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Research Article

In vitro Antifungal Activity of *Mentha piperita* and *Thymus vulgaris* Essential Oils against Ochratoxigenic *Aspergillus carbonarius* Isolated from Bozcaada Çavuş Grape

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Abstract: In this study, the antifungal properties of *Mentha piperita* and *Thymus vulgaris* essential oils against an isolate of ochratoxin A producer, *Aspergillus carbonarius*, isolated from Bozcaada Çavuş grape, were evaluated in three steps. By GC-MS of *M. piperita* and *T. vulgaris* essential oils, the main components were determined to be menthol (39.911%) and carvacrol (49.042%). Antifungal activity was first evaluated by the agar well diffusion method, and it was determined that the tested essential oils completely inhibited the growth of *A. carbonarius* and were as effective as fluconazole antifungal. In the second step, the MIC and MFC values of the tested essential oils were determined; both values were 1 µL mL^{-1}. Finally, it was determined that *M. piperita* and *T. vulgaris* essential oils completely inhibited the radial growth of *A. carbonarius* at the MIC value. These results show that *M. piperita* and *T. vulgaris* essential oils may be a good strategy to control ochratoxigenic *A. carbonarius* contamination.

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1. Introduction

Mycotoxins are harmful substances produced by certain moulds that grow in various agricultural products (Dammak et al., 2018; Nurtjahja et al., 2022). These toxins have been causing significant health and economic problems since their discovery. So far, around 300-400 different mycotoxins have been identified. *Aspergillus*, a genus of mould, produces several mycotoxins, such as aflatoxins, fumonisins, gliotoxin, ochratoxins, patulin, sterigmatocystin, etc., that can have adverse effects on human health (Pócsi et al., 2020; Ráduly et al., 2020). When humans or animals consume food or feed contaminated with mycotoxins, it can lead to acute and chronic toxicity. According to the Food and Agriculture Organization (FAO), approximately 25% of food products are contaminated by mycotoxins. The World Health Organization (WHO) and FAO have set guidelines and restrictions to address the problem of mycotoxin contamination in feeds and foods (Navale et al., 2021).

Recently, researchers have identified at least 20 analogues of ochratoxin, one of the most important mycotoxins. Ochratoxin A (OTA) is the most toxic and common among these analogues. OTA is immunosuppressive, immunotoxic, embryotoxic, genotoxic, neurotoxic, and teratogenic and is rated as a potential carcinogen (group 2B) by the International Agency for Research on Cancer (IARC, 1993). The European Union and China have recommended a maximum tolerable OTA level in cereal

grains of 5 µg kg^{-1}. OTA is produced by some species such as *Aspergillus niger, Aspergillus carbonarius, Aspergillus ochraceus, Aspergillus westerdijkiae, Penicillium verrucosum,* and *Penicillium nordicum*. *A. ochraceus* is responsible for producing OTA in rice, oats, wheat, coffee, and other beverages. On the other hand, *A. niger* and *A. carbonarius* also produce OTA in grapes, raisins, and wine (Chiotta et al., 2013; Hua et al., 2014; Pantelides et al., 2017). *A. carbonarius* is the primary ochratoxigenic fungus found on grapes, especially in Mediterranean countries, and contaminates grapes during ripening (Pantelides et al., 2017; Dammak et al., 2018; Özcan Ateş and Zorba, 2020).

With global climate change, the spread of mycotoxigenic *Aspergillus* species has increased and is expected to continue to do so, leading to a greater possibility of mycotoxin contamination in the food and feed supply chain. Currently, measures are taken to ensure food safety and prevent the development of moulds, mycotoxin accumulation, and mycotoxin formation in the food chain (Ráduly et al., 2020). Chemical-based control is a standard method used to minimize post-harvest contamination in different types of food. This involves using antifungal chemicals, for example, aromatic hydrocarbons, benzimidazoles, and sterol biosynthesis inhibitors. However, applying these chemicals enhances the risk of toxic residues in foods and can have side effects, such as carcinogenic and teratogenic effects and producing residual toxicity. Due to these concerns, researchers are exploring natural alternatives to synthetic fungicides. The aim of current research is to find effective natural preservatives to limit the need for chemical fungicides. One area of focus is the use of natural antibacterial and antifungal agents. Essential oils (EO) and their components are being extensively researched for their antifungal and anti-toxigenic effects (Hua et al., 2014; Thippeswamy et al., 2014; Boukaew et al., 2017; Dammak et al., 2018 and 2019; Kapetanakou et al., 2019; Rodrigues et al., 2019; Achar et al., 2020; Bouderba et al., 2020; Kalagatur et al., 2020; Nerilo et al., 2020; Laaziz et al., 2022). Therefore, the study evaluated the antifungal activity of *Mentha piperita* and *Thymus vulgaris* EOs against the *A. carbonarius* isolate, which is known to produce ochratoxin.

