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Suppression of the interaction damage of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici* to tomato plants by some plant oils

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ABSTRACT

In vitro effect of four plant oils namely roquette, onion, eucalyptus and aloe applied at 25, 50 and 100 % on egg hatching of *Meloidogyne incognita* and on the mycelium growth of *Fusarium oxysporum* f. sp. *Lycopersici* was investigated. Tomato root dip technique was also used to study the suppression of damage produced by the interaction between two organisms. Data showed that all the tested plant oils at selected concentrations caused significant reduction on the numbers of hatched juvenile nematodes with dose dependent manner. The highest reduction was obtained by roquette oil followed by onion, eucalyptus and then aloe oils. Moreover, the results showed that there were significant differences between the various plant oils corresponding to their *in vitro* inhibitory effect on the mycelial growth of *F. oxysporum* f. sp. *lycopersici* comparing to control value. The maximum percentage of inhibition was recorded by onion oil (71.8 %) followed by roquette (67.7 %), eucalyptus (60.6 %) and then aloe oils (46.2 %). All the tested plant oils reduced both the interaction damage produced by the interaction of *M. incognita* and *F. oxysporum* f. sp. *lycopersici*. The highest reduction was obtained with onion followed by eucalyptus, roquette and aloe, whereas roquette was found the most potent oil to reduce the number of nematode galls followed by onion, eucalyptus and aloe. It could be concluded that some plant oils such as roquette, onion and eucalyptus can be used in tomato plants as protectant from the infection with fungus and/or nematode under greenhouse conditions instead of using the traditional pesticides.

INTRODUCTION

Tomato plant is one of the most worldwide economic and nutritive importance. According to Adejuwon and Olutiola (2005), tomato Fusarium wilt disease (*Fusarium oxysporum* Schl. f. sp. *lycopersici* (Sacc.) Synder & Hansen) is one of the most damaging diseases either in nurseries or in the

open fields causing considerable reduction in tomato yield. This disease is widely spread particularly through early summer and/or autumn (Nili) tomato plantations in Upper Egypt Governorates and newly reclaimed sandy regions in Egypt (Awad, 1990).

Root-knot nematode disease (*Meloidogyne incognita* Chitwood) is widespread and economically important in a range of field and vegetable crops (Ploeg, 2002). Symptoms associated with root-knot nematodes are non-specific and may include stunting, chlorosis, wilting under sufficient soil moisture, and increased susceptibility of plants to other diseases (Taylor and Sasser, 1978).

Natural products that possess antagonistic activity against noxious nematodes have received special attention during the last few decades. The crude extracts of *Ocimum basilicum* (Yadav, 1970 and Chatterjee *et al.*, 1982); *O. sanctum* (Chatterjee *et al.*, 1982); *Ambrosia maritime* and *Origanum najorana* (Hashim, 1993 and Hashim *et al.*, 1999) and *Mentha longifolia* and *Thymus vulgaris* (Hashim *et al.*, 1999) were found to have nematicidal activity against *M. incognita*. Moreover, some of these extracts adversely affect the hatching and penetration of *M. incognita* (Hashim, 1993). Plant extracts and essential oils also showed antifungal activity against a wide range of fungi (Wilson *et al*, 1997 and Ibrahim and Al-Mahna, 2002). In this respect, *Aristea ecklonmii* and *Agapathus inapertus* were found to have antifungal activity against *Botrytis cinerea*, *F. oxysporum* and *Rhizoctonia solani* (Pretorius *et al.*, 2002), and oil of *Mentha spicanta* against *F. oxysporum* f. sp. *Lentis* (Singh *et al.*, 1994). In addition, the essential oils of java citronella, eucalyptus, cinnamon and peppermint were found to inhibit the growth of *F. moniliforme* (Baruah *et al.*, 1996).

The present investigation aimed to examine the effect of some plant oils namely roquette (*Eruca sativa*), onion (*Allium cepa*), eucalyptus (*Eucalyptus globules*) and aloe (*Aloe vera*) as safe alternative control strategy to suppress the interaction damage results from *M. incognita* and *F. oxysporum* f. sp. *lycopersici* to tomato plants.

MATERIALS AND METHODS

Isolation and identification of the causal organisms:

The causal organisms of tomato wilt diseases (*F. oxysporum* f. sp. *lycopersici*) was isolated from the vascular bundles of tomato plants collected from Alexandria and El-Behira Governorates fields and showed typical external wilt symptoms and internal brown xylem discoloration. The purified fungal culture was identified according to cultural and morphological diagnostic criteria following the key of genus *Fusarium* (Booth, 1971). The juveniles of root-knot nematode (*M. incognita*) was cultured from natural infected tomato plants collected from Alexandria and El-Behira Governorates fields and showed typical symptoms of root-knot nematode disease. Individual egg-masses with their mature adult females were placed in fresh water inside small glass vials and identified by the perineal pattern system according to Taylor *et al.*, (1955). The identified egg-masses were transferred to pots filled with sterilized sandy clay soil, cultivated with 30-day old Supermarmande tomato seedling and kept under greenhouse conditions for 40 days. Infected roots with a considerable number of mature females and egg-masses were chopped and used as a source of infection.

