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Antifungal Activity of Spearmint and Peppermint Essential Oils against Macrophomina Root Rot of Cotton

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ABSTRACT

Essential oils as natural antifungal substances one of the alternative methods for plant disease control. The present study was conducted during 2015 and 2016 to investigate the antifungal activity and oil constituents of volatile oils from spearmint (*Mentha viridis* L.) and peppermint(*Mentha piperita* L.) against cotton root rot pathogen (*Macrophomina phaseolina*). Gas chromatographic analysis revealed that spearmint volatile oil was constituted by carvone (60.16%) as a major component followed by 1,8 cineole (8.67%), limonene (7.40 %), dihydro carvone (5.86 %), β- ocimene (4.29%) and pulegone (3.23%). While peppermint volatile oil was rich in menthone (46.52%), menthol (25.88%), limonene (7.72%) menthyl acetate (3.90%), iso menthol (2.10%) and sabinene (2.03%). Both essential oils with different concentrations were evaluated *in vitro* against three fungus isolates. The two tested oils exhibited 89.55 inhibition percent for the crude oils, against all the tested fungal isolates. Moreover, it was noticed that as oil concentrations decreased, the inhibitory effect also decreased. At the same time, a highly significant effect of oils at all concentrations was observed during sclerotial formation (number and size). The use of essential oils as seed treatment exhibited a highly significant reduction in disease incidence of cotton which has been artificially infested with root rot pathogen, compared to fungicide and untreated control treatments under the greenhouse conditions. This reduction was calculated to be between 4.56 and 100% compared with a 26.67% reduction with the utilization of Topsin M treatment at the pre-emergence stage. At the post-emergence stage, all applied treatments were able to decrease the percentage of root-rot incidence. Reduction ranged between 66.67 and 100% over the untreated control. Reduction in disease incidence was reflected in a survival plants increase of 34.62–96.17% and 73.09–126.9% for spearmint and peppermint volatile oils, receptively. Results in the current study demonstrated, that application of peppermint essential oils has an observer influence on the plant growth (plant height), which differ significantly from this of spearmint oil.

Keywords: spearmint; peppermint; essential oil; cotton; *Macrophomina phaseolina*

INTRODUCTION

Macrophomina phaseolina (Tassi) Goid is one of the most important soil borne pathogens, has a wide host range and infected over 500 plant species in more than 100 plant families around the world(Khan, 2007). The fungus responsible for causing a major disease of cotton (*Gossypium barbadense*) and has been cause severe losses from seedling to maturity of cotton. Despite its wide host range, the genus *Macrophomina* includes only one species, *M. phaseolina*. Variation in morphology and virulence between isolates of *M. phaseolina* was described as polyphagous and cosmopolitan fungus which attack several species of cultivated plants, comprising sorghum, soybean, cotton, and corn (Su *et al*., 2001).

Due to the development of resistance to the synthetic fungicide and accumulation of residues, the utilization of natural products is deemed one of the better substitutes for fungal disease management (Gujar and Talwankar, 2012). Many scholars are trying to find effective natural products for controlling plant diseases replacing synthetic pesticides (Kim *et al*., 2005). Various plant extracts have been reported as a source of bio-pesticide because it's inhibited the growth of plant pathogens and reduced the hazard impacts to human health and the environment. Various medicinal plants which have antifungal properties can also be employed as a source of plant bio pesticides (Aslam *et al*., 2010). Plant bio-pesticides are cheap, locally available, nontoxic, and easily degradable (Hadizadeh *et al*., 2009). Furthermore, its display structural diversity, complexity and rarely include halogenated atoms. These can act completely as pesticides (Neerman, 2003).

Lamiaceae family involves more than 4000 species in 200 genera. Numerous species in lamiaceae family are medicinal plants that apply in human disease remedy as well as food in a raw and cooked form (Dhifi *et al*., 2011). *Mentha piperita* is a perennial herbaceous plant belonging to the family Lamiaceae. It is indigenous of the Mediterranean region. Peppermint oil is the greatest popular and extensively utilized essential oil in food, pharmaceutical and cosmetic industries (Baser 1993). The biological activity of peppermint essential oil against fungi and bacteria have been formerly reported by Jirovetz *et al.,* (2007) and Moghaddam *et al*., (2013) Furthermore, the antifungal and antibacterial activities of the *Mentha viridis* volatile oil components have been defined in the previous literature (Singh, *et al*., 1994 and Mkaddem *et al*., 2009).

