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Chemical composition and antifungal activity of marjoram, peppermint and thyme essential oils on cumin seed pathogens

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Abstract

Cumin seeds suffer a significant loss in yield and viability due to attacks by several soil-borne fungal pathogens. This study aimed to analyze of chemical composition of marjoram, peppermint and thyme essential oils and evaluate their antifungal activity on the cumin fungal pathogens *Fusarium oxysporum*, *Macrophomina Phaseolina and Rhizoctonia solani*. We also studied their effect on seed germination. The essential oils of marjoram, peppermint and thyme were extracted by hydro distillation and their chemical constituents were analyzed with gas chromatography /mass spectrometry (GC/MS). We identified 47, 40, and 36 different compounds in thyme, marjoram, and peppermint, respectively. The most common constituents in each were thymol (33.09%), and β-Pinene (9.47%) in thyme oils, (±)-p-Menth-1-en-4-ol (14.70%), Sabinene hydrate, trans (13.44%), 1,3,8-p-Menthatriene (13.41%), psi-Limonene (9.71%) in marjoram, and p-Menthan-1-ol (35.91%), Eucalyptol (11.9%), p-Menth-4(8)-en-3-one (10.79%) in peppermint. Minimum inhibitory concentration of the identified oils was determined. Thyme essential oil emulsion showed the highest reduction on mycelial growth of all fungal pathogens followed by peppermint at the concentration 6000 ppm, whereas, marjoram oil gave a germination rate of 95% for the seeds at the lowest concentration of 2000 ppm.

Keywords: Marjoram, Peppermint, Thyme, Essential oil, *Fusarium oxysporum*.

Full length article **Corresponding Author*, e-mail: na944193@gmail.com

1. Introduction

Numerous crops are seriously harmed by fungal infections. One of the most significant soil-borne pathogens that causes wilt disease in cumin plants is *Fusarium oxysporum* [1,2]. The pathogen has the capacity to survive for much extended periods of time in soil without a host [3]. According to research by [4], isolates of the fungi *F. oxysporum*, *R. solani*, and *M. phaseolina* were the most common in cumin plants and pathogenic to cumin plants. Since the chemical fungicides used to control the fungal pathogens cause pollution in the environment and also kill beneficial organisms, the search for alternative sources of environmentally ecofriendly and antifungal chemicals in plants. These substances are thought to be useful for managing various plant diseases since they are biodegradable and poisonous in a selective manner [5]. Essential oils are aromatic liquids that are produced by steam distilling plant. These aromatic molecules are pure chemical compounds that are volatile under normal conditions. Mono- and sesquiterpenes, as well as other types of chemicals like allyl and isoallyl phenols, may also be found. They are mostly made up of lipophilic and highly volatile secondary plant metabolites. Other molecules that have been found in volatile oils include coumarins, anthraquinones, and alkaloids, which are frequently distillable. various nonvolatile compounds, such as fat and various diterpenes, can also be extracted from essential oils using techniques other than distillation. Essential oils are widely utilized in cosmetics and fragrances, and they also have medical purposes due to their therapeutic qualities and agro-dietary uses due to their antibacterial and antioxidant activities. Less toxicity for people and environmental safety are two characteristics of essential oils. According to [6,7], chemicals generated from essential oils have a wide spectrum of antibacterial properties and are less hazardous to humans and the environment. Lipophilic monoterpens like thymol, carvacrol, linalool, citral, geraniol, and 1, 8-cineole is among the powerful antibacterial chemicals identified from essential oils. Numerous studies have been conducted on their potential uses as antiseptics and disinfectants or as food preservatives [8,9]. Essential oils have been shown to have antifungal effect against phytopathogens in the past. In the case of Fusarium oxysporum, for example, marjoram, peppermint, and thyme

oils served as anti-fungal agents [10,11]. The activity of thyme essential oil as anti-fungal agent against species of Aspergillus, Penicillium, Alternaria, Cladosporium, Rhizopus and Trichoderma was mainly attributed to p-cymene, 1,8-cineole and other thymol constituens [12]. The aim of this study was to estimation of chemical composition of essential oils of marjoram, peppermint and thyme and evaluate the antifungal activity of essential oils emulsions against some soil fungi isolated from cumin plants and their effects on germination of cumin seeds under *in vitro* conditions.

