry stem flower yield per decare ranged from 10.62 to 60.87 kg, and dry stemless flower yield per decare ranged from 4.15 to 49.17 kg. These findings highlight the variability in dry flower yield in lavender, which can be influenced by a range of factors including variety attributes, harvest timing, environmental conditions, cultivation methods, and planting density.

Conclusion

Medicinal and aromatic plants have not only found their place in the food industry but have also been increasingly utilized in the paint industry, landscaping and ornamental plant cultivation, as well as insecticide production. Their usage continues to grow year by year, reflecting their expanding significance. These plants, which have maintained their importance from the past to the present, have now become essential commodities in many countries worldwide, including our own, especially with the rise of natural nutrition and natural treatment methods, often referred to as "going natural.". In this study, conducted to assess the morphological characteristics of various lavender (Lavandula spp.) cultivars in the ecological conditions of Kahramanmaraş, several significant differences were observed among these characteristics. Notably, based on the two-year average, the GRASSO variety displayed the most impressive results in terms of fresh branched flower yield (672.81 kg/da), dry branched flower yield (240.40 kg/da), and dry flower yield (59.04 kg/da). Consequently, the GRASSO variety consistently outperformed other cultivars across many parameters. These findings underscore the influence of environmental factors, especially in regions like Kahramanmaraş with temperate climates, where irrigated conditions play a significant role in enhancing certain morphological parameters.

Table 3. Average dry stemless flower yield (%) of Lavandula spp for 2019-2020 for varieties and applications in the study

Applications	Cultivars (%)							Application
	Ingiliz	Seguret	Akmeşe	Grasso	Süper-A	Abrial	Dutch	Average
No Irrigated (Dry)	223.67	210.61	220.41	228.21	213.43	214.19	219.62	218.59
Irrigated	248.80	235.24	246.05	252.59	239.57	239.58	248.12	244.28
Average	236.24	222.93	233.23	240.40	226.50	226.89	233.87	231.44
F Application	3773							
F cultivars.	0.59							
F application x cultivars	0.007							
CV %	9.81							

n.i: no important; *: 0.05 important; **: 0.01 important

Table 4. Average dry stemless flower yield (kg/da) of Lavandula spp. for 2019-2020 for varieties and applications

Applications	Cultivars (kg/da)							Application
	Ingiliz	Seguret	Akmeşe	Grasso	Süper-A	Abrial	Dutch	Average
Dry	57.41	53.44	55.26	58.41	53.47	53.84	55.80	55.38
Irrigated	58.51	54.36	56.25	59.66	54.95	55.00	56.83	56.51
Average	57.96	53.90	55.76	59.04	54.21	54.42	56.32	55.94
F Application	289.68 n.i							
F cultivars	0.64 n.i							
F application x cultivars	0.001							
CV %	12,37							

n.i: no important; *: 0.05 important; **: 0.01 important

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Evaluation of Some Reproductive Performance of Ewes, Livability and Growth Traits of Lambs of Akkaraman in Breeder Flocks in Niğde/Bor Province

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ABSTRACT

Conducted within the National Sheep and Goat Breeding "subproject: Akkaraman Sheep Breeding Project under farmer conditions in Nigde's Bor district" between 2018 and 2022, the current study evaluated the reproductive characteristics of ewes as well as the livability and growth traits of lambs. This study investigated some 'reproductive traits' of an average of 6000 ewes per year and 'growth performance and 'survival traits' of a total of 30051 head lambs. The birth weight (LBW), 60th-day body weight (BW60), 120th-day body weight (BW120), and 120th-day body weight gain (BWG120) of Akkaraman lambs during the study periods were 4.14±0.19 kg, 18.58±0.03 kg, 31.31±0.02 kg, and 246±2.23 g/lamb/day, respectively. In the study, among the factors affecting the growth characteristics of Akkaraman lambs, only the effect of gender on BWG120 and birth type on BW120 was found to be insignificant. In contrast, the other factors were found to be found to be statistically significant. In the study, it was determined that the number of lambs per Akkaraman ewe giving birth between 2018-2022 varied between 1.03-1.10. The highest livability in Akkaraman lambs was determined in 2018 (96.61%) and the lowest in 2020 (83.21%). As a result, it was found that liveability in Akkaraman lambs was dependent on birth year, the age of the ewe, gender, and birth type.

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Introduction

Sheep breeding has a significant role in the Turkish economy as well as all over the world (Küçük and Akçapınar, 1999; Özmen et al., 2015). Obtaining high-value-added products especially meat and milk from sheep increases the importance of sheep breeding every year (Yıldız and Denk, 2006a; Şireli, 2021). Among these products, lamb meat production constitutes the primary source of income for sheep breeders (Akçapınar et al., 2000; Yakan et al., 2012; Aksoy et al., 2023).

Turkiye's sheep population is around 44,687,888 heads. While 91,10% (40.728.954) of the sheep population is composed of indigenous sheep breeds, 8,90% (3.958.934) is composed of cross-breed Merino sheep. Sheep has a share of 22.32% in total red meat production among cattle, sheep, goat, and buffalo species (TurkStat, 2023). It is obvious that sheep contribute significantly to red meat production in Turkiye. However, when evaluating lamb carcass weight (approximate 13-22 kg), it is clear that Turkiye falls behind the average weight of many developed countries (about 21-27 kg) (Esen and Yıldız, 2000a; Kul and Akcan, 2002; Yarali et al., 2015; TurkStat, 2023).

From time to time, the red meat industry in Turkiye faces various challenges. Increasing the animal number for slaughter can be a short-term solution to the red meat problem. Despite the sufficient number of sheep to increase per capita red meat production in Turkiye, the productivity of local breeds which constitute a large part of the sheep population is low (Akçapınar et al., 2000). Therefore, to solve the problem in the long term, it is necessary to increase the carcass yield per animal in local breeds (Bingöl and Aygün, 2013; Turkyilmaz and Esenbuga, 2019; Türkyilmaz et al., 2021). Assuming that a 1 kg increase in lamb live weight will increase carcass yield by approximately 42-50% (for Akkaraman) (Boztepe, 2015;

Aksoy et al., 2018), lamb carcass weight will increase by roughly 420-500 g.

Akkaraman sheep, which is the subject of the research, has approximately 40-50% of the sheep population in Turkiye (Öztürk, 2000; Tufan and Akmaz, 2001; Arık et al., 2002; Yaranoğlu and Özbeyaz, 2019; Noyan and Ceyhan, 2021). Akkaraman sheep, which is resistant to harsh climatic conditions, is one of the local fat tail sheep breeds. Akkaraman sheep are bred across a wide geographical area that includes Central Anatolia and nearby provinces (Boztepe, 2015; Kaymakçı, 2016). Many researchers have reported the following results for Akkaraman sheep: live weight is 35-50 kg (Boztepe, 2015), milk yield is 40-99.57 kg (Esen and Özbey, 2002; Mundan and Özbeyaz, 2004; Özmen et al., 2015; Kaymakçı, 2016; Kahraman and Özkul, 2020), lactation length is 120-155.80 days (Kaymakçı et al., 2001; Esen and Özbey, 2002; Mundan and Özbeyaz, 2004; Sönmez et al., 2009; Özmen et al., 2015; Kahraman and Özkul, 2020), twinning rate is 4.00-20.80% (Akçapınar et al., 2000; Kaymakçı et al., 2001; Esen and Özbey, 2002; Özmen et al., 2015; Ceyhan et al., 2019), fleece yield is 1.5-2.87 kg (Arık et al., 2002; Yıldız and Denk, 2006b; Boztepe, 2015; Kaymakçı, 2016; Tuncer and Cengiz, 2018), birth rate is 0.69-0.94 (Akçapınar et al., 2000; Özbey and Akçan, 2000a; Esen and Özbey, 2002; Ünal et al., 2006; Yakan et al., 2012; Özmen et al., 2015; Güngör and Ünal, 2020; Türkmen and Çak, 2021; Aksoy et al., 2023), the number of lambs born per ram is 0.93-1.03 (Esen and Özbey, 2002; Ceyhan et al., 2019; Aksoy et al., 2023), litter size at birth is 1-1.39 (Akçapınar et al., 2000; Özbey and Akçan, 2000a; Ünal, 2002; Ünal et al., 2006; Yıldız and Denk, 2006a; Esen and Bozkurt, 2001; Yakan et al., 2012; Büyüktekin and Öztürk, 2018; Ceyhan et al., 2019; Türkmen and Çak, 2021), birth weight is 3.56-4.91 (Çolakoğlu and Özbeyaz, 1999; Özbey and Akcan 2000b; Yakan et al., 2012; Ceyhan et al., 2019; Güngör and Ünal, 2020; Sakar and Ünal, 2021; Türkmen and Çak, 2021; Aksoy et al., 2023), 60th day weight is 12.84-22.11 kg (Odabaşıoğlu et al., 1996; Akçapınar et al., 2000; Esen and Yıldız, 2000b; Kucuk and Erduran, 2009; Aktaş and Doğan, 2014; Özmen et al., 2015; Sakar and Erişek, 2019; Türkmen and Çak, 2021; Aksoy et al., 2023; Tüfekci, 2023), 120th day weight is 25.51-34.95 kg (Akçapınar et al., 2000; Kucuk and Eyduran, 2009; Yakan et al., 2012; Aktaş and Doğan, 2014; Aktaş et al., 2014; Özmen et al., 2015; Sakar and Erişek, 2019; Aksoy et al., 2023; Tüfekci, 2023). The survival rate from birth to weaning (up to the 120th day of age) is 88.80-100.00 (Mundan and Özbeyaz, 2004; Aktaş and Doğan, 2014; Aktaş et al., 2014; Özmen et al., 2015; Aksoy et al., 2023), daily live weights gains between the birth day and the 120th day of age is between 231-264 g lamb^{-1} day^{-1} (Aktaş et al., 2014; Sakar and Erişek, 2019; Aksoy et al., 2023).

Within the scope of National Sheep and Goat Breeding in Nigde, many scientific studies have been published on the determination of some reproductive characteristics of Akkaraman sheep and the livability and growth characteristics of lambs under breeder conditions in five-year periods (Ceyhan et al., 2019; Sekeroglu et al., 2019; Noyan and Ceyhan, 2021; Aksoy et al., 2023). This research aimed to evaluate some reproductive characteristics of Akkaraman sheep and the livability and growth characteristics of their lambs within the scope of the National Sheep and Goat Breeding Project (number of project: 51AKK2012-02) in the Bor district of Nigde between 2018 and 2022.

Materials and Methods

The study was conducted on sheep and lambs bred by the public in the Bor district of Nigde between 2018 and 2022 as part of a project that began in 2013. The data was collected annually from approximately 6000 head of Akkaraman sheep and their total 30051 head offspring. Although there are differences between the feeding methods in enterprises, sheep generally meet their nutrition requirements in pens during winter months. After winter, sheep feed on pasture from March to November (Ceyhan et al., 2019).

Ram mating in the study was between August and September. It was planned at one ram per 30 ewes (class mating) in elite flocks and one ram per 25 ewes (free mating) in base flocks. . In the herds covered by the project, ram mating was limited to 45 days. Once the lambs were born, breeders recorded their birth date, birth type, gender, ewe's age, and birth weight (LBW). Besides LBW, the study has examined the growth characteristics of lambs: 60th-day weight (BW60), 120th-day weight (BW120), and lamb's daily weight gain (BWG120) between birth and 120th day. The growth characteristics of the lambs were examined by evaluating the birth records in the two months following the birthday. The live weights of lambs BW60 and BW120 were adjusted using the interpolation method (Aksoy et al., 2023).

The research investigated some reproductive performance traits according to mating, lambing and weaning results in Akkaraman ewes. Table 1 shows the formulas that determine the fertility characteristics of Akkaraman lambs discussed in the study (Kaymakçı, 2016; Aksoy et al., 2023).

In the research, the 120-day-Akkaraman lambs' livability (SR120) = Number of lambs born / Number of lambs weighted on the 120th day × 100) was determined according to the year of lamb birth (2018, 2019, 2020, 2021, and 2022), the age of the ewe (2, 3, 4, 5, 6, and 7 years old and above), gender (male and female), and the type of birth (single and twin).

The following general linear model was used to determine the environmental factors affecting LBW, BW60, BW120, and BWG120 (Model 1).

Table 1. Equations used in calculation of some reproductive performance traits according to mating, lambing and weaning results in Akkaraman ewes

Mating and lambing results	Equality for calculation
Litter size at birth (n)	Number of lambs born / Number of ewes lambing
Weaning results	
Litter size at weaning (n)	Number of lambs at weaning / Number of ewes lambing

Table 2. The least squares means (LSM) and standard errors (kg) of birth (LBW) and 60th day weight (BW60) of Akkaraman lambs

Factor	N	LBW		N	BW60	
		LSM	SE		LSM	SE
Birth year		P<0.01			P<0.01	
2018	5228	4.33a	0.011	5061	17.60c	0.060
2019	6420	3.95d	0.012	5940	19.52b	0.067
2020	5881	4.13b	0.010	5349	17.38d	0.060
2021	5971	4.30a	0.094	5632	21.68a	0.014
2022	6551	4.06c	0.085	6282	16.71e	0.013
Ewe's age		P<0.01			P<0.01	
2	4332	4.14b	0.082	3924	18.11b	0.018
3	4519	4.15b	0.074	4293	18.24b	0.012
4	3688	4.22a	0.071	3469	19.94a	0.011
5	6811	4.21a	0.094	6538	18.28b	0.015
6	5428	4.00c	0.077	5117	19.14a	0.013
7 and more	5273	4.16b	0.083	4923	18.10b	0.013
Gender		NS			P<0.01	
Male	14759	4.17	0.136	13684	18.72	0.021
Female	15292	4.12	0.140	14580	18.45	0.022
Birth type		P<0.01			P<0.01	
Single	26527	4.28	0.198	24940	18.74	0.028
Twin	3524	3.11	0.092	3324	17.34	0.011
Overall mean	30051	4.14	0.195	28264	18.58	0.030

LBW: Lamb birth weight; BW60: Live weight of lamb at day 60; SE: Standard error of the mean; a, b, c, d, e: The observed differences between the mean denoted by different letters in the same column are significant (P<0.01); NS: Nonsignificant

$y_{ijklm} = \mu + A_i + B_j + C_k + D_l +$ interactions between factors $+ e_{ijklm}$ Model 1

In the model;

y_{ijklm} = Individual yield record of m^{th} lamb in i^{th} lambing year, j^{th} ewe-age, k^{th} birth-type, and l^{th} gender

μ = Overall mean of population

A_i = The effect of the lambing year (in five categories: 2018, 2019, 2020, 2021, and 2022)

B_j = The effect of ewe's age at lambing (in six categories: 2, 3, 4, 5, 6, and 7+ years old)

C_k = The effect of lamb's birth type (in two categories: twin and single)

D_l = The effect of lamb's gender (in two categories: female and male)

e_{ijklm} = Random error

In this study, Duncan multiple comparison tests were used to determine the differences between more than two subgroups in Akkaraman lambs (Düzgüneş et al., 1983). In this current study on the factors affecting lamb survival, Chi-square (X^2) test was used to determine the dependence on these factors (breeding age, gender, type of birth and year of birth) and SPSS (2015) package programme was used for all data analyses.

Results

Table 2 shows the least squares mean (LSM) and standard errors for the LBW and BW60 weights of lambs. In this study, LBW in Akkaraman lambs in 2018, 2019, 2020, 2021, and 2022 were 4.33±0.01, 3.95±0.01, 4.13±0.01, 4.30±0.09, and 4.06±0.08 kg, respectively. In terms of ewe's age, the highest LBW was in lambs born to 4-year-old mothers (4.22±0.07 kg), and the lowest LBW was in lambs born with a maternal age of six (4.00±0.07 kg). Although the LBWs of males and females in Akkaraman lambs were similar (P>0.05), the difference of 1.17 kg observed between singleton and twin lambs was significant (P<0.01).

All environmental factors considered in this study had a significant effect on BW60 (Table 2; P<0.01). In Akkaraman lambs, BW60 varied between 16.71±0.01 and 21.68±0.01 kg (LSM = 18.58±0.03 kg) between 2018-2022. In Akkaraman lambs, the highest BW60 was determined in males (18.72±0.02 kg), single-born (18.74±0.02 kg) with ewe ages 4 (19.94±0.01 kg) and birth years in 2021 (21.68±0.01 kg).

The gender, age of the ewe, and year of birth affected the BW120 of the lambs (P<0.01). BWG120 were higher in male lambs (249±0.58 g day^{-1} $lamb^{-1}$) compared to female lambs (243±0.54 g day^{-1} $lamb^{-1}$) (P>0.05), and in single lambs (246±0.42 g day^{-1} $lamb^{-1}$) compared to twins (242±1.12 g day^{-1} $lamb^{-1}$) (Table 3; P<0.01).

Litter size at weaning (LSW) and litter size at birth (LSB) was 0.95 and 1.06, respectively (Table 4). The year 2022 had the highest LSB (1.10), and the lowest was in 2021 (1.03). SR120 had the lowest value (86.56%) in lambs born to 2-year-old ewes and the highest value (95.34%) in lambs born to 5-year-old ewes. The current study concluded that SR120 (mean = 91.88%) was dependent on the environmental factors examined (P<0.01).

Discussion and Conclusion

Birth Weight

Birth weight is also a significant criterion in evaluating the growth characteristics of lambs. Since the heritability of lamb birth weight is medium and high (h^2 = 0.33-0.77), it is a criterion for improving the growth characteristics of

lambs through selection. Many researchers reported that birth weight generally had a positive relationship between a lamb's livability and future growth characteristics (Assan et al., 2002; Hatcher et al., 2009; Everett-Hincks et al., 2014; Ptáček et al., 2017; Juengel et al., 2018).

In Akkaraman lambs, the birth weight of male lambs was higher than that of female lambs. Previous studies have explained this situation with a higher number of cotyledons of ewes giving birth to male lambs than that of ewes giving birth to female lambs. It has also been reported that male lambs secrete growth hormone at an earlier stage, thus possibly increasing their birth weight. In addition, some studies have reported that birth weight in female lambs is lower than in males due to the estrogen hormone weakening bone development in female lambs (Rashidi et al., 2008; Jawasreh et al., 2009; Bancheva et al., 2022). Babar et al. (2004) reported that male lambs generally had higher birth weights because they stayed in the uterus longer than female lambs.

Table 3. The least squares means (LSM) and standard errors of live weights (BW120) and daily live weight gains (BWG120) of Akkaraman lambs at 120 days

Factor	N	BW120 (kg)		BWG120 (g day^{-1})	
		LSM	SE	LSM	SE
Birth year		P<0.01		P<0.01	
2018	5051	28.78d	0.087	240d	0.730
2019	5754	33.11b	0.112	276a	0.940
2020	4894	30.36c	0.103	253c	0.860
2021	5632	35.54a	0.107	260b	0.890
2022	6282	28.67d	0.080	205e	0.670
Ewe's age		P<0.01		P<0.01	
2	3750	30.26c	0.121	239d	1.010
3	4236	31.24b	0.116	239d	0.990
4	3401	32.96a	0.140	256b	1.130
5	6494	30.10c	0.089	238d	0.750
6	4990	32.46a	0.118	262a	1.030
7 and more	4742	31.38b	0.115	244c	0.970
Gender		P<0.01		NS	
Male	13397	31.69	0.069	249	0.580
Female	14216	30.96	0.064	243	0.540
Birth type		NS		P<0.01	
Single	24416	31.43	0.050	246	0.420
Twin	3197	30.45	0.134	242	1.120
Overall mean	27613	31.31	0.021	246	2.230

BW120: Live weight of lamb at day 120, BWG120: Daily live weight gains of lambs between birth and 120 days of age; SE: Standard error of the mean; a, b, c, d, e: The observed differences between the mean denoted by different letters in the same column are significant (P<0.01); NS: Nonsignificant

Table 4. Livability of Akkaraman lambs at 120-days postpartum (SR120) and some reproductive traits

Factor	Livability					Reproductive traits	
	NB (n)	NB120 (n)	SR120 (%)	X2 Value	P	LSB (n)	LSW (n)
Birth year				982.3	0.000		
2018	5228	5051	96.61			1.04	1.01
2019	6420	5754	89.62			1.08	0.97
2020	5881	4894	83.21			1.05	0.88
2021	5971	5632	94.32			1.03	0.97
2022	6551	6282	95.89			1.10	0.95
Ewe's age				322.2	0.000		
2	4332	3750	86.56				
3	4519	4236	93.73				
4	3688	3401	92.21				
5	6811	6494	95.34				
6	5428	4990	91.93				
7 and more	5273	4742	89.92				
Gender				48.4	0.000		
Male	14759	13397	90.77				
Female	15292	14216	92.96				
Birth type				7.2	0.007		
Single	26527	24416	92.04				
Twin	3524	3197	90.72				
Overall mean	30051	27613	91.88			1.06	0.95

NB: The number of live-born lambs, NB120: The number of lambs that survived to 120 days of age; SR120: The livability of lambs at 120 days of age; LSB: Litter size at birth; LSW: Litter size at weaning

The birth weight of single-born lambs was higher than that of twin lambs. A previous study reported that the birth weight of lambs in multiple births is lower than in single-born lambs due to the finite capacity of the uterine space of sheep giving birth to multiples and the limited nutrition of the lambs during the gestation period (single-born lambs have no competition for nutrition) (Bancheva et al., 2022).

Although the effect of the age factor decreases until 8 ages, some researchers reported that the birth weight of older sheep was higher than that of two-year-old sheep. Previous studies attribute this to the fact that older ewes can transfer their energy to productivity as they have completed their development and produce heavier lambs. In addition, many researchers report that with increasing age, the weight of the placenta, uterus, and the amount of nutrients transferred from the mother to the lamb cause an increase in lamb birth weight (Babar et al., 2004; Wu et al., 2006; Jawasreh et al., 2009; Bancheva et al., 2022). In this study, lowest birth weight was detected in born lambs from 6-year-old ewes (4.00 kg). The differences observed in Akkaraman lambs in birth weight in 2-year-old (4.14 kg), 3-year-old (4.15 kg), and 7-year and more (4.16 kg) lambs were insignificant (Table 2).

Various studies report that lamb birth weight is affected by the following factors: genetic factors (heterosis effect and breed) (Bancheva et al., 2022), non-genetic factors (production year (Kleemann et al., 1990; Mellado et al., 2016; Sudan et al., 2018; Sveinbjörnsson et al., 2021), lamb birth type (Sudan et al., 2018; Bancheva et al., 2022), weight and body condition of ewes (Kleemann et al. 1990; Bancheva et al., 2022), lamb gender (Mellado et al., 2016; Sudan et al., 2018; Bancheva et al., 2022), litter size (Hinch et al., 1985; Kleemann et al., 1990; Mellado et al., 2016) weight of ewes at pairing (Hinch et al., 1985), inbreeding (Alsheikh, 2005), Ewes' feeding (Bancheva et al., 2022), herd (Baneh and Hafezian, 2009), season of lambing (Mellado et al., 2016). Similar to many studies in Akkaraman sheep, this study found that environmental factors such as ewe's age (Aktaş et al., 2014; Noyan and Ceyhan, 2021; Aksoy et al., 2023), birth type (Aktaş and Doğan, 2014; Aktaş et al., 2014; Ceyhan et al., 2019; Sakar and Erişek, 2019; Noyan and Ceyhan, 2021; Sakar and Ünal, 2021; Aksoy et al., 2023; Tüfekci, 2023), and birth year (Ceyhan et al., 2019; Behrem, 2021; Noyan and Ceyhan, 2021; Aksoy et al., 2023; Tüfekci, 2023) significantly affected birth weight in Akkaraman lambs.

The direct effect of lamb birth weight on lamb survival has been reported in previous studies. Therefore, researchers have announced that the ideal birth weight of lambs is between 3.5 and 6.0 kg, but for maximum lamb survival, it should be approximately 4.50 kg (Oldham et al., 2011).

The birth weight value determined by this study for Akkaraman lambs (4.14 kg) was lower than the values found by Çolakoğlu and Özbeyaz (1999), Kucuk and Eyduran (2009), Yakan et al. (2012), Ceyhan et al. (2019), and Aksoy et al. (2023) (4.23-4.91 kg). Akkaraman birth weight values documented by the previous studies were as follows: Özbey and Akcan (2000b) 3.57 kg, Tüfekci (2023) 3.71 kg, Esen and Yıldız (2000b) 3.73 kg, Özmen et al. (2015) 3.74 kg, Yıldız and Denk (2006b) 3.81 kg. The birth weight value of Akkaraman lambs in this study was compatible with the reports of Aktaş and Doğan (2014) and Behrem (2021). Past studies conducted on the Akkaraman male and female lambs in Nigde province found birth weight values as 4.44 and 4.28 kg (Aksoy et al., 2023); 4.32 and 4.14 kg (Ceyhan et al., 2019); 4.10 and 4.04 kg (Noyan and Ceyhan, 2021), respectively. Besides, the birth weight in singleton and twin lambs were 4.51 and 3.82 kg (Aksoy et al., 2023), 4.44 and 4.02 kg (Ceyhan et al., 2019), 4.26 and 3.11 kg (Noyan and Ceyhan, 2021), respectively. In this study, the birth weight determined for male and singleton lambs (4.17 and 4.28 kg, respectively) was higher than the value reported by Noyan and Ceyhan (2021) and lower than the value reported by Ceyhan et al. (2019) and Aksoy et al. (2023). The birth weight value determined by this study in Akkaraman twin lambs in Nigde province was similar to the value stated by Noyan and Ceyhan (2021).

The differences observed between the results of this research and previous studies in terms of birth weight in Akkaraman sheep may be due to the ram effect, pasture vegetation status of the growing region, climatic conditions, sheep's genetic capacity and breeding system, and the herds' regeneration level.

