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In vitro Antifungal Effects of Various Essential Oils against Aspergillus Crown Rot of Peanut

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A R T I C L E I N F O

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A B S T R A C T

Synthetic fungicides that combat plant pathogenic fungi can enhance crop yields, ensuring stable crop production and market quality. However, the increase in the use of fungicides may cause to development of fungicide-resistant pathogen strains and the accumulation of fungicide residues in the food chain above safe limits. This situation underscores the need for improved fungal disease management through alternatives to synthetic fungicides. These alternatives include plant-derived compounds such as essential oils and extracts. Essential oils are known to be potent antifungal compounds against both human and plant pathogens. *Aspergillus niger* is a toxin-producing fungal disease agent that causes Aspergillus crown rot in peanuts. In this study, the antifungal activities of nine different essential oils from *Foeniculum vulgare, Lippia citriodora, Origanum majorana, Origanum minutiflorum, Origanum onites, Origanum syriacum, Origanum vulgare, Salvia aramiensis* and *Thymus syriacus* plants were evaluated against *A. niger* under *in vitro* conditions by using disc diffusion test. Among the nine essential oils tested, the highest antifungal activities were displayed by *O. vulgare* essential oil (with an inhibition zone diameter of 49.33 mm) which was followed by *T. syriacus*, *O. onites, O. syriacum* and *O. minutiflorum* essential oils (48.67, 47.00, 46.33 and 43.33 mm, respectively). The essential oils of *F. vulgare, L. citriodora*, and *O. majorana* showed relatively lower antifungal effects. The essential oil of *S. aramiensis* did not show antifungal effect against pathogen. The results indicated that plant essential oils could be valuable in promoting research aimed at developing new antifungal agent(s) for fungal disease management. However, further studies are needed to optimize the *in vivo* application conditions of essential oils against *A. niger*.

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Yer Fıstığında Aspergillus Kök Boğazı Çürüklüğüne Karşı Çeşitli Uçucu Yağların *in vitro* Antifungal Etkileri

M A K A L E B İ L G İ S İ

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Ö Z

Bitki patojeni funguslarla mücadelede sentetik fungisitler, ürün verimini artırarak istikrarlı üretim ve pazar kalitesi sağlayabilir. Ancak fungisit kullanımındaki artış, fungisite toleranslı patojen türlerinin gelişmesine ve gıda zincirinde güvenli sınırların üzerinde fungisit kalıntılarının birikmesine yol açmıştır. Bu durum, sentetik fungisitlere alternatif olarak geliştirilen fungal hastalıklarla mücadele yöntemlerine olan ihtiyacı doğurmuştur. Bu alternatifler arasında, uçucu yağlar ve ekstraktlar gibi bitkisel kaynaklı bileşikler yer almaktadır. Uçucu yağların, hem insan hem de bitki patojenlerine karşı güçlü antifungal etkiye sahip olduğu bilinmektedir. *Aspergillus niger,* yer fıstığında Aspergillus kök boğazı çürüklüğüne neden olan, toksin üreten bir fungal hastalık etmenidir. Bu çalışmada, *Foeniculum vulgare, Lippia citriodora, Origanum majorana, Origanum minutiflorum, Origanum onites, Origanum syriacum, Origanum vulgare, Salvia aramiensis* ve *Thymus syriacus* bitkilerinden elde edilen dokuz farklı uçucu yağın antifungal etkileri disk difüzyon testi kullanılarak *in vitro* koşullar altında *A. niger*'e karşı değerlendirilmiştir. Test edilen dokuz uçucu yağ arasından en yüksek antifungal etkiyi *O. vulgare* uçucu yağı (49.33 mm engelleme zon çapı ile) göstermiş, bunu *T. syriacus, O. onites, O. syriacum* ve *O. minutiflorum* uçucu yağları (sırasıyla 48.67, 47.00, 46.33 ve 43.33 mm) izlemiştir. *F. vulgare, L. citriodora* ve *O. majorana* uçucu yağları nispeten daha düşük antifungal etkiler göstermiştir. *S. aramiensis* uçucu yağı patojene karşı herhangi bir antifungal etki göstermemiştir. Bu çalışmanın sonuçları, bitki uçucu yağlarının fungal hastalık yönetimi için yeni antifungal etmen(ler) geliştirmeyi amaçlayan araştırmaları teşvik etmede değerli olabileceğini göstermektedir. Bununla birlikte, *A. niger*'e karşı uçucu yağların *in vivo* uygulama koşullarını optimize etmek için daha fazla çalışmaya ihtiyaç vardır.