2. Materials and Methods

2.1. Source of fungi

The isolates of *Fusarium oxysporum*, *Macrophomina phaseolina* and *Rhizoctonia solani* used in this study were isolated from cumin plants and obtained from the fungal collection of Ornamental, Medicinal and Aromatic Plant Dis. Dept., Plant Pathol. Res. Inst. ARC, Giza, Egypt [4].

2.2. Plant materials

Fresh herb of marjoram (*Majorana hortensis* L.), peppermint (*Mentha piperita* L.) and thyme (*Thymus vulgaris* L.) were collected at the beginning of the flowering stage from El-Kanater El- Khairia Farm, Medicinal and Aromatic Plants Research Department, Horticulture Research Institute (HRI), ARC, Egypt. Samples from each plant were then shade dried and subjected for essential oil extraction.

2.3. GC-MS analysis of the essential oils

The essential oils were extracted by hydro-distillation using a Clevenger apparatus for 3 hours, then dried with anhydrous sodium sulphate and stored at 4º C until use [13]. The Gas Chromatography/Mass Spectrometry (GC/MS) technique was used to separate and identify the components of marjoram, peppermint, and thyme essential oils by comparing their mass spectra and retention times to those of the authentic compounds and by computer matching with the NIST and WILLEY libraries. Analyses were carried out with the use of a G.C (Agilent Technologies 7890A) interfaced with a mass-selective detector (MSD, Agilent 7000) fitted with a polar Agilent HP-5ms (5%phenyl methyl poly siloxane) capillary column (30 m 0.25 mm I. d. and 0.25 m film thickness) [14].

2.4. Evaluation the effect of the essential oils emulsions on the mycelial growth of the tested fungi in vitro

Essential oil emulsions were made by gently adding 10 mL of each essential oil and 5 mL of the non-ionic surfactant Tween 80 until a homogenous liquid was created. Then, 85 ml of water was added to the final mixture of each oil to help disperse and completely include the essential oils, and the mixture was agitated for 30 minutes using a magnetic stirrer. The effectiveness of volatile compounds in inhibiting fungal growth was investigated. To reach the proposed concentrations of 2000, 4000, 6000, and 8000 ppm, essential oils of marjoram, peppermint, and thyme emulsions were introduced to sterilized PDA flasks before solidifying. To prevent bacterial contamination, a bactericide (0.1mg/l chloramphenicol) was added to the medium. Three plates of potato dextrose agar (PDA) media were inoculated with 5 mm discs of *F. oxysporum*, *M. phaseolina*, and *R. solani*. At 252°C, petri dishes were incubated. Percentages of fungal growth inhibition were estimated when the fungal growth of the control plates (without treatments) entirely covered the plates using the [15] formula:

$$\text{Inhibition \%} = \frac{A - B}{A} \times 100$$

A= The linear growth (cm) in control treatment.
B= The linear growth (cm) of treated fungus.

2.5. Germination test

`Sets of 300 cumin seeds were soaked for 20 minutes in marjoram, peppermint, and thyme essential oil emulsions at rates of 2000, 4000, 6000, and 8000 ppm. At a rate of 100 seeds per plate, seeds treated with each concentration were deposited in Petri dishes on layers of wet blotters. Dishes were then incubated for 15 days in a controlled environment (27 °C) with alternating cycles of 12 hours light and 12 hours dark. Finally, the percentage of germination for each treatment was calculated as follows:

$$\text{Germination \%} = \frac{\text{No. of germinated seeds}}{\text{Total number of experimented seeds}} \times 100$$

2.6. Statistical analysis

Analysis of significant differences according to [16] that significant differences in means were assessed using one-way ANOVA followed by LSD to compare treatment means at a probability level of 0.05.

