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Determination of Antifungal Activities of Some Plant Fixed and Essential Oils Against Three *Penicillium* Species

Bazı Bitkisel Sabit ve Uçucu Yağların Üç *Penicillium* Türüne Karşı Antifungal Aktivitelerinin Belirlenmesi

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ABSTRACT

Some members of the *Penicillium* genus are storage molds that cause spoilage of agricultural products during storage and distribution. It is crucial to control *Penicillium digitatum* and *Penicillium italicum*, which are green-blue molds that cause spoilage, especially of citrus fruits, during storage. While synthetic chemical preservatives can be used for this purpose, the increasing concern over synthetic chemicals and the rising consumer interest in natural products make it important to explore alternative natural options for protecting products during storage. Therefore, in this study, the antifungal activities of 135 plant fixed and essential oils against *Penicillium digitatum* (CECT 2954), *Penicillium italicum* (CECT 2294), and *Penicillium notatum* (NRRL 807) were evaluated using agar well diffusion, microdilution, and radial growth inhibition methods. Nine oils showed antifungal activity against the tested *Penicillium* species using the agar well diffusion method. Thyme oil (77) demonstrated both MIC and MFC values of 1 µL/mL and exhibited fungicidal activity. Lemon oil (84) and peppermint oil (93, 94) were found to have MIC values of 1 µL/mL and MFC values of 20 µL/mL, respectively, and exhibited fungistatic properties. When the effect of thyme oil on the radial growth of *Penicillium* species was examined over 21 days, it was observed that 100% inhibition was achieved, while the effects of lemon and peppermint oils decreased over time. In conclusion, plant fixed, and essential oils can act as natural antifungal agents. However, their effectiveness may decrease over time during storage due to the volatility of their components. As a result, creating various controlled release or delivery systems is essential.

Keywords: Antifungal activity, Essential oil, *Penicillium*, Plant fixed oil, Storage microfungus

ÖZ

Penicilium cinsinin bazı üyeleri depo küfü olup, tarımsal ürünlerin depolama ve taşıma sürecinde bozulmaya neden olmaktadır. Yeşil-mavi küf olan ve depolama sürecinde özellikle narenciye ürünlerinin bozulmasına neden olan *Penicillium digitatum ve Penicillium italicum* türlerinin kontrol altına alınması oldukça önemlidir. Bu amaçla sentetik kimyasal koruyucular kullanılabilmekle birlikte, günümüzde sentetik kimyasal maddelerin kullanılmasına yönelik tepkilerin artması ve tüketicilerin doğal ürünlere olan ilgisinin artması nedeniyle depolama sürecinde ürünlerin korunması amacıyla alternatif doğal ürünlerin araştırılması önem arz etmektedir. Bu nedenle bu çalışmada *Penicillium digitatum* (CECT 2954), *Penicillium italicum* (CECT 2294) ve *Penicillium notatum* (NRRL 807) türlerine karşı 135 adet bitki sabit ve esansiyel yağının antifungal etkileri agar kuyu difüzyon, mikrodilüsyon ve radyal büyüme inhibisyonu yöntemleri kullanılarak değerlendirilmiştir. 9 tane yağın agar kuyu difüzyon yöntemiyle test edilen *Penicillium* türlerine karşı antifungal aktiviteye sahip olduğu tespit edilmiştir. Özellikle test edilen kekik yağının (77) hem MİK hem de MFK değerinin 1 µL/mL olduğu ve fungisidal aktiviteye sahip olduğu belirlenmiştir. Test edilen limon yağı (84), nane yağı (93) ve nane yağı (94)'nın ise MİK değerinin 1 µL/mL iken MFK değerinin 20 µL/mL fungistatik olduğu belirlenmiştir. Kekik yağının 21. gün süre ile test edilen *Penicillium* türlerinin radyal büyümesine olan etkisi incelendiğinde ise %100 inhibisyon sağladığı, buna karşın limon ve nane yağlarının zamanla etkilerinde azalma gözlemlenmiştir. Sonuç olarak bitki sabit ve esansiyel yağları doğal antifungal ajanlar olarak kullanılabilir, fakat sahip oldukları uçucu bileşenler nedeniyle etkinlikleri depolama sürecinde azalabilir. Bu nedenle kontrollü salınım sağlayan veya taşıyıcı farklı sistemlerin geliştirilmesine ihtiyaç vardır.

Anahtar Kelimeler: Antifungal aktivite, Uçucu yağ, *Penicillium*, Bitkisel sabit yağ, Depo küfü

This study is Ahsen ÖZDEMİR's master's thesis.

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Introduction

The rapidly increasing global population has driven a surge in food demand, requiring higher agricultural output. This has resulted in the extensive use of chemicals to fight diseases, pests, and weeds, aiming to protect and boost crop productivity. However, both intentional and accidental application of these chemicals have harmed the environment and pose risks to human health (Chen et al., 2023; Maurya et al., 2021).

Fruit and vegetables are fresh foods that contain multiple nutrients essential for human health and are consumed worldwide. Freshly harvested foods go through a storage period until they are eaten. During this storage time, physical, chemical, and microbial spoilage can occur. These spoilage processes result in both product loss and economic damage. Microbial spoilage in fresh foods is mainly caused by fungal pathogens such as *Alternaria* spp., *Aspergillus* spp., *Botrytis* spp., *Colletotrichum* spp., *Fusarium* spp., and *Penicillium* spp. These fungi not only cause product losses and economic damage but also pose food safety concerns due to the production of mycotoxins, which are toxic to humans (Bhunia, 2018; Chen et al., 2023).

Green mold, caused by *Penicillium digitatum* Sacc, accounts for about 90% of postharvest losses and produces mycotoxins, which are heat-generating alkaloids like tryptophan A and tryptophan C. Blue mold, caused by *Penicillium italicum* Wehmer, can infect fresh food even during cold storage and low water activity, leading to significant economic losses globally (Bhatta, 2022). *Penicillium* notatum is also a microfungus found in indoor air and food, exhibiting both allergenic and toxigenic effects (Li et al., 2023). Mycotoxins, which adversely affect human health, are secondary metabolites produced by microfungi that cause significant damage to agricultural products during and after harvest, as well as during storage. Chemicals are used to inhibit the growth of these microfungi in agricultural products. However, these chemicals can leave environmental residues and, when overused, promote the development of resistance in microorganisms to antimicrobial agents. Additionally, growing consumer preference for natural products, along with reluctance to consume synthetic and chemically contaminated items, raises concerns about environmental harm. Consequently, researchers are now exploring sustainable, reliable alternatives that do not harm humans or the environment. Plants, with their naturally occurring active compounds, are emerging as a promising option (Chen et al., 2023)..