Plant oils and antimicrobial assay:

Four plant oils namely roquette (*Eruca sativa*), onion (*Allium cepa*), eucalyptus (*Eucalyptus globules*) and aloe (*Aloe vera*) were obtained from Alexandria Pharm. Chem. Ind. Company, Egypt. Disk diffusion technique was used to evaluate these oils against *F. oxysporum* f. sp. *lycopersici* according to Ibrahim and Al-Mahna, (2002). In this method, five filter paper disks (Whatman no. 1, 9 mm diameter) were carefully loaded in different concentrations and then placed on potato dextrose agar (PDA) plate. The tested concentrations of plant oils, 25, 50 and 100 % were prepared in water contained Tween-20 as an emulsifier. Control sample inoculated with *F. oxysporum* f. sp. *lycopersici* only. Disk of inoculated *F. oxysporum* f. sp. *lycopersici* was put on the center of the plate and then incubated at 25 °C for full growth. The inhibition zones were daily measured and compared with control values. Each treatment was replicated four times.

Egg-masses of *M. incognita* were harvested from infected roots and then ten egg-masses were suspended in test tube containing 3 ml of each concentration of the tested plant oils. At the same time another 10 egg-masses were suspended in sterilized distilled water and served as control.

Each treatment was replicated four times. Tubes were incubated at 25 °C for 14 days and then the numbers of hatched juveniles and percent of inhibition were counted.

Bare root dip technique:

Supermarmande tomato seeds cultivar susceptible to root-knot nematode, *M. incognita* and *F. oxysporum* f. sp. *lycopersici* were sown in seedbeds containing autoclaved sandy loam soil. Tomato seedlings were removed carefully after 30 days and then roots were washed gently in running tap water to remove soil particles and dipped for 5 minutes in plant oils freshly prepared by mixing 20 ml of each plant oil with 1 L of water and 1ml of Tween-20. Eggs of nematode were harvested from infected root using sodium hypochlorite (Hussey and Barker, 1973), suspended in sterilized distilled water and the inoculum was standardized at 5000 eggs per pot. The microconidia of the fungus isolate growing on PDA plates at 25 °C for 14 days were harvested by flooding the plates with sterilized distilled water. The resulting suspension was strained through cheesecloth and then the inoculum potential was adjusted to $1x10^6$ spores/ml using haemocytometer. Each pot containing 2 tomato plants received 25 ml of the spore suspension and treatment were arranged as following: (1) control, uninfested soil and non-treated seedling; (2) soil infested with *M. incognita* and seedling treated with plant oils; (3) soil infested with *M. incognita*, *F. oxysporum* f. sp. *lycopersici* and seedling treated with plant oils and (4) soil infested with *F. oxysporum* and seedling treated with plant oils. Each treatment was replicated 4 times. The pots were randomly arranged in the greenhouse, watered every other day for 40 days. Fusarium wilt, root-knot nematode diseases and dry weight of shoot plants were investigated. Fusarium wilt was rated a scale of 0 to 4 according to the percentage of foliage with yellowing or necrosis (0= 0 %, 1= 1-33 %, 2= 34-66 %, 3= 67-100 % and 4= death of plant) (Jimenez *et al.*, 1991).

Root-knot nematode disease was counted as number of galls / 2 g root system. The experiments were carried out twice.

Statistical analysis:

Statistical analysis was carried out using a computer software program of statistical analysis system, SAS (SAS Institute, 1988)

RESULTS

Data presented in Table 1 illustrate that all the tested plant oils at selected concentrations exhibited significant reduction on the numbers of hatched juveniles of *M.incoinita* with dose dependent manner. The highest reduction was obtained by roquette oil followed by onion, eucalyptus and then aloe oils. Moreover, the data showed that all the hatched juveniles were found dead following the using concentration of either 50 or 100 % of all the tested oils.

In vitro anti fungal properties of the tested plant oils were evaluated by disk diffusion technique against *F. oxysporum* f. sp. *lycopersici* (Table 1). The results showed that there were significant differences between the various plant oils corresponding to their inhibitory effect on *F. oxysporum* f. sp. *lycopersici* mycelial growth comparing to control value. The maximum percentage of inhibition was recorded by onion oil (71.8 %) followed by roquette (67.7 %), eucalyptus (60.6 %) and then aloe oils (46.2 %).

The percentage of Fusarium wilt in tomato cultivar was recorded after dipping tomato seedling in oils is presented in Table 2. The roquette oil treatment was found to be the most potent one to reduce Fusarium wilt by 67.7 %, while the treatment with eucalyptus, onion and aloe oils reduced Fusarium wilt by 65, 63 and 40.5 %, respectively, when compared with soil infested with *F. oxysporum* f. sp. *lycopersici* plus *M. incognita*. In addition, the data illustrate that the shoot dry weights increased by 83.5, 75.4, 61.2 and 29.6 % following treatment with roquette, onion, eucalyptus and aloe oils, respectively. On the other hand, the number of nematode galls were reduced by 32.4, 32.1, 30.1 and 22.3 % following treatment with onion, eucalyptus, roquette and aloe, respectively, when compared with soil infested with *F. oxysporum* f. sp. *lycopersici* plus *M. incognita* (Table 2). Also, all tested oils increased the percentages of shoot dry weight by 65.4, