Evaluation of Some Essential Oils for Controlling Some Pathogenic Fungi Associated With Broad Bean Seeds

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Abstract: Two essential oils were tested for their antifungal activity against damping-off disease (which causes with *Rizoctonia solani* and *Fusarium solani* as a pathogenic fungi associated with broad bean seeds, name clove (Syzygium aromaticum L.), and thymy. Clove oil was the most effective followed by thyme oil. Also, inhibition activity of the two tested essential oil evidently increased by increasing their concentration.the most sensitive fungus to clove oil was R. *solani*, also, the most sensitive fungus to thyme was R. *solani* under greenhouse conditions, oil treatments significantly reduced the damping-off of broad bean when compared with chemical treatment, the highest reduction in damping-off was observed on socked seeds in clove oil or thymy oil at 24h. The best crop parameter were recorded from plants resulted from seeds soaked with clove oil. It gave the length (stem-root) in soil infested with R. solani, F. solani and mixed (R+F) being (38.2 - 22.9 cm), (37.0 - 23.7 cm) and (31.6 - 21.4 cm), and improved number, fresh weight and dry weight of nodules compared with control.

Keywords: Damping-off disease, Broad bean, Clove oil, Thyme oil

INTRODUCTION

Broad bean (*Vicia faba* L.) is one of the most important legume crops in Egypt. It is grown mostly to fulfill food and feed requirements for human and animal consumption. Seeds of broad bean are rich in protein 26:28 % and some other compounds (El-Sayed *et al.*, 1982).

Broad bean is attacked by certain seed borne fungi causing damping-off and root rot diseases which seriously affected both plant stand and yield production (El-Wakil, et al., 2009). In this respect damping-off and root rot diseases are the most important disease affecting broad bean production in Egypt. In this respect (Omar1986., El-Morsy et al., 1997, Ying, et al., 2001, Hugar, 2004, and Ali, et al., 2009) isolated Fusarium. oxysporum, Fusarium solani Rhizoctonia solani, Sclerotium rolfsii Sacc, and Macrophomina phasiolina.

Fungal plant diseases are usually controlled by application of fungicides (Maloy, 1993). Among the different management strategies used in controlling fungal plant diseases, chemicals rule the roost. However extensive use of chemicals as antifungal agents might lead to severe side effects such as carcinogenicity terotogenecity, oncogenecity and other genotoxic properties (Basilico and Basilico, 1999). Further extensive use of chemicals leads to biohazordous effects on ecosystem. Further, their persistent applications lead to resistance in pathogens against these fungicides (Brent, 1995). Thus alternative approaches are preferred which are ecofriendly (Anandraj and Leela, 1996).

Essential oils from plant edible parts which are ecofriendly in nature have been used by several workers for controlling fungi, bacteria, viruses and insect pests (Singh and Upadhyaya, 1993; Singh, 1996). The main reasons for using essential oils as antifungal agents is their natural origin and low chance of pathogens developing resistance. The complexicity of essential oils are attributed to their terpene hydrocarbons and their oxygenated derivatives such as alcohols, aldehydes, ketones, acids and esters (Tzortazakis *et al.*, 2007). Antifungal properties of plant essential oils have been reported by researchers throughout world (Bouchra et al., 2003; Daferera et al., 2003). Even researchers in Egypt have reported a number of essential oils for their antifungal activity (Ismayl et al., 2006). A good number of essential oils are reported to be effective against many phytopathogenic fungi (Srivatsava and Singh, 2001). A lot of researchers have documented the antimicrobial activity of essential oils including, clove and thyme oils against different fungal species (Srivatsava and Gouda,2001., Bouchra et al., 2003; Chami et al. 2005., Taha 2005 and Viuda-Martos, et al., 2007). Am to hence, in the present study, some important essential oils of aromatic plant species have been screened for their antifungal activity against seed borne and damping-off diseases of broad bean. The nontoxic, non-pollutive and biodegradable nature of these essential oils prompted to exploit these natural products of higher plants against Rhizoctonia solani, and Fusarium solani.

