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BIOLOGICAL CONTROL OF PEA ROOT ROT DISEASE IN EGYPT

By

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ABSTRACT: Pea root rot caused by *Fusarium oxysporum, F. solani Pythium debaryanum* and *Rhizoctonia solani* is a major yield – limiting factor for field pea production in Egypt. Eleven plant extracts, five plant essential oils, four bioagent control and three fungicides were compared for their antifungal effects on pea root rot pathegens.

In Vitro all tested plant extraxts significantly decreased the mycelial growth of pathogenic fungi. Giant milk weed, Blue gum, Datura, Morella, Sapodilla, Bengal fig, Mint and Periwinkle extracts were highly effective on mycelial growth of four pathogenic fungi. While Lantana, Leaved goose and Bamboo extracts were the less effective on the same fungi. Mycelial growth of *P.debaryanum* was more affected by most of the plant extracts, while *F. Solani* was the less affected. All the essential oils under study completely inhibited the mycelial growth of *P. debaryanum* and *R. solani* at 75% concentration, while thyme and Blue gum essential oils completely inhibted the mycelial growth of all tested fungi at 50% concentration and 25% concentration on *P. debaryanum* mycelial growth.

In vivo pre - and post-emergence damping- off were reduced and healthy plants increased when pea seeds were treated by plant extracts before planting in infested soil. All essential oil concentrations significantly reduced disease incidence and increases the survived plants. When the four bioagent used as controlling the diseases Rizo. N was more effective on disease incidence which gave highly percentage of survival plants.

The best plant extracts, essential oils, biocontrol agent and fungicides were used to controlling pea root rot pathogens in the open field. Essential oils gave the best results in minimizing the pre and post emergence damping off as compared with another treatments, while the best yield was giving with biocontrol agents than the other treatments. Therefore, the results of this study suggest that essential oils, biocontrol agent ant and plant extracts is an effective in controlling pea root rot pathogens.

INTRODUCTION

Root rots occur wherever peas are grown in the world. Crop losses are probably greater from root diseases than from any other type of pea disease. Root rot may start when the pea plant is the pre-or post – emergent seedling stage. The most common pathogens are *Pythium ultimum, Fusarium solani* f. sp. *pisi, F. oxysporum, Rhizoctonia solani* and *Sclerotinia sclerotiorum. (Babadoost 2002, Allen 2003 and El Shazly 2007), Fusarium solani, Rhizoctonia solani, Macrophomina phaseolina* and *Sclerotium rolfsii* are considered the most pathogenic fungi attacking legume crops in Egypt *(Nofal, et al., 1982, El Gantiry, et al., 1994 & Omar and Abd – Alla 2000 and Eisa 2003).* The pathogens individually or synergistically cause symptoms such as seed decay, root rot and foot rot.

For decades, fungicide seed treatment have been the only means to combat pea root rot. However, fungicide seed treatment is not desirable for disease control due to some adverse effects on the environment and ecosystem, such as harm to nontarget organisms, animals, and plants; residues in soil; and contamination of the water and food chains. Fungicides also may induce pathogen resistance, making their variable effects and short lived. In addition fungicides are expensive in comparison with the relatively low commodity price of field pea. Hence, there is a need for an improved pea root rot management system with reduced fungicide use (Baker and Paulitz 1996).

Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods (Allen, 2003). Accordingly it is desirable to identify a microorganism that displays antagonistic effects against target pathogens and it is capable of survival and propagation in target locations. Several fungi and bacteria have been reported to have antagonistic effects to pea root rot pathogens. Specifically, Walter and Cindral (1988), Sabet, *et al.,* (1991) and Abd El–Moity (1992) noted that application of antagonistic microorganisms to the seeds before sowing has been successfully used for cotrolling damping-off and root rot diseases of several plants. Youssef (1991), Agha (1992) Arab (1994), Zedan *et al* (1994) and El- Shazly (1996 & 2000) showed some plant extracts (Limon Scented, Mulberry, Ficus, Mint, Guava, Thyme and Blue gum), and essential oils of some medicinal plants which exhibited antifungal properties.

In this investigation five essential oils, eleven plant extracts, four spices as bioagent control and three fungicides were examined on four pathogenic fungi i.e., *F. oxysporum*, *F. solani*, *Pythium debaryanum* and *R. solani* which isolated from pea roots in four governorates. The ability of the materials to prevent the growth of the fungi was also studied.

MATERIALS AND METHODS

Microorganisms culture. Pure cultures of *Fusarium oxysporum*, *F. solani*, *Pythium debaryanum* and *Rhizoctonia solani* were used in various experiments in this study. Those pathogens were isolated from naturally infected pea plants in 2003 and 2004 seasons. All isolates were grown on (PDA) medium. Cultures of pathogens were stored at 4^0C and subcultured every 6 months.

a) Effect of plant extracts:

Eleven medicinal plants, i.e. bamboo (*Arundo donax* L.), bengal fig (*Ficus bengalensis* L.), blue gum *(Eucalyptus gelobulus* Labill), datura (*Datura stramonium* L.), giant milk weed (*Calotropis procera* Ait), lantana (*Lantana camara* L.), leaved goose (*Chenopodium ambrosioides* L.), mint (*Mentha piperita* L.), morella (*Solanum nigrum* L.) periwinkle (*Vinca rosea* L.) and sapodilla (*Achras sapota* L.) were collected from El-Kanater El- Khairia and Qaha (Kalubia). The aerial parts (leaves, flowers, young fruits and shoots) of medicinal plants were separately grinded in a mortar. The ground materials were squeezed, the fluid was centrifuged at 4000 rpm for 20min and sterilized using Seitz's filter. The sterilized fluids were used to study their effect on fungal growth *in vitro* and disease incidence *in vivo*.