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Introduction

Groundnut (*Arachis hypogaea* L.) is an agricultural crop of great importance in both human nutrition and economic terms worldwide. Thanks to its high oil and protein content, it is both consumed directly as food and used in various fields such as animal husbandry and industrial oil production (FAO, 2020). Fungal disease agents such as *Aspergillus niger, A. oryzae, Penicillium georgiense, P. polonicum, P. glabrum* and *P. expansum* cause rot diseases in agriculturally important crops before and after harvest (Kara & Soylu, 2023). Peanuts are attacked by fungal pathogens in post-harvest processes, causing significant quality and yield losses. *A. niger*, one of these pathogens, multiplies rapidly on peanuts, causing both physical deterioration and food safety hazards with mycotoxin production (Pitt & Hocking, 2009).

A. niger grows under favorable moisture and temperature conditions, especially during storage, and produces toxic compounds such as aflatoxins. Aflatoxins are extremely dangerous for both humans and animals and can cause serious health problems such as liver cancer (Williams et al., 2004). The World Health Organization (WHO) and the Food and Agriculture Organisation (FAO) emphasize that aflatoxins pose a serious public health risk in food products and should therefore be checked regularly (WHO, 2018). Therefore, control of *A. niger* and prevention of damage caused by this fungus in groundnut products is of critical importance.

Traditionally, chemical fungicides have been widely used to control fungal pathogens. However, overuse of chemical fungicides leads to problems such as environmental pollution, loss of biodiversity, and fungicide resistance in pathogens (Fernández-Baldo et al., 2013). In addition, chemical residues can accumulate in foods and this situation creates risks for consumer health (Pimentel, 2009). In this context, environmentally friendly, non-toxic, and safe alternative solutions are of great interest.

Plant essential oils (EOs) have been increasingly investigated as natural antifungal agents in recent years. Essential oils are rich in components such as terpenes, phenols, and aldehydes and show broad-spectrum biological activity against pests and pathogenic microorganisms (Bakkali et al., 2008). For example, essential oils of plants such as thyme (*Origanum vulgare*), peppermint (*Mentha piperita*) and tea tree (*Melaleuca alternifolia*) have shown significant antifungal activity on various microorganisms (Soković et al., 2010). The natural and multicomponent nature of essential oils attracts attention as an alternative control method against not only fungal pathogens but also plant pests (Sertkaya et al., 2010).

Several studies have been conducted to investigate the antifungal effects of essential oils against *A. niger.* Especially essential oils obtained from *Origanum* species attract attention due to their broad-spectrum antimicrobial effects. For example, essential oils of species such as *O. vulgare* and *O. syriacum* have been reported to exhibit strong antifungal properties against different microorganisms (Fikry et al., 2019; Žitek et al., 2021). Active components such as carvacrol and thymol in these oils were found to inhibit the growth of pathogens by damaging the cell membrane (Sakkas & Papadopoulou, 2017). Similarly, essential oils of plants such as *Foeniculum vulgare* (fennel) and *Salvia aramiensis* (sage) have also been shown to be effective against *Aspergillus* species. While fennel oil shows antifungal properties thanks to its high amount of anethole, components such as salvin and rosmarinic acid in sage oil have been reported to inhibit the growth of pathogens (Sharma et al., 2019; Ghasemian et al., 2020; Zulu et al., 2023). Recently, *in vitro* antifungal effects of different concentrations of essential oils of different thyme species such as *T. vulgaris*, *T. spicata* and *O. syriacum* L., fennel (*F. vulgare*), laurel (*Laurus nobilis*) and eucalyptus (*Eucalyptus camaldulensis*) were investigated against pepper fungal disease agents *Alternaria alternata* and *A. niger* (Atay & Soylu, 2023). The highest antifungal activities (100% inhibition) against fungal isolates were shown by essential oils of *T. spicata*, *O. syriacum* and *T. vulgaris*. *E. camaldulensis* displayed the lowest antifungal activity against tested fungal isolates. Previous studies showed that essential oils not only inhibit the growth of pathogens but also contribute to making plants more resistant by enhancing the overall immune response (Valdivieso-Ugarte et al., 2019; Sandner et al., 2020). These findings emphasize that essential oils can be used as an environmentally friendly alternative to chemical fungicides.

