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Mentha pulegium L. (Yarpuz) Ekstrelerinin Fitokimyasal İçeriği, Antioksidan Kapasitesi ve Bazı Bitki Patojenlerine Karşı *in vitro* Antimikrobiyal Etkisi

Phytochemical Composition, Antioxidant Capacity, and *in vitro* Antimicrobial Activity of *Mentha pulegium* L. (Pennyroyal) Extracts Against Selected Plant Pathogens

Özet

Amaç: Bu çalışmanın amacı, tarımsal üretimde önemli kayıplara neden olan fitopatojenik mikroorganizmaların kontrolünde *Mentha pulegium* L. (yarpuz) bitkisinin biyopestisit potansiyelini araştırmaktır. Seçilmiş bitki patojenlerine karşı *in vitro* antimikrobiyal etkileri değerlendirilmiş ve bu amacı desteklemek üzere ekstraktların toplam fenolik içeriği ile antioksidan kapasiteleri belirlenmiştir.

Materyal ve Yöntem: *M. pulegium*'un kurutulmuş toprak üstü kısımları metanol ve hekzan ile ekstraksiyon işlemine tabi tutulmuştur. Toplam fenolik içeriği ve antioksidan kapasitesi sırasıyla Folin-Ciocalteu yöntemi ve DPPH radikal süpürme yöntemi kullanılarak belirlenmiştir. Numunenin fitokimyasal profili GC-MS ile karakterize edilmiştir. Antimikrobiyal aktivite, üç farklı konsantrasyonda disk difüzyon yöntemi ile test edilmiştir .

Bulgular: Metanol ekstresinin toplam fenolik bileşik içeriği, 5 mg/mL konsantrasyonda 1458 µg GAE/mL olarak ölçülmüştür. Aynı ekstrenin DPPH radikal temizleme aktivitesi konsantrasyonla birlikte artmış ve en yüksek konsantrasyonda %86,31'e ulaşmıştır. GC-MS analizi, ekstrelerde toplam 50 bileşik tanımlamıştır; öne çıkan bileşikler arasında dihidrokarvil asetat (%9,66), piperiton (%4,84) ve *p*-menton (%3,5) bulunmaktadır. GC-MS analizi, ekstrelerde toplam 50 bileşik tespit etmiştir; öne çıkan bileşikler arasında dihidrokarvil asetat (%9,66), piperiton (%4,84), p-menton (%3,57), limonen (%3,43), dihidrokarveol (%3,34) ve mentol (%3,18) bulunmaktadır. Antimikrobiyal testlerde, metanol ekstresi *C. michiganensis* (20 mm) ve *F. oxysporum* (19 mm) üzerinde önemli bir etki gösterirken, hekzan ekstresi aynı koşullar altında daha düşük bir inhibisyon zonu göstermiştir. Her iki ekstre de *Pestalotiopsis* sp. (yaklaşık 10–12 mm) karşı benzer aktivite seviyeleri göstermiştir.

Sonuç: *M. pulegium* ekstreleri yüksek fenolik içeriğe ve güçlü antioksidan kapasiteye sahiptir. Özellikle, metanol ekstresinin hem fungal hem de bakteriyel fitopatojenlere karşı önemli antimikrobiyal aktivite göstermesi, *M. pulegium* bitkisinin çevre dostu bir biyopestisit olarak kullanılma potansiyelini göstermektedir. Tarımda görülen fitopatojenlere karşı *M. pulegium*'un çözücü bazlı ekstrelerinin incelenmesine dair bilgiler oldukça sınırlıdır. Mevcut çalışma bu boşluğun doldurulmasına katkı sağlamakta ve bitkinin potansiyel uygulamalarına yönelik yeni veriler sunmaktadır.

Anahtar kelimeler: Mentha pulegium, fenolik içerik, antioksidan, GC-MS, antimikrobiyal aktivite, biyopestisit

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Abstract

Objective: The aim of this study was to investigate the biopesticidal potential of *Mentha pulegium* L. (pennyroyal) against phytopathogenic microorganisms that cause significant agricultural losses. Their *in vitro* antimicrobial effects against selected plant pathogens were evaluated, and to support this aim, the total phenolic content and antioxidant capacity of the extracts were determined.

Materials and Methods: The dried aerial parts of *M. pulegium* were extracted with methanol and hexane. The total phenolic content and antioxidant capacity were determined using the Folin-Ciocalteu method and the DPPH radical scavenging method, respectively. The phytochemical profile of the sample was characterized by GC-MS. Antimicrobial activity was tested by the disk diffusion method at three different concentrations.