2. Material and Methods

2.1. Culture and essential oil

A. carbonarius PP264185 (NCBI gen bank number) (closely related species accession number MK778845.1), isolated from grape samples taken from the Çavuş vineyard in Bozcaada Çayır location in 2015, was used in the study (Özcan Ateş and Zorba, 2020; Özcan Ateş et al., 2024). It was determined through HPLC analysis that the isolate produced 49.448 ± 0.354 ppm of OTA in the medium (Özcan Ateş et al., 2024).

M. piperita (MPEO) and *T. vulgaris* (TVEO) EOs were obtained from Altın Toroslar A.Ş. (Adana, Türkiye).

2.2. Preparation of spore suspension

The stock culture was first revived in the Potato Dextrose Agar (PDA) medium (Biolife, Italy). Then, it was planted in Malt Extract Agar (MEA) (Oxoid, England) medium and incubated at 25°C for 7-10 days for sporulation. Sterilized Tween 80 (Merck, Germany) solution (0.1%, v v^{-1}) was added to the MEA, and spores were collected. The spore solution was vortexed for 15-30 seconds for a homogeneous mixture and adjusted to 0.5 McFarland density (Özcan Ateş, 2023).

2.3. Agar well diffusion method

To determine the antifungal activity of *MPEO* and *TVEO*, a modified version of the NCCLS M44-A method was used (NCCLS, 2004; Özcan Ateş, 2023). A spore solution was prepared to 0.5 McFarland density and inoculated on the PDA medium using the spreading plate method. A well with a diameter of 6 mm was created on a PDA medium using a cork borer set instead of a disc. Then, 20 µL of *MPEO* and *TVEO* were added to the well. Plates were incubated at 25°C for 3-5 days, and zone diameters were measured.

As a positive control, amphotericin B (AMB, 20U) (Bioanalyse, Türkiye) and fluconazole (FLU, 25 mcg) (SD232-5CT, Himedia, India) antifungal disks were used. Amphotericin B is a polyene produced by *Streptomyces nodosus* and binds to ergosterols in the fungal cell wall, disrupting wall permeability (Erdem et al., 2018). Conversely, fluconazole is effective against some species of the

Aspergillus genus and inhibits the synthesis of ergosterol in the fungal cell wall (Öncel and Keçeli, 2018).

2.4. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

The MIC value was determined with a minor modification by the method in NCCLS M38-A2 (NCCLS, 2008; Özcan Ateş, 2023). An RPMI 1640 medium (Himedia, India) containing 0.165 M MOPS was prepared to determine the MIC value. To ensure a homogeneous mixture of EOs in the medium, dimethylsulfoxide (DMSO) (Merck, Germany) was added to a final concentration of 3%. A double-content RPMI 1640 medium containing EO and DMSO was distributed as 100 µL into each well of 96-well U-bottom microplates, with decreasing concentrations in rows B and G. Then, 100 µL of the spore solution adjusted to 0.5 McFarland and diluted 1:10 was added to each well, and the microplates were incubated at 25°C for 24-48 hours. The final concentration of EOs in the study was 40, 20, 10, 5, 2.5, and 1 µl ml^{-1}. It was used as a positive control, and 200 µL of RPMI 1640 medium containing 40 µl ml^{-1} EO and 3% DMSO was added to row A of the microplates. In the H row of the microplates, 100 µL of RPMI 1640 medium containing 3% DMSO and 100 µL of culture were added and used as a negative control. After incubation, the MIC value was determined as the first well in which no growth was observed by visual evaluation. The well was defined as the MIC value, and the subsequent two wells were inoculated into PDA medium using the drip seeding method and incubated at 25°C for 24-48 hours. The MFC value was determined as the lowest concentration at which no growth was observed after incubation.

2.5. Effect of EOs on *A. carbonarius* mycelium radial growth

The effect of EOs on the radial growth of mould mycelium was carried out according to the method specified in Özcan Ateş (2023). PDA media were prepared to contain EO at different concentrations (1⁄(4) x MIC, 1⁄(2) x MIC, MIC, 2 x MIC, 4 x MIC) and 3% DMSO. A 7-10-day-old *A. carbonarius* isolate was grown in a PDA medium and inoculated in a single spot in the middle of the petri dish using a needle loop. A PDA medium containing 3% DMSO was used as a control. Plates then incubated at 25°C for 7 days. After incubation, the colony diameters were measured, and radial growth inhibition was calculated using the equation (1).

$$I\% = \frac{C - T}{C} * 100 \quad (1)$$

C: the growth diameter in the control petri dish (mm), T: the growth diameter in the petri dish containing EOs (mm), and I: inhibition (%).

