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ANTIFUNGAL ACTIVITY OF SOME PLANT EXTRACTS AGAINST DAMPING-OFF DISEASE OF LUPIN AND CHICKPEA SEEDLINGS

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The antifungal activity of five plant extracts i.e., clove, cinnamon, jojoba, eucalyptus and christ's thorn in comparison with the fungicide vitavax captan were evaluated *In vitro* and *In vivo* against damping- off fungi in lupin and chickpea seedlings. The tested fungi were *Macrophomina phaseolina*, *Fusarium solani*, *Rhizoctonia solani* and two isolates of *Fusarium semitectum.* Clove and eucalyptus oils strongly reduced the mycelial growth of the tested fungi, meanwhile the other three oils gave amoderate reduction. Dilution of oils variably reduced the mycelial growth of the tested fungi. Clove, jojoba and christ's thorn oils significantly reduced pre-emergence damping-off in lupin and chick pea seedlings and increased the survival plants.

Keywords: Lupin, chickpea, damping-off disease, plant extracts.

تأثير بعض المستخلصات النباتية كمبيدات فطرية ضد اصابة كل من الترمس والحمص بمرض موت البادرات

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تم دراسة تأثير مستخلصات خمس نباتات كمبيدات فطرية لمعاملة بذور الترمس والحمص ضد مرض موت البادرات وهى القرنفل ، القرفة ، الهوهوبا ، الكافور والسدر بالمقارنة بالمبيد الفطرى فيتافاكس كابتان. والفطريات المختبرة كانت ماكروفومينا فاسيولنيا وفيوزاريوم سولانى للترمس وعزلتين من الفطر فيوزاريوم سميتيكتم للحمص واستخدم الفطر ريزوكتوينا سولانى لكلا المحصولين. استخدم فى الدراسة المعملية خمس تركيزات من المستخلصات النباتية ١٠٠ ، ٥٠ ، ٢٥ ، ١٢,٥ ، ٦,٢٥%. وقد وجد ان مستخلصات القرنفل والكافور لهم تأثير قوى فى تقليل النمو الطولى للفطريات المختبرة بينما الثلاثة مستخلصات الاخرى فكان تأثيرها متوسط وذلك بالنسبة لتركيز ١٠٠% . أما التخفيفات المستخدمة من هذه المستخلصات فقد اعطت نتائج متباينه. للدراسة فى الصوبة استخدم تركيز ١٠٠% فقط من المستخلصات وقد وجد ان مستخلصات القرنفل والهوهوبا والسدر كان لها تأثير كبير فى تقليل الإصابة بمرض موت البادرات فى كل من الترمس والحمص مما ادى الى زيادة عدد النباتات السليمة.

INTRODUCTION

Efficiency of the medicinal and aromatic plants against the mycelial growth and spore germination of different plant pathogens was documented (Zedan *et al*., 1994 and Hassanein and EL-Doksch, 1997). The modern approach in plant diseases control is directed toward minimizing the fungicidal use to avoid environmental pollution (Baron and Tansey, 1977; Omar and abdel-Halim , 1992 and Oliveira *et al*., 1999).

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 Tripathi, 1987; Youssef, 1991; Agha 1992 and Jaspal *et al.*, 1994).

Dwivedi and Singh (1998) evaluate the aqueous extracts and essential oils of leaves and seeds of 15 angiospermic plants against the mycelial growth of *M. phaseolina in vitro* and *in vivo*. They found that the essential oil from seeds of *Trachy spermum ammi* exhibited absolute (100%) inhibition of mycelial growth. The fungicidal activity of volatiles from selected cruciferous plants against resting propaguls of soil borne fungal pathogens was studied by Smolinska and Horbowicz (1999). They found that the highest fungicidal activity was that of indian mustard against *F. oxysporum f. sp. radicis-lycopersici* and *Selerotinia sclerotiorum*. Ismail (1998) reported that the percentage of pre and post emergence damping off caused by *Sclerotium rolfesii* and *M. phaseolina* in tomato seedlings was decreased and the survival plants increased by aqueous extracts of eucalyptus, salinin and acacia leaves as soil drench. Oily extracts of chamomile, thyme and majoran caused great reduction of the *Leveillula taurica* the causative fungus of pepper powdery mildew (El-Naggar, 1997).

The present investigation was designed to study the antifungal effect of clove, cinnamon, jojoba, eucalyptus and christ's thorn oils in comparison with the

vitavax-captan fungicide against damping - off disease in lupin and chickpea seedlings *In vitro* and under green house conditions.