3. Results

3.1. Chemical composition of essential oils

Chemical composition of thyme (*Thymus vulgaris* L.) essential oil analyzed by gas chromatography /Mass Spectrometry (GC/MS), their percentage compositions, calculated as the ratio of peak area to the total chromatographic area in Table (1). Forty-seven compounds were identified in thyme oil. Thymol (33.09%) at the retention time (10.85 min), (-) -β-Pinene (9.47%) at the retention time (6.08 min), 4-Terpinenyl acetate (5.96%) at the retention time (7.29 min), O-Methylthymol (4.51%) at the retention time (9.51 min), α-Pinene (4.48%) at the retention time (5.44 min), β-Caryophyllen (3.67%) at the retention time (11.87 min), 3-p-Menthene (3.09%) at the retention time (9.96 min) and p-Cimene (3.01%) at the retention time (6.92 min) were identified as the major constituents of thyme essential oil. The essential oil also contained on several smaller quantities of compounds. Chemical composition of marjoram (Majorana hortensis L.) essential oil analyzed by GC/MS, their percentage compositions, calculated as the ratio of peak area to the total chromatographic area in Table (2). Forty compounds were identified in marjoram oil. (±)-p-Menth-1-en-4-ol (14.70%) at the retention time (9.27 min), Sabinene hydrate, trans (13.44%) at the retention time (8.09 min), 1,3,8-p-Menthatriene (13.41%) at the retention time (6.88 min), psi.-

Limonene (9.71%) at the retention time (6.07 min), γ-Terpinene (6.99%) at the retention time (7.38 min), β-Caryophyllene (4.72%) at the retention time (11.86 min), Linalool acetate (3.39%) at the retention time (9.87 min) and α-Pinene (3.13%) at the retention time (5.40 min) were identified as the major constituents of marjoram essential oil. The essential oil also contained on several smaller quantities of compounds. Chemical composition of peppermint (Mentha piperita L.) essential oil analyzed by GC/MS, their percentage compositions, calculated as the ratio of peak area to the total chromatographic area in Table (3). Thirty six compounds have been identified in peppermint oil.p-Menthan-1-ol(35.91%) at the retention time (9.27 min), Eucalyptol (11.9%) at the retention time (6.91 min), p-Menth-4(8)-en-3-one (10.79%) at the retention time (9.86 min), α-Pinene (6.65%) at the retention time (5.44 min), Menthyl acetate (5.68%) at the retention time (10.35 min), (+)-Camphene (4.16%) at the retention time (6.07 min), (-)-α- Terpineol (2.87%) at the retention time (9.36 min), Caryophyllene (2.33%) at the retention time (11.80 min) and p-Menth-1-en-3-one (2.12%) at the retention time (10.03 min) were identified as the major constituents of peppermint essential oil. The essential oil also contained on several smaller quantities of compounds.

3.2. Antifungal activity

Under in vitro circumstances, the antifungal activity of three essential oil emulsions at doses of 2000, 4000, 6000, and 8000 ppm against F. oxysporum, M. phaseolina, and R. solani isolated from diseased cumin plants was examined. After seven days of incubation, the fungal isolates showed dose-dependent growth suppression (Table 4) and (Fig. 1,2,3). The statistical analysis revealed that essential oil emulsions at various concentrations had a substantial effect on fungal growth. Fungi growth inhibition increased linearly with increasing content of essential oil emulsions. All essential oil emulsions at 8000 ppm concentration totally suppressed mycelial development, according to the data. The treatment of isolates of F. oxysporum, M. phaseolina and R. solani with thyme essential oil emulsion at 6000 ppm significantly reduced fungal growth. Thyme essential oil emulsion, on the other hand, was the maximum impact on mycelial development of all fungi, followed by peppermint essential oil emulsion at 6000 ppm concentration.

3.3. Germination test

3.3.1. Effect of three essential oils emulsions at different concentrations on germination of cumin seeds

The results showed the effect of emulsions of essential oils with different concentrations on the germination of cumin seeds, as shown in Table (3). In general, there was a significant increase in germination percentage up to 6000 ppm and then it decreased at 8000 ppm. Germination (%) was increased gradually with increasing concentration. The maximum germination percentage (100%) was recorded with all essential oil emulsions tested at 6000 ppm. On the other hand, the best emulsion that had an effect on seed germination was the marjoram oil emulsion, which gave a germination rate of 95% for the seeds at the lowest concentration of 2000 ppm, while the treatment with both the marjoram and thyme oil emulsions at the concentration of 6000 ppm gave a germination rate of 100%. Thus, the emulsions of marjoram and thyme oils were the best at a concentration of 6000 ppm.