Body weights of lambs at 60 days

The BW60 value determined for Akkaraman lambs in the study was higher than the value reported Esen and Yıldız (2020b), Türkmen and Çak (2021), Aksoy et al. (2023), and Tüfekci (2023) (12.84-18.43 kg), similar to the value announced by Akçapınar et al. (2000) (18.51 kg), and lower than the value documented by Sakar and Erişek (2019) (22.11 kg). In the study, the effects of lamb gender, ewe's age, birth year, and type on BW60 were significant. This finding was consistent with the reports of Aksoy et al. (2023). In an earlier study conducted under breeder conditions in Yozgat, similar to the research findings, the effect of birth year on BW60 was significant; however, the effects of birth type and gender were insignificant (Tüfekci, 2023). In another study conducted under breeder conditions in Çankırı province, the impact of gender on BW60 in Akkaraman lambs was negligible, but the birth type was significant (Sakar and Erişek (2019). In their study on Akkaraman sheep in Van, Türkmen and Çak (2021) found the differences as influential in male and female lambs and singleton and twin lambs in terms of BW60. On the BW60 of Akkaraman lambs bred in Konya, Aktaş and Doğan (2014) reported a significant effect of lamb birth type and gender and an insignificant impact of the ewe's age.

Body weights of lambs at 120 days

In this research, the BW120 value detected in the male (31.69 kg) and singleton (31.43 kg) Akkaraman lambs was similar to the value reported by Özmen et al. (2015). The BW120 value reported by Özmen et al. (2015) (30.76 kg) for Akkaraman lambs under Elazığ conditions was lower than the value found in this study. In the study conducted in Konya, the values reported by Aktaş et al. (2014) for Akkaraman ewes and twin lambs are compatible with the findings of this study. The value reported by the same researcher for male and twin lambs was higher than our

research findings. The BW120 value reported by Yakan et al. (2012) for Akkaraman lambs (30.38 kg) was similar to the value found in this study in twin lambs (30.45 kg). In this study, the BW120 value was lower than the value reported by Sakar and Erişek (2019) and Aksoy et al. (2023) (34.95 and 32.62 kg, respectively). The BW120 value reported by Tüfekci (2023) (31.08 kg) for Akkaraman lambs in Yozgat breeder conditions was similar to the effect of lamb gender, year of birth, and ewe's age on BW120 as significant. This result is compatible with Aktaş et al. (2014) and Aksoy et al. (2023) for Akkaraman lambs. Unlike the research findings, Sakar and Erişek (2019) and Tüfekci (2023) reported that the effect of gender and birth type on BW120 in Akkaraman lambs was negligible.

Live weight gain at 120 days

Aktaş et al. (2014) determined BWG120 in Akkaraman lambs as 218 and 244 g day^{-1} lamb^{-1} in female and male lambs and 220 and 241 g day^{-1} lamb^{-1} (LSM = 231 g day^{-1} lamb^{-1}) in twin and single-born lambs. Aksoy et al. (2023) reported BWG120 in Akkaraman lambs in Nigde as 255 and 275 g day^{-1} lamb^{-1} in female and male lambs and 253 and 268 (LSM = 264 g day^{-1} lamb^{-1}) in twin and singleton lambs. In this study, the BWG120 value detected in Akkaraman male and female and singleton and twin lambs was higher than that reported by Aktaş et al. (2014) and lower than that reported by Aksoy et al. (2023). A study conducted in Çankırı determined the daily weight gain in Akkaraman lambs from birth to the 90th day and from birth to the 120th day as 287 and 255 g day^{-1} lamb^{-1}, respectively (Sakar and Erişek, 2019). Ceyhan et al. (2019) reported daily live weight gains between birth and 90 days in Akkaraman lambs as 208 g day^{-1} lamb^{-1}; Türkmen and Çak (2021) reported 171 and 170 g day^{-1} lamb^{-1} in male and female lambs, respectively; Noyan and Ceyhan (2021) found it to be 225 and 222 g day^{-1} lamb $^{-1}$ (LSM = 222 g day^{-1} lamb^{-1}) in singletons and twins, respectively, and Odabaşıoğlu et al. (1996) found it to be 226 g day^{-1} lamb^{-1}. In this study, the BWG120 value detected in Akkaraman lambs was lower than the value reported by Sakar and Erişek (2019) at the same age.

Similar to the research findings, Aksoy et al. (2023) reported that the effect of ewe's age, birth type, and year on BWG120 was significant in Akkaraman lambs. The study by Aktaş et al. (2014) on Akkaraman sheep in Konya revealed similar results to this research on the powerful effect of birth year and lamb birth type on BWG120. Unlike the research findings, Sakar and Erişek (2019) reported that the BWG120 in Akkaraman, singleton and twin lambs were similar.

Livability of lambs

Lamb livability has an impact on farm profitability through changes in the number of lambs that are retained after weaning. (Ünal et al., 2006; Aktaş and Doğan, 2014). In this study, the lowest survival rate was in the lambs born from 2-year-old mothers (86.56%). Similar to this research finding, Aktaş and Doğan (2014) found the lowest SR120 value in Akkaraman lambs born from 2-year-old ewes (87.00%). This situation can reveal the low maternal abilities of 2-year-old mothers, low milk yield, and hypothermia occurring in lambs due to low lamb birth weight. The current study has found SR120 as 91.88%. Özmen et al. (2015) determined the SR120 value in Akkaraman lambs in Elazığ as 87.83 and 92.36% in male and female lambs and 90.82 and 92.59% (mean = 90.06%) in singleton and twin lambs. This study found the SR120 value determined in Akkaraman male and singleton lambs to be higher than the value reported by Özmen et al. (2015) for the same breed. In their study conducted on Akkaraman lambs in Nigde, Aksoy et al. (2023) announced that females (93.93%) and singletons (93.75%) had higher SR120 survival rates than males and (93.27%) and twins (93.10%) (mean = 93.60%). In the current study, similar to the report of Aksoy et al. (2023), SR120 in Akkaraman lambs was higher in female lambs than in male lambs. Aktaş et al. (2014) found an average of 91.40% of SR120 in Akkaraman sheep raised under breeding conditions in Konya in 2007 (88.30%), 2008 (89.00%), 2009 (94.50%) and 2010 (93.70%). In the study conducted in the same province, Aktaş and Doğan (2014) reported that SR120 in lambs born from 2, 3, 4, 5, and 6+ year-old sheep varied between 87.00-90.60% (mean = 88.80%). This research revealed that the SR120 value in Akkaraman lambs was compatible with the value reported by Aktaş et al. (2014) and higher than that reported by Aktaş and Doğan (2014). Çolakoğlu and Özbeyaz (1999) determined the survival rate of Akkaraman sheep on the 105th day (at weaning) as 97.67%, 96.05%, 97.06% and 96.50% in 1992, 1993, 1994, and 1995, respectively. Again, the 90th-day survival rate in Akkaraman lambs was determined as 89.52% by Akçapınar et al. (2000), 95.92% by Türkmen and Çak (2021), and 91.02% by Ünal et al. (2006). This research found the SR120 value for Akkaraman lambs to be lower than the value reported by Türkmen and Çak (2021). This difference can be explained by the higher age of the lamb in the study.

Some Reproductive Traits of Ewes

The litter size and the number of lambs reaching marketing age measure sheep productivity. In the study, while the LSB varied between 1.03-1.10 (mean = 1.06), the LSW was between 0.88-1.01 (mean = 0.95). A study conducted in Nigde found that the LSB was 1.13, and the LSW was 1.05 (Aksoy et al., 2023). In the study, both fertility traits were lower than the value reported by Aksoy et al. (2023). The current study found the number of Akkaraman lambs per birthing ewe lower (1.12-1.39) than many studies conducted on the same breed in the past (Akçapınar et al., 2000; Özbey and Akçan, 2000a; Yakan et al., 2012; Büyüktekin and Öztürk, 2018; Ceyhan et al., 2019). Although the number of Akkaraman lambs per birthing ewe determined in the study was similar to the value reported by Esen and Bozkurt (2001) (1.06), it was higher than the values reported by Türkmen and Çak (2021) (1.03) and Yıldız and Denk (2006a) (1-1.02) for Akkaraman sheep raised in Van.

In conclusion, in this study, the growth characteristics, survival and litter size of Akkaraman ewes raised in Bor district of Nigde were analysed , within the scope of the National Sheep and Goat Breeding Project. This research found the highest BW60 and BW120 in male and single lambs born to 4-year-old ewes in 2021. The birth weight, one of the growth characteristics considered in the study, was lower or higher than the value reported by many

previous researchers. This research found the birth weight slightly lower than in two studies but higher than in one study conducted within the scope of the same project in Nigde. This research found that BW60 and BW120 among the growth characteristics of lambs were similar or higher than most studies. In the study, among the factors whose effects were examined on the growth performance of Akkaraman lambs, only the impacts of gender on BWG120 and birth type on BW120 were insignificant. It could be concluded that adequate care, nutrition and routine health and protection measures during the gestation and calving season in Akkaraman ewes could improve the yield traits examined in the study within the next five years in Nigde.

We foresee that adequate care, nutrition and routine health and protection measures during the gestation and parturition season in Akkaraman ewes can improve the productivity traits examined in the study over the next five years in Nigde.

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Extraction of Bioactive Compounds from Yellow Onion Peels: Taguchi-SAW Hybrid Optimization

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ABSTRACT

The aim of this study was to obtain an extract rich in bioactive components from yellow onion peels, which are generally considered waste material. Accordingly, a three-factor three-level Taguchi (L9) experimental design with three factors, namely ethanol concentration (A; 50%, 75%, 100%), extraction temperature (B; 30, 40, 50 °C), and sonication time (C; 10, 20, 30 min) was used to optimize the ultrasound-assisted extraction process of onion peel powders. Through Taguchi optimization, the optimum extraction conditions were determined as $A_2B_3C_2$ to obtain the extract with the highest total phenolic matter (TPM) content and antioxidant activity (DPPH (%)). In addition, the extract produced under $A_2B_1C_2$ conditions was found to be the richest in total monomeric anthocyanin (TMA) content with the highest level of color pigments. In order to determine the overall optimization conditions and to reduce the three-response optimization process to a single response, the simple sum weighting (SAW) method was used as a multi-criteria decision-making method. As a result of the optimization, it was concluded that an extract rich in bioactive components with optimal TPM and TMA contents and DPPH (%) value could be obtained as a result of sonication at 30 °C for 20 min to onion peel powders mixed with a solvent containing 75% ethanol ($A_2B_1C_2$). The extraction conditions of bioactive components from yellow onion peels were successfully optimized by the Taguchi-SAW hybrid optimization method.

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Introduction

Biomass waste is recognized as one of the significant global issues throughout the world and is becoming increasingly important in developing countries due to its detrimental impact on the environment. Fruit and vegetable waste and associated by-products are produced in substantial quantities through industrial processes, posing a serious environmental threat when not appropriately managed. However, these waste materials are rich in bioactive compounds known for their potential health benefits. In recent years, considerable efforts have been made to develop techniques for the efficient utilization of fruit and vegetable waste (Pal and Jadeja, 2019).

Onions (*Allium cepa* L.), a member of the Allium family, are not only known for their delicious taste but also serve as a valuable source of various beneficial compounds. Onions, particularly their peels, are rich in phenolic compounds and exhibit antioxidant properties (Bordin Viera et al., 2023). Red, yellow, and white onions contain significant amounts of anthocyanins, which are responsible for the coloration of onion peels. These anthocyanins have found applications in treating various diseases, including cancer, atherosclerosis, diabetes, and cardiovascular disease (Jeya Krithika et al., 2022).

Bioactive components are used as additives in foods due to their positive effects on human health. These functional compounds, which are already used in various applications such as pharmaceuticals, cosmetics, and textiles, are usually obtained by extraction from different parts of plants (stems, leaves, bark, seeds, fruits, etc.). Studies on different extraction methods have focused on the separation of these components in order to increase extraction efficiency, reduce costs, and maximize profits. Ultrasound (US) technique is an alternative to supercritical fluid extraction and microwave-assisted extraction for the extraction of active substances from plant samples. This technique is economical, environmentally friendly, and time-saving compared to conventional extraction methods. The US disrupts cell tissues in a short time, accelerating mass transfer and thus increasing extraction efficiency (Alves Filho et al., 2021).

Taguchi method is one of the optimization techniques that can reduce the number of experiments, improve product quality, and determine design solutions. Taguchi method, which is generally used in single response optimization, can be integrated with multi-criteria decision-making methods and applied to obtain optimal conditions for multiple responses (Güldane, 2023). Taguchi-PROMETHEE (Crnjac et al., 2019), Taguchi-TOPSIS (Singh et al., 2011), and Taguchi-AHP (Salari et al., 2019) hybrid optimization techniques have been applied successfully in various process operations. Pal and Jadeja (2019) applied the Taguchi method in single response optimization to maximize polyphenolic antioxidant extraction from onion peel.

In the literature, there is no study in which the Taguchi-SAW method was applied in multiple response optimization for the extraction of bioactive compounds from yellow onion peels. In this study, it was aimed to determine the optimal extraction conditions of yellow onion peels by ultrasonically assisted extraction method. Extraction conditions, ethanol concentration (A; 50%, 75%, 100%), extraction temperature (B; 30, 40, 50 °C), and sonication time (C; 10, 20, 30 min) were optimized by Taguchi-SAW hybrid optimization method to maximize total phenolic and total monomeric anthocyanin contents, and DPPH radical scavenging activity.

Materials and Methods

Materials

The waste onion peels used in this study were obtained from a local grocery store in the Pamukova region of Sakarya. The chemicals used in the analysis were of analytical purity and were obtained from Merck (Germany).

Extraction of Bioactive Compounds from Yellow Onion Peels

Onion peels were washed with distilled water and dried in an oven at 40 °C for 4 hours (Santos and Martins, 2022). After cooling the onion peels to room temperature, they were ground with a blender. Then, 1 g of the powdered samples was transferred to a beaker and 10 ml of 96% (v/v) ethanol was added at different concentrations (50, 75, and 100%). The pH of the mixtures was adjusted to 2.0 using 0.1N HCl (Santos and Martins, 2022). The samples were sonicated for different times (10, 20, and 30 min) in a 150 kW ultrasonic water bath (CALISKAN, LAB ULT 4045, China) set at various temperatures (30, 40, and 50 °C). The mixtures were then filtered through a water tromb using filter paper (Whatman no:1). The filtrates were stored at 4°C until analysis.

Experimental Design

Taguchi method was used in the optimization of bioactive component extraction from onion peel. Minitab 19.0 software was utilized in the planning of the study. Experiments were carried out according to Taguchi L9 experimental design which consists of 3 factors and 3 levels (Table 2). Ethanol concentration (50-100%), extraction temperature (30-50 °C), and sonication time (10-30 min) were selected as control parameters (Table 1). Literature data was used to determine the extraction factors and the levels of each extraction factor.

Taguchi Optimization Method

Taguchi method is an optimization technique that is characterized by determining the optimum levels of extraction parameters with a minimum number of experiments. This procedure is specifically preferred for optimizing a single process characteristic. In the Taguchi technique, optimal parameters are obtained by comparing the S/N ratios corresponding to each test parameter. In this study, the "larger is better" (Equation 1), which corresponds to the maximum levels of S/N ratios for each of the responses was preferred since the aim was to obtain an extract rich in bioactive components (Roy, 2010).

$$S/_N = -10log\left[\frac{1}{n}\sum_{i=1}^{n}\frac{1}{y_{ij}{}^2}\right] \quad (1)$$

Table 1. Control parameters and levels

Symbol	Factors	Unit	Level 1	Level 2	Level 3
A	Ethanol concentration	%	50	75	100
B	Sonication temperature	°C	30	40	50
C	Sonication time	min	10	20	30

Table 2. Taguchi L9 orthogonal design, experimental results, and S/N ratio values

Run	Factors and levels			Responses					
	A	B	C	TPM (mg GAE/g)		TMA (mg C3G/100 g)		DPPH (%)	
				Mean	S/N	Mean	S/N	Mean	S/N
1	50	30	10	29.54	29.41	10.75	20.63	47.53	33.54
2	50	40	20	30.91	29.80	7.86	17.91	62.46	35.91
3	50	50	30	33.29	30.45	12.31	21.81	64.43	36.18
4	75	30	20	33.36	30.47	17.63	24.92	61.24	35.74
5	75	40	30	32.49	30.24	9.30	19.37	53.67	34.59
6	75	50	10	33.83	30.59	10.30	20.26	58.71	35.37
7	100	30	30	31.05	29.84	9.56	19.61	45.98	33.25
8	100	40	10	24.80	27.89	10.70	20.58	36.88	31.34
9	100	50	20	33.12	30.40	9.80	19.83	46.08	33.27

SAW Method

Simple additive weighting (SAW) method was used for multiple response optimization in the extraction of bioactive components from onion peels. In this approach, the runs are scored between 0 and 1, and the sample equal to or closest to 1 is considered as the most ideal sample. The SAW method involves 4 steps (Muddineni et al., 2017):

- Construction of decision matrix (D_{mxn}) (Equation 2)

$$D_{mxn} = \begin{Bmatrix} x_{11} & x_{12} \dots & x_{1n} \\ x_{12} & x_{22} \dots & x_{2n} \\ \vdots & \vdots & \vdots \\ x_{m1} & x_{m2} \dots & x_{mn} \end{Bmatrix}, \quad (2)$$

- Normalization of the parameters using Equation 3

$$n_{ij} = \frac{r_{ij}}{\max(r_{ij})} \quad (3)$$

- Calculation of the weighted decision matrix using Equation 4. The weighting value for each parameter was determined by principal component analysis (PCA). The details of this method are described by Guldane and Dogan (2022).

$$A_j = \sum w_i x_{ij} \quad (4)$$

- The sample with the highest score is identified as the ideal.

Analysis

Total phenolic matter (TPM)

The TPM content of onion peel extracts was determined using the Folin-Ciocalteu spectrophotometric method described by Pal and Jadeja (2019). Firstly, 1 ml onion peel extract, 5 ml distilled water, and 0.5 ml Folin Ciocalteu reagent were added to the tubes and vortexed. After 5 minutes, 1.5 ml of 20% sodium carbonate (Na_2CO_3) and 2 ml of distilled water were added to the tubes and kept in the dark for 2 hours and the absorbance at 760 nm wavelength was measured in a spectrophotometer (Shimadzu UV-1240, Japan). The phenolic content of the samples was calculated using the calibration curve equation prepared using standard gallic acid solutions (25-500 mg/L). The total phenolic content of the extracts was calculated as mg GAE/g extract. These measurements were performed in 3 replicates and the results were given as the average.

Total monomeric anthocyanin (TMA)

The total anthocyanin content of onion peel extracts was determined according to the pH differential method reported by Stoica et al. (2020). This method is based on the absorbance values obtained spectrophotometrically at different pH ranges. Buffer solutions of pH 1.0 prepared with potassium chloride and pH 4.5 prepared with sodium acetate were used to prepare the dilutions of the prepared extracts. The absorbance values of the samples were measured in a UV-VIS spectrophotometer at 520 and 700 nm wavelengths. The TMA content of the samples was calculated by Equation 6 in terms of cyanidin-3-glycoside (C3G) equivalents based on the absorbance differences determined by Equation 5. Results are expressed as mg C3G/ 100 g sample.

$$\text{Abs} = (A_{520\,nm} - A_{700\,nm})_{pH\,1.0} - (A_{520\,nm} - A_{700\,nm})_{pH\,4.5} \quad (5)$$

$$\text{TMA}\left(\frac{\text{mg}}{100\,\text{g}}\right) = \frac{\text{Abs} \times M_w \times DF \times 10}{\varepsilon \times l} \quad (6)$$

where M_w= 449.2 g/mol for cyanidin-3-glucoside (C3G); DF= dilution factor; 1000= factor for conversion from g to mg; ε= 26.900 L/mol x cm, molar extinction coefficient for C3G; l= path length in cm.

Antioxidant activity (DPPH(%))

The total antioxidant activity of onion peel extracts was determined by using the radical scavenging activity of the extracts against DPPH radical according to the method proposed by Viera et al. (2023). For this purpose, 0.5 ml of ethanolic extract was added to 2.5 ml of 0.1 mM DPPH solution. The mixture was shaken gently by hand and kept at room temperature in the dark for 30 minutes. The absorbance of the samples was determined by measuring at 517 nm wavelength. The percent radical scavenging activity values of the samples were expressed as DPPH (%). The results were calculated using Equation 7.

$$\text{DPPH}(\%) = \frac{(A_0 - A_{sample})}{A_0} \times 100 \quad (7)$$

Results and Discussion

Taguchi Optimization

Experimental studies were carried out according to the L9 experimental design (Table 2) to determine the influence of extraction parameters on the responses in ultrasound-assisted extraction of onion peel. The studies were carefully conducted to determine the optimal extraction process for each response variable. Ultrasonic extraction aimed to maximize the total phenolic matter, total monomeric anthocyanin content, and antioxidant activity of the extracts. Therefore, the individual optimization of all three responses was based on the "larger is better" criterion to determine the optimal conditions.

Effect of Process Parameters on Total Phenolic Matter (TPM)

The TPM content of onion peel extracts varied between 24.80 and 33.83 mg GAE/g (Table 2). Viera et al. (2023) stated that the TPM content of red onion peels varied between 117.50 and 822.87 mg GAE/g. JK et al. (2022) also reported that red onion was richer in bioactive compounds than yellow one. The main effect plot for total phenolic content during ultrasonic extraction of bioactive phenolic compounds is given in Figure 1. From the response plot, it can be seen that the phenolic content of extracts obtained with 75% ethanol is higher than those extracted with 50% and 100% ethanol. Also, it can be concluded from Figure 1 that the TPM contents of the samples extracted at 50°C were higher than the others. The positive effect of temperature on TPM content can be attributed to keeping the extraction temperature at a moderate level. Short-term (10 min) ultrasound treatment had little effect on the phenolic content of the extracts. Increasing the sonication time to 20 min significantly increased the TPM content of the extracts. However, longer (30 min) ultrasound treatment had no significant effect on the TPM content of the extracts.

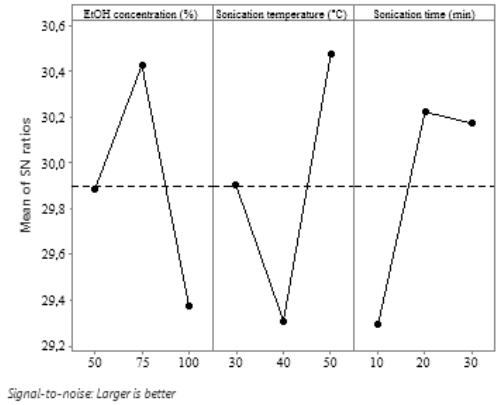

Figure 1. Main effect plot for total phenolic matter (TPM)

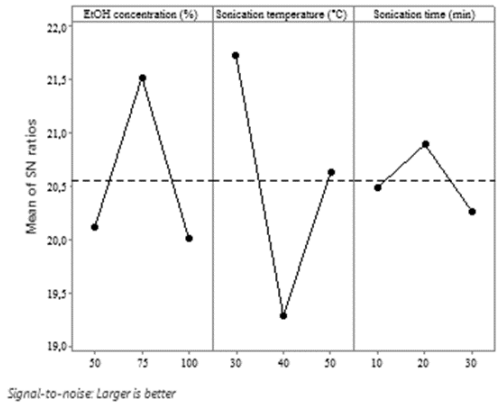

Figure 2. Main effect plot for total monomeric anthocyanin (TMA)

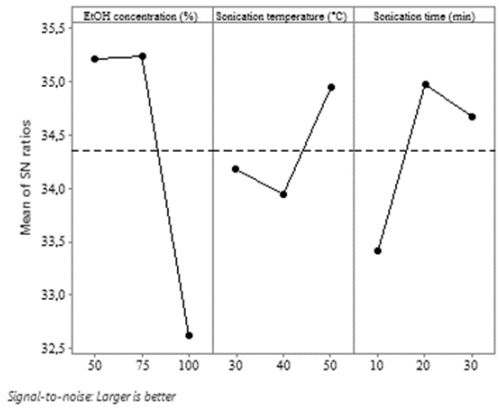

Figure 3. Main effect plot for DPPH (%)

The S/N ratios response table in the optimization study carried out to maximize the TPM content of onion peel extracts is given in Table 3. The optimal levels of each parameter are indicated in italics. As a result, the optimum process parameters for higher phenolic extraction were determined as $A_2B_3C_2$. This means that the optimal combination of process variables for better extraction performance is 75% for ethanol concentration, 50°C for extraction temperature, and 20 min for sonication time. Furthermore, according to delta values, extraction temperature was also found to be the most effective parameter for phenolic extraction, followed by ethanol concentration and sonication time, respectively.

Effect of Process Parameters On Total Monomeric Anthocyanin (TMA)

The TMA content of onion peel extracts varied between 7.86 and 17.63 mg C3G/100g sample (Table 2). Stoica et al. (2022) reported that the TMA content of red onion peels varied between 45-143 mg C3G/100 g. It was observed that the anthocyanin content of yellow onion peels was lower than that of red onion peels. The main effect graph of TMA content in ultrasonic extraction of onion peel is given in Figure 2. According to the graph, it is clear that the anthocyanin content of extracts containing 75% ethanol is significantly higher than the other solvents. Viera et al. (2023) reported that the TMA content of red onion peel extracts increased as the concentration of ethanol in the solvent increased from 20% to 80%. They also reported that the maximum TMA content was obtained at 60% and 80% ethanol concentrations. This result is in agreement with our study. However, it can be concluded that TMA contents are low in samples extracted at higher temperatures. The effect of sonication time on the extraction of color substances from onion peel was similar to that of TPM extraction. The ultrasound application for 20 min resulted in the highest yield of TMA extraction.

Taguchi method was performed for the TMA content extracted from onion peel and the S/N ratios response table is presented in Table 4. In the table, the optimal levels of each parameter are indicated in italics. Parameter levels with higher average S/N ratios can be expressed as optimum process parameters $A_2B_1C_2$ for TMA content. In terms of TMA content, it can be concluded that extracts sonicated for 20 min at 30 °C with 75% ethanol-containing solvent (Example 4) have the maximum TMA content. However, extraction temperature, which had the highest delta value (2.431) in TMA extraction, was found to be the most effective parameter in the extraction of color substances, followed by ethanol concentration and sonication time, respectively.