The hazardous influences of synthetic chemicals fungicides result in a growing attention in exploiting alternative and harmless treatments is a great challenge. Previous reports elucidated the antifungal activity of essential oils involving lemongrass, citronella, clove, peppermint, thyme and oregano oils against various fungal species (Viuda-Martos *et al*. 2007). Antifungal activities of specific essential oils were noticed effective against *Rhizoctonia solani, Fusarium moniliforme* and *Sclerotinia sclerotiorum* (Muller *et al.,* 1995), *F. oxysporum* (Bowers and Locke, 2000), and *F. solani, R. solani, Pythium ultimum* and *Colletotrichum lindemuthianum* (Zambonelli *et al.*, 1996). The mechanism of action of these compounds against fungi may be correlated to their capability to dissolve or disrupt the integrity of cell walls and membranes (Isman and Machial, 2006). Also, essential oils from eleven medicinal plants were effective in controlling several soilborne cotton pathogenic fungi (*Fusarium poae, F. oxysporum, F. moniliforme, F. solani, Rhizoctonia solani, Macrophomina phaseolina, and Sclerotium rolfsii*) under the greenhouse and field conditions (El-Shoraky, 2016). Moreover, essential oils of thyme and spearmint diminished *in vitro* growth of the pathogenic fungi, *R. Solani*, *Pythium ultimum* var. *ultimum*, *Fusarium solani* and *Colletotrichum lindemuthianum* (Zambonelli *et al.*, 1996). New biocides-based methods have advanced by using formulations of essential oils. These formulations, obtained from fennel, peppermint, caraway, oregano, rosemary, and ginger, are emulsified with diverse fixed oils (sesame, olive, cotton and soybean oils) to be operated as a carrier (Bowers & Locke, 2000 and Mario *et al.,* 2002). Formulations derived from ginger has been utilized in treating black rot caused by *Alternaria alternata* in tomato fruits (Helal & Abdeldaiem, 2009).

In relation to *M. phaseolina* chemical control, there are no fungicides registered for this pathogen. Thus, it is necessary to evaluate natural alternatives as a fungicide and their efficiency for controlling it. So, the present study aimed to evaluate the antifungal potential of *Mentha viridis* L. and *Mentha piperita* L. essential oils against three isolates of *M. phaseolina* and to determine both essential oil constituents. At the same time, to assess the essential oils effectiveness as cotton seed treatments in controlling Macrophomina root rot in cotton.

MATERIALS AND METHODS

Essential oils extraction and oil constituents

The tested plants were acquired from Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agriculture Research Center (ARC), Ministry of Agriculture, Egypt. Fresh herb of spearmint (*Mentha viridis*) and peppermint (*Mentha piperita* L.) Family Labiatae. These plants were chosen on the basis of earlier knowledge on their antifungal activities (Singh, *et al*., 1994 and Moghaddam *et al*., 2013). The essential oils were extracted by steam distillation using Clevenger type apparatus for 3 hours. The extracted oils were dried over anhydrous sodium sulfate. Gas Chromatographic analysis (GC) was determined for spearmint and peppermint essential oils at Medicinal and Aromatic Plants Research Department lab., Horticulture Research Institute, Agriculture Research Center, Giza, Egypt which were investigated by DsChrom 6200 Gas Chromatograph prepared with a flame ionization detector for separation of volatile oil constituents. The analysis circumstances were as follows: The chromatograph apparatus was installed with capillary column BPX-5, 5% phenyl (equiv.) polysillphenylene-siloxane 30 m x 0.25mmID x 0.25 µm film. Temperature program ramp increase with a rate of 10 Cº / min from 70 to 200 Cº. Flow rates of gases were nitrogen at 1 ml/min, hydrogen at 30 ml/min and 330 ml/min for air. Detector and injector temperatures were 300 Cº and 250 Cº, respectively. The obtained chromatogram and report of GC analysis were analyzed to calculate the percentage of essential oils main components. The essential oils were then evaluated for antifungal activity (El-Shoraky& Rashed, 2012).