MATERIALS AND METHODS

I- Plant materials:-

Extracts of five plants were tested for their antifungal activity. Leaves of eucalyptus (*Eucalyptus globulus*). jojoba (*Simmonlia chinesis* L.) and christ's thorn (*Zizyphus lotus* L.); stems of cinnamon (*Cinnamomum zylanicum* L.) and buds of clove (*Syzygium aromaticum* L.) were extracted.

II- Preparation of oil crude extracts:

The extraction technique was carried out according to Bhowmick *et al.* (1981). Samples of 100 gm of dried plant materials were ground into powder and extracted by petroleum ether (boiling range 60-80^{0}C). The extracts were then concentrated under vacuum at 40^{0}C in rotary vacuum apparatus to dryness to obtain oily crude extracts. Crude extracts were considered as the 100% concentration. Dilutions 6.25, 12.5, 25 and 50% of these plant extracts, were prepared by using sterilized distilled water with tween 40 to make emulsion.

III-The tested fungi :

Two isolates of *Fusarium semitectum* (Berk.) were isolated from rotted chickpea (*Cicer arietinum* L.) seedlings and one isolate of *Fusarium solani* (Mart.) and *Macrphomina phaseolina* (Tassi) Goid were isolated from rotted lupin (*Lupinus termis* L.) seedlings grown at the Research station of Faculty of Agriculture Alexandria University. *Rhizoctonia Solani* was isolated from rotted soybean seedlings grown at Noubaria Research Station ARC. Pathogenicity of these fungi was previously detected in another work (El-Safwani 2002).

IV- Inhibitory effect of plant extracts on mycelial growth:

The tested fungi were grown on PDA medium for 7 days. Each fungus was scratched gently with sterilized cutter from the surface of PDA plate and added to milted PDA medium at 40^{0}C and shaken gently before solidification and poured in petri-dishes. Sterilized filter paper disks (Whatman No.1, 9 mm diameter) were saturated with 50 µl of plant extract. Such treated filter papers were then placed on surface of the plats, which previously inoculated, with one of the tested fungi. Four replicates were used for each treatment. The plates were incubated at 25^{0}C for 7 days and the percentage of reduction in the mycelial growth was calculated. The obtained data were statistically analyzed according to Snedecor and Cochran (1967).

V- Green house experiment:

Two isolates of *F. semitectum* and *R. solani* were checked with chickpea (cv. Giza 195) and *F. solani, M. phaseolina* and *R. solani* were checked with lupin (cv Giza 2). The tested fungi were grown for two weeks at 25^{0}C in 500 ml bottles containing autoclaved cornmeal medium (50 gm corn + 40 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) were filled with inoculated soil at the rate 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. Lupin and chickpea seeds were sterelized with sodium hypochlorite 1% for 2 minutes and then washed for three times with sterelized water. Seeds of lupin and chickpea were soaked in tested oil (crude extracts) for one hour (Zedan *et al.* 1994). Ten treated

seeds were sown in each pot. The plants were weekly inspected for 30 days. Data were statistically analysed as Randomize Complete Block Design suggested by Snedecor and Cochran (1967). Least significant difference (L.S.D. at 5% probability) was used to compare between treatment averages.

RESULTS AND DISCUSSION

I- Inhibitory effect of plant extracts on the mycelial growth:

The antifungal properties of five oily extracts (clove, cinnamon, jojoba, eucalyptus and christ's thorn) against phytopathogenic isolates of *M. Phaseolina, F. solani*, two isolates of *F. semitectum* and *R. solani* were evaluated *in vitro* by disk diffusion assay. The results presented in (Table 1) showed that the tested plant oils exhibited a variable degree of antifungal activity against the tested fungi.

Clove oil exhibited the strongest fungicidal action against *F. solani, F. semitectum* (isolate no 1and 2) and R. Solani 80.37, 80.0, 72.29 and 74.07%, respectively and a moderate effect against *M. phaseolina* (56.85%). The efficiency of oils as antifungal decreased with their dilutions. It is the only tested oil, which reduced the mycelial growth of *R. solani* with all dilutions.

Concentrations of cinnamon oil showed different antifungal activity with all tested fungi. In case of *R. solani* the dilutions 6.25, 12.5% has no antifungal effect. Cinnamon oil gave a good activity against *M. phaseolina* and *F. semitectum* isolate no (2), 68.15 and 64.82%, respectively and a moderate effect against the other tested fungi.