4. Discussion

Chemical composition of thyme essential oil analyzed by (GC/MS). Forty-seven compounds were identified in thyme oil. Thymol, (-) -β-Pinene, 4-Terpinenyl acetate, O-Methylthymol, α-Pinene, β-Caryophyllen, 3-p-Menthene and p-Cimene were identified as the major constituents of thyme essential oil. The essential oil also contained on several smaller quantities of compounds. The results were similar with [12] who found that the thyme essential oil contains p-cymene (36.5%), thymol (33.0%) and 1,8-cineole (11.3%) as main components. Also, [11] demonstrated that a major constituent of thyme essential oil was identified asρ-cymene (52.36%), thymol (23.62%) and borneol (5.80%). Forty compounds were identified in marjoram oil. (±)-p-Menth-1-en-4-ol, Sabinene hydrate, trans, 1,3,8-p-Menthatriene, psi.-Limonene, γ-Terpinene, β-Caryophyllene, Linalool acetate and α-Pinene were identified as the major constituents of marjoram essential oil. The essential oil also contained on several smaller quantities of compounds. The results were similar with [17] who mentioned that Chromatographic analysis of marjoram oil was show that trans-sabinene hydrate (22%) and terpinen-4-ol (17.61%). Components with significant concentrations in the volatile fraction included also γ-terpinene (10.29%), α-terpinene (6.61%), α-terpineol (5.85%), sabinene (5.51%) and cis-sabinene hydrate (5.39%). Also, [11] demonstrated that a major constituents of marjoram essential oil was characterized by the presence of terpinene-4-ol (30.28%), γ-terpinene (13.32%), α-terinene (12.46%) and sabinene (10.49%). Thirty-six compounds have been identified in peppermint oil. p-Menthan-1-ol, Eucalyptol, p-Menth-4(8)-en-3-one, α-Pinene, Menthyl acetate, (+)-Camphene, (-)-α- Terpineol, Caryophyllene and p-Menth-1-en-3-one were identified as the major constituents of peppermint essential oil. The essential oil also contained on several smaller quantities of compounds. [11] reported that the main constituents of peppermint essential oil were identified as menthol (30.51%), menthyl acetate (23.06%) and menthone (14.45%). On the other hand, [18] stated that drying methods caused significant variations in the main constituents of the peppermint leaves essential oil including menthol, menthone, menthofuran, 1,8-cineole, and menthyl acetate. The minimum (35.01%) and maximum (47.50%) concentrations of menthol, as the major compound of the oil, were found in hot air-dried leaves at temperature of 50°C and microwave-dried leaves at power of 400 W, respectively. The percentage of menthone, as the second constituent in the essential oil, was significantly lost ($p < 0.01$) under microwave drying. The differences among reported values and components could be attributed to the environmental and experimental conditions to which plants were submitted. The efficacy of essential oils of marjoram, peppermint and thyme emulsions at four concentrations (2000, 4000, 6000 and 8000 ppm) against F. oxysporum, M. phaseolina and R. solani isolated from infected cumin plants were confirmed, since decreases than the control in this respect were significant in most cases.

Table 1: List of the compounds present in thyme essential oil analyzed by GC–MS to show the retention time (RT) and the area percentage of each compound