Plant fixed and essential oils have been used since the Middle Ages for treating diseases and preserving and flavoring food. As is true worldwide, in Türkiye and everywhere, the use of medicinal and aromatic plants, as well as their essential oils and extracts, has been increasingly popular among the public for centuries. In recent years, increased awareness of the potential risks associated with synthetic additives has led consumers to favor naturally sourced ingredients, resulting in a rise in the use of essential oils across various sectors, including food, beverage, pharmaceutical, sanitation, perfumery, cosmetics, and agriculture. As a result, the significance of natural additives continues to grow daily (Abers et al., 2021; Maurya et al., 2021).

Research results on the antibacterial, antifungal, antiviral, antimutagenic, and antioxidant properties of plant fixed and essential oils are generally positive. Therefore, it is widely accepted that using essential oils and plant extracts can be one of the most effective solutions. Since plant essential oils typically lack mutagenic effects, they are regarded as a natural and reliable alternative to many synthetic food additives (Maurya et al., 2021). Thus, this study examined the antifungal activity of plant fixed oils (FO) and essential oils (EO) obtained from online shops and herbalists against *Penicillium digitatum* (CECT 2954), *Penicillium italicum* (CECT 2294), and *Penicillium notatum* (NRRL 807), which cause post-harvest fungal infections in fruits and vegetables.

Methods

Cultures

In the study, *P. digitatum* CECT 2954 and *P. italicum* CECT 2294 strains from the culture collection of Prof. Dr. Burhan ŞEN, Department of Biology, Faculty of Science, Trakya University, and *P. notatum* NRRL 807 strains from the culture collection of Dr. Gülçin ÖZCAN ATEŞ, Vocational School of Health Services, Çanakkale Onsekiz Mart University, were used.

Plant Fixed and Essential Oils

FO and EO, whose antifungal activity against *Penicillium* strains will be determined, were obtained from online shopping and herbalists and are given in Table 1.

Table 1.

Details about FO and EOs were examined for their antifungal properties in the study