In this study, the antifungal activities of nine different essential oils (EOs) obtained from *Foeniculum vulgare, Lippia citriodora, Origanum majorana, Origanum minutiflorum, Origanum onites, Origanum syriacum, Origanum vulgare, Salvia aramiensis* and *Thymus syriacus* plants were investigated *in vitro* conditions against *A. niger*. This study aimed to investigate the potential of using plant essential oils as a natural solution for the protection of agricultural products and to determine whether these oils can be effective in the control of *A. niger.* Thus, environmentally friendly alternatives that can replace chemical fungicides were evaluated.

Materials and Methods

Isolation and Identification of Fungal Disease Agent

Fungal disease agent *Aspergillus niger* was isolated from the rind and inner tissues of infected peanut plants growing in Hatay province. Small pieces were taken from infected peanut samples with a sterile scalpel and surface sterilised with 70% alcohol. In a sterile environment, the samples were placed on potato sucrose agar (PSA) medium and incubated at 25°C for 7 days. Fungal disease agent was tested for pathogenicity and identified based on morphological characteristics (Ahmed & Ravinder Reddy, 1993) and MALDI-TOF (Bruker Daltonics GmbH, Bremen, Germany) analysis (Kara & Soylu, 2023). Spore suspension (10^3 spores/ml) of *A. niger* was prepared from the actively growing culture (5 days old) in distilled sterile water. These suspensions were used to inoculate PSA in antifungal activity studies.

Determination of Antifungal Effects of Essential Oils

The essential oils used in the study (*Foeniculum vulgare, Lippia citriodora, Origanum majorana, Origanum minutiflorum, Origanum onites, Origanum syriacum, Origanum vulgare, Salvia aramiensis* and *Thymus syriacus*) were obtained from the stocks of Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Plant Protection, Phytopathology Laboratory. The hydrodistillation method with a Neo-Clevenger device (Ildam, Ankara, Turkey) was used to extract the EOs. The main compounds and % ratios of essential oils were analysed using a GC-MS device (Thermo Scientific ISQ Single Quadrupole, Milan, Italy) and the results have been reported in previous studies (Kara et. al., 2020; Soylu et al., 2020; Kara et. al., 2021; Kara et. al., 2022a). The antifungal effect of each essential oil under *in vitro* conditions was determined by using disc diffusion technique in petri dishes. On the surface of the petri dishes containing PSA medium were inoculated 200 µl of fungal suspension prepared as previously described was spread. Sterile filter paper discs (6 mm in diameter) were placed on the surface of the petri dishes, 5 µl of essential oils were added, and the petri dishes were sealed with sterile parafilm and incubated at 25°C for 48 hours. In negative control petri dishes, sterile filter paper discs amended with distilled water were used. The antifungal effect of essential oils was evaluated by measuring the diameter of the inhibition zone around the filter paper disc 48 hours after treatment.

Statistical Analysis

In vitro antifungal activity trials were carried out in at least three replicates for each essential oil/fungal isolate and the experiment was repeated at two different times. One-way analysis of variance was performed on the values obtained in the trials using SPSS statistics software (SPSS Statistics 17.0) and the difference between the treatments was analysed by Duncan Multiple Comparison Test ($P \leq 0.05$).

Results and Discussion

The fungal disease agent was isolated from infected groundnut shells. Preliminary identification of the fungal isolate was based on the morphological characteristics of the isolate grown on PDA medium and confirmed by MALDI-TOF MS.