NO.	Retention time (min)	Thyme essential oil	
		Compound name	**Area Sum %**
1	5.30	α-Thujene	1.30
2	5.44	α-Pinene	4.48
3	5.64	Camphene	0.47
4	5.71	γ-Terpinene	0.38
5	6.08	(-)-β-Pinene	9.47
6	6.47	α-Phellandrene	1.15
7	6.62	α-Terpinene	0.37
8	6.76	β-Cymene	1.34
9	6.92	*p*-Cimene	3.01
10	7.29	4-Terpinenyl acetate	5.96
11	7.66	*p*-Cymenene	1.27
12	7.80	Linalool	1.40
13	8.38	L-Pinocarveol	0.54
14	8.86	4-Carvomenthenol	0.72
15	9.09	Camphol	1.70
16	9.51	O-Methylthymol	4.51
17	9.96	3-*p*-Menthene	3.09
18	10.85	Thymol	33.09
19	11.12	Carvacrol	1.14
20	11.87	β-Caryophyllen	3.67
21	12.05	β-Eudesmene	0.54
22	12.23	Geranyl propionate	0.47
23	12.38	γ-Muurolene	0.70
24	12.57	β-Copaene	0.58
25	12.61	α-Muurolene	0.67
26	12.79	γ-Cadinene	0.73
27	12.84	(+)-δ-Cadinene	1.07
28	13.15	Elemol	2.09
29	13.54	(+)-Spathulenol	2.7
30	13.60	Caryophyllene oxide	1.1
31	14.04	epi-α-Cadinol	0.92
32	14.18	β-Eudesmol	1.34
33	14.33	Cedrenol	0.74
34	14.96	7-Isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-one	0.52
35	16.06	Farnesol	0.48
36	16.3	7-Methoxychromanone	0.59
37	16.5	1,2-Dipalmitoleoyl-sn-glycero-3-phosphoethanolamine	0.46
38	16.64	m-Camphorene	0.39
39	16.69	trans-Geranylgeraniol	0.36
40	16.87	Squalene	0.39
41	16.93	3,7,3',4'-Tetrahydroxyflavone	0.62
42	17.10	Glyceryl tripalmitoleate	0.61
43	17.31	1-Stearoyl-2-linoleoyl-sn-glycero-3-phosphoethanolamine	0.45
44	17.60	1-Linoleoyl-2-oleoyl-rac-glycerol	0.8
45	19.12	3'-Methoxyapigenin	0.53
46	20.08	Teprenone	0.43
47	21.66	Eudesm-7(11)-en-4-ol	0.67

Table 2: List of the compounds present in marjoram essential oil analyzed by GC–MS to show the retention time (RT) and the area percentage of each compound

NO.	Retention time (min)	Marjoram essential oil	Area Sum %
		Compound name	
1	5.28	α-Thujene	1.84
2	5.40	α-Pinene	3.13
3	6.07	psi.-Limonene	9.71
4	6.45	α-Phellandrene	1.00
5	6.88	1,3,8-p-Menthatriene	13.41
6	7.38	γ-Terpinene	6.99
7	7.50	4-Thujanol	1.98
8	7.74	Terpinolene	2.15
9	8.09	Sabinene hydrate, trans	13.44
10	8.44	cis-*p*-Mentha-2-en-1-ol	1.00
11	8.73	trans-*p*-Menth-2-ene-1-ol	2.78
12	9.27	(±)-*p*-Menth-1-en-4-ol	14.70
13	9.36	*p*-Menth-1-en-8-ol	2.64
14	9.45	*p*-Menth-1-en-3-ol, trans-	0.96
15	9.87	Linalool acetate	3.39
16	10.71	cis-Menthone	1.42
17	10.83	(-)-γ-Elemene	0.82
18	11.01	Geranyl acetate	0.69
19	11.24	all-trans-Farnesyl acetate	0.75
20	11.86	β-Caryophyllene	4.72
21	12.01	Eremophilene	0.39
22	12.16	Humulene	0.60
23	12.34	Aristolone	0.33
24	12.43	(1S,2S,4S)-Trihydroxy-*p*-menthane	0.45
25	12.62	β-Eudesmene	2.60
26	13.46	(+)-Spathulenol	0.95
27	13.52	Caryophyllene oxide	0.81
28	14.01	(-)-Spathulenol	0.97
29	14.32	1,2-Dilinolenoylglycerol	0.32
30	14.79	(+)-Isospathulenol	0.37
31	15.03	Lutein	0.16
32	15.82	1-Linoleoyl-2-oleoyl-rac-glycerol	0.27
33	16.65	3',5'-Dimethoxy-3,5,7,4'-tetrahydroxyflavone	0.66
34	16.83	Glycerol trioleate	0.7
35	16.92	Farnesol	0.5
36	17.06	trans-Geranylgeraniol	0.3
37	17.33	3,5,7-Trihydroxy-4'-methoxyflavone	0.2
38	18.15	1-Palmitoyl-2-oleoyl-sn-glycerol	1.26
39	20.34	4',5,7-Trihydroxy 3,6,8-trimethoxyflavone	0.2
40	23.11	11,14-Eicosadienoic acid	0.44

Table 3: List of the compounds present in peppermint essential oil analyzed by GC–MS to show the retention time (RT) and the area percentage of each compound