FO or EO code	Producted by	FO or EO Product name	Ingredients	Oil Type	FO or EO code	Producted by	FO or EO Product name	Ingredients	Oil Type
1	A	Bitter almond oil	*Prunus amygdalus* var. *amara*	FO	69	K	Black pepper oil	*Eugenia caryophyllata*	EO
2	A	Bitter apple (sage) oil	*Salvia triloba*	EO	70	H	Clove oil	*Eugenia caryophyllata*	EO
3	B	Hot pepper oil	*Capsicum annuum* oleum, *Helianthus annuus* seed oleum	FO*	71	O	Clove oil	*Prunus armeniaca* kernel	FO
4	C	Adiyaman mint	*Mentha pulegium*, orange oil, lemon oil	EO	72	H	Apricot kernel oil	*Prunus armeniaca* kernel	FO
5	D	Anise oil	*Pimpinella anisum*	FO	73	F	Apricot kernel oil	*Prunus armeniaca* kernel	FO
6	A	Anise oil	*Pimpinella anisum*	FO	74	A	Apricot kernel oil	*Prunus armeniaca* kernel	FO
7	A	Juniper tar	*Juniperus oxicedrus*	Tar	75	T	Apricot oil	*Prunus armeniaca* kernel	FO
8	A	Juniper essential oil	*Juniperus oxicedrus*	EO	76	A	Apricot oil	*Prunus armeniaca*	FO
9	E	Safflower oil	*Carthamus tinctorious*	FO	77	H	Thyme oil	*Thymus* spp.	EO
10	F	Safflower oil	*Carthamus tinctorious*	FO	78	D	Kiwi oil	*Actinidia deliciosa*	FO
11	A	Safflower oil	*Carthamus tinctorious*	FO	79	P	Mitten melon oil	*Momordica chantia fruit*	FO
12	J	Horse chestnut oil	*Aesculus hippocastanum* seed	FO	80	F	Mitten melon oil	*Momordica chantia fruit*	FO
13	A	Horse chestnut oil	*Aesculus hippocastanum*	FO	81	K	Lavender oil	*Lavandula angustifolia*	EO
14	G	Horse chestnut oil	*Aesculus hippocastanum*	FO	82	A	Lavender oil	*Lavandula angustifolia*	EO
15	H	Avocado oil	*Persea gratissima*	FO	83	T	Lemon oil	*Citrus limon*	EO
16	A	Avocado oil	*Persea gratissima*	FO	84	F	Lemon oil	*Citrus limon*	EO
17	K	Calendula oil	*Calendula officinalis* flower	EO	85	A	Lemon oil	*Citrus limon*	EO
18	F	Calendula oil	*Calendula officinalis* flower	EO	86	A	Tangerine oil	*Citrus reticulata*	EO
19	L	Calendula oil	*Calendula officinalis* flower	FO*	87	L	Parsley seed oil	*Petroselinum sativum* seed	FO
20	A	Calendula oil	*Calendula officinalis* flower	EO	88	F	Parsley oil	*Petroselinum sativum*	EO
21	E	Almond oil	*Prunus amygdalus dulcis*	FO	89	A	Melissa oil	*Melissa officinalis*	EO
22	A	Rosemary oil	*Rosmarinus officinalis*	EO	90	A	Violet oil	*Viola odorata*	EO
23	A	Wheat germ oil	*Triticum* spp.	FO	91	A	Myrtle oil	*Myrtus communis*	EO
24	H	Wheat oil	*Triticum* spp.	FO	92	A	Banana oil	*Musa paradisiaca*	EO
25	H	Walnut oil	*Juglans regia*	FO	93	H	Peppermint oil	*Mentha piperita*	EO
26	A	Walnut oil	*Juglans regia*	FO	94	A	Peppermint oil	*Mentha piperita*	EO
27	A	Pine turpentine essential oil	Terebinthiniae	EO	95	F	Pomegranate seed oil	*Punica granatum*	FO
28	E	Pine turpentine oil	Turpentine	EO	96	A	Pomegranate seed oil	*Punica granatum*	FO
29	M	Pine oil	*Pinus palustris*	EO	97	A	Calendula oil	*Narcissus* spp.	EO
30	A	Pine oil	*Pinus sylvestris*	EO	98	A	Eucalyptus oil	*Eucalyptus camaldulensis*	EO
31	A	Tea tree oil	*Melaleuca alternifolia*	EO	99	L	Chamomile oil	*Chamomilla recutita*	FO*
32	A	Strawberry oil	*Fragaria vesca*	EO	100	A	Chamomile oil	*Chamomilla recutita*	EO
33	A	Cayenne oil	Menthe-chn oil	EO	101	F	Rosewood oil	-	FO
34	N	Black seed oil	*Nigella sativa* seed	FO	102	A	Rosewood oil	-	FO
35	A	Black cumin oil	*Nigella sativa* seed	FO	103	P	Orange oil	*Citrus aurantium*	EO
36	A	Evening primrose oil	*Primula elatior*	EO	104	A	Orange oil	*Citrus aurantium*	EO
37	K	Bay gar (seed) oil	*Laurus* spp. (semen)	EO	105	U	Fennel oil	*Foeniculum vulgare*	FO
38	A	Bay gar (seed) oil	*Laurus* spp. (semen)	EO	106	A	Fennel oil	*Foeniculum vulgare*	FO
39	A	Bay essential oil	*Laurus* spp.	EO	107	A	Sandalwood oil	*Santalum album*	EO
40	P	Bay seed oil	*Laurus* spp. seed	FO	108	K	Garlic oil	*Allium sativum*	EO
41	F	Basil oil	*Ocimum basilicum*	FO	109	P	Garlic oil	*Allium sativum*	EO
42	A	Hazelnut oil	*Corylus avellana*	FO	110	A	Garlic oil	*Allium sativum*	FO
43	A	Rose oil	*Rosa* spp.	EO	111	F	Cedarwood oil	*Cedrus libani*	EO
44	R	Mustard seed oil	*Sinapsis alba*	FO	112	A	Cedarwood oil	*Cedrus libani*	EO
45	A	Mustard oil	*Sinapis* spp.	EO	113	V	Black garlic oil	*Allium sativum* (black) bulb oil %50 + Sunflower seed oil %50	FO*
46	A	Poppy seed oil	*Papaver somniferum*	EO	114	L	Black garlic oil	*Allium sativum* (black)	FO
47	D	Galangal oil	*Alpinia officinarum*	EO	115	A	Onion oil	*Oleum cepa*	FO
48	O	Carrot oil	*Daucus carota sativa* seed	FO	116	A	Soybean oil	*Glycine max*	FO
49	A	Carrot oil	*Daucus carota*	FO	117	O	Sesame oil	*Sesamum indicum* seed	FO
50	A	Coconut oil	*Cocos nucifera*	FO	118	H	Sesame oil	*Sesamum indicum* seed	FO
51	S	Castor oil	*Ricinus communis* seed oil, tocopheryl acetate	EO	119	A	Sesame oil	*Sesamum indicum* seed	FO
52	A	Castor oil	*Ricinus communis*	EO	120	O	Peach oil	*Prunus persica* kernel	FO
53	A	Borage oil	*Borago officinalis*	FO	121	H	Cinnamon oil	*Cinnamomum* spp.	EO
54	T	Nettle seed oil	*Urtica* spp.	FO	122	A	Sweet almond oil	*Amygdalus dulcus*	FO
55	A	Nettle seed oil	*Urtica* spp.	FO	123	A	Sweet apple oil	*Pyrus malus*	EO
56	A	Oleaster oil	*Elaegnus angustifolia*	FO	124	Y	Taurus mint	*Mentha pulegium* oil, eucalyptus oil, orange oil	EO
57	A	Jojoba oil	*Simmondsia chinesis*	FO	125	T	Grape seed oil	*Vitis vinifera*	FO
58	O	Pumpkin seed oil	*Cucurbita pepo seed*	FO	126	A	Grape seed oil	*Vitis vinifera*	FO
59	H	Pumpkin seed oil	*Cucurbita pepo seed*	FO	127	A	Vanilla oil	*Vanilla* spp.	EO
60	F	Pumpkin seed oil	*Cucurbita pepo seed*	FO	128	A	Sour cherry oil	*Prunus creasus*	EO
61	A	Pumpkin seed oil	*Cucurbita pepo seed*	FO	129	A	Jasmine oil	*Jasminum* spp.	EO
62	A	Cocoa butter	*Theobroma cacao*	FO	130	A	Peanut oil	*Arachis hypogae*	FO
63	A	St. John's Wort oil	*Centaurium minus*	FO*	131	Z	Ylang ylang oil	*Cananga oderata*	EO
64	F	Lavandula oil	*Lavandula cariensis*	FO	132	A	Ylang ylang oil	*Cananga oderata*	EO
65	A	Lavandula oil	*Lavandula cariensis*	EO	133	A	Algae oil	Algea	EO
66	D	Lavandula oil	*Lavandula cariensis*	EO	134	A	Lily oil	*Lilium candidum*	EO
67	F	Black pepper oil	*Piper nigrum*	FO	135	A	Ginger oil	*Zingiber officinale*	EO
68	A	Black pepper oil	*Piper nigrum*	FO					

FO*: obtained by infusion.

Preparation of Penicillium Spore Solutions

Penicillium strains stored as stock cultures were revived on Potato Dextrose Agar (PDA) (Merck, Germany). Revived *Penicillium* strains were then inoculated with three spots in PDA and incubated at 25°C for 7-10 days. To collect the developing spores, 10 mL of sterile Tween 80 (Merck, Germany) solution (0.1% v/v) was added to the PDA medium, and the spores were scraped with a Drigalski loop. The collected spore-containing solution was transferred to a sterile empty tube. Analyses of antifungal activity were performed with the prepared fresh spore solution (Özcan Ateş, 2023; 2024).

