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Chemical Composition, Antioxidant and Antifungal Activities of Three Essential Oils against Fungal Pathogens Causing Damping-off and Root-rot diseases in Pea

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ABSTRACT

Plant essential oils are being taken into greater consideration because their constituents have unique antioxidant and antimicrobial properties which makes it a good alternative to synthetic antioxidant and chemical pesticides. The present study was conducted to evaluate the antioxidant and antifungal activities of lemongrass (Cymbogon schoenanthus L.), sage (Salvia officinalis L.) and thyme (Thymus vulgaris L.) essential oils. The chemical compositions of three hydrodistilled essential oils were analyzed by Gas Chromatography-Mass Spectrometry (GC/MS) system. The major components in lemongrass, sage and thyme essential oils were D-limonene (52.34%), eucalyptol (43.17%) and thymol (27.94%), respectively. The antioxidant activities of three essential oils were evaluated by using reducing power assay and DPPH methods. The highest antioxidant activity was recorded for lemongrass, followed by sage and thyme essential oils, respectively. The effect of thyme, sage and lemongrass essential oils at two concentrations on the control of pea root-rot and damping-off diseases were determined in vitro and in vivo. The results showed that application of oils significantly reduced the mycelial growth, disease severity and increase plant growth parameters. Thyme essential oil (10% conc.) gave the best results followed by lemongrass oil (10% conc.) while sage oil (5% conc.) gave the least effect. These data denoted that the three essential oils possess antimicrobial and antioxidant properties, so these essential oils can be used as natural antioxidant in food and pharmaceuticals and also in biological control.

Key words: Essential oils, Antioxidant, Antifungal, Damping-off, Pea

INTRODUCTION

Pea (*Pisum sativum* L.) is one of the most important cool season crops in many parts of the world, including Egypt. Green peas are rich in protein, crude fiber and carbohydrates as well as, minerals and B complex vitamins [1] which used for fresh meals and food industry. Pea plants suffer from diseases during all stage of growth. Damping-off is one of the most important diseases which cause economic lost annually in different countries [2,3]. Damping-off is soil borne diseases causing considerable loss in pea yield [4].

Seed treatment with fungicide is a common method to control soil borne fungi [5]. However, fungicidal treatments are hazardous to human health, increase environmental pollution and increasing resistance to antifungal compounds. These reasons led us to search for the new alternatives among aromatic plants and their essential oils, used for their antifungal properties. Previous researches showed that essential oil were effective against pest and disease which could be developed as safety control method [6].

Nowadays, medicinal and aromatic plants showed high activity as antimicrobial and antioxidant agents in both *in vitro* and *in vivo* models [7-9]. Essential oils are the most effective components in these plants [10]. In the study made by Adam et al. [11] it was demonstrated that the essential oils could be used as effective antifungal agents. Found that, in greenhouse and field experiment, 7 essential oil of camphor, thyme, anise, lettuce, ground-net, rocket and caraway were effective in controlling damping-off and root-rot diseases in sugar beet [12].

Chemically the essential oils consist of terpene compounds (mono-, sesqui- and diterpenes), alcohols, acids, esters, epoxides, aldehydes, ketones, amines and sulfides [13]. The components of essential oils can be divided into two groups: (i) Terpene compounds and (ii) Aroma compounds [13,14]. The antioxidant activities of essential oils have been investigated using various model systems and assays. Both *in vitro* and *in vivo* studies have demonstrated how essential oils act as antioxidant [15,16]. Essential oil is natural antimicrobial widespread in plant kingdom which makes it appropriate alternative to antibiotics [17,18]. The essential oils of sage species exert many various pharmacological activities; the main and significant pharmacological activities of sage essential oils, including antioxidant, antimicrobial, antimutagenic, anticancer and anti-inflammatory activities [19].

Many researchers studied chemical components, antimicrobial and antioxidant activities of different essential oils [20-23]. Therefore, the objective of this study were to identification essential oil components, *in vitro* estimation of antioxidant activity and examined the antifungal activity of sage (*Salvia officinalis* L.), thyme (*Thymus vulgaris* L.) and lemongrass (*Cymbopgon schoenanthus* L.) against *Fusarium solani* and *Rhizoctonia solani* which causing damping-off and root-rot disease and plant quality characters in pea.