Jojoba oil gave a moderate reduction on the mycelial growth of *F. solani, M. phasoolina* and *F. semitectum* (isolate no 2) 45.52, 42.22 and 36.29%, respectively and a slight activity against *F. semitectum* isolat no (1) 24.07%. Jojoba oil and its dilutions did not affect on the mycelial growth of *R. solani*.

Eucalyptus oil exhibited the strongest antifungal activity against *M. phaseolina, F. semitectum* isolate no (2), *R. solani* and *F. semitectum* isolate no (1) 88.89, 87.7, 73.7 and 69.63%, respectively. The reduction in the mycelial growth of *F. solani* was 60.74% .The dilutions gave variable degrees of mycelial growth reduction ranged between 34.82 and 71.11%. Christ's thorn oil showed a moderate inhibitory effect on the mycelial growth of *F. solani, F. semitectum* (isolates 1&2) and *M. phaseolina* 54.82, 50.0, 35.93 and 30.37%, respectively and gave slight inhibitory effect against mycelial growth of *R. solani* (19.62).

Table (1): Inhibitory effects of oily extracts certain plants on the mycelial growth of five phytopathogenic fungi.

Oils	Dilutions %	Reduction percentage in the mycelial growth				
		F. solani	*M. phaseolina*	*F. semitectum* (1)	*F. semitectum* (2)	*R. solani*
Clove(*S. aromaticum*)	6.25	18.85	24.08	37.4	38.88	11.11
	12.5	71.85	36.03	47.41	47.97	19.62
	25.0	74.08	46.29	68.89	59.00	24.06
	50.0	79.26	56.3	72.22	72.97	42.22
	100.0	80.37	56.85	80.00	72.29	74.07
Cinnamon(*C. cinnamomum*)	6.25	45.93	37.77	44.45	33.33	0.0
	12.5	48.89	40.37	46.67	28.40	0.0
	25.0	49.26	52.22	51.49	41.49	36.29
	50.0	52.22	64.08	50.01	60.37	39.25

	100.0	50.74	68.15	57.78	64.82	48.51
Jojoba (*S. chinesis*)	6.25	45.15	16.30	19.26	13.71	0.0
	12.5	44.45	23.71	19.63	14.82	0.0
	25.0	44.78	29.66	18.52	14.45	0.0
	50.0	44.45	37.78	22.96	18.15	0.0
	100.0	45.52	42.22	24.07	36.29	0.0
Eucalyptus (*E. globulus*)	6.25	48.15	44.45	34.82	37.78	0.0
	12.5	47.78	47.04	41.86	40.37	0.0
	25.0	47.04	49.63	48.52	40.74	0.0
	50.0	46.26	71.11	48.15	53.71	0.0
	100.0	60.74	88.89	69.63	87.70	73.7
Christ's thorn (*Z. lotus*)	6.25	45.12	44.45	41.11	21.11	0.0
	12.5	47.78	46.30	42.22	22.22	0.0
	25.0	48.15	39.67	40.67	27.04	0.0
	50.0	52.22	44.45	46.74	37.03	0.0
	100.0	54.82	30.37	50.00	35.93	19.62
L.S.D. 5% Oil		2.02	2.31	2.14	2.31	1.06
Dilutions		2.02	2.31	2.14	2.31	1.06
Oil x Dilution		4.53	5.61	4.79	5.17	2.38

- **The growth of control treatments are considered 100%**

The present work is similar to that reported by Pattnaik *et al.* (1996). They found that eucalyptus and peppermint oils were effective against *F. solani*, *F. oxysporum* and *M.phaseolina in vitro*. Also Zambonelli *et al.*, (1996) reported that the mycelial growth of *R.solani*, *Pythium ultimum, F. solani* and *Colletotrichum lindemuthianum* was strongly inhibited by certain compound in thymol oils.

II- Control of lupin and chickpea damping-off with five plant extracts:

The effect of five plant oils on pre and post-emergence damping-off incidence caused by *M.phasealina. F. solani* and *R. solani* and percentages of survived plants on lupin seedling was studied. Data presented in (Table 2) showed that the pre-emergence damping-off of lupin seedlings in treatments infested with *M. phaseolina. F. solani* and *R. solani* were 66.66, 55 and 65%, respectively. Differences between plants showed that post-emergence damping-off caused by those fungi were not significant.