Agar Well Diffusion Method

The antifungal activity of plant FO and EO was determined using the method described by Özcan Ateş (2023; 2024). A 0.5 McFarland inoculum suspension was prepared from freshly made spore solution in sterile 0.1% Tween 80, measured with a McFarland densitometer (Biosan, DEN-1, Latvia). Then, 0.1 mL of this spore suspension was evenly spread on the PDA medium using the spread plate technique. Wells with a diameter of 6 mm were punched into the medium using a cork borer. Each well received 20 µL of FO or EO. The plates were incubated at 25°C for 3 to 5 days. Zone diameters were measured with a caliper after incubation. Amphotericin B (20 IU, Bioanalyze) disc served as a positive control.

Microdilution Method

FO and EOs that were determined to have antifungal activity were studied using the agar well diffusion method. The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) were determined using the microdilution method outlined by Özcan Ateş (2023; 2024). This method was used with 96-well flat-bottom microplates and RPMI 1640 medium containing 0.165 M MOPS (Himedia, India). To ensure an even distribution of the oils, 3% dimethylsulfoxide (DMSO, 99% purity, Isolab, Germany) was added to the medium. The medium containing oils was added to the wells, 100 µL of each concentration. Then, fresh spore suspensions were diluted 1:10 in distilled water to achieve a final inoculum concentration of 2-5×10^5spore/mL, with 100 µL added to each well. The oil concentrations tested were 40, 20, 10, 5, 2.5, and 1 µL/mL. Negative controls consisted of RPMI 1640 broth with 3% DMSO and 1 µL/mL FO or EO, while positive controls used RPMI 1640 broth with 3% DMSO and Penicillium spore solutions. The plates were incubated at 25°C for 24-48 hours, and the MIC was identified as the first well showing no growth. To determine MFC, the wells corresponding to MIC and the following two higher concentrations were subcultured onto PDA medium using the drop-in method after incubation.

FO and EOs determined to have antifungal activity were evaluated as fungicidal (MFC/MIC ratio ≤ 4) and fungistatic (MFC/MIC ratio > 4) according to the MFC/MIC ratio (Gatsing, et al., 2009; Mseddi, et al., 2020; Özcan Ateş, 2023; 2024; Snoussi, et al., 2018).

Determine of Radial Growth Inhibition

The effect of *Penicillium* strains on mycelial radial growth was assessed based on the MIC values of FO and EO, following the method in Özcan Ateş (2023, 2024). *Penicillium* strains were initially incubated on PDA medium at 25°C for 7-10 days. FO and EO, with a known MIC value, were added to PDA containing 3% DMSO at concentrations of 1/4 MIC, 1/2 MIC, MIC, 2 MIC, and 4 MIC, then poured into 90 mm petri dishes. Cultures grown on PDA were inoculated at the center of each dish with a needle loop. PDA with 3% DMSO served as the control. Dishes were incubated at 25°C, and colony diameters were measured with calipers on days 5, 14, and 21. Radial growth inhibition was calculated as: % inhibition = [(control growth - growth in FO or EO) / control growth] × 100.

Statistical Analyses

All experiments were performed in triplicate. Data analysis of zone diameter was performed using the SPSS Package Program (v23.0, IBM SPSS Corp., Armonk, NY, USA), and results are presented as mean (m) ± standard deviation (SD). Radial growth inhibition was calculated using the Excel Program (Microsoft Office 365).

Results

This study assessed the antifungal effects of 135 commercial FOs and EOs against storage molds: *P. digitatum* (CECT 2954), *P. italicum* (CECT 2294), and *P. notatum* (NRRL 807). Nine oils exhibited antifungal activity in the agar well diffusion method (Table 2). Seven oils were effective against all three molds: juniper tar (7), thyme oil (77), lemon oil (83, 84), and peppermint oil (93, 94, 124). Conversely, avocado oil (15) was only effective against *P.* notatum (NRRL 807), showing no activity against the other *Penicillium* spp. Moreover, violet oil (90) was active against *P. digitatum* (CECT 2954) and *P. italicum* (CECT 2294), but ineffective against *P. notatum* (NRRL 807).

Table 2

FO and EOs determined to have antifungal activity by agar well diffusion method

FO or EO	*Penicillium italicum* (CECT 2294)		*Penicillium digitatum* (CECT 2954)		*Penicillium notatum* (NRRL 807)	
	Inhibition zone (mm)	%*	Inhibition zone (mm)	%	Inhibition zone (mm)	%
Juniper tar (7)	15.86 ± 0.23	48.92	22.68 ± 1.43	81.73	16.67 ± 0.17	-28.39
Avocado oil (15)	6.00 ± 0.01	-43.66	6.00 ± 0.01	-51.92	10.09 ± 0.20	-56.66
Thyme oil (77)	90.00 ± 0.01	745.07	90.00 ± 0.01	621.15	90.00 ± 0.01	286.60
Lemon oil (83)	10.81 ± 0.37	1.50	11.09 ± 0.28	-11.14	10.18 ± 0.10	-56.27
Lemon oil (84)	28.05 ± 0.43	163.38	30.76 ± 0.81	146.47	30.13 ± 0.63	29.42
Violet oil (90)	10.47 ± 0.26	-1.69	14.39 ± 0.12	15.30	6.00 ± 0.01	-74.23
Peppermint oil (93)	16.15 ± 0.29	51.64	32.57 ± 0.96	160.98	25.01 ± 1.30	7.43
Peppermint oil (94)	11.75 ± 0.75	10.33	18.48 ± 0.49	48.08	10.78 ± 0.21	-53.69
Toros mint oil (124)	15.88 ± 0.29	49.11	16.29 ± 0.15	30.53	13.38 ± 0.47	-42.53
Amphotericin B	10.65 ± 0.45	0.00	12.48 ± 0.44	0.00	23.28 ± 0.25	0.00

*Effect of EO and FO against antifungal disc

Table 3

MIC and MFC values of FO and EO were determined to have antifungal activity (in µL/mL)