MATERIALS AND METHODS

Preparation of essential oils

Essential oils of three plants (lemongrass, sage and thyme) were obtained by hydrodistillation method. The plant materials (about 100 g) were ground into small pieces and were placed in a flask (2 L) together with double distilled water (1 L). The mixture was boiled for 4 h. The extract was condensed in cooling vapour to collect the essential oil. The extracted oil dried over anhydrous sodium sulphate and filtered. All essential oils were kept at freezing temperature until used [20-24].

GC/Ms analysis of lemongrass, sage and thyme essential oils

The GC-MS analysis of the three essential oils samples (lemongrass, sage and thyme) were carried out using gas chromatography-mass spectrometry instrument with the following specifications, Instrument: a Trace GC ultra-gas chromatographs (THERMO Scientific Corp., USA), coupled with a thermo mass spectrometer detector (ISQ Single Quadruple Mass Spectrometer). The GC/MS system was equipped with a TG-5MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 ml/min and a split ratio of 1:10 using the following temperature program: 80°C for 2 min; rising at 5°C/min to 300°C and held for 5 min. The injector and detector were held at 280°C. Diluted samples (1:10 hexane, v/v) of 0.2 µl of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 35-500.

In vitro antioxidant activity

Protocol for reducing power

A spectrophotometric method [25] was used for the measurement of reducing power. For this determination 2.5 ml of each of the essential oils were mixed with 2.5 ml of 200 mmol/l sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. After adding 2.5 ml of 10% trichloroacetic acid (w/v), the mixture was centrifuged at 650 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml deionized water and 1 ml of 0.1% of ferric chloride, and the absorbance was then measured at 700 nm: higher absorbance indicates higher reducing power (vitamin C was used as standard).

Antioxidant activity 2,2-diphenyl-1-picrylhydrazyl (DPPH) protocol

This spectrophotometric assay uses the stable radical (DPPH) as a reagent [26]. 50 microliters of various concentrations of essential oils in methanol were added to 5 ml of a 0.004% (w/v) methanol solution of DPPH. After a 30 min incubation period at room temperature the absorbance was read against a blank at 517 nm. Inhibition of the free radical DPPH in percent (I %) was calculated in the following way:

$$\text{Inhibition of the free radical DPPH in percent } I\% = (A_{control} - A_{sample}/A_{control}) \times 100$$

Where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. IC_{50} values (concentration of sample required to scavenge 50% of free radicals) were calculated from the regression equation, prepared from the concentration of the essential oil and the percentage inhibition of free radical formation/percentage inhibition of the DPPH was assayed. L-ascorbic acid were used as positive controls.

Causal organisms

Samples of pea plants showing root-rot symptoms were collected from different pea fields at four locations in Menoufia province. All samples were subjected to isolation trials for the causal organisms. The purified isolated fungi were identified according to cultural and microscopically characters [27].

Laboratory experiment

The antifungal assay of three essential oils was carried out in petri dishes (9 cm in diameter) containing PDA according to Tripathi et al. [28]. Essential oils diluted in Dimethyl Sulphoxide (DMSO) to get concentrations 5 and 10% (v/v). After that oils were sterilized by 0.2 µm filters (Sartorius) oils were added to Potato Dextrose Agar (PDA) media and mixed thoroughly before solidification. The pathogenic fungi (*R. solani* and *F. solani*) were inoculated immediately by placing a disk of mycelial growth (0.5 cm diameter) in the center, taken from the rim of 7-day-old cultures on PDA plates. Then, the petri dishes were kept at a temperature of 24°C. The reduction of mycelial growth was measured when the complete growth of fungi in control plates (without oils) reaches to maximum growth (29 cm) by using the following formula:

$$\text{Reduction } \% = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

Greenhouse experiment

The experiment was carried out to evaluate the efficiency of seed coating with three essential oils and fungicide Rhizolex-T in controlling pea damping-off and root-rot disease under artificially infested soil. Master-B cultivar seeds were disinfected with 2% sodium hypochlorite solution for 2 min then dried and soaked overnight in oils [12], then seeds were air-dried plastic pots 25 cm in diameter filled with 4 kg of sterilized clay-sand mixed soil (1:1, v/v) were used. Pots were inoculated with *F. solani* and *R. solani* prepared on sand/wheat bran medium at rate 3% of soil weight, while control pots were inoculated with the same medium without fungus. After that, pots were irrigation and left for one week for the establishment of fungal inoculums. Later, four treated pea seeds were planted in each pot seed soaked in sterilized water were sown as a control. Treatments were arranged in a completely randomized design with four replicates the percentage of pre- and post-emergence damping-off was calculated aster 15 and 45 days as follows:

Pre emergence damping-off=Number of non-germinated seeds/Number of sown seeds × 100

Post emergence damping-off=Number of dead seedlings/Number of sown seeds × 100

Survival plants=Number of survived healthy plants/Number of sown seeds × 100

Field experiments

A field experiment was carried out during two successive seasons: 2014/2015 and 2015/2016 in a pea field with a history of damping-off and root-rot disease on an experimental Farm of Faculty of Agric. Menoufia University, Shibin El-Kom, Egypt. Master-B cultivar seeds were disinfected with 2% sodium hypochlorite solution for 2 min then dried and soaked overnight in oils, and then seeds were air-dried [12]. The experiment consisted of 24 plots (three plot for each replicate), each plot comprised five rows (row 5 × 75 cm) in randomly complete block design plots received the used agricultural practices, i.e., NPK fertilizer and irrigation etc. The percentages of pre- and post-emergence damping-off were recorded after 15 and 45 days respectively and the percentage of surviving plants in each treatment was calculated as follows:

Pre emergence damping-off=Number of non-germinated seeds/Number of sown seeds × 100

Post emergence damping-off=Number of dead seedlings/Number of sown seeds × 100

Moreover, some vegetative growth and yield of pea plants were investigated.

Statistical analysis

Average data were subjected to Analysis of Variance (ANOVA) using CoStat Software, Version 6.4 (2008). The mean differences were compared to Duncan's Multiple Range Test (DMRT).

RESULTS

GC-MS analysis of essential oils

A total of 28 compounds representing 97.54% of the lemongrass essential oil was identified (Table 1); D-limonene was the main constituent (54.34%), followed by β-citral (17.09%), α-citral (13%), α-pinene (3.3%) and eucalyptol (2.9%).

Table 1: The main constituents of the essentials oil of lemongrass grown in Egypt

S. No.	**Compound name**	**RT**	**Area %**
1	α-Thujene	4.58	0.09
2	α-Pinene	4.77	3.3
3	Camphene	5.22	0.67
4	Sabinene	5.8	2.69
5	β-Pinene	5.97	2.3
6	β-Myrcene	6.23	0.73
7	Limonene 1,2-epoxide	6.32	0.51
8	α-Phellandrene	6.83	0.07
9	β-Cymene	7.44	0.03
10	D-Limonene	7.55	52.34
11	Eucalyptol	7.67	2.9
12	Dihydrocarveol	8.21	0.03
13	γ-Terpinene	8.55	0.05
14	cis-β-Terpineol	9.09	0.03
15	p-Mentha-1,4(8)-diene	9.52	0.03
16	Linalool	10.18	0.39
17	(E)-p-Menth-2,8-dien-1-ol	11.12	0.08
18	cis-Limonene oxide	11.49	0.43
19	trans-Limonene oxide	11.67	0.22
20	Camphor	12.2	0.04
21	Isopulegol	12.38	0.04
22	Limonene oxide	13.58	0.09
23	α-Terpineol	14.13	0.08
24	Isopulegol	14.29	0.14
25	cis-Carveol	15.19	0.07
26	β-Citral	15.96	17.09
27	cis-Verbenol	16.33	0.1
28	α-Citral	17.27	13
Monoterpene hydrocarbons			62.3
Oxygen Monoterpenes			35.64
Sesquiterpene Hydroc			0
Oxygen Sesquiterpenes			0
Total			97.54

The major components in sage essential oil were: eucalyptol (43.17%), caryophyllene (12.53%), β-pinene (9.53%) and α-pinene (9.05%) (Table 2).

