

Studies on Seedlings Damping-off and Root Rot Diseases of Pea

III. Control by Essential Plant Oils

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ABSTRACT

Spearmint, cinnamon, clove and lemon oils significantly reduced the radial growth of *Pythium debaryanum* (Pd), *Rhizoctonia solani* (Rs) and *Fusarium solani* (Fs), the tested damping-off and root rot agents of pea. Garlic, onion and thyme oils were relatively ineffective. Spearmint oil exhibited the highest antagonistic effect, while *Pythium debaryanum* was the most sensitive to treatments among the other tested disease pathogens. In greenhouse experiments, treatment of pea seeds with spearmint oil gave the highest reduction in pre-, and post-emergence damping-off and root rot incidence and increased survived seedlings values compared to control. Cinnamon, clove and lemon oils gave significant reduction in disease incidence only at high concentrations. The effect was more pronounced in Pd and Rs experiments. The relatively resistant Sinnary pea cultivar exhibited more response to oil seed treatments than the more susceptible Lincoln cv.

INTRODUCTION

Peas (*Pisum sativum* L.) is considered one of the most important leguminous cultivated in Egypt. Pea plants are commonly exposed to attack by many serious soilborne fungi, especially those related to *Pythium*, *Rhizoctonia* and *Fusarium* (King and Parke, 1993 and Chen and McBeath, 1993), causing in most cases damping-off and root rot diseases (Abada *et al.*, 1992); leading to great economic losses in crop yield and quality. Great success in controlling damping-off diseases in pea and other crops realized by seed or soil treatment with different fungicides (Kraft and Papavizas, 1983; Bowers and Parke, 1993; Ibrahim *et al.*, 1997 a, b; El-Awadi *et al.*, 1997). However, the modern approach in disease control was directed toward minimizing the fungicidal use to avoid environmental pollution (Barons and Tansey, 1977; Omar and Abdel-Halim, 1992; Oliveira *et al.*, 1999). Thus, integrated pest management (IPM) was applied, employing physical, mechanical, cultural and biological control, as a supplementary programs with chemical control.

One important compound of these strategies includes seed or soil treatment with extracts of some aromatic and medicinal plants which proved to be highly efficient in suppressing mycelial growth and spore germination of many plant pathogens (Hassanein and El-Doksch, 1997 and Zedan *et al.*, 1994). Different organs of certain plants contain relatively high amounts of chemical compounds such as alkaloids, essential oils and phenolic substances, which showed inhibitory effects to various fungi (Saksena and Tripath, 1987; Youssef, 1991 and Agha, 1992). Many of the essential plant oils were proved to be efficient in suppressing different pathogens wether *in Vitro* or *in Vivo*. Among these oils were spearmint (Yagen *et al.*, 1992; Jaspal, *et al.*, 1994; Zedan *et al.*, 1994 and El-Korashy, 1997), clove oil (Chatterjee, 1990; Ismail, 1998; El-Safwani and Nadia, 2002), onion and garlic oils (Dubey and Dwivedi, 1991, Hammad and Youssef, 1994 and Heweidy *et al.*, 1997) and thyme oil (El-Naggar, 1997). However, little informations are available on controlling damping-off and root rot of pea by seed or soil treatment with plant essential oils and extracts.

Therefore, this investigation aimed to (1) study the antagonistic effect of some plant oils at different concentrations on the radial growth of the tested damping-off and root rot pathogens; (2) investigate the effect of these oils on damping-off and root rot disease incidence under greenhouse conditions.

MATERIALS AND METHODS

Source of disease agents

Among 6 fungal isolates isolated from damped-off and root rotted pea seedlings, purified, identified and tested for pathogenicity by the authors (under publishing), three fungal isolates were only used throughout this study; namely, *Pythium debaryanum* (Pd), *Rhizoctonia solani* (Rs) and *Fusarium solani*.

I. Antagonistic effect of some plant oils

(A) Sources of plant oils

Several essential oils were tested for their antifungal activity against damping-off and root-rot diseases in pea seedlings, i.e. cinnamon (*Cinnamomum zylanicum* L.), clove (*Syzygium aromaticum* L.), spearmint (*Mentha viridis* L.), lemon (*Citrus aurantifolia*), onion (*Allium cepa* L.), garlic (*Allium sativum* L.) and thyme (*Thymus vulgaris* L.). These plant oils were obtained from Horticulture Research Station, Aromatic and Medicinal Plants Department, Sabahia, Alex., Egypt.

(B) *In vitro* experiments

Different concentrations of the tested oils i.e., 0, 12.5, 25, 50, 75 and 100% were prepared for checking their antagonistic effect. Acetone and tween 40 were added to the crude oil before dilution with sterilized distilled water. Petri plates with PDA media were used. Five mm disc of 7 days old culture of the tested fungus was placed at the edge of the Petri plate. On the opposite side 5 mm sterilized filter paper discs (Whatman No. 1) were saturated with 50 µl of plant oil and placed. The tested oil and the fungal discs were placed in Petri dish in the same time. Control treatment was carried out using sterilized water instead of plant oils. Four replicates were used for each treatment. The plates were incubated at $25 \pm 2^\circ\text{C}$ for 7 days. Linear growth and the percentage of reduction in mycelial growth was calculated according to formula proposed by Ferreira *et al.* (1991) :

$$R = \frac{A - B}{A} \times 100$$

Where:

R = Percentage of growth reduction.

A = The distance of mycelial growth of the pathogenic fungus apart from the tested oil.

B = The distance of mycelial growth of the pathogenic fungus toward the tested oil.

(C) *In vivo* experiments

According to the results obtained *in Vitro*, two concentrations 50 and 100% from each of the four plant oils (cinnamon, clove, spearmint and lemon) were tested against *P. debaryanum*, *R. solani* and *F. solani*. Two pea cvs. Lincoln and Sinnary were checked in this experiment. The tested fungi were grown for 7 days at $25 \pm 2^\circ\text{C}$ in 500 ml bottles containing autoclaved barley grains-sand medium (30 gm barley grains + 10 gm sand + 30 ml water). For soil infestation contents of the bottles were thoroughly mixed with autoclaved sterilized soil at the rate of 40 gm/kg soil. Plastic pots (20 cm in diameter) were filled with inoculated soil with the quantity of one kg/pot. Four replicates were used for each treatment. The infested soil was left for 7 days to secure establishment of the inoculated fungi. Sterilized pea seeds were soaked in the tested oils (at different concentrations) for two hours (Zedan *et al.*, 1994). Ten treated seeds were sown in each pot. The plants were weekly inspected for 45 days. Pre-, and post-emergence damping-off were recorded 20 days after planting. Determination of percentage of survived seedlings and root rot percentage was carried out 30 and 45 days after planting, respectively. Determination of the root rot disease severity index (DSI) was carried out based on a

scale from 0 (non visible damage) to 5 (completely destroyed roots) according to Salt (1981). Percentage of root rot was recorded according to the formula :

$$\% \text{ Root rot} = \frac{\text{No of infected plants}}{\text{Total plant number}} \times 100$$

Statistical analysis

A completely randomized design with 4 replicates was used in the present study. Percentage data were transformed into arcsine angles (Snedecor and Cochran, 1981) before carrying out analysis of variance (ANOVA) to produce approximately constant variance. Least significant difference (LSD) at 5% level of probability was applied for comparing treatment means (Duncan, 1955).

RESULTS AND DISCUSSION

(1) Antagonistic effect of some plant oils *In vitro*

This investigation was carried out to study the antagonistic effect of seven plant oils, namely cinnamon, clove, spearmint, lemon, onion, garlic and thyme against, *Pythium debaryanum*, *Rhizoctonia solani* and *Fusarium solani*, pea damping-off and root rot pathogens. Preliminary test using 100% oil concentrations was applied. Linear growth of the tested fungi was determined in each treatment. Data were statistically analyzed and presented in Table (1) and illustrated in Fig. (1).

According to the obtained results in Table (1), crude spearmint oil proved to be the most effective against all the tested pathogens. In this respect, hyphal growth of both *P. debaryanum* and *Rhizoctonia solani* was completely inhibited. Moreover, it was efficient in reducing the hyphal growth of each of *Fusarium solani* (88.9%). Cinnamon oil completely inhibited the hyphal growth of *R. solani*. Moreover, it significantly reduced growth of *P. debaryanum* by 68.5%, while growth of *F. solani* was affected but in a relatively less rate (50%). Clove oil in its crude state was very effective in reducing hyphal growth of *P. debaryanum* (72.29%), followed by *R. solani* (66.7%). Less antagonistic effect was exhibited against *F. solani* (52.6%). Crude lemon oil exhibited a highly antagonistic effect against *P. debaryanum* (88.9%). Both *R. solani* and *F. solani* were less sensitive to lemon oil, where reduction rates did not exceed 33.3%, compared with the control (Table 1). Crude oils of thyme, onion and garlic plants were absolutely ineffective against most of the tested pathogens. Hence, these oils were completely neglected in successive experiments.

Generally, crude spearmint oil was the most effective in reducing hyphal growth of the tested fungi. Besides, both *P. debaryanum* and *R. solani* were more sensitive to the tested oils and their growth was greatly reduced compared with the other tested pathogens.

Antagonistic effect of different concentrations of the selected plant oils

This experiment was carried out *in vitro* to determine the effective antagonistic doses of cinnamon, clove, spearmint and lemon oils against the tested damping-off causal agents. Results were presented in Table (2). Thyme, onion and garlic oils were neglected according to their ineffective antagonistic effect obtained from the preliminary experiments (Table 1 & Fig. 1).

The obtained results (Table 2) showed highly significant antagonistic effect of spearmint at concentrations up to 50%. Both *P. debaryanum* and *R. solani* were more sensitive than *F. solani*. Lemon, cinnamon and clove oils had also a pronounced antagonistic effect but at relatively higher concentrations

These findings were in agreement with those of Pattnaik *et al.* (1996), who found that spearmint oil was among the most effective oils tested against *F. solani*, *F. oxysporum* and *Macrophomina phaseolina* *in vitro*. Onion, garlic and thyme oils were ineffective against the tested pathogens throughout our experiments *in vitro*. In contrast to that, Zambonelli *et al.* (1996) reported that the mycelial growth of *R. solani*, *Pythium ultimum*, *F. solani* and *Colletotrichum lindemuthianum* strongly inhibited by certain compounds in thymol oils. Similar results were found on peanut damping-off by El-Korashy (1997), who reported that extract of *Mentha spicata* at concentration of 50% and 100% inhibited the growth of *R. solani*, *F. solani* and *S. rolfsii*.

Efficacy of medicinal and aromatic plants against mycelial growth and spore germination of different pathogen was documented by Zedan *et al.* (1994) and Hassanein and El-Doksch (1997). The inhibitory effects of many plant extracts were attributed in most cases to alkaloids, essential oils and phenolic substances (Saksena and Tripath, 1987; Youssef, 1991; Agha, 1992 and Jaspal *et al.*, 1994). The present *in vitro* results on the antifungal activity of clove and cinnamon against some damping-off fungal pathogens on pea were in agreement with those obtained by El-Safwani and Nasif (2002) on the effect of these oils on the growth of damping-off fungal pathogens.

Table (1) : Antagonistic activity of the tested crude oils on mycelial growth of the tested damping-off agents

Treatment	<i>P. debaryanum</i>		<i>R. solani</i>		<i>F. solani</i>	
	Linear growth (mm)	% Reduction	Linear growth (mm)	% Reduction	Linear growth (mm)	% Reduction
Cinnamon (<i>Cinnamomum zylanicum</i> L.)	28.33 ^d	68.5	0.10 ^a	99.9	45.00 ^c	50.0
Clove (<i>Zizyphus lotus</i> L.)	25.00 ^c	72.2	30.0 ^b	66.7	42.67 ^b	52.6
Spearmint (<i>Mentha viridis</i> L.)	0.100 ^a	99.9	0.10 ^a	99.9	29.00 ^a	67.8
Lemon oil (<i>Citrus aurantifolia</i>)	10.00 ^b	88.9	60.0 ^c	33.3	60.00 ^d	33.3
Thyme (<i>Thymus vulgaris</i> L.)	90.00 ^e	0.00	90.0 ^d	0.00	90.00 ^f	0.00
Onion (<i>Allium cepa</i> L.)	90.00 ^e	0.00	90.0 ^d	0.00	90.00 ^f	0.00
Garlic (<i>Allium sativum</i> L.)	90.00 ^e	0.00	90.0 ^d	0.00	80.00 ^e	11.1
control	90.00 ^e	0.00	90.0 ^d	0.00	90.00 ^f	0.00

* values are means of 4 replicates.

* values within column followed by the same letter(s), are not significantly different at (P = 0.05).

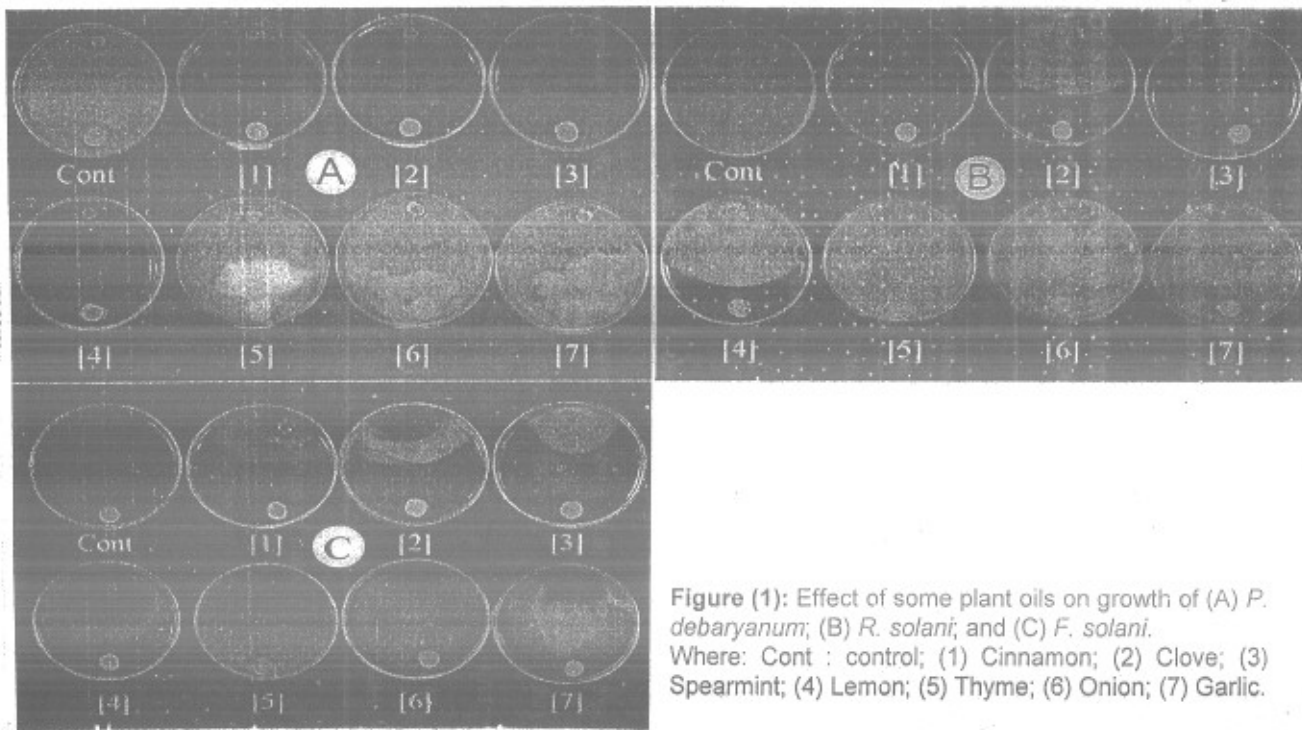


Figure (1): Effect of some plant oils on growth of (A) *P. debaryanum*; (B) *R. solani*; and (C) *F. solani*.
 Where: Cont : control; (1) Cinnamon; (2) Clove; (3) Spearmint; (4) Lemon; (5) Thyme; (6) Onion; (7) Garlic.

Table (2): Effect of different concentrations of four tested oils on the mycelial growth of the tested damping-off agents.

Treatment (Oils)	Concentration %	<i>P. debaryanum</i>		<i>R. solani</i>		<i>F. solani</i>	
		Mycelial growth (mm)	% Reduction	Mycelial growth (mm)	% Reduction	Mycelial growth (mm)	% Reduction
1- Cinnamon	100	28.3	68.5	0.1	99.9	45.0	50.0
	75	32.7	63.7	61.0	32.2	64.0	28.9
	50	40.0	55.6	70.7	21.5	65.0	27.8
	25	60.0	33.3	75.0	16.7	70.0	22.2
	12.5	70.0	22.2	90.0	0.0	75.0	16.7
	0.0	90.0	0.0	90.0	0.0	90.0	0.0
2- Clove	100	25.0	72.2	30.0	66.7	43.7	51.5
	75	42.0	53.3	34.3	61.9	60.0	33.3
	50	44.0	51.1	41.3	54.1	65.0	27.8
	25	46.0	48.9	58.7	34.8	75.0	16.7
	12.5	60.0	33.3	84.3	6.3	80.0	11.1
	0.0	90.0	0.0	90.0	0.0	90.0	0.0
3- Spearmint	100	0.1	99.9	0.1	99.9	29.0	67.8
	75	0.1	99.9	0.1	99.9	30.0	66.7
	50	0.1	99.9	40.0	55.6	40.0	55.6
	25	55.0	38.9	73.3	18.5	60.0	33.3
	12.5	70.0	22.2	80.7	10.4	70.0	22.2
	0.0	90.0	0.0	90.0	0.0	90.0	0.0
Lemon	100	10.0	88.9	60.0	33.3	60.0	33.3
	75	26.7	70.4	73.7	18.1	65.0	27.8
	50	48.3	46.3	80.0	11.1	70.0	22.2
	25	90.0	0.0	90.0	0.0	75.0	16.7
	12.5	90.0	0.0	90.0	0.0	80.0	11.1
	0.0	90.0	0.0	90.0	0.0	90.0	0.0
L.S.D _{0.05} (Conc.)		0.8918		1.2576		0.3869	
L.S.D _{0.05} (Treat.)		0.7282		1.0268		0.3159	

* Values are means of 4 replicates

(2) Antagonistic effect of some plants oils *In vivo*

This experiment was carried out to test the *in vivo* effect of two concentrations, i.e. 100% and 50%, of the four tested oils on the incidence

of damping-off and root rot diseases of two pea cultivars, namely Lincoln and Sinnary. Results were presented in Tables (3, 4 & 5).

I. Effect on damping-off incidence

a. Pre-emergence damping-off (PRD)

From Tables 3, 4 and 5, it is evident that seed treatment with any of the tested oils at both tested concentrations significantly reduced PRD incidence. Reduction % attained maximum levels when seeds were treated with spearmint oil at 100% conc. The other tested oils were less active in reducing PRD values than spearmint oil. Moreover, lemon oil proved to be the least efficient among the tested oils, especially at 50% conc. Reduction % in PRD incidence due to seed treatment of Sinnary cv. with spearmint oil at 50% compared to the infected untreated control was higher in *P. debaryanum* (57.57%) than in *R. solani* (46.77), whereas the least reduction % in PRD was realized by *F. solani* (34.57%). At higher oil concentration, PRD values were significantly lower. At 50% oil concentration, cinnamon and clove oil were inefficient in reducing PRD values especially in case of *F. solani*.

b. Post-emergence damping-off (PTD)

Data presented in Table 3, 4 and 5 showed that PTD incidence was significantly reduced as a result of pea seed treatment with any of the tested oils. However, the decrease in PTD incidence significantly varied according to the applied oil, its concentration and the tested pea cultivar. Spearmint oil was the most effective in reducing PTD, followed by clove oil, whereas cinnamon and lemon oils were less effective especially at 50%. Higher reduction in PTD incidence due to seed treatment with spearmint at 50% compared with infected untreated control was reported in *R. solani* (47.67 %), followed by *P. debaryanum* (26.54 %), whereas, *F. solani* exhibited the lowest response to the treatment (19.75%). In general, reduction in PTD incidence due to seed treatment with the tested oils was more pronounced in Sinnary than Lincoln cv. At 100% oil conc., reduction rates were significantly higher in all tested pathogens.

c. Survived seedlings (SS)

From the obtained results (Table 3, 4 and 5), it is evident that all the tested treatments significantly decreased SS values compared to infected untreated control. At 50% oil conc., spearmint was the most effective among the tested oils. SS values in *R. solani* were higher than those in *P. debaryanum* followed by *F. solani* (86, 58.3 and 42.72% more than control, respectively). Moreover, SS values were higher in Sinnary than Lincoln pea cv.

Table (3): Effect of two concentrations of the four tested plant oils on the incidence of pre-, and post-emergence damping-off of pea seedlings caused by *Pythium debaryanum*.

Treatments		Pre-emergence		Post-emergence		Survivor	
Plant oils	Concentration %	Lincoln	Sinnary	Lincoln	Sinnary	Lincoln	Sinnary
1- Cinnamon	100	15.32	12.21	28.78	23.86	54.99	63.02
	50	21.14	21.14	26.57	28.78	44.74	51.38
2- Clove	100	18.43	12.21	23.86	23.86	51.88	63.02
	50	21.14	18.43	26.57	28.78	51.38	51.88
3- Spearmint	100	12.21	9.10	21.14	15.32	65.74	74.67
	50	15.32	12.21	23.86	21.14	52.78	65.74
4- Lemon	100	15.32	15.32	28.78	23.86	59.91	63.02
	50	21.14	18.43	33.21	26.57	44.74	53.66
5- Infected control	0.0	33.21	28.78	33.21	28.78	32.67	41.53
6- Uninfected control	0.0	12.21	9.097	9.097	9.097	77.79	80.9
L.S.D _{0.05} (Treatment)		5.457		4.716		3.958	
L.S.D _{0.05} (Varieties)		2.440		2.109		1.770	
L.S.D _{0.05} (Interaction)		4.677		4.042		3.392	

* values are means of 4 replicates.

Table (4): Effect of two concentrations of the four tested plant oils on the incidence of pre-, and post-emergence damping-off of pea seedlings caused by *Rhizoctonia solani*.

Treatments		Pre-emergence		Post-emergence		Survivor	
Plant oil	Concentration %	Lincoln	Sinnary	Lincoln	Sinnary	Lincoln	Sinnary
1- Cinnamon	100	18.43	15.32	26.57	23.86	54.09	59.91
	50	23.86	23.86	28.78	26.57	46.45	48.66
2- Clove	100	23.86	18.43	23.86	18.43	51.37	62.23
	50	26.57	23.86	26.57	18.43	45.95	56.80
3- Spearmint	100	21.14	15.32	18.43	9.097	59.50	74.68
	50	23.86	15.32	26.57	18.43	48.66	65.34
4- Lemon	100	23.86	21.14	26.57	26.57	48.66	51.38
	50	26.57	26.57	33.21	28.78	39.31	43.74
5- Infected control	0.0	33.21	28.78	46.92	35.22	18.96	35.09
6- Uninfected control	0.0	12.21	9.097	9.097	9.097	77.79	80.9
L.S.D _{0.05} (Treatment)		4.757		3.706		3.849	
L.S.D _{0.05} (Varieties)		2.127		1.657		1.721	
L.S.D _{0.05} (Interaction)		4.076		3.176		3.298	

* values are means of 4 replicates.

Table (5) : Effect of two concentrations of the four plant oils on the incidence of pre-, and post-emergence damping-off of pea seedlings caused by *Fusarium solani*.

Treatments		Pre-emergence		Post-emergence		Survivor	
Oily plant extracts	Concentration %	Lincoln	Sinnary	Lincoln	Sinnary	Lincoln	Sinnary
1- Cinnamon	100	21.14	18.43	26.57	28.78	51.38	51.88
	50	26.57	23.86	28.78	30.99	43.74	44.24
2- Clove	100	23.86	21.14	28.78	23.86	46.45	54.09
	50	26.57	23.86	30.99	26.57	41.53	48.66
3- Spearmint	100	18.43	15.32	26.57	21.14	54.09	62.63
	50	23.86	18.43	28.78	26.57	46.45	54.09
4- Lemon	100	26.57	23.86	30.99	28.78	41.53	47.15
	50	28.78	26.57	35.22	30.99	35.09	41.53
5- Infected control	0.0	33.21	28.08	39.21	33.11	26.67	37.90
6- Uninfected control	0.0	12.12	9.097	9.097	9.097	77.79	80.90
L.S.D _{0.05} (Treatment)		5.090		4.707		3.504	
L.S.D _{0.05} (Varieties)		2.276		2.105		1.567	
L.S.D _{0.05} (Interaction)		4.362		4.034		3.002	

* values are means of 4 replicates.

II. Effect on root rot incidence

Data illustrated in Fig. (2) clearly showed that all the tested plant oils significantly decreased root rot incidence incited by the three tested pathogens compared to the infected untreated control. Spearmint oil was the most effective in reducing percentage of infection especially at 100% conc. However, pronounced reduction was obtained at even 50% oil concentration, where reduction rates were 85%, 76.9% and 73.3% less than the control in *P. debaryanum*, *R. solani* and *F. solani*, respectively. Generally, root rot incidence in Sinnary cv. was significantly lower than those of Lincoln cv.

It could be concluded that seed treatment with the tested oils significantly reduced the incidence of PRD, PTD and root rot and increased the seedling survivals. Moreover, spearmint oil was the most effective among the other tested oils. Moreover, application of *Mentha viridis* successfully reduced damping-off diseases in different crops (Chatterjee, 1990; Sardud *et al.*, 1992; Vimala *et al.*, 1993; Pandey and Dubey, 1994 and Rahhal, 1997). Muller-Riebau *et al.* (1995) believed that the fungitoxic component in the applied oil was attributed to a phenolic compound.

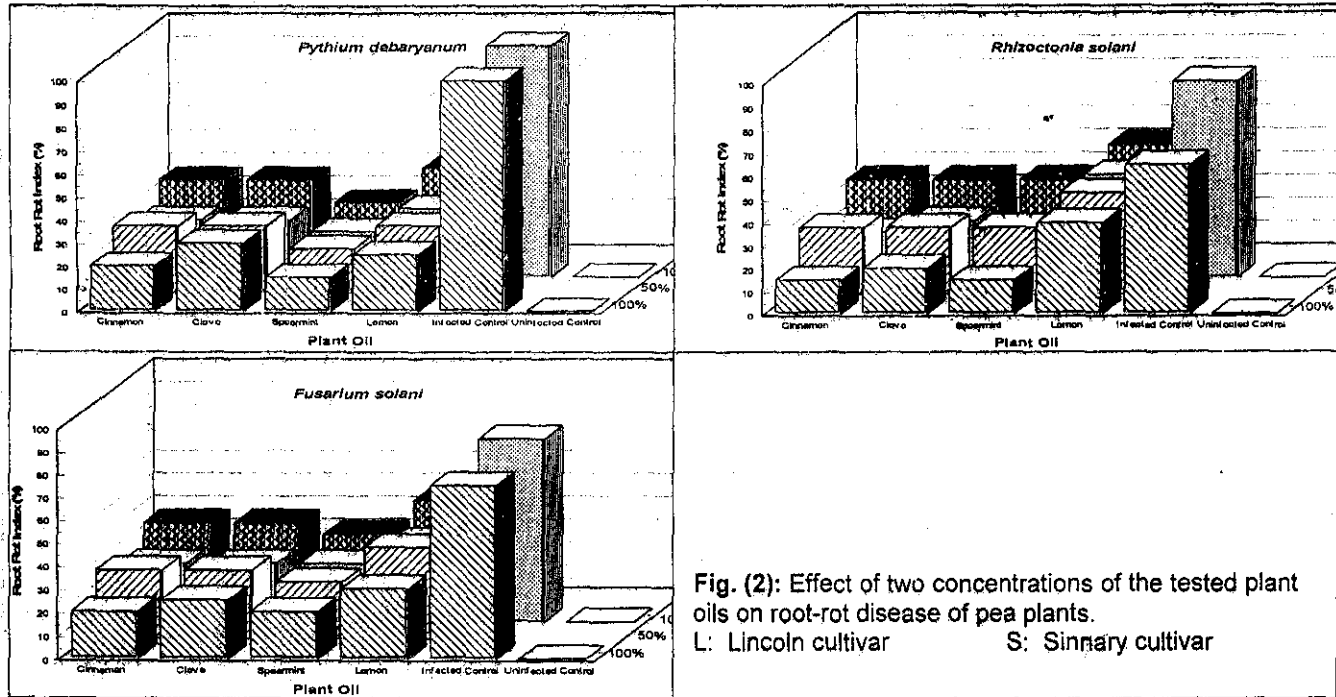


Fig. (2): Effect of two concentrations of the tested plant oils on root rot disease of pea plants.
 L: Lincoln cultivar S: Sinnary cultivar

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الملخص العربى

دراسيات عن امراض الذبول الطرى وأعفان الجذور

فى بادرات البسلة

III. المقاومة بالزيوت النباتية

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أوضحت النتائج أن زيوت النعناع ، القرفة ، القرنفل والليمون قد أدت إلى إنخفاض معنوى فى النمو القطرى لفطريات البيثيوم ديباريانم ، ريزوكتونيا سولانى، فيوزاريوم سولانى والمسببة لأمراض الذبول الطرى وأعفان الجذور فى نبات البسلة. وكانت زيوت الثوم والبصل والزعتر غير فعالة فى تثبيط الفطريات المختبرة بالمقارنة بالزيوت الأخرى. ولقد كان زيت النعناع هو أكثر الزيوت المستخدمة فعالية فى تثبيط نمو الفطريات المختبرة بالمقارنة بالزيوت الأخرى ، كما كان الفطر بيثيوم ديباريانم أكثر للفطريات المختبرة حساسية للمعاملات بالمقارنة بالمسببات المرضية الأخرى. وفى تجربة الصوبة ، أدت معاملة بذور البسلة بزيت النعناع إلى أعلى نسبة إنخفاض فى قيم ذبول ما قبل وما بعد ظهور البادرات وقيم عفن الجذور ، كما أدت إلى زيادة معنوية فى نسبة البادرات الناجية من الإصابة وذلك بالمقارنة بتجربة المقارنة المعده والغير معاملة. ولقد نجحت زيوت القرفة ، القرنفل والليمون فى خفض الإصابة بشكل معنوى وذلك عند التركيزات العالية فقط. وكان إنخفاض الإصابة نتيجة المعاملة أكثر وضوحاً فى تجارب الذبول الطرى المتسبب عن البيثيوم والريزوكتونيا. بالإضافة إلى ذلك أشارت النتائج أن صنف سنارى أكثر مقاومة حيث أظهر إستجابة للمعاملة أكثر من صنف لנקولن.