

## CONTROL OF WILT AND ROOT ROT INCIDENCE IN *PHASEOLUS VULGARIS* L. BY SOME PLANT VOLATILE COMPOUNDS

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**Abstract:** Essential oils from four plants, *i.e.* geranium, rosa, lemon and mint were tested for their activity *in vitro* and *in vivo* against *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *phaseoli*, the cause of root rot and wilt of beans. *In vitro*, they were found to have an inhibitory effect against the mycelial growth of *R. solani* and *F. oxysporum* f. sp. *phaseoli*. Complete inhibition in fungal growth was observed at a concentration of 4% of each essential oil and Topsin M at 400 ppm as well. In greenhouse the four essential oils were tested as seed coating and/or foliar spray. Results of seed coating at a concentration of 1% clearly demonstrate a good protection of emerged bean seeds against invasion of *R. solani* and *F. oxysporum* f. sp. *phaseoli* compared with the fungicide treatment. A similar trend was observed in a lower extent when the essential oils were applied as bean seeds coating followed by seedlings foliar spray under field conditions. Obvious yield increase as bean green pods, in all treatments, was significantly higher than in the control.

**Key words:** bean, *Fusarium oxysporum* f. sp. *phaseoli*, *Rhizoctonia solani*, geranium, lemon, mint, rosa oils, root rot, wilt

### INTRODUCTION

Green bean (*Phaseolus vulgaris* L.) is attacked by certain soil borne fungi causing root rot and wilt diseases. The main pathogens responsible for root rot and wilt incidence of bean were reported to be *Rhizoctonia solani* (Kühn) and *Fusarium oxysporum* f. sp. *phaseoli*, respectively (Abdel-Kader 1997; Burnchara and Camacho 2000; El-Mougy 2001). Recently there has been a considerable concern in discovering plant-derived anti-microbial agents (Sagdic *et al.* 2003) for alternative application in the strategy of

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preventing bacterial and fungal growth (Lanciotti *et al.* 2004). Plant extracts are characterized for a wide range of volatile compounds and essential oils used as alternatives for anti-bacterial and anti-fungal treatments (Jenny 2000; Michael 2000). Kumar and Tripathi (1991) mentioned that the extracts of *Eupartrium cannabinum* completely inhibited the mycelial growth of *Pythium debaryanum*, *R. solani* and *Sclerotium rolfsii*. Furthermore, Juglal *et al.* (2002) studied the effectiveness of nine essential oils to control the growth of mycotoxins producing moulds and observed that clove, cinnamon and oregano were able to prevent the growth of *Aspergillus parasiticus* and *Fusarium moniliforme*. Benkeblia (2004) observed an inhibitory effect of onion essential oil at different concentrations on the growth of *F. oxysporum*, *Aspergillus niger* and *Penicillium cyclopinum*.

The objective of the present work was aimed to determine the efficacy of some plant-derived essential oils against the growth of *F. oxysporum* f. sp. *phaseoli* and *R. solani* *in vitro*. Application of essential oils as seed treatment and/or foliar spray against wilt and root rot incidence of bean was also evaluated under greenhouse and field conditions.

## MATERIALS AND METHODS

### Inoculum preparation

Pathogenic isolates of *F. oxysporum* f. sp. *phaseoli* and *R. solani* were isolated from diseased beans with wilt and root rot symptoms. These isolates were highly aggressive on beans (Abdel-Kader 1997; El-Mougy 2001). This work was carried out in the Department of Plant Pathology laboratory and open greenhouse, National Research Centre, Giza, Egypt. The field experiment was conducted on natural infested field with root rot and wilt pathogens at Al-Aiat county, Giza governorate, Egypt.

### Essential oils and fungicide

Commercial geranium (extracted from the plant *Pelargonium odorantissimum*, of the *Geraniaceae* family and from *Pelargonium graveolens*), rosa (extracted from the damask rose – *Rosa damascena* of the *Rosaceae* family also known as Bulgarian and Turkish rose, otto of rose and attar of rose.), mint (extracted from *Mentha piperita* of the *Labiatae* family also known as brandy mint and balm mint) and lemon (extracted from the *Citrus limonum* [also known as *Citrus Limon*], of the *Rutaceae* family) essential oils used in the present study were obtained from CID Company, Egypt. The major components of geranium, rosa, lemon and mint oils are citronellol geraniol (27%), citronellol (33%), citral (63%) and menthol (30%), respectively. The fungicide, Topsin M 70 WP, dimethyl 4,4-(*o*-phenylene)bis(3-thioallophanate), Agrichemicals group, Cerexagri, Inc. was used as a positive control to the essential oils.

### Inhibition of mycelial growth *in vitro* tests

The essential oils were added individually to conical flasks containing sterilized PDA medium before solidification to obtain concentrations of 0.5, 1.0, 2.0 and 4%. In addition, different volumes of stock solution of the fungicide Topsin M 70 WP were added to other conical flasks containing PDA medium to obtain concentrations of 50, 100, 200 and 400 ppm. A separate PDA flask free of either essential oils or fungicide was used as check (control) treatment. The supplemented media were poured into Petri dishes (9 cm Ø) nearly 20 ml per each. Mycelial discs (5 mm Ø) were taken from

the periphery of actively growing PDA cultures of *F. oxysporum* f. sp. *phaseoli* and *R. solani*, placed at the centre of the Petri dishes, then quickly sealed with parafilm and incubated for seven days at  $25 \pm 2^\circ\text{C}$ . For each treatment, oil and fungicide and the concentrations tested, five replicate Petri dish cultures were used. The diameter of the colonies was measured and reduction in fungal growth was calculated in relation to its growth in check treatment.

### Disease control in the greenhouse

Root rot and wilt of beans (*P. vulgaris*) caused by *R. solani* and *F. oxysporum* f. sp. *phaseoli*, were used as a model system in greenhouse studies. In greenhouse experiments, the efficacy of geranium, rosa, mint and lemon essential oils against root rot and wilt incidence of bean was evaluated. Essential oils were applied as seed coating and/or foliar sprays. Disinfected bean seeds were soaked for 3 h in the tested essential oils at the rate of 1% (v/w), and then left to air dry before sowing. Stock solutions (1000 ml) of each of the tested essential oils were prepared in concentrations of 0.25, 0.50 and 1.0% by dissolving in sterilized distilled water and used for foliar spray. Two mL of Tween 80 (0.2% of water volume) were added to the essential oils solutions to obtain an aqueous emulsion. Tween 80 also known as polysorbate 80 is a nonionic surfactant and emulsifier derived from sorbitol obtained from various types of fruit. Polysorbate 80 is a water-soluble somewhat yellowish amber liquid that is used as a dispersing agent to mix oil and water and to solubilize fragrances and essential oils (Anonymous 2007). All stock solutions were prepared in black glass bottles kept in refrigerator until used.

Foliar spray with essential oils was applied at the second true leaves stage of emerged bean seedlings. Treated and untreated bean seeds (Giza, 3 cv.) were sown in plastic pots (40 cm Ø) in artificially infested loamy soil with inocula of either *R. solani* or *F. oxysporum* f. sp. *phaseoli* at the rate of 5% of soil weight (Abdel-Kader 1997). Comparison treatment included a set of bean seeds coated with fungicide Topsin M at the rate of 3 g/kg. Untreated plastic pots were left for general check. Five bean seeds were sown in each pot, and five pots were used as replicate for each particular treatment. Percentages of root rot and wilt incidence of bean at pre- and post-emergence stages were calculated after 10 and up to 60 days of the experimental period, respectively. Pre-emergence (%) was based on the number of un-emerged seeds in relation to the number of sown seeds, while Post-emergence (%) was based on the number of plants showing disease symptoms in relation to the number of emerged seedlings.

### Field experiment

Field experiment was carried out at Al-Aiat county, Giza governorate during 2006 growing season to evaluate the efficacy of the promising treatment obtained under greenhouse trail for controlling root rot and wilt diseases of bean. This field is well known by the authors as naturally heavily infested with soilborne pathogens. The fungicide Topsin M was used in this study as a comparison to the other treatments.