FO or EO	*Penicillium italicum* (CECT 2294)			*Penicillium digitatum* (CECT 2954)			*Penicillium notatum* (NRRL 807)		
	MIC	MFC	MFC/MIC	MIC	MFC	MFC/MIC	MIC	MFC	MFC/MIC
Juniper tar (7)	10	>40	-*	40	>40	-	10	>40	-
Avocado oil (15)	40	>40	-	40	>40	-	40	>40	-
Thyme oil (77)	1	1	1	1	1	1	1	1	1
Lemon oil (83)	10	>40	-	10	>40	-	5	>40	-
Lemon oil (84)	1	20	20	1	20	20	1	20	20
Peppermint oil (93)	1	20	20	1	20	20	1	20	20
Peppermint oil (94)	1	20	20	1	20	20	1	20	20
Toros mint oil (124)	40	>40	-	40	>40	-	20	>40	-

*Could not be calculated because the MFC value could not be determined.

Table 3 lists the MIC values of 11 oils identified as effective through the agar well diffusion method. Thyme oil (77) showed MIC and MFC values of 1 µL/mL against the tested *Penicillium* species, indicating a fungicidal effect. Lemon oil (84) and peppermint oil (93, 94) exhibited MIC values of 1 µL/mL and MFC values of 20 µL/mL, indicating that they are fungistatic. Lemon oil (83) had MICs of 5 and 10 µL/mL, with an MFC value exceeding 40 µL/mL, indicating it is fungistatic. The MIC values for juniper tar (7), avocado oil (15), and taurus mint oil (124) ranged from 10 to 40 µL/mL, while their MFC values were above 40 µL/mL and it could not be determined whether it was fungicidal or fungistatic.

Five oils identified by microdilution as having fungicidal and fungistatic properties were selected to evaluate their ability to inhibit radial growth. Radial growth inhibition was determined over 21 days, taking into account the storage time of the citrus products. The inhibition rates of radial growth of selected oils are given in Table 4. Thyme oil (77) completely inhibited radial growth on days 5, 14, and 21 at all concentrations tested, except for the 1/4x MIC concentration (Figure 1, 2, and 3). For lemon oil (83 and 84) and peppermint oil (94), the inhibition rate increased with increasing concentration, but when the incubation time reached day 21, the inhibition rate decreased compared to day 5. Peppermint oil (93) completely inhibited all isolates tested on day 5 at 4x MIC; however, inhibition rates decreased with increasing incubation time for all isolates except *P. notatum* (NRRL 807). Therefore, when it comes to the use of essential oils for long-term food preservation, the use of thyme oil (77) is more suitable as it provides complete inhibition.

Table 4
Inhibition of Radial Growth (%) on Days 5, 14, and 21 of incubation

FO or EO		*Penicillium italicum* (CECT 2294)			*Penicillium digitatum* (CECT 2954)			*Penicillium notatum* (NRRL 807)		
		5	14	21	5	14	21	5	14	21
Thyme oil (77)	¼ x MIC	100.00	86.92	74.02	100.00	58.77	35.55	76.39	81.97	66.60
	½ x MIC	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	MIC	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	2 x MIC	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	4 x MIC	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Lemon oil (83)	¼ x MIC	34.52	8.03	16.55	4.88	-33.43	-44.70	-8.41	-13.87	-8.21
	½ x MIC	51.91	15.06	22.85	13.09	-28.11	-22.68	3.45	9.33	-3.76
	MIC	63.01	37.69	39.19	30.68	8.90	5.22	31.23	32.49	22.68
	2 x MIC	65.40	63.35	61.13	49.17	15.97	20.07	39.60	41.21	35.70
	4 x MIC	68.09	64.70	61.78	49.70	37.07	38.81	66.16	70.42	54.41
Lemon oil (84)	¼ x MIC	-6.46	-3.11	1.56	-35.35	-57.58	-48.35	27.06	-3.49	-6.04
	½ x MIC	5.08	3.51	8.62	-15.48	-27.17	-26.49	27.07	-1.74	-2.02
	MIC	18.71	6.00	9.20	0.17	-14.18	-11.34	1.12	0.04	1.08
	2 x MIC	26.24	11.60	9.61	4.78	-12.69	-6.30	1.59	1.38	3.60
	4 x MIC	32.18	18.90	19.06	22.11	18.78	20.65	19.69	19.40	18.53
Peppermint oil (93)	¼ x MIC	41.90	13.80	19.35	9.06	-17.34	-30.86	3.53	-4.57	6.76
	½ x MIC	43.65	17.52	13.81	6.38	-43.60	-4.74	7.77	14.45	5.33
	MIC	59.50	22.43	18.15	24.87	-23.14	-4.02	23.05	31.33	15.85
	2 x MIC	100.00	58.74	43.06	37.49	-0.14	7.44	48.44	42.14	38.40
	4 x MIC	100.00	74.74	49.34	100.00	30.18	23.76	100.00	100.00	100.00
Peppermint oil (94)	¼ x MIC	-17.55	-15.91	-11.98	-12.21	-116.22	-83.45	-8.20	-7.84	-7.79
	½ x MIC	8.30	4.55	6.92	-21.85	-82.98	-60.50	-5.87	-2.70	-1.40
	MIC	11.15	6.57	8.38	-6.08	-36.54	-36.21	7.35	12.67	7.58
	2 x MIC	18.21	13.24	13.42	14.05	-23.65	-15.58	13.44	12.51	16.26
	4 x MIC	31.95	31.45	27.48	30.03	-4.82	13.05	44.12	47.69	39.62

Figure 1.
Radial growth inhibition of thyme oil (80) against Penicillium digitatum (CECT 2954) after 21 days