Findings: The total phenolic compound content of the methanol extract was measured as 1458 µg GAE/mL at a concentration of 5 mg/mL. The DPPH radical scavenging activity of the same extract increased with concentration, reaching 86.31% at the highest concentration. GC-MS analysis identified a total of 50 compounds in the extracts; the prominent compounds included dihydrocarvyl acetate (9.66%), piperitone (4.84%), *p*-menthone (3.57%), limonene (3.43%), dihydrocarveol (3.34%), and menthol (3.18%). In antimicrobial tests, the methanol extract showed a significant effect on *C. michiganensis* (20 mm inhibition zone) and *F. oxysporum* (19 mm), while the hexane extract produced a lower inhibition under the same conditions. Both extracts showed similar levels of activity against *Pestalotiopsis* sp. (approximately 10–12 mm).

Conclusion: *M. pulegium* extracts have high phenolic content and strong antioxidant capacity. In particular, the fact that the methanol extract exhibits significant antimicrobial activity against both fungal and bacterial phytopathogens indicates the potential of the *M. pulegium* plant to be used as an environmentally friendly biopesticide. There is limited information regarding the evaluation of solvent-based extracts of *M. pulegium* against agricultural phytopathogens. The present study contributes to addressing this gap and offers new insights into its potential applications.

Keywords: *Mentha pulegium*, phenolic content, antioxidant, GC-MS, antimicrobial activity, biopesticide

1. Introduction

Plant diseases, pests, and weeds in agriculture pose serious threats to crop yield, quality, and food safety on a global scale. Fungal, bacterial, and viral plant pathogens, as well as entomological pests and weeds, cause yield losses of 20% to 40% in major field crops [1,2]. These losses not only affect yield quantities but also negatively impact product quality and the economic sustainability of agricultural production [3]. Therefore, the effective control of plant diseases and harmful organisms is of strategic importance in ensuring global food security.

Although synthetic pesticides have been effective tools in combating plant diseases for many years, their intensive and uncontrolled use poses serious environmental and health risks. Many chemical pesticides break down slowly in nature or become persistent, leaching into soil and water sources, causing long-term toxic effects on ecosystem components, and disrupting the biological balance in beneficial insects, soil microorganisms, and other non-target organisms [4]. Repeated pesticide uses leads to the development of resistance in some insect and pathogen species, which has become a major problem reducing the effectiveness of pesticides [5]. Additionally, pesticide residues have chronic effects not only on ecosystems but also on human health, particularly being associated with cancer, neurological disorders, and endocrine system irregularities [6].

The use of natural resources such as medicinal and aromatic plants (MAP) in plant protection has become a research area of intense interest in recent years. These plants stand out among innovative biological control agents known as "green pesticides" due to their environmentally friendly, biodegradable nature and their ability to contain effective components against target organisms [7]. In particular, MAP exhibits strong

antimicrobial effects against various phytopathogens thanks to secondary metabolites [8,9].

Mentha pulegium L. (pennyroyal) is an aromatic plant belonging to the Lamiaceae family that grows naturally in Türkiye and the Mediterranean basin. This species, widely used in traditional medicine, is notable for its distinct mint aroma and rich secondary metabolite content. In particular, monoterpene components such as pulegone and menthol, which are present in high concentrations in its essential oil, exhibit strong antifungal and antimicrobial effects against various phytopathogens [10,11].

Although the effects of *M. pulegium* extracts and essential oils on microbial pathogens have been investigated in various studies in literature, these studies have focused mainly on microorganisms of foodborne or clinical importance. However, data on the *in vitro* effects of M. pulegium on agricultural pathogens such as Fusarium oxysporum, *Clavibacter michiganensis,* and *Pestalotiopsis* species are limited [12,13]. This situation highlights an important knowledge gap in evaluating *M. pulegium* as a potential biopesticide. Phenolic compounds are widely recognized for their dual role as antioxidants and antimicrobial agents. Consequently, the present study not only examined the direct antimicrobial effects of *M. pulegium* extracts but also evaluated their antioxidant properties. This further analysis provides additional support for the observed bioactivity and strengthens the main objective of the study.