2.6. Determination of *M. piperita* and *T. vulgaris* EOs volatile chemical composition by GC-MS

MPEO and *TVEO* volatile component compositions were determined according to Özcan Ateş and Kanbur (2023). In the study, a gas chromatograph 7890 A coupled to the mass spectrometer series MSD 5975 C (Agilent Technologies) was used, and the integrations were made with MSDCHEM software.

2.7. Statistical analysis

Microbiological analysis was carried out in three parallel; the results were evaluated using the SPSS (v23.0, IBM Corp., Armonk, NY, USA) program and presenting the results as mean (M) ± standard deviation (sd).

3. Results

The antifungal activity of *MPEO* and *TVEO* against the OTA producer *A. carbonarius* isolated from the Bozcaada Çavuş grape was first evaluated by the agar well diffusion method, and the results of the inhibition zone diameters are given in Table 1. It was determined that *MPEO* and *TVEO* were as effective as the positive control fluconazole, and the inhibition zone diameter was 90.00 ± 0.01.

Table 1. Inhibition zone diameters (in mm), MIC (µL mL^{-1}), MFC (µL mL^{-1}), and MFC/MIC ratio of MPEO and TVEO

	***Aspergillus carbonarius* PP264185**			
	Zone diameter (in mm)	**MIC**	**MFC**	**MFC/MIC**
MPEO	90.00 ± 0.01	1 µL mL^{-1}	1 µL mL^{-1}	1
TVEO	90.00 ± 0.01	1 µL mL^{-1}	1 µL mL^{-1}	1
FLU	90.00 ± 0.01	-*	-	-
AMP	14.72 ± 1.21	-	-	-

-*: not determined.

MIC and MFC methods were used to evaluate the fungistatic and fungicidal properties of *MPEO* and *TVEO* against the ochratoxigenic *A. carbonarius* isolate. The MIC and MFC values (Table 2) of *MPEO* and *TVEO* were determined to be 1 µL mL^{-1}. It was found that the tested EOs had an MFC/MIC ratio of 1; therefore, they were fungicidal (MFC/MIC ≤ 4) (Snoussi et al., 2018; Mseddi et al., 2020).

The effect of *MPEO* and *TVEO* on the radial growth of ochratoxigenic *A. carbonarius* was also evaluated. While determining the effect on radial growth, the radial growth inhibition of EOs at 1⁄(4) x MIC, 1⁄(2) x MIC, MIC, 2 x MIC, and 4 x MIC concentrations was examined. Results are given in Table 2. It was determined that *TVEO* completely inhibited the radial growth of the tested *A.carbonarius* isolate starting from 1⁄(2) x MIC concentration, and *MPEO* inhibited the radial growth of the isolate at the MIC value and all concentrations thereafter.

Table 2. *A. carbonarius* radial growth inhibition rate of tested EOs (in %)

EO Concentration	**MPEO**	**TVEO**
1⁄(4) x MIC	72.49 ± 3.72	100.00 ± 0.01
1⁄(2) x MIC	91.05 ± 0.10	100.00 ± 0.01
MIC	100.00 ± 0.01	100.00 ± 0.01
2 x MIC	100.00 ± 0.01	100.00 ± 0.01
4 x MIC	100.00 ± 0.01	100.00 ± 0.01

GC-MS analyses of *MPEO* and *TVEO* identified 51 and 41 different components, respectively, and the volatile chemical composition in the EOs is given in Table 3. The main components of *MPEO* are menthol (39.911%), menthone (17.933%), isomenthone (8.986%), neomenthol (5.942%) and limonene (5.644%), while *TVEO* is carvacrol (49.042%), p. -cymene (11.681%), gamma-terpinene (11.607%) and linalool (7.084%).

Table 3. Volatile chemical compositions of *MPEO* and *TVEO*

Components	**MPEO**		**TVEO**	
	Abundance %	**RT**	**Abundance %**	**RT**
1,3,8-p-Menthatriene	-	-	0.007	26.430
1,5,8-p-Menthatriene	-	-	0.038	34.327
1,8-Cineole	0.629	11.584	-	-
1-[1-Methyl-1-(4-methyl-cyclohex-3-enyl)-ethyl]-1H-pyrrole	-	-	0.065	27.451
1-C42Nonen-3-ol	0.034	23.178	-	-
1-okten-3-ol	-	-	0.324	23.12
1-terpinen-4-ol	-	-	0.804	31.512
2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, cis	-	-	0.041	32.914
2-Hexen-1-ol, (E)-	-	-	0.013	20.776
3-(1'Isopropenyl-2',2'-dimethyl cyclopentyl)-1-propanol	0.03	44.089	-	-

-*: not determined.