Effect of Process Parameters on Antioxidant Activity

The total antioxidant activities of the extracts obtained from ultrasonic extraction of onion peel were determined based on the DPPH radical scavenging activity of the extracts and expressed as DPPH (%). The DPPH radical scavenging activities of the samples ranged from 36.88% to 64.43% (Table 2). The S/N ratios average plot for DPPH (%) values of the samples extracted according to Taguchi L9 experimental design is given in Figure 3. In the S/N ratios response graph, it was determined that the antioxidant activities of the samples extracted at 75% ethanol concentration were higher than the other samples. DPPH (%) values of the samples extracted at high temperatures (50 °C) were also higher. Increasing the sonication time to 20 min significantly increased the DPPH (%) value of the extracts. Also, there was no positive effect of a longer ultrasonication time on the antioxidant activity of the samples observed.

The S/N responses of Taguchi optimization performed to maximize the antioxidant activities of onion peel extracts are given in Table 5. The data in the table shows that the extraction conditions for the maximum DPPH (%) value were found as $A_2B_3C_2$.

Table 3. S/N ratio response table for total phenolic matter

Level	A	B	C
1	29.89	29.90	29.29
2	30.43	29.31	30.22
3	29.38	30.48	30.17
Delta	1.05	1.17	0.93
Range	2	1	3

*Italic values indicate optimal levels.

Table 4. S/N ratio response table for total monomeric anthocyanin (TMA)

Level	A	B	C
1	20.12	21.72	20.49
2	21.52	19.29	20.89
3	20.01	20.63	20.26
Delta	1.51	2.43	0.63
Range	2	1	3

*Italic values indicate optimal levels.

Table 5. S/N ratio response table for DPPH (%)

Level	A	B	C
1	35.21	34.18	33.42
2	35.24	33.95	34.97
3	32.62	34.94	34.68
Delta	2.62	0.99	1.56
Range	1	3	2

Table 6. SAW results for optimization of bioactive component extraction

Sample	Normalized values			Weighted normalized values			Results	
	TPM	TMA	DPPH	TPM (0.419)*	TMA (0.146)*	DPPH (0.436)*	Score	Range
1	0.873	0.610	0.738	0.366	0.089	0.322	0.776	7
2	0.914	0.446	0.969	0.383	0.065	0.423	0.871	4
3	0.984	0.698	1.000	0.412	0.102	0.436	0.950	2
4	0.986	1.000	0.950	0.413	0.146	0.414	0.974	1
5	0.961	0.528	0.833	0.402	0.077	0.363	0.843	5
6	1.000	0.584	0.911	0.419	0.085	0.397	0.902	3
7	0.918	0.542	0.714	0.385	0.079	0.311	0.775	8
8	0.733	0.607	0.572	0.307	0.089	0.250	0.645	9
9	0.979	0.556	0.715	0.410	0.081	0.312	0.803	6

*Weight values determined by principal component analysis (PCA).

The optimum extraction variables for the extract with higher antioxidant activity were determined as 75%, 50 °C, and 20 min for ethanol concentration, extraction temperature, and sonication time, respectively. The most effective process parameter on DPPH (%) values was found to be ethanol concentration. This parameter was followed by sonication time and extraction temperature, respectively.

Multi-Response Optimization Through SAW Method

In the extraction of bioactive substances from onion peel, there were no common optimization conditions for the responses. $A_2B_3C_2$ for TPM, $A_2B_1C_2$ for TMA, and $A_2B_3C_2$ for DPPH (%) were found to be the optimum conditions. In these conditions, the sample providing the best-targeted properties among the available specimens was determined by the SAW method and the results are given in Table 6. The results show that sample 4 ($A_2B_1C_2$), which has the highest score (0.974) in the ranking, represents the optimal extraction process for all three responses.

Conclusion

In this study, extraction conditions were optimized by the Taguchi-SAW hybrid method for an extract rich in phenolics and anthocyanins and high antioxidant activity from onion peel rich in bioactive components. Individual responses in the extraction process were successfully optimized by Taguchi optimization. Multiple response optimization was also performed successfully with the SAW ranking method. It is recommended to verify the success of this hybrid technique by comparing it with alternative optimization methods such as the response surface method, genetic algorithm, and fuzzy logic.

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Effectiveness of Phosphorous acid, *Bacillus subtilis* and Copper Compounds on Apple cv. Gala with M9 Rootstock in the Control of Fire Blight

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ABSTRACT

Necrogenic Gram-negative bacterium *Erwinia amylovora*, which causes economic losses especially in pome fruits such as apple, pear, quince and loquat, some berries and many ornamental plants, causes fire blight disease. Copper compounds are used extensively in disease control programs and they can cause toxicproblems in terms of plant and environmental health. In addition, the formation of resistance to copper in the pathogen is frequently observed. In this study, plant activator phosphorous acid and biological control agent *Bacillus subtilis*, and 3 different copper compounds, Copper sulfate, Copper oxychloride and Copper hydroxide which are commonly used against *E. amylovora*, on apple cv. Gala with M9 rootstock were evaluated comparatively. When the new season shoot lengths of 3-year-old plants with homogeneous growth reached 20-25 cm, chemicals and *B. subtilis* were applied first time before one week ago from the pathogen inoculation, and after 2 times with 1 week intervals. The youngest two leaves at tips of actively growing terminal plant shoots were inoculated by cutting off using scissors dipped in suspension of *E. amylovora* str. EaARADY5 containing 10^8 CFU ml^{-1}. Disease assessments were made after the disease severity (%) was determined on the basis of shoot blight after symptom development of the disease stopped, and the results were found to be statistically significant ($p<0.05$). According to the findings, while the highest effect was obtained by copper oxychloride with 69% effectiveness among all applications, the lowest effectiveness was obtained with 43.5% copper sulfate. It has been revealed that the use of the most effective of the copper compounds in field applications will result in less exposure to chemicals in terms of human and environmental health, and that *B. subtilis* and phosphorous acid can be used significantly in the integrated control of fire blight.

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Introduction

Apple (*Malus* x *domestica* L.) is the third most widely produced fruit worldwide, following bananas and grapes (FAO, 2018). In Turkey, it accounts for approximately 4% of global apple production, with a total of 3.6 million tons (Anonymous, 2020). The highest production area in Turkey is in Niğde, with 235,150 hectares dedicated to apple cultivation. Niğde, Isparta, Karaman, Antalya, and Konya provinces collectively constitute 53.4% of Turkey's total apple production areas, covering approximately 912,000 hectares (Anonymous, 2020).

Diseases and pests lead to significant yield losses in apple production. Fire blight disease, caused by *Erwinia amylovora*, was first observed in the United States in 1780. The pathogen is a highly serious and destructive disease reported in more than 50 countries worldwide, causing significant issues, especially in apple trees and many ornamental plants belonging to the Rosaceae family (Zhao et al., 2019). The disease also causes symptoms of wilting and cankers in plants, in addition to shoot, leaf, and fruit blight. Fire blight leads to significant economic losses worldwide. While a permanent effective method for combating the disease has not been identified, every year, the size of infected apple and pear orchards decreases due to quarantine-related measures (Deckers and Schoofs, 2007). Controlling this disease is only possible by discovering and implementing the most suitable control methods.

Copper compounds, especially in countries like the European Union where antibiotic use is restricted, are still widely used in the fight against fire blight disease. This application is one of the most common methods to control bacterial plant diseases, but it has led to the development of various strategies by many bacteria against copper ions. To date, there is very little information about the interaction between *E. amylovora* and Cu^{2+} ions (Ordax et al., 2006).

Globally, various copper-based preparations with different compositions are used in the current chemical control programs for the disease. The limited use of effective copper compounds against the disease is becoming important due to issues such as the rapid development of resistance to copper preparations, resulting in a loss of effectiveness, harm to beneficial bacteria, and phytotoxicity (Saygılı, 2008).

Plant activators also provide protection against bacterial diseases in addition to fungal diseases (Yüce et al., 2020). Phosphorous acid is rapidly absorbed by the plant and can be transported to all parts of the plant through the phloem and xylem, thereby strengthening the plant's immune system.

Bacillus subtilis is a commonly used biological control agent in the management of bacterial diseases (Abbasi and Weselowski, 2014; Fousia et al., 2016; Ibrahim et al., 2016). It has been reported that *B. subtilis* induces systemic acquired resistance (SAR) in plants (Borriss, 2011).

In this study, in addition to determining the most effective copper compound in Gala apple variety with M9 rootstock against fire blight disease and allowing the use of a small number of copper compounds in terms of human and environmental health, the usability of *B. subtilis* and phosphorous acid applications in integrated fire blight control programs was investigated.

Materials and Methods

Materials

Three-year-old apple cv. Gala seedlings with M9 rootstock, known to be susceptible to *Erwinia amylovora*, were used as the plant material for this study.

The *E. amylovora* str. EaARADY5 was provided from the Molecular Bacteriology Laboratory collection of Selçuk University, Department of Plant Protection. The biological agent, copper compounds and phosphorous acid used in the experiment were obtained from commercial companies (Table 1).

Methods

In vitro experiments

The chemicals provided in Table 1 were added to Nutrient Agar (NA) medium at the specified doses and intensities after autoclaving and cooling. The pathogen suspensions were prepared from 48-hour fresh cultures of *E. amylovora* str. EaARADY5 at a concentration of 10^8 CFU ml^{-1}, determined by measuring an optical density of 0.15 at 600 nm using a spectrophotometer (Eppendorph Bioplus). These suspensions were then spread on NA medium containing chemicals compounds used in the experiments.

The application of *B. subtilis* was carried out after streaking the *E. amylovora* str. EaARADY5 onto Petri dishes.

After incubating the Petri dishes at 27°C for 48-72 hours, the bacterial population densities were determined by counting the colonies that developed on the medium following the method described by Klement et al. (1990).

Bacterial population densities (cell ml^{-1}) = NC × DS × 10

(NC; number of the colony, DS; dilution series)

In vivo experiments

The experiments were conducted under controlled greenhouse conditions with a temperature of 23-25°C and 80% relative humidity, following a 16-hour light / 8-hour dark cycle. The potting soil for the plants was prepared as a mixture of soil, composted animal manure, and peat in a 1:1:1 ratio. During the growing season; the plants were fertilized with NPK (20,20,20) once time to growth healthy, and sulfur (WP, 40g L^{-1}) was applied twice to protect against powdery mildew disease.

Application of chemicals and biological agent

The chemicals and B subtilis used in the experiments were applied twice; first when the new season shoots of homogeneous growing Gala apples with M9 rootstock plants reached 20-25 cm in length, and the second three days after *E. amylovora* inoculation. The inoculation of *B. subtilis* was carried out by spraying the leaves of the plants with a pressurized hand sprayer, prepared from a 48-hour fresh culture with a concentration of 10^8 CFU ml^{-1}, as measured by a biophotometer (Eppendorph bioplus, OD: 660 nm; 0.15). Chemicals were applied to the leaves of the plants using a pressurized hand sprayer at the recommended doses by the manufacturer.

Pathogen inoculation

Inoculation of the pathogen was performed by cutting the two youngest leaves at the tips of actively growing terminal plant shoots, using sterilized scissors dipped in a suspension of *E. amylovora* str. EaArady5 containing 10^8 CFU ml^{-1}. Inoculations were carried out one week after the initial applications. The surfaces of the inoculated shoots were covered with a polyethylene bag for the first 24 hours following inoculation (Bonasera et al., 2006).

Re-isolation of the pathogen

Before disease assessments were made, in accordance with Koch's postulates, pathogen re-isolation and diagnosis were conducted using samples taken from shoots displaying disease symptoms (Koch, 1884). The diagnosis of the pathogen was carried out through biochemical, morphological, and physiological tests according to Schaad et al. (2001).

Table 1. Chemicals, biological agent, formulations, application method, and usage rates used in the experiments

Application	Commercial Name	Formulation	Application	Usage Dosage
Erwinia amylovora	-	S	leaf	10^8 CFU ml^{-1}
Copper sulfate	Mastergold	SC	leaf	125 ml / 100 liters of water
Copper oxychloride	Cuprocol	SC	leaf	150 ml / 100 liters of water
Copper hydroxide	Kocide 2000	WG	leaf	175 g/ 100 liters of water
Phosphorous acid	Fosfogard	SC	leaf	200 ml / 100 liters of water
Bacillus subtilis QST713	Serenade	S	leaf	10^8 CFU ml^{-1}

Table 2. The effects of copper compounds (copper hydroxide, copper oxychloride and copper sulfate), phosphorous acid, and *Bacillus subtilis* QST713on bacterial populations *in vitro*.

Application	Bacterial growth (CFU ml^{-1})
Erwinia amylovora (Control)	1.60×10^6
Phosphorous acid	1.50×10^4
Bacillus subtilis QST713	1.40×10^2
Copper sulfate	1.20×10^1
Copper hydroxide	0.20×10^1
Copper oxychloride	0.19×10^1

Table 3. Determination of disease severity and effectiveness of phosphorous acid, *Bacillus subtilis*, and commonly used copper compounds against *Erwinia amylovora*.

Application	Disease Severity (%)	Effectiveness of the Treatment (%)
Erwinia amylovora (Control)	70.67 a	-
Copper sulfate	39.86 ab	43.59
Bacillus subtilis QST713	38.94 ab	44.89
Phosphorous acid	32.75 ab	53.65
Copper hydroxide	32.18 ab	54.46
Copper oxychloride	21.72 b	69.26

These tests included growth at 36°C, gelatin hydrolysis test, KOH test, growth in 5% SNA, fluorescence pigment formation on King B medium, oxidative-fermentative test, reduction of compounds from sucrose, esculin hydrolysis, cysteine H2S production, and acid production from carbohydrates.

$$DS\ (\%) = a / b \times 100$$

(DS = disease severity, a = length of the blighted of the shoot (cm), b = total length of the shoot (cm))

The percentage effectiveness of the applications used in the experiments was determined using the Abbott (1925) formula, shown in the following lines:

$$E\ (\%) = (K - U / K) \times 100,$$

(E = effectiveness, K = percentage disease severity of the control plant, U = percentage disease severity of the treated plant).

Molecular diagnosis of the pathogen using specific primer base pairs A/B (A: 5'- CGGTTTTTAACGCTGGG-3' and B: 5'- GGGCAAATACTCGGATT- 3') and using the PCR protocol (at 95°C for 3 sec (1 cycle), followed by 94°C for 1 sec, 52°C for 1 sec, and 72°C for 2 sec (35 cycles), a final extension at 72°C for 10 sec (1 cycle)) suggested by Bereswill et al. (1992) was performed by a thermal cycler (Eppendorph Personal). The PCR products were evaluated by using a Prizma imaging device after electrophoretic seperation (Russell and Sambrook, 2001).

Evaluation of treatments

After disease symptoms ceased (on the 30th day after the pathogen inoculation), the effects of the treatments on the disease severity percentage (DS, %) were determined by dividing the length of necrotic tissue by the total shoot length, as shown in the following formula (Fernando and Jones, 1999; Aldwinckle et al., 2002).

Statistical Analyses

The data obtained from the study were subjected to variance analysis using the MINITAB version 14 program, and statistical evaluations were conducted using the MSTAT program, where the Tukey multiple comparison test was applied to determine the interactions between the treatments and the disease (Düzgüneş et al., 1983).

Results

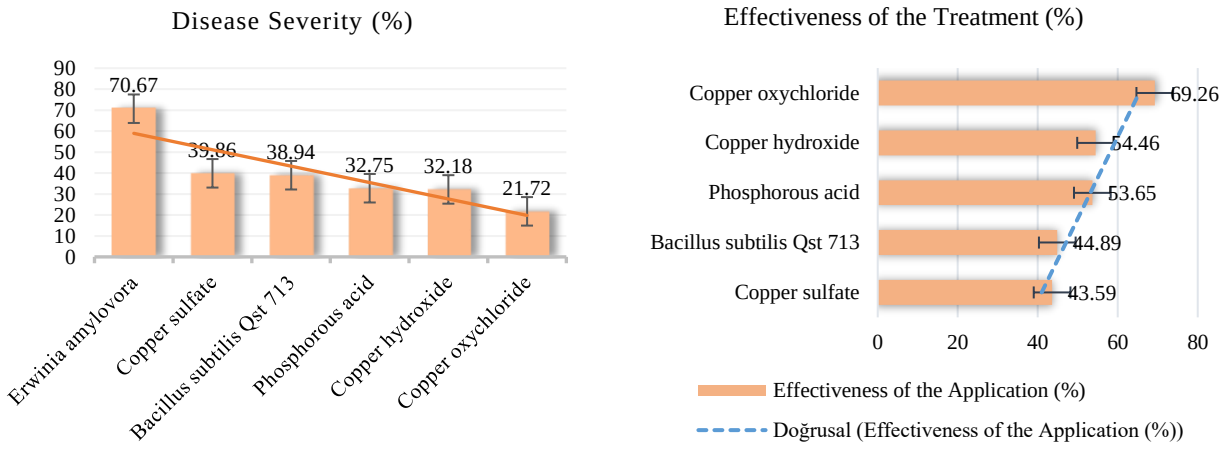
In this study, the effectiveness of copper sulfate, copper oxychloride, copper hydroxide, phosphorous acid, and *Bacillus subtilis* QST 713 against fire blight disease, which significantly impacts the yield and quality of Gala apple trees on M9 rootstock, was determined under *in vitro* and *in vivo* conditions.

In vitro experiments

The copper compounds (copper hydroxide, copper oxychloride, and copper sulfate) used in the experiments, phosphoric acid and *B. subtilis* were used at commercial application doses to determine the development of *E. amylovora* bacterial populations on NA medium and data were shown in Table 2. Accordingly, the most successful copper compounds were identified as copper hydroxide and copper oxychloride, while the lowest effectiveness was obtained by phosphorous acid on fire blight.

In vivo experiments

Copper-based compounds, phosphorous acid and *B. subtilis* were applied under in vivo conditions according to the dosages recommended by commercial companies, and the percentage of disease severity caused by *E. amylovora* on apple cv Gala with M9 rootstock plants was determined. According to the findings, the highest efficacy, with a 69% effectiveness, was achieved with copper oxychloride, statistically, all treatments (copper sulfate, copper hydroxide, phosphorous acid and *B. subtilis* QST713) had the close to effectiveness, despite numerical differences (Table 3; Figure 1; Figure 2).



a b

Figure 1. Disease severity and effectiveness of phosphorous acid, *Bacillus subtilis* QST713, and commonly used copper compounds (Copper sulfate, Copper oxychloride, Copper hydroxide) against *Erwinia amylovora* (a) disease severity (%) and (b) effectiveness of the treatments (%).

a b c

d e f

Figure 2. Shoot blight caused by *Erwinia amylovora* on apple plants cv. Gala with M9 rootstock, a) Control plants with only pathogen inoculation, Plants treated with b) Copper oxychloride, c) Copper sulfate, d) Copper hydroxide, e) Phosphorous acid, f) *Bacillus subtilis* QST713.

Table 4. Results of biochemical and molecular diagnostic tests conducted for the re-isolates.

Tests	RI	RZ
Gram reaction	G(-)	G(-)
Growth at 36°C	-	-
Hydrolysis of gelatin	+	+
Levan formation	+	+
Fluorescent pigment on KB agar	-	-
Oxidative-Fermentative Test	+	+
Esculin hydrolysis	-	-
H_2S production from Cysteine	-	-
Acid production from carbohydrates		
Sorbitol	+	+
Mannitol	-	-
Maltose	-	-
Tobacco hypersensitivity reaction (HR) test	+	+
PCR assay with specific A/B primers (1 kb)	+	+

RI: Reference isolate (ARADY5), RZ: Isolates obtained through re-isolation, (+) positive reaction, (-) negative reaction

Re-Isolation and Diagnosis of the pathogen

Bacterial isolations were conducted to confirm that the disease symptoms in the treated and control plants were caused by *E. amylovora*. The pathogen responsible for the disease was identified as *E. amylovora* through the biochemical, morphological, physiological, and molecular diagnostic tests provided in Table 4.

Discussion

One of the major quality and economic losses in apple production is caused by fire blight disease, which is attributed to *E. amylovora* (Deckers and Schoofs, 2007).

The management of the disease is quite challenging due to the lack of specific synthetic compounds that directly affect the pathogen (Myung et al., 2016). Additionally, *E. amylovora* can rapidly spread and develop resistance to antibiotics (McGhee and Sundin, 2011). Antibiotics not only have adverse effects on the environment and soil but also pose challenges in clinical medicine since commensal microorganisms serve as reservoirs for antibiotic resistance genes (McManus, 2014; Lamichhane et al., 2018). For these reasons, their use is banned in a lot of country.

It has been reported that the use of copper-based preparations in disease management can lead to the development of resistance in the pathogen and, at the same time, cause phytotoxicity in the plant (Saygılı, 2008). In Turkey, copper-based preparations, biological agents, and plant growth regulators are commonly used. Therefore, it is of great importance to limit the use of effective copper compounds against the disease to a minimum.

Copper compounds are effective bactericides in some areas, but they can lead to phytotoxic effects on leaves and fruits. Therefore, they are generally used in strategies combined with other compounds or reduced spraying programs, often applied before flowering (Ninot et al., 2002). Momol et al. (1991) reported that a combination of maneb and copper allowed for successful results without causing any phytotoxicity in plants when applied following the flowering period.

Saygılı and Üstün (1995) conducted research on the effectiveness of various chemicals against *E. amylovora in vitro* and on pear fruits. They determined that the most effective chemicals were, in order, streptomycin, copper salts + mancozeb, kasugamycin, and copper oxychloride + maneb. They also found that copper hydroxide was more effective than copper oxychloride, and copper sulfate showed lower activity compared to other copper compounds. In our study, we tested on apple plant shoots, and copper oxychloride was identified as the most effective copper compound with an effectiveness rate of 69.26%, while copper sulfate showed the lowest effectiveness at 43.59%. The difference in the results obtained with the copper hydroxide used in our study, which shows parallels with Saygılı and Üstün (1995), suggests that different results can be achieved by using different formulations of the chemical in different plants and different climates.

Recently, Butt and Bastas (2021) determined the antagonistic effect of Bacillus spp against Erwinia amylovora. They found that Bacillus subtilis QST713 had a success rate of 54.75%, while *B. amyloliquefaciens* MBI600 had a success rate of 47.01%. *Bacillus subtilis* QST713 has been reported to provide an average of 60% control and to yield the best results when used as part of an integrated program (Johnson and Temple 2013; Smith 2012, 2015). According to Aldwinckle et al. (2002), the commercial strain Bacillus subtilis QST713 had a success rate of 64.3% in controlling flower infections of fire blight disease. In our study, the Bacillus subtilis QST713 has become a success rate of 44.84%.

Considering all the results, it is thought that it may be beneficial to use *B. subtilis* together with other antagonistic factors after preliminary trials to increase the success of *B. subtilis* in the combat against fire blight.

Phosphorous acid, also known as phosphonic acid with the chemical formula H_3PO_3, is one of the colorless oxyacids of phosphorus. It is rapidly absorbed by plants and can be transported throughout the plant's various parts via the phloem and xylem, strengthening the plant's immune system. It can be used to prevent fungal diseases as well as bacterial diseases (Yüce et al., 2020).

Norman et al. (2006) tested the effectiveness of phosphorous acid against *Ralstonia solanacearum*, which causes bacterial wilt in geranium (*Pelargonium hortorum*). They found that commonly used phosphorus-containing products in the industry, such as phosphorus pentoxide (P_2O_5) and phosphoric acid (H_3PO_4), were unable to protect the plant from infection. In contrast, they determined that phosphorous acid (H_3PO_3) was successful (Norman et al., 2006).

The phosphorous acid used in our study caused similar successful results with some copper compounds and *B. subtilis* applications. These results suggest that phosphorous acid will be included in programs that can be applied both in plant development and in the combat against fire blight disease.

This study has demonstrated that copper oxychloride and copper hydroxide stand out due to their success in field conditions and they can be used alternately and fewer in numerical terms in combat against the disease. Additionally, it highlights that *B. subtilis* and phosphorous acid can be significantly employed in the integrated and eco-friendly control of fire blight.

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Potential Biological Control Agents against Soft Rot Diseases Caused by Pectobacteria on Some Sugar Beet Cultivars

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ABSTRACT

Sugar beet is one of the most economically important agricultural crops cultivated in many provinces of Turkey. Especially in recent years, there has been an increase in bacterial tuber rot due to factors related to climate change. In preliminary trials, soft rot disease by *Pectobacterium caratovorum* subsp. *caratovorum* (Pcc) and *Pectobacterium betavasculorum* (Pb) were detected predominantly in sugar beets in Central Anatolia. Today, some cultural measures and copper compounds are used against soft rot agents in sugar beet, but successful results cannot be obtained in preventing the disease. In this study, a total of 270 soil samples were taken from the rhizosphere of 10 different fields in 3 different periods in 3 different ecologically diverse districts (Çumra, Altınekin and Seydişehir) of Konya, one of the provinces with the highest amount of sugar beet production in Turkey. As a result of the isolations, a total of 3064 bacterial isolates were purified and 262 of them showed antibacterial activity against Pcc and Pb *in vitro* conditions. In addition, 15 antagonist bacteria with the highest inhibitory effect on the development of both pathogens were tested in greenhouse conditions, and according to the results obtained from here, 3 antagonists with the highest effect were tested in field conditions in the cultivation areas of 3 different districts named above. Biochemical, morphological and molecular diagnoses of antagonist bacteria with high efficacy were made. According to the results obtained, it has been concluded that rhizospheric bacteria with antagonistic effect have a success rate of 33-90% against Pcc and Pb pathogens, and that the biological products to be prepared in future studies can be used in ecological, climate friendly and within sustainable agricultural practices in sugar beet production areas.