In vitro* antagonists against *M. phaseolina:

Paper disc plate method (Loo *et al*., 1945) was used. Circular disc (5 mm dia.) of Whatman filter (No. 1) were cut and after dipping in different oils were located 1 cm inward from the periphery of Petri dishes at four equidistance places, having in the center the inoculum of the pathogen (*M. phaseolina*). Then, a 5 mm plug taken from 7 days old culture of the test fungus was placed on the center of PDA medium in a Petri dish and sealed with parafilm, plates were incubated at 25+3 Cº for 5 days. At the completion of the incubation period, the linear growth of the mycelium was measured. Radial growth of *M. phaseolina* was recorded and the inhibition percentage was calculated using the following formula:

$$\text{Percent growth inhibition} = \frac{C - T}{C} \times 100$$

C = Radial growth of *M. phaseolina* in control (mm)
T= Radial growth of *M. phaseolina* in presence of treatment (mm)

Based on the preliminary screening results the selected essential oils were prepared in paraffin oil and four doses viz., 1:1, 1:2, 1:3 and 1:4 (crude oil: paraffin oil) and applied on Whatman no.1 filter papers (5mm diameter), control papers were treated with paraffin oil only, another one was treated with Topsin M fungicide (Thiophenat methyl 70%). The antifungal activity of essential oil was evaluated on the mycelia growth of *M. phaseolina* according to Boyraz and Ozcan (2006). Five-millimeter mycelia discs taken from the borders of a 7 days old culture were located in the middle of a (PDA) plate. Circular disc (5 mm dia.) of Whatman filter (No. 1) were cut and after dipping in different oils were placed 1 cm inward from the periphery of Petri dishes at four equidistance places, having in the center the inoculum of the pathogen (*M. phaseolina*). The Petri plates were sealed with parafilm and incubated at 24 Cº for 5 days (Arya, *et al*., 2017).

Sclerotial formations:

The number and size of fungus sclerotia were counted in fungal culture suspensions under the microscope (at low power 10X). The fungal culture suspension was prepared by vigorously shaking the 5 mm mycelial disc of the fungus in 5 ml (FAA) (Parmar *et al*., 2017).

***In vivo* experiments (pot experiments):**

The present study was conducted at Cotton Pathology Department, Sakha Agric. Res. St. Seeds of a local cotton variety cv. Giza 96 were treated with different concentrations of the tested oils. From the preliminary trial, these concentrations hadn't harmful impacts on seeds germination. For comparison, some seeds were treated with Topsin M fungicide at the recommended dose (3g/kg seeds). Greenhouse experiments have been performed to specify the efficiency of plant essential oils as a seed treatment to manage cotton root rot (caused by *Macrophomina phaseolina*). The potting mixture (soil) infested with *M. phaseolina* was used. In this experiment three agent's viz., two essential oils (spearmint and peppermint) and one fungicide (Topspin M) were used individually for seed treatment. Essential oils were used at 1ml kg^{-1} seed while fungicide was used at 3g/kg seed. In case of control, seeds were sown in *Macrophomina* inoculated soil without any agents.

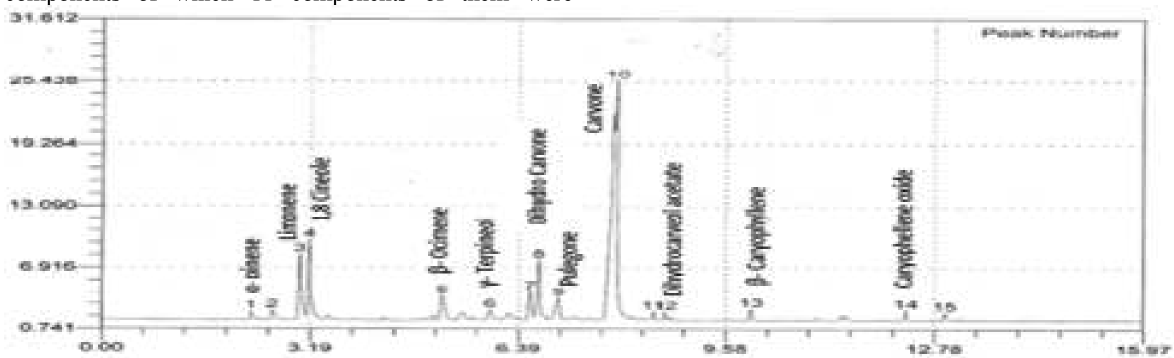
Preparation of fungal inoculum and soil infestation: Substrate for the growth of each isolate of *M. phaseolina*, was prepared by used autoclaved sorghum medium (50 g of sorghum grains and 40 ml of tap water) in 500ml glass bottles. Every bottle was inoculated with five discs (0.5 cm in diameter) of the 5-day-old culture of fungus. Bottles were incubated at 25±1 Cº for 15 days. The inoculums which used for soil infestation was a mixture of equal amounts (w/w) of 3 isolates. 5% formalin solution was used for soil and pots sterilizing for 15 min. Sterilized soil was covered with a polyethylene sheet for 7 days to maintain the gas then left to dry for 2 weeks till all formaldehyde traces were disappeared. Inoculum was added at a rate of 3% (w/w) and mixed carefully with the soil one week prior planting. The infested soil was dispensed in diameter 25 cm plastic pots and these were planted with treated seeds (10 seeds/pot) and kept in the greenhouse. Pre- and post-emergence damping- off were registered after 15 and 45 days from planting.