The GC/MS analysis of thyme essential oil led to the identification of 31 different components, representing 97.99% of the oil constituents (Table 3). A total of 31 constituents representing 97.99% of the oil were identified; thymol (27.94%), β-cymene (20.25%) and terpinen-4-ol (8.95%) were the main components comprising 57.14% of the oil.

Table 2: The main constituents of the essentials oil of sage grown in Egypt

S. No.	Compound name	RT	Area %
1	α-Thujene	4.58	0.4
2	α-Pinene	4.77	9.05
3	Camphene	5.22	2.31
4	β-Pinene	5.97	9.53
5	β-Myrcene	6.23	3.52
6	α-Phellandrene	6.83	0.14
7	p-Mentha-1,4(8)-diene	7.13	0.54
8	β-Cymene	7.44	1
9	D-Limonene	7.55	1.3
10	Eucalyptol	7.67	43.17
11	γ-Terpinene	8.55	0.7
12	β-Thujone	10.5	0.44
13	Thujone	10.95	0.39
14	Camphor	12.2	3.26
15	trans-3-Pinanone	12.7	0.69
16	Isoborneol	13.15	0.59
17	Isocamphopinone	13.38	0.15
18	Terpinen-4-ol	13.49	0.19
19	α-Terpineol	14.13	0.57
20	Isoledene	21.1	0.13
21	α-Gurjunene	22.62	0.15
22	Caryophyllene	23.19	12.53
23	Guaia-1(10),11-diene	23.52	0.36
24	α-Bergamotene	23.76	0.27
25	(-)-Alloaromadendrene	23.96	2.39
26	α-Guaiene	24.3	0.2
27	Humulene	24.7	1.26
28	(+)-Ledene	26.12	0.54
29	γ-Muurolene	30.35	0.12
Monoterpene hydrocarbons			28.49
Oxyg. Monoterpenes			49.45
Sesquiterpene Hydroc			17.95
Oxig. Sesquiterpenes			0
Total			95.89

***In vitro* antioxidant activity**

Reducing power activity for lemongrass, sage and thyme essential oils

Data in Figure 1 showed reducing power assay results for lemongrass, sage and thyme essential oils (5 and 10% concentration). For 5% concentration lemongrass essential oil appeared highest activity (32.28 mMol ascorbic Eq) followed by sage essential oil (23.14 mMol ascorbic Eq) and finally thyme essential oil (18.14 mMol ascorbic Eq) and also the same trend appeared in 10% concentration.

DPPH for lemongrass, sage and thyme essential oils

The inhibition of the free radical DPPH in percent (I%) of thyme, sage and lemongrass essential oils in three concentrations (250, 500 and 750 µg/ml) as shown in Figure 2, these data evident that lemongrass essential oil appeared the highest (I%) values (40.23, 58.45 and 67.22 respectively) followed by sage essential oil (39.57, 54.33 and 61.18 respectively) while thyme essential oil showed the lowest (I%) values (38, 49.12 and 58.77 respectively).

Data in Figure 3 showed the IC_{50} values of three essential oils compared with the IC_{50} values of ascorbic acid. A lower IC_{50} value indicates greater antioxidant activity. The IC_{50} values of lemongrass essential oil, sage essential oil and thyme essential oil were found to be 401.67, 463.26 and 527.37, respectively.

Identification of fungal disease agents from infected pea roots

Samples of infected pea roots showing root-rot symptoms were collected from 4 districts in Menoufia Governorate. Fifty one fungi isolates in pure cultures were isolated from samples. Data presented in Table 4 indicated that, *F. solani* and *R. solani* were the most frequent fungi that were isolated from roots of pea plants.

Antifungal effect of essential oils on fungal mycelial growth *in vitro* conditions

In Table 5 data shown that, all essential oils significantly reduced the linear growth of *F. solani* and *R. solani*. Thyme oil (10% conc.) was more effective (81.11 and 78.88 in *F. solani* and *R. solani*, respectively) followed by sage oil (10% conc.) (77.77 and 7 4.44 in *R. solani* and *F. solani*, respectively) while the least effective observed by sage (5% conc.) (68.88 and 66.66 in *R. solani* and *F. solani*, respectively).