This study aims to evaluate the *in vitro* effects of extracts obtained from the *M. pulegium* plant on the aforementioned phytopathogens. In the study, the total phenolic compound content, antioxidant capacity, and phytochemical profile of the plant were comprehensively analyzed; then, the inhibitory potential of extracts obtained with different solvents on the development of target pathogens was investigated. The data obtained is expected to shed light on the potential use of *M. pulegium* as a natural agent in plant protection applications.

2. Materials and Methods

2.1. Plant Material and Extract Preparation

The plant material used in this study was purchased from a local market in Ereğli Province (Türkiye). The taxonomic identification was carried out by the authors with reference to standard floras. As the material was obtained from a commercial source, no herbarium voucher specimen was prepared. The plants were dried in the shade in a well-ventilated environment; the above-ground parts were used in the analysis. Two grams of dried plant samples were weighed, ground in a mortar, and homogenized with 30 mL of methanol or hexane (Daihan HG 15D, 8000 rpm, 5 min). The resulting mixtures were left to stand at room temperature overnight, then subjected to ultrasonication at 40°C for 30 min. The suspensions were filtered through Whatman No. 1 filter paper, the solvent was removed using a rotary evaporator (HeiVap Value), and the remaining residue was dissolved in an appropriate solvent to prepare 100 mg/mL stock solutions. The extracts were stored at +4°C for further use.

2.2. Determination of Total Phenolic Content and Antioxidant Activity

Total phenolic content was determined using the Folin-Ciocalteu method [14]. 100 µL of extract was incubated with 1 mL of Folin-Ciocalteu's phenol reagent, which was diluted tenfold with distilled water, for 5 minutes; then 1 mL of 7.5% $NaHCO_3$ was added and the mixture was left to stand in the dark for 90 minutes. Absorbance of the blueish color formed in the reaction tube was measured at 765 nm. All measurements were conducted as triplicates and the results were expressed as mean µg GAE/mL± standard deviation.

The antioxidant capacity of the methanol extract was evaluated using the DPPH radical scavenging method [15]. 100 µL of extract or methanol-for blank solution was mixed with 2.9 mL of 0.1 mM DPPH solution and incubated in the dark for 15 minutes. Absorbance values were measured spectrophotometrically at 517 nm; % inhibition was calculated with the following formula;

DPPH Scavenging (%) = $[(A_{blank} - A_{sample})/A_{blank}]*100$

where A_{blank} is the absorbance of the blank solution at 517 nm, A_{sample} is the absorbance of the sample solution at 517 nm. All measurements were performed as triplicates and the data were expressed as mean DPPH scavenging percentage ± standard deviation.

2.3. GC-MS Analysis

Hexane extract of *M. pulegium* was analyzed using a GC-MS system (Shimadzu QP2010 Ultra). A 1 µL sample was injected using a Rxi-5MS column.

The column temperature program and instrument parameters were optimized; compound identifications were performed by comparing with the Wiley and FFNSC 1.2 libraries.

2.4. Antimicrobial Activity Test

The antimicrobial activities of the extracts were tested using the disk diffusion method. *Fusarium oxysporum, Pestalotiopsis* sp., and *Clavibacter michiganensis* subsp. *michiganensis* strains were used as phytopathogens. Fungal pathogens were inoculated into PDA medium, while bacterial pathogens were inoculated into LB agar medium. Disks prepared with extracts at concentrations of 25, 50, and 100 mg/mL were placed in Petri dishes and incubated at 27°C for 96–120 hours (for fungi) and at 37°C for 18–24 hours (for bacteria). The zones of inhibition were measured in millimeters. All experiments were conducted in triplicate and the data was expressed as mean inhibition zone diameter (mm) ± standard deviation.

2.5. Statistical Analysis

The data were analyzed using SPSS v24.0 software. One-way ANOVA was used to evaluate the differences between groups, and the Tukey HSD test was used to determine significant differences ($p<0.05$).

3. Results and Discussion

3.1. Phenolic Compound and Antioxidant Activity

In this study, the total phenolic compound (TPC) content in the methanol extract of *M. pulegium* at a concentration of 5 mg/mL was determined to be 1458±57.17 µg GAE/mL. This value indicates that the plant is rich in phenolic compounds and may be associated with its potential antioxidant capacity.

In the DPPH free radical scavenging analysis conducted with methanol extracts of *M. pulegium* at different concentrations, it was observed that antioxidant activity exhibited a dose-dependent increase. At the lowest concentration of 0.5 mg/mL, it was 14.61%, while it reached 34.22% at 1 mg/mL. Antioxidant activity reached its maximum value of 86.31% at a concentration of 5 mg/mL (Figure 1).

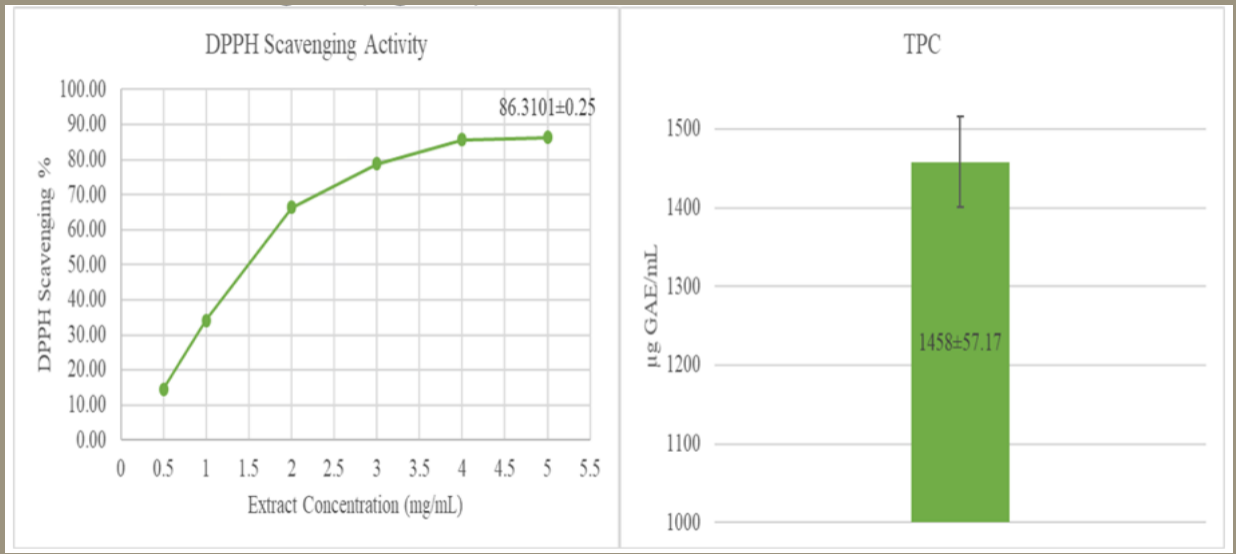

Figure 1: Figure 1: DPPH scavenging activity and total phenolic compound content of *M. pulegium* methanol extract.

Phenolic compounds are one of the main components of plant-derived antioxidants and are known for their ability to scavenge reactive oxygen species. The TPC value of 1458 µg GAE/mL obtained in this study indicates that *M. pulegium* is a phenol-rich plant.

Barros et al. [16] have reported that plants belonging to the Lamiaceae family have high phenolic content and, consequently, high antioxidant potential. The value of 1458 µg GAE/mL found in our study is much lower than the methanol extract findings of Necip and Durgun [17]; this difference is probably due to plant and methodological variables.

In this study, it was determined that the antioxidant capacity of *M. pulegium* extract is directly related to its phenolic compound content. At a concentration of 5 mg/mL, both the DPPH inhibition percentage (86.31%) and the TPC value (1458 µg GAE/mL) were measured at the highest levels. This parallel increase observed between TPC and DPPH suggests a significant positive correlation between the two parameters. The hydroxyl groups present in the structure of phenolic compounds have the ability to stabilize these molecules by transferring electrons or hydrogen to free radicals. In this context, it is considered that the DPPH scavenging percentage obtained is largely derived from TPC. Similarly, many studies in the literature, such as Kähkönen et al. [18] and Gülçin [19], have reported a high degree of correlation between TPC and DPPH radical scavenging capacity. This shows that our findings are not specific to the sample but reflect a general biochemical trend.

Hariri et al. [20] reported that *M. pulegium* essential oils showed a free radical scavenging capacity of approximately 90.54 ± 1.5%. This is largely consistent with our findings. Additionally, a study by Gulluce et al., 2007 [21] reported that plants belonging to the Lamiaceae family have high antioxidant capacity, and this effect is largely due to phenolic compounds.

The results observed in *M. pulegium* sample support the phenolic-based mechanisms underlying the biological effects of this species, which is traditionally used in folk medicine; they also reinforce its potential for use as a natural antioxidant in the food, cosmetic, and pharmaceutical industries.