Statistical analyses:

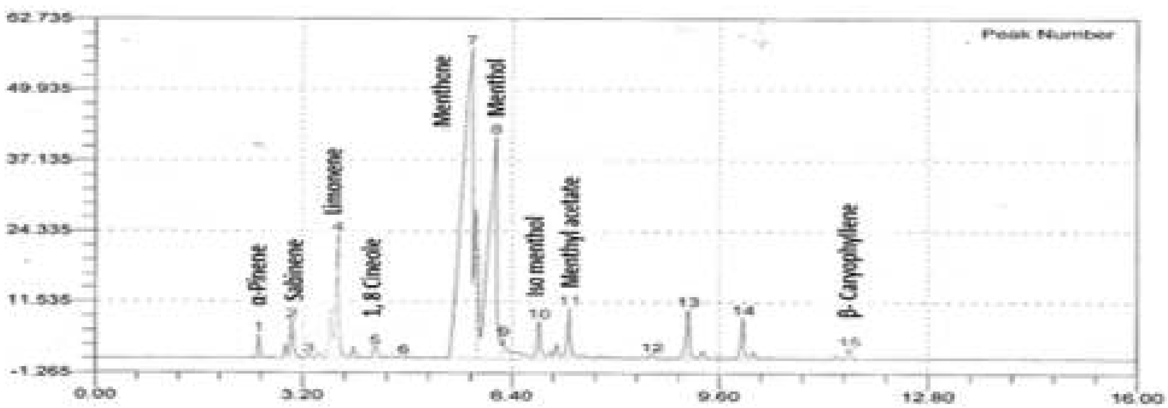
Statistical analyses were performed using Assistat-7.7 beta software for windows (Silva & Azevedo, 2009.). A complete randomized design was used in these experiments. The collected data was statistically analyzed by Duncan´s Multiple Range Test for comparing means.

RESULTS

Gas chromatographic analysis for spearmint essential oil (Chromatogram 1) revealed the presence of 15 components of which 11 components of them were identified by the retention times obtained from pure reference compounds. The identified components were α-pinene (0.55 %), limonene (7.40 %), 1,8 cineole (8.67 %), β- ocimene (4.29%) , γ- terpineol (1.23%), dihydro carvone (5.86 %), pulegone (3.23%), carvone (60.16 %), dihydrocarveol acetate (0.93 %), β- caryophyllene (0.95 %) , caryophyllene oxide (0.88 %) and the rest of numbers are unknown these results were in accordance with Baser , (1993) and Mkaddem *et al.*, (2009). While chromatographic analysis for peppermint essential oil revealed the presence of 15 components of which 9 components of them were identified (Chromatogram 2). The identified components were α-pinene (0.80%), sabinene (2.03%), limonene (7.72%), 1,8 cineole (0.83%), menthone(46.52 %), menthol (25.88 %), iso menthol (2.10%), menthyl acetate (3.90%) and β-caryophyllene (0.71 %), whereas the rest of numbers are unknown these results were in harmony with the previous studiesJirovetz *et al.,* (2007) and Moghaddam *et al.*, (2013) .



Chromatogram 1. Spearmint essential oil components



Chromatogram 2. Peppermint essential oil components

Antifungal activity

The antifungal activity of two essential oils (spearmint and peppermint) against mycelial growth of three isolates of *Macrophomina phaseolina* is displayed in (Table1). Statistical analysis (p = 0.05) showed that both essential tested oils had antifungal activity against *M. phaseolina*. The obtained results showed that all concentrations of tested oils gave a reduction of fungus growth which was found significantly superior over or equal of the check fungicide. However, the two tested oils showed more than 88 percent inhibition in crude oils against all the tested fungal isolates. It was additionally detected that when concentrations of oil reduced, the inhibitory effect also reduced.

Sclerotial formation

ANOVA of the influence of two essential oils at different concentrations and their interactions on the sclerotial formation of three isolates of *M. phaseolina* is shown in Table 2. Analysis of variance showed significant influences of isolates and oils in addition to its concentrations on sclerotial numbers. At the same time, ANOVA revealed a non- significant effect of isolates and oils on sclerotial size. A highly significant effect of oils concentrations was observed on sclerotial formation (number and size). Based on the significant effect of interaction between oil concentrations and the other factors (isolates and oils), (Fig. 1). Similarly; the tested two oils at all concentrations decreased sclerotial formation. It was also noticed that when concentrations of oil decreased, the inhibitory effect also decreased.