NO.	Retention time (min)	Peppermint essential oil Compound name	Area Sum %
1	5.44	α-Pinene	6.65
2	5.64	cis-Geraniol	0.98
3	6.07	(+)-Camphene	4.16
4	6.20	(-)-β-Pinene	0.38
5	6.91	Eucalyptol	11.9
6	7.22	4-Terpinenyl acetate	0.57
7	9.27	*p*-Menthan-1-ol	35.91
8	9.36	(-)-α-Terpineol	2.87
9	9.54	Phytol	1.19
10	9.62	cis-3-Hexenyl isovalerate	0.54
11	9.67	trans-Carveol	0.48
12	9.86	*p*-Menth-4(8)-en-3-one	10.79
13	10.03	*p*-Menth-1-en-3-one	2.12
14	10.12	2'-Hydroxy-2,4,4',5,6'-pentamethoxychalcone	0.55
15	10.13	Isomenthol acetate	0.38
16	10.35	Menthyl acetate	5.68
17	10.43	Verbenone	0.68
18	10.59	Menthyl valerate	1.97
19	10.72	5,7-Dimethoxyflavanone	0.84
20	11.41	(-)-β-Bourbonene	1.60
21	11.80	Caryophyllene	2.33
22	11.87	β-Copaene	0.57
23	12.15	Aromandendrene	0.52
24	12.34	Cadinene	1.00
25	12.58	Eremophilene	0.36
26	12.83	α-Ionene	0.72
27	13.44	Caryophyllene oxide	0.51
28	13.48	trans-β-Ionone	0.44
29	13.51	1,2-Dilinolenoylglycerol	0.42
30	13.60	d-Viridiflorol	0.38
31	14.88	L-α-Terpineol	0.36
32	14.92	Isomethyl-α-ionol	0.43
33	15.00	Corymbolone	0.38
34	15.69	9-Octadecen-1-ol, (Z)-	0.42
35	17.71	5,8,11,14-Eicosatetraenoic acid	0.51
36	18.26	6-Hydroxy-2'-methoxyflavone	0.4

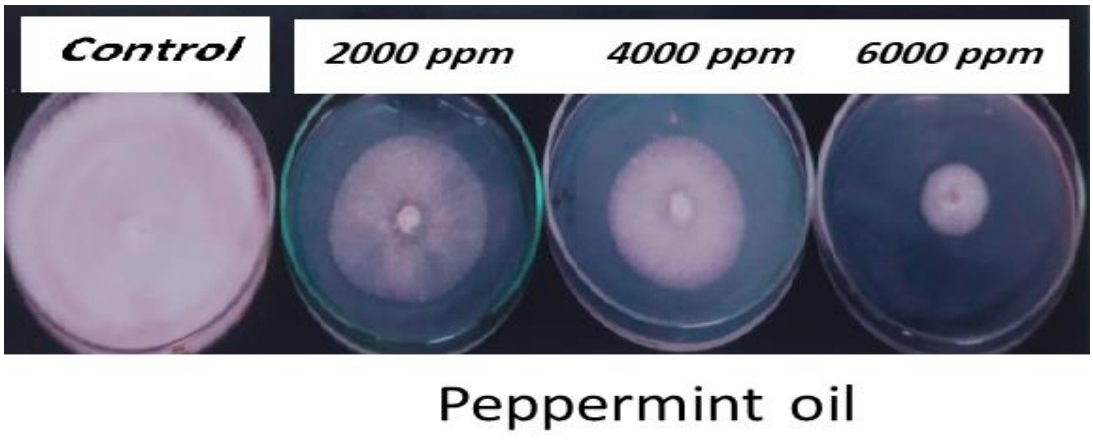

Figure 1: Effect of different concentrations from Pappermint essential oil emulsion on *F. oxysporum* growth *in vitro*.