The antifungal effects of the essential oils obtained from each plant species were determined using the disc diffusion technique and the diameters of the inhibition zones of these essential oils against the fungal disease agent were measured and given in Table 1. The findings of this study showed that plant essential oils have a significant antifungal potential against *A. niger*. The essential oils of different plant species showed significantly varying levels of antifungal activity. The essential oils of *F. vulgare, L. citriodora, O. majorana, O. minutiflorum, O. onites, O. syriacum, O. vulgare,* and *T. syriacus* significantly inhibited the growth of *A. niger* (Figure 1). According to the inhibition zone diameter values, the highest antifungal activities were displayed by *O. vulgare* EO (with an inhibition zone diameter of 49.33 mm) which was followed by *T. syriacus, O. onites, O. syriacum* and *O. minutiflorum*, EOs (48.67, 47.00, 46.33 and 43.33 mm, respectively). The EOs of *F. vulgare, L. citriodora, and O. majorana* showed relatively lower antifungal effects. The EO of *S. aramiensis* did not show antifungal effect against the pathogen.

EOs of different thyme and oregano plant species are known to contain high levels of phenolic compounds, such as carvacrol and thymol (Burt, 2004; Soylu et al., 2006). These compounds have been also reported to disrupt the fungal cell membrane, increasing cell wall permeability and weakening the cellular structure (Burt, 2004; Soković et al., 2010; Soylu et al., 2006). Thyme and oregano EOs have been reported to show high antifungal activity against other common fungal species such as *Candida albicans, P. infestans, Botrytis cinerea, Aspergillus flavus* and *Penicillium* spp. (Rasooli & Abyaneh, 2004, Soylu et al., 2005; Soylu et al., 2006; Soylu et al., 2010; Kara et al., 2022a). This study shows that oregano oil can be used against fungi, especially *A. niger*, which damage agricultural products during storage. However, there are some practical difficulties for EOs to be used for the large-scale protection of agricultural products. Large-scale production and application of essential oils may increase production costs. Furthermore, factors such as the stability of essential oils, application methods, and possible flavour or aroma on products should be taken into account (Isman, 2000). Therefore, future research could be focussed on the development of formulations of oregano oil and improving its efficacy. *T. syriacus*, one of the thyme species, is known for its high content of thymol and carvacrol.

Table 1. Antifungal activities of essential oils on the mycelial growth of *Aspergillus niger*

Essential oils	Inhibition Zone[a]
Origanum vulgare	49.33[d]
Thymus syriacus	48.67[d]
Origanum onites	47.00[cd]
Origanum syriacum	46.33[cd]
Origanum minutiflorum	43.33[c]
Foeniculum vulgare	18.67[b]
Lippia citriodora	16.67[b]
Origanum majorana	16.67[b]
Salvia aramiensis	0.00[a]
Control	0.00[a]

[a]The values obtained are the mean values of fungal growth zone diameter (mm) developed in 3 different petri dishes. Similar lower-case letters next to the mean values in the same column or similar upper-case letters next to the mean values in the same row indicate that the difference between treatments is not statistically significant (Duncan's Multiple Range Test, $P \leq 0.05$).