Table 1. The antifungal activity of two essential oils (spearmint and peppermint) against three *Macrophomina phaseolina* isolates (cotton root rot pathogen)

Oils	Isolates		Oil concentrations (v:v) Crude	1:1	1:2	1:3	1:4	0	Topsin M	Control
Spearmint	M1	Linear growth	0.47 C	0.70 BC	0.70 BC	0.83 BC	1.00 C	4.50 A	1.10 B	4.50 A
		In% [A]	89.56	84.44	84.44	81.56	77.78	0.0	75.56	--
	M2	Linear growth	0.53 E	0.97 DE	1.40 CD	1.80 C	1.97 C	3.63 B	0.60 E	4.50 A
		In %	88.22	78.44	68.89	60.00	56.22	19.33	86.67	--
	M3	Linear growth	0.47 F	1.20 DE	1.50 CDE	1.70 CD	1.93 C	3.73 B	0.93 EF	4.50 A
		In %	89.56	73.33	66.67	62.22	57.11	17.11	79.33	--
Peppermint	M1	Linear growth	0.47 C	0.53 BC	0.53 BC	0.60 BC	0.63 BC	4.50 A	1.10 B	4.50 A
		In%	89.56	88.22	88.22	86.67	86.00	0.00	75.55	--
	M2	Linear growth	0.47 C	0.57 C	0.73 C	0.83 C	0.87 C	3.57 B	0.60 C	4.50 A
		In%	89.56	87.33	83.78	81.56	80.67	20.67	86.67	--
	M3	Linear growth.	0.47 D	1.50 BC	1.63 B	1.83 B	1.97 B	4.50 A	0.93 CD	4.50 A
		In %	89.56	66.67	63.78	59.33	56.22	0.00	79.33	--
Mean of Linear growth			0.48 f	0.91 e	1.08 de	1.27 cd	1.39 c	4.07 b	0.88 e	4.50 a

***mean values within rows followed by the same letter are not significantly different at $p < 0.05$**
[A] inhibition percent in fungal growth under different treatments used, calculated relative to fungal growth in untreated control
[B] Concentrations of essential oils were calculated as (v: v) to the carrier oil

Table 2. Analysis of variance of interactions between isolates of *Macrophomina phaseolina* and essential oils at different concentrations *in vitro*

Parameters and Source of variation	DF	SS	MS	F
Sclerotial number				
Isolates (a)	2	59.89575	29.94788	1175.0483 **
Error-a	6	0.15292	0.02549	
Oils (b)	1	1.11446	1.11446	397.4597 **
Int. a x b	2	1.21798	0.60899	217.1879 **
Error-b	6	0.01682	0.00280	
Concentrations (c)	6	313.1977	52.19962	22048.7848 **
Int. a x c	12	15.81619	1.31802	556.7214 **
Int. b x c	6	5.31679	0.88613	374.2960 **
Int. a x b x c	12	5.30286	0.44190	186.6577 **
Error-c	72	0.17046	0.00237	
Sclrtotial size				
Isolates (a)	2	0.12968	0.06484	3.8000 ns
Error-a	6	0.10238	0.01706	
oils (b)	1	0.02032	0.02032	5.2245 ns
Int. a x b	2	0.69063	0.34532	88.7959 **
Error-b	6	0.02333	0.00389	
Concentrations (c)	6	4.20762	0.70127	259.8824 **
Int. a x c	12	0.09810	0.00817	3.0294 **
Int. b x c	6	0.10190	0.01698	6.2941 **
Int. a x Tb x c	12	0.34381	0.02865	10.6176 **
Error-c	72	0.19429	0.00270	

**** Significant at a level of 1% of probability ($p < .01$)**
*** Significant at a level of 5% of probability ($.01 =< p < .05$)**
ns Non-significant ($p >= .05$)

