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ESSENTIAL OILS AND *TRICHODERMA HARZIANUM* AS AN INTEGRATED CONTROL MEASURE AGAINST FABA BEAN ROOT ROT PATHOGENS

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Abstract: Carnation, caraway, thyme, peppermint and geranium essential oils have been found to have inhibitory effects against the mycelial growth of *Fusarium solani, Rhizoctonia solani, Sclerotium rolfsii* and *Macrophomina phaseolina* under *in vitro* conditions. Complete inhibition of fungal growth was observed with the use of 4% carnation and geranium oils. Mycelial growth of the tested fungi showed more sensitivity to high concentrations of thyme than to caraway and peppermint oils. Moreover, essential oils used to coat seeds resulted in a significant reduction of root rot incidence of bean, at both pre- and post-emergence stages under greenhouse conditions. Under field conditions seeds coated with essential oils at a concentration of 4% sown in soil treated with the bio-agent *Trihoderma harzianum,* gave pronounced protection to emerged bean seeds against the invasion of root rot pathogenic fungi. Compared to the control, the above treatment resulted in a reduction of disease incidence at the pre-emergence stage. This reduction was calculated to be between 47.3 and 55.4% compared with a 16.1% reduction with the use of the Rizolex-T treatment. At the post-emergence stage, all applied treatments were able to reduce the percentage of root-rot incidence. Reduction ranged between 41.4 and 47.1% over the untreated control. Reduction in disease incidence was reflected in a yield increase of 15.1–28.8% and 40.1–50%, in seeds coated with one of the different essential oils, or combined with *T. harzianum* soil treatments, in the respective order. Seeds coated with the fungicide Rhizolex-T caused a yield increase estimated as 11.3% over the check treatment. These results show that application of essential oils in integration with the bio-agent *T. harzianum* may be considered as an applicable, safe and cost-effective method for controlling such soilborne diseases.

Key words: carnation oil, caraway oil, essential oil, faba bean, *Fusarium solani*, Geranium oil, peppermint oil, root rot, *Rhizoctonia solani* , Rhizolex-T, *Trichoderma harzianum,* thyme oil

INTRODUCTION

Legumes are of paramount importance in the Egyptian diet. Faba bean (*Vicia fabae*) is one of the most important of the leguminous crops, being cultivated on an area of ~160,000 ha and yielding ~640,000 tons (Anonymous 2006). Dry seeds of high quality are used for meals and in the food industries. Low quality seeds, in addition to plant wastes, are used for animal feed. Faba bean is attacked by certain soilborne fungi, *i.e. Rhizoctonia solani*, *Fusarium solani, Sclerotium rolfsii* and *Macrophomina phaseolina* causing root rot diseases. This disease appears during the growing season at the seedling stage of plant growth. Many soilborne fungi attack earlier at the pre-emergence stage, thus forcing the farmer to replant the missed hills or dead plants. Due to the economic importance of faba bean, the farmers plant repeatedly on the same land. This practice leads to a high build-up of pathogens, causing serious losses that could reach up to 12% (Anonymous 2006). The management strategy followed by the farmers included an unwise, intensive use of fungicides. This strategy was not a satisfactory solution for controlling root rot disease. An investigation of root rot disease is considered particularly important due to its wide prevalence in Egypt, particularly in sandy soils. Thus far, because of scientific and practical difficulties, there is no economic way to control root rot disease in many crops. The application of biological controls using antagonistic microorganisms has proved to be successful for controlling various plant diseases in many countries (Sivan 1987). However, this is not an easy method, and it is costly to apply. It is possible to use biological controls as the best control measure under greenhouse conditions. Abdel-Kader (1997) reported that *Trichoderma harzianum* introduced to the soil, was able to reduce root rot incidence of faba bean plants significantly more than the fungicide Rizolex-T. In recent years, several attempts have been made to overcome this obstacle by applying antagonistic microorganisms. *Trichoderma* spp. are well documented as effective biological control agents of plant diseases caused by soilborne fungi (Sivan and Chet 1986; Whipps and Lumsden 2001; McLean *et al*. 2004). Hadar *et al*. (1979, 1984) and Elad *et al*. (1980) observed that the application of wheat bran colonised by *T. harzianum* to soil infested with *R. solani* and *S. rolfsii*, reduced the incidence of root diseases caused by these pathogens in beans.