Geranium, rosa, mint and lemon essential oils were used as bean seed dressing before sowing, next, the emerged plants (at 2–3 true leaves age) were sprayed with the same essential oils at concentration of 1% as stated above. Topsin M was applied only as seed dressing at the recommended dose (3 g/kg seeds).

A field experiment consisted of plots (7×6 m) each comprised of 12 rows and 30 holes/row which were conducted in completely randomized block design with five plots as replicates for each particular treatment as well as untreated check treatment. Bean seeds Giza, 3 cv. were sown in all treatments at the rate of 3 seeds/hole. All plots received the traditional agricultural practices. Average percent of root rot infection and wilt at pre-emergence stage was recorded after 15 days after sowing. At post-emergence stage bean plants showing root rot or wilt symptoms were recorded and the average accumulated disease incidence was calculated three times after 30, 45 and 60 days after sowing throughout the growing season. The bean pathogens isolated from plants that showed root rot or wilt symptoms were identified as *R. solani* and *F. oxysporum* f. sp. *phaseoli* according to Barnett and Hunter (1972). At harvest time, the average accumulated yield was calculated for all applied treatments and the control as well.

### Statistical analysis

One way analysis of variance (ANOVA) was used to analyze differences between toxic concentrations of essential oils, the fungicide and the linear growth of pathogens as well as differences between toxicity of essential oils, the fungicide and root rot or wilt incidence at different applied concentrations under laboratory and greenhouse conditions. MSTAT-C program (V2.1) was used to perform the analysis of variance between toxicity of essential oils, fungicide and root rot or wilt incidence at different applied concentrations under field conditions. Duncan's Multiple Range Test was used for means separation (Winer 1971).

## RESULTS

### Inhibition of mycelial growth *in vitro* tests

Fungicidal activity against the myceliae growth of *R. solani* and *F. oxysporum* f. sp. *phaseoli* was observed at a concentration of 4% of the geranium, rosa and lemon essential oils (Table 1). This activity was also observed at 2% and 4% mint essential oils. Fungal mycelial growth decreased significantly with the increase in concentrations of essential oils and Topsin M to reach minimum growth with the highest concentration used. Complete inhibition in fungal growth was observed at 4% of all essential oils and Topsin M at 400 ppm. Mycelial growth of *R. solani* showed more sensitivity to rosa and mint oils, than *F. oxysporum* f. sp. *phaseoli*, while the opposite feature was observed with geranium and lemon oils. The fungicide Topsin M caused complete inhibition of the growth of *F. oxysporum* f. sp. *phaseoli* at 200 and 400 ppm and at 400 ppm against *R. solani*.

### Greenhouse studies

The essential oils were able to reduce significantly the incidence of both root rot and wilt of bean compared with the fungicide and untreated control treatments (Table 2a, b). At the pre-emergence stage, seed coating with the essential oils at a concentration of 1% gave a clear protection to the emerged bean seeds against *R. solani* invasion (Table 2a) resulting in the reduction in disease incidence by 75.0% when treated with rosa, lemon or mint essential oils. This protection reached 87.5% when treated with geranium essential oils. The use of the commercial fungicide Topsin M as 3 g/kg treatment gave protection only by 25%. A similar trend in disease reduction

during the pre-emergence stage was observed in case of the *F. oxysporum* f. sp. *phaseoli* seed invasion (Table 2b). The reduction of 66.7% in disease incidence was recorded when treated with the rosa, lemon or mint essential oils. The protection was the highest (83.3%) when the seeds were treated with geranium essential oils, while protection of the seeds treated with Topsin M was significantly lower (33.3%).