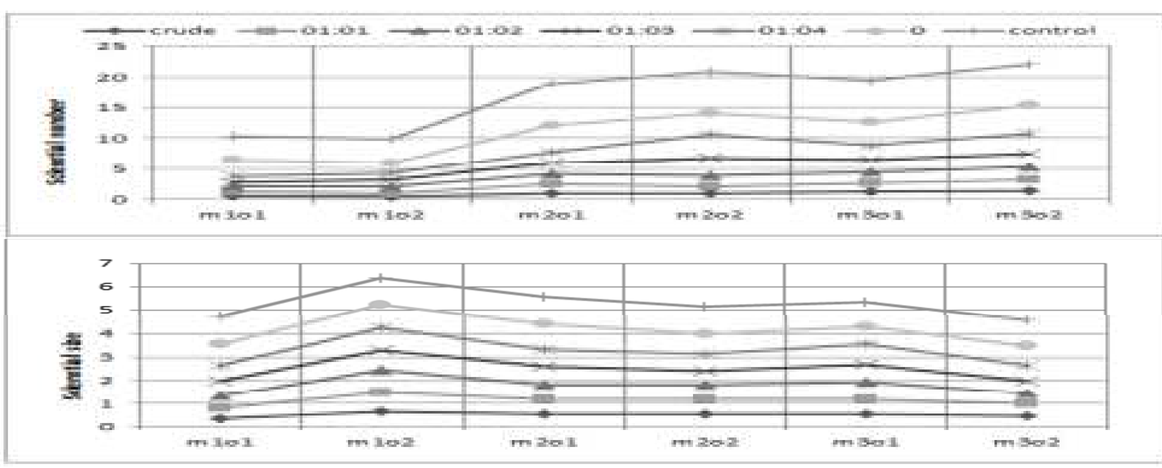


Fig .1. Effect of two essential oils {spearmint (o1) and peppermint (o2)} at different concentrations on sclerotial formation (number and size µm) of three *Macrophomina phaseolina* isolates (m1-m3), cotton root rot pathogen

Greenhouse experiments

The tested essential oils as seed treatment exhibited a highly significant reduction in disease incidence of cotton which has been artificially infested with root rot pathogens, compared to fungicide and untreated control treatments (Table 3). Seed treatment with spearmint essential oil gave a significant reduction to emerged cotton seeds against the invasion of pathogenic fungi at the pure oil and the first two concentrations, at the pre-emergence stage. Peppermint oil at all concentrations recorded from 100% to 50% protection compared with 27.27% protection when Topsin M treatment was used, over the untreated control.

At the post-emergence stage, data also displayed that all used treatments reduced the percentage of root-rot incidence from 100% to 66.67% compared with the untreated control. The oil treated seeds had a superior impact on disease incidence by recorded plant survival percentage over cent percent (126.9% for pure peppermint oil). Results in the existing table demonstrated that all concentrations of the tested oils caused an increase in the survival plant percentage. The spearmint oil recorded survival plant percentage ranged from 96.66 % to 34.62% at the low concentration, while the peppermint oil recorded survival percentage ranged from 126.9 to 73.09%. In this regard, *M. phaseolina* appeared further sensitivity against seed treatments through using both spearmint and peppermint oil than fungicide.

Results in the current study revealed that applying of peppermint essential oil have an observer effect on the plant growth (plant height), which differ significantly from spearmint oil.

Table 3. Incidence of cotton root rot caused by *M. phaseolina* and plant height in response to seed dressing with two essential oils (spearmint and peppermint) under greenhouse conditions.

Oils & parameters			Oil concentrations						Topsin M	control	average
			Pure	1:1	1:2	1:3	1:4	0:1			
Pre emergence %	Spearmint	D I	15.00 aB	18.33 aB	26.67 aAB	35.00 aA	38.33 aA	36.67 aA	26.67 aAB	36.67 aA	29.17 a
		R%	59.1	50.01	27.27	4.55	-4.53	0.0	27.27		
	Peppermint	D I	0.00 bD	6.67 bCD	13.33 bC	18.33 bBC	18.33 bBC	36.67 aA	26.67 aAB	36.67 aA	19.58 b
		R%	100	81.81	63.65	50.01	50.01	0.0	27.27		
	average		7.50 d	12.50 cd	20.00 bc	26.67 b	28.33 ab	36.67 a	26.67 b	36.67 a	
Post emergence %	Spearmint	D I	0.00	3.33	0.00	3.33	3.33	16.67	6.67	20.00	6.67 a
		R%	100	83.35	100	83.35	83.35	16.65	66.67		
	Peppermint	D I	1.67	0.00	3.33	3.33	6.67	16.67	6.67	20.00	7.29 a
		R%	91.65	100	83.35	83.35	66.67	16.65	66.67		
	average		0.84 b	1.67 b	1.67 b	3.33 b	5.00 b	16.67 a	6.67 b	20.00 a	
Stand %	Spearmint	D I	85.00 bA	78.34 bAB	73.33 bABC	61.67 bCD	58.34 bDE	46.66 aEF	66.66 aBCD	43.33 aF	64.16 b
		E%	96.17	80.78	69.24	42.33	34.62	7.69	53.87		
	Peppermint	D I	98.33 aA	93.33 aAB	83.33 aBC	78.33 aCD	75.00 aCD	46.67 aE	66.67 aD	43.33 aE	73.13 a
		E%	126.93	115.39	92.31	80.78	73.09	7.69	53.87		
	average		91.66 a	85.83 ab	78.33 bc	70.00 cd	66.66 d	46.67 e	66.67 d	43.33 e	
Plant height	Spearmint	D I	44.50 bC	56.77 bAB	59.00 bAB	61.67 bA	57.00 bAB	49.33 aBC	50.10 aABC	50.67 aABC	53.63 b
		R%	-12.18	12.04	16.44	21.71	12.49	-2.65	-1.13		
	Peppermint	D I	84.67 aA	78.33 aA	80.60 aA	89.00 aA	81.67 aA	49.33 aB	50.10 aB	50.67 aB	70.55 a
		R%	67.10	54.59	59.07	75.65	61.18	-2.65	-1.13		
	average		64.58 b	67.55 ab	69.80 ab	75.334 a	69.34 ab	49.33 c	50.10 c	50.67 c	

