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Efficacy of plant oils and their major monoterpenes in addition to powder formulation and seed cake of caraway oil on bacterial wilt incidence in potato

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ABSTRACT

Pot experiments were conducted to determine the efficiency of plant essential oils and monoterpenoides, carvone, thymol and menthol to control bacterial wilt in potato caused by *Ralstonia solanacearum* as potato seed tubers or soil treatments. Soil infested with *R.. solanacearum* was treated with the plant oils or their main components by the drench method at 0.5 and 1% concentrations using 200 ml of aqueous emulsion/pot. Caraway and thyme oils and the monoterpenes; carvone, thymol and menthol (1%) gave complete protection of potato plants from the infection by *R. solanacearum*, 6 weeks after inoculation. Peppermint oil (1%) gave reasonable effect in reducing wilt infection by 83% relative efficiency, whereas the neem oil showed no bactericidal activity as all treated plants wilted. Seed tuber treatment by powder formulation of caraway oil (1.3% carvone) at 3 and 5 g/kg seed tubers showed significant reduction in wilt severity with reduction percentages of 92 and 100%, respectively compared with the untreated inoculated control. Treatment of the soil with caraway seed cake at 10, 15 and 20 g/kg soil resulted in reduction of bacterial wilt severity by 58, 75 and 100%, respectively. Complete protection of potato from bacterial wilt incidence was obtained by the joint treatment of both soil treatment by caraway seed cake (15 g/kg soil) and seed tubers treatment by powdered caraway oil at 3 g/kg seeds. The observed high efficacy of these plant oils and their main components, in reducing bacterial wilt infection and increasing percentage of healthy plants, pointed out that these plant products have important roles in bacterial disease management.

Keywords: Essential oils, monoterpenes, natural bactericide, bacterial wilt control, *Ralstonia solanacearum*, potato.

INTRODUCTION

Potato ranks the third among food crops after wheat and rice and the fifth in total production in Egypt. This crop is affected by many pests and diseases and of these the potato brown rot disease (bacterial wilt) caused by *Ralstonia solanacearum* which is causing serious problems for potato exportation to Europe. *R.. solanacearum*, a soil borne pathogen, causes a lethal welting of more than 200 plant species world-wide including potatoes (Kucharek, 1998). This bacterium has a wide distribution in the tropics, subtropics and warm temperate regions, where it is one of the main factors limiting potato production and exportation. The pathogen is able to survive in soils and is carried along with water.

Infection of the potato plant by brown rot disease commonly occurs via the soil where bacteria enter the root system of the plant at root emergence points through wound sites, e.g., caused by nematode activity or via infected mother tubers and then colonizing the stem vascular tissue. The vascular tissues in the stem of the wilted plants exhibit a brown discoloration. The wilt disease of many solanaceous crop plants caused by *R.. solanacearum* has been controlled primarily by the development of disease resistant cultivars (Thurston, 1976). Several researchers studied the effect of certain bactericides from different groups on the *in vitro* and *in vivo* growth of *R.. solanacearum* (El-Goorani *et al.*, 1978 and Hassanein, 1987), but residues from applied chemicals in the soil are known to cause environmental problems. Furthermore, cultural practices and crop rotation showed limited effect (Kucharek, 1998). So, control of plant bacterial diseases remains difficult due to the limited availability of bactericides. In addition, the use of copper compounds, which are widely used for the control of plant bacterial diseases, will be limited in many countries, especially the European Union countries, by rule 473/2002 due to their impact on the environment. As a result, measures to control plant bacterial diseases are mostly limited to prevention. It is, therefore, necessary to find out more selective and safe materials to be used in management of bacterial wilt disease on potatoes.

Natural products and essential oils from plant sources have proved to be promising in pest control management with diverse biological activities as insecticides, fungicides and growth regulators (El-Sebae *et al.*, 1986; El-Sebae, 1987; Duke, 1991; El-Gendy *et al.*, 2004 and Eldoksch and El-Sebae, 2005). Essential oils are known for their antimicrobial activity (Maiti *et al.*, 1985; Hassanien and Eldoksch, 1997 and Cowan, 1999) and

proved to have the potential to control plant diseases caused by bacteria and, in particular, eradicate bacteria from seeds. Preliminary *in vitro* experiments conducted with several plant essential oils and some of their basic components exhibited bactericidal activity against seven of phytopathogenic bacteria including *R.. solanacearum* in laboratory tests (Hassanien and Eldoksch, 1997) and also showed fungicidal activity against soil-borne fungi (Eldoksch and Abdel-Mouty, 1997).

The aim of the present study was to evaluate the efficacy of selected plant essential oils of caraway, thyme, peppermint and some of their main components namely carvone, thymol and menthol in reducing wilt incidence caused by *R.. solanacearum* in pot experiments. The effects of powder formulation of caraway oil as seed tuber treatment and caraway seed cake as soil treatment on the bacterial wilt incidence were also studied. The joint effect of potato seed treatment with powder formulation of caraway oil plus soil treatment by seed cake on its potential in reducing bacterial wilt incidence in potato plants was investigated.

MATERIALS AND METHODS

Test oils and monoterpenoids: Three plant oils and three monoterpene compounds; carvone, menthol and thymol, were tested for their potential bactericidal activity against *R.. solanacearum*. The selected essential oils were extracted from the following medicinal plants: caraway, *Carum carvi* L. (fruits); peppermint, *Mentha piperata* L. (leaves); thyme, *Thymus vulgaris* L. (leaves) in addition to the commercial neem seed oil which was used as Neem Azal-T 5%, EC, produced by Bayer AG, Germany. Samples of about 100 g each of dried plant materials (peppermint and thyme) were ground into fine powder and extracted by soaking for three days with 80% EtOH. The combined ethanol extract was filtered and concentrated under reduced pressure at 40-45°C and then it was partitioned with petroleum ether 40-60°C. The petroleum ether extract was filtered and evaporated to dryness to obtain the oily crude extracts (Meisner *et al.*, 1981). Caraway oil has been pressed out of the raw fruits (seeds) by using cold press equipment. Caraway oil seed cake, which was remaining after pressing the seeds was tested as potential soil protectant agent against infection by *R. solanacearum*. Menthol 98.5% and thymol 99% (LOBA CHEMIE) were purchased from El-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt. Carvone 98% was provided from Schwarz's Essence-Fabrieken, Zaandam, Holland.

Chromatographic analysis of caraway oil: Essential oil of caraway was diluted by diethyl ether and 1 µl was injected to a gas chromatography HP 5890 coupled to flame ionization detector (FID). Authentic carvone was used as standard material. The GC analytical conditions were as follows: injector temperature, 220°C; program, 60°C (held for 10 min), ramped to 80°C at 1°C/min and then at 2.5°C/min to 120°C. Detector temperature was 280°C.

Bacterial culture and inoculum preparation: *Ralstonia solanacearum* (race 3, biovar 2), isolated from infested potato samples, was used in this study. Pathogenecity of the strain on potato was confirmed as part of fulfilling Kock's Postulates. The bacterium was grown on glycerol agar medium (GAM), containing 0.005% triphenyl tetrazolium chloride (TTC) in Petri dishes and kept at 30°C for 48-72 h.

Soil infestation with *R.. solanacearum*: Healthy tubers of potato *Solanum tuberosum* L. (cv. Draga) were obtained from the Brown Rot Disease Project, Ministry of Agriculture, Dokki, Egypt. The tubers were surface-disinfected in 75% ethanol solution for 2 min, then rinsed two times with sterile water and placed at room temperature to stimulate germination. Germinated tubers were sown (one/pot) in autoclaved sandy loam soil (1500 g) which was placed in sterile 15 cm diameter plastic pots. Soil was infected directly after planting by drenching soil of each pot with about 200 ml 10^8 CFU/ml of bacterial cell suspension of highly virulent *R.. solanacearum.*

Soil and potato tuber treatments: Pots with inoculated soils were treated with aqueous emulsions of essential oils of caraway, peppermint, thyme and their major components carvone, menthol and thymol, respectively in addition to neem oil by drench method using 200 ml of formulated tested oil or pure compound after 2 h of soil inoculation with *R.. solanacearum.* Two concentrations were applied for each selected oil; 5 ml/l (0.5%) and 10 ml/l (1%). Formulated essential oil required the addition of an emulsifier Tween-40 (1%) and water to get a stable essential oil emulsion. The untreated inoculated control consisted of the same amount of emulsifier and water.