3.2. GC-MS Results

As a result of GC-MS analysis, a total of 50 compounds were detected in the M. pulegium sample. Some of these compounds were found in high concentrations and are considered to be dominant components in the chemical profile. Some of the prominent compounds and their relative concentrations are given in Table 1.

Table 1. Phytochemical profile of *M. pulegium* hexane extract according to GC-MS analysis

No.	Compound Name	RT	MW-CF	SI	Concentration
1.	α -Pinene	7.718	136($C_{10}H_{16}$)	94	0.54
2.	2,3,3-Trimethyloctane	10.129	156($C_{11}H_{24}$)	88	0.51
3.	**Limonene**	**10.589**	**136($C_{10}H_{16}$)**	**97**	**3.43**
4.	Eucalyptol	10.679	154($C_{10}H_{18}O$)	93	0.61
5.	5-Isobutylnonane	11.499	184($C_{13}H_{28}$)	92	1.81
6.	Linalool	12.807	154($C_{10}H_{18}O$)	95	1.21
7.	4,6-Dimethyldodecane	12.888	198($C_{14}H_{30}$)	88	0.50
8.	Caprylic acid methyl ester	13.567	158($C_9H_{18}O_2$)	84	0.71
9.	***p*-Menthone**	**14.479**	**154($C_{10}H_{18}O$)**	**96**	**3.57**
10.	**Menthol**	**15.007**	**156($C_{10}H_{20}O$)**	**97**	**3.18**
11.	**Dihydrocarveol**	**15.731**	**154($C_{10}H_{18}O$)**	**94**	**3.34**
12.	Hexadecane	17.175	226($C_{16}H_{34}$)	84	0.54
13.	**Piperitone**	**17.511**	**152($C_{10}H_{16}O$)**	**84**	**4.84**
14.	Tetradecane	17.754	212($C_{15}H_{32}$)	87	0.59
15.	Octadecane	18.232	254($C_{18}H_{38}$)	91	1.55
16.	Menthol acetate	18.659	198($C_{12}H_{22}O_2$)	95	1.35
17.	Carvacrol	18.867	150($C_{10}H_{14}O$)	89	0.98
18.	Tridecanol	19.220	200($C_{13}H_{28}O$)	83	0.62
19.	4,6-Dimethyldodecane	19.540	198($C_{14}H_{30}$)	87	0.72
20.	**Dihydrocarvyl acetate**	**19.627**	**196($C_{12}H_{20}O_2$)**	**94**	**9.66**
21.	Carvyl acetate	20.587	194($C_{12}H_{18}O_2$)	90	0.57
22.	Pentatadecane	21.557	212($C_{15}H_{32}$)	93	0.65
23.	Hexadecane	23.205	282($C_{20}H_{42}$)	91	0.51
24.	Docosane	24.100	310($C_{22}H_{46}$)	87	1.72
25.	Phthalic acid, diethyl ester	26.557	222($C_{12}H_{14}O_4$)	94	3.93
26.	2,6-Bis(1,1-dimethylethyl)-4-(methoxymethyl)phenol	30.028	250($C_{16}H_{26}O_2$)	90	2.40
27.	Heptacosane	30.485	380($C_{27}H_{56}$)	90	1.47
28.	Myristic acid, methyl ester	30.917	242($C_{15}H_{30}O_2$)	93	2.86
29.	Lauric acid butyl ester	32.651	256($C_{16}H_{32}O_2$)	88	0.72
30.	Tetracosane	33.041	338($C_{24}H_{50}$)	89	0.66
31.	Palmitic acid methyl ester	36.143	270($C_{17}H_{34}O_2$)	94	12.8
32.	Linoleic acid, methyl ester	39.955	294($C_{19}H_{34}O_2$)	94	1.25
33.	Linolenic acid, methyl ester	40.094	292($C_{19}H_{32}O_2$)	91	4.25
34.	Phytol	40.341	296($C_{20}H_{40}O$)	93	1.56
35.	Stearic acid, methyl ester	40.543	298($C_{19}H_{38}O_2$)	95	7.32
36.	Dotriacontane	42.026	450($C_{32}H_{66}$)	95	8.99
37.	Hexatriacontane	46.931	506($C_{36}H_{74}$)	85	1.03
	Total Identified (%)				**92.95**

*RT: Retention time (min); MW: Molecular weight; CF: Chemical formula; SI: Similarity index (%); Concentration: Relative concentration (%).