MATERIALS AND METHODS

Source of Fungal Pathogens

Rhizoctonia solani and Fusarium solani were isolated during a course of ph.D. Study of Mr. H, A. A. Abd El-Wahab (Unpublished data), and these pathogens were selected for further investigation

Preparation of Two Essential Oils

Two essential oils were tested for their antifungal activity against damping-off disease (which causes with *Rizoctonia solani* and *Fusarium solani* as a pathogenic fungi associated with broad bean seeds, name clove (Syzygium aromaticum L.),and thymy (These essential oils were obtained from horticulture research Station, Aromatic and Medicine plants, Department of Sabahia, Alex., Egypt.

In vitro Experiments

Different concentration of the tested plant oils, i.e., 0, 12.5, 25, 50, 75 and 100% were dissolved using acetone and adding tween 40 plus sterilized distilled 78

water. Petri-dishes (9 cm in diam.) with PDA media were used. Five mm disc of 7days old culture of the tested fungus was placed at the edge of the Petri-dish. On the opposite side 5mm sterilized filter paper discs (What man No.1) were saturated with 50Ul of plant oils and placed. Control treatment was carried out using sterilized water instead to plant oils four replicates were used for each treatment.

Preparation of Pathogenic Fungal Inocula

Corn meal-sand medium (3:1, w/w) were autoclaved in 500 ml glass bottles at 121 °C for 20 min. The sterilized bottles were then inoculated with disks (5 mm) of 7 days old culture of any of *R* solani and *F*. solani and incubated at 25 ± 1 °C for 15 days. Broad bean (*Vicia faba* L.) seeds: variety (Giza 2) of broad bean was provided by Unit, Field Crops Research Institute, Agric. Res. Center (ARC), Giza, Egypt.

Greenhouse Experiment

The experiment was conducted in plastic pots 25 cm diameter. The pots were sterilized with 5% formalin solution and left to dry before use. Fungal inocula were mixed with the potted sterilized soil at the rate of 2 and 5% for *R. solani* and *F. solani*, respectively. The infested soils were watered daily for one week to enhance growth and distribution of the inocula.

Seed Treatment

Seeds of broad bean cv. (Giza2) were soaking in Two essential oils 6, 12 and 24 houres. Treated seeds were sown in infested soil with tested pathoginic fungi (*Rizoctonia solani* and *Fusarium solani*) at the rate 2-5% (W/W) in pots 15 cm in diameter, respectivly (El-Garhy, 2000). and then they were planted at the rate of 10 seeds per pot. Three pots were, however, used per each treatment. Percentages of pre- and post-emergence damping-off and survivals 15, 45 and 60 days after planting, respectively, were calcualated as follows:

% pre-emergence damping-off = $\frac{No. of non-emerged seedlings}{No. of planted seedl} \times 100$ % post-emergence damping-off = $\frac{No. of dead seedlings}{No. of planted seedls} \times 100$ % survivals = $\frac{No. of healthy seedlings}{No. of planted seedls} \times 100$

% SUIVIVAIS No. of planted seeds X 10

Data were taken after 50 days, number of nodules per plant and the dry weight of nodules (mg), were recorded. The number of plants were estimated, while in addition 60 days, plant height (cm), the fresh and dry weights of root and shoots (g) were determined.

RESULTS

Effect of Different Concentrations of Two Tested Plant Containing Oils on The Mycelial Growth of Two Pathogenic Fungi Associated with Broad Bean Seeds

Data in Table (1) indicated that clove oil inhibited significantly ($P \le 0.5$) growth of *R. solani* and *F. solani* at all tested concentrations. While, thyme oil inhabited the liner growth of *R. solani* and *F. solani* at concentrations 25%, 50%, 75% and 100%, respectively. Clove oil was the most effective caused the highest significant reduction on mycelial growth of *R. solani* followed by *F. solani* (88.89% and 85.55%) to the highest concentrations (100%) respectively. On the other hand, the inhibition of the pathogens growth was correlated with the increase of essential oil concentration.