Table 3: The main constituents of the essentials oil of thyme grown in Egypt

S. No.	Compound name	RT	Area %
1	α-Thujene	4.58	0.95
2	α-Pinene	4.77	1.16
3	Camphene	5.22	0.67
5	β-Pinene	5.97	0.35
6	1-Octen-3-ol	6.1	0.33
7	β-Myrcene	6.23	0.9
8	α-Phellandrene	6.83	0.4
9	p-Mentha-1,4(8)-diene	7.13	2.35
10	β-Cymene	7.44	20.25
11	Eucalyptol	7.67	2.75
12	γ-Terpinene	8.55	6.28
13	cis-β-Terpineol	9.09	0.36
14	p-Mentha-1,4(8)-diene	9.52	0.49
15	Linalool	10.18	6.74
16	trans-β-Terpineol	10.23	0.68
17	Camphor	12.2	1.17
18	l-Menthone	12.51	0.46
19	Isoborneol	13.15	2.87
20	Terpinen-4-ol	13.49	8.95
21	α-Terpineol	14.13	2.15
22	Thymol methyl ether	15.46	1.21
23	Isothymol methyl ether	15.84	0.88
24	Bornyl acetate	17.74	0.65
25	Thymol	18.26	27.94
26	Carvacrol	18.6	0.79
27	Caryophyllene	23.19	3.65
28	α-Bergamotene	23.76	0.93
29	γ-Elemene	26.34	0.35
30	γ-Cadinene	27.1	0.7
31	tau.-Cadinol	32.23	0.63
	Monoterpene hydrocarbons		33.8
	Oxyg. Monoterpenes		57.93
	Sesquiterpene Hydroc		5.63
	Oxig. Sesquiterpenes		0.63
	Total		97.99

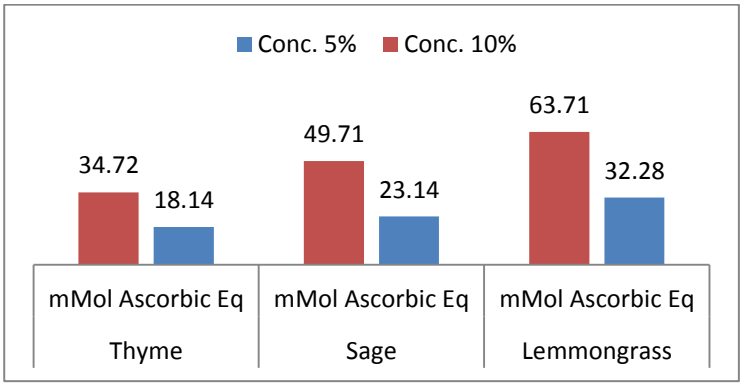

Figure 1: Reducing power activity for different essential oils (5 and 10% concentration)

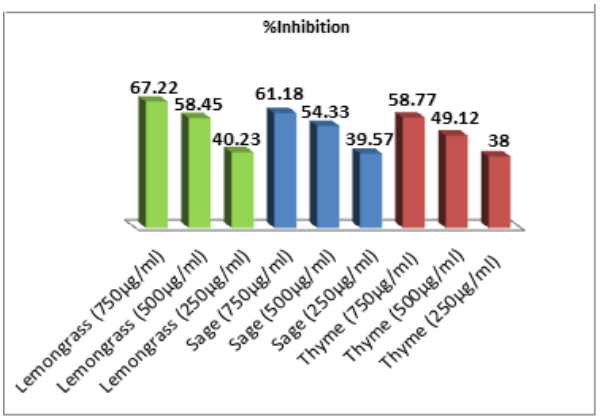

Figure 2: Scavenging activity of the DPPH radical of thyme, sage and lemongrass essential oils in different concentrations

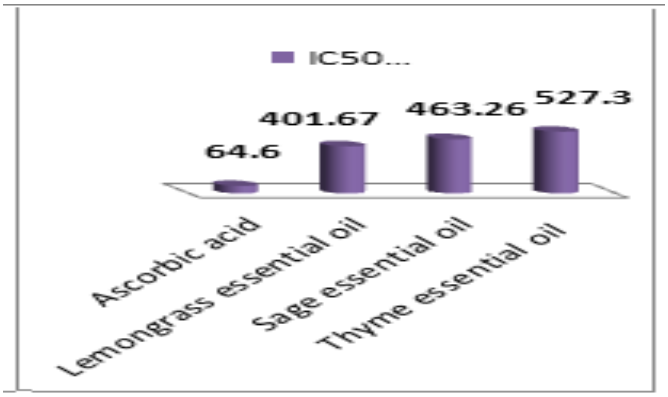

Figure 3: IC_{50} values of lemongrass, sage and thyme essential oils compared with ascorbic acid

Table 4: Frequency of fungi isolated from infected roots of pea plants collected from different districts of Menoufia Governorate

Districts	Total No. of isolate	*Fusarium oxysporum*		*Fusarium solani*		*Rhizoctonia solani*		*Pythium* spp.	
		No. of isolate	Freq.	No. of isolate	Freq.	No. of isolate	Freq.	No. of isolate	Freq.
Shibin El-Kom	14	3	21.42	5	35.71	4	28.57	2	14.28
Tala	12	2	14.28	4	33.33	6	50	0	0.0
Quesina	15	4	26.66	6	40	3	20	2	13.33
Berket El-Sabae	10	0	0	5	50	4	40	1	10

Freq.=Frequency

Table 5: Effect of some essential oils on liner growth of *Fusarium solani* and *Rhizoctonia solani* from infected pea rot under laboratory condition

Essential oil	Conc. (%)	*Rhizoctonia solani*		*Fusarium solani*	
		Mycelial growth (mm)	Growth reduction (%)	Mycelial growth (mm)	Growth reduction (%)
Thyme	5	27^{d}	70.00	24^{c}	73.33
	10	19^{a}	78.88	17^{a}	81.11
Lemongrass	5	25^{c}	72.22	21^{b}	76.66
	10	22^{b}	75.55	20^{b}	77.77
Sage	5	30^{e}	66.66	28^{d}	68.88
	10	23^{b}	74.44	20^{b}	77.77
Demso	-	90^{f}	0.0	88^{e}	1.11
Control	-	90^{f}	0.0^{f}	90^{e}	0.0

*Duncan's multiple range tests were used. Values followed by the same letters are not significantly differed ($P \leq 0.05$)

Antifungal effect of essential oils on disease development in greenhouse conditions

Seed treatment with essential oils suppressed the incidence of damping-off disease compared with control treatment under greenhouse and field experiment. As showed in Table 6, the highest reduction in pre- and post-emergence damping-off was attributed to thyme oil (10% conc.) followed by lemongrass oil (10% conc.). This was expressed in higher percentage of survival plants (80.00 and 72.00%, respectively). The lowest reduction was attributed to sage (5% conc.) which lead to (52% conc.) of survival plants.

Table 6: Effect of some essential oils on damping-off disease (%) and survival of pea plants under artificially infested soil in greenhouse

Essential oil	Conc. (%)	*Fusarium solani*			*Rhizoctonia solani*		
		Pre-emergence (%)	Post-emergence (%)	Plant survival (%)	Pre-emergence (%)	Post-emergence (%)	Plant survival (%)
Thyme	5	24^{c}	16^{c}	60^{g}	24^{b}	20^{b}	56^{g}
	10	12^{f}	8^{e}	80^{c}	12^{e}	12^{d}	76^{c}
Lemongrass	5	20^{d}	16^{c}	64^{f}	20^{c}	12^{d}	68^{e}
	10	16^{e}	12^{d}	72^{d}	16^{d}	12^{d}	72^{d}
Sage	5	28^{b}	20^{b}	52^{h}	24^{b}	20^{b}	56^{g}
	10	16^{e}	16^{c}	68^{e}	20^{c}	16^{c}	64^{f}
Demso	-	0^{h}	0^{f}	100^{a}	0^{f}	0^{f}	100^{a}
Rhizolex-T	-	8^{g}	8^{e}	84^{b}	12^{e}	8^{e}	80^{b}
Pathogenic fungi only	-	48^{a}	36^{a}	16^{i}	44^{a}	32^{a}	24^{h}
Control (non-treated)	-	0^{h}	0^{f}	100^{a}	0^{f}	0^{f}	100^{a}

*Duncan's multiple range test was used. Values followed by the same letters are not significantly differed ($P \leq 0.05$)

Antifungal effect of essential oils on disease development in field conditions

Data presented in Table 7 revealed that seed treatment with oils decreased the disease severity and increase the survival plants. Thyme oil (10% conc.) showed the superior inhibitory effect (75.3 and 73.7% reduction and 83.5 increases in plants survival), while the sage oil gave the least effect (50.0 and 67.0% reduction and 67.0% increase in plants survival).

Table 7: Effect of seed treatment with some essential oil on damping-off disease (%) and survival of pea plants under in infested soil under field condition

Essential oil	Conc. (%)	Pre-emergence (%)	Reduction (%)	Post-emergence (%)	Reduction (%)	Plant survival (%)
Thyme	5	18.8^{b}	53	12.9^{b}	45.1	68.2^{e}
	10	10.5^{f}	73.7	5.8^{d}	75.3	83.5^{a}
Lemongrass	5	16.4^{c}	59	11.7^{b}	50.2	71.7^{d}
	10	14.11^{d}	64.7	9.4^{c}	60	76.4^{c}
Sage	5	20^{b}	50.0	12.9^{b}	45.1	67^{e}
	10	12.9^{de}	67.7	7^{d}	70.2	80^{b}
Rhizolex-T	-	11.7^{ef}	70.7	7.0^{d}	70.2	81.1^{b}
Control (non-treated)	-	40^{a}	-	23.5a	-	36.4^{f}

*Duncan's multiple range test was used. Values followed by the same letters are not significantly differed ($P \leq 0.05$)

Moreover, all treatments increased the vegetative growth and yield quality of pea. Result in Table 8 showed that thyme oil (10% conc.) was the best treatment followed by lemongrass oil (10% conc.) whiles the sage (5% conc.) gave the lowest effect compared to the control.

Table 8: Effect of seed treatment with essential oil on some vegetative growth and yield parameters of pea plants under field condition

Essential oil	Conc. (%)	Plant height (cm)	Average No. of branches/plant	Average No. of pods/plant	Average pod weight (g)	Total yield of pods (kg)
Thyme	5	66.3^{de}	6.2^{a}	17.6^{b}	4.9^{a}	48^{cd}
	10	72.4^{a}	7.1^{a}	20.1^{a}	5.4^{a}	52.1^{a}
Lemongrass	5	68.0^{cd}	6.5^{a}	17.9^{b}	5^{a}	49.3^{bc}
	10	68.4^{c}	6.8^{a}	18.8^{ab}	5.3^{a}	51.4^{a}
Sage	5	65.5^{e}	6^{a}	17.0^{bc}	4.7^{a}	46.4^{d}
	10	67.5^{cd}	6.7^{a}	18.0^{b}	5.3^{a}	51^{ab}
Rhizolex-T	-	70.6^{b}	6.9^{a}	18.9^{ab}	5.2^{a}	52^{a}
Control (non-treated)	-	54.7^{f}	5.5^{a}	15.5^{c}	4^{a}	41.6^{e}

*Duncan's multiple range test was used. Values followed by the same letters are not significantly differed ($P \leq 0.05$)

DISCUSSION

Our data indicated that D-limonene (54.34%), eucalyptol (43.17%) and thymol (27.94%) were the main components in lemongrass, sage and thyme essential oils respectively; these results are in line with many authors [29-31] whose studied the same essential oils and analyzed these essential oils by GC/MS. The reduction of Fe^{+3} is often used as an indicator of electron donating activity, which is an important mechanism in phenolic compounds antioxidant action [32]. In reducing power assay, the reduction of Fe^{+3} to Fe^{+2} by donating an electron indicated the presence of antioxidants in tested essential oils. Data in Figure 1 showed reducing power assay results for lemongrass, sage and thyme essential oils at two different oils concentration (5 and 10%). We notice that lemongrass essential oil showed the highest activity followed by sage, while thyme essential oil appeared the lowest activity. In previous studies [33,34] suggested that phenolic compounds act as antioxidant and scavengers of free radicals.