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Introduction

Sugar, a leading agricultural food resource, has been a fundamental food item with numerous applications of strategic importance for many countries for centuries. In Turkey, 95% of the sugar produced is derived from sugar beets. Sugar beet is a strategically important agricultural product in our country, both in terms of production and industry. Sugar beet (*Beta vulgaris* var. *saccharifera*) is an industrial plant that belongs to the Chenopodiaceae family, with origins in Central Europe. It is grown as an annual crop for sugar production and as a biennial crop for seed production. Additionally, sugar beet yields various by-products such as molasses, alcohol, yeast, antibiotics, bioethanol, apart from sugar (Sunulu and Sunulu, 2016). According to the Sugar Beet Product Report published by the Institute of Agricultural Economics and Policy Development in 2022, it is reported that approximately 18 million tons of sugar beet were produced in an area of about 300,000 hectares during the 2021-2022 production season, resulting in the production of 2.52 million tons of sugar (Anonymous, 2023).

According to the 2022 data in Turkey, sugar beet was cultivated in 56 provinces. The province of Konya, where the highest sugar beet production takes place, accounts for 25% of Turkey's cultivation area and covers 29% of the production. In a study that investigated the susceptibility of some commonly cultivated sugar beet varieties in Turkey to bacterial soft rot pathogens of the Pectobacterium genus, Bastas and Kaya (2019) obtained soft rot bacteria from diseased sugar beets during the 2016-2017 growing season in Konya and Karaman provinces. Biochemical, physiological, morphological, and PCR-based molecular analyses were performed for the identification and characterization of the isolates, and it was determined that the main pathogens causing soft rot disease in sugar beet plants are Pectobacterium caratovorum subsp. caratovorum and Pectobacterium betavasculorum.

Pectobacterium betavasculorum and *Pectobacterium carotovorum*, both of which are soft rot pathogens in sugar beets, are among the most destructive bacterial disease agents in sugar beet root diseases. These pathogens can also be transmitted through seeds and cause significant losses in plant growth, particularly in tuber yield and quality when suitable moisture and temperature conditions are present for pathogen development (Bastas and Kaya, 2019).

Bacteria that secrete a high amount of pectolytic and hydrolytic cell wall-degrading enzymes such as polygalacturonases, pectinases, and cellulases, develop intracellularly, leading to the softening and complete rotting of root tissues (Fassihiani and Nedaeini, 2008). Symptoms of the disease can be noticed after the roots have rotted and turned wet, dark brown, and the leaves have wilted. The disease can occur at any time during the growing season when environmental conditions are favorable. In the later stages of vegetation, soft and wet darkened tissue develops within the root structure. In the final stage of infection, sugar beet roots are completely deteriorated, resulting in a structure that has no value in terms of yield and quality. Sugar beets infected with soft rot agents continue to cause infections in silos after harvest.

These diseases occurring in sugar beet roots, which are of great agricultural and economic importance, not only result in yield losses but also cause a significant reduction in sugar content. Despite the use of certain cultural measures and copper compounds against these pathogens, successful results in disease prevention have not been achieved. Therefore, biological control studies aimed at effective, environmentally friendly, and sustainable agricultural production against *Pectobacterium caratovorum* subsp. *caratovorum* and *Pectobacterium betavasculorum*, which cause soft rot in sugar beet, are of great importance.

In this study, antagonist bacterial agents isolated from soil samples collected from sugar beet cultivation areas in Konya, a central hub for sugar beet cultivation in our country, were investigated in vitro and in vivo to explore the possibilities of biological control against soft rot pathogens *Pectobacterium caratovorum* subsp. *caratovorum* and *Pectobacterium betavasculorum*.

Materials and Methods

Isolation of Bacterial Soft Rot Pathogens and Antagonistic Bacteria

In this study, a total of 270 soil samples were collected from the rhizosphere of both diseased and healthy sugar beet plants at three different critical growth stages of sugar beets, marked with GPS coordinates, from 10 different sugar beet fields in the Altınekin, Çumra, and Seydişehir districts. The samples were collected from each field at three different time periods corresponding to three critical growth stages, including post-thinning, the beginning of tuber formation, and pre-harvest periods. The first period, post-thinning samples were collected from May 26th to June 1st, 2021, the second period, tuber formation samples were collected from July 30th to August 5th, 2021, and the third period, pre-harvest samples were collected from October 8th to October 14th, 2021. The collected samples were transferred to sterile paper bags for the isolation of antagonist isolates and then transported to the laboratory under cold chain conditions.

In addition, reference pathogenic isolates, *Pectobacterium caratovorum* subsp. *caratovorum* S1 isolate, were obtained from Selçuk University Faculty of Agriculture Molecular Plant Bacteriology Laboratory (Prof. Dr. Kubilay Kurtulus Bastas), and *Pectobacterium betavasculorum* isolate were sourced from Bozok University Faculty of Agriculture, Department of Plant Protection (Assoc. Prof. Dr. Murat Öztürk).

Isolation of Bacterial Agents

During the isolation of bacterial isolates, 10 grams of soil sample was transferred to 90 ml of sterile water, and the mixture was horizontally shaken at 150 rpm for 30 minutes. After the shaking process, dilutions of 10^{-3} CFU ml^{-1} and 10^{-5} CFU ml^{-1} were prepared from the suspensions of each sample. Subsequently, 100 µl from each suspension was triple streaked onto nutrient agar (NA) culture plates. Petri dishes were incubated at 25°C for 24-48 hours, and the developed bacterial colonies were grouped based on color, shape, and distinct colony characteristics. The purified bacteria were stored at -80°C in nutrient broth (NB) containing 25% glycerol until further use.

Pathogenicity Test of Pathogens

Isolates of *Pectobacterium betavasculorum* (Pb) and *Pectobacterium caratovorum* subsp. *caratovorum* (Pcc), cultured in NA medium for 48 hours, were adjusted to an absorbance of 0.2 at 600 nm using a spectrophotometer. Prior to application, a 2 mm diameter wound was created in the root area using a metal punch, and 25 ml of bacterial suspension was inoculated into the wound tissue. The inoculated plants were left for two weeks at 25°C and 75% humidity along with negative control groups. The reference culture Pcc isolate was used as a positive control, and sterile distilled water was applied as a negative control (Khan and Siddiqui 2020).

Determination of Hiper-Sensitivity Reaction in Tobacco by Antagonists

Before pot experiments with antagonist isolates found to be effective in in vitro efficacy tests, over-sensitivity tests were conducted in tobacco to determine the possibility of being a phytopathogen. Suspensions of 10^{8} CFU ml^{-1}density in saline buffer were prepared from the isolates with antibacterial activity developed in NA medium for 48 hours. Each prepared suspension was injected into the interveinal area between two veins of the tobacco plant (*Nicotiana tabacum* cv. Samsun). Necrosis formation in the inoculated area was considered a positive reaction 24-48 hours after inoculation (Lelliot and Stead, 1987). Sterile water was applied as a negative control, and Pcc isolate was used as a positive control.

Identification of Soft Rot and Antagonistic Bacterial Agents

For all bacterial agents obtained in the study, biochemical, morphological, and physiological tests were conducted according to Sachaad et al. (2001), and preliminary identifications of pathogenic and antagonistic bacteria were performed. In molecular diagnosis, bacterial

DNA isolation was carried out following the method of De Boer and Ward (1995), and the PCR primers used for Pb and Pcc are listed in Table 1.

After conducting biochemical, morphological, and biochemical tests for antagonist bacterial agents, molecular level MALDI-TOF-MS diagnoses were performed at the Mustafa Kemal University Plant Health Application and Research Center, and 16SrRNA diagnoses were obtained through service procurement from Subgenomic Analysis company.

Determination of in vitro Antagonistic Activity of Antagonist Bacterial Isolates

The biocontrol activities of candidate antagonist bacterial isolates, isolated from the sugar beet rhizosphere microbiota, were determined under in vitro conditions using a binary comparison test in NA medium. In the experiments, the effectiveness values for each isolate were calculated by dividing the zone diameter formed around the bacterial colony by the diameter of the bacterial colony (Bora and Özaktan, 1998; Aysan et al., 2003; Bozkurt and Soylu, 2019; Bitgen and Mirik, 2021). The trial was conducted with three replications. After incubation, suspensions of Pb and Pcc pathogens at a density of 10^8 CFU ml^{-1} were sprayed onto petri dishes at equal distances around the bacterial colonies using a hand sprayer for each application. Blank disks containing 25 µg streptomycin sulfate were used as a positive control (Umarusman et al., 2019). After 48 hours of incubation at 25°C, the inhibition zones formed around the isolates and the colony diameter of the isolates were measured in millimeters and recorded. All isolated isolates were tested separately for both pathogens.

In vivo Experiments of Antagonists

Greenhouse Experiments

In controlled greenhouse conditions, pot experiments were set up in a randomized complete block design. In 3-liter pots containing a 1:1:1 mixture of sterile peat, perlite, and soil, 3 sugar beet seeds belonging to the Mohican (sensitive) and Rodeo (resistant) varieties were sown. Two weeks after the cotyledon stage of sugar beet seedlings, thinning was performed to leave one plant per pot. Each treatment was planned with 5 replications, with one plant in each replication. Separate experiments were conducted for Pb and Pcc applications, resulting in a total of 340 pots. Fifteen antagonist isolates with the highest combined effectiveness ratio against Pb and Pcc, as determined in *in vitro* efficacy tests, were selected and tested in pot experiments.

Fifteen common antagonist isolates were prepared at a concentration of 10^9 CFU ml^{-1} in the NA medium. Prior to antagonist applications, a 1 cm wound tissue with a thickness of 2 mm was created in the root-shoot of each plant using a metal punch, 3 cm below the soil surface. Then, 25 ml of antagonist applications were made to the root area. Negative control applications included 25 mg/liter of 25 ml streptomycin sulfate, and positive control applications consisted of 25 ml of water (Ganiyu et al., 2023).

One week after the antagonist application, Pectobacteria cultured for 48 hours in NA medium were inoculated into slices at a density of 10^9 CFU ml^{-1} using a syringe. After inoculation, 25 ml of pathogen bacterial suspensions were applied to the root area of each plant. Negative control applications included 25 mg/liter of 25 ml streptomycin sulfate, and positive control applications involved the inoculation of pathogen bacteria into sugar beet seedlings that had been watered one week earlier (Ganiyu et al., 2023).

Field Experiments

Field experiments were set up in the Altınekin, Çumra, and Seydişehir districts where antagonist isolates were obtained. These field trials were established to compare the disease suppression potential of the same antagonists in areas with ecological differences.

Seeds of sugar beet varieties Mohican and Rodeo were sown with row spacing of 1×0.75 meters, with 20 cm between rows (6 plants) and 25 cm between rows (4 plants), resulting in a total of 24 plants per plot. As the basal fertilizer, DAP (Diammonium Phosphate) was applied at a rate of 20 kg/da. Herbicide treatment with Stomp Extra herbicide was conducted prior to crop emergence for weed control. The field trials were planned with a randomized complete block design, with three replications, and a total of 24 plants in each replication.

In the greenhouse experiments, three antagonists were selected that were effective against both pathogens and exerted the highest disease suppression. The same method and bacterial inoculum density used in the greenhouse experiments were applied in field experiments. Similarly, bacterial pathogens were applied using the same method and bacterial density, but two applications were made to each plant, with a one-week interval between them, using 50 ml of pathogen bacterial suspensions each time.

Effectiveness Evaluations

All plants in the experiment were harvested, and the lesion length in the longitudinally cut tubers was measured with the help of a digital caliper and expressed as a ratio to tuber length. Disease severity (%) was calculated accordingly. The effectiveness of antagonist isolates was determined by comparing them to the positive control using the Abbott formula (% efficacy: (control-treatment/control) ×100) (Karman, 1971).

Statistical Analysis

The % efficacy of different treatments was analyzed using the Anova statistical program with the LSD multiple comparison test at a significance level of $P \leq 0.05$ (Umarusman et al., 2019).

Table 1. PCR Primers Used for Pb and Pcc

Primer	Pathogen	PCR Products (bp)	Reference
L1(5'CAAGGCATCCACCGT3') G1(5'GAAGTCGTAACAAGG3')	Pb	540 620	(Toth et al., 2011)
Y1('TTACCGGACGCCGAGCTGTGGCGT') Y2('CAGGAAGATGTCGTTATCGCGAGT')	Pcc	434 434	(Darrasse et al., 1994)

Table 2. PCR Protocols for Pb and Pcc

L1 G1 Primers			Y1 Y2 Primers		
Initial denaturation	94°C - 5 dk		Initial denaturation	94°C - 5 dk	
Denaturation	94°C –1 dk		Denaturation	94°C –30 sn	
Annealing	55° C –2 dk	28 Cycles	Annealing	55° C –45 sn	34 Cycles
Extension	72° C –2 dk		Extension	72° C –45 sn	
Final extension	72°C - 7 dk		Final extension	72°C - 7 dk	

Table 3. Reactions of isolated Pb and Pcc isolates in diagnostic tests

Analysis	Pb (Ref.)	Pcc S1 (Ref.)	Pb A2	Pb A5	Pcc Ç4	Pcc Ç11	Pb Sy9	Pcc Sy4	Pcc Sy7	Pcc Sy9
Gram Reaction	-	-	-	-	-	-	-	-	-	-
CVP Deepening	+	+	+	+	+	+	+	+	+	+
Pectolytic Activity	+	+	+	+	+	+	+	+	+	+
Bacterial Growth at 37°C	+	+	+	+	+	+	+	+	+	+
Bacterial Growth in 5% NaCl	+	+	+	+	+	+	+	+	+	+
Oxidase Reaction	-	-	-	-	-	-	-	-	-	-
Levan Production	+	+	+	+	+	+	+	+	+	+
Presence of Catalase	+	+	+	+	+	+	+	+	+	+
Hypersensitivity Reaction (HR)	+	+	+	+	+	+	+	+	+	+
Erythromycin Sensitivity	-	-	-	-	-	-	-	-	-	-
Utilization of a-Methyl-Glucoside	+	+	+	+	+	+	+	+	+	+
KB Fluorescent Pigmentation	-	-	-	-	-	-	-	-	-	-
Reduction of Substances from Sucrose	+	+	+	+	+	+	+	+	+	+
Indole Production	-	-	-	-	-	-	-	-	-	-
Phosphatase Production	-	-	-	-	-	-	-	-	-	-
Acid Production from Lactose	+	+	+	+	+	+	+	+	+	+
Acid Production from Maltose	+	+	+	+	+	+	+	+	+	+
Acid Production from Trehalose	+	+	+	+	+	+	+	+	+	
Acid Production from Sorbitol	-	-	-	-	-	-	-	-	-	-
Acid Production from Malonate	-	-	-	-	-	-	-	-	-	-

Results

Identification of Soft Rot Pathogens

The soft rot isolates obtained were identified as Pb and Pcc based on biochemical, morphological, physiological, HR, and pathogenicity tests (Sachaad et al., 2001). Diagnostic tests of pathogens are listed in Table 3.

In molecular diagnosis, specific bands of approximately 540 and 620 bp were obtained for the Pb isolates using L1/G1 primers, and for Pcc isolates, approximately 434 bp bands were obtained using Y1/Y2 primers. According to the results obtained, it was determined that 2 isolates from the Altınekin region were Pb, 2 isolates from the Çumra region were Pcc, 3 isolates from the Seydişehir region were Pcc, and 1 isolate was Pb.

Isolation of Antagonist Bacterial Agents

In this study, a total of 3064 bacterial isolates were obtained from 270 soil samples collected from Altınekin, Çumra, and Seydişehir districts. According to their regional distribution, Altınekin region obtained 378 isolates from the post-thinning period, 356 isolates from the beginning of tuber formation period, and 315 isolates from the pre-harvest period samples. Çumra region obtained 318 isolates from the post-thinning period, 360 isolates from the beginning of tuber formation period, and 345 isolates from the pre-harvest period samples. Seydişehir region obtained 316 isolates from the post-thinning period, 328 isolates from the beginning of tuber formation period, and 348 isolates from the pre-harvest period samples.

Determination of Antagonist Bacterial Isolates' Antagonistic in vitro Activity

In the *in vitro* conditions with Petri dishes containing NA agar, a total of 3064 bacterial isolates from sugar beet rhizosphere were tested against Pb and Pcc separately. As a result of these tests, 64 isolates from the I. period, 136 isolates from the II. period, and 60 isolates from the III. period showed antagonistic effects. Of all antagonist isolates, 45% were effective against Pcc, 50% against Pb, and 28% showed antagonistic effects against both pathogens (Figure 1).

The results of the tests showed that the IS.Ç.5.1.C isolate had a higher efficacy value than the streptomycin antibiotic used as the positive control. The most successful 15 antagonists that showed antagonistic effects against both pathogens are listed in Table 4.

Effect of Antagonists on Soft Rot Disease in Greenhouse Trials

Sugar beet plants of the Mohican and Rodeo varieties that underwent antagonist and pathogenic bacterial inoculations were observed for typical soft rot disease symptoms around the inoculation points three weeks after inoculations, and the study was terminated.

According to the results of the sugar beet pot trials established under greenhouse conditions with the 15 antagonist isolates that had the highest efficacy values in in vitro effectiveness tests and showed common effects on Pb

and Pcc (Table 4), Pb caused a disease rate of 97.27% in the susceptible Mohican variety. In the antagonist applications, the IS.A.4.3.O isolate was found to suppress the disease by 89.99%. Pb caused a disease rate of 56.67% in the resistant Rodeo variety. Among the applications, YB.S.7.2.F isolate showed the most successful effect with a suppression rate of 88.00% (Figure 1). According to the results of the Pcc greenhouse trials, Pcc caused a disease rate of 87.39% in the susceptible Mohican variety. In the antagonist applications, the IS.Ç.5.1.C isolate was found to suppress the disease by 90.00%. Pcc caused a disease rate of 95.30% in the resistant Rodeo variety. Similar to the susceptible variety, the IS.Ç.5.1.C isolate showed the most successful effect with a suppression rate of 84.09%.

Table 4. *In vitro* activity value of antagonistic bacterial agents against soft rot agents in sugar beet and their effectiveness under greenhouse conditions (%)

İzolat Kodu	Pcc *In vitro* Activity	Pcc Mohican		Pcc Rodeo	
		Disease Incidence (%)	Disease Control (%)	Disease Incidence (%)	Disease Control (%)
N.C.		0		0	
P.C.	60	87.39a		95.3a	
İS.Ç. 5.1.C	63	8.74h	90.00	15.17f	84.09
HÖ.A.9.2.G	40	43bc	50.80	17.23ef	81.92
İS.A.4.3.O	40	26.16defg	70.07	27.92bc	70.71
YB.A.9.3.D	30	25.16defg	71.22	26.59bcd	72.09
YB.A.8.1.N	23	27.41def	68.64	15.98ef	83.23
YB.A.9.2.O	23	22.23efg	74.56	19.64def	79.39
YB.A.10.2.L	23	14.65gh	83.24	20.16cdef	78.84
YB.S.7.2.F	23	19.68fgh	77.48	28.72b	69.86
YB.S.7.2.N	23	28.7def	67.17	16.99ef	82.17
YB.A.4.1.H	22	25.3defg	71.05	13.62f	85.71
İS.Ç.8.3.A	22	20.56fgh	76.48	25.6bcd	73.13
YB.A.7.3.G	20	20.37fgh	76.69	29.81b	68.72
YB.A.3.1.N	20	36.96bcd	57.71	20.81cdef	78.16
YB.Ç.2.1.A	20	34.21cde	60.85	23.75bcde	75.08
YB.S.7.2.K	20	47.82b	45.29	25.29bcd	73.46
İzolat Kodu	Pb *In vitro* Activity	Pb Mohican		Pb Rodeo	
		Disease Incidence (%)	Disease Control (%)	Disease Incidence (%)	Disease Control (%)
N.C.		0		0	
P.C.	60	97.27a		56.67a	
İS.Ç. 5.1.C	68	16.09f	83.46	23.73b	58.13
HÖ.A.9.2.G	30	18.57f	80.92	12.21bc	78.46
İS.A.4.3.O	35	9.74f	89.99	10.15bc	82.09
YB.A.9.3.D	25	18.85f	80.63	13.96bc	75.36
YB.A.8.1.N	23	49.31b	49.33	7.97bc	85.94
YB.A.9.2.O	23	35.22cd	63.80	11.4bc	79.89
YB.A.10.2.L	23	37.42bcd	61.54	19.62bc	65.38
YB.S.7.2.F	22	33.11de	65.97	6.8c	88.00
YB.S.7.2.N	20	41.06bc	57.80	19.21bc	66.10
YB.A.4.1.H	20	19.22f	80.24	12.24bc	78.41
İS.Ç.8.3.A	20	13.03f	86.61	9.72bc	82.85
YB.A.7.3.G	18	16.11f	83.45	13.2bc	76.70
YB.A.3.1.N	20	36.64cd	62.34	10.54bc	81.40
YB.Ç.2.1.A	17	22.1ef	77.29	9.94bc	82.45
YB.S.7.2.K	18	46.29bc	52.42	9.89bc	82.55

Means followed by the same letter within a column are not significantly different from each other at $P \leq 0.05$ according to Duncan's Multiple Range Test

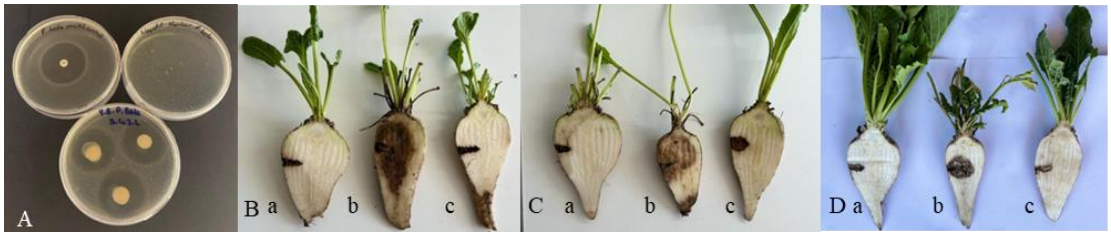

Figure 1. Biological activity assays on sugar beet

A. Antibacterial activity of antagonists against pathogens in *in vitro* effectiveness tests, B. Effect of YB.S.7.2.F isolate on disease severity of *Pectobacterium betavasculorum* in sugar beet variety Rodeo under greenhouse conditions, C. Effect of IS.Ç.5.1.C isolate on disease severity of *Pectobacterium caratovorum* in sugar beet variety Rodeo under greenhouse conditions, D. Effect of IS.Ç.5.1.C isolate on disease severity of *Pectobacterium betavasculorum* in sugar beet variety Rodeo under field conditions (a: Negative Control, b: Positive Control, c: Antagonist Application).

Table 5. Effectiveness of antagonistic bacterial agents against soft rot agents in field conditions in Altınekin, Çumra and Seydişehir sugar beet cultivation areas in Konya province (%)

Treatments	Altınekin Field Trials							
	Pb Mohican		Pb Rodeo		Pcc Mohican		Pcc Rodeo	
	DI	DC	DI	DC	DI	DC	DI	DC
N.C.	0.0		0.0		0.0		0.0	
P.C.	31.84a		40.41a		72.36a		73.38a	
İS.Ç.5.1.C	10.07c	68.38	9.53c	76.42	16.5b	77.18	12.07b	83.55
YB.A.4.1.H	16.13b	49.35	15.26b	62.24	18.52b	74.39	13.42b	81.71
İS.Ç.8.3.A	16.88b	46.98	11.4bc	71.79	10.46b	85.54	16.46b	77.57
Treatments	Çumra Field Trials							
	Pb Mohican		Pb Rodeo		Pcc Mohican		Pcc Rodeo	
	DI	DC	DI	DC	DI	DC	DI	DC
N.C.	0.0		0.0		0.0		0.0	
P.C.	20.84a		34.07a		35.73a		42.88a	
İS.Ç.5.1.C	6.23c	70.12	5.66bc	83.38	7.3c	79.56	8.68b	79.75
YB.A.4.1.H	7.1c	65.91	10.45b	69.33	12.17b	65.94	7.29b	83
İS.Ç.8.3.A	13.92b	33.18	7.75bc	77.24	8.72b	75.6	7.99b	81.37
Treatments	Seydişehir Field Trials							
	Pb Mohican		Pb Rodeo		Pcc Mohican		Pcc Rodeo	
	DI	DC	DI	DC	DI	DC	DI	DC
N.C.	0.0		0.0		0.0		0.0	
P.C.	15.5a		14.59a		62.78a		44.02a	
İS.Ç.5.1.C	6.19c	60.04	6.9c	52.7	16.9ab	73.07	16.61b	62.28
YB.A.4.1.H	8.71b	43.8	10.64ab	27.08	8.33b	86.73	8.92bc	79.74
İS.Ç.8.3.A	7.67bc	50.53	8.79b	39.74	8.77b	86.02	6.14c	86.05

DI: Disease Incidence (%); DC: Disease Control (%); Means followed by the same letter within a column are not significantly different from each other at $P \leq 0.05$ according to Duncan's Multiple Range Test

Effect of Antagonister on Soft Rot Disease in Field Trials

In the study, field trials with Mohican and Rodeo varieties were conducted in Altınekin, Çumra and Seydişehir districts, where the antagonist isolates were collected, and the study was terminated when typical soft rot symptoms were observed in the positive control plants four weeks after the inoculations.

According to the results of Altınekin field trials; Pb caused disease at a rate of 31.88% in the Mohican sensitive variety. Pb caused disease at a rate of 40.55% in the Rodeo resistant variety. Among the treatments, the IS.5.1.C isolate showed the most successful effect with a suppression level of 76.38%, similar to the sensitive variety. IS.C.8.3.A isolate was found to suppress Pcc disease by 84.03%. Pcc caused disease at a rate of 72.28% in the Rodeo resistant variety.

According to the results of Cumra field trials; Pb caused disease at a rate of 20.50% in the Mohican sensitive variety. It was observed that the IS.C.5.1.C isolate suppressed the disease by 70.14%. Pb caused disease at a rate of 34.08% in the Rodeo resistant variety. Similarly, among the treatments, the most successful effect on the sensitive variety was the IS.Ç.5.1.C isolate, with a suppression level of 72.74%. Pcc caused disease at a rate of 35.85% in the Mohican sensitive variety. It was observed that the application of IS.C.5.1.C isolate suppressed the disease by 79.26%. Pcc caused disease at a rate of 44.10% in the Rodeo resistant variety. Among the applications, unlike the sensitive variety, the YB.A.4.1.H application showed the most successful effect, with a suppression level of 83.39%.

According to the results of Seydişehir field trials; Pb caused disease at a rate of 15.72% in the Mohican sensitive variety. It was observed that the application of IS.C.5.1.C isolate suppressed the disease by 60.31%. Pb caused disease at a rate of 14.57% in the Rodeo resistant variety. Among the treatments, the IS.C.5.1.C isolate showed the most successful effect with a suppression level of 55.98%, similar to the sensitive variety. Pcc caused disease at a rate of 64.24% in the Mohican susceptible variety. Pcc caused disease at a rate of 44.24% in the Rodeo resistant variety. Among the applications, unlike the sensitive variety, the most successful effect was the IS.Ç.8.3.A isolate application, with a suppression rate of 90.38% (Table 5).

Re-isolation Studies

In accordance with Koch's postulates, reisolation of soft rot bacteria from sugar beet plants was carried out following in vivo greenhouse and field trials. Samples were collected from plant tissues displaying typical soft rot symptoms, and reisolation was performed. The obtained agents were identified as the disease agents in plants as Pb and Pcc through biochemical, morphological, physiological, and molecular tests.

Discussion and Conclusion

Sugar beet soft rot disease agents *Pectobacterium betavasculorum* and *Pectobacterium carotovorum* subsp. *caratovorum*, which have great economic importance in our country as well as in the world, cause significant yield and quality losses. While the disease generally causes around 15-30% damage (Agrios, 2006), Pb, specific to sugar beet, has been reported to cause over 40% crop loss

in some growing areas (Thomson et al., 1981). As a matter of fact, it is known that bacterial soft rot disease is increasing in the Konya region, which has the largest cultivation and production area in our country (Bastas and Kaya, 2019).

In the managements against the plant pathogens, apart from cultural measures, the recommended copper preparations and limited chemical control practices are insufficient in the management against these diseases. For this reason, the development of alternative methods such as biological control is of great importance in terms of producing effective biopesticides and protecting environmental health (Li et al., 2018; Safara et al., 2022; Kim et al., 2023). In our country, there are successful studies on the use of antagonist bacteria in the fight against many pathogenic bacterial disease factors (Bora and Özaktan, 1998; Aysan et al., 2003; Bozkurt and Soylu, 2019; Bitgen and Mirik, 2021; Aktepe and Aysan, 2023).

In biological control studies of sugar beet root rot disease agents in different regions of the world, bacteria (Xiao et al., 2011), fungus (Bagy et al., 2019), virus (Kim et al., 2023) and yeast (Hassan et al., 2019). It has been reported that different prokaryotic and eukaryotic microorganisms such as) give successful results. However, to date, no information has been found on biological control studies against soft rot disease in the sugar beet production areas of our country. With increasing temperatures due to global climate change, the emergence or infections of some plant pathogenic bacterial agents have begun to increase. Especially heat-loving bacterial factors, one of which is Pectobacterium spp. It causes significant yield and quality problems (Schaad et al., 2001).

In our study, for the first time in our country, the effectiveness of antagonistic bacterial factors isolated from Konya sugar beet cultivation areas against soft rot disease was investigated. For this purpose, 3064 bacterial isolates were obtained from 270 soil samples collected from 3 different districts with the highest production amounts and different ecological conditions (Altınekin, Çumra and Seydişehir), 15 of which showed antagonistic effects on both Pcc and Pb.

In *in vitro* tests against the sugar beet soft rot disease agents Pb and Pcc of antagonist isolates specific to our country and Konya province, the effectiveness value of the streptomycin antibiotic was 60%, while İ.S. Ç.5.1.C isolate showed 68% effectiveness for Pb and 63% effectiveness for Pcc. The disease severity suppression rates of 15 antagonist isolates applied in greenhouse trials showed effectiveness varying between 49-86% depending on resistant and sensitive varieties.

In *in vivo* experiments conducted in Altınekin, Çumra and Seydişehir districts where the antagonist isolates were collected, the isolate's disease suppression rates were 33-90%, depending on the resistant and susceptible varieties and the districts. It has been demonstrated by many studies that biochemical substances, secondary metabolites, enzymes or volatile organic compounds produced by friendly microorganisms used in biological control studies of plant pathogenic bacteria significantly reduce disease severity by targeting the growth, proliferation, biofilm formation or quorum-sensing mechanisms of pathogenic bacteria (Garge et al. Nerurkar, 2016; Kumar et al., 2016; Zhang et al., 2019; Vesuna and Nerurkar, 2020; Safara et al., 2022).

Studies are continuing on the mechanism of action of bacterial antagonists, which were determined to be highly effective against soft rot disease in our trials, in preventing the disease. The findings to be obtained can be used effectively in the production of biological preparations. The methods of antagonist microorganisms of application, their high potential to colonize the applied area, and their survival time are important factors affecting the success of biological control. In addition, determining the virulence mechanisms of the target pathogen and using antagonist microorganisms that have a specific effect on these mechanisms increases the success of biological control.

While the results we obtained in this study form the basis for the development of effective bioformulations in the control against soft rot factors, which have caused significant losses in sugar beet cultivation in our country in recent years, it is thought that these data will enable the development of biopesticides within the scope of sustainable, organic and environmentally friendly control practices.

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Insecticidal Effect of *Thymus citriodorus* (Pers.) Schreb (Lamiaceae) Essential Oil on *Sitophilus granarius* (Linnaeus, 1758) (Coleoptera: Dryophthoridae) and *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae)

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ABSTRACT

In the current study, the essential oil of *Thymus citriodorus* (Pers.) Schreb (Lamiaceae) was evaluated for its ability to control adults of two significant pests of stored products, *Sitophilus granarius* (Linnaeus, 1758) (Coleoptera: Dryophthoridae) and *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae), under laboratory conditions. Using a microapplicator, test insects were exposed to 0.025, 0.05, 0.1 and 0.15 µl/insect concentrations of plant essential oil in order to assess contact toxicity. At 24, 48, 72, and 96 hours following applications, deaths were noted. The experiment revealed that, depending on the insects and dosages, the essential oil showed varying degrees of contact activity. The essential oil of *T. citriodorus* generally had low effect on adult *T. castaneum*, with the greatest effect was 15.32% at 0.15 µl/insect dose after 96 hours. Adults of *S. granarius* showed greater sensitivity to the essential oil of *T. citriodorus*. After 48 hours, doses of 0.1 and 0.15 µl/insect concentrations of the essential oil resulted in over 95% of *S. granarius* adult mortality. The findings of the study indicate that *T. citriodorus* essential oil has the potential to be used in the control of *S. granarius*.

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Introduction

The red flour beetle, scientifically known as *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae), and the granary weevil, *Sitophilus granarius* (Linnaeus, 1758) (Coleoptera: Dryophthoridae), have been recognized as the primary insect pests with significant economic implications in storage facilities (Guru-Pirasanna-Pandi et al., 2018; Demeter et al., 2021). These insects are responsible for significant reductions commercial and nutritional value of stored products over the storage period. Insect pests in stored products are controlled by cultural, mechanical and chemical means. According to Teke and Mutlu (2020), chemical control approaches are extensively employed due to their ability to yield rapid outcomes. Chemicals used intensively and unconsciously cause adversely affect the environment and human health, residue problems in the product, and develop resistance (Demeter et al., 2021). The presence of environmental issues, along with the imperative for ensuring food safety, has underscored the requirement for alternative research. Aromatic plants produce secondary metabolites known as plant essential oils, which have gained attention in recent years due to their potential applications as alternatives for various purposes, including antifungal, herbicidal, and insecticidal uses (Nazzaro et al., 2017; Nikolova and Berkov, 2018; Budak et al., 2022; Alkan et al., 2023).

Thymus (Lamiaceae) species exhibit powerful antifungal and antibacterial properties (Šegvić Klarić et al., 2007; Karami-Osboo et al., 2010). Furthermore, their extracts and essential oils have been shown to have different impacts on insect pests, including stored-product insects (Saroukolai et al., 2010; Küçükaydın et al., 2021). Among the *Thymus* species, the perennial subshrub medicinal plant known as lemon thyme [*T. citriodorus* (Pers.) Schreb.] is native to southern Europe and is grown in the Mediterranean region. The plant has strong and distinct aromas ranging from lemon to orange (Toncer et al., 2017; Golparvar and Hadipanah, 2023)

A limited number of studies have been carried out in Türkiye to determine the insecticidal effect of *Thymus* essential oils against different stored product pests. Küçükaydın et al. (2021) reported the insecticidal activities of *T. cariensis* Hub.-Mor. & Jalas and *T. cilicicus* Boiss. & Bal essential oils against *Rhizopertha dominica* (F.) Coleoptera: Bostrichidae) and *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Additionally, Bozhüyük et al. (2016) tested *T. sipyleus* Boiss. against *C. maculatus*, and Yıldırım et al. (2011) conducted tests on the efficacy of *T. fallax* Fisch. & Mey. and *T. sipyleus* against *S. granarius*. In the present study, the contact effect of the essential oil of *T. citriodorus* has been investigated against *T. castaneum* and *S. granarius*.

Materials and Methods

Plant Material and Extraction of Essential Oils

Thymus citriodorus plant used in the study was obtained from the Central Research Institute of Field Crops (Ankara-Türkiye). The cultivation of the plants was carried out by Dr. Reyhan BAHTİYARCA. The vegetative parts of the plants were collected during flowering and dried in an area away from direct sunlight and with air circulation. The desiccated plants were fragmented into diminutive segments and preserved within containers until the extraction of the oil was accomplished. The Neo-clavanger device was employed to extract essential oil from the plant. In order to achieve the intended objective, a plant sample weighing 100 g was carefully measured and thereafter exposed to the process of hydrodistillation using a Clevenger apparatus for 3 hours. The condenser component of the apparatus was linked to microfilters and the temperature of the cooling water was maintained at a constant value of +4°C. The essential oil derived from the plant was subjected to purification using water on Na2SO4 and thereafter stored in amber-colored containers at a temperature of -20 ºC until the designated day of utilization in the experimental procedure.

Insect Rearing

The initial cultures of *Tribolium castaneum* and *Sitophilus granarius* to be used in the study were obtained from Yozgat Bozok University, Faculty of Agriculture, Department of Plant Protection, Entomology laboratory (Yozgat-Türkiye). Approximately 750 mixed sex adults of *T. castaneum* were released into one litre jars containing a mixture of flour and yeast (70%-30%). *T. castaneum* eggs, which were separated from the jar containing the adults by sieving (with a hole size of 60 mesh) at certain intervals, were transferred to the jars containing food. The procedure for rearing *S. granarius* eggs involved the use of one litre glass jars with about 300 grams of wheat in each jar. A total of approximately 750 adult individuals of varying genders were introduced into the enclosed containers and allowed to remain for 24 hours to facilitate the process of oviposition. Subsequently, the adults were taken out of the nutrient-rich medium. The process of adult emergence was observed daily to collect individuals within the necessary age range of 14-21 days for both test insects. The jars were maintaned at a temperature of 25±2ºC and a humidity level of 60±5% throughout the duration of the study (Karakoç et al., 2006).

Contact Toxicity Tests

The contact activity of the essential oil derived from *Thymus citriodorus* was assessed using the methodology described by Alkan et al. (2021) in the conducted research. Consequently, the applications were topically administered using a microapplicator (Hamilton Company, PB-600, Reno, NV, USA). To perform the contact activity tests, the essential oils were diluted with acetone at concentrations of 0.025, 0.05, 0.1 and 0.15 µl/insect to form solutions and applied to the ventral part of the abdomen of each insect. To serve as a negative control, pure acetone was applied at a dose of 1 µl. The positive control utilized in the study was K-Obiol® EC 25 (25 g/l Deltamethrin + 250 g/l Piperonyl Butoxide). The trials were conducted under controlled laboratory circumstances, using a completely randomized design with nine repetitions. In every experimental trial, a total of 10 individuals of mixed sex were utilized, including the control group. Following the completion of the treatment, the insects were subsequently relocated to Petri plates containing food and subjected to incubation at a temperature of 25 ± 2ºC. Dead insects were recorded through the implementation of 24-hour interval counts over four consecutive days. The experiments were carried out in August 2022 in the entomology laboratory of Tokat Gaziosmanpaşa University, Faculty of Agriculture, Department of Plant Protection (Tokat-Türkiye).

Statistical Analyses

The data acquired from the study were initially transformed into mortality percentages, followed by an examination of their normal distribution. Once it was shown that the data had a normal distribution, an ArcSin transformation was employed. Subsequently, the interactions between the various treatments were assessed using the Tukey multiple comparison test at a significance level of 5% ($P<0.05$). The determination of interactions between treatments was conducted using the General Linear Model (GLM). The statistical analyses were conducted using the MINITAB 19 statistical software suite.

Results and Discussion

The findings of the investigation indicated that the essential oil derived from *Thymus citriodorus* exhibited notable effect against *Sitophilus granarius*, as demonstrated in Table 1. The contact activity of the 0.025 µl/insect dose was found to have a low mortality rate of 1.14% after 24 hours. However, when the applied dose rose, the activity also increased, reaching a mortality rate of 36.82% at a dose of 0.15 µl/insect ($F=46.81$; $df=5.53$; $P<0.05$). When the activity of the essential oil at the end of 48 hours was analyzed, it was concluded that the activity increased with time and the mortality rate was calculated as 99.87% at the highest application dose. In this period, the closest mortality rate to this mortality rate was 96.98% at 0.1 µl/insect dose ($F=262.40$; $df=5.53$; $P<0.05$). At the highest application dose, almost all insects were dead by 48 hours. At 0.1 µl/insect application dose, the mortality rate at 48 and 72 hours is the same, while the mortality rate increased to 98.49% at 96 hours ($F=277.27$; $df=5.53$; $P<0.05$). At 0.05 µl/insect application dose, the mortality rates at 72 and 96 hours were 32.55% and 39.02%, respectively (Table 1).

Table 1. Contact activity of *Thymus citriodorus* essential oil against *Sitophilus granarius*

Treatment	Mortality (%) ±StDev			
	24 h[1]	48 h	72 h	96 h
Control	0.00±0.00c[2]	0.00±0.00c	0.00±0.00c	0.00±0.00c
0.025 µl/insect	1.14±2.56c	1.14±2.56c	1.14±2.56c	1.14±2.56c
0.05 µl/ insect	18.99±1.92b	32.55±2.47b	32.55±2.47b	39.02±4.11b
0.1 µl/ insect	28.45±18.02b	96.98±4.46a	96.98±4.46a	98.49±3.53a
0.15 µl/ insect	36.82±11.77b	99.87±1.14a	99.87±1.14a	99.87±1.14a
K-Obiol	100.00±0.0a	100.00±0.0a	100.00±0.00a	100.00±0.00a

[1]h: Hours after treatment; [2]Means followed by the same lowercase letter within each column are not significantly different using Tukey test at P<0.05.

Table 2. Contact activity of *Thymus citriodorus* essential oil against *Tribolium castaneum*

Treatment	*Mortality(%)±StDev*			
	24 h[1]	48 h	72 h	96 h
Control	0.00±0.00c[2]	0.00±0.00c	0.00±0.00c	0.00±0.00d
0.025 µl/insect	1.24±5.64c	1.24±5.64c	1.50±7.12c	1.50±7.12cd
0.05 µl/insect	1.14±2.56c	1.05±4.11c	1.05±4.11c	2.28±5.48bcd
0.1 µl/insect	3.73±3.56bc	4.35±4.22bc	5.02±4.81bc	6.69±4.17bc
0.15 µl/insect	14.49±3.14b	14.49±3.14b	15.32±3.75b	15.32±4.17b
Kobiol	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a

[1]h: Hours after treatment; [2]Means followed by the same lowercase letter within each column are not significantly different using Tukey test at P<0.05.

Table 3. ANOVA parameters for main effects of variables for the adults of *Sitophilus granarius* and *Tribolium castaneum* in the study

Source	DF	F-Value	P-Value
Dose	4	330.48	P<0.05
Insect	1	662.27	P<0.05
Time	3	31.40	P<0.05
Dose x Insect	4	139.97	P<0.05
Dose x Time	12	7.72	P<0.05
Insect x Time	3	26.13	P<0.05
Dose x Insect x Time	12	7.31	P<0.05
Error	356		
Total	395		

The investigation of the impact of *T. citriodorus* essential oil on *T. castaneum* revealed that the activity of the oil varied based on both duration and dosage, similar to the findings observed in *S. granarius* (Table 2). Nevertheless, the *T. citriodorus* essential oil did not exhibit noteworthy contact insecticidal action against this pest. After 24 hours, the highest mortality rate was 14.49% in the essential oil treatment at 0.15 µl/insect dose. However, no significant effect was seen for the remaining application doses during this time period (F=124.4; df =5.53; P<0.05). The highest activity was determined at the dose of 0.15 µl/insect after 96 hours with a mortality rate of 15.32% (F=87.03; df=5.53; P<0.05). In the study in which the effect of *T. citriodorus* essential oil on *T. castaneum* was determined, no dose was statistically similar to K-Obiol used as positive control at the time intervals used (Table 2).

As a result of the interaction analyses, it was concluded that the effects of the treatments alone were statistically significant. In addition, dose x insect, dose x time, insect x time and dose x insect x time inetraction were also significant (Table 3).

The present study investigated the contact insecticidal activity of the essential oil derived from *T. citriodorus* against two significant stored product pests. The efficacy of the essential oil varied based on the insect species, dosage, and exposure duration. Previous studies have examined the biological activities of *Thymus* species essential oils (Moazeni et al., 2014; Saroukolai et al., 2010; Jarrahi et al., 2016; Lu et al., 2021; Lazarević et al., 2020; Barros et al., 2022; Rozman et al., 2007; Demeter et al., 2021; Moutassem et al., 2021) and specific compounds found in these oils (Papachristos et al., 2004; Chu et al., 2010; Maga et al., 2000; Huang et al., 2021; Liska et al., 2010; Jiang et al., 2016) for their efficacy against stored product pests in controlled laboratory conditions.

Upon analysis of the contact activity results, it was shown that the essential oil of *T. citriodorus* exhibited noteworthy efficacy against *S. granarius*, however it did not demonstrate substantial activity against *T. castaneum*. The observed phenomenon can exhibit variability contingent upon the chemical makeup of the essential oil, as well as the physiological and biochemical characteristics of the insect. Numerous studies have previously demonstrated variations in the effects of a particular plant essential oil on diverse insect species, including those within the same genus (Ayvaz et al., 2010; Vera et al., 2014; Ma et al., 2020; Papachristos et al., 2004; Cheng et al., 2009). Kimani and Sum (1999) experimented to evaluate the contact activity of the essential oils derived from *Tanacetum cinerariifolium* (Trevir.) against adult specimens of *S. oryzae* and *T. castaneum*. The efficacy of essential oils was assessed through the use of topical applications, and it was observed that *S. oryzae* exhibited more resistance compared to *T. castaneum*. In a separate

study, the researchers examined the contact, fumigant, and anti-feeding properties of essential oils derived from nutmeg tree seeds using the process of steam distillation against *T. castaneum* and *S. zeamais*. The research conducted on contact effect studies revealed that *S. zeamais* adults exhibited a sensitivity level around ten times higher than that of *T. castaneum* adults (Huang et al., 1997). In the study in which the effect of *Artemisia vulgaris* essential oil on *S. granarius* and *T. castaneum* was determined, 92.9% effect on *S. granarius* was determined at the end of 72 hours, while the essential oil of the plant showed no significant activity for *T. castaneum* (Evlice et al., 2023). In a study conducted by Alkan (2020), the efficacy of plant essential oils from the genus *Origanum* was examined against four distinct stored product pests. The findings of the study indicated that the effectiveness of the essential oils varied based on factors such as the plant essential oil used, dosage, time of application, and the type of insect being targeted.

In conclusion, the results of this study showed that the essential oil of *T. citriodorus* showed a significant and remarkable contact activity on *S. granarius*. This study is a basic study and additional studies are needed. Especially when we examine the recent studies, it is seen that studies on the formulation of pure essential oils as well as their applications have gained intensity. In fact, it is understood that studies on the application of oils obtained from plants belonging to different families and oils with different main components in mixtures are gaining momentum. In this context, it is thought that it is important to carry out formulation studies of *T. citriodorus* essential oil and to carry out researches on different application methods.

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Investigation of Selcuk University Alaeddin Keykubat Campus in Terms of Xeriscape Design

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ABSTRACT

Drought and thirst are among the most important problems in today's world where water resources are depleted more rapidly due to global warming and climate change. The use of plant species with high water needs in landscaping causes an increase in the need for irrigation and more water consumption. For this reason, the need for xeriscape design in landscape areas has increased in recent years. The aim of this study is to examine and evaluate the xeriscape landscaping in Selcuk University Alaeddin Keykubat Campus in line with the xeriscape principles. As a result of the examinations, it was determined that the xeriscape design areas in the campus were generally created in accordance with xeriscape principles.

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Introduction

The need for green space in urban settlement areas has emerged as a result of man's dependence on nature. Green areas have many functions, such as creating an aesthetic and healthy environment and reducing the effects of some gases " will increase in direct proportion to the population in the future, the amount of water to be used in these areas will increase compared to today. For this reason, it is extremely important that the water required for irrigation of green areas is not met from mains water, but is made with water obtained through recycling, and the correct irrigation method is chosen in the field. Because it is obvious that our need for water will increase in the future (Şahin ve Güngör, 2008).

In recent years, drought caused by global warming has caused global climate changes. Excessive consumption resulting from rapid urbanization has reached unconscious levels, and this is one of the most important factors triggering drought (Bradley et al, 2012). Water is one of the important natural resources that is indispensable for living life, cannot be reproduced and has no alternative. In recent years, design approaches that use water effectively have been preferred in order to reduce water consumption, especially in landscaping in cities. One of these approaches is "Xeriscape Design". Xeriscape design is generally defined as a type of landscape design that adopts the principle of minimizing the use of water and protecting water resources and the environment (Sezen et al., 2018).

Xeriscape was derived from the Greek word 'xeros' meaning dry and 'landscape' (Sovocool and Morgan, 2005). The term "Xeriscape" was first used by the Colorado Xeriscape Council, which was established in Denver, Colorado, USA, in 1978, and continues to spread today with different interpretations (Çorbacı et al, 2011).

The first garden designed in line with xeriscape principles was designed in the 1970s. Located in a semi-arid region in Colorado, this garden was implemented as an exhibition area where vegetal areas were created using little or no water. This garden was designed around a non-classical plan, using similar but different materials. Thus, it has become an instructive garden on how to use water more effectively. This garden, opened in 1980, consists of more than five thousand plants of ninety different species. Most of these plants are local plants of the region they belong to (Yazgan et al, 2010).

The main purpose of xeriscape design is to protect water resources by minimizing water use. Areas designed with xeriscape do not need regular maintenance. Since regular maintenance is not required, the sustainability of

these areas is easily ensured. Additionally, by minimizing water use, water resources are used effectively. It is very important for xeriscape design that the plants used in the design are drought resistant (Çetin, 2016).

The maintenance costs of gardens designed according to xeriscape design are less than gardens designed with other methods. Large grass areas are not included in the designed areas. These designs are often used for many years. In addition, xeriscape design saves time as it is applied to the area in a shorter time than other landscape design applications. Efficient use of water and long life of the design are among the most important features of xeriscape design (Taner, 2010). In addition, xeriscape design approaches not only reduce water use by 50%, but also demonstrate an approach that is compatible with the environment, requires little use of chemicals, and requires low maintenance and cost. Instead of completely changing the existing design, it is based on how to evaluate the current situation for the effective use of water. Xeriscape planning provides many economic and ecological benefits. It is expected to provide drought-resistant herbal solutions by reducing water use (Becca Rodomsky-Bish, 2015; Pouya et al, 2016).

Principles of Xeriscape

In addition to saving water, xeriscape principles contribute to nature with their nature-friendly approach, and by reducing water consumption, they contribute to fresh water resources lost as a result of global warming in the long term (Metin ve Koçan, 2020). There are seven xeriscape principles (Welsh et al., 2007):

- Planning and design
- Soil analysis and preparation
- Practical turf areas
- Appropriate plant selection
- Efficient irrigation
- Use of mulches
- Appropriate maintenance

Planning and Design

Planning and design stages are very important for successful xeriscape application. Proper planning saves time, money and prevents work from being done twice (Çorbacı et al., 2011). In addition, determining the direction of the design and creating a framework that suits the project goals is also extremely important (Akay and Polat, 2023).

Creating a water-efficient landscape starts with a well-thought-out landscape design. The outline of the garden must first be drawn by determining the locations of buildings, trees, bushes and grass areas. Next, consideration should be given to how various areas of the garden will be used, how the garden will look, the amount of maintenance planned and the budget. In addition, the areas of the landscape that need the most water must be determined. The goal of planning is to design a landscape that will have the desired appearance and function while conserving water. Landscape design can also be implemented gradually over several years (Welsh et al., 2007).

Soil Analysis and Preparation

First of all, soil analysis should be done. Analysis results tell you what type and amount of fertilizer the soil needs and whether organic matter is required. Most soils benefit greatly from organic matter. Adding organic matter to the soil of flower beds and shrubs makes plants healthier. Organic matter also helps soil absorb and store water. It is not necessary to add organic matter for trees (Welsh et al., 2007).

Soil analysis is extremely important for a successful xeriscape design. Soil analysis plays an important role in determining the plant species to be used in the area. It is necessary to improve the soil before installing the irrigation and drainage system and planting plants. Soil reclamation forms the basis of a good landscaping work. In a reclaimed soil, the water retention capacity of the soil increases, it becomes easier for plants to absorb water, and sufficient air spaces are formed to allow plant roots to get enough air (Çorbacı et al., 2011).

Practical Turf Areas

When designing the landscape, it should not be forgotten that grasses need more water and care than other plants. To preserve water, the amount of lawns should be reduced by incorporating patios, terraces, shrubs and ground cover plants into the landscape design. It is also necessary to consider the ease of watering lawn areas. Long and narrow, small or oddly shaped areas are difficult to irrigate efficiently. Lawns should be confined to blocky, square-like areas that are easier to maintain. Ground cover plants can also be used as alternative plants instead of grass (Welsh et al., 2007).