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On the other hand, there has been a constant search for alternative and efficient compounds for the control of plant pathogens. The aim is a partial or total replacement of antimicrobial chemical fungicides. Essential oils are promising alternative compounds which have an inhibitory activity on the growth of pathogens. It is possible that essential oils could be used in plant disease control as the main or as adjuvant antimicrobial compounds. Recently, there has been considerable demand for the discovery of new natural antimicrobials (Sagdic *et al.* 2003). Plant products with antimicrobial properties have notably obtained attention as possible applicants in order to prevent bacterial and fungal growth (Lanciotti *et al.* 2004). Plant products are characterized as having a wide range of volatile compounds. This means that essential oils could be used as alternative anti-bacterial and anti-fungal treatments (Jenny 2000). It is evident from reviews by Karapinar (1985) and Nanir and Kadu (1987) that some plant extracts and essential oils exhibited antifungal properties. Nirmala *et al.* (1988) showed that essential oil of *Juniperus communis* may be applicable against a range of damping-off diseases. Kumar and Tripathi (1991) mentioned that extracts of *Eupartrium cannabinum* completely inhibited the mycelia growth of *Pythium de-baryanum, R. solani* and *S. rolfsii.* Furthermore, Juglal *et al*. (2002) studied the effectiveness of nine essential oils to control the growth of mycotoxins producing moulds and noted that, clove, cinnamon and oregano were able to prevent the growth of *Aspergillus parasiticus* and *Fusarium moniliforme.* Benkeblia (2004) observed the inhibitory effect of onion essential oil at different concentrations, on the growth of *F. oxysporum, Aspergillus niger* and *Penicillium cyclopinum.* Furthermore, El-Mougy *et al.* (2007) reported that Geranium, rose, lemon and mint essential oils were tested as seed coating and/or foliar spray. A significant reduction in root rot and wilt incidence of bean at both pre- and post-emergence stages were recorded under greenhouse conditions.

Application of essential oil is a very attractive method for controlling plant diseases. Essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional uses (Ormancey *et al*. 2001). Recently, interest has been shown in combining microbial biocontrol agents with other chemical components to increase their activity against plant pathogens. Essential oils have been used successfully in combination with a variety of treatments, such as antibacterial agents, mild heat and salt compounds (Karatzas *et al.* 2000).

The increasing interest in pesticide alternatives was due to the toxicity implication of pesticides for humans. Therefore, there will be an increasingly driven demand motivated by different priorities such as health benefits, cost, ecological benefits, ethical issues, food safety and sustainability of supply. Against this background, and the demand for natural products as raw material for new antifungal agents, the objective of the present work was aimed to determine the efficacy of some plant -derived essential oils against the growth of of faba bean root rot pathogens *F. solani, R. solani, S. rolfsii* and *M. phaseolina* under *in vitro* conditions. Application of essential oils as seed treatments in combination with the bio-agent *T. harzianum* as a soil treatment, was also evaluated against root-rot incidence of faba bean under field conditions.

MATERIALS AND METHODS

The present work was developed at the laboratory and greenhouse of Plant Pathology of National Research Centre (NRC), Egypt. The confirmed field experiment was carried out at Al-Aiat territory, Giza Governorate, Egypt. Tests were performed with the following plant pathogenic fungi: *F. solani, R. solani, S. rolfsii* and *M. phaseolina* as well as the antagonistic fungus *T. harzianum* which are kept at the fungi collection unit of the same laboratory. Pure-grade of the essential oils, *i.e.* carnation (*Dianthus caryophyllus*), caraway (*Carum carvi*), thyme (*Thymus vulgaris*), peppermint (*Mentha piperita*) and geranium (*Geranium viscosissimum*) were obtained from Cairo Company for oils and aromatic extractions Cario Industrial Development (CID), Egypt. The essential oils were stored in dark glass bottles.

Laboratory tests

Five essential oils (Table 1) at concentrations of 1, 2 and 4% were evaluated for their inhibitory effect on fungal radial growth, through *in vitro* tests.