These results show that the chemical profile of *M. pulegium* plant consists largely of monoterpene derivatives and derivative esters.
The chemical compounds identified by GC-MS in *M. pulegium* sample reveal both the aromatic and pharmacological value of the plant. In particular, the presence of compounds such as carvacrol and *trans*-carveyl acetate supports the antimicrobial and antioxidant potential of this species.

Carvacrol is a compound that stands out in literature for its phenolic monoterpene structure and has been described in many studies for its antimicrobial and anti-inflammatory effects [25]. Its presence at a rate of 0.98% in our study makes it possible to evaluate it as a commercially viable source of essential oil.

Compounds such as dihydrocarvyl acetate (9.66%), piperitone (4.84%) *p*-menthone (3.57%) limonen (3.43%) and dihydrocarveol (3.34%), which were detected in significant amounts in the volatile oil profile of *M. pulegium*, are important indicators of the plant's biochemical defense strategies against environmental stress factors. The high levels of these compounds indicate *M. pulegium's* increased capacity for secondary metabolite production under abiotic stress conditions (drought, UV, oxidative stress, etc.) [26,27]. The dominance of oxygenated monoterpenes (carveol derivatives, dihydrocarveol, etc.) in the volatile oil content is consistent with the stress responses of *Mentha* species reported in the literature [28,29].

3.3. Determination of Antimicrobial Activity

The disk diffusion method was used to determine the antimicrobial effect of methanol and hexane extracts obtained from plant samples against phytopathogenic microorganisms. The antimicrobial activity of *M. pulegium* methanol and hexane extracts against three phytopathogens at different extract volumes using the disk diffusion method is presented in Table 2.

Table 2. Antimicrobial activity of *M. pulegium*

Microorganisms	Inhibition zone diameters (mm)					
	Methanol			Hexane		
	25	50	100	25	50	100
F. oxysporum	15.50±0.71	17.00±0.00	19.00±1.41	7.50±0.71	8.50±0.71	11.00±1.41
Pestalotiopsis sp.	7.50±0.71	8.50±0.71	10.00±0.71	8.50±0.71	10.50±0.71	12.50±0.71
C. michiganensis	12.50±0.71	16.50±0.71	20.00±0.71	nd	7.00±0	9.50±0.71

*All data presented in the table are expressed as means±standard deviations of three independent experiments.
nd: not detected

The methanol extract exhibited a stronger effect against all pathogens. Against *F. oxysporum,* the methanol extract produced an inhibition zone of 19.00 ± 1.41 mm at 100 mg/mL concentration, while the hexane extract yielded a value of 11.00 ± 1.41 mm. Against *Pestalotiopsis* sp., the methanol extract showed 10.00 ± 0.71 mm inhibition at 100 mg/mL concentration, while the hexane extract showed 12.50 ± 0.71 mm inhibition. For *C. michiganensis*, inhibition zones of 20.00 ± 0.71 mm were measured in the methanol extract and 9.50 ± 0.71 mm in the hexane extract.

It was observed that the methanol extract exhibited a significantly higher inhibition effect on *C. michiganensis* and F. *oxysporum*. The data obtained in this study clearly demonstrate that extracts obtained from the *M. pulegium* plant have antimicrobial potential. In particular, the methanol extract showed a significant inhibitory effect on both gram-positive bacterial (*C. michiganensis*) and fungal pathogens (*F. oxysporum, Pestalotiopsis* sp.).

The biological activities of compounds such as carvacrol, dihydrocarveol, and *trans*-carveyl acetate,

which are present in high concentrations in the methanol extract, can be explained by their lipophilic structures, which confer membrane interaction advantages. In particular, carvacrol binds to the phospholipid layer of the bacterial cell membrane through hydrophobic interactions [30] and increases membrane permeability, leading to ion leakage; this process, combined with the inhibition of ATP synthesis, results in a bactericidal effect [30,31].

Similarly, in the literature, the high antimicrobial activity of the methanolic extract of *M. pulegium* has been strongly reported, particularly in studies on Gram-positive and Gram-negative bacteria [32,33]. However, the relatively low activity in the hexane extract may be related to the extraction efficiency of volatile and lipophilic compounds. In particular, the limited solubility of polar phenolic compounds in hexane may have reduced the antimicrobial activity of this extract.

4. Conclusion

In this study, the phytochemical contents, antioxidant capacities, and *in vitro* antimicrobial effects of extracts obtained from *Mentha pulegium* L. against selected plant pathogens (*Fusarium oxysporum, Pestalotiopsis* sp.*, Clavibacter michiganensis*) were evaluated.