Appropriate Plant Selection

Trees, shrubs and ground cover plants suitable for the soil and climate of the region where xeriscape design will be made should be selected. One of the best ways to preserve water is to choose grass species that are suitable for the region where xeriscape design will be applied and have low water demand (Welsh et al., 2007).

In xeriscape design studies, the selection of plant species is very important. Imported species that will be chosen instead of plant species belonging to the local habitat will require more water than endemic and locally suitable species, so the use of such plants will result in the use of additional water and fertilizer (Wade et al., 2010).

Appropriate plant selection means choosing plants that not only match the design, but also suit the planting site and local environment. Plants should be selected according to the soil type and light level of the area. The plants you choose should be able to adapt to local fluctuations in temperature and soil moisture (Wade et al., 2010).

Efficient Irrigation

Huge amounts of water are supplied to lawns and gardens, but most of the water is never absorbed by the plants. Some of the water flows away because it is given too quickly, and some of it evaporates from the exposed, unmulched soil. However, the biggest water waste comes from watering too frequently. When too much water is supplied to the land, nutrients can leach from plant roots deep into the soil and possibly contaminate groundwater. In addition, fertilizers and pesticides are transported to

streams and lakes in this way, causing pollution. These problems can be easily eliminated with correct irrigation techniques (Welsh et al., 2007).

The goal of any irrigation system is to provide plants with sufficient water without wasting it. By installing an irrigation system, lawns, ground cover plants, shrubs and trees can be watered individually and more frequently. Sprinkler and drip irrigation systems are used together to save water in the landscape. If a permanent sprinkler system is used, sprinkler heads must be positioned correctly to prevent watering sidewalks and driveways. In addition, it is essential to adjust the sprinkler heads to spray large water droplets rather than those that evaporate quickly and can be carried away by the wind. Drip irrigation system is more efficient and beneficial for plants than sprinkler irrigation system. Drip irrigation systems are also safer for landscapes in areas where water quality is poor. Drip irrigation slowly applies water to the soil. Water flows under low pressure through emitters, sprinklers, or spray heads placed throughout each facility. There is very little chance that water applied via drip irrigation will be wasted through evaporation or runoff (Welsh et al., 2007).

Use of Mulches

Mulch is a layer of non-living material that covers the soil surface around plants. Mulches can be of organic materials, such as pine bark, compost, and sawdust, or inorganic materials, such as lava rock, limestone, or permeable plastic. Mulch should be used wherever possible. Good mulching saves water by significantly reducing moisture evaporation from the soil. It also reduces weeds, prevents soil compaction and maintains soil temperature (Welsh et al., 2007).

Mulch up to 5-7.5 cm thick can be used under trees and shrubs. If this layer is thicker than necessary, plant roots may be damaged. Fine-textured organic mulches retain water better than coarse-textured mulches (Çorbacı et al., 2011).

Appropriate Maintenance

Mowing lawns at the appropriate height saves water because it encourages root systems to grow deeper and be more water efficient. Fertilizing the lawn at the right time and in the right amount saves time, effort and money by reducing mowing and watering. It is necessary to fertilize the lawn once in spring and once in autumn. While slow-release nitrogen form fertilizer should be used in spring application, fast-release nitrogen form fertilizer should be used in autumn application. For efficient operation, the irrigation system should be checked and maintained periodically. Insect and disease controls should be carried out and weeds should be eliminated. Maintenance costs for a well-designed landscape using xeriscape principles can be reduced by up to 50% by mowing less frequently, mulching once a year, eliminating incompatible plants that need a lot of water, and efficient irrigation methods (Welsh et al., 2007).

Maintenance works, as in all other landscape areas, are extremely important in terms of preserving the features of the area and ensuring its continuity. Depending on climatic factors and the characteristics of the plants used, maintenance work such as pruning, fertilization, weeding, disease and pest control should be carried out on time and in accordance with the technique, and attention should be paid to the irrigation system, in order to preserve and increase the quality of the xeriscape design (Çorbacı et al., 2011).

Materials and Methods

The main material of the study consists of all xeriscape design areas within Selçuk University Alaeddin Keykubat Campus, located in Selçuklu district of Konya province. These areas are: Rectorate garden, Museum garden, Faculty of Agriculture garden and areas with xeriscape designs covering certain parts of Celal Bayar Street.

The aim of this study is to examine and evaluate the xeriscape designs in Selçuk University Alaeddin Keykubat Campus in line with xeriscape principles. For this purpose, first the study areas were determined. Then, a literature review was made and the study areas were visited and examined. Photographs were obtained from the areas. The suitability of the areas for xeriscape design was evaluated in the light of literature sources. As a result of the evaluations, conclusions and recommendations are given.

Results and Discussion

Examination of Xeriscape Design in the Rectorate Garden in Terms of Xeriscape Principles

The Rectorate building was built on November 2, 2017. It is located on Celal Bayar Street of Alaeddin Keykubat Campus.

When the xeriscape designs applied in the Rectorate garden are examined according to xeriscape principles, it is seen that appropriate planning and design has been made in the area. The necessary soil reclamation for the xeriscape design in the garden was done beforehand and appropriate plant species were used in the design. Water is given to the plants using the drip irrigation method and in this way the water needs of the plants are met. Effective irrigation is done using the drip irrigation method.

White dolomite stones, pumice stones and pebbles were used instead of grass plants in the xeriscape areas in the Rectorate garden (Figure 1). Therefore, it can be said that these areas comply with the principle of sustainable grass areas. In this design, thanks to the sulzer fabric base cover used under the stones, the soil is prevented from losing moisture and the temperature of the soil is maintained. Mulching was done with this fabric. Due to the tearing of the sulzer fabric base cover used under the pebbles, soil moisture emerged and weeds grew in the area. Therefore, it can be said that mulching is not fully done in this area.

Using plant species that grow naturally in the region in xeriscape design ensures that the plant's maintenance needs such as watering and fertilization are low. Plant species suitable for soil and climate conditions were selected in the Rectorate garden. In this respect, it can be said that the maintenance need of the area is low. However, in a part of the xeriscape area, the text “Selçuk” was written with boxwood (*Buxus sempervirens*) plants (Figure 2). The fact that this plant always needs pruning and that the area is invaded by weeds due to the worn-out mulch material used in this area shows that the principle of appropriate and sustainable care is not fully fulfilled.

Figure 1. Xeriscape design applied at the entrance of the Rectorate building (Original, 2022).

Figure 2. Xeriscape design applied on the garden of the Rectorate building (Original, 2022).

Figure 3. Xeriscape design applied in the front garden of the museum building (Original, 2022).

Figure 4. Xeriscape design applied in the front garden of the museum building (Original, 2022).

Examination of Xeriscape Design in the Museum Garden in Terms of Xeriscape Principles

The museum was opened in 2016. It is located on Celal Bayar Street of Alaeddin Keykubat Campus.

When the xeriscape designs applied in the museum garden are examined according to xeriscape principles, it is seen that appropriate planning and design has been made in the area. The necessary soil reclamation for the xeriscape design in the garden was done beforehand and appropriate plant species were used in the design. Water is given to the plants using the drip irrigation method and in this way the water needs of the plants are met. Effective irrigation is done using the drip irrigation method (Figure 3).

White dolomite stones were used in some of the xeriscape area in the museum garden, and in some parts, ground cover plants (especially *Sedum* species) were used instead of grass plants (Figure 4). Therefore, it can be said that these areas comply with the principle of sustainable grass areas. It was observed that the sulzer fabric base cover used under the stones in this design was torn. Thus, soil moisture emerged and weeds grew in the area, so the mulching process could not be done correctly.

Maintenance work is required to protect the area and ensure continuity, as in all other landscape areas. The aim of the principle of appropriate and sustainable maintenance is to carry out maintenance activities such as irrigation, pruning, fertilization and weeding in a complete and sustainable manner. The inadequate development and drying of the plants used in the area, the bareness in the soil and the deterioration in the design indicate that the maintenance work is not sufficient.

Examination of Xeriscape Design in Agricultural Faculty Garden in Terms of Xeriscape Principles

Faculty of Agriculture was opened on July 20, 1982. It moved to its current building in 1992. It is located on Suleyman Nazif Street of Alaeddin Keykubat Campus.

When the xeriscape designs applied in the faculty garden are examined according to xeriscape principles, it is seen that appropriate planning and design has been made in the area. The necessary soil reclamation for the xeriscape design in the garden was done beforehand and appropriate plant species were used in the design. Water is given to the plants using the drip irrigation method and in this way the water needs of the plants are met. Effective irrigation is done using the drip irrigation method.

Pebbles were used in some of the xeriscape area in the faculty garden, and ground cover plants (especially *Berberis thunbergii "Atropurpurea"* and *Cerastium tomentosa*) were used instead of grass plants in some parts (Figure 5 and 6).

Figure 5. Xeriscape design applied in the front garden of the Faculty of Agriculture (Original, 2022).

Figure 6. Xeriscape design applied in the front garden of the Faculty of Agriculture (Original, 2022)

Figure 7. Xeriscape design applied on Celal Bayar street (Original, 2022).

Figure 8. Xeriscape design applied on Celal Bayar street (Original, 2022).

Therefore, it can be said that these areas comply with the principle of sustainable grass areas. In this design, thanks to the sulzer fabric base cover used under the stones, the soil is prevented from losing moisture and the temperature of the soil is maintained. Mulching was done with this fabric. Using plant species that grow naturally in the region in xeriscape design ensures that the plant's maintenance needs such as watering and fertilization are low. Plant species suitable for soil and climate conditions were selected in the faculty garden. In this respect, it can be said that the maintenance need of the area is low. The fact that there is no need for pruning since no grass is used in the xeriscape area, the area is mulched and there are no weeds shows the existence of the principle of appropriate and sustainable maintenance.

Examination of Xeriscape Design in Celal Bayar Street in Terms of Xeriscape Principles

When the xeriscape designs applied on Celal Bayar street are examined according to xeriscape principles, it is seen that appropriate planning and design has been made in the area. The necessary soil reclamation for the xeriscape design in the garden was done beforehand and appropriate plant species were used in the design. Water is given to the plants using the drip irrigation method and in this way the water needs of the plants are met. Effective irrigation is done using the drip irrigation method. In the xeriscape areas on Celal Bayar street, pebbles and ground cover plants (especially *Berberis thunbergii "Atropurpurea"*) were used instead of grass plants (Figure 7). Therefore, it can be said that these areas comply with the principle of sustainable grass areas. In this design, thanks to the sulzer fabric base cover used under the stones, the soil is prevented from losing moisture and the temperature of the soil is maintained. Mulching was done with this fabric (Figure 8).

Using plant species that grow naturally in the region in xeriscape design ensures that the plant's maintenance needs such as watering and fertilization are low. Plant species suitable for soil and climate conditions were selected in the Celal Bayar street. In this respect, it can be said that the maintenance need of the area is low. The fact that there is no need for pruning in these areas since grass is not used, the area is mulched and there are no weeds indicate the existence of the principle of appropriate and sustainable maintenance.

Conclusion and Recommendations

Conclusion

In the table above, xeriscape designs are evaluated whether they comply with xeriscape principles. It has been determined that the use of appropriate mulch and sustainable maintenance efforts are not sufficient in the xeriscape designs applied in the Rectorate and Museum gardens, and other xeriscape principles are sufficient. In xeriscape designs applied in other areas, it has been determined that all xeriscape principles are sufficient (Table 1).

Table 1. Evaluation of Xeriscape Designs according to Xeriscape Principles

Xeriscape Principles	Rectorate Garden	Museum Garden	Agricultural Faculty Garden	Celal Bayar Street
1. Planning and design	√	√	√	√
2. Soil analysis and preparation	√	√	√	√
3. Practical turf areas	√	√	√	√
4. Appropriate plant selection	√	√	√	√
5. Efficient irrigation	√	√	√	√
6. Use of mulches	X	X	√	√
7. Appropriate maintenance	X	X	√	√

Recommendations

Since a large part of Turkey has semi-arid climate characteristics, it has become necessary to take some precautions against changing climate conditions and increasing drought. It is important to use water economically and protect water resources. Drought is considered one of the most risky disasters for humanity among natural disasters. The availability of water, a natural resource that has no alternative, is decreasing day by day. When the climate data of Konya province is examined, it is seen that Konya has an arid climate. For this reason, it is obvious that especially xeriscape designs should be at the forefront in landscape design studies to be carried out throughout Konya province.

Recommendations for the study areas are as follows: It was observed that there were weeds in the area because the sulzer fabric base cover used in the xeriscape design applied in the Rectorate garden was torn. This base cover needs to be replaced. In addition, plant species that will need less pruning should be determined and these plants should be included in the area.

The bare soil appearance resulting from the drying of the plants used in the xeriscape design applied in the museum garden and the wear and tear of the plants due to lack of care negatively affects the design. To cover the appearance of bare soil, ground cover plants suitable for soil and climate conditions can be used in the area. In addition, the pipes in the drip irrigation system used to irrigate areas should be hidden as they affect the aesthetic appearance. The sulzer fabric base cover used for mulching should be replaced because it is torn.

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Landscape Design in Hospital Gardens: The Example of Selcuk University Medical Faculty Hospital

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ABSTRACT

Hospital gardens, located within urban open-green areas, are places created for patients coming to the hospital, their relatives and the staff working in the hospital to spend time in an environment where they can renew themselves. The importance of these places is increasing day by day. The purpose of this study is to examine the landscape design of the Selcuk University Faculty of Medicine hospital garden located on the Selcuk University Alaeddin Keykubad Campus. The findings show that the hospital garden was generally not designed in accordance with landscape design principles. With the suggestions developed, this hospital garden can be redesigned in accordance with landscape design principles and made more comfortable for people using the hospital garden.

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Introduction

Throughout history, people have used nature for various purposes to find health. It is possible to define the garden, which is a part of nature, as safe areas they choose to relax, have fun and engage in activities (Bulut ve Göktuğ, 2006).

From the perspective of the landscape architecture discipline, every detail that forms the city and affects the city image and identity should be considered and evaluated as a research subject. Hospital gardens, which are a part of public outdoor spaces, are one of the important issues to be evaluated in landscape design. Hospital gardens are important not only for their impact on people's psychological health, but also for the meaning and image they add to the environment and urban architecture (Bulut ve Göktuğ, 2006).

Hospital gardens are open areas where passive or semi-passive activities are carried out, providing effects such as physical relaxation, stress reduction, increasing the feeling of well-being, memory renewal, increasing physical mobility and motivation (Elings, 2006).

In order to reduce the stress factor, which is the leading cause of all diseases, more peaceful, comfortable and safe spaces should be created outside the building. In other words, hospital gardens need to be planned in light of certain design criteria. In this way, patients treated in the hospital recover faster and the staff can do their job better (Ayan, 2009).

While the outdoor features and designs of hospitals have a positive impact on patients, they are also important in spiritual, physical and social terms during the treatment of patients. Well-designed hospital gardens create a social environment by saving patients from the monotony of the clinical environment and positively affect patients' clinical outcomes by reducing stress (Sakıcı, 2009).

The landscape is part of the land, as perceived by local people or visitors, which evolves through time as a result of being acted upon by natural forces and human beings according to the European Landscape Convention (Anonymus, 2023a). Landscape design focuses on both the integrated master landscape planning of a property and the specific garden design of landscape elements and plants within it (Anonymus, 2023b).

Landscape design, which is the field of study of landscape architecture at lower scales, is the process in which outdoor spaces are shaped in line with planning decisions. In landscape design, the use of the space is revealed in line with its needs. The main purpose of landscape design is to present the best possible spatial composition of the work area in the context of sustainability and in the light of design principles. Features such as the designer's vision, aesthetic understanding, and cultural background offer an endless number of solutions in landscape design (Korkut ve ark., 2010).

Landscape Design in Hospital Gardens

Structural Landscape Design

Entrances

The functionality of the entrance points to the garden and whether there are security booths, entrance gates and entrance arches at these entrances should be evaluated in terms of their adequacy and aesthetics. There must be a suitable entrance unit, an arch or door defining the entrance, directional signs identifying the institution, and a security unit. The entrance system is very important as it is a structure that reflects the general design approach and corporate structure of the garden and the institution to the outside (Atabeyoğlu ve Bulut, 2007).

Orientation

Orientation should be provided through construction, gardens, plant materials, and roads connecting spaces that create a sense of space. Aesthetic images should be created with vegetative and structural designs that will provide an emphasis effect by drawing the direction line (Atabeyoğlu ve Bulut, 2007).

Car park

It covers the existence and adequacy of areas designed directly for parking purposes that can serve the employees and visitors of the institution. Parking areas should be established in shaded areas as much as possible by using plant or structural materials. The floors of parking lots must be paved and drained. In addition, car parks should be located at distances that allow easy access to buildings and functions (Atabeyoğlu ve Bulut, 2007).

Reinforcement elements

Erdem (1995) stated the reinforcement elements as base elements, top cover elements, screening and surrounding elements, water elements, urban furniture, children's playgrounds, sports areas and equipment requiring technical infrastructure. Aksu (2012) classified the reinforcement elements as floor coverings, seating areas, lighting elements, information signs, delimiters, water elements, floor covering elements, sales units, artistic objects, children's playgrounds and other elements.

Uses for disabled people

Uses for disabled people in hospital gardens include garden activities and uses that cover the entire area (Atabeyoğlu ve Bulut, 2007).

Physical movement spaces

According to Hazen (1995), exercise provides many physical and psychological benefits, as well as having a positive effect on the cardiovascular system and reducing the risk of depression. For this reason, a horticultural therapy program should be created in hospitals and a suitable activity program should be created. In this way, visitors can also enjoy the garden.

Vegetative Landscape Design

Balance

It is defined as the distribution of landscape design elements at equal intervals within the framework of a layout (Austin, 1982). The principle of balance in design is equality or parity of visual impact (Hansen, 2010). In design, the use of a central axis is a frequently applied method. Design elements such as size, form, shape, color and texture to be created on both sides of this axis ensure balance (Gültekin, 1994).

Repetition

The principle of repetition is defined as the character or quality of an object whose color, texture, form, size and line elements are repeated (Engstrom, 2005;Uzun, 2020). Repetition is the use of an object in the design in more than one or similar ways (Güngör, 1969; Korkut vd., 2010).

Emphasis and Sovereignty

Emphasis, which can be achieved through contrasts of design elements such as color, form, texture and line, is a design principle used to draw attention to a point in the space (Uzun, 1999).

In design, the superiority of a certain plant or group of plants over other objects around it is called sovereignty. There may be sovereignty in terms of size, texture, color and texture. Sovereignty is also an emphasis (Önder, 2020).

Contrast

It is done by making an important and distinct feature of a living or inanimate object stronger with another living or inanimate object that is opposite to it. Any of the features of nature such as size, shape, color, texture may be the most obvious. This distinctive feature becomes even more prominent and more noticeable when a contrasting material is used together (Korkut vd., 2010).

Simplicity

In vegetative design studies, it is very difficult to create a purposeful, functional, easily perceptible, creative, original, but simple composition by using design principles. Simplicity is one of the elusive design principles. Because, when evaluating many design principles together, creating a simple composition that is free from complexity depends on the knowledge, experience and skills of the designer (Önder, 2020).

Proportion

Proportion is defined as finding an appropriate balance in terms of area, mass and volume between the sections that make up the order in the field of plant design. For example; Living elements used in parks and gardens are trees, shrubs, grass areas and flower beds. These must be used in a certain proportion. It is wrong in terms of proportion to give too much space to one and not the other (Önder, 2020).

Variety

Variety is the difference of design elements in a composition. In other words, variety is the contrast or change in line, form, texture and color to attract the eye's attention, hold the observer's attention and satisfy the mind's need for change. It is the state of lack of uniformity and uniformity. An orderly and interesting landscape can be created with variety. The dose of variety in the design should be adjusted well. Too little causes monotony, too much causes complexity (Önder, 2020).

Hierarchy

It is a design principle that provides a regular change, mobility or transition in design features. The transition from one part of the design composition to another can be created by changes in colors, textures, forms and dimensions (Nelson, 2004). Hierarchy is called cascading. While successive elements are in harmonic relationships, there is a deep contrast between the beginning and the end (Özbilen, 2000).

Unity

It is the combination of structural and vegetative elements in the design to create a balanced integrity. Unity ensures that the designed space is perceived holistically. Displaying different design models or concepts that are not related to each other throughout the space creates a situation that contradicts the concept of unity. Unity means that the elements in the space complement each other and create a holistic organization (Korkut ve ark., 2010).

Materials and Methods

The main material of the study is the garden of the Faculty of Medicine hospital located in Selcuk University Alaeddin Keykubat Campus. The study area is located in Selcuklu district of Konya province.

First, the study area and the purpose of the study were determined. Afterwards, a literature review was conducted on the concepts that form the basis of the research. Selcuk University Faculty of Medicine hospital garden was visited and the materials forming the structural and vegetal landscape designs in the area were examined and photographs were obtained from the area (Güngör, 2016). The landscape design of the garden was examined and evaluated in the light of literature sources. As a result of the evaluations, conclusions and recommendations on the subject were developed.

Results and Discussion

Evaluation of the Structural Landscape Design of Selcuk University Faculty of Medicine Hospital Garden

Entrances

Selcuk University Faculty of Medicine hospital has five hospital building entrances: Chief physician entrance (Figure 1), B block entrance, D block entrance, E block entrance and emergency entrance. However, at the entrance to the hospital garden, there is neither a suitable entrance unit, nor an arch or gate defining the entrance, nor directional signs and security unit identifying the institution. Security units are inside the hospital building.

Orientation

Direction in the garden of Selcuk University Faculty of Medicine hospital is provided by roads connecting the spaces. In addition, the emphasis effect that draws the direction line is supported by the plant and structural designs that will ensure this.

Car park

The parking area in the garden of Selcuk University Faculty of Medicine hospital was designed with hospital staff, patients and visitors in mind. The car park is designed to accommodate 1600 vehicles.

Reinforcement elements

Seating area: There are 24 benches and 23 gazebos in the seating areas in the garden of Selcuk University Faculty of Medicine hospital (Figure 2). There are also 40 garbage bins in the garden.

Children's play area: There is a children's playground in the garden of Selcuk University Faculty of Medicine hospital (Figure 3). There are two slides, two swings and three seesaws in the children's playground.

Figure 1. Selcuk University Faculty of Medicine hospital chief physician entrance

Figure 2. Seating areas in the garden of Selcuk University Faculty of Medicine hospital

Figure 3. Children's playground in the garden of Selcuk University Faculty of Medicine hospital

Figure 5. Water elements in the garden of Selcuk University Faculty of Medicine hospital

Figure 6. Ibn-i Sina statue in the garden of Selcuk University Faculty of Medicine hospital

Figure 4. Floor covering in the garden of Selcuk University Faculty of Medicine hospital

Figure 7. Example of balance principle in the garden of Selcuk University Faculty of Medicine hospital

Lighting element: 11 large lighting elements and 7 luminaire lighting elements were used in the garden of Selcuk University Faculty of Medicine hospital.

Floor covering: Square rubber was used as floor covering material in the children's playground located in the garden of Selcuk University Faculty of Medicine hospital. Additionally, interlocking paving stone flooring was used on all pedestrian paths in the area (Figure 4). There are stepping stones on the grass to reach the gazebos around the children's playground.

Water element: There is an ornamental pool in the garden of Selcuk University Faculty of Medicine hospital. There is also a fountain in the hospital garden (Figure 5).

Artistic object: There are two statues in the garden of Selcuk University Faculty of Medicine hospital. One of the statues is located next to the children's playground, while the other is located near the emergency entrance (Figure 6).

Uses for disabled people

There are ramps for the use of disabled people at the B block and emergency entrances of Selcuk University Faculty of Medicine hospital, but the slope of the ramps is not suitable for disabled people to use. Because the ramps are very steep, disabled people have difficulty or cannot use the ramps. Materials for the use of disabled people are not included in children's playgrounds and seatting areas. Some of the perceivable surfaces used on pedestrian paths have become worn, deformed or even disintegrated. For this reason, it can be said that disabled people were not taken into consideration when designing the hospital garden.

Physical movement spaces

Gardens for horticultural therapy have not been created in the garden of Selcuk University Faculty of Medicine hospital. Additionally, there are no areas where people using the garden can do physical exercise. For this reason, it can be said that there are no places for physical movement in the hospital garden.

Evaluation of the Vegetative Landscape Design of Selcuk University Faculty of Medicine Hospital Garden

Balance

In the garden of Selcuk University Faculty of Medicine hospital, the principle of balance is achieved with various plant designs. In Figure 7, the balance principle applied with *Lavandula angustifolia* (Lavender) and *Robinia pseudoacacia 'Umbraculifera'* used on both sides of the road is given as an example. This design which features symmetry is balanced.

Repetition

In the garden of Selcuk University Faculty of Medicine hospital, the principle of repetition is achieved with various plant designs. In the example in Figure 8, the principle of repetition was applied by creating a repeating design with the plants *Lavandula angustifolia* (Lavender) and *Prunus cerasifera "Pisardi Nigra"*.

Emphasis and Sovereignty

The principle of emphasis in the garden of Selcuk University Faculty of Medicine hospital was tried to be achieved through plants, water elements and sculptures. In the example in Figure 9, the emphasis principle was used with the color effect of *Acer platanoides "Crimson King"* (Maple with red sycamore leaves). Evergreen coniferous plants were used as the dominant species in the hospital garden. Especially *Pinus nigra* (Black Pine), *Cupressus arizonica* (Arizona Cypress) and *Cedrus libani* (Lebanon Cedar) are the dominant species.

Figure 8. Example of repetition principle in the garden of Selcuk University Faculty of Medicine hospital

Figure 9. Example of emphasis principle in the garden of Selcuk University Faculty of Medicine hospital

Contrast

In the garden of Selcuk University Faculty of Medicine hospital, the principle of contrast is achieved by using plants used together in different colors and sizes. In the example in Figure 10, the principle of contrast is realized with the plants *Salix babylonica* (Willow) and *Lavandula angustifolia* (Lavender).