Emulsified stocks at high concentrations of tested essential oils were prepared by dissolving in sterilized distilled water. A few drops of the emulsifier Tween 20 (Sigma Co.) were added to the essential oil volumes to obtain an emulsion feature. Different volumes of the essential oil emulsion were added to conical flasks containing 100 ml of sterilized PDA medium before its solidification, to obtain the proposed concentrations. The supplemented media were poured into Petri-dishes (9 cm) about 20 ml each. The control check treatment was PDA medium which was free of essential oils.

Table 1. Botanical plant classification and main active principles of their essential oil*

Common name	Scientific name	Family	Major active component
Carnation	*Dianthus caryophyllus*	Umbelliferae	eugenol, α-pinene, myrcene
Caraway	*Carum carvi*	Umbelliferae	carvone, limonene, carveol, pinen, thujone
Thyme	*Thymus vulgaris*	Labiatae	thymol, carvacrol, geraniol, thymol methyl ether, α -pinene
Peppermint	*Mentha piperita*	Lamiaceae	menthol, menthone, menthyl acetate, viridiflorol, ledol
Geranium	*Geranium viscosissimum*	Geraniaceae	geraniol, Citronellol, Tannins including gallic acid

*herb information (www.holisticonline.com/Herbal-Med/_Herbs/h280.htm)

Disks (5 mm-diameter) of each pathogenic fungi taken from seven day-old cultures were placed on the centre of Petri dishes. All plates were incubated at 25±2°C until the tested fungi reached full growth in the check treatment. Reduction in mycelial growth was calculated as the percentage of fungal growth diameter in the treatment, relative to the growth diameter in the control.

Greenhouse experiments

Effect of the essential oils, *i.e.* carnation, caraway, thyme, peppermint and geranium on root rot disease incidence of faba bean was studied in a pot experiment. The root pathogens: *F. solani*; *R. solani; S. rolfsii* and *M. phaseolina* were used in the present experiment as a model of faba bean root rot pathogens. Loamy soil was artificially infested individually (at the rate of 5% w:w and $10x10^8$ cfu/gram medium) with the inoculum of each tested fungus which had which previously been growing for two weeks on sand barley medium (1:1, w:w and 40% water) at 25±2°C.

Faba bean seeds (cv. Giza 3) were surface disinfected by immersing in sodium hypochlorite (2%) for 2 min, and washed several times with sterilized water, then dried between two sterilized layers of filter paper.

The disinfected faba bean seeds were coated with a different essential oil tested at the rate of 4 ml/kg seeds. Seed dressing was carried out by applying the tested essential oil to the gum moistened seeds in polyethylene bags and shaking well to ensure even distribution of the added materials. The treated seeds were then left on a plastic tray to air dried. The fungicide Rizolex-T 50 WP at the recommended dose (3 g/kg) was applied as the seed dressing as stated before. In addition, disinfected, untreated faba bean seeds were sown as a comparison treatment.

Varied infested soils were filled in plastic pots (30 cm in diameter) and sown with treated faba bean seeds relevant to the specific treatment. Five faba bean seeds were sown in each pot and six replicated pots were used for each particular treatment. The percentage of root rot disease incidence was calculated as the pre- and post-emergence infection after 15 and 40 days of sowing date, respectively. This test was carried out twice during the 2007/2008 growing season, with each period lasting 60 days. Recorded data as the obtained results of the two experiments were calculated as the average percentages of pre- and post-emergence root rot incidence.

Field experiment

The activity of integrated treatment of bio-agent *T. harzianum* and soaked faba bean seeds took place with 2% of any of the following essential oils (v:w) carnation, caraway, thyme, peppermint and geranium against root rot incidence. Activity of the treatments was evaluated under field conditions. This study was performed in a naturally heavily infested field with faba bean root rot pathogens, at the Al-Aiat territory, Giza Governorate, Egypt during the two successive growing seasons of 2008/2009 and 2009/2010. A field experiment was established which consisted of (3.5x6.0 m) plots, composed of 12 rows and a 25 cm spacing between plants within a row. Inoculum of *T. harzianum* grown on sand-barley medium at a ratio of 120 g/m^2 and $10x10^8$ cfu/gram medium (Abdel-Kader 1997) were incorporated into the top 20 cm of the soil surface at planting row sites. Three replicates (plots) per each relevant treatment were used in a completely randomized block design. Three seeds of faba bean per hole were used in all the treatments. Plots received the usual agricultural practices, *i.e.* NPK fertilizer and irrigation *etc*. Percentage of root rot incidence at the pre- and post-emergence of growth stages was investigated and calculated 15 and 45 days after the sowing date. The obtained yield was estimated for each particular treatment as kg/plot at the end of experiment. Recorded data as the obtained results of the two growing seasons, were calculated as the average percentages of pre-, and post-emergence root rot incidence as well as the produced yield.

Statistical analysis

All *in vitro* and *in vivo* experiments were set up in a complete randomized design. One way analysis of variance (ANOVA) was used to analyze the obtain results concerning the following: 1. Differences between the tested essential oil inhibitor effect and linear growth of pathogenic fungi *in vitro*, 2. Differences between tested essential oils on the incidence of root rot infection under greenhouse conditions, 3. Differences between integrated treatments of seed soaked with essential oils, as bio-agent against root rot pathogens, under field conditions. General Linear Model option of the Analysis System SAS (SAS 1996) was used to perform the analysis of variance. Duncan's Multiple Range Test at $p \leq 0.05$ level was used for means separation (Winer 1971).

RESULTS AND DISCUSSION

Laboratory tests

The essential oils evaluated in this work have a great variety of phytochemicals (Table 1) that could be considered as responsible for a larger or smaller antifungal activity. Those phytochemicals are as follows: 1. Eugenol, α-pinene, myrcene in carnation essential oil; 2. Carvone, limonene, carveol, pinen, thujone in caraway essential oil; 3. Thymol, carvacrol, geraniol, thymol methyl ether, α -pinene in thyme essential oil; 4. Menthol, menthone, menthyl acetate, viridiflorol, ledol in peppermint essential oil; 5. Geraniol, Citronellol, Tannins including gallic acid in geranium essential oil (C.f. herb information: www.holisticonline.com/Herbal-Med/_Herbs/h280.htm).

The inhibitory effect of carnation, caraway, thyme, peppermint and geranium essential oils against the mycelia growth of plant pathogenic fungi *F. solani, R. solani, S. rolfsii* and *M. phaseolina* are presented in table 2. Fungal mycelia growth decreased significantly as the concentrations of essential oils were increased, to reach the fungal growth's minimum at the highest concentration used. Complete inhibition in fungal growth was observed with 4% of carnation and geranium essential oils. Mycelial growth of *S. rolfsii* showed more sensitivity to all tested essential oils, meanwhile *F. solani, R. solani,* and *M. phaseolina* showed more tolerance, but the tolerance was not significant, against the effects of caraway; thyme and peppermint essential oils. Similar results were also

Table 2. Growth reduction[A] of faba bean root rot pathogens in response to different concentrations of essential oils, *in vitro*

Essential oils	Concentration [%][B] (v:v)	Growth reduction [%] in response to essential oil concentration			
		F. solani	*R. solani*	*S. rolfsii*	*M. phaseolina*
Carnation	1.0	48.1 e	91.1 a	72.2 b	72.2 b
	2.0	83.0 a	100 a	100 a	100 a
	4.0	100 a	100 a	100 a	100 a
Caraway	1.0	34.7 e	37.8 e	72.2 b	38.8 e
	2.0	64.2 d	47.8 e	100 a	42.2 e
	4.0	85.4 a	78.8 b	100 a	52.2 bc
Thyme	1.0	28.6 f	100 a	100 a	43.3 e
	2.0	40.8 e	100 a	100 a	53.3 bc
	4.0	80.4 a	100 a	100 a	97.5 a
Peppermint	1.0	46.7 e	36.8 e	48.8 e	37.7 e
	2.0	57.7 bc	44.8 e	76.6 b	56.4 bc
	4.0	82.2 a	76.4 b	83.3 a	85.5 a
Geranium	1.0	83.3 a	76.6 b	56.4 bc	48.3 e
	2.0	88.8 a	100 a	85.5 a	56.3 bc
	4.0	100 a	100 a	100 a	97.5 a

*mean values within columns followed by the same letter are not significantly different at $p < 0.05$

[A] reduction in fungal growth under different treatments used, calculated relative to fungal growth in untreated control