According to the results obtained, the methanol extract of *M. pulegium* stood out due to its high phenolic content (1458 µg GAE/mL) and strong antioxidant capacity (86.31% DPPH inhibition). In antimicrobial tests, the methanol extract was observed to have significant effects, particularly on *C. michiganensis* (20±0.71 mm inhibition zone) and *F. oxysporum* (19±1.41 mm inhibition zone). The hexane extract, however, showed relatively low efficacy against the same pathogens. These results indicate that the antimicrobial activity of *M. pulegium* extracts may vary depending on the type of solvent used, and that the methanol extract is more effective due to its phenolic and polar components.

In conclusion, *M. pulegium* extracts have the potential to serve as an effective and environmentally friendly biopesticide alternative against both fungal and bacterial phytopathogens, thanks to their rich phenolic compound profile and strong antioxidant capacity. This study lays the groundwork for evaluating *M. pulegium* as a natural agent in agricultural disease management and sheds light on advanced field studies.

References

[1] Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., & Nelson, A. (2019). The global burden of pathogens and pests on major food crops. Nature Ecology & Evolution, 3(3), 430–439.

[2] Cullen, E. M., Sánchez-Retuerta, V., Matías-Hernández, L., & Zhang, B. (2024). Plant disease dynamics in a changing climate: Impacts, molecular adaptations and ecosystem consequences. Discover Agriculture, 3, 257.

[3] Junaid, M. D., & Gökçe, A. F. (2024). Global agricultural losses and their causes. Bulletin of Biological and Allied Sciences Research, 9, 66.

[4] Geissen, V., Wessel, L., & Vervoort, R. (2022). Pesticide effects on soil bacterial, fungal and protist communities and associated functions. Environmental Microbiology Reports, 14(1), 67–78.

[5] Onilude, E., & Adeyemi, F. (2022). Pesticide resistance in arthropods: Ecology matters too. Functional Ecology, 36, 1529–1542.

[6] Sawyer, C., Li, J., Zhang, Y., & Patel, R. (2024). Public health implications of pesticide residues in food: Risks of chronic exposure linked to cancer, endocrine disruption and neuro developmental effects. Global Journal of Public Health, 12(4), 102–114.

[7] Gupta, I., Singh, R., Muthusamy, S., Sharma, M., Grewal, K., Singh, H. P., & Batish, D. R. (2023). Plant essential oils as biopesticides: Applications, mechanisms, innovations, and constraints. Plants, 12(16), Article 2916.

[8] Shan, M. I., Ali, R. M. M., Zeshan, A., Bilal, S., & Haider, S. (2024). Essential oils as biopesticides in pest management: Safer alternatives to synthetic pesticides. European Chemical Bulletin, Regular Issue 1, 246–267.

[9] Hernández Bolaños, E., Sánchez Retuerta, V., Matías Hernández, L., et al. (2025). Promising applications on the use of medicinal and aromatic plants in agriculture. Discover Agriculture, 3, Article 36.

[10] Montenegro, I., Said, B., Godoy, P., Besoain, X., Parra, C., Díaz, K., & Madrid, A. (2020). Antifungal activity of essential oil and main components from *Mentha pulegium* growing wild on the Chilean central coast. Agronomy, 10(2), 254.

[11] Allali, A., Rezouki, S., Fadli, A., & Eloutassi, N. (2021). Chemical characterization and antimicrobial activities of essential oil from *Mentha pulegium* L. (Morocco) against food-borne pathogens and phytopathogenic fungi. Journal of Food and Agriculture Research, 20, 81–90.

[12] Belkhir, H., Talbi, M., & Bouhdid, S. (2021). Antifungal evaluation of essential oils from *Mentha pulegium* growing in Algeria against *Fusarium oxysporum albedinis*. Algerian Journal of Medicinal Plants, 10(3), 47 56.

[13] Daferera, D. J., Ziogas, B. N., & Polissiou, M. G. (2003). The effectiveness of plant essential oils on the growth of Botrytis cinerea, *Fusarium* sp. and *Clavibacter michiganensis* subsp. *michiganensis*. Crop Protection, 22(1), 39–44.

[14] Singleton, V.L. and Rossi, J.A., "Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents", American Journal of Enology and Viticulture, 16(3), 144-158, 1965.