Effect of Some Essential Oils on Damping-Off and Survival of Broad Bean Was Evaluated in Greenhouse

Data presented in Table (2) show that all treatment significantly reduced damping-off broad bean at both pre and post-emergence stages in comparison with check treatment, data also show that oil treatments significantly reduced the damping-off of broad bean when compared with chemical treatment, the highest reduction in damping-off was observed on socked seeds at 24h where the recorded pre and post-emergence damping-off stage were 6.7, 3.3 and 6.7% as well as 0.0, 0.0 and 10.0% for *F solani*, *R solani* and mixed F+R, in respective order.

Thymy oil recorded 6.7, 0.0 and 10.0% as well as 3.3, 6.7 and 20.0% for F solani, R solani and mixed F+R. On the other hand, seeds treatment with Rhizolex-T 3g/kg resulted in significantly reduction in damping-off caused by all pathogens tested comparing with the cheek treatment at pre and post-emergence damping-off stage.

 Table 1. Effect of different concentration of two tested oils on the mycelial growth-cm of two pathogenic fungi associated broad bean seeds.

			L	inear grov	wth of myc	elium (cm)		_	
Concentration	Fusariu	sarium solani Rhizoctonia solani							
%	Clove	ve thyme Clove thy							
	L. G.*	R%	L. G.	R%	L. G.	R%	L. G.	R%	
0.0	9.0	0.0	9.0	0.0	9.0	0.0	9.0	0.0	
12.5	7.9	12.2	9.0	0.0	7.9	12.2	9.0	0.0	
25.0	6.0	33.3	8.7			37.8	8.6	4.4	
50.0	4.1	54.4	7.1	21.1	4.0	55.6	7.6	15.6	
75.0	2.5	72.2	6.0	33.3	2.3	74.4	5.3	41.0	
100.0	1.3	85.6	3.7	58.9	1.0	88.9	3.6	60.0	
L. S. D.at 5%	0.69		0.49		0.48		0.62		

*L.G = Linear growth, R% = G1 – G2 / G1 x100, (G1: growth of control, G2 Growth of treatment)

Effect of Soaking Seeds of Broad Bean in Essential Oils on Some Growth Parameter

Table (3) show that vegetative parameter significantly increased in plants grown from seeds soaked in any of the six tested essential oil over the control. Maximum stem length, fresh weight (stem-root) and dry weight was obtained from seeds soaked in clove oil at (24h) in soil infested with R solani whereas, treatment seeds soaked in clove oil (24h) in soil infested with R solani significantly increased length (stemroot)and fresh weight of (stem-root) per plant as well as dry weight (stem-root), compared with control. Maximum length (being 38.2 23.7cm) (26.1 and 14.09) for length and fresh weight respectively and dry weight (being 4.0 and 0.35) respectively. Generally clove oil (24h) gave the length (stem-root) in soil infested with R solani, F solani and mixed (R+F) being (38.2-2.29cm), (37.0-23.7cm) and 31.6-21.4cm). The best crop parameter were recorded from plants resulted from seeds soaked with clove oil.

Effect of Some Essential Oils for Controlling Some Pathogenic Fungi Associated with Broad Bean Seeds on Number, Fresh and Dry Weight of Nodules Under Greenhouse Conditions

Table (4) Data indicated that Clove oil or Thymy oil significantly improved number, fresh weight and dry weight of nodules compared with control. Essential oils was the more effective on F solani than R solani and mixed on socked seeds at 24h in increasing nodules fresh weight and dry weight of nodules, 1n case clove oil (31.7, 42.2 and 11.39), (27.7, 39.5 and 10.66) and (21.7, 35.0 and 9.45) respectively.

DISCUSSION

The objective was to study the effect of essential oils on the mycelial growth of R. solani, F. solani. The large scale application of synthetic fungicides has been cautioned due to their non-biodegradability, pollutive nature and residual toxicities. Chemicals also considered being deleterious for associated soil microbiota (Bunker and Mathur, 2001). Synthetic chemicals are also known to possess carcinogenic, teratogenic, oncogenic and genotoxic properties. Further many plant pathogens can develop resistance to synthetic fungicides with continuous exposure (Brent, 1995). Most of these chemical fungicides have been condemmened by environmentalists and are considered to be the most important man-made pollutant (Khoshoo, 1980). This has led to finding ecofriendly alternative approaches for management of plant diseases (Lyon et al., 1967; Cook and Baker, 1983; Ahmed et al., 1999; Parveen and Kumar, 2004). Softer biological measures for the control of plant diseases are gaining popularity in recent years Biocontrol agents are considered are better alternatives with different mechanisms of action than chemical pesticides. A lot of researchers have documented the antimicrobial activity of essential oils including, clove and thyme oils against different fungal species (Mishra and Dubey, 1994; Viuda-Martos, et al.,

2007). The present study has evaluated the effect of two essential oils on R. solani, F. solani F oxyspoum isolated from broad bean seeds

These results confirm the antimicrobial activity of all the essential oils used in the present study. Clove oil showed excellent growth inhibition of the fungus followed by the essential oils of thyme, the levels of essential oils and their compounds necessary to inhibit fungal growth are higher in practical condition than in culture media. This can be due to interaction between the phenolic compounds and other environmental factors (Nuchas and Tassou, 2000) and so, should be considered for commercial applications (Tzortzakis et al., 2007). This study indicated that plant essential oils possess antifungal activity and can be exploited as an ideal treatment for future plant disease management programs eliminating fungal spread. Recently, there has been great interest in essential oils from aromatic plants for controlling plant pathogens (Soliman and Badeaa, 2002; Valero and Salmeron, 2003).

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	Fusarium sola	ni		Rhizoctonia so	olani		Fusarium sola	ni + Rhizoctonia s	solani
Trees a form a met	Damping-off	%		Damping-off	%		Damping-off ⁴	%	
Treatment	Pre-	Post-	Survivals %	Pre-	Post-	Survivals %	Pre-	Post-	Survivals %
	emergence	emergence		emergence	emergence		emergence	emergence	
Clove oil (6hr.)	10.0	3.3	86.7	6.7	10.0	83.3	13.3	16.7	70.0
Clove oil (12hr.)	10.0	0.0	90.0	0.0	6.7	93.3	13.3	6.7	80.0
Clove oil (24hr.)	6.7	0.0	93.3	3.3	0.0	96.7	6.7	10.0	83.3
Thyme oil (6hr.)	10.0	6.7	83.3	10.0	16.7	73.3	23.3	16.7	60.0
Thyme oil (12hr.)	10.0	3.3	86.7	6.7	13.3	80.0	13.3	16.7	70.0
Thyme oil (24hr.)	6.7	3.3	90.0	0.0	6.7	93.3	10.0	20.0	70.0
Rizolex T	0.0	0.0	100	0.0	0.0	100	3.3	3.3	93.4
Control	23.3	6.7	70.0	16.7	30.0	53.3	30.0	36.7	33.3
L.S.D.at 5%	N.S	N.S	N.S	N.S	12.89	12.46	N.S	16.38	21.74

Table 2. Effect of clove and thyme essential oils on incidence of damping-off and survivals plants of broad bean under greenhouse conditions.

Table 3. Effect of clove and thyme essential oils on some growth parameter under greenhouse conditions.

	Fusar	ium sold	ani				Rhizo	ctonia s	olani				Fusar	ium sold	ani + Rh	izoctonia se	olani	
Treatments	Lengt	h cm.	Fresh	weight g.	Dry w	eight g.	Lengt	h cm.	Fresh	weight g.	Dry w	eight g.	Lengt	h cm.	Fresh	weight g.	Dry w	eight g.
	stem	root	stem	root	stem	root	stem	root	stem	root	stem	root	stem	root	stem	root	stem	root
Clove oil (6hr.)	30.6	19.4	20.3	14.0	2.8	0.30	29.8	19.1	21.5	10.9	3.0	0.27	25.3	15.3	19.2	8.4	2.5	0.17
Clove oil (12hr.)	32.5	20.9	21.7	14.2	3.0	0.35	31.5	19.6	23.0	12.5	3.4	0.29	30.1	18.0	21.0	9.0	2.8	0.18
Clove oil (24hr.)	37.0	23.7	24.8	19.6	3.5	0.45	38.2	22.9	26.1	14.0	4.0	0.35	31.6	21.4	23.5	13.5	3.1	0.18
Thyme oil (6hr.)	30.0	16.1	20.1	10.8	2.6	0.25	30.2	17.8	22.7	10.3	3.2	0.25	24.0	15.2	20.5	8.4	2.5	0.15
Thyme oil (12hr.)	34.0	20.1	20.4	12.6	2.4	0.27	31.4	19.5	22.9	11.9	3.3	0.25	30.0	17.6	22.4	10.2	2.9	0.16
Thyme oil (24hr.)	35.7	21.3	22.3	14.8	3.1	0.39	34.6	20.6	25.6	12.7	3.8	0.35	30.8	20.0	22.9	11.6	3.2	0.17
Rizolex T	35.9	24.5	28.4	16.4	4.5	0.45	34.5	23.1	27.4	16.0	4.1	0.45	33.1	20.7	26.3	15.1	3.8	0.42
Control	27.3	13.5	14.7	7.8	2.3	0.15	19.4	12.0	13.8	6.4	2.1	0.11	15.7	9.0	10.3	5.7	1.5	0.09
L.S.D.at 5%	N.S	5.7	4.9	4.4	0.95	0.08	5.54	4.52	4.35	4.74	0.63	0.06	4.45	5.43	4.17	4.87	0.46	0.08

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E	Fusarium solani			Rhizoctonia solani	ni	-	Fusarium solani + Rhizoctonia solani	i + Rhizoctonia	solani
Ireautient	No. of nodules	Wet weight	Dry weight	No. of nodules	Wet weight	Dry weight	No. of nodules	Wet weight	Dry weight
Clove oil (6hr.)	30.7	41.7	11.8	27.3	38.3	10.34	20.7	34.6	9.34
Clove oil (12hr.)	30.7	41.3	11.15	27.7	39.2	10.58	21.7	34.6	9.34
Clove oil (24hr.)	31.7	42.2	11.39	27.7	39.5	10.66	21.7	35.0	9.45
Thyme oil (6hr.)	30.3	40.8	11.01	27.0	38.1	10.28	20.0	32.6	8.80
Thyme oil (12hr.)	30.7	41.4	11.18	27.7	38.7	10.42	21.3	33.5	9.04
Thyme oil (24hr.)	31.3	41.7	11.26	27.7	38.6	10.42	21.7	33.3	8.99
Rizolex T	30.7	41.0	11.07	30.7	40.5	10.93	30.3	41.2	11.12
Control	12.3	23.9	6.45	0.6	17.9	4.83	3.7	7.8	2.10
L.S.D.at 5%	5.71	7.24	0.70	4.88	5.02	0.29	4.35	3.22	0.65

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تقييم بعض الزيوت الطيارة في مقاومة الفطريات الممرضة المصاحبة لبذور الفول البلدي

متولى على بركة *، عبد العزيز أحمد سلام *، وليد ابر اهيم شعبان * وحسن عبد المنعم عبد الوهاب ** *كلية الزراعة جامعة قناة السويس ** معهد بحوث امراض النباتات مركز البحوث الزراعية

تم استخدام بعض الزيوت النباتيه (زيت القرنفل- زيت الزعتر) ومعرفه تاثيرها على الفطريات (ريزوكتونيا سولاني و فيوزاريم سولاني) تحت ظروف المعمل بتركيزات مختلفه وجد ان كلما زاد التركيز ادي الي نقص شديد في النمو الميسيليومي وكمان اكثر الفطريات تأثر ا بالزيوت فطر ريزوكتونيا سولاني، وزيت القرنفل كان اكثر تأثيرا عن زيت الزعتر. تحت ظروف الصوبة ادت معامله البذور بزيت القرنغل الي تقليل نسبة الأصابه بمرض موت البادرات وزيادة نسبه النباتات السليمة، مما اثر ذلك على الفريات المحصولية من عدد العقد الجذرية ووزن النباتات الرطب والجاف اكثر من المعامله بزيت الزعتر وخاصة عند معاملة البذور بزيت المعامين المعام ال ١٢ او ٦ ساعات.