[B] concentrations of essential oils were calculated as (v:v) to the growth medium

reported concerning the efficacy of essential oils as antifungal inhibitors. Akgul and Kivanc (1988**)** studied antifungal activity of selected Turkish spices (black cumin, coriander, cumin, dill, laurel, oregano, parslely, spearmint, white mustard) on some foodborne fungi. They found that ground (1.0, 1.5, 2.0% w/v) plus essential oil (0.05%, 0.025%) showed an inhibitory effect on *Aspergillus flavus, A. niger, Geotricum candidum, Mucor* spp., *Penicillium roqueforti.* Also, El-Mougy *et al*. (2007) reported that 4% of the tested essential oils geranium, rosa, lemon and mint have an inhibitory effect against the mycelial growth of *R. solani* and *F. oxysporum* f. sp. p*haseoli* under *in vitro* , causing complete inhibition in fungal growth.

Furthermore, El-Toony *et al.* (2003) studied the biochemical reaction of onion, garlic, eucalyptus, caraway, fennel**,** black cumin**,** mustard**,** carnation**,** neemix and trilogy essential oils against mycelial growth of *R. solani* and *Pythium debaryanum in vitro.* They found that complete inhibition of both fungi was obtained by only carnation oil at 4%, however, considerable inhibition (more than 90%) was obtained with neemix and trilogy oils.

Greenhouse experiments

Data in table 3 show that essential oils could highly significantly reduced disease incidence of faba beans which have been artificially infested with root rot pathogens, compared to fungicide and untreated control treatments. Seed coating with any of the tested essential oils gave significant protection to emerged bean seeds against invasion of pathogenic fungi at the pre-emergence stage. Seed coating recorded more than 50% protection compared with 100% protection when Rhizolex-T treatment was used, over the untreated control. In this regard, *F. solani* and *R. solani* showed more sensitivity against seed treatments using carnation, caraway and thyme oils than treatments using peppermint and geranium oils. Both peppermint and geranium oil treatments reflected a superior effect on pre-emergence reduction recorded as (71.2–85.8%) and (57.1–71.2%), respectively. The opposite effect was observed as concerned the sensitivity of *S. rolfsii* and *M. phaseolina* against peppermint and geranium oil treatments the same essential oils whereas the percentage of disease reduction ranged between 50.1–69.9% and 74.9–79.9%, in respective order at the pre- emergence stage.

At the post-emergence stage, data also showed that all applied treatments could reduce the percentage of root-rot incidence more than 22.3% compared with the untreated control. Seed coating with fungicide showed a superior effect on disease incidence. Treatment with essential oils caused reduction in the percentage of root-rot incidence recorded as 43.3–60.5%, 22.3–55%, 27.8–59.3% and 38.3–52.3% in soil infested with *F. solani, R. solani, S. rolfsii* and *M. phaseolina,* respectively. Seed treatment with fungicide had a superior effect on disease incidence whereas it caused root rot reduction recorded between 44.3–61.7% at different infested soils with disease pathogens. Similar results, that essential oils inhibit large number of soilborne pathogens were reported by many researchers (Nirmala *et al.* 1988; Kumar and Tripathi 1991; Singh *et al.* 1992, 1994). El-Toony *et al*. (2003) reported that soil application with carnation oil concentrations (1, 2, 3 and 4 ml/l) significantly reduced the percentage of pre- and post-emergence damping-off of tomato. They added that all concentrations showed the same activity when applied once, twice or three times. These results are in agreement with the present observation which showed that increasing essential oils concentration more than 0.5% had no significance in disease incidence.

Table 3. Incidence of faba bean root rot caused by different soilborne pathogens in response to seed dressing with essential oils[A] under greenhouse conditions

Plant growth stage	pathogens	Root rot[D]	Seed dressing with essential oil						
			carnation	caraway	thyme	peppermint	geranium	Rizolex-T	control
pre-emergence[B]	*F. solani*	DI	3.3 e	6.7 d	3.3 e	6.7 d	10.0 bc	0.0 f	23.3 a
		R	85.8	71.2	85.8	71.2	57.1	100	–
	R. solani	DI	6.7 d	6.7 d	6.7 d	10.0 bc	10.0 bc	0.0 f	30.0 a
		R	77.7	77.7	77.7	66.7	66.7	100	–
	S. rolfsii	DI	13.3 b	13.3 b	13.3 b	6.7 d	6.7 d	0.0 f	26.7 a
		R	60.0	50.1	50.1	74.9	74.9	100	–
	M. phaseolina	DI	10.0 bc	13.3 b	10.0 bc	6.7 d	6.7 d	0.0 f	33.3 a
		R	69.9	60.0	69.9	74.9	79.9	100	–
post-emergence[C]	*F. solani*	DI	10.3 g	13.8 ef	13.6 ef	10.7 g	14.8 e	10.0 g	26.1 a
		R	60.5	47.1	47.9	59.0	43.3	61.7	–
	R. solani	DI	10.7 g	10.7 g	14.3 e	14.8 e	18.5 b	13.3 ef	23.8 a
		R	55.0	55.0	39.9	37.8	22.3	44.1	–
	S. rolfsii	DI	14.3 e	11.1 g	19.2 b	17.9 bc	17.9 bc	13.3 ef	27.3 a
		R	47.6	59.3	29.7	27.8	27.8	51.3	–
	M. phaseolina	DI	14.8 e	15.4 d	18.5 b	18.5 b	14.3 e	16.7 cd	30.0 a
		R	50.7	48.7	38.3	38.3	52.3	44.3	–

Mean values within columns followed by the same letter are not significantly different (p = 0.05); DI – Disease incidence, R – reduction in disease incidence; [A]essential oils at 4 ml/kg were used for faba bean seed dressings; [B]percentage of pre-emergence was calculated as the number of emerged seedlings in relative to the total number of sown seeds; [C]percentage of post-emergence was calculated as the number of diseased plants in relative to the total number of emerged seedlings; [D]recorded data as the obtained results of the two growing seasons were calculated as the average percentages of pre- and post-emergence root rot incidence

The mode by which microorganisms are inhibited by essential oils and their chemical compounds seems to involve different mechanisms. It has been hypothesized that inhibition involves phenolic compounds, because these compounds sensitize the phospholipid bilayer of the microbial cytoplasmic membrane causing increased permeability, unavailability of vital intracellular constituents (Juven *et al*. 1994). Reports have indicated that essential oils containing carvacrol, eugenol and thymol (phenolic compounds) had the highest antibacterial performances (Kim *et al*. 1995). Many authors have emphasized that the antimicrobial effect of essential oil constituents has been dependent on their hydrophobicity and partition in the microbial plasmatic membrane. Effect of specific ions due to their addition in/on plasmatic membrane had a great effect on the motive force of protons, intracellular ATP content and overall activity of microbial cells, including turgor pressure control, solutes transport and metabolism regulation (Lanciotti *et al*. 2004).

Results in the present study showed that carnation, caraway, thyme, peppermint and geranium essential oils have an inhibiting effect on the growth of root rot pathogens of faba bean under *in vitro*, and their efficacy are extended to protect seed germination or plant invasion under *in vivo* conditions. The presented data make it clear that the percentage of root rot infection at the pre-emergence stage was less than that recorded at the post emergence stage in all the essential oil treatments, while seed treatment with the fungicide Rhizolex-T showed a superior significant effect. These results led to the suggestion that another supporting factor is needed to raise the efficacy of the applied essential oils. Therefore, application of the bioagent *T. harzianum* as a soil treatment in integration with seed coatings of essential oils were evaluated against root rot incidence of faba bean in field trails.

Field experiment

The efficacy of essential oils as seed coating sown together with the bioagent *T. harzianum* in treated soil, against the incidence of root rot diseases of faba bean, was evaluated under field conditions. Data in table 4 clearly demonstrate that all treatments significantly reduced disease incidence compared with the control. Peppermint, carnation, geranium, thyme and caraway in descending order could reduce the incidence of root rot at both pre- and post-emergence stages by 16.1–34.4% and 17.2–31.7% respectively, compared to 14.5–16.1% in case of the fungicide Rhizolex-T. Higher protective effect against root rot incidence at both pre- and post-emergence growth stages when essential oils integrated with *T. harzianum* as an applied soil treatment, were noted. A similar trend was observed regarding the applied treatments. Treatment of peppermint plus *T. harzianum* had the greatest effect on disease incidence; the reduction in root rot was recorded as 55.4% and 47.1% at pre- and post-emergence stages, respectively. Carnation, geranium, thyme and caraway plus *T. harzianum* recorded disease reduction which ranged between 47.3–52.7% and 41.4–41.6% at both growth stages, respectively. A lower significant effect was observed when using the Rhizolex-T treatment (14.5–16.1%),