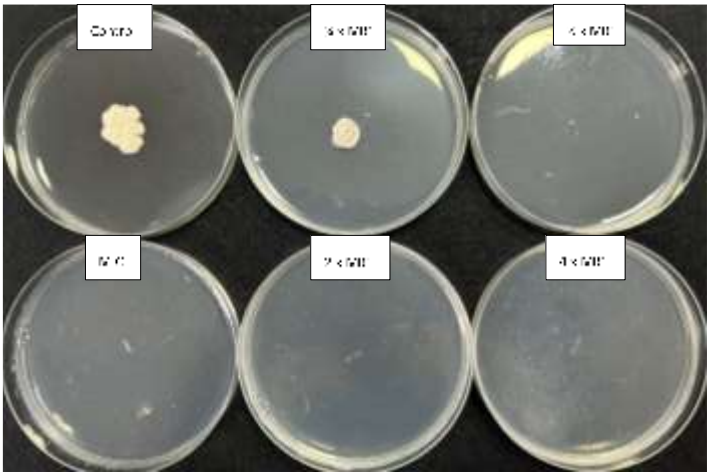


Figure 2.
Radial growth inhibition of thyme oil (80) against Penicillium italicum (CECT 2294) after 21 days

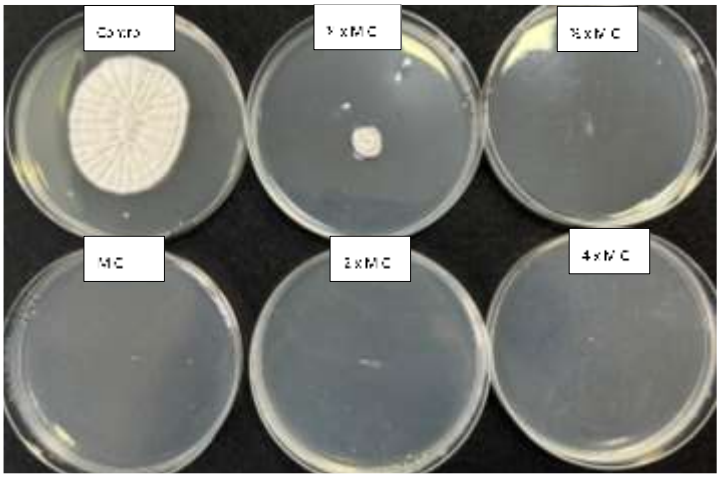
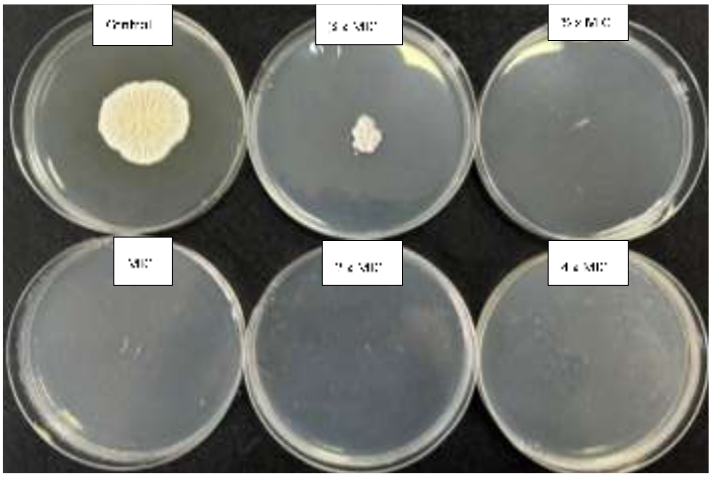


Figure 3.
Radial growth inhibition of thyme oil (80) against Penicillium notatum (NRRL 807) after 21 days



Discussion

This study evaluated 135 commercially available fixed and essential plant oils for their antifungal activity against *P. digitatum* (CECT 2954), *P. italicum* (CECT 2294), and *P. notatum* (NRRL 807), which are commonly found during the storage of agricultural products. Using agar diffusion, microdilution, and radial growth inhibition techniques, nine oils showed antifungal effects against at least one of these fungi. In this study, thyme, peppermint, and lemon essential oils were determined to exhibit high levels of antifungal activity, particularly against storage molds such as *P. digitatum, P. italicum, and P. notatum*. Thyme oil (77) stood out, offering the strongest inhibition across all tested *Penicillium* species, with both MIC and MFC values of 1 µL/mL. The complete inhibition observed over 21 days confirms its fungicidal properties and potential as a natural, long-term antifungal agent. These results align with Yahyazadeh et al. (2008), who reported a potent inhibitory effect of *Thymus vulgaris* on *P. digitatum*. Also, these findings are consistent with the data reported by Mutlu-İngök et al. (2020). In that study, essential oils from *Thymus* spp. were reported to inhibit *P. digitatum* and *P. italicum* at concentrations as low as <500 µL/L. This finding supports the high antifungal effects of thyme oil, as observed in this study. The antifungal effects of oils are generally attributed to the disruption of cell membrane permeability by volatile compounds, including phenolic compounds, terpenoids, and aldehydes, which leads to the leakage of cell contents and inhibition of enzymatic processes (Nazzaro et al., 2017). In this study, it is believed that microscopic cellular damage occurred, particularly in samples where complete inhibition was achieved with thyme oil.

The inhibitory effects of lemon oils (83 and 84) and peppermint oils (93 and 94) against three *Penicillium* species varied depending on concentration and time. It was determined that these EOs exhibited fungistatic properties, and their inhibitory effect decreased, particularly with prolonged storage periods. The volatile components of EOs are responsible for their biological activity, and these volatile compounds with biological activity may have evaporated during storage, which leads to a decrease in their inhibitory effect. Indeed, studies by Tao et al. (2014) and Trabelsi et al. (2016) also indicated that the antifungal activity of essential oils extracted from citrus fruits may have possible limitations related to time. Felšöciová et al. (2020) reported that the effectiveness of peppermint oils may vary depending on the species and environment, as in our study.

Violet oil (90) was found to have antifungal activity against *P. italicum* and *P. digitatum* but was inactive against *P. notatum*. This suggests that the biological activities of EOs may be species-selective. Moreover, while studies on the antibacterial and anticandidal activities of violet oil are available in the literature (Doğan et al., 2023; Orchard et al., 2023), no research has been conducted on its antifungal activity. Therefore, this study is the first data on the antifungal activity of violet oil against *Penicillium* species. The study also found that juniper tar (7) and avocado oil (15) have antifungal activity and fungistatic properties, but no MFC value was determined. Bangulzai et al. (2022) reported similar findings, noting that plant-fixed oils, especially, have limited antifungal activity. Although the literature states that various plant FOs and EOs, such as clove, cumin, bay laurel, etc., possess antifungal activity (Hu et al., 2019; Nazzaro et al., 2017), the oils tested in this study showed no antifungal activity. The main reason for this discrepancy is the variability in the concentrations of the active components in the oils.