Table 1. Growth reduction<sup>A</sup> of *F. oxysporum* f. sp. *phaseoli* and *R. solani* in response to different concentrations of some essential oils *in vitro*

Treatment <sup>B</sup>	Concentration	Average growth reduction % <sup>C</sup>	
		<i>F. oxysporum</i> f. sp. <i>phaseoli</i>	<i>R. solani</i>
Geranium oil	0.5%	72.2b	55.5 bc
	1.0%	83.3a	76.6 b
	2.0%	88.8a	100 a
	4.0%	100 a	100 a
Rosa oil	0.5%	35.6 d	44.4 d
	1.0%	44.4 d	66.6 c
	2.0%	77.7 b	88.8 a
	4.0%	100 a	100 a
Mint oil	0.5%	46.6 d	72.2 b
	1.0%	68.8 c	83.3 a
	2.0%	100 a	100 a
	4.0%	100 a	100 a
Lemon oil	0.5%	55.5 bc	55.5 bc
	1.0%	71.1 b	66.6 c
	2.0%	92.2 a	83.3 a
	4.0%	100 a	100 a
Topsin M (70%)	50 ppm	75.6 b	64.4 c
	100 ppm	88.8 a	75.6 b
	200 ppm	100 a	84.4 a
	400 ppm	100 a	100 a

<sup>A</sup>reduction of fungal growth in different treatment, calculated relatively to its growth in untreated control

<sup>B</sup>concentrations of essential oils were calculated as (v:v) to the growth medium, while based on the active ingredient of the fungicide

<sup>C</sup>mean values within columns followed by the same letter are not significantly different at  $p = 0.05$

During the post-emergence stage, the infection with *R. solani* (Table 2a) was inhibited by treatment with the geranium, rosa and mint essential oils by 85.2% and to a lesser extent by the lemon essential oils (70.1%), while the Topsin M was at a further distance in its compatibility against infection (55.2%). The reduction in the incidence of *F. oxysporum* f. sp. *phaseoli* (Table 2b) during the post-emergence stage was variable for the different treatments ranging between 85.6%, 76.4%, 62.4% and 77.4% in case of geranium, rosa, lemon and mint essential oils, respectively, comparing with Topsin M

Table 2a. Root rot incidence of bean in response to seed and/or foliar spray with essential oils<sup>A</sup> under greenhouse conditions

Treatment		Concentration	Average disease incidence [%] <sup>E</sup>			
			pre-emergence <sup>B</sup>	reduction [%] <sup>C</sup>	post-emergence <sup>D</sup>	reduction [%] <sup>C</sup>
Seed treatment only	geranium	1%	4.0 bc	87.5	8.6 e	85.2
	rosa		8.0 c	75.0	8.6 e	85.2
	lemon		8.0 c	75.0	17.3 cd	70.1
	mint		8.0 c	75.0	8.6 e	85.2
	Topsin M	3g/kg	24.0 a	25.0	26.3 b	55.2
Seed treatment (1%) + foliar spray	geranium	0.25	8.0 c	75.0	13.0 d	77.8
		0.50	8.0 c	75.0	8.6 e	85.2
		1.00	4.0 d	87.5	4.1 f	92.9
	rosa	0.25	8.0 c	75.0	17.3 cd	70.1
		0.50	4.0 d	75.0	8.6 e	85.3
		1.00	8.0 c	87.5	8.3 e	85.8
	lemon	0.25	8.0 c	75.0	17.3 cd	70.1
		0.50	4.0 d	75.0	13.0 d	77.8
		1.00	8.0 c	87.5	8.3 e	85.3
	mint	0.25	8.0 c	75.0	13.0 d	77.8
		0.50	8.0 c	75.0	8.6 e	85.3
		1.00	8.0 c	75.0	8.6 e	85.3
Foliar spray only	geranium	0.25	16.0 ab	50.0	18.1 c	69.0
		0.50	12.0 b	62.5	18.1 c	69.0
		1.00	12.0 b	62.5	13.6 d	76.8
	rosa	0.25	16.0 ab	50.0	22.7 b	61.3
		0.50	12.0 b	62.5	18.1 c	69.0
		1.00	12.0 b	62.5	14.2 d	75.8
	lemon	0.25	16.0 ab	50.0	19.0 c	67.6
		0.50	16.0 ab	50.0	19.0 c	67.6
		1.00	16.0 ab	50.0	14.2 d	75.7
	mint	0.25	16.0 ab	50.0	19.0 c	67.6
		0.50	12.0 b	62.5	18.1 c	69.0
		1.00	12.0 b	62.5	13.6 d	76.8
Untreated			32.0 a	–	58.8 a	–

<sup>A</sup>essential oils applied as seed treatment and/or foliar spray<sup>B</sup>pre-emergence (%) based on the number of not emerged seeds in relation to the number of sown seeds<sup>C</sup>reduction at different treatment, calculated relatively to untreated control<sup>D</sup>post-emergence (%) based on the number of plants showing disease symptoms in relation to the number of emerged seedlings<sup>E</sup>mean values within columns followed by the same letter are not significantly different at  $p = 0.05$

Table 2b. Wilt incidence of bean in response to seed and/or foliar spray with essential oils<sup>A</sup> under greenhouse conditions

Treatment		Concentration	Average disease incidence [%] <sup>E</sup>			
			pre-emergence <sup>B</sup>	reduction [%] <sup>C</sup>	post-emergence <sup>D</sup>	reduction [%] <sup>C</sup>
Seed treatment only	geranium	1%	4.0 d	83.3	8.3 h	85.6
	rosa		8.0 c	66.7	13.0 fg	76.4
	lemon		8.0 c	66.7	21.7 cd	62.4
	mint		8.0 c	66.7	13.0 fg	77.4
	Topsin M	3g/kg	16.0 ab	33.3	28.5 b	50.6
Seed treatment (1%) + foliar spray	geranium	0.25	8.0 c	66.7	17.3 f	69.9
		0.50	8.0 c	66.7	8.6 h	84.9
		1.00	4.0 d	83.3	4.1 i	92.7
	rosa	0.25	8.0 c	66.7	17.3 f	69.9
		0.50	8.0 c	66.7	13.0 fg	77.4
		1.00	4.0 d	83.3	8.3 h	85.6
	lemon	0.25	8.0 c	66.7	17.3 f	69.9
		0.50	8.0 c	66.7	8.6 h	84.9
		1.00	4.0 d	83.3	4.1 i	92.7
	mint	0.25	8.0 c	66.7	16.3 f	71.7
		0.50	8.0 c	66.7	12.6 fg	78.2
		1.00	8.0 c	66.7	8.4 h	85.4
Foliar spray only	geranium	0.25	16.0 ab	33.3	23.8 c	58.8
		0.50	12.0 b	50.0	18.1 de	67.5
		1.00	12.0 b	50.0	13.6 fg	76.4
	rosa	0.25	12.0 b	50.0	22.7 c	60.7
		0.50	12.0 b	50.0	22.7 c	60.7
		1.00	12.0 b	50.0	18.1 de	67.5
	lemon	0.25	16.0 ab	33.3	20.7 cd	64.1
		0.50	12.0 b	50.0	19.2 de	66.7
		1.00	12.0 b	50.0	18.3 de	68.3
	mint	0.25	16.0 ab	33.3	23.8 c	58.8
		0.50	16.0 ab	33.3	19.0 de	67.1
		1.00	12.0 b	50.0	14.2 fg	75.3
Untreated			24.0 a	–	57.8 a	–

<sup>A</sup>essential oils applied as seed treatment and/or foliar spray<sup>B</sup>pre-emergence (%) based on the number of not emerged seeds in relation to the number of sown seeds<sup>C</sup>reduction at different treatment, calculated relatively to untreated control<sup>D</sup>post-emergence (%) based on the number of plants showing disease symptoms in relation to the number of emerged seedlings<sup>E</sup>mean values within columns followed by the same letter are not significantly different at  $p = 0.05$

(50.6%). Also data in Tables (2a, b) showed that when the essential oils were used as 1% seed treatment in addition to foliar spray (at different concentrations) during the pre-/or post-emergence stage, the protection against root rot reached more than 70.1%. The efficacy of the essential oils when similarly applied against wilt disease was found to reduce wilt disease incidence during the pre-emergence stage by more than 66.7%, while the post-emergence stage data recorded reduction in the incidence of wilt disease above 69.9% and reached 92.7%. The use of geranium, rosa, lemon and mint essential oils as foliar spray alone had a similar significant trend in its efficacy against disease incidence, although lower than seed treatment alone or combined with foliar spray, yet significantly higher than those treated by the commercial fungicide Topsin M.