Table 4. Incidence of faba bean root rot caused by different soilborne pathogens in response to seed dressings with essential oils[A] and *T. harzianum*[B] and the influence of seed dressings with essential oils[A] and *T. harzianum*[B] on yield production under field conditions[F]

Treatment	Root rot disease of faba bean				Yield	
	pre-emergence[C]		post-emergence[D]		[kg/plot[E]]	increases [%]
	incidence [%]	reduction [%]	incidence [%]	reduction [%]		
Carnation	16.5 c	26.3	18.3 bc	19.4	26.7 d	25.9
Caraway	18.8 b	16.1	18.8 bc	17.2	24.4 c	15.1
Thyme	16.5 c	26.3	18.3 bc	19.4	25.4 cd	19.8
Peppermint	14.7 e	34.4	15.5 d	31.7	27.3 de	28.8
Geranium	16.5 c	26.3	17.3 c	23.8	26.8 d	26.4
Carnation+*T. harzianum*	11.8 f	47.3	13.3 e	41.4	29.7 f	40.1
Caraway+*T. harzianum*	11.8 f	47.3	13.3 e	41.4	30.2 fg	42.5
Thyme+*T. harzianum*	10.6 f	52.7	13.1 e	42.3	31.3 g	47.6
Peppermint+*T. harzianum*	10.0 f	55.4	12.0 f	47.1	31.8 g	50.0
Geranium+*T. harzianum*	11.8 f	47.3	13.2 e	41.6	30.6 fg	44.3
Rizolex-T (3 g/kg)	18.8 b	16.1	19.4 b	14.5	23.6 b	11.3
Untreated – control	22.4 a	–	22.7 a	–	21.2 a	–

Mean values within columns followed by the same letter are not significantly different (p = 0.05); [A]essential oils at 2 ml/kg were used for faba bean seed dressings; [B]*T. harzianum* inoculum was used as soil treatment at the rate of 120 g/m^2; [C]percentage of pre-emergence was calculated as the number of emerged seedlings relative to the total number of sown seeds; [D]percentage of post-emergence was calculated as the number of diseased plants relative to the total number of emerged seedlings; [E]yield increase using different treatments, calculated relatively to the untreated control; [F]recorded data as the obtained results of the two growing seasons, were calculated as the average percentages of pre- and post-emergence root rot incidence as well as produced yield

although Rhizolex-T treatment could significantly reduce disease incidence over the control treatment.

The harvested yield as green pods, in all treatments, was significantly higher than that in the control treatment. The treatments of essential oils plus the bioagent *T. harzianum* showed higher yield production than those treatments using an essential oil.

Highly effective treatments which helped increase faba bean yield were peppermint (50.0%), followed by thyme (47.6%), geranium (44.3%), caraway (42.5%) and carnation (40.1%), respectively. Seeds coated with essential oils only, caused a yield increase estimated between 15.1–28.8%. The lowest increase in bean yield (11.3%) was observed with seed coated with the fungicide Rhizolex-T over the check control treatment.

The present investigation has demonstrated the antifungal activity of all treatments tested. This work proves that some essential oils have potential and could be useful when integrated with bioagents against faba bean fungal pathogens. From the earlier reports (Dabur *et al.* 2007; Bindu and Kumar 2009) it is evident that some of the plant products have antifungal compounds which do have the capacity to inhibit the fungal pathogens. Therefore, there is increasing interest in obtaining alternative antimicrobial agents for use in plant disease control systems. One of the main procedures used in the research of biologically active substances is a systematic screening for the interaction between microorganisms and plant products. This procedure has been a source of useful agents to control the microbial survival (Salvat *et al*. 2001). Plant products of a recognized antimicrobial spectrum could appear in food conservation systems as main antimicrobial compounds or as adjuvant to improve the action of other antimicrobial compounds (Kaur and Arora 1999). Among other chemical products, aromatic plants possess essential oils resulting from secondary metabolism. These substances have a great economic potential, especially in the food, pharmaceutical and perfumery sectors. Thus, the number of studies on the chemical composition and biological properties of these oils, as well as the taxonomic, environmental and cultivation factors that lead to variation in their quantity and quality, has been increasing (Simões *et al.* 2003). In the present study the introduction of the bioagent *T. harzianum* to the soil increased the efficacy of essential oils against root rot incidence under field conditions. Similar results were reported by Abdel-Kader (1997). He stated that *T. harzianum* introduced to the soil was able to reduce root rot incidence of faba bean plants significantly more than the fungicide Rizolex-T. Moreover, the application of biological controls using antagonistic microorganisms, has proved to be successful for controlling various plant diseases in many countries (Chao *et al*. 1986; Sivan 1987; El-Mougy 2001; Wright *et al.* 2003; El-Mougy and Abdel-Kader 2008).