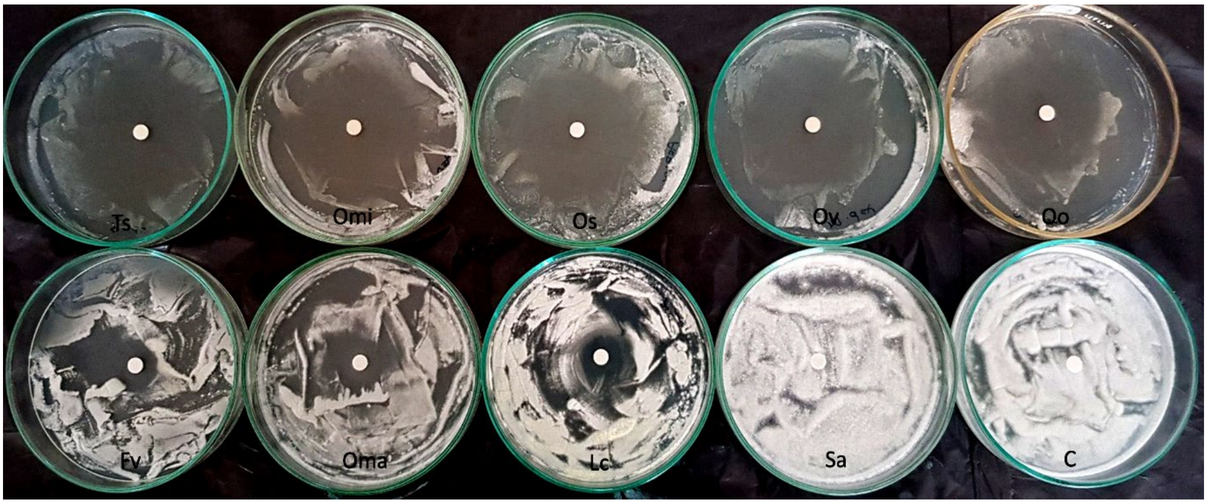

Figure 1. Antifungal activity of essential oils against *A. niger*, characterised by the formation of inhibition zones around filter discs (arrow). (Ts: *Thymus syriacus, Omi: Origanum minutiflorum, Os: Origanum syriacum, Ov: Origanum vulgare, Oo: Origanum onites, Fv: Foeniculum vulgare, Oma: Origanum majorana, Lc: Lippia citriodora, Sa: Salvia aramiensis,* C: Control)

These two components show antifungal effect by destroying the fungal cell membrane. Studies show that *Thymus* species have broad-spectrum antifungal effects (Soković et al., 2010; Atay & Soylu, 2023). This species, which is rarer than other thyme species, attracts attention especially with its high carvacrol content. Its antifungal effect is largely dependent on the concentration of active compounds it contains (Burt, 2004).

Fennel oil contains compounds such as anethole and fenchon and showed weaker milder antifungal effects. In the literature, it has been reported that this essential oil is more prominent with its mild antibacterial and antioxidant properties (Soylu et al., 2009; Bozkurt et al., 2020; Soylu et al., 2024), but its antifungal effect is lower compared to thyme species (Isman, 2000; Soylu et al., 2007; Soylu et al., 2010; Kara et al., 2020). Lemongrass oil contains components such as citral and limonene. Its antifungal effect is particularly associated with the weakening of the fungal cell wall by these components. However, the effect of this oil against fungal species is reported to be moderate (Kalemba and Kunicka, 2003). Marjoram oil shows mild antibacterial and antifungal properties. However, its antifungal effect is generally more limited compared to thyme species (Burt, 2004). Sage oils contain high levels of cineole and camphor. Their antifungal effects may be milder than other oils; however, they have shown suppressive effects on fungal growth (Bakkali et al., 2008). The lowest antifungal effect of *S. aramiensis* oil may be related to the fact that the active components it contains are not effective on *A. niger* at the expected level. It is thought that this plant oil contains a lower proportion of antifungal components compared to the others or that it causes less damage to the fungal cell wall. Some studies have reported that this species shows particular antibacterial activity, but antifungal activity may be limited (Pitarokili et al., 2003). This may explain why *S. aramiensis* is less effective compared to other more potent essential oils from *Origanum* or *Thymus* species. The low antifungal activity of *S. aramiensis* suggests that the use of this essential oil as a stand-alone antifungal agent in agriculture is limited. However, its efficacy may increase when used in synergistic combinations or combination with other essential oils (Kara et al., 2022b; Kara, 2024).

Conclusion

This study shows that oregano and thyme oils can be used as a potent antifungal agents against *A. niger* in groundnut. This essential oil, which offers a more environmentally friendly and natural alternative to chemical fungicides, is promising in reducing fungal losses, especially in organic farming practices.

Declarations

Author Contribution Statement

All the authors contributed equally for analyses, writing and interpretation of the article. The authors read and approved the final version of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest between them.

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