### Field experiment

The efficacy of essential oils as seed coating followed by emerged seedlings foliar spray against the incidence of root rot and wilt diseases of bean was evaluated under field conditions. Data in Table 3 clearly demonstrate that all treatments significantly reduced disease incidence comparing with control. Rosa, mint, geranium, lemon in

Table 3. Root rot and wilt incidence of bean in response to essential oils<sup>A</sup> treatment and their influence on yield production under field conditions

Disease incidence % <sup>G</sup>		Treatment					
		geranium	rosa	lemon	mint	Topsin M 3g/kg	untreated
Root rot	pre-emergence <sup>B</sup>	13.2 d	12.1 d	14.1 c	12.4 d	15.2 c	21.3 a
	reduction <sup>C</sup>	38.0	43.2	33.8	41.8	28.6	–
	post-emergence <sup>D</sup>	11.8 cd	10.7 cd	12.5 d	11.7 cd	13.3 c	18.6 a
	reduction <sup>C</sup>	36.6	42.4	32.8	37.1	28.5	–
Wilt	pre-emergence <sup>B</sup>	14.4 c	12.8 d	14.1 c	12.7 d	16.2 bc	19.7 a
	reduction <sup>C</sup>	26.9	35.0	28.4	35.5	17.8	–
	post-emergence <sup>D</sup>	16.8 bc	15.7 c	17.5 ab	18.2 a	21.4 a	26.8 a
	reduction <sup>C</sup>	37.3	41.4	34.7	32.1	20.1	–
Yield	kg/plot <sup>E</sup> (42 m <sup>2</sup> )	33.7 d	34.2 d	29.8 c	31.3 cd	24.6 b	20.7 a
	increase % <sup>F</sup>	62.8	65.2	43.9	51.2	18.8	–

<sup>A</sup>essential oils applied as seed treatment (1%) plus foliar spray (1%)

<sup>B</sup>pre-emergence (%) based on the number of not emerged seeds in relation to the number of sown seeds

<sup>C</sup>reduction at different treatment, calculated relatively to untreated control

<sup>D</sup>post-emergence (%) based on the number of plants showing disease symptoms in relation to the number of emerged seedlings

<sup>E</sup>yield rated as average of accumulated kg/plot green bean at different treatments

<sup>F</sup>yield increase at different treatment, calculated relatively to untreated control

<sup>G</sup>mean values within columns followed by the same letter are not significantly different at  $p = 0.05$



descending order could reduce the incidence of root rot at both pre-, and post-emergence stages by 33.8–43.2% and 32.8–42.4% respectively, comparing with 28.6% in case of Topsin M. Variable order was observed regarding the protective effect of essential oils against wilt disease, which ranged between 26.9–35.5% and 32.1–41.4% at the both growth stages. Lesser significant effect was observed Topsin M seed treatment (17.8–20.1%), although it could significantly reduce wilt incidence over the control treatment. The accumulated harvested yield as green pods, in all treatments, was significantly higher than in the control. The highly effective treatments which demonstrated the increase of bean yield were rosa (65.2%), followed by geranium (62.8%), mint (51.2%) and lemon (43.9) respectively. The lowest increase in bean yield (18.8%) was observed for fungicide treatment.

## DISCUSSION

Within the large reservoir of natural fungicides that exist in plants examples exist that would serve as safe and effective alternatives to synthetic fungicides. Such compounds (volatile components, essential oils), if properly formulated and applied, could be used directly or could serve as templates for synthetic analogs.