The present study demonstrated that carnation, caraway, thyme, peppermint and geranium essential oils were found to have an inhibitory effect against the mycelial growth of *F. solani, R. solani, S. rolfsii* and *M. phaseolina* under *in vitro* conditions. Moreover, application of an essential oil as a seed coating,, under greenhouse conditions, or sown in treated soil with the bioagent *T. harzianum* as integrated treatment under field trails, resulted in a significant reduction in root rot incidence of faba bean. It may be concluded that application of essential oils combined with bioagent *T. harzianum* is considered an applicable, safe and cost-effective method for controlling such soilborne diseases.

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POLISH SUMMARY

ROŚLINNE OLEJKI ETERYCZNE ORAZ GRZYB *TRICHODERMA HARZIANUM* JAKO BIOLOGICZNE CZYNNIKI INTEGROWANEGO ZWALCZANIA SPRAWCÓW ZGNILIZNY KORZENI BOBU

Stwierdzono, że olejki eteryczne pochodzące z roślin: goździka ogrodowego, kminku zwyczajnego, tymianku, mięty pieprzowej i geranium wykazywały właściwości inhibujące wzrost grzybni *Fusarium solani*, *Rhizoctonia solani*, *Scerotium rolfsii* i *Macrophomina phaseolina* w warunkach *in vitro*. Całkowite zahamowanie wzrostu grzybów obserwowano po zastosowaniu 4% olejku z roślin goździka i geranium. Grzybnie testowanych patogenów okazały się bardziej wrażliwe na wysokie koncentracje olejku z tymianku niż z goździka ogrodowego czy mięty pieprzowej. Ponadto olejki eteryczne zastosowane do otoczkowania nasion powodowały istotne ograniczenie występowania zgnilizny korzeni bobu, zarówno przed jak też po wschodach roślin w warunkach szklarniowych. W warunkach polowych otoczkowanie nasion olejkami eterycznymi w koncentracji 4% oraz siew do gleby potraktowanej grzybem *T. harzianum* jako czynniki biologicznego zwalczania zapewniło wyraźną ochronę kiełkujących nasion przed porażeniem przez grzyby patogeniczne powodujące zgniliznę korzeni. Powyższy zabieg wpływał na ograniczenie występowania choroby przed wschodami roślin rzędu 47,3 – 55% w porównaniu do obiektu kontrolnego z zaprawą Rizolex-T, gdzie procent ograniczenia występowania choroby wynosił 16%. Po wschodach roślin wszystkie zastosowane zabiegi ograniczały występowanie zgnilizny korzeni od 41,4 – 47,1% w porównaniu do nie traktowanej kombinacji kontrolnej. Ograniczenie występowania choroby spowodowało wzrost plonu roślin 15,1- 28,8% i 40,1 – 50% odpowiednio dla nasion otoczkowanych jednym z testowanych olejków oraz zabiegami łączonymi z glebowym stosowaniem grzyba *T. harzianum*. Nasiona bobu potraktowane fungicydem Rhizolex-T dawały wyższy plon o 11,3% w porównaniu do obiektu kontrolnego. Wyniki prezentowanych badań pokazują, że wykorzystanie roślinnych olejków eterycznych w połączeniu grzybem *T. harzianum* jako biologiczne czynniki, należy uznać jako odpowiednią, bezpieczną i opłacalną metodę zwalczania chorób pochodzenia odglebowego.