Table 3. Volatile chemical compositions of *MPEO* and *TVEO* (continued)

Components	MPEO		TVEO	
	Abundance %	RT	Abundance %	RT
3-Decyne	0.01	19.098	-	-
3-Octanol	0.884	20.064	-	-
3-Octanone	0.023	13.43	-	-
4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, (R)-	0.54	35.738	-	-
Alcanfor	0.018	42.048	-	-
Alpha-amorphene	-	-	0.019	35.996
Alpha-cadinol	-	-	0.135	60.227
Alpha-humulene	-	-	0.031	34.877
Alpha-pinene	1.725	6.605	3.875	6.633
Alpha-terpinene	-	-	2.757	10.594
Alpha terpineol	0.974	36.783	0.121	39.731
Alpha-terpinolene	0.01	14.667	0.525	14.679
Amorphane	0.031	20.591	-	-
Benzene, methyl(1-methylethenyl)	-	-	0.022	22.416
Beta-bisabolene	-	-	0.616	38.194
Beta Bourbonene	0.116	26.714	0.033	26.685
Beta-myrcene	0.426	9.96	2.841	9.972
Beta-phellandrene	-	-	0.036	22.578
Beta-pinene	2.899	8.47	0.269	8.448
Bicyclo[4.1.0]heptane, 3,7,7-trimethyl- Carane	0.073	28.445	-	-
Borneol	-	-	1.414	36.919
Camphene	-	-	0.651	7.473
Camphor	-	-	0.015	26.572
Carane, trans	0.072	27.405	-	-
Carvacrol	-	-	49.042	62.72
Carvacrol methyl ether	-	-	0.599	31.638
Carveol	0.047	39.719	-	-
Caryophyllene	-	-	1.124	31.022
Caryophyllene oxide	0.077	51.027	-	-
Cis-2-alpha-methylbicyc[4.3.0]nonan-1 beta-ol	0.034	43.515	-	-
Cis-3a-methyl-3a,4,5,6,7,7a-hexahydro-1H-inden-1-one	0.016	21.000	-	-
Citronella	0.055 0.502	28.575 31.699	-	-
Cyclohexanol, 3-methyl0	0.009	22.316	-	-
Cyclohexanol, 3-methyl-2-(1-methylethyl)-, (1.alpha.,2.alpha.,3.alpha.)	0.603	32.339	-	-
Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-	1.035	33.018	-	-
Cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1R-(1.alpha.,2.beta.,5.alpha.)]-	0.963	35.146	-	-
Cyclohexanone, 2-methyl-5-(1-methylethyl)-, trans	0.048	40.332	-	-
Cyclohexanone, 3-methyl-, (R)-	0.286	16.839	-	-
Delta-3-carene	0.016	13.252	0.156	9.58
Dihydro-neoclovene	0.027	20.78	-	-
Ethyl amyl carbinol	-	-	0.023	20.024
Gamma-terpinene	-	-	11.607	13.171
Isomenthone	8.986	25.431	-	-
Isopulegol	1.309	29.731	-	-
Isopulegone	0.295	30.474	-	-
Leaf alcohol	0.011	19.599	-	-
Ledene	-	-	0.027	36.331
Limonen-6-ol, pivalate	-	-	0.025	26.864

-*: not determined.

Table 3. Volatile chemical compositions of *MPEO* and *TVEO* (continued)

Components	MPEO		TVEO	
	Abundance %	RT	Abundance %	RT
Limonene	5.644	11.302	0.408 0.034	11.245 24.159
Linalool	-	-	7.084	28.67
Linalyl acetate	-	-	0.431	29.032
Menthol	39.911	33.872	-	-
Menthone	17.933	23.973	-	-
Menthyl acetate	3.727	29.318	-	-
Naphthalene, decohydro-2-methyl	0.015	41.788	-	-
Naphthalene, 1-isocyano	0.034	47.288	-	-
Neomenthol	5.942	31.312	-	-
p-Cymen-8-ol	0.028	44.875	-	-
p-Cymene	0.234	14.14	11.681	14.225
Pentane, 3-methyl-	0.019	17.949	-	-
Phellandrene	-	-	0.652	10.102
Piperitenone	0.083	48.158	-	-
Piperitone	0.705	38.127	-	-
Piperitone-oxide	0.039	50.025	-	-
Pulegone	2.245	34.258	-	-
Sabinene	-	-	0.508	11.608
Salvene	0.012	22.597	-	-
Spathulenol	-	-	0.124	58.015
Terpineol, Z-.beta.-	-	-	0.910	23.902
Trans-3-Methylcyclohexanol	0.041	23.286	-	-
Trans-Caryophyllene	0.422	31.005	-	-
Thymol	-	-	0.572	61.35
Zingiberene	-	-	0.056	25.266

-*: not determined.