In the present study, preliminary data suggested that the evaluated essential oils are capable of fungal growth inhibition *in vitro* when tested in direct contact. Geranium, rosa, lemon and mint essential oils were found to have an inhibitory effect against the mycelial growth of *R. solani* and *F. oxysporum* f. sp. *phaseoli* under *in vitro* conditions. Moreover, application of essential oils as seed coating and/or foliar spray revealed their efficacy against seed or plant invasion under *in vivo* conditions which resulted in a significant reduction in root rot and wilt incidence of bean under greenhouse and field conditions. The suppression of wilt and root rot development under greenhouse and field condition seem to corresponds with the ability of these essential oils to reduce disease incidence. Geranium oil had a superior effect in this concern out of all applied treatments followed by the other oils. The major components of the used geranium, rosa, lemon and mint oils are citronellol geraniol (27%), citronellol (33%), citral (63%) and menthol (30%), respectively. These results could be correlated with the stability and concentration as well as volatility of the active component in the essential oil used. In this regard, although lemon oil contains 63% of citral as active component, its low effectively against the radial growth of *R. solani* and *F. oxysporum* f. sp. *phaseoli* and their disease incidence in greenhouse and field could support this hypothesis. The used essential oils, geranium, rosa, lemon and mint are reported to contain many volatile compounds (Singh 1980) so, it seems that the antifungal effects are the result of compounds acting synergistically. This means that the individual components by themselves are not sufficiently effective. Similar results were also reported by many researchers indicating the efficacy of essential oils as antifungal inhibitors for a large number of soilborne pathogens (Akgul and Kivanc 1988; Nirmala *et al.* 1988; Kumar and Tripathi 1991; Singh 1992, 1994). The mode by which microorganisms are inhibited by essential oils and their chemical compounds seems to involve different mechanisms. It has been hypothesized that the inhibition involves phenolic compounds, because these compounds sensitize the phospholipid bilayer of the microbial cytoplasmic membrane causing increased permeability and unavailability of vital intracellular constituents (Juven 1994). Many authors empha-

sized 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 great a effect on the protons motive force, intracellular ATP content and overall activity of microbial cells, including turgor pressure control, solutes transport and metabolism regulation (Lanciotti 2004). Topsin M did not give an expected sufficient reduction on disease incidence in greenhouse and field, although it caused a complete inhibition of fungal radial growth at 400 ppm. This could be explained by the probable presence of fungal strains resistant to the fungicidal action under natural conditions, especially as the present experiment was carried out in heavily naturally infested field, where the fungicides were intensively used as regular agriculture practice. In this context, uncontrolled use of chemicals has been an inducing factor for the appearance of microbial strains more and more resistant to classic fungicides. Many years of increasing use of chemicals have created a situation leading to an ecological imbalance and the increase of multiples multi-resistant pathogenic microorganisms (Levy 1997).

In the light of these results it could be concluded that the application of essential oils is applicable, safe and cost-effective method for controlling soilborne diseases. Also, the use of essential oils in agriculture as fungicides has the advantages as they disintegrate in nature and do not leave a toxic residue of the product.

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## POLISH SUMMARY

### ZWALCZANIE WYSTĘPOWANIA WIĘDNIĘCIA I ZGNILIZNY KORZENI *PHASEOLUS VULGARIS* L. PRZEZ NIEKTÓRE LOTNE ZWIĄZKI ROŚLINNE

Badano aktywność *in vitro* i *in vivo* 4 olejków eterycznych z geranium, róży, cytryny i mięty przeciwko *Rhizoctonia solani* i *Fusarium oxysporum* f. sp. *phaseoli*, sprawcom zgnilizny korzeni oraz więdnienia roślin fasoli. *In vitro* związki te inhibowały wzrost grzybni obydwóch patogenów. Całkowitą inhibicję wzrostu grzybni obserwowano stosując każdy z badanych olejków eterycznych w stężeniu 4% oraz fungicyd Topsin M w stężeniu 400 ppm. W doświadczeniu szklarniowym olejki eteryczne stosowano do zaprawiania nasion i/lub jako oprysk nalistny wzeszłych roślin fasoli. Uzyskane wyniki zaprawiania nasion olejkami eterycznymi użytymi w stężeniu 1% jasno wskazują na dobry efekt ochronny przed zakażeniem grzybami *R. solani* i *F. oxysporum* f. sp. *phaseoli*, w porównaniu do zaprawiania fungicydem. Podobny, lecz nieco niższy efekt zaobserwowano w warunkach polowych w wyniku zaprawiania nasion olejkami eterycznymi, a następnie opryskiwania wzeszłych roślin roztworem tych olejków. Plon zielonych strąków fasoli uzyskany we wszystkich kombinacjach doświadczalnych był istotnie wyższy w porównaniu z plonem kombinacji kontrolnej, ale najniższy w przypadku zaprawiania nasion fungicydem.