Effect on fungal growth:

Fusarium oxysporum, *F. solani*, *Pythium debaryanum* and *Rhizoctonia solani* were grown on PDA. Four discs (5mm) from the PDA around the sides of each Petri-dish were cut and replaced by the plant extracts, then a disc of each fungus was placed in the center of the plates. Four replicates were prepared for each treatment and for each fungus and incubated at 25^0C for 7 days. In the control treatments, sterilized water was used instead of the plant extract. The effect of extracts was expressed as the average width of inhibition zones for each treatment.

Effect on disease incidence:

Sterilized soil was infested by pea pathogenic fungi in green house, while natural infested soil was used in the open field (The fields which the same fungi were isolated from it) to study the effect of medicinal plant extracts on disease incidence. Pea seeds lencolin c.v. (susceptible. Pea cultivar) were soaked in medicinal plant extracts for three hours before sowing. Ten seeds were planted in each pot (40 cm^2 diameter) and four pots were used for each treatment. All pots were kept under green house conditions.

In the open field, ten proximate plots, each 0.7x5 meter were used for each treatment. Each plot contained 100 pea seeds. Untreated seeds were used as a control. The percentages of pre – and post – emergence damping – off were recorded after 2 weeks and 5 weeks from planting. The atmospheric temperature renged between 25-30^{0}C. daily and 13-19^{0}C. in the night during 2006 growing season.

b) Effect of essential oils:

Essential oils of Black cumin *(Nigella sativa* L.*)*, Blue gum *(Eucalyptus globules* L.*)*, Peppermint *(Mentha piperita* L.*)*, Rosemary *(Rosmarinus officinales* L.*)* and Thyme (*Thymus vulgaris* L.) were obtained from medicinal and Aromatic plant pathology Research Dept. Plant Pathology Res. Institute, Agric. Rec. Center, Giza, Egypt.

Effect on the mycelial growth:

Effect of essential oils at different concentrations on mycelial growth was tested of all tested fungi in this experiment. For preparation of the desired concentrations 5 ml of each oil was mixed in 0.5ml acetone to emulsion in water. Four plates containing P D A medium were inoculated with each of the tested fungi the mycelial disk of 5mm diameter of each tested fungus (taken from a 7 days old culture) was put into the center of each plate. Essential oils (1ml) of 100%, 75%, 50% and 25% concentration were immediately introduced individually into lid of a Petri dish containining sterilized filter paper. Control treatment was made by the 1ml of sterilized distilled water with 2 drops of acetone instead of the essential oils. The treated and control sets were incubated at 25 ± 1^{0}C. The colony's diameter was measured when the growth was covered the plates from any treatment.

The percentage of reduction in colony diameter was calculated

following the method of Dixit *et al.*, (1978) as follows:

Inhibition percentage of mycelial growth = $d_e - d_l / d_e \times 100$

Where, d_e = average increase in mycelial growth in control set and d_l = average increase in mycelial growth in treatment set.

Effect on disease incidence:

To study the effect of essential oils on disease incidence of pre-emrgence damping – off on pea plants caused by, *F.oxysporum, F.solani, P. debaryanum* and *R. solani* in green house and in the open field. The same methods which used with plant extracts were used with this experiment at the same season.

c) Effect of biocontrol agents.

In order to study the efficacy of some biocontrol agents, prepared by the commercial companies, namely Plant Guard (*Trichoderma harzianum*, $300x10^6$ cells per ml), Promot (*Trichoderma harzianum* and *T. konigii*, $50x10^6$ cells/g). Rizo – N (*Bacillus subtilis*, $30x10^6$ cells per/g) and *Pseudomonas fluorescens* $30x10^6$ cells/g) in controlling pre- and post – emergence damping – off of pea lencolin cv. previously infested with either *F. oxysporum., F. solaui, P. debaryanum* and *R. solani,* under greenhouse conditions. These biocontrol agents individually added to the pots (40 cm in diameter) at the rate of 500ml solution prepared from biocontrol agents as follows:

Plant Guard, Promot and Rizo-N (were 5g per liter of water) but *P. fluorescens* (was 5ml per liter of water) directly at sowing time as recommended doses of the producing company for each biocontrol agent. Ten seeds were sown per pot and each treatment contained four pots. Control treatment was carried out without adding biocontrol agent. Disease assessment was recorded as mentioned before.

d) Effect of fungicides:

Three systemic fungicides, namely Benlate, Rizolex and Sumisclex were used to compare with effect of medicinal plant extracts, essential oils and biocontrol agents. Fungicides, at recommended dose was based on their active ingredients. Pea seeds Lencolin cv. were treated by fungicides than planted in natural infested soil with the other treatments (best medicinal extracts, essential oils and biocontrol agents) as the same mentioned before methods.

Table (1): Trade mark, common name, % at active ingredient, chemical name and rate of application of tested fungicides.

Trade mark	Common name & % of active ingredient	Chemical name	Rate of application g/L
Benlate	50% Benomyl	Methyl-1- (butylcarbonyl) -2-benzimidazole carbonate.	2.0
Rizolex-T	50% Tolclofos methyl + Thiram	0.0–dimethyl–0-2,6 dichloro-4-methylphenyl)-phosphorothioate	.2-0
Sumisclex	50% Procymidone	N(3,5- dichloro phenyl) 1,2 dimethyl cyclopropane – 1,2 dicarboximide.	10.0

RESULTS AND DISCUSSION

I- In vitro:

Effect of some medicinal plant extracts on mycelial growth:

Out of eleven plant screened, the extracts of Giant milk weed, Blue gum, Datura, Morella, Sapodilla, Bengal fig, Mint and Periwinkle were highly effected on mycelial growth of *F. oxysporum, F. solani, P. debaryanum* and *R. solani*, which give the large inhibition zone in (Table 2), while Bamboo, Leaved goose and Lantana were low effected on the mycelial growth. On the other hand, all extracts were effected on all tested fungi mycelial growth. Giant milk weed extract was highly effect on mycelial growth of *P. debaryanum* and *F. oxysporum*, while the effect on *F. solani* was equal with Blue gum extract, but the effect was equal with Datura on mycelial growth of *R. solani.*

Bengal fig, Datura and Morella give the same affect on *F. oxysporum* and *F. solani* while this affect was changed with *P. debaryanum* and *R. solani.* In the same respect Abhay and Lal (1997) reported that *Calotropis procera, Lantana Camara* and *Ocimum basilicum* stopped the mycelial growth of *A. alternata* and several other fungi.

Singh and Dwivedi (1990) Youssef (1991) Agha (1992) Zedan *et al.,* (1994) El Shazly (1996) and Harsh (2001) reported that *Lantana camara, Datura stramonium, Calotropis procera* extractes inhibited mycelial

growth of *R. solani, F. solani, M. phaseolina, Pythium* sp., and *F. moniliforme.* Abhy and Lal (1997) reported that, thymol and phenolic compounds present in *Lantana camara* and *Ocimum basilicum* are toxic substances reported to inhibit the growth of many fungi and bacteria.

Table (2): effect of some medicinal plant extracts on mycelial growth of pathogenic fungi.

Treatments	Inhibition zone (mm)				
	F. oxysporum	*F. solani*	*P. debaryanum*	*R. solani*	Main
Bamboo	12	12	14	12	12.5
Bengal fig.	20	20	18	15	18.25
Blue gum	24	22	20	22	22.0
Datura	20	20	22	24	21.5
Giant milk weed	26	22	28	24	25.0
Lantana	8	8	10	15	10.25
Leaved goose	10	8	15	14	11.75
Mint	18	16	20	16	17.5
Morella	20	20	24	20	21.0
Periwinkle	18	16	20	16	17.5
Sapodilla	22	20	22	18	20.5
Control	0	0	0	0	0.0

- L.S. D at 0.05 for Treatments = 4.82
  Fungi = 3.56
  Fungi x Treatments = 5.21

Effect of essential oil on mycelial growth:

Data in Table (3) indicate that all the essential oils completely inhibited the mycelial growth of all tested fungi at 100% concentration except peppermint oil with *F. solani* and *F. oxysporum* which give 94.5% and 92% inhibition at 100% concentration.

Mycelial growth of the tested fungi was completely inhibited when it was exposed to thyme and Blue gum volatile oils at 25% concentration. *P. debaryanum* and *R. solani* were completely inhibited with all essential oil at 75% concentration, and highly affected when it treated by all

Tabel (3): Effect of different concentrations of some essential oils on mycelial growth of pea pathogenic fungi.

Essential oil	% of Concentration	Percentage of inhibition			
		F.oxysporum	*F. solani*	*P.debaryanum*	*R. solani*
Black cumin	100	100	100	100	100
	75	100	94	100	100
	50	90.2	86.4	94.0	92.0
	25	86.0	74.7	85.6	80.0
Blue gum	100	100	100	100	100
	75	100	100	100	100
	50	100	100	100	100
	25	90.1	92.6	100	89.7
Peppermint	100	92.0	94.5	100	100
	75	80.2	86.4	100	94
	50	64.0	60.0	92.5	80.2
	25	48.6	42.5	77.7	62.5
Rosemary	100	100	100	100	100
	75	90.2	92.5	100	100
	50	70.6	76.5	94	90
	25	62.5	62.5	88.7	77.2
Thyme	100	100	100	100	100
	75	100	100	100	100
	50	100	100	100	100
	25	90	95	100	92.5
Control		0.0	0.0	0.0	0.0

L.S. D at 0.05 for treatments = 12.61
L.S. D at 0.05 for fungi = 5.04

essential oil at all concentrations. These results are in harmony with the works of Saksena and Tripathi (1987), Agha (1992) Zedan *et al.*(1994) El-Shazly (1996 and 2000) and Jaspal and Tripathi (1999) who mentioned that the pure essential oils completely inhibted the mycelial growth of many pathogenic fungi, and the fungal sensitivity to the previous essential oils differed from one fungus to another this may be due to the capability of essential oils to penetrate in to the fungal cell.

Another aspect of the activity of this volatile oils is that it causes a reductions in hyphal diameter that may be due to alterations in fungal metabolism caused by the mutagenic activity of many essential oils. Zani *et al.*, (1991).

II) In green house:

Effect of plant extracts on pea damping - off:

Data presented in Table (4) show that all plant extracts were effective on pea pathogenic fungi. It reduced percentage of pre – and post – emergence damping – off with all fungi on plants. Blue gum, Giant milk weed and sapodilla were highly effective on tested fungi, while Bamboo and leaved goose were give low effective on pea pathogenic fungi. On the other hand, *P. debaryanum* and *F. solani* were the most affected as percentages of damping-off caused by both fungi were highly reduced (7.5% and 10% with blue gum, 10% and 12.5% with giant milk weed and 10% & 10% with sapodilla extracts) compared with control (52.5% and 60%), respectively.

Manasi-Mishra and Tewari (1990) concluded that extracts from leave of *calotropis procera, Azadirachia indica* and *Datura stramonium* possessed toxic principles against one or more of *Pyricularia oryzae*, *Rhizoctonia solani* and *Fusarium moniliforme*. Yossry *et al.* (2001) found that plant extracts of Datura, River-redgum, Limon and o shar were more effective on soil born fungi. While, El-shazly (1996) Stated that the crude juices of *C. procera, D. stramonium* and margosa leaves were reduced percentage of pre- and post- emergence damping off on mint caused by *Fusarium lateratium, F. tabacinum, F. sporotrichioides, F. semitectum, R. solani* and *C. maydis* when rhizomes of mint were dipped in juices for 30 minuts before planting in infested soil. Variation among tested extracts for their antifungal effect may be due to the different amounts of phenolic or glycosides and other compounds in the plant extract of these medicinal plants.

Effect of essential oil:

Data listed in table (5) indicate thate thyme and blue gum essential oils showed the best result in minimizing of the percent of pre–emergence damping–off with all concentrations. All treatments reduced the percent of damping off. Rosemary, peppermint and black cumin essential oils give highly effect at 100% concentration with pre–emergence damping

Table (4): Effect of some medicinal plant extracts on pea damping – off in green house:

Treatments	*F. oxysporum*			*F.solani*			*P. debaryanum*			*R. solani*			Control		
	Pre-%	Post-%	Surv..%	Pre-%	Post-%	Surv.%	Pre-%	Post-%	Surv.%	Pre-%	Post-%	Surv.%	Pre-%	Post-%	Surv.%
Bamboo	10.0	20.0	70.0	20.0	10.0	70.0	10.0	10.0	80.0	20.0	10.0	70.0	0.0	0.0	100
Bengal fig	5.0	15.0	80.0	15.0	12.5	72.5	10.0	7.5	8.2	17.5	7.5	75.0	0.0	0.0	100
Blue gum	2.5	10.0	87.5	0.0	10.0	90.0	0.0	7.5	92.5	10.0	7.5	82.5	0.0	0.0	100
Datura	5.0	15.0	80.0	5.0	15.0	80.0	2.5	10.0	87.5	10.0	5.0	85.0	0.0	0.0	100
Giant milk weed	5.0	10.0	85.0	0.0	12.5	87.5	0.0	10.0	90.0	7.5	5.0	87.5	0.0	0.0	100
Lantana	10.0	17.5	72.5	10.0	10.0	80.0	7.5	10.0	82.5	17.5	10.0	72.5	0.0	0.0	100
Leaved goose	15.0	15.0	70.0	20.0	10.0	70.0	10.0	10.0	80.0	20.0	10.0	70.0	0.0	0.0	100
Mint	10.0	15.0	75.0	10.0	12.5	77.5	5.0	10.0	85.0	12.5	10.0	77.5	0.0	0.0	100
Morella	10.0	17.5	72.5	10.0	10.0	80.0	5.0	7.5	87.5	10.0	7.5	82.5	0.0	0.0	100
Periwinkle	7.5	20.0	72.5	15.0	10.0	75.0	5.0	10.0	85.0	12.5	10.0	77.5	0.0	0.0	100
Sapodilla	5.0	10.0	82.5	0.0	10.0	90.0	2.5	7.5	90.0	10.0	7.5	82.5	0.0	0.0	100
Control	30.0	35.0	35.0	40.0	20.0	40.0	35.0	17.5	47.5	42.5	15.0	42.5	0.0	0.0	100
L.S.D. at 0.05	3.12	4.21	7.17	3.6	4.1	6.91	4.75	2.6	5.62	4.11	3.7	6.14			

L.S.D at 0.05 for Treatments = 11.71

L.S.D at 0.05 for Fungi = 9.45

Table (5): Effect of essential oil concentrations on pea damping – off in green house

Essential oil	% of	*E. oxysporum*			*F. solani*			*P. debaryanum*			*R. solani*			*Control*		
		Pre-%	Post-%	Surv%	Pre-%	Post-%	Surv%	Pre-%	Post-%	Surv%	Pre-%	Post-%	Surv%	Pre-%	Post-%	Surv%
Black cumin	100	0.0	20.0	80.0	7.5	12.5	80.0	7.5	10.0	82.5	5.0	12.5	82.5	0.0	0.0	100
	50	2.5	22.5	75.0	17.5	12.5	70.0	15.0	15.0	70.0	15.0	12.5	72.5	0.0	0.0	100
	25	15.0	25.0	60.0	30.0	15.0	55.0	22.5	15.0	62.5	22.5	15.0	62.5	0.0	0.0	100
Blue gum	100	0.0	7.5	92.5	0.0	10.0	90.0	0.0	7.5	92.5	0.0	7.5	92.5	0.0	0.0	100
	50	0.0	10.0	90.0	0.0	10.0	90.0	2.5	7.5	90.0	0.0	10.0	90.0	0.0	0.0	100
	25	2.5	10.0	87.5	2.5	12.5	85.0	2.5	7.5	90.0	5.0	10.0	85.0	0.0	0.0	100
Peppermint	100	2.5	17.5	80.0	7.5	15.0	77.5	5.0	10.0	85.0	7.5	7.5	85.0	0.0	0.0	100
	50	10.0	20.0	70.0	12.0	15.0	72.5	7.5	15.0	77.5	15.0	10.0	75.0	0.0	0.0	100
	25	20.0	20.0	60.0	25.0	15.0	60.0	17.5	15.0	67.5	25.0	15.0	60.0	0.0	0.0	100
Rosemary	100	0.0	15.0	85.0	5.0	10.0	85.0	2.5	10.0	87.5	2.5	10.0	87.5	0.0	0.0	100
	50	5.0	17.5	77.5	15.0	10.0	75.0	15.0	10.0	75.0	10.0	10.0	80.0	0.0	0.0	100
	25	12.5	20.0	67.5	25.0	12.5	62.5	20.0	12.5	67.5	20.0	10.0	70.0	0.0	0.0	100
Thyme	100	0.0	5.0	95.0	0.0	7.5	92.5	0.0	5.0	95.0	0.0	5.0	95.0	0.0	0.0	100
	50	0.0	7.5	92.5	0.0	7.5	92.5	0.0	7.5	92.5	0.0	10.0	90.0	0.0	0.0	100
	25	0.0	10.0	90.0	2.5	10.0	87.5	0.0	10.0	90.0	5.0	12.5	82.5	0.0	0.0	100
Control		30.0	35.0	35.0	40.0	20.0	40.0	35.0	17.5	47.5	42.5	15.0	42.5	0.0	0.0	100
L.S.D at 0.05		4.81	6.5	9.82	4.97	5.74	8.85	4.31	5.86	9.86	3.51	4.9	9.37			

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off caused by *F. oxysporum* only, while the essential oils of black cumin and peppermint at 25% concentration did not show any positive results, where the percent of post emergence damping – off was approximately equal or near with untreated seeds sown in infested soil by *P. debarysnum* and *R. solani*. Generaly pre-emergence damping-off was highly affected by all essential oil concentrations while the post emergence damping off was low affected by the same concentrations. Thyme and blue gum essential oils significantly reduced the percentage of pre and post emergence damping off at 25% concentration and recorded highly survival plants with all tested pathogenic fungi. This fungicidal activity of blue gum essential oil most probably due to phenolic compounds and other inhibitory substances present in the oil, such as thymol concentration in thyme essential oil and menthol, isomenthol and sabinene in mint essential oil. The inhibitor compounds in these oils may be effect on tested fungi when it vapours from treated seeds through planting and may be due to gave highly protection to seedling stage. The same result was obtained by El-Shazly (2000) and Nachman *et al* (1994) who found that thyme, blue gum and mint essential oils have fungicidal activity, with thyme essential oil was superior in controlling mycelia and spores of some phytopathogenic fungi. The antifungal activity of thyme oil already widly described in the literature of Maruzzella and Sicurella (1960). The fungicdal activity attributable to thymol is found in the oil. The high antifungal activity of thyme oil against the pathogenic fungi tested as stressed by Zambonelli *et al.* (1996), who reported that this effect is probably a result to chitin penetration of the hyphal wall which damages the lipoprotein cytoplasmic membrane, leading to escape of cytoplasm.

Effect of biocontrol agents:

Data in Table (6) indicate that all the tested biocontrol agents significantly decreased pre and post emergence damping off incited by any of the four pathogenic fungi in comparison with the control (untreated seeds). The lowest percentage of pre–emergence damping off was obtained when Plant Guard was used with *F. solani* (5%), while promot and Rizo. N. gave 7.5% with *F. oxysporum*. The lowest percentage of post – emergence damping–off was happened when Rize–N was used with *P. debaryanum*. *R. solani* gave the equal percentage of post emergence damping off with all treatments. In addition to *P. debaryanum* was the most affected pathogen by these treatments followed by *R. solani*

Table (6): Effect of biocontrol agents on pea damping off in green house

Treatment	*F.oxysporum*			*F.solani*			*P.debaryanum*			*R. solani*			Control		
	Pre-%	Post-%	Surv%	Pre-%	Post-%	Surv%	Pre-%	Post-%	Surv%	Pre-%	Post-%	Surv%	Pre-%	Post-%	Surv%
Plant Guard	10.0	15.0	75.0	5.0	15.0	80.0	10.0	10.0	80.0	12.5	10.0	77.5	0.0	0.0	100
P. fluorescens	15.0	15.0	70.0	20.0	10.0	70.0	17.5	10.0	72.5	15.0	10.0	75.0	0.0	0.0	100
Promot	7.5	20.0	72.5	12.5	15.0	72.5	12.5	7.5	80.0	20.0	10.0	70.0	0.0	0.0	100
Rizo. N	7.5	15.0	77.5	10.0	12.5	77.5	15.0	0.0	85.0	10.0	10.0	80.0	0.0	0.0	100
Control	30.0	35.0	35.0	40.0	20.0	40.0	35.0	17.5	47.5	42.5	15.0	42.5	0.0	0.0	100

L. S. D at 0.05 for pre-emergence = 2.8
L. S. D at 0.05 for post emergence = 2.25
L. S. D at 0.05 for survival = 4.17

and *F. solani*. Whereas *F. oxysperum* was the least affected.

These results were confirmed by the results obtained by many investigators (Benhamou and Chet, 1993. and Eisa, 2003). Therefore, it was though that the use of biological control, either singly or combined in an integrated control program, will be more success in controlling the plant diseases. Reddy *et al.* (1994) obtained promising results on controlling bean damping. off diseases when promot was applied in the from of wheat barn to soil infested with *R. solani*. Promot *(T.harzianum* and *T. konigii)* was the superior bioagent followed by Rizo.N, Plant Guard and *P. fluorescens*. This may be due to the presence of containing two species in Promot of *Trichoderma* spp. which were more effective than each antibiotic agent used singly. Hibar *et al* (2006) recorded that *T. harzianum, Bacillus subtilis* and *B. pumilus* were more effective on damping- off disease caused by *Fusarium* sp. and *Pythium oligandrum.*

III) In the open field:

Data listed in Table (7) indicate that all the treatments were reduced the percentage of pre-and post – emergence damping- off in comparison with control in the natural infested soil of open field conditions. Giant milk weed, blue gum and sapodilla extracts were recorded the lowest percentage of pre-emergence damping-off (0.0, 0.0 and 0.5% respectively), while the lowest percentage of post- emergence damping-off was obtained when giant milk weed and Morella extracts were used (10% and 11.6% respectively). Giant milk weed extract gave the highest affect on damping – off disease, while Datura extract recorded the lowest affective on damping off disease. On the other hand all essential oils were completely affected on the pathogens, which did not give any percentage of pre-damping-off, but it gave low percentage of post-emergence damping–off.

All Bioagent gave low percentages of pre-emergence damping off than the post–except Plant Guard which gave high percentage of pre-emergence damping–off and low percentage of post–emergence damping–off. Benlate, Rhizolox and sumisclex fungicides were used as compared with other treatments control. Fresh yield was increased with all control treatments. These increases of yield approximately one and half or more than yield control (untreated seeds). The best fresh weight of yield was obtained when seeds were treated by promot bioagent, also the survival plants was 90.5% it gave 2780 k.g./Fe while thyme essential oil

gave 2700 kg/Fe. with 99.8% survived plants. Generally all bio agent treatments gave increases of yield weight than another treatments specially treatments of essential oils which gave the best control of disease 99.8%, 97.0% and 94.0% survived plants. This results may be due to the toxicity of essential oils on useful microbial soil while the bioagent were used as biofertilizers.

Table (7): Effect of medicinal plant extracts, essential oils, biocontrol agents and some fungicides on pea damping off and yield in open field.

Treatments	Percentage of damping off			Fresh yield
	Pre -	Post-	Survival %	K.g. /fedan
Plant extracts:				
Blue gum	0.0	13.5	86.5	2230
Datura	5.0	19.0	76.0	2150
Giant milk weed	0.0	10.0	90.0	2500
Morella	4.4	11.6	84.0	2280
Sapodilla	0.5	14.2	85.3	2310
Essential oils:				
Blue gum	0.0	3.0	97.0	2610
Rosemary	0.0	6.0	94.0	2580
Thyme	0.0	0.2	99.8	2700
Bioagent:				
Plant Guard	6.0	5.2	88.8	2700
P.fluorescens	6.0	7.4	86.6	2600
Promot	2.4	7.1	90.5	2780
Rizo. N	0.7	9.1	90.2	2700
Fungicides:				
Benlate	0.2	4.2	95.6	2700
Rizolex	4.2	7.1	88.7	2540
Sumisclex	3.6	7.7	88.7	2540
Control	20.8	26.3	52.9	1600
L. S.D at 0.05	1.21	2.7	4.52	

The results of plant extracts as biocontrol of pre-postemergenc damping off was described in the literature of Youssef (1991), Agha (1992), Arab (1994) Zedan *et al.* (1994), El–Shazly (1996) and Harsh (2001). The effect of plant extracts may be due to phenolic compounds,

essential oils, glycosides, saponin, and other inhibitory substances present in the leaves, shoots or young fruits and flowers, which have induce synergistic toxicity effect.

On the other hand, the effect of essential oils on the disease incidence was recorded by Maruzzella and Sicurella (1960), Zambonelli *et al.* (1996) and El-Shazly (2000) who found that, thyme, blue gum and mint essential oils have fungicidal activity, and reduced the disease incidence with many pathogens. Zedan *et al.*, (1994) recorded that the fungicidal activity of essential oil against pathogenic fungi may be due to increase the permeability of the cell of pathogens or due to essential oil which inhibit the fungal detoxification enzymes of antifungal compounds in the essential oil.

The bioagent control results were confirmed by the results obtained by many investigators Benhamou and Chet 1993, El-Shazly 1996 and Eisa 2003). Hibar *et al* (2006) found that 10 Bacillus strains have significantly reduced the disease incidence when bio fungicides were added one week before inoculation with the pathogen. Therefore, antagonists may induce systemic resistance against *F. oxysporum* and, *F. solani* with *T. harzianum, B. subtilis, B. pumilus* and soil microorganisms earlier applied to roots can effectively protect plants against soilborne pathogens. *B. subtilis* or *P. fluorescens* were formed a 0.4 – 0.8 mm thick film around seeds and roots of plants when seeds were previously treated with pathogen. Jha and Jalal (2006) found thate *T. viride* and *A . niger* applied individually at 15g/5kg sick soil recorded the disease severity of 33.3% and 40% while the disease severity under untreated sick soil was 96% - of root rot on pea plants caused by *Fusarium solani.*

REFERENCES

Abd El-Moity. T.H. (1992): The use of *Trichoderma* spp. to control soilborne plant pathogens in Egypt. In Diamos *et al.,* (Eds.) Biological control of plant Diseases. New york. Pp. 255-258.

Abhay, K. S. and Lal, B. (1997). Studies on bio fungicidal properties of leaf extract of some plants. Indian Phytopath. 50 (3): 408-411.

Agha, M. S. (1992): Studies on antifungal agents in certain ornamental, medicinal and aromatic plants with special reference to control seedling damping – off disease of seasame. Egypt. Appl. Sci., 7 (1): 104-114.

Allen G.Xue (2003): Biological control of pathogens causing root rot

complex in field pea using *Clonostachys rosea* strain ACM 941. phytopathology. 93 (3) 329-335.

Arab, Y. A. (1994): Water hyacinth as adepressor to some fungi with special reference to *Verticillium* will. Egypt J. Appl. Sci. 9 (4): 1-13.

Babadoost, M.E. (2002): Root rots of pea. Plant Dis. 86: 452-458.

Baker, R. and paulitz, T.C. (1996): Theoretical basis for microbial interactions leading to biological control of soilborne plant pathogens. Can. J. Plant Sci. 76: 583-591.

Benhamou, N. and Chet, I. (1993): Hyphal interactions between *Trichoderma harzianum* and *R. solani*. Ultra structure and gold cytochemistry of the my co parasitic process. Physiopathology, 83 (10): 1062-1071.

Dixit, S.N., Tripathi, N.N. and Tripathi, S. C. (1978). Natl. Acad. Sci. letters, l, 287. c. f. jaspol, S. and tripath, N.N. (1999).

Eisa, Nour-Jehan M.M. (2003): Biological control of peas (*Pisum sativam* L.) damping-off disease caused by four fungi. J. agric. Sci. Mansoura univ., 28 (11): 6633-6642.

El- Gantiry, S. M., Omar S.A., Salem E. Dorreiah and. Rahal M.M (1994): Survey, host response and fungicidal treatment of root rot disease in faba been. Egypt J. Appl. Sci., 9 (7): 366-375.

El- Shazly, A. M. A. (1996). Studies on certain fungal diseases on some medicinal plants of Family Labiatea. Ph. D. thesis, Fac. of Agric., Al-Azhar Univ. pp 136.

El- Shazly, A. M. A. *(2007):* Study of prevalence and Pathogenicity of root rot pathogens of pea in Egypt Al-Azher J. Agric. Sci. Sector Res., 2: 61-76..

El-Shazly, A. M. A. *(2000).* Antifungal activity of some essential oils on fungi causing damping- off disease of maize. Al-Azher J. Agric. Res., 31: 95-107.

Harsh, N. S.K. (2001*).* Biological control of damping. off and wilt of *Albizia lebbek* seedlings using plant extracts Indian forester. 124:11, 962-966.

Hibar, K., Daami- Remadi, M., Hamada W., and El-Mahjoub, M. (2006): Bio- fungicides as an Alternative for tomato *Fusarium* crown and root rot control. Tunisian Journal of Plant Protection 1: 19-29.

Jaspal, S. and Tripathi, N.N. (1999): Inhibition of storage fungi of black gram (*Vigna mungo* L.) by some essential oils. Flavour Fragr. J. 14: 1-4.

Jha, P. K. and Jalal, B. L. (2006): Biocontrol of pea root rot incited by *F. solani* with rhizosphere mycoflora. Indian Phytopathology 59 (1): 41-43.

Manasi- Mishra and Tewari, S.N. (1990). Ethanolic extract toxicity of three botanicals against five fungal pathogens of rice. National Academy Science Letters 13 (11): 409-412.

Maruzzella, J.C. and Sicurella, N.A. (1960): Antibacterial activity of essential oil vapors. J. Am. Pharm. Assoc. Sci. Ed. 49: 662-694. c.f. Inouye et al., (1998).

Nachman paster; Mazal, M., Uzi, R. and Benjamin, J. (1994): Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. Journal of Food Protection Vol. 58(1): 81-85.

Nofal, M.A., A. F. Sahab, M.M. Diab and A. A. Morsy (1982): Response of broad been plants infected with root rot fungi to fuli-fertile application. Egypt. J. phytopathology., 14: 67-74.

Omar, S. A. and M.H. Abd-Alla (2000): physiological aspects of fungi isolated from root nodules of faba been (*Vicia faba.* L) Egypt J. Appl. Sci., 15 (4): 339-347.

Reddy, M.S., R.K. Hynez and G. Lazarovits (1994): Relation ship between *invitro* growth inhibition of pathogens and suppression of pre- and post- emergence damping off and root rot of white been seedlings in the greenhouse by bacteria. Can. J. Microbiol., 40 (2): 113-119.

Sabet, K.K., Om- Hashem, I. El-Banna, Mostafa and Ebtisam M.El-Sherif. (1991): *Pseudomonas lindbergii* and *Coniothyrium minitans* as biocontrol agents offective against some soil fungi pathogenic to peanut. Egypt J. Agric, Res. 70: 403-414.

Saksena, N. and Tripathi, H.H.S. (1987). Antifungi substance in the essential oil of anise (*Pimpinella anisum* L.). Agricultural and Biological Chemistry, 51 (7): 1991-1993.

Singh, R, K and Dwivedi, R. S. (1990). Fungicidal properties of calotropis prosera and babul gum against *Fusarium* sp. Acta. Bot. Indica 14: 104-110.

Walter, D. and D. Cindral (1988): Biological control of damping –off of sugar beet and cotton with *Chaetomium globosum* or *Pseudomonas flurescent*. Can. J. microbial., 34: 631-637.

Yossry, A.A, El-All S.M.A and El-Imery S.M. (2001): Fungi toxic properties of some plant extracts against growth of soil borne fungi. Annals. Of Agricultural Science- Cairo, Special Issue, Volume. 3:891-909.

Youssef, R.M. (1991): A comparative study of the use of plant juice and fungicides for controlling damping off disease of lentil. Al-Azhar. J. Agric. Res. 13: 63-78.

Zambonelli, A., Biancchi, A. and Albasini, A. (1996): Effect of essential oils on phytopathogenic fungi in vitro Phytopatholgy 144: 491-494.

Zani, F. G., Massimo, S. and Vampa, A. (1991): Studies on the genotoxic properties of essential oils with *Bacillus subtilis*. Planta Med. 57: 237 – 241.

Zeedan, A. M., El-Toony, A.M. and Awad, N.G.H. (1994). A comparative study on antifungal activity of certain plant extractes, essential oils and fungicides on tomato wilt pathogens. Al-Azhar. J. Agric. Rec., 20: 217-236.

المقاومة الحيوية لمرض عفن جذور البسلة في مصر

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أجرى هذا البحث بهدف اختيار أفضل وسائل المقاومة الحيوية لمرض عفن الجذور في البسلة المتسبب عن الفطريات (فيوزاريوم أكسيسبورم، فيوزاريوم سولاني، بيثيوم دبريانم ورايزوكتونيا سولاني) ويعتبر هذا المرض هو الأساس في خفض إنتاجية المحصول في الحقل. لذا فقد تم اختيار أحد عشر مستخلصاً نباتياً وخمسة زيوت طياره وأربع مركبات حيوية مقارنة بثلاث مبيدات فطرية في مقاومة عفن الجذور في البسلة.

أثبتت التجارب المعملية أن جميع المستخلصات النباتية كان لها تأثير معنوي في تثبيط النمو الميسيليومي للفطريات المختبرة وكان أكثرها تأثيراً مستخلصات كل من نبات العشار - الكافور - الداتورة - عنب الديب - السبوت - التين البنغالي - النعناع والونكا بينما أعطي مستخلص نباتات اللانتانا والزربيح والغاب (البوص) تأثيرا أقل على النمو الميسيليومي لنفس الفطريات. وكان الفطر بيثيوم دبريانم هو أكثر الفطريات تأثراً بمعظم المستخلصات النباتية وكان أقل الفطريات تأثراً بالمستخلصات هو الفطر فيوزاريوم سولاني.

وجد أن جميع الزيوت الطيارة المختبره ذات تأثير قوى على النمو الميسيليومي لجميع الفطريات تحت الدراسة وذلك باستخدامها في صورة نقيه بتركيزها الطبيعي ١٠٠% وكان التأثير الأقوى على النمو الميسيليومي للفطر بيثيوم دبريانم والفطر رايزوكتوينا سولاني حيث تم وقف نموها تماماً مع كل الزيوت المختبرة عند تركيز ٧٥%. بينما كان الزيت الطيار لكل من الزعتر والكافور من أفضل الزيوت الطياره في التأثير على النمو الميسيليومي حيث أنهما أوقفا النمو الميسيليومي لجميع الفطريات المختبرة حتى تركيز ٢٥%.

عند معاملة بذور البسلة بالمستخلصات النباتية قبل زراعتها في التربة المعداه بالفطريات المختبرة في الصوبة وجد أن جميع المستخلصات قد خفضت نسبة الإصابة مع اختلاف تلك النسب بين المستخلصات المختلفة. كما ثبت أن الزيوت الطيارة بتركيزاتها المختلفة ذات تأثير قوى على الفطريات الممرضة حيث قللت نسبة الإصابة وزاد معها نسب النباتات السليمة.

عند استخدام المبيدات الحيوية لمعاملة البذور قبل الزراعة بالصوبه في التربة المعداه بالفطريات وجد أنها جميعاً خفضت نسب الإصابة وكان أفضلها مركب Rhiz. N الذي أعطي أكبر نسبة من النباتات السليمة.

تم استخدام أفضل خمس مستخلصات نباتية وثلاث زيوت طياره مع المركبات الحيوية ومقارنتها بثلاث مبيدات فطرية لمقاومة المرض في أحد الحقول التي ظهرت بها الإصابة بشدة في السنوات الماضية. أعطت الزيوت الطيارة أفضل نتيجة في مقاومة المرض يليها المبيد الفطري بنليت بينما أعطت المركبات الحيوية نتائج أفضل من المبيدات الكيماوية ريزولكس والسوميسلكس. وأعطت المستخلصات النباتية نتائج جيده في المقاومة قريبة من نتائج بعض المبيدات الكيماوية.

جميع المعاملات أعطت زيادة في المحصول الخضري للبسلة وكان أفضلها المركبات

الحيوية بالرغم من أن النباتات السليمة كانت أقل محصولاً من المعاملة بالزيوت الطيارة.

لذلك فإن هذه الدراسة تشير إلي إمكانية استخدام كلا من الزيوت الطيارة، المركبات الحيوية والمستخلصات النباتية في مقاومة مرض عفن الجذور في البسلة بديلاً عن المبيدات الفطرية الكيماوية التي تحدث تلوثاً كبيراً للبيئة وتأثيرها الضار على المستهلك.