In the study, it was determined that peppermint oil (94) at low concentrations, such as ¼ x MIC, increased radial growth rather than inhibiting it compared to the control group. Allagui et al. (2023) and Zulu et al. (2023) reported that essential oils stimulate radial growth at low concentrations and act as nutrients.

Thyme oil mainly contains the phenolic monoterpenes thymol and carvacrol (Wang et al., 2019). Their antifungal action is not completely understood, but theories suggest they may disrupt the cell wall or membrane, or cause morphological changes in conidia and hyphae (Lambert, 2001; Neri et al., 2006; Pérez-Alfonso et al., 2012; Wang et al., 2019). Peppermint oil's key component is menthol. Although its antifungal mechanism is still unclear, it likely involves disrupting the lipid part of the cell membrane, interacting with ergosterol, increasing membrane fluidity and permeability, leading to ion transport issues, leakage of intracellular materials, alterations in mitochondrial structure, reduced proton-motive force, and hindering cellular respiration (de Castro Teixeira et al., 2024). Lemon

oil's main ingredient, limonene, inhibits fungi through its lipophilicity, allowing it to penetrate membranes, cause membrane expansion, increase fluidity and permeability, disrupt membrane proteins, inhibit respiration, interfere with ion transport, and promote leakage of ions and cellular contents (Tao et al., 2014; Tao et al., 2019).

The effects of EOs on molds can vary from cellular to macromorphological levels. At the cellular level, similar to how antifungal agents work, they can inhibit the production of DNA, RNA, proteins, and polysaccharides. They may also disrupt the structure of the cell membrane and organelles. Furthermore, they can interfere with enzymatic reactions involved in cell wall synthesis, which impacts cell growth and shape, and can cause cytoplasmic retraction in hyphae, leading to mycelium death. In macromorphological studies, these oils can influence sporulation, pigmentation, conidia count, and hyphal branching (Nazzaro et al., 2017). As shown in Figures 1, 2, and 3, alterations in the morphology of *Penicillium* spp. tested in the study, especially in sporulation color, were observed.

A key strength of this study is its thorough evaluation, involving a large variety of plant oil samples and multiple testing methods. This enables a detailed assessment of the extent and longevity of antifungal activity. Nonetheless, there are some limitations. The chemical makeup of the oils tested was not examined, making it impossible to pinpoint which components drive the antifungal effects. Additionally, the research was limited to *in vitro* conditions, which restricts how directly the findings can be applied to real-world situations.

Conclusion and Recommendations

In conclusion, this study demonstrates that certain essential oils, particularly thyme oil, exhibit strong antifungal properties against storage molds, including *Penicillium digitatum*, *Penicillium italicum*, and *Penicillium notatum*. This research offers valuable insights into the potential of natural antifungal agents and provides a foundation for more extensive and practical investigations in the future. These oils are promising as natural antifungal agents for use in agriculture and food preservation. Nonetheless, since the effectiveness of essential oils may fade over time, developing advanced formulations, carrier systems, and controlled-release methods is advisable. Additionally, synergistic effects with different ingredients or combinations of essential oils can be tested. *In vivo* evaluation of commercial storage conditions is particularly important for applicability. Additionally, safety, sensory, and stability evaluations are important. Future research should focus on analyzing the chemical makeup of these oils, exploring their synergistic interactions, and conducting *in vivo* tests.

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References

Abers, M., Schroeder, S., Goelz, L., Sulser, A., St. Rose, T., Puchalski, K., & Langland, J. (2021). Antimicrobial activity of the volatile substances from essential oils. *BMC Complementary Medicine and Therapies, 21*(1), 124. https://doi.org/10.1186/s12906-021-03285-3

Allagui, M. B., Moumni, M., & Romanazzi, G. (2023). Antifungal activity of thirty essential oils to control pathogenic fungi of postharvest decay. *Antibiotics, 13*(1), 28. https://doi.org/10.3390/antibiotics13010028

Bangulzai, N., Ahmed, S., Kashif, M., Fatima, M., Ahmed, M., & Mushtaq, N. (2022). Antifungal Activity of Essential Oils Extracted from Different Plants Against. International Journal of Agriculture and Biosciences *Penicillium digitatum Causing Green Mold of Citrus, 11*(2), 75-83. https://doi.org/10.47278/journal.ijab/2022.011

Bhatta, U. K. (2022). Alternative management approaches of citrus diseases caused by *Penicillium digitatum* (green mold) and *Penicillium italicum* (blue mold). *Frontiers in Plant Science, 12*, 833328. https://doi.org/10.3389/fpls.2021.833328

Bhunia, A. (2018). Molds and Mycotoxins. *Foodborne Microbial Pathogens (2nd ed.)*, 167-174. Springer. https://doi.org/10.1007/978-1-4939-7349-1_8

Chen, Y., Xing, M., Chen, T., Tian, S., & Li, B. (2023). Effects and mechanisms of plant bioactive compounds in preventing fungal spoilage and mycotoxin contamination in

postharvest fruits: A review. *Food Chemistry, 415*, 135787. https://doi.org/10.1016/j.foodchem.2023.135787

de Castro Teixeira, A. P., de Sousa Melo, F. M., Lima, I. O., de Luna Freire Pessôa, H., & de Cássia da Silveira e Sá, R. (2024). Do menthol and its derivatives present biological activity with antifungal potential? *Journal of Essential Oil Research, 36*(4), 291-320. https://doi.org/10.1080/10412905.2024.2376673

Doğan, M., Mohammed, F. S., Uysal, İ., Mencik, K., Kına, E., Pehlivan, M., & Sevindik, M. (2023). Total antioxidant status, antimicrobial and antiproliferative potentials of *Viola odorata* (Fragrant Violet). *Journal of Faculty of Pharmacy of Ankara University, 47*(3), 784-791. https://doi.org/10.33483/jfpau.1161440