4. Discussion

A. carbonarius contamination, found during the ripening process of grapes in vineyards in Mediterranean countries, is still a critical problem. Because *A. carbonarius* produces OTA, especially in grapes and products, it endangers human health and causes economic losses to producers. Synthetic pesticides with different chemical structures control this mould type and other OTA producers. However, these synthetic chemical fungicides negatively affect human and environmental health. Therefore, their use becomes limited (Dammak et al., 2019). In addition, fungal infection agents are increasing and becoming resistant to synthetic chemicals. This makes it difficult to control and treat. For this reason, researchers have focused on the antifungal activities of natural substances for various reasons, such as identifying natural and safe products as alternatives to synthetic chemical fungicides. EOs from plants have important biological (antibacterial and antifungal) activities and are a potentially helpful source of antifungal compounds (Moghaddam and Mehdizadeh, 2020). The study aimed to explore the potential of *MPEO* and *TVEO* as natural alternatives to synthetic fungicides for controlling *A. carbonarius*, a fungus that produces OTA.

The composition of EO is affected by numerous factors, such as the geographical conditions where the plant grows, abiotic and biotic factors during the growing phase, the age of the plant, the condition of the plant, which part of the plant is collected, the genotype of the plant, and the method of obtaining the EO. Therefore, before recommending an EO as a food preservative or alternative to drugs, issues such as the composition of EOs and the concentration of active ingredients need to be standardized. As a result, the chemical composition of the EO and the change in the concentration of the active ingredient will affect its biological activities. Thus, synergistic effects can be seen, as well as antagonistic effects (Dammak et al., 2019). In this study, *MPEO*'s main components were menthol (39.911%) and menthone (17.933%). In the literature, Moghaddam et al. (2013) reported that the main components of *MPEO* obtained from Tehran, Iran, were menthone (30.63%), menthol (25.16%), and

Beigi et al. (2018) also reported that the main components of MPEO obtained from Isfahan in Southwest Iran were found to be menthol (44.39%), menthone (15.36%). In addition, Camele et al. (2021) determined that the main components of *MPEO* obtained in Slovakia were menthol (70.08%) and menthone (14.49%). As a result, it was determined that the main components were the same as in the literature, but the percentage of presence changed. Also, the main component of *TVEO* was determined to be carvacrol (49.042%). It has a series of multicomponent chemotypes, including (1)linalool, (2)borneol, (3)geraniol, (4) sabinene hydrate, (5)thymol, (6)carvacrol based on the main components of *TVEO* (Satyal et al., 2016). Even Satyal et al. (2016) stated that *TVEO* has 20 different chemotypes through a cluster analysis based on 85 different TVEO compositions. Therefore, TVEO's main components vary widely.

Kostik et al. (2015) reported that MPEO, the main component of which is 34.3% menthol, gave 20 ± 2 and 28 ± 1 mm inhibition zones against *Aspergillus flavus* and *A. niger* isolates, and the MIC values were 115.4 ± 3.9 and 65.4 ± 3.1 µg ml^{-1}, respectively. Ambindei et al. (2017) reported that the main components of MPEO and TVEO were menthol (33.59%) and thymol (35.12%), respectively, and the MIC values against the *Aspergillus tamarii* isolate were 0.13 µl ml^{-1} and 0.33 µl ml^{-1}, respectively. Císarová et al. (2016) stated that TVEO, whose main component is 40±3% ρ-cymene, completely inhibited the growth of *A. niger* and *Aspergillus tubingensis* at a concentration of 0.625 µl cm^{-3}. The study determined that MPEO and TVEO gave an inhibition zone of 90.00 ± 0.01 mm for the ochratoxigenic *A.carbonarius* isolate, and the MIC and MFC values were 1 µl ml^{-1}. Additionally, MPEO and TVEO were found to completely inhibit radial growth at MIC, 2xMIC, and 4xMIC concentrations. Our study results are similar to the data in the literature. It is important to measure the antifungal activity of plant extracts, especially MIC. A lower MIC value means that a lower dose is needed to control the growth of foodborne fungi. This means that extracts with a lower MIC are more effective as antifungal agents. As a result, plant essential oil can be applied to both fields and stored crops to control fungal growth and mycotoxin production as a potential alternative to synthetic chemicals. Thus, food safety can be ensured to a large extent.