Table (2): Effect of five oily plant extracts on pre and post-emergence damping – off of lupin seedlings caused by *M. phaseolina, F. solani* and *R. solani.*

Oily plant extracts (100%)	Pre and post- emergence damping-off (%)								
	M. phaseolina			*F. solani*			*R. solani*		
	% pre emergence	% post-emergence	% survivor plants	% pre emergence	% post-emergence	% survivor plants	% pre emergence	% post-emergence	% survivor plants
Clove	10.0	6.66	83.34	10.0	3.33	86.67	23.33	3.33	73.34
Cinnamon	40.0	3.33	56.67	23.33	0.0	76.67	60.0	0.0	40.0
Jojoba	25.0	0.0	65.0	15.0	0.0	85.0	26.66	0.0	73.34
Eucalyptus	55.0	3.33	41.67	18.33	11.66	70.01	45.0	6.66	48.34
Christs thorn	6.66	10.0	83.34	3.33	0.0	96.67	23.33	0.0	76.67
Vitavaxcaptan	10.0	0.0	90.0	3.33	6.66	90.01	10.0	6.66	83.34
Infested control	66.66	10.33	23.01	55.0	3.33	41.67	65.0	0.0	35.0
Uninfested control	0.0	0.0	10.0	3.33	0.0	96.67	3.33	0.0	96.67
L.S.D. 5%	14.38	N.S	24.61	14.22	N.S	20.36	17.72	N.S	28.38

Oil extracts (100%) of four plants, i.e. clove, cinnamon, jojoba and christ's thorn significantly reduced lupin pre-emergence damping-off caused by *M.*

phaseolina 10, 40, 35 and 6.66 respectively. Oil extracts of clove and christ's thorn plants were as effective as the tested fungicide vitavax captan in reducing lupin pre-emergence damping-off. There was no significant difference between obtained data from eucalyptus oil treatment and infested control. Data also revealed that differences in post-emergence damping-off incidence between all treatments and infested control were not significant. As a result of the reduction in disease incidence the survived plants were increased. Data also showed that the tested oils and the fungicide vitavax captan significantly reduced the percentage of pre-emergence damping-off caused by *F. solani* 10, 23.33, 15, 18.33, 3.33 and 3.33% for clove, cinnamon, jojoba, eucalyptus, christ's thorn and vitavax captan respectively. Post-emergence damping-off caused by *F. solani* was negligible. Due to the reduction in pre-emergence damping-off as a result of oil extract treatments and vitavax captan the percentage of survived plants was increased.

Clove, jojoba, christ's thorn and the fungicide vitavax captan significantly reduced pre-emergence damping-off of lupin seedlings caused by *R. solani* 23.33, 26.66, 23.33 and 10% respectively, while in infested control it was 65%. The reduction in disease incidence increased the survived plant. There was no significant differences between all treatments and infested control in case of post-emergence damping-off caused by *R. solani.*

These results are in harmony with that reported by Martini *et al.* (1996). They evaluate the antifungal effect of eugonol and carvacol in ethanolic extract of clove against *Cladosporium herbarum and Penicillium glubram.* Both compounds were exclusively responsible for the antifungal action against the tested fungi. Moreover the aqueous extract of garlic, ginger, eucalyptus, salinin and acacia inhibited mycelial growth of *M. phaseolina, S. rolfsii* and *F. oxysporum* f. sp *lycopersici* with no phytoxicity observed on seedlings of cotton and tomato (Ismail 1998 and Raja and Kurucheve 1999).

The effect of five plant oils on pre and post-emergence damping-off incidence caused by two isolates of *F. semitectum* and *R. solani* and the percentages of survived plants in chickpea seedlings was studied.

Data presented in (Table 3) showed that oil extracts of four tested plants, i.e clove, jojoba, eucalyptus and christ's thorn significantly reduced chickpea pre-emergence damping-off caused by *F. semitectum* isolate no (1) 18.33, 3.33, 11.66 and 3.33%, respectively while in infested control pre-emergence damping-off was 60%. The fungicide vitavax captan significantly reduced the pre-emergence damping-off. Data also, revealed that differences in post-emergence damping- off were not significant. As a result of the reduction in disease incidence the survived plants were increased.

All the tested oils and the fungicide vitavax captan significantly reduced the percentage of pre-emergence damping-off caused by *F. semitectum* isolate no (2) 6.66, 28.33, 26.66, 18.33, 26.66 and 15% for clove, cinnamon, jojoba, eucalyptus, christ's thorn and the fungicide respectively, while infested control was 65%.