Felšöciová, S., Vukovic, N., Jeżowski, P., & Kačániová, M. (2020). Antifungal activity of selected volatile essential oils against *Penicillium* sp. *Open Life Sciences, 15*(1), 511-512. https://doi.org/10.1515/biol-2020-0045

Gatsing, D., Tchakoute, V., Ngamga, D., Kuiate, J.-R., Tamokou, J., Nji-Nkah, B., Tchouanguep,. F. M., & Fodouop, S. (2009). In Vitro antibacterial activity of *Crinum purpurascens* herb leaf extract against the Salmonella species causing typhoid fever and its toxicological evaluation. *Iranian Journal of Medical Sciences, 34*(2), 126-136. http://ijms.sums.ac.ir/files/PDFfiles/34_2_08-Dr_Gatsing.pdf

Hu, F., Tu, X. F., Thakur, K., Hu, F., Li, X. L., Zhang, Y. S., Zhang, J. G., & Wei, Z. J. (2019). Comparison of antifungal activity of essential oils from different plants against three fungi. *Food and Chemical Toxicology, 134*, 110821. https://doi.org/10.1016/j.fct.2019.110821

Lambert, R. J. W. (2001). Advances in disinfection testing and modelling. *Journal of Applied Microbiology, 91*(2), 351-363. https://doi.org/10.1046/j.1365-2672.2001.01393.x

Li, P., Xie , D., Chen, H., Qiu, Y., Zhang, X., Zhang, S., Wang L., Lin, H., Li, X., & Liu, K. (2023). Secondary metabolites from marine derived fungus *Penicillium chrysogenum* Y19-1 with proangiogenic and antithrombotic activities. *Biochemical Systematics and Ecology, 107*, 104625. https://doi.org/10.1016/j.bse.2023.104625

Maurya, A., Prasad, J., Das, S., & Dwivedy, A. K. (2021). Essential oils and their application in food safety. *Frontiers in Sustainable Food Systems, 5*, 653420. https://doi.org/10.3389/fsufs.2021.653420

Mseddi, K., Alimi, F., Noumi, E., Veettil, V., 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

Mutlu-Ingok, A., Devecioglu, D., Dikmetas, D., Karbancioglu-Guler, F., & Capanoglu, E. (2020). Antibacterial, Antifungal, Antimycotoxigenic, and Antioxidant Activities of Essential Oils: An Updated Review. *Molecules, 25*(20), 4711. https://doi.org/10.3390/molecules25204711

Nazzaro, F., Fratianni, F., Coppola, R., & Feo, V. (2017). Essential Oils and Antifungal Activity. *Pharmaceuticals, 10*(4), 86. https://doi.org/10.3390/ph10040086

Neri, F., Mari, M., & Brigati, S. (2006). Control of *Penicillium expansum* by plant volatile compounds. *Plant pathology, 55*(1), 100-105. https://doi.org/10.1111/j.1365-3059.2005.01312.x

Orchard, A., Moosa, T., Motala, N., Kamatou, G., Viljoen, A., & Vuuren, S. V. (2023). Commercially available Viola odorata oil, chemical variability and antimicrobial activity. *Molecules, 28*(4), 1676. https://doi.org/10.3390/molecules28041676

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. (2024). In vitro Antifungal Activity of *Mentha piperita* and *Thymus vulgaris* Essential Oils against Ochratoxigenic *Aspergillus carbonarius* Isolated from Bozcaada Çavuş Grape. *Yuzuncu Yıl University Journal of Agricultural Sciences, 34*(1), 166-175. https://doi.org/10.29133/yyutbd.1403366

Pérez-Alfonso, C. O., Martínez-Romero, D., Zapata, P. J., Serrano, M., Valero, D., & Castillo, S. (2012). The effects of essential oils carvacrol and thymol on growth of *Penicillium digitatum* and *P. italicum* involved in lemon decay. *International Journal of Food Microbiology, 158*(2), 101-106.

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*, 2824-2832.

https://doi.org/10.1007/s13197-018-3219-6

Tao, N., Jia, L., & Zhou, H. (2014). Anti-fungal activity of Citrus reticulata Blanco essential oil against *Penicillium italicum* and *Penicillium digitatum*. *Food Chemistry, 153*, 265-271. https://doi.org/10.1016/j.foodchem.2013.12.070

Tao, N., Chen, Y., Wu, Y., Wang, X., Li, L., & Zhu, A. (2019). The terpene limonene induced the green mold of citrus fruit through regulation of reactive oxygen species (ROS) homeostasis in *Penicillium digitatum* spores. *Food Chemistry, 277*, 414-422. https://doi.org/10.1016/j.foodchem.2018.10.142

Trabelsi, D., Hamdane, A., Said, M., & Abdrrabba, M. (2016). Chemical Composition and Antifungal Activity of Essential Oils from Flowers, Leaves and Peels of Tunisian *Citrus aurantium* Against *Penicillium digitatum* and *Penicillium italicum*. *Journal of Essential Oil Bearing Plants, 19*(7), 1660-1674. https://doi.org/10.1080/0972060X.2016.1141069

Wang, K., Jiang, S., Pu, T., Fan, L., Su, F., & Ye, M. (2019). Antifungal activity of phenolic monoterpenes and structure-related compounds against plant pathogenic fungi. *Natural Product Research, 33*(10), 1423-1430. https://d0192efdd77670f7012d3fece2c7c0fc720c897f.vetisonline.com/10.1080/14786419.2017.1419232

Yahyazadeh, M., Omidbaigi, R., Zare, R., & Taheri, H. (2008). Effect of some essential oils on mycelial growth of *Penicillium digitatum* Sacc. *World Journal of Microbiology and Biotechnology, 24*, 1445-1450. https://doi.org/10.1007/s11274-007-9636-8

Zulu, L., Gao, H., Zhu, Y., Wu, H., Xie, Y., Liu, X., Yao, H., & Rao, Q. (2023). Antifungal effects of seven plant essential oils against *Penicillium digitatum*. *Chemical and Biological Technologies in Agriculture, 10*(1), 82. https://doi.org/10.1186/s40538-023-00434-3