It is well known that the antioxidant activity of essential oils is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals. DPPH analysis is one of the tests used to prove the ability of the components of the essential oils to act as donors of hydrogen atoms. The principal of DPPH method is based on the reduction of alcoholic DPPH solution by antioxidant agent due to the formation of the non-radical form DPPH–H [25]. DPPH method has been widely used to assess the free radical scavenging efficiency of different antioxidant substances [25,35].

The results illustrated in Tables 1-3 showed that the three essential oils (lemongrass, sage and thyme) contained more than 90 % monoterpene hydrocarbons, oxygenated monoterpenes and sesquiterpenes, these findings are in a close agreement with that presented by Tepe et al. [36] who suggested that the essential oils which contain high amount of monoterpene hydrocarbons, oxygenated monoterpenes and sesquiterpenes appeared greater antioxidant activities. Many authors [37,38] correlate antioxidant activity of essential oils and its terpene hydrocarbons content, and explain the mode of action by break free-radical chain reactions, which could be joining by the irreversible oxidation into inert compounds. It is worth mentioning that the differences in antioxidant activities appeared in three essential oils results (in both methods) can be referred to the different functional groups whish found in each essential oils.

Pea root-rot and damping-off disease are important disease which causing huge loss in yield [2,3]. The present study was started by collecting samples showing typical symptoms of root-rot and damping-off disease from different locations in Menoufia governorate. The results obtained revealed that, there is a variation in the occurrence of fungi according to growing area. The variation between climate, environment and soil conditions may explain this result. *F. solani* and *R. solani* are the most frequent fungi. The same results were clearly evident by Abd El-Kareem [39], who reported that *F. solani* and *R. solani* were the most severe pathogens of pea plants in Egypt.

According to the results obtained to anti-fungal effect of essential oils used, whether in the lab or in the greenhouse or field, leading to increased crop yield in seeds treated with essential oils, compared to the control group that did not treat with tested essential oils, this increase may be explained by the antifungal effect of these essential oils against fungi caused crop loses.

Essential oils of medicinal plants have antifungal and antibacterial effect. The antifungal activity can be attributed to the presence of some components such as: Carvacrol, α-terpinly acetate, cymene, thymol, pinene, linalool which are already known to exhibit antimicrobial activity [40]. Therefore, these oils of medicinal plants have antifungal and antibacterial effect; therefore, these oils should be used to control plant disease as a safe alternative method of fungicides. In the previous study, thyme oil has antifungal activity against *R. solani*, *P. ultimum* and *F. solani* [41,42] reported that, lemongrass and thyme oils significantly reduced the linear growth and spore germination of *B. cinerea*. Also, Arrebola et al. [43] recorded that thyme and lemongrass oils showed over 50 and 25% inhibition of radial growth, respectively.

Essential oils caused morphological changes in hyphae and plasma membrane [7]. Hypha appeared to collapse and become flexuous, cytoplasm was lacked, folding of the nuclear membrane and thickened cell wall [44]. Phenolic compounds of essential oils sensitize the phospholipid bilayer of the microbial cytoplasmic membrane which led to increase of permeability and inhibition of intercellular and extracellular enzymes [45-47]. According to Chami et al. [47] scanning electron microscopy analysis revealed that the surface of *Saccharomyces cerevisiae* cells that had been treated with oregano and clove oils was significantly damaged. On the same trend [48], suggest that components of the essential oils cross the cell membrane, interacting with the enzymes and proteins of the membrane, producing a flux of protons towards the cell exterior which induces changes in the cells and, ultimately, their death.

The present study demonstrated that some essential oils were found to have antifungal effect on some causal agent of pea root-rot and damping-off. So the application of medicinal plant oils is applicable, safe and cost effective method for controlling disease which leads to minimize the risk of fungicides.

CONCLUSION

The results presented in this study highlighted the importance of lemongrass, sage and thyme essential oils as antioxidant and antifungal compounds. Our findings pointed to important three essential oils which possess strong antioxidant and antifungal substances.

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