The Tukey Test at a level of 5% of probability was applied
Lower case letters for columns   upper case letters for rows
The averages followed by the same letter do not differ statistically between themselves

DISCUSSION

Success in controlling plant diseases has been documented from the use of artificial fungicides. It has side effects, such as possible toxicity to plants, animals, and man as well as adverse environmental impact. Fungicides can also be costly, and indiscriminate application could result in resistance, which can counteract their effectiveness. Depending on the previously mentioned constraints, alternative approaches such as using natural products applications (plant extracts and essential oils) may have a potential role in crop protection. Plant essential oils are commercially produced from numerous botanical sources. In our study, the growth inhibition and sclerotial formation of *M. phaseolina* causing root rot in cotton have been tested at various concentration of two essential oils *in vitro*. The obtained results showed that the mycelial growth of *M. phaseolina* isolates has been influenced differently by the two tested essential oils. The highest efficiency against *M. phaseolina* was registered for peppermint oil at all tested concentrations, followed by spearmint oil, with the same efficiency.

Effect of tested oils at various concentrations on sclerotial formation was noticed negatively associated with the inhibition of growth. The essential oils have the capability to penetrate and disrupt the fungal cell wall and cytoplasmic membranes, permeablise them and finally damage mitochondrial membranes. Then changes in electron flow through the electron transport system in the mitochondria damage the lipids, proteins and nucleic acid contents of the fungal cells (Arnal-Schnebelen *et al*., 2004). Essential oils could also reduce the membrane potential resulted into the leakage of radicals, cytochrome C, calcium ions and proteins. Thus, permeabilization of outer and inner mitochondrial membranes leads to cell death by apoptosis and necrosis (Yoon *et al*., 2000). Sharma and Tripathi (2008) stated that essential oils perform on the hyphae of the mycelium, provoking the exit of constituents from the cytoplasm, the loss of rigidity of the hyphae cell wall and resulting in its collapse. The activity of essential oils not only in their capability to cross the cell wall, but also their capacity to damage cellular enzyme system, including that relating to energy production (Conner and Beuchat 1984). Thus, there has been grown interest in research on, natural antifungal substances utilization, which might substitute synthetic fungicides or participate to the development of new disease control agents.

In the current study, it is well confirmed the control impact of essential oils from peppermint and spearmint at different concentrations against important root rot pathogen of cotton (*M. phaseolina*). Inhibition of the fungal growth might be due to the main components such as menthone, menthol, and limonene in peppermint essential oil in addition to carvone, 1,8 cineole, limonene, dihydro carvone, and β- ocimene in spearmint essential oil. Furthermore, it is possible that the other minor components may act together synergistically in each oil as has previously been recommended (Moghaddam *et al*., 2013).

The obtained data demonstrated that use of both essential oils as seed treatment for cotton with the same or supreme anticipated output which obtained with Topsin M. Therefore, it is concluded that the tested –essential oils have potential bio-fungicides against the major root rot pathogen of cotton and as such contributed to characterize these essential oils as "broad spectrum" bio-fungicidal chemicals. Nguefack *et al*., (2008), concluded that essential oils from *Cymbopogon citratus*, *Ocimum gratissimum* and *Thymus vulgaris*, has potential bio-fungicides against the major seed-borne pathogens of rice. Helal, (2017) revealed that soaking squash seeds in biocides formulating peppermint oil leads to a significant decline of damping off and wilt diseases and caused a 10-fold increase in the plants survival which grown in non-infested soil, compared with the non-treated plants. In our study, the obtained results showed that peppermint and spearmint essential oils have an inhibiting impact on the pathogens growth of cotton root rot *in vitro*, and their efficacy is prolonged to protect seed germination or plant invasion *in vivo* test.