Table (3): Effect of five oily plant extracts on pre and past-emergence damping –off of chickpea seedlings caused by two isolates of *F. semitectum* and *R. solani.*

Oily plant extracts (100%)	Pre and post- emergence daming-off (%)		
	F. semitectum isolate (1)	*F. semitectum isolate* (2)	*R. solani*

	% pre emergence	% post-emergence	% survivor plants	% pre emergence	% post-emergence	% survivor plants	% pre emergence	% post-emergence	% survivor plants
Clove	18.33	10.0	71.67	6.66	6.66	86.68	10.0	3.33	86.67
Cinnamon	45	0.0	55.0	28.33	5.0	66.67	65.0	0.0	35.0
Jojoba	3.33	3.33	93.34	26.66	5.0	68.34	15.0	0.0	85.0
Eucalyptus	11.66	10.0	78.34	18.33	5.0	76.67	15.0	3.33	81.67
Christs thorn	3.33	3.33	93.34	26.66	3.33	70.34	18.33	3.33	78.34
Vitavax captan	10.0	10.0	80.0	15.0	6.66	78.34	25.0	0.0	76.0
Infested control	60.0	0.0	40.0	65.0	10.0	25.0	75.0	3.33	21.67
Uninfested control	3.33	3.33	93.34	0.0	3.33	96.67	0.0	0.0	100.0
L.S.D. 5%	16.36	N.S.	28.38	17.07	N.S.	20.98	19.76	N.S.	25.4

The post-emergence damping-off was negligiale and the survived plants were increased as a result of the reduction in pre- emergence damping-off.

Clove, jojoba, eucalyptus, christ's thorn and the fungicide exhibited good antifungal activity against *R. solani* in chickpea seedlings. The percentages of pre-emergence damping-off incidence were 10, 15, 15, 18.33 and 25%, respectively. The reduction in disease incidence increased the survived plants. There was no significant difference between all treatments and infested control in case of post-emergence damping-off caused by *R. solani.*

These results are in agreement with that reported by Khan *et al.*(1996). They made a comparative study of the efficacy of some plant extracts and fungicides on mycoflora of chickpea seeds. The results revealed that the plant extracts of *Calotropis procera* and the fungicide benlate reduced the number *of F. oxysporum* and *M. phaseolina* in chickpea seeds and increased the germination percentages. Also EL-Korashy (1997) reported that the extract of *Mentha spicata* at concentrations of 50 and 100% inhibited the growth of *R. solani, F. solani* and *S. rolfsii,* which cause damping-off disease of peanut plants. The determination of the fungitoxic component in the essential oil of *Thymbra spicat, Satureja thymbra, Salvia fruticosa, Laurus nobilis, Mentha pulegium* and *Eucalyptus comldulensis* was studied by Muller-Riebau *et al.* (1995). They found that fungitoxicity of the essential oils against the soil borne fungi, i.e. *F. moniliform, R. solani, S. sclerotiorum* and *Phytophthora capsici* was due to different concentrations of the phenolic fraction (especially thymol and /or carvacrol).It could be concluded that use of natural plant extracts to control fungal plant diseases may be extended in the future, instead of fungicides which destroy the natural equilibrium and cause sever environmental pollution.

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تأثير بعض المستخلصات النباتية كمبيدات فطرية ضد اصابة كل من الترمس والحمص بمرض موت البادرات

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تم دراسة تأثير مستخلصات خمس نباتات كمبيدات فطرية لمعاملة بذور الترمس والحمص ضد مرض موت البادرات وهى القرنفل ، القرفة ، الهوهوبا ، الكافور والسدر بالمقارنة بالمبيد الفطرى فيتافاكس كابتان. والفطريات المختبرة كانت ماكروفومينا فاسيولنيا وفيوزاريوم سولانى للترمس وعزلتين من الفطر فيوزاريوم سميتيكتم للحمص واستخدم الفطر ريزوكتوينا سولانى لكلا المحصولين. استخدم فى الدراسة المعملية خمس تركيزات من المستخلصات النباتية ١٠٠ ، ٥٠ ، ٢٥ ، ١٢,٥ ، ٦,٢٥%. وقد وجد ان مستخلصات القرنفل والكافور لهم تأثير قوى فى تقليل النمو الطولى للفطريات المختبرة بينما الثلاثة مستخلصات الاخرى فكان تأثيرها متوسط وذلك بالنسبة لتركيز ١٠٠% . أما التخفيفات المستخدمة من هذه المستخلصات فقد اعطت نتائج متباينه. للدراسة فى الصوبة استخدم تركيز ١٠٠% فقط من المستخلصات وقد وجد ان مستخلصات القرنفل والهوهوبا والسدر كان لها تأثير كبير فى تقليل الإصابة بمرض موت البادرات فى كل من الترمس والحمص مما ادى الى زيادة عدد النباتات السليمة.