[15] Blois, M.S., "Antioxidant determinations by the use of a stable free radical", Nature, 181(4617), 1199-1200, 1958.

[16] Barros, L., Carvalho, A. M., & Ferreira, I. C. F. R. (2011). Comparing the composition and bioactivity of *Crataegus monogyna* flowers and fruits used in folk medicine. Phytochemical Analysis, 22(2), 181–188

[17] Necip, A., & Durgun, M. (2022). Antioxidant properties, total phenolic content and LC MS/MS analysis of *Mentha pulegium, Lepidium draba* and *Centaurea solstitialis*. Journal of the Institute of Science and Technology, 12(4), 2375–2385

[18] Kähkönen, M.-P., Hopia, A.-I., Vuorela, H.-J., Rauha, J.-P., Pihlaja, K., Kujala, T. S., & Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural and Food Chemistry, 47(10), 3954–3962.

[19] Gülçin, İ. (2006). Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid). Toxicology, 217(2–3), 213–220.

[20] Hariri, A., Ouis, N., Bouhadi, D., & Benatouche, Z. (2020). *In vitro* antioxidant activity of essential oil of aerial parts of *Mentha pulegium* L. Acta Agriculturae Serbica, 25(50), 193–197.

[21] Güllüce, M., Şahin, F., Sökmen, A., Özer, H., Daferera, D., Sokmen, M., Polissiou, M., Adıgüzel, A., & Özkan, H. (2007). Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. *longifolia*. Food Chemistry, 103(4), 1449–1456

[22] Messaoudi, M., Rebiai, A., Sawicka, B., Atanassova, M., Ouakouak, H., Larkem, I., Ferhat, M. A. (2022). Effect of extraction methods on polyphenols, flavonoids, mineral elements, and biological activities of *Mentha pulegium* L. Molecules, 27(1), 11.

[23] Kızıl, S., Haşimi, N., Tolan, V., Kılınç, E., & Yüksel, U. (2010). Mineral content, essential oil components and biological activity of two *Mentha* species (*Mentha piperita* L., *Mentha spicata* L.). Turkish Journal of Field Crops, 15(2), 148–153

[24] Ebrahimzadeh, M. A., Eslami, S., Nabavi, S. M., Nabavi, F. S., & Hajizadeh Moghaddam, A. (2011). Estimation of essential and toxic mineral elements in *Mentha* species. Asian Journal of Chemistry, 23(4), 1648–1650.

[25] Lambert, R. J. W., Skandamis, P. N., Coote, P. J., & Nychas, G. J. E. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. Journal of Applied Microbiology, 91(3), 453 462

[26] Loreto, F., & Schnitzler, J. P. (2010). Abiotic stresses and induced BVOCs. Trends in Plant Science, 15(3), 154–166.

[27] Holopainen, J. K., & Gershenzon, J. (2010). Multiple stress factors and the emission of plant VOCs. Trends in Plant Science, 15(3), 176–184.

[28] Figueiredo, A. C., Barroso, J. G., Pedro, L. G., & Scheffer, J. J. C. (2008). Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour and Fragrance Journal, 23(4), 213–226.

[29] Göger, F., Kökdil, G., Yılmaz, G., & Yalçın, F. N. (2018). Chemical characterization and biological activity of *Mentha longifolia* subsp. *typhoides* essential oil from Turkey. Journal of Essential Oil Research, 30(5), 332–340.

[30] Ben Arfa, A., Combes, S., Preziosi-Belloy, L., Gontard, N., & Chalier, P. (2016). Antimicrobial activity of carvacrol related to its chemical structure and its ability to disrupt membrane integrity and ion gradients. Journal of Applied Microbiology, 120,(4), 994–1006.

[31] Marchese, A., Orhan, I. E., Daglia, M., Barbieri, R., Di Lorenzo, A., Nabavi, S. F., Gortzi, O., Izadi, M., & Nabavi, S. M. (2017). Carvacrol and its antibacterial activity on the cell membrane. Frontiers in Microbiology, 8, 1932.

[32] Mahboubi, M., & Haghi, G. (2008). Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil. Journal of Ethnopharmacology, 119(2), 325–327.

[33] Messaoudi, M., Rebiai, A., Sawicka, B., Atanassova, M., Ouakouak, H., Larkem, I., & Ferhat, M. A. (2021). Effect of extraction methods on polyphenols, flavonoids, mineral elements, and biological activities of *Mentha pulegium* L. Molecules, 27(1), 11