Simplicity

The use of plants irregularly and intertwined in the designs made in a part of the garden of Selcuk University Faculty of Medicine hospital caused the garden to move away from the principle of simplicity. The designs created with plants in many parts of the garden are complex.

Proportion

An appropriate balance has been achieved in terms of area, mass and volume in the Selcuk University Faculty of Medicine hospital garden. Trees, shrubs, grass and flowers were used in a certain proportion.

Figure 10. Example of the principle of contrast in the garden of Selcuk University Faculty of Medicine hospital

Figure 11. Example of variety principle in the garden of Selcuk University Faculty of Medicine hospital

Table 1. Evaluation of the Structural Landscape Design of Selcuk University Faculty of Medicine Hospital Garden

Structural Landscape Design	Suitable	Not suitable
1- Entrances		X
2- Orientation	√	
3- Car park	√	
4- Reinforcement Elements	√	
5- Uses for Disabled People		X
6- Physical Movement Spaces		X

Table 2. Evaluation of the Vegetative Landscape Design of Selcuk University Faculty of Medicine Hospital Garden

Vegetative Landscape Design	Suitable	Not suitable
1. Balance	√	
2. Repetition	√	
3. Emphasis and Sovereignty	√	
4. Contrast	√	
5. Simplicity		X
6. Proportion	√	
7. Variety	√	
8. Hierarchy		X
9. Unity		X

Variety

Although there are the same plants in the garden of Selcuk University Faculty of Medicine hospital, this principle has been achieved by the juxtaposition of different species. In the example in Figure 11, the variety principle has been created with *Lavandula angustifolia* (Lavender), *Acer platanoides "Crimson King"*, *Picea pungens glauca* (Blue Spruce), *Prunus cerasifera "Pisardi Nigra"* and grasses.

Hierarchy

In the vegetative design of the hospital garden of Selcuk University Faculty of Medicine, designs were not made in accordance with the principle of hierarchy.

Unity

The structural and vegetative elements in the design of the Selcuk University Faculty of Medicine hospital garden could not come together to create a balanced unity. The elements in the space could not complement each other and become a whole.

Conclusion and Recommendations

Conclusion

When the structural landscape design applied in the garden of Selcuk University Faculty of Medicine hospital was examined, it was noted that the garden entrances were

not created considering sufficient criteria. Additionally, it has been observed that there are almost no uses for disabled people and that appropriate designs for physical movements are not made (Table 1). When the vegetative landscape design applied in the garden was examined, it was revealed that the vegetative design was made without considering the principles of simplicity, hierarchy and unity (Table 2).

Recommendations

Although the equipment elements in the garden of Selcuk University Faculty of Medicine hospital are generally sufficient, it has been determined that there are no lighting elements at the entrances of D and E blocks and around the children's playground and seating areas. Lighting systems that will increase the usage time of the area in the evening and ensure the safety of the users should be placed in the area. The existing lighting system in the area is insufficient (Benliay and Soydan, 2021). Additionally, a more effective landscape design can be made in the area by increasing the number of water elements and artistic objects. Trees that provide shade should be used in outdoor seating areas. Broken and neglected floors were found in the garden. These floors need to be maintained and repaired regularly. Thorny and poisonous plants should never be used in floral designs in children's playgrounds.

The garden should be used for disabled people, ramps should be built in accordance with standards, and tactile surfaces should be used as flooring. Plant designs in the parking lot are insufficient. Trees that provide shade should be included in the parking lot.

Since there are many faculties, food and beverage areas and dormitories around the hospital garden, the plant design of the garden should be screened with plants to prevent noise, thus reducing the noise. Allergic conditions should also be considered when planting. It was determined that allergenic plants such as Populus alba (White Poplar) were used in the hospital garden. Such plants should never be used in the field. To minimize allergies, female plants should be used and maintenance work such as pruning should be done regularly. Plants with strong scents should also be avoided.

The structural and vegetative landscape design in the garden of Selcuk University Faculty of Medicine hospital should be reconsidered, taking these suggestions into consideration.

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Educational Venue from Design to Implementation Process; A Project by Faculty of Fine Arts, Selçuk University

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ABSTRACT

Throughout history, there has been a reciprocal relationship between humans and space. Even though there are numerous spaces covered by this ongoing relationship process, it has integrated and gained value with educational venues. Mainly because they closely monitor social, cultural, technological, and economic developments and pass on knowledge to future generations, educational spaces play a crucial role in the development of individuals as well as society. It is feasible to conclude from research on educational spaces that the physical environment has a major positive or negative impact on education. To solve the issue that the current Faculty of Fine Arts at Selçuk University could not sufficiently respond to user needs physically, it was decided to construct a new Faculty of Fine Arts building. The primary goal of the study is to design the building using user-oriented techniques that promote social interaction and showcase artistic identity throughout the design phase. A qualitative research approach, based on inspection and observations, was used in the study to gather data, documents, and reports on the topic and to ensure that the architectural programming stages advanced correctly and received ongoing feedback. Studies have confirmed the results, which show that artistic education in structures that provide users with distinct experiences is different from that provided in faculty buildings with a type plan scheme. The process, which involves collaboration between stakeholders from various disciplines and necessitates coordination, is also maintained in a coordinated fashion as a consequence of the study. Because of this, it is believed that buildings with comparable features can offer direction by offering a set of data that may also be reliable for design procedures that are prearranged.

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Introduction

Design is a methodically constructed phenomenon that is broadly defined as the art of problem-solving and involves a creation process at its core. In architectural design, the problem area or all of the data combined defines the form, and all of the problems the designer must solve make up the design's content (Bilir, 2013). It is impossible to foresee beforehand what the process's contents, design outputs, and effects on the final product will be, even though rationally planned design processes are necessary to expect the best possible outcome. Though the phases of the process are generally agreed upon, it should be assumed that each situation will present a different process depending on the nature of the problem or how it is expressed, as well as the preferences, ideas, opinions, and experiences of the designer (Osmanoğlu, 2022).

University buildings serve as significant social and cultural places in addition to being educational establishments. These spaces are crucial for social interaction between instructors and employees because they are where students from diverse cultural, social, and ethnic backgrounds spend a significant amount of their after-school hours during their academic careers. This makes it necessary to take a variety of factors into account when planning the university's educational spaces. As the planning process moves forward, so does the design process, which shapes areas and reveals the best spatial compositions in accordance with planning decisions. Planning and design are therefore physical processes that come after one another (Korkut and Üstün Topal, 2015).

Physical planning is becoming increasingly important as it is rather costly to create the spaces that a university requires (Öner, 1999). University structures have a distinct meaning in terms of education and training as well as integrating people into society, in contrast to primary and secondary education. Within these frameworks, an individual produces original works and grows in areas such as selecting a career and related matters. An individual can succeed in such work if there are social areas that are cozy and practical as well as ergonomically designed. The greater the desire to produce knowledge and successful

original works can emerge, the more the individual masters the space and feels uniquely designer (Güner, 2019).

There are many studies showing that the social and cultural activity areas of university spaces are directly related to the individual and social development of students (Erçevik and Önal, 2011; Yılmaz, 2015; Sıramkaya and Çınar, 2012). However, Jarvis (2005) emphasizes that the learning environment created in educational buildings, which are the primary places where learning takes place, is the set of affective, cognitive and social interactions that each individual who uses that space establishes with the spaces. Therefore, educational buildings appear as one of the most basic design problems in which the quality of the spatial order is important as well as the fulfillment of the basic requirements of the physical space (Tunçok Sarıberberoğlu, 2020).

The goal of creating a planned and programmed university structure is to improve the perceptions of the university among academics, administrative personnel, and students to raise expectations (Özyurt, 2019). Spatial disconnections prevent areas from being used efficiently and make it impossible to meet user demands in poorly managed design processes (Begeç, 2002). Faculties are complexes with various educational policies, and managing the faculty's units in a methodical manner is the only way to construct physical spaces that can adapt to these policies (Erkman, 1990).

In this sense, the study covers the entire process of designing and constructing a faculty building, taking into account educational policies, involving users in the design phase, and ensuring that all teams—including architects, engineers, educators, administrators, and construction workers—follow coordinated protocols.

The Selçuk University Faculty of Fine Arts building was selected as an example, and the design, application, and construction processes are all covered in the study. The study did not include the post-use evaluation phase. The content analysis method was employed as the study methodology. This method gathers files and documents related to the structure and gathers data based on observations and conclusions. The information gathered through interviews and descriptive analysis within the required definitions was put to use. A descriptive and content analysis component of a qualitative research methodology was applied. It covers the construction process as well as the phase of construction, which entails evaluating all of the findings and preparing preliminary and application projects, drafts, and sketches. In the course of the construction phase, field observations, data collection, and detection studies were conducted. The conclusion section included recommendations for resolving the research problem. Even though the events and phenomena experienced in the study were complex, interwoven, and involved many variables, an attempt was made to define and make sense of the whole by looking at a case study in which the authors participated and experienced. Therefore, it is believed that publications about the shared creative endeavors of designers as well as the process of designing and implementing built structures will be valuable resources for other designers as they share firsthand knowledge of analogous design processes. Furthermore, there is a distinction in the scientific platform regarding the significance of the study due to the fact that the sample structure can be used as a model for other structures in terms of educational spaces with identity and that project applications of this type should be dropped.

Conceptual Background

Design is defined as the form that emerges from the act of designing in a person's mind. The term "design" becomes a concept that is challenging to define in terms of content when it is combined with other meanings like "planning," "making sketches," "editing," and "designing." A plan or an idea for solving a problem is a brief definition of design found in a variety of literary sources (Demirarslan, 2006). Design is an extensive process with multiple phases. The term "design process" refers to the sequence of steps that include the methods and equipment utilized in the design action. Stated differently, the design process encompasses all the actions taken from the moment the design problem arises until it is resolved. This process may involve one or more considerations, forming a decision sequence, depending on the nature of the design problem (Bayazıt,1994).

There are four steps in the design process. These consist of the pre-design, final, implementation, and construction phases of a project. Various types of information about the structure are gathered during the pre-design stage. These details are crucial for determining the location and effective use of the building that will be designed. Because of this, the needs of the users and the specific environmental factors of the place where the space will be used must come first in the design process. User requests are taken into account when creating a needs program. Stain studies and handwritten notes, known as sketches, are used to create alternative designs based on the requirements and program. At the sketch stage, the design philosophy needs to be correctly constructed. Because every design needs to have a foundation, or its primary theme, or its philosophy (Demirarslan, 2006). During the sketching stage, offering a range of alternative suggestions within the framework of design philosophy helps to foster the development of design thinking. Writing down the thought process is a feedback process in design (Asıliskender, 2004). One of the crucial stages in the pre-design stage is coming up with alternate solutions. By choosing the best design from a range of potential solutions and developing it in two and three dimensions, the project is first scaled up at *the final project stage*. At this point, designers also select the material and size in addition to looking for form and function. The budget is another element that influences the design. During the implementation stage, it is possible to lose money and time by disregarding the opportunities and/or constraints given by the budget. *The implementation project phase* is the last step of the final project process, during which the design is turned into an application project, expert consultant reports from other disciplines and final engineering projects are used, the design's structural details and technical specifications are drawn, and the project's cost is determined. During the construction phase, the prepared implementation project is made available for use. The final stage, known as the construction phase, deals with the transformation of the design process into a physical building form. (Figure 1).

All of these procedures have distinct steps even though they are all similar in various building groups. Many researchers stress how important it is to build the stages of the design process correctly to improve the environment for learning, teaching, and socializing—especially in educational buildings (Gabrielsen & Saugstad, 2007; Temple, 2008; Çalışkan, 2023). Dutch architect Herman Hertzberger highlights the role of the architect in the education system expressing "The role of the architect is not to determine the education system, the architect should create a physical environment by following the ideas and philosophy underlying the education system" regarding educational buildings (Al Şensoy, 2019). The growth of the university and the caliber of the faculty are not as crucial to an effective educational process as the buildings in which architectural education is offered. This quality can be effectively determined by a variety of factors, including spatial features, data on the physical environment, and characteristics of residential areas (Çalışkan, 2021).

Material and Methods

The study's primary issues are the addition of new departments to Selçuk University's Faculty of Fine Arts and the inadequacy of the current structure to accommodate socialization and education-training programs. It was decided to construct a new faculty of fine arts building to address the issue. This building would house art education across multiple disciplines as well as exhibition and socialization spaces. The goal is to accurately schedule each step in the phases of the architectural design process for this reason. Studies on educational spaces have shown that physical space can either help or hinder learning (Uludağ, 2008). Specifically, faculty buildings are social and cultural spaces in addition to being places of learning. The quality of education and socialization is directly impacted by establishing the conceptual framework for the design, implementation, and construction processes of university education buildings and by correctly constructing the working method as a result of the information obtained. The design, implementation, and construction process of a sample area that the authors experienced are covered in the study. The study's methodology is based on qualitative research, which makes comprehensive use of interviewing, photography, on-site observation, and examination methods. The study's implementation, final, construction, and pre-design are the four stages of the project process. Every step comprises linked procedures.

Findings and Discussion

Established on December 4, 1999, the Faculty of Fine Arts at Selçuk University, situated in the Selçuklu district of Konya province, welcomed its inaugural students to the traditional Turkish arts and ceramics department in 2001. Admissions for the departments of interior architecture and environmental design began in 2003, while admissions for the painting department began in 2007. The departments of sculpture, graphics, industrial product design, and cartoon animation were empty at the time. In the 2650 m^2 building, educational activities were still in progress (Figure 2)

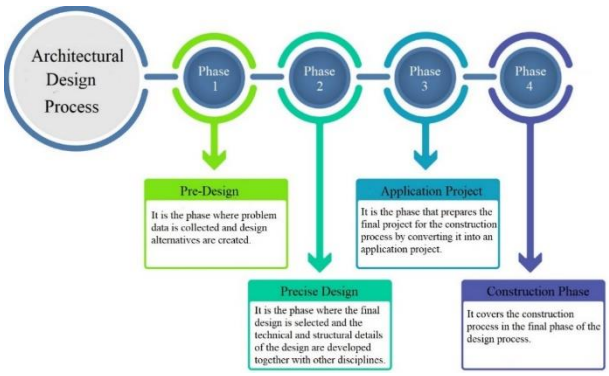


Figure 1. Architectural Design Process (Created by authors)

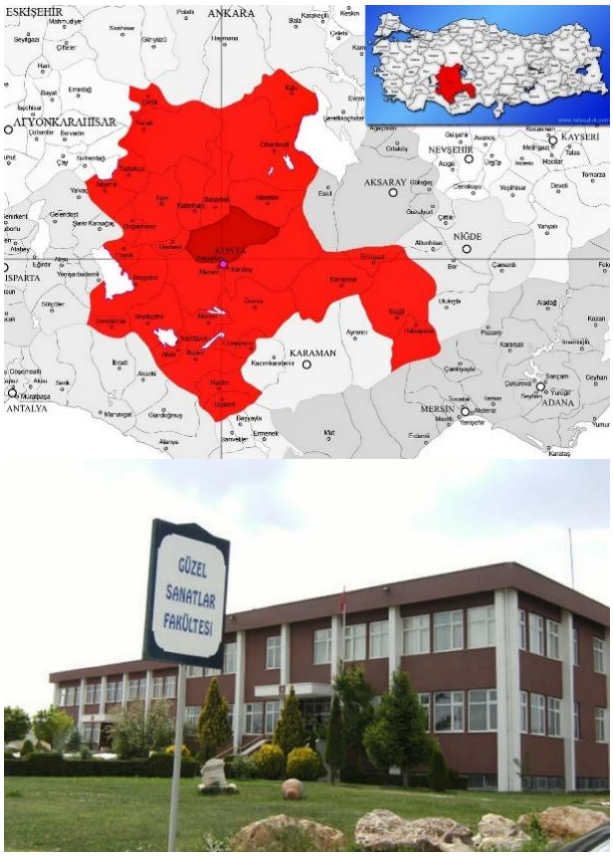


Figure 2. Location and old building of the faculty of fine arts

Figure 3. Location of the planned fine arts faculty building

In 2007, it was decided to build a new faculty of fine arts building after the admission of students to the painting department. This decision was made due to the insufficient physical space of the existing building, which would not be enough to accommodate the departments planning to admit more students in the future. The study's authors were tasked with planning the new building, while Selçuk University's Technical Department and Construction Works managed and supervised the project. According to the faculty dean's office, as of 2007, there are 191 students and 27 academic staff members. It was determined that the issue would be resolved by creating a unique, cost-effective structure that is adaptable, development-ready, and capable of offering the best possible opportunities for art education as well as comfortable surroundings that are conducive to socializing and have highly performing, qualified spaces, while also taking into consideration the sections that are scheduled to open in the future.

The location where the Olympic Swimming Pool is located in the west, the Dilek Sabancı Conservatory Building in the east, and the university football field in the north were determined by the construction Works (Figure 3).

The designers and the department of construction works worked together to design the necessary planning and organization work, as the design process is made up of interconnected decision steps and stages of deciding on different contents. Administrative staff, mechanical, electrical, and civil engineers, as well as material suppliers, would all be involved in the design process, with decisions made jointly by the Technical Department of Construction Works, and the designers, employer, users, and trainers appointed by department heads or professors to represent each department. The team in question was the subject of frequent meetings, and steps were taken to guarantee that the project process was executed in a coordinated fashion. The fact that students, the structure's primary user group, were left off of the team was a serious shortcoming, even though instructors were involved in the design process as users. There was a contentious design process that involved all of the experts. The architectural design process was carried out in stages for the project, which included the construction phase, the implementation phase, the final project phase, and the preliminary design phase. Processes for information flow, feedback, evaluation, and control were planned for each stage of the system.

Preliminary Design Phase

It is generally accepted that preliminary design, which is thought to be the introduction phase of design, is a meaning-making process that can be characterized as a type of mental representation. In this context, the form related to function and construction, functional benefit and spatial efficiency, reality, direct expression, environmental harmony, transformability, and flexibility potential depending on the space-time relationship are expressed in the structure. These are all accepted as fundamental concepts that should be adhered to in the early stages of design. Conceptual design is defined as the place where architecture begins. Based on the idea of spatial continuity, the design created a comprehensive design fiction that combined traditional Turkish architectural elements like courtyards, covered canopies, and dead-end streets with contemporary methods for roof lanterns and intersections. A plan featuring a courtyard connecting two major arteries to secondary roads was solved with the help of sketch studies (Figure 4).

By examining their subunits, the administrative and educational blocks are bundled. Originally, the building was designed as a ground floor, first floor, and basement. The talks led to the inclusion of the second floor in the design, which took the building's potential for expansion into consideration. The building works department approved the proposal to partially subterranean the building to save costs.

Final Project Phase

The final project phase, which involves developing the building in two and three dimensions and scaling it down, started with the completion of the building's sketch works, divided into the education and deanery blocks. Furthermore, three-dimensional studies are becoming more and more important in the field of a space design to guide, explain, and inform users—especially those who have difficulty envisioning the space they are designing. In this way, the project's mass and interior space were expressed during the final project stage using AutoCAD and 3d Max software (Figure 5-6).

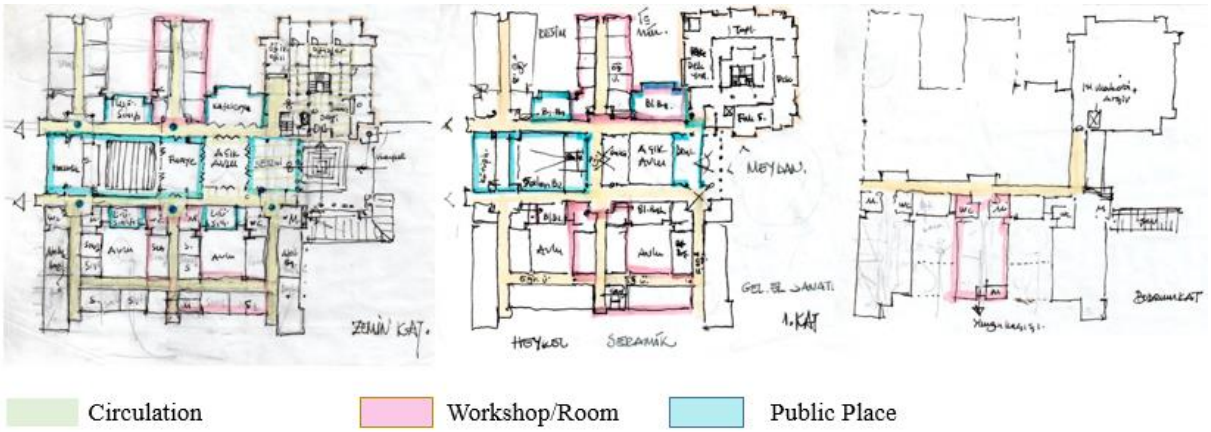


Figure 4. Sketches of the new fine arts faculty building (Images belong to the authors)

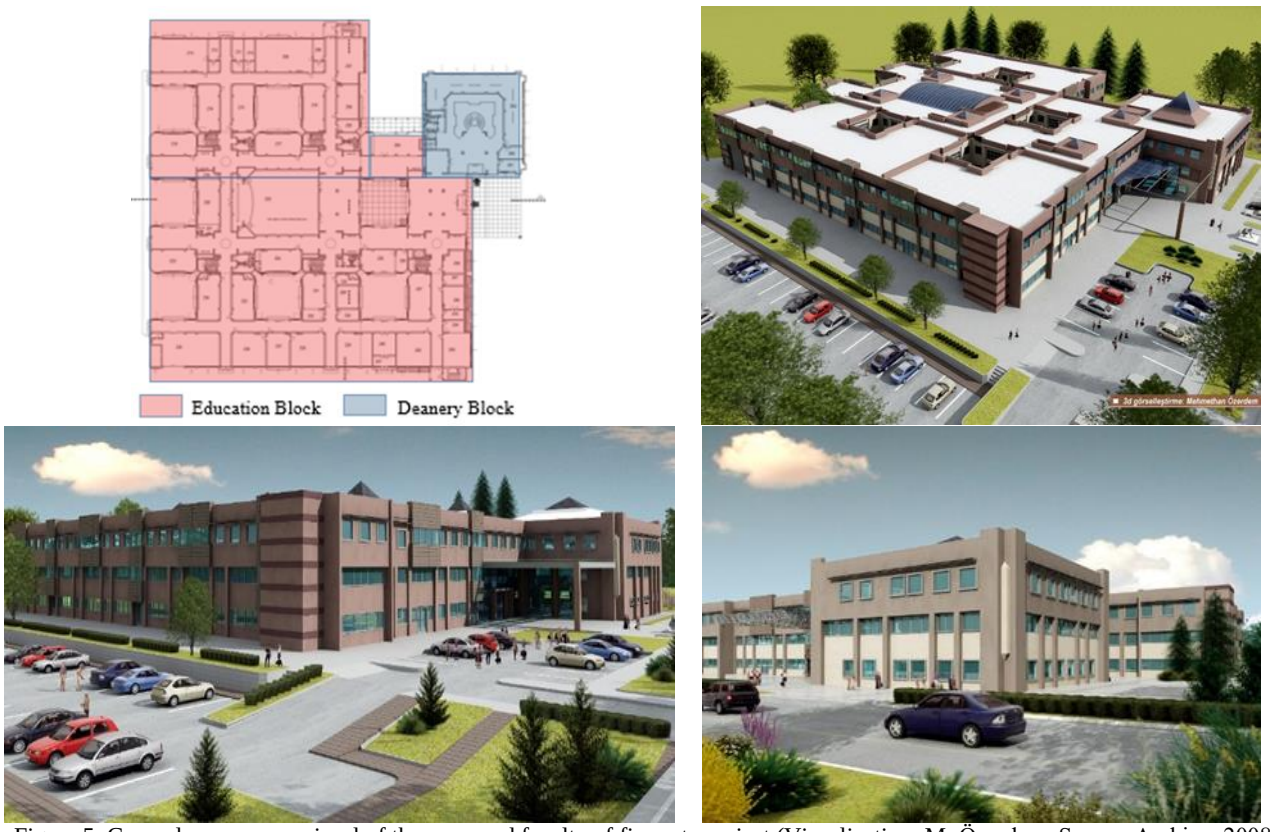


Figure 5. General appearance visual of the proposed faculty of fine arts project (Visualization: M. Özerdem, Sungur Archive, 2008)

Figure 6. Interior visuals of the proposed faculty of fine arts project (Visualization: M. Özerdem, Sungur Archive, 2008)

Implementation Project Phase

Selçuk University Construction Works and Technical Department organized the preparatory services, analytical studies of the building's final engineering projects, and expert consultant reports prior to the construction phase of the design. Following the final implementation project process, where the design's structural details and technical specifications were drawn up and a cost estimate was made, the construction process got underway.

Construction Project Phase

Following the 2008 comprehensive tender and 2009 contract signing, the Faculty of Fine Arts construction was underway. The building's floor height is 3.20 meters, and its approximate area is 14,000 square meters. It features a beamless hollow block flooring system with a reinforced concrete carcass structure. The interior partition walls were made of baked brick, while the exterior walls were made of aerated concrete. On a 260 cm axis, 50 cm x 50 cm square columns are constructed. The facades of the inner courtyards are covered in travertine stone, while the exterior is covered in mechanically assembled andesite stone (Figure 7-8-9).

Information about the Structure

Information about the Ground Floor

There are two entrances to the ground floor of the building: the deanery entrance from the east and the student entrance from the north. The student entrance greets visitors with a sizable exhibition space. Solutions that complement the Faculty of Fine Arts building have been developed for the student exhibition. The entrance exhibition area is flanked by two primary arterial horizontal circulations that run to the right and left. From these two principal arterial circulations, there are auxiliary corridors. By serving as streets, secondary corridors help to define sections. The interior architecture street is home to the interior architecture department's workshops and instructor rooms. Yet, the streets cannot be clearly distinguished from one another due to the usage of shared classrooms by departments. The building contains seven courtyards which create an open area inside the enclosed structure because they are directly connected to the corridors. As a result, the courtyards provide users with a variety of affairs for relaxation, mingling, and activities. The main arteries' open ends that extend southward give the structure flexibility by accounting for its potential for expansion.