Table 4: Effect of three essential oils emulsions at different concentrations on mycelial growth of three fungi isolated from cumin plants

Tested essential oils emultions	Fungi	Linear growth (cm) of mycelial growth at different concentrations (ppm)					Mean
		Control	2000	4000	6000	8000	
Marjoram	*F. oxysporum*	9.0	9.0	7.6	5.0	0.0	6.1
	M. phaseolina	9.0	8.1	7.3	4.6	0.0	5.8
	R. solani	9.0	5.8	3.2	1.7	0.0	3.9
Mean		**9.0**	**7.6**	**6.0**	**3.8**	**0.0**	**5.3**
Peppermint	*F. oxysporum*	9.0	7.0	4.6	3.0	0.0	4.7
	M. phaseolina	9.0	8.3	5.8	2.7	0.0	5.2
	R. solani	9.0	6.2	4.1	2.1	0.0	4.3
Mean		**9.0**	**7.2**	**4.8**	**2.6**	**0.0**	**4.7**
Thyme	*F. oxysporum*	9.0	2.8	0.0	0.0	0.0	2.4
	M. phaseolina	9.0	5.7	2.8	0.0	0.0	3.5
	R. solani	9.0	2.5	0.0	0.0	0.0	2.3
Mean		**9.0**	**3.7**	**0.9**	**0.0**	**0.0**	**2.7**
L.S.D. at 5% :		Essential oils (A) = 0.6, Fungi (B) = 0.2, Concentrations (C) = 0.1 , A × B = 0.05, A × C = 0.3, B × C = N.S. , A × B × C = 0.3					

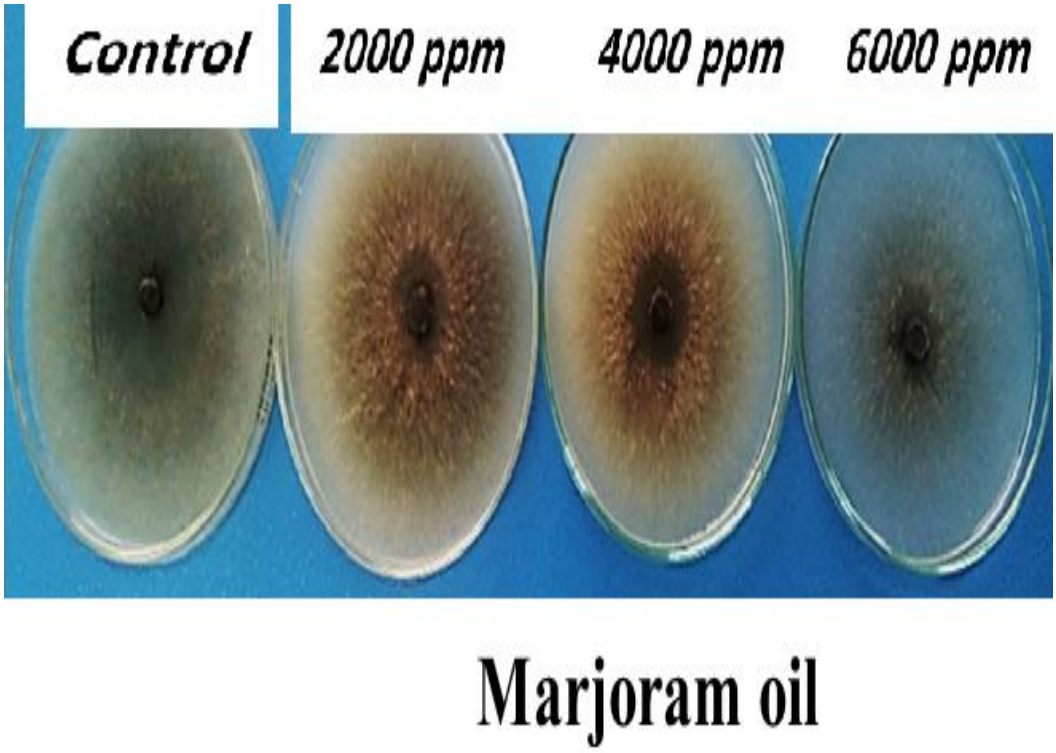

Figure 2: Effect of different concentrations from Marjoram essential oil emulsion on *M. phaseolina* growth *in vitro*.