Conclusion

In conclusion, it is clearly seen from this study that the radial growth of the ochratoxigenic *A. carbonarius* isolate is completely inhibited at concentrations as low as 1 µl ml^{-1}, and accordingly, it provides inhibition of ochratoxin production. Mould species contaminated with foods and the mycotoxins they produce cause economic losses and endanger human and animal health. Therefore, it can be concluded that it can be used as an alternative and therapeutic agent to pesticides and synthetic food preservatives to limit mould contamination and mycotoxin formation in foods. In addition, there are a few limited studies in the literature on the growth of ochratoxigenic moulds and ochratoxin production inhibition. Therefore, the study will contribute to developing safer natural products and an effective strategy to control the growth of ochratoxigenic moulds and ochratoxin contamination, especially in foods.

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References

Achar, P. N., Quyen, P., Adukwu, E. C., Sharma, A., Msimanga, H. Z., Nagaraja, H., & Sreenivasa, M. Y. (2020). Investigation of the antifungal and anti-aflatoxigenic potential of plant-based essential oils against *Aspergillus flavus* in peanuts. *Journal of Fungi*, *6*(4), 383. https://doi.org/10.3390/jof6040383

Ambindei, W. A., Jazet, P. M. D., Tatsadjieu, L. N., Priya, P., Manilal, V. B., Krishnakumar, B., & Zollo, P. H. A. (2017). Effect of the essential oils of *Thymus vulgaris, Cinnamomum zeylanicum* and *Mentha piperita* on fungal growth and morphology. *African Journal of Biotechnology*, *16*(9), 388-399. https://doi.org/10.5897/AJB2017.15881

Beigi, M., Torki-Harchegani, M., & Ghasemi Pirbalouti, A. (2018). Quantity and chemical composition of essential oil of peppermint (*Mentha× piperita* L.) leaves under different drying methods. International *Journal of Food Properties*, *21*(1), 267-276. https://doi.org/10.1080/10942912.2018.1453839

Bouderba, N. N., Elhussain, K. M. K., & Kadi, H. (2020). In-Vitro Antifungal Activity of Citrullus Colocynthis Schard Against Aflatoxigenic *Aspergillus Flavus* and Ochratoxigenic *Aspergillus Ochraceus*. *Biomed. J. Sci. Tech. Res*, *26*, 19759-19764. http://dx.doi.org/10.26717/BJSTR.2020.26.004316

Boukaew, S., Prasertsan, P., & Sattayasamitsathit, S. (2017). Evaluation of antifungal activity of essential oils against aflatoxigenic *Aspergillus flavus* and their allelopathic activity from fumigation to protect maize seeds during storage. *Industrial Crops and Products*, *97*, 558-566. https://doi.org/10.1016/j.indcrop.2017.01.005

Camele, I., Gruľová, D., & Elshafie, H. S. (2021). Chemical composition and antimicrobial properties of *Mentha× piperita* cv. 'Kristinka'essential oil. *Plants*, *10*(8), 1567. https://doi.org/10.3390/plants10081567

Chiotta, M. L., Ponsone, M. L., Sosa, D. M., Combina, M., & Chulze, S. N. (2013). Biodiversity of *Aspergillus* section Nigri populations in Argentinian vineyards and ochratoxin A contamination. *Food Microbiology*, *36*(2), 182-190. https://doi.org/10.1016/j.fm.2013.04.003

Císarová, M., Tančinová, D., & Medo, J. (2016). Antifungal activity of lemon, eucalyptus, thyme, oregano, sage and lavender essential oils against *Aspergillus niger* and *Aspergillus tubingensis* isolated from grapes. *Potravinarstvo*, *10*(1), 83-88. https://doi.org/10.5219/554

Dammak, I., Hamdi, Z., El Euch, S. K., Zemni, H., Mliki, A., Hassouna, M., & Lasram, S. (2019). Evaluation of antifungal and anti-ochratoxigenic activities of *Salvia officinalis, Lavandula dentata* and *Laurus nobilis* essential oils and a major monoterpene constituent 1, 8-cineole against *Aspergillus carbonarius*. *Industrial Crops and Products*, *128*, 85-93. https://doi.org/10.1016/j.indcrop.2018.11.006

Dammak, I., Lasram, S., Hamdi, Z., Moussa, O. B., Hammi, K. M., Trigui, I., Houissa, H., Mliki, A. & Hassouna, M. (2018). In vitro antifungal and anti-ochratoxigenic activities of *Aloe vera* gel against *Aspergillus carbonarius* isolated from grapes. *Industrial Crops and Products*, *123*, 416-423. https://doi.org/10.1016/j.indcrop.2018.07.023

Erdem, İ., Doğan, M., Karaali, R., Ardinç, E., & Elbasan Omar, Ş. (2018). İnvaziv Aspergilloz Tedavisi. *Namık Kemal Tıp Dergisi, 6*(2), 64–82. https://cms.galenos.com.tr/Uploads/Article_40406/nkmj-6-64-En.pdf

Hua, H., Xing, F., Selvaraj, J. N., Wang, Y., Zhao, Y., Zhou, L., Liu, X. & Liu, Y. (2014). Inhibitory effect of essential oils on *Aspergillus ochraceus* growth and ochratoxin A production. *PloS One*, *9*(9), e108285. https://doi.org/10.1371/journal.pone.0108285

IARC. (1993). International Agency for Research on Cancer, The evaluation of carcinogenic risk to human. (IARC monograph N 56), Lyon France, vol. 56, pp. 257-263.