Figure 7. Images from the construction process (Images belong to the authors)

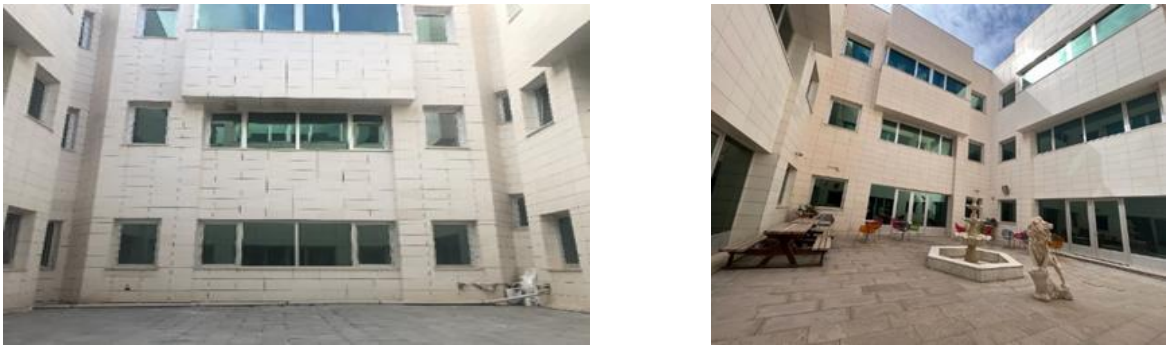


Figure 8. Travertine covered courtyard wall surfaces (Images belong to the authors)

Figure 9. Mechanically assembled andesite cladding exterior facades (Image belongs to the authors)

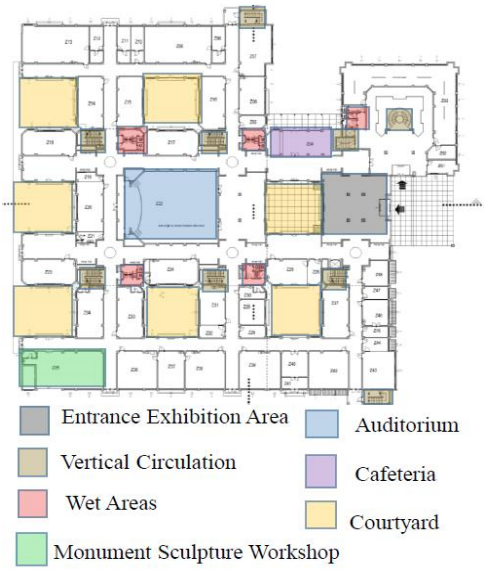
The building's cores, which consist of a wet area across from the stairs, are located at the intersection of the corridors. The focal point of the Faculty of Fine Arts project is a 240-person conference hall that can accommodate gatherings for meetings, events, and graduations. The courtyard is connected to the foyer area in front of the conference hall entrance. On the ground floor, there's a cafeteria as well, which can adjust to users' eating and drinking habits. Shuttle service between events and meetings is made easier by placing the cafeteria adjacent to the conference foyer area. During the preliminary design phase of the Faculty of Fine Arts project, the sculpture department specifically recommended the placement of a monument-sculpture workshop inside the building. The workshop should have a height of roughly 6 meters. The ground and first floors were joined in this direction to form a workshop that stands 6.40 meters tall. A door that is four meters wide and six meters high and has a direct opening to the outside was made to transfer the monumental sculptures that were prepared in the workshop from the faculty to a different location. As a result, a vehicle equipped with a rail crane system that can enter the workshop can move the enormous sculpture to different locations. A sizable exhibition space with the option to display in natural or artificial light can be found in the Dean's Office building. From the very beginning of the design process, the Dean's Office and the education block have included accessible restrooms and elevators for individuals with disabilities (Table 1).

Information about the First Floor

The ground floor's entrance exhibition area is 6.40 meters high, with a steel bridge that connects the main arteries on the first floor. Thus, the building's high entrance area added volumetric richness. The first and second floors of the building, where the corridors and cores are located, have spaces opened up from the floor. To maintain the vertical integrity between floors, these spaces are crucial. It draws interest as an exhibit tool as well. Students can work, interact, and create in the "design kitchen" located above the conference hall foyer area in between classes. The design kitchen makes it possible for the area to face the courtyard and provides upper-floor viewing for any exhibitions or events taking place in the foyer.

Table 1. Ground floor plan and images from different spaces (Images belong to the authors)

Ground Floor Plan



Entrance Exhibition Area

Vertical Circulation

Wet Area

Auditorium

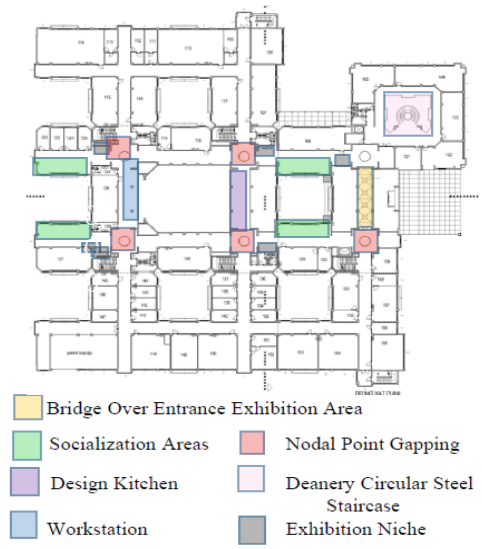
Monument Sculpture Workshop

Cafeteria

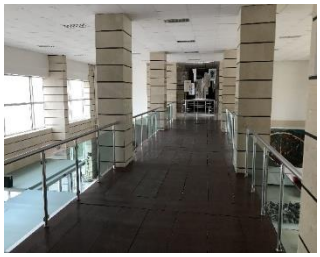
Courtyard

Table 2. First floor plan and images from different spaces (Images belong to the authors)

First Floor Plan



Bridge Over Entrance Exhibition Area



Nodal Point Gapping

Design Kitchen

Workstation

Exhibition Niches

Socialization Areas

Deanery Circular Steel Staircase

The design kitchen makes it possible for the area to face the courtyard and provides upper-floor viewing for any exhibitions or events taking place in the foyer. Once more, a workstation was suggested for the first floor, on the opposite side of the conference hall, allowing students to design throughout the structure. Artworks can be displayed in exhibition niches that have been created on both sides of the corridor junctions. The main arteries are designated as socialization areas, which facilitate exhibition and socialization and offer users experiences beyond education. Additionally, the first floor of the deanery block houses offices for student affairs, accounting, and meeting spaces. A circular steel staircase connects the floors (Table 2).

Information about the Second Floor

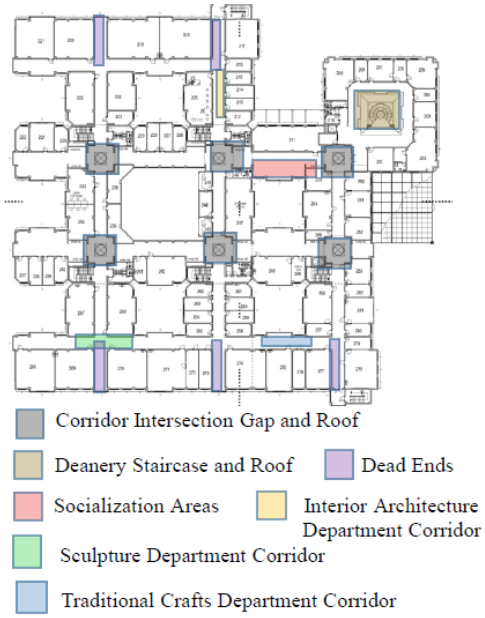
The corridor intersection space on the first floor is also located on the second floor. The top cover material of these spaces is chosen as glass so that the daylight coming from the roof descends to the ground as light filtering. The roof of the circular steel staircase in the deanery block was treated with the same method. The first and second-floor classrooms and workshops, as well as the socializing and exhibition areas across from the instructor rooms, are crucial in capturing the essence of the fine arts faculty. The choice of colors, textures, and materials to symbolize the section defines the corridors, which are likewise divided into sections (Table 3).

Information about the Basement Floor

During the design process of the Fine Arts faculty building, academic staff users of departments such as painting, ceramics and sculpture requested that the materials be stored in appropriate environments. Additionally, administrative staff requested large warehouse areas to store official documents. However, the Selçuk University Department of Construction Works recommended constructing a partial basement floor in light of the expense of constructing a full basement floor. As a result, the education block and the deanery block were set up as basement floors. This floor has been designed with storage spaces to accommodate every department and administrative unit's requirements. Among the units on the basement floor are technical units and shelter areas. The shelter area has been rearranged for exhibition today due to its large size. The basement floor's ceiling design, which features traditional motifs at the exhibition's entrance, created a different viewpoint (Table 4).

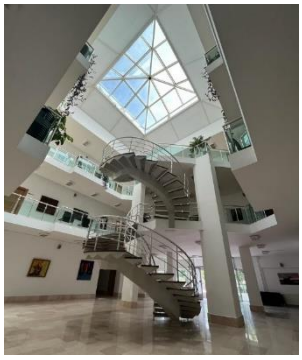
Table 3. Second floor plan and images from different spaces (Images belong to the authors)

Second Floor Plan



Corridor Intersection Gap and Roof

Deanery Staircase and Roof



Socialization- Exhibition Areas

Sculpture Department Corridor

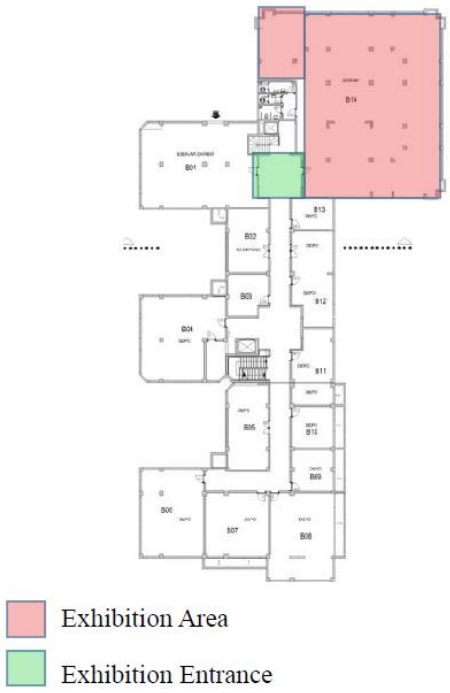
Interior Architecture Department Corridor

Traditional Crafts Department Corridor

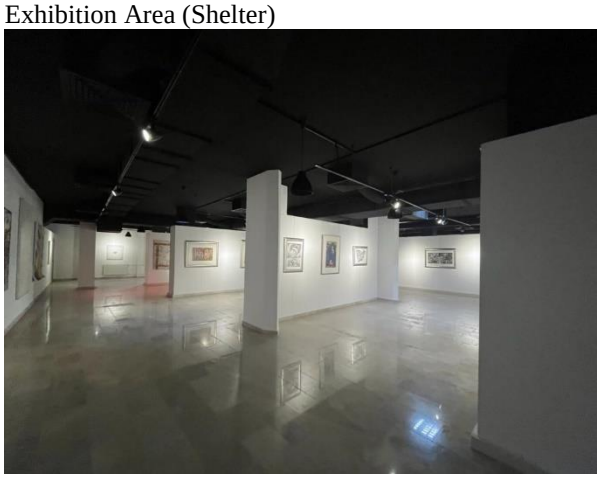
Dead Ends

Table 4. Basement floor plan and images from different places (Images belong to the authors)

Basement Floor Plan



Exhibition Area (Shelter)



Exhibition Area (Shelter)

Exhibition Entrance

Conclusion

Solution-oriented methods are a part of architectural design, which is founded on the creation and reorganization of the physical environment. Thus, the most crucial components of the physical environment are the establishment of an accurate and effective design process as well as the structures that are constructed and house the institutional system. Universities are one of the institutions where sociological and social developments occur in addition to knowledge production. The university has instructional spaces where experts in a range of arts and sciences receive training. The needs of various disciplines, such as engineering, education, and the arts, can influence these learning environments, known as faculties.

Unlike other faculties, the fine arts faculty serves as a training ground for aspiring artists like sculptors, painters, and graphic designers. Being creative is essential to creating art. Originality is dependent on various elements as well as areas where design is allowed to flow freely. Studies have shown that artistic education in structures that provide users with unique experiences is different from that provided in faculty buildings with standard floor plans. Scholars underscore that the quality of education is also impacted by physical space. The study's findings support the notion that improving functional benefit expectations and user needs and expectations can have a substantial positive impact on both spatial quality and academic performance. One of the most significant results of this study in terms of saving time, energy, and money is the planning of the preliminary design, final project, implementation project, and construction phases from the very beginning of the design process and leaving as few issues for the construction phase. The process, which involves collaboration between stakeholders from various disciplines and necessitates coordination, is systematically maintained, which is another outcome of the study.

In a nutshell, it has been established that, depending on the content and the final product, a methodical approach to design preparation can and may result in favorable outcomes. The results of the design process, which are derived from the correlation of all the data gathered from the case study's research and the process's experience, are believed to be able to offer direction by offering a range of data that may also be applicable to the pre-planned design processes of buildings with comparable features.

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The Declaration of Conflict of Interest

The authors declare that there are no competing financial and non-financial interest.

The Declaration of Ethics Committee Approval

This study does not require ethics committee permission or any special permission.

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An Example of an Application Project on Contemporary Office Design

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ABSTRACT

This study aims to define the concept of office spaces and their requirements, as well as the contemporary interior design approaches. It will also cover the interior design and application process for an architecture office in Konya. The company, originally named Çınar Architecture and active in Konya between 1985 and 1990, has been operating as Kerimler Planning Construction Consultancy Company in Konya since 1995. The company requested an interior architecture project for their new location. In the current environment, the office is starting to become outdated and is unable to offer users enough physical amenities. The main goal of the new office's interior design was to create areas where employers and employees could collaborate in a welcoming and adaptable setting without facing any kind of discrimination. In Konya Meram Pirebi District, the Menekşe Apartment's ground floor, which is currently a street-level store, is the site of the project, which occupies a total of 280 m2 and consists of a ground + basement. The office occupies 145 square meters on the ground floor of a recently constructed building, with glass extending to the open floor on the south, north, and west facades. The room has a very large opening because the columns are concealed by the walls, making the two stories easily visible from the entrance. The office, which occupies a 135 m2 basement floor, is connected to the elevator and staircase at the back. Natural light enters the basement through the gallery area that is attached to the staircase. Within the parameters of the study, two- and three-dimensional architectural design tools, including phases of the interior design process, were employed in conjunction with a qualitative research method based on inspection and observation. The architectural office put it into practice about half a year after the design stage. The outcome was the creation of a dynamic, modern office analysis that values teamwork and permits the emergence of unique and creative ideas throughout the project.

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Introduction

"All things considered, offices are systems made up of people, technology, interpersonal and organizational relationships, and physical surroundings. Workplaces and offices are assemblages of social and physical systems. The social system consists of human relationships, task perception and meaning, expected needs of individuals, and organizational culture. The intended environment, technology, job specifications, and activities are all part of the physical system" (Goodrich, 1982). According to this viewpoint, offices serve as both practical places where information is exchanged and environments with social and physical elements where people spend the majority of their working hours. Offices should better be viewed as locations where time and space are occasionally broken for social activities, according to Çimen (2008), rather than as the primary location for solitary activities. It is also highlighted that while the office should be primarily designed as a social space, there should also be private areas available for concentration, thinking, and privacy (Çimen, 2008). Taking into account these definitions and details, the office should be conceptualized as a place where various hierarchical relationships are established and common areas offer workers physically and socially productive working environments. The design setup should be based on these specifics.

Office spaces are dynamic environments where there is a constant flow of intense and ever-changing information. These environments establish hierarchical relationships of varying dimensions. With the advancement of technology, offices are no longer perceived as just working spaces, but also as living spaces. As a result, the interior design approaches of today's offices have changed. Concepts such as the importance of time, teamwork, providing prestige to clients, and corporate image necessitate an examination of contemporary office designs with today's approaches (Noraslı and Köse Doğan, 2020). This study explains interior design approaches for modern offices, the concept of an office and its requirements, and interior design and application for the architectural office in Konya.

Interior Design Approaches in Contemporary Offices

Office design approaches have changed due to factors like the speed at which technology is developing, the idea that time is important in business, and the desire of employees to work in more comfortable environments. The idea of the "Contemporary Office" was developed as a result of evolving office logic and the blending of technology and production-consumption relations.

In today's highly technologically-influenced offices, there are several requirements for interior design that boost user satisfaction and create a more productive work environment. Personal space, social boundaries or togetherness, aesthetics, visual and auditory privacy, and the need for flexible space designs that can accommodate changes and innovations are some of these user-oriented needs (Kayan and Tuncel, 2012). The needs of the users have also changed as a result of the evolving perception of the office. As a result of technological advances; adaptable, modular, and privacy-conscious designs and solutions have been created to satisfy these shifting requirements.

Innovative approaches are used in contemporary office interior designs, such as workspaces that support both individuality and togetherness simultaneously with flexible and mobile modules, units that support group workspaces that will support brainstorming, common social use areas that encourage communication, and cell-type individual workspaces that promote focus while respecting privacy. In conclusion, modern offices should be viewed as a collection of areas created with a comprehensive approach, fusing technology and physical space, where creative thinking and novel viewpoints can emerge and connect to facilitate both solo and collaborative work.

Materials and Methods

After operating as Çınar Architecture in Konya from 1985 to 1990, the company has been operating as Kerimler Planning Construction Consultancy Company in Konya since 1995. The company asked for an interior architecture project for the new headquarters of the business. Given the current state of conditions, the office is beginning to become obsolete and unable to offer its users adequate physical amenities. The main objective of the new office's interior design was to create areas where employers and employees could collaborate in a welcoming and adaptable setting, free from discrimination. Within the parameters of the study, two- and three-dimensional architectural design tools, including phases of the interior design process, were employed in conjunction with a qualitative research method based on inspection and observation. The author created the modern interior design and application within the parameters of the study, taking into account the demands of business owners. The design and application procedures are outlined below.

The project area

The Menekşe Apartment's ground floor in Konya Meram Prebi District, which is currently a street-level store, is the site of the project, which occupies a total of 280 m2 and consists of a ground + basement. The office occupies 145 square meters on the ground floor of a recently constructed building, with glass extending to the open floor on the south, north, and west facades. The room has a very large opening because the columns are concealed by the walls, making the two stories easily visible from the entrance. The office, which occupies a 135 m2 basement floor, is connected to the elevator and staircase at the back. Natural light enters the basement through the gallery area that is attached to the staircase (Figures 1-2-3).

Office Interior Design Process

The project phase has begun using the Autodesk-based AutoCAD program, taking into account the data of the projected area and the desired need program. First and foremost, a free and organized layout was produced in the project by keeping large openings and minimizing the use of closed dividers. There is multipurpose seating, an exhibition space (for material-project promotion), and a work area that can accommodate three persons or more on the ground floor. The basement floor is set up with kitchens, wet areas, archives, meeting rooms, and areas for seating and exhibition (Figures 4-5).

On the left side of the entry section, there is a cozy seating area that visitors and staff members can use. This area serves to greet incoming clients and provides a first impression of the office. The floor in this area, which is about 25 m2, is divided with wooden laminate, while the floor material in other office areas is micro concrete with matte varnish and diagonal lines applied. The remaining walls were covered with a material that looked like concrete. Wire grids on the ceiling, certain wall surfaces, and dividers were taken into consideration for corporate identity works and project exhibitions (Figure 6).

To accommodate busy workers, the designers have created versatile communal areas situated side by side. The display boards showcasing the products for sale are neatly arranged, while the glass front on the other side of the workspace is closed. Computer support has been provided for controlling the daylight in the workspace. The back wall of the area features an open shelving system that staff members can use freely. The floor is covered with micro cement, a material that is widely used throughout the office. To complement the space's dynamic lines, pendant lighting has been installed over the work desk, and hidden LED lighting has been incorporated into the plasterboard wall and suspended ceiling. On the product display panels, rail spotlights were also utilized (Figure 7-8).

The basement floor features a transparent meeting area designed to foster idea exchange and brainstorming, as well as serve as a social space for in-office celebrations and coffee breaks on special occasions. The area is partitioned from other spaces by a glass wall, and covered in carpet tiles on the floor and concrete-looking material on the walls. Whiteboards, shelves, and LCD televisions are also mounted on the walls for use during meetings (Figure 9).

Office Interior Application Process

The office's plumbing and electricity were mostly replaced during the implementation phase, which was marked by the completion of the project design and 3D visualizations. After pouring the leveling screed for the basement floor, the wall masonry for the restroom was finished, and 60 by 60 ceramic tiles that resembled natural stone were placed on top (Figures 10-11-12).

Figure 1-2-3. General Views of Office Floors

Figure 4-5. Basement and Ground Floor Layout Plans.

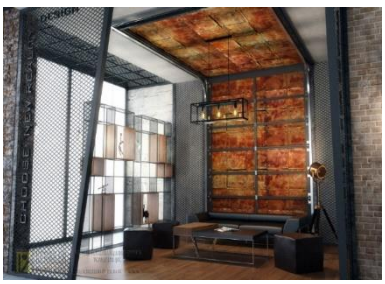

Figure 6. Ground Floor Seating Area

Figure 7-8. Views from the Workspace

Figure 10-11-12. Renovation of Electrical and Plumbing Systems, Wall Construction in Basement and Ground Floor.

On the ceiling, the 3×4 metal profiles meant for the ground floor ceiling design were arranged in a grid pattern spaced 100 by 200 apart. It was covered with 100×200 sheet metal panels that were laser-cut. Solar shading panels were installed and the exterior door's aluminum joinery was replaced in accordance with the Project (Figures 13-14-15).

In compliance with the project, the plasterboard work for the basement floor's space divisions has been finished. The wallpaper chosen per the design was laid after the rough plaster and plaster works of the ground floor and basement were completed. Ceramic tiles measuring 60 by 120 were used to lay the ground floor (Figures 16-17-18).

A static paint that matched the wall paint color was used to paint the radiators in the basement and ground floor. Both the gallery area and the stairwell's edge have glass railings installed (Figures 19-20-21). Iron profile structure of kitchen was constructed on-site using 3×4 metal profiles in compliance with the drawing. After their frames were made from 2×2 metal profiles and painted with static paint, the bookcases that were designed for the ground floor and basement were put together on location (Figures 22-23-24). On the ground floor, the aluminum joinery measurements of the divided rooms (manager, meeting room and kitchen) were installed and glass was installed. General and local lighting enclosures were installed (Figures 25-26-27).

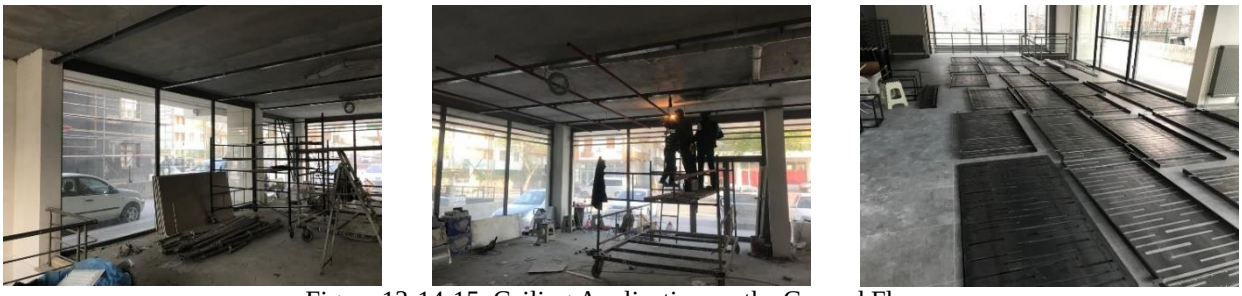

Figure 13-14-15. Ceiling Application on the Ground Floor.

Figure 16-17-18. Applications in Basement and Ground Floor.

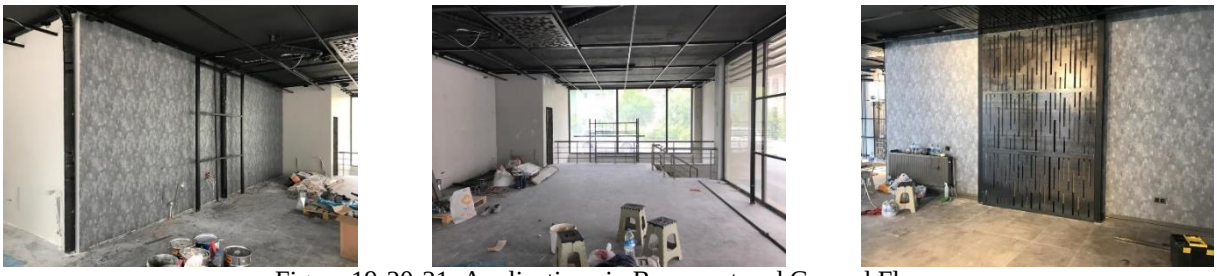

Figure 19-20-21. Applications in Basement and Ground Floor.

Figure 22-23-24. Views from the Workspace.

Figure 25-26-27. Views from the Workspace.

Table 1. Office Design Result Products.

Ground Floor Visuals

Entrance Reception Area | Open Office Working Areas | Rest Areas

Basement Floor Visuals

Lobby | Manager's Office | Kitchen | Gallery Void and Meeting Room

Findings and Conclusion

After the design phase, the author implemented and delivered the architectural office in about six months. Throughout the project, a dynamic, modern office solution that values teamwork and fosters the emergence of creative, original ideas is stressed. Together with general and local lighting and accessory parts, the wooden furniture works, which were finished in the final stage of the design application created in compliance with the project, are displayed in Table 1.

The entrance hall, open office design areas, waiting-rest area, manager's room, meeting room, gallery space, kitchen, and WC areas were all created with the demands and requirements of the workforce in mind. These areas have been evaluated based on the physical indoor atmosphere criteria. The design concept applied in this direction is contemporary, inventive, and dynamic industrial, with a clear plan to ensure ease of use and to satisfy all user needs. As a result, a new perception of the space atmosphere has been created.

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