In the present study, *M. phaseolina* revealed more sensitivity against seed treatments by applying peppermint oil than treatments by spearmint oil and Topsin M. The application of peppermint oil leads to a significant reduction of damping- off diseases and induced an increase in the survival of the plants compared with the non-treated plants. The present observation displayed that increasing essential oils concentration led to a significant decline in disease incidence.

CONCLUSION

The results of this study have shown that volatile oil from *M. piperita* has strong remarkable antifungal activity against *M. phaseolina* than *M. viridis*. Gas Chromatographic analysis of volatile oil from *M. viridis* was determined, and its main component was carvone. While the main component of *mentha piperita* was menthone. The study results can be used in the management of plant pathogenic fungi.

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فاعلية الزيت الطيار للنعناع البلدى والفلفلى ضد فطر الماكروفومينا المسبب لعفن الجذور في نبات القطن

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[١]قسم بحوث امراض القطن ومحاصيل الالياف ـ معهد بحوث امراض النباتات ـــ مركز البحوث الزراعية

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تعتبرالزيوت الطياره كمواد طبيعية مضادة للفطريات أحد الطرق البديلة لمقاومة الأمراض النباتية. وقد أجريت الدراسة في ٢٠١٥ , ٢٠١٦ بهدف تقدير المكونات الكيميائية للزيوت الطيارة لنباتى (النعناع البلدي – والنعناع الفلفلى) والقدرة التضادية ضد فطرماكروفومينا فاصيولينا المسبب لمرض عفن جذور نبات القطن. وقد أثبت التحليل الكروماتوجرافى لزيت النعناع البلدى أنه يتكون من الكارفون (٦٠,١٦%) كمكون رئيسى يليه ١,٨ سنيول (٨,٦٧%) والليمونين (٧,٤٠%) والداى هيدروكرفون (٥,٨٦%) والبيتا أوسيمين (٤,٢٩ %) والبوليجون (٣.٢٣%) بينما كان زيت النعناع الفلفلى غنيا بالمنتون (٤٦,٥٢%) والمنتول (٢٥,٨٨%) والليمونين (٧,٧٢%) والمنتول أسيتات (٣,٩٠%) والأيزومنتول (٢,١٠%) والصابنين (٢,٠٣%). تم تقييم الزيوت النباتية الطيارة بتركيزات مختلفة ضد ثلاثة عزلات للفطر. وقد ابدت الزيوت نسبة تثبيط تصل إلى ٨٨,٥٥ % ضد جميع العزلات المختبرة وذلك في حالة الزيوت الخام. وقد كان من الملاحظ انه بانخفاض تركيز الزيت يقل التأثير التثبيطى أيضا. وفى نفس الوقت لوحظ أن للزيوت بكل التركيزات تأثير عالى المعنوية على تكوين الاجسام الحجرية للفطر (العدد والحجم). إستخدام الزيوت الطيارة في معاملة البذور أبدى نقصا عالي المعنوية في نسبة الاصابة في نبات القطن المعدى صناعياً باستخدام المسبب المرضى وذلك بالمقارنة بالنباتات المعاملة بالمبيد أو غير المعاملة تحت ظروف الصوبة. وقد تراوحت نسب النقص لسقوط ما قبل الانبات ما بين ٤,٥٦ الى ١٠٠ % في مقابل ٢٦,٦٧ % للمعاملة بالمبيد توبسين ام. أما سقوط ما بعد الانبات فان كل المعاملات كانت لها القدرة على تقليل نسبة الاصابة. وقد تراوحت نسب النقص ما بين ٦٦,٦٧ الى ١٠٠ % بالمقارنة بالنباتات غير المعاملة. أما تقليل نسبة الاصابة بالمرض فقد إنعكس في زيادة نسبة النباتات الباقية بنسب تراوحت بين ٣٤,٦٢ إلى ٩٦,١٧ % لزيت النعناع البلدى و٧٣,٠٩ إلى ١٢٦,٩ % لزيت النعناع الفلفلى. وقد أوضحت نتائج هذه الدراسة أن لزيت النعناع الفلفلى تأثير ملحوظ على نمو النباتات (ارتفاع النبات) والذي يختلف معنويا مع ذلك الناتج من زيت النعناع البلدي.