Bioassay of powder formulation and seed cake of caraway oil: Both the powder formulation of caraway oil (1.3% carvone) which was prepared in Central Agric. Pesticide Lab. (CAPL) and caraway oil seed cake were

evaluated for their potential efficacy as potato tuber treatment and soil treatment, respectively against bacterial wilt incidence. Powder formulation of caraway oil was applied as seed treatment at the rates of 3, 5 and 10 g/kg potato seed tubers, whereas caraway seed cake was applied to soil at the rates of 10, 15 and 20 g/kg soil. The combined effect of both potato seed treatment using powdered caraway oil at the rate of 3 g/kg seeds plus soil treatment with seed cake at 15 g/kg soil against bacterial wilt incidence was also studied. Treated pots were kept under the open air conditions at temperature ranging from 22-30°C for about 42 days. Pots were arranged in a randomized block design with six replications. Wilted plants were recorded for each treatment, 6 weeks after inoculation. The data obtained were statistically analyzed according to Steel and Torrie (1980). Treatment efficacy (TE) was calculated according to the following suggested equation: TE = B - A / B x 100, where; A = wilt severity (no. of wilted plants) in treatment, B = wilt severity in control, taking into consideration that each potato plant which exhibited the symptoms of bacterial wilt incidence through the entire experiment, 6 weeks after inoculation with any degree of wilt severity on leaves and stems is accounted as wilted plant in comparison with the untreated control and inoculated control.

RESULTS AND DISCUSSION

Soil treatment with essential oils and monoterpene compounds: Data of the effects of formulated essential oils or their main monoterpene components on reducing bacterial wilt severity are presented in Table (1). The data showed that soil treatment with 1% aqueous emulsions of carvone and thymol, the basic components of caraway and thyme oils, respectively, significantly reduced bacterial wilt severity, 6 weeks after inoculation and all plants in the treated pots were healthy compared with the untreated infested control. Soil treatment with menthol, the main component of peppermint oil, showed 75% reduction in wilt incidence compared with the untreated control. Results showed significant differences between formulated plant oils and oil concentrations. Treatment of the soil with 0.5% (5 ml/liter) of aqueous emulsion of caraway oil resulted in the most effective treatment against *R.. solanacearum* with about 75% reduction in bacterial wilt severity, 6 weeks after inoculation followed by thymol oil (70%) and then peppermint oil (58%), while neem oil (5% azaderachtin) had no effect on reducing bacterial wilt. Treatment of the soil with 1% aqueous emulsion of caraway and thyme oils exhibited high efficacy in reducing bacterial wilt incidence and showed 100% healthy plants, followed

by peppermint oil with 83% reduction in wilt severity and neem oil treatment which exhibited no efficacy in reducing brown rot infection, where all the plants wilted indicating that neem oil has no bactericidal activity on *R.. solanacearum* (Table 1).

Table (1): Effect of soil treatments with the tested plant oils and their major components on potato bacterial wilt incidence caused by *Ralstonia solanacearum.*

Treatments	Application rate (ml or g/l)	Soil drench[1] (2 h after inoculation)	
		Bacterial[2] wilt severity (%)	Efficacy (%)
Caraway oil	5 ml	25 [c]	75
	10 ml	0	100
Peppermint oil	5 ml	42 [b]	58
	10 ml	17 [c]	83
Thyme oil	5 ml	30 [bc]	70
	10 ml	0	100
Neem oil	5 ml	100 [a]	0
(Neem Azal-T 5%)	10 ml	100 [a]	0
Carvone	10 ml	0	100
Menthol	10 g	25 [c]	75
Thymol	10 g	0	100
Control[3]	-	100 [a]	-

L.S.$D_{0.05}$ for bacterial wilt severity = 13.7

[1] Soil treatment by 200 ml/pot of prepared aqueous emulsions of oil or pure compound.

[2] Bacterial wilt severity based on 12 plants, 6 weeks after inoculation.

[3] Untreated inoculated control.