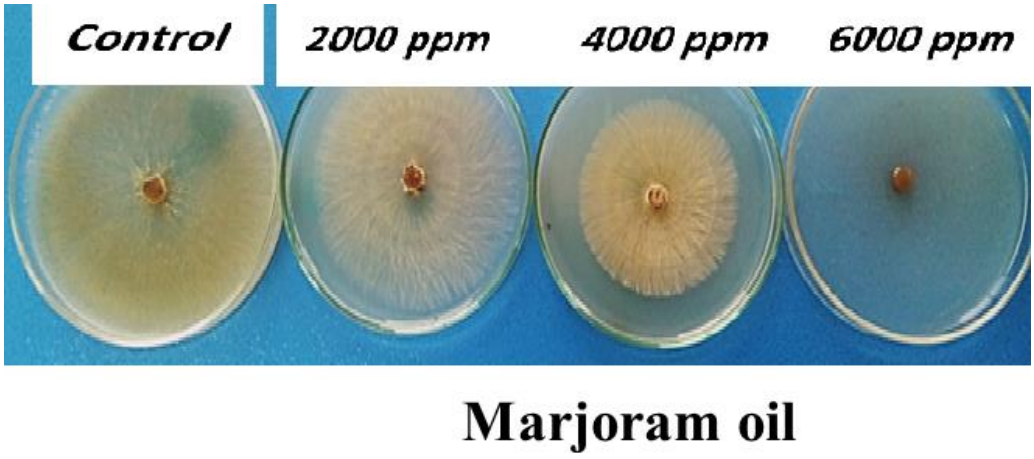

Fig. 3: Effect of different concentrations from Marjoram essential oil emulsion on *R. solani* growth *in vitro*.

Table 5: Effect of essential oils emulsions at different concentrations on cumin seed germination percentage

Tested essentials oils emulations	Concentrations (ppm)				
	Control	2000	4000	6000	8000
Marjoram	100	95	100	100	90
Peppermint	100	67	95	100	85
Thyme	100	66.7	100	100	92.3
L.S.D. at 5%: Tested essentials oils emultions (A) = 3.4, Concentrations (C) = 1.0 and (A) ×(C) = 3.6.					

Thyme essential oil emulsion at 6000 ppm completely inhibited the radial growth of isolates of F. oxysporum, M. phaseolina and R. solani. The presence of antifungal chemicals in essential oils may explain their inhibitory impact. The variances could be attributed to differences in the nature, quality, and quantity of inhibitory compounds found in essential oils. According to [12], these chemicals have antibacterial action against a variety of fungus. Greater antimicrobial potential could be ascribed to the oxygenated terpenes, especially phenolic compounds such as thymol [19]. However, thyme oil activity might be due to its chitin penetration of cell wall which damages the lipoprotein cytoplasmic membrane, leading to escape of cytoplasm [20]. The efficacy of marjoram, peppermint and thyme essential oil emulsions at the rates of 2000, 4000, 6000 and 8000 ppm on germination of cumin seeds were confirmed. Germination (%) was gradually increased by increasing concentration. Maximum germination percentage (100%) was recorded with all tested essential oils emulsions at 6000 ppm. The obtained results were in agreement with different results [11] who found treating cumin seeds with each of the concentrations of essential oil emulsions of sweet basil, marjoram, peppermint, spearmint and thyme did not affect germination, while seed germination percentage sharply decreased at high concentrations of nanoemulsions treatments (Table 5).

5. Conclusions

The current study looked at the chemical makeup of marjoram, peppermint, and thyme essential oils, as well as their efficacy against some soil fungi isolated from cumin plants and also, their impact on cumin seed germination in vitro. Thymol and (-) --Pinene were discovered to be the primary volatile compounds of thyme essential oil in the study. The primary volatile compounds of marjoram essential oil were identified as ()-p-Menth-1-en-4-ol, Sabinene hydrate, trans, 1,3,8-p-Menthatriene, and psi.-Limonene. The primary volatile compounds of peppermint essential oil were discovered to be p-Menthan-1-ol, Eucalyptol, and p-Menth-4(8)-en-3-one. Thyme essential oil emulsion was the most effective on mycelial development of all fungi, followed by peppermint essential oil emulsion at 6000 ppm concentration. On the other hand, the best emulsion that had an effect on cumin seed germination was the marjoram oil emulsion at 2000 ppm. From the results of the study, we recommend with the possibility of using essential oils as alternative fungicides agents against some soil fungi and that do not affect cumin seeds germination at the low concentrations.

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