Kalagatur, N. K., Gurunathan, S., Kamasani, J. R., Gunti, L., Kadirvelu, K., Mohan, C. D., Rangappa, S., Prasad, R., Almeida, F., Mudili, V., & Siddaiah, C. (2020). Inhibitory effect of *C. zeylanicum, C. longa, O. basilicum, Z. officinale*, and *C. martini* essential oils on growth and ochratoxin A content of *A. ochraceous* and *P. verrucosum* in maize grains. *Biotechnology Reports*, *27*, e00490. https://doi.org/10.1016/j.btre.2020.e00490

Kapetanakou, A. E., Nestora, S., Evageliou, V., & Skandamis, P. N. (2019). Sodium alginate–cinnamon essential oil coated apples and pears: Variability of *Aspergillus carbonarius* growth and ochratoxin A production. *Food Research International*, *119*, 876-885. https://doi.org/10.1016/j.foodres.2018.10.072

Kostik, V., Gjorgjeska, B., & Petkovska, S. (2015). *Mentha* L. essential oils composition and in vitro antifungal activity. *IOSR Journal of Pharmacy*, *5*(7), 1-7. http://www.iosrphr.org/pages/volume5-issue7.html

Laaziz, A., El Hammoudi, Y., Qjidaa, S., Hajjaji, A., Hajjaj, H., Haesaert, G., & Bouseta, A. (2022). Activity of essential oils from *Syzygium aromaticum* and *Rosmarinus officinalis* against growth and ochratoxin A production by *Aspergillus tubingensis* and *Aspergillus luchuensis* from Moroccan grapes. *Phytopathologia Mediterranea*, *61*(2), 299-310. https://doi.org/10.36253/phyto-12841

Moghaddam, M., & Mehdizadeh, L. (2020). Chemical composition and antifungal activity of essential oil of *Thymus vulgaris* grown in Iran against some plant pathogenic fungi. *Journal of Essential Oil Bearing Plants*, *23*(5), 1072-1083. https://doi.org/10.1080/0972060X.2020.1843547

Moghaddam, M., Pourbaige, M., Tabar, H. K., Farhadi, N., & Hosseini, S. M. A. (2013). Composition and antifungal activity of peppermint (*Mentha piperita*) essential oil from Iran. *Journal of Essential Oil Bearing Plants*, *16*(4), 506-512. https://doi.org/10.1080/0972060X.2013.813265

Mseddi, K., Alimi, F., Noumi, E., Veettil, V. N., Deshpande, S., Adnan, M., Hamdi, A., Elkahoui, S., Alghamdi, A., Kadri, A., Patel, M., Snoussi, M., (2020). *Thymus musilii* Velen. as a promising source of potent bioactive compounds with its pharmacological properties: In vitro and in silico analysis. *Arabian Journal of Chemistry*, *13*(8), 6782-6801. https://doi.org/10.1016/j.arabjc.2020.06.032

National Committee for Clinical Laboratory Standards. (2008). *Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; Approved Standard-Second Edition* (NCCLS Standard No. M38-A2). https://clsi.org/standards/products/microbiology/documents/m44/

National Committee for Clinical Laboratory Standards. (2004). *Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline*. (NCCLS Standard No: M44-A). https://clsi.org/standards/products/microbiology/documents/m44/

Navale, V., Vamkudoth, K. R., Ajmera, S., & Dhuri, V. (2021). *Aspergillus* derived mycotoxins in food and the environment: Prevalence, detection, and toxicity. *Toxicology Reports*, *8*, 1008-1030. https://doi.org/10.1016/j.toxrep.2021.04.013

Nerilo, S. B., Romoli, J. C. Z., Nakasugi, L. P., Zampieri, N. S., Mossini, S. A. G., Rocha, G. H. O., Micotti da Gloria, E., Alves de Abreu Filh, B., & Machinski Jr, M. (2020). Antifungal activity and inhibition of aflatoxins production by *Zingiber officinale* Roscoe essential oil against *Aspergillus flavus* in stored maize grains. *Ciência Rural*, 50, 1-10. https://doi.org/10.1590/0103-8478cr20190779