The data of the current study clearly indicated that soil treatment with aqueous emulsions of caraway, thyme and peppermint essential oils or their active components, carvone, thymol or menthol were found to be effective in reducing bacterial wilt severity on potato caused by *R.. solanacearum.* These plant oils and their main components have been shown to have bactericidal activity in *in vitro* experiments against several phytopathogenic bacteria including *R.. solanacearum* (Hassanien and Eldoksch, 1997). Also, thymol and thyme oil have been shown to effectively reduce populations of *R.. solanacearum* in *in vitro* tests and to reduce bacterial wilt incidence on tomato in greenhouse experiments (Momol *et al.*, 1999). Furthermore, caraway, thyme and peppermint oils in addition to carvone and menthol have also exhibited antifungal activity

against *Rhizoctonia solani* and *Fusarium oxysporium* in *in vitro* studies (Eldoksch and Abdel-Mouty, 1997). They found also that essential oil of thyme in addition to carvone and menthol suppressed soil borne disease development caused by *R. solani* on mungbean. Lack of effectiveness of the neem oil in the present study indicates that plant oils differ in their bactericidal activity against *R.. solanacearum*.

Effect of powder formulation and seed cake of caraway oil on potato bacterial wilt incidence: Seed treatment of potato with powder formulation of caraway oil showed protection of potato plants against bacterial wilt incidence through the entire experiment, 6 weeks after inoculation and all plants were healthy when potato seeds were treated at the rates of 5 and 10 g/kg seeds with 100% reduction in wilt severity, as shown in Table (2). In addition, promising efficiency was obtained at the rate of 3 g/kg seeds with 92% reduction in wilt severity compared with the untreated inoculated control. Incorporation of soil with caraway seed cake at the rate of 10, 15 and 20 g/kg soil resulted in reduction percentages of bacterial wilt incidence, 6 weeks after inoculation by about 58, 75 and 100% reduction, respectively. These data indicate that caraway seed cake has such compounds with bactericidal activity and may be including carvone and other minor compounds. Results also indicated that the joint action treatment of both soil treatment by caraway seed cake plus seed tubers treatment with powdered caraway oil showed highly effective treatment and all potato plants were healthy and free from wilt after 6 weeks of inoculation (Table 2). Gas chromatography analysis of caraway oil revealed that carvone, the main monoterpene compound of caraway oil, constitutes 23.5% of the oil. Seed tuber treatment with powdered caraway oil (1.3% carvone) exhibited significant efficacy as potato seed protectant against bacterial wilt infection caused by *R.. solanacearum* (Table 2). This bactericidal activity was attributed to the main component carvone and can be also attributed to the resulting synergistic or antagonistic activity of minor components such as limonene, carvacrol and dihydrocarvone (Bouwmeester *et al.*, 1998). Iacobellis *et al.*(2005) reported that the antimicrobial activity of caraway essential oil is apparently due to the presence of carvone (23.3%), limonine (18.2%), carvacrol (6.7%) and linalool (0.3%), which inhibit the growth of fungi and bacteria (Farag *et al.*, 1989; Scortichini and Rossi, 1991; Oosterhaven *et al.*, 1995 and Dorman and Deans, 2000). In general, the results of the current study indicated the antibacterial activity of essential oils of caraway, thyme, peppermint and their main components against *R.. solanacearum* the causal agent of bacterial wilt disease of potato. In

addition, powder formulation of caraway oil as seed tuber treatment and seed cake as soil treatment have proved to be effective in reducing bacterial wilt severity in potato plants demonstrating the bactericidal activity of caraway products.

Table (2): Effect of powder formulation and seed cake of caraway oil on potato bacterial wilt incidence caused by *Ralstonia solanacearum*.

Treatments	Method of application	Rate of application	Bacterial[1] wilt severity (%)	Efficacy (%)
Caraway oil Powder[2]	Seed treatment	3 g/kg seeds	8 [d]	92
		5 g	0	100
		10 g	0	100
Caraway seed cake	Soil treatment	10 g/kg soil	42 [b]	58
		15 g	25 [c]	75
		20 g	0	100
Caraway oil powder + Caraway seed cake	Seed treatment + Soil treatment	3 g/kg seeds + 15 g/kg soil	0	100
Control[3]	-	-	100 [a]	-

L.S.$D_{0.05}$ for bacterial wilt severity = 16.2

[1] Bacterial wilt severity based on 12 plants, 6 weeks after inoculation.

[2] Caraway oil powder formulation contains 1.3% carvone.

[3] Untreated inoculated control.

Since these essential oils or their major components, carvone, thymol and menthol have been reported to have bactericidal, fungicidal and nematicidal activities (Eldoksch and Abdel-Mouty, 1997; Hassanien and Eldoksch, 1997; Oka *et al.*, 2000 and Pradhanang *et al.*, 2003), they could be used in integrated management of soil-borne diseases in potatoes. It should be mentioned also that these natural plant oils and their active monoterpene molecules have no adverse effects to humans or the environment when it is used in a manner consistent with label (Liu *et al.*, 2002). In addition, chemical structures of these bioactive monoterpene molecules such as carvone, thymol and menthol could be used as template molecules for future synthesis of new and safe bactericides with potential novel and safe modes of action.

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تأثير الزيوت النباتية ومكوناتها التربينية الأساسية وأيضا تجهيزة مسحوق وكسب زيت الكراوية على مدى الإصابة بالذبول البكتيرى فى البطاطس

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تم إجراء تجارب بزراعة تقاوى البطاطس فى أصص لتقدير كفائة بعض الزيوت الأساسية ومكوناتها التربينية النقية بالاضافة إلى تجهيزة مسحوق وكسب زيت الكراوية فى مكافحة مرض الذبول البكتيرى (العفن البنى) فى البطاطس المتسبب عن البكتيريا رالستونيا سولاناسيرم وذلك عن طريق معاملة تقاوى البطاطس قبل الزراعة أو معاملة التربة أو تطبيق المعاملتين معا. تم إجراء عدوى صناعية للتربة بالبكتيريا المسببة لمرض ثم أجرى تبليل للتربة بالزيوت الأساسية أو مركباتها التربينية النقية عند تركيز ٠,٥% ، ١% باستخدام ٢٠٠ ملى مستحلب مائى/أصيص. وأوضحت النتائج أن زيوت الكراوية والزعتر وأيضا المركبات التربينية النقية كارفون، ثيمول ومنثول أظهرو وقاية كاملة لنباتات البطاطس من الاصابة بالذبول البكتيرى بنسبة ١٠٠% وذلك بعد ٦ أسابيع من العدوى الصناعية باستخدام تركيز ١%. ولقد أعطى زيت النعناع تأثير جيد فى خفض نسبة الاصابة بمقدار ٨٣% بالمقارنة بالكنترول، بينما أظهر زيت النيم (٥% أزادراختين) عدم فعالية ضد الذبول البكتيرى حيث أن جميع النباتات أصيبت بالذبول.ولقد وجد أن معاملة تقاوى البطاطس قبل الزراعة بمسحوق زيت الكراوية (١,٣% كارفون) عند تركيزات ٣ و ٥ جم/كجم تقاوى أدى إلى خفض معنوى فى شدة الذبول بنسبة ٩٢، ١٠٠% على التوالى. ولقد وجد أن معاملة التربة بكسبة بذور الكراوية بعد الاستخلاص (seed cake) بمعدل ١٠، ١٥، ٢٠ جم/كجم تربة أعطى خفض فى شدة الذبول البكتيرى بنسبة ٥٨، ٧٥، ١٠٠% على التوالى.

تم الحصول على وقاية كاملة لتقاوى البطاطس من العدوى بالذبول البكتيرى باستخدام المعاملة المزدوجة للتربة باستخدام كسب بذور الكراوية (١٥ جم/كجم تربة) والتقاوى بمسحوق زيت الكراوية بمعدل (٣ جم/كجم تقاوى). وبناءاً عليه فإن الفعالية العالية لهذه الزيوت النباتية المختبرة ومكوناتها الأساسية فى خفض شدة الذبول البكتيرى وزيادة عدد النباتات السليمة يشير إلى أن هذه المنتجات النباتية قد يكون لها دور هام وفعال فى مكافحة الأمراض البكتيرية وخاصة مرض العفن البنى فى البطاطس.