Nurtjahja, K., Hastuti, L., Purnamasari, N., & Silitonga, G. N. (2022). Fungal Contamination and Toxigenicity of *Aspergillus flavus* on Postharvest Cacao Beans in Northern Sumatera, Indonesia. *Yuzuncu Yıl University Journal of Agricultural Sciences*, 32(3), 448-454. https://doi.org/10.29133/yyutbd.1071092

Öncel, S., & Keçeli, S. A. (2018). Flukonazol. *Mantar Dergisi*, *9*(1), 67-75. https://doi.org/10.30708/mantar.371030

Özcan Ateş, G. & Kanbur, S. (2023). Evaluation of In-vitro Anticandidal Activity of 99 Different Commercial Plant Extract, Fixed and Essential Oils against Vaginal *Candida albicans* Isolates. *Kahramanmaraş Sütçü İmam Üniversitesi Fen ve Mühendislik Dergisi*, *26*(5), 1034-1047. https://doi.org/10.18016/ksutarimdoga.vi.1211862

Özcan Ateş, G. & Zorba, N. N. D. (2020). Determination of mycobiota and microbial loads of grapes grown in Bozcaada, Turkey. *The Journal of Microbiology, Biotechnology and Food Sciences*, *9*(5), 899. https://doi.org/10.15414/jmbfs.2020.9.5.899-906

Özcan Ateş, G. (2023). Antifungal activity of *Eugenia caryophyllata, Cinnamomum sp., Mentha piperita*, and *Thymus vulgaris* essential oils against *Aspergillus niger*. *Anatolian Journal of Botany*, *7*(2), 135-139. https://doi.org/10.30616/ajb.1337945

Özcan Ateş, G., Zorba, N. N. D., & Şen, B. (2024). Biodiversity of ochratoxigenic *Aspergillus* species isolated from çavuş and karalahna grapes in Bozcaada, Türkiye. *Anatolian Journal of Botany*, 8(1), 39-45. https://doi.org/10.30616/ajb.1395701

Pantelides, I. S., Aristeidou, E., Lazari, M., Tsolakidou, M. D., Tsaltas, D., Christofidou, M., Kafouris, D., Christou, E., & Ioannou, N. (2017). Biodiversity and ochratoxin A profile of *Aspergillus* section Nigri populations isolated from wine grapes in Cyprus vineyards. *Food Microbiology*, *67*, 106-115. https://doi.org/10.1016/j.fm.2017.06.010

Pócsi, I., Giacometti, F., Ambrus, Á., & Logrieco, A. F. (2020). *Aspergillus*-derived mycotoxins in the feed and food chain. *Frontiers in Microbiology*, *11*, 606108. https://doi.org/10.3389/fmicb.2020.606108

Ráduly, Z., Szabó, L., Madar, A., Pócsi, I., & Csernoch, L. (2020). Toxicological and medical aspects of *Aspergillus*-derived mycotoxins entering the feed and food chain. Frontiers in Microbiology, *10*, 2908. https://doi.org/10.3389/fmicb.2019.02908

Rodrigues, M. P., Astoreca, A. L., Oliveira, Á. A. D., Salvato, L. A., Biscoto, G. L., Keller, L. A. M., Rosa, C. A. .R., Cavaglieri, L. R., Azevedo, M. I., & Keller, K. M. (2019). *In vitro* activity of neem (*Azadirachta indica*) oil on growth and ochratoxin A production by *Aspergillus carbonarius* Isolates. *Toxins*, *11*(10), 579. https://doi.org/10.3390/toxins11100579

Satyal, P., Murray, B. L., McFeeters, R. L., & Setzer, W. N. (2016). Essential oil characterization of *Thymus vulgaris* from various geographical locations. *Foods*, *5*(4), 70. https://doi.org/10.3390/foods5040070

Snoussi, M., Noumi, E., Punchappady-Devasya, R., Trabelsi, N., Kanekar, S., Nazzaro, F., Fratianni, F., Flamini, G., De Feo, V., Al-Sieni, A. (2018). Antioxidant properties and anti-quorum sensing potential of *Carum copticum* essential oil and phenolics against *Chromobacterium violaceum*. *Journal of Food Science and Technology*, *55*(8), 2824-2832. https://doi.org/10.1007/s13197-018-3219-6

Thippeswamy, S., Mohana, D. C., Abhishek, R. U., & Manjunath, K. (2014). Inhibitory activity of plant extracts on aflatoxin B1 biosynthesis by *Aspergillus flavus*. *Journal of Agricultural Science and Technology*, *16*(5), 1123-1132. http://jast.modares.ac.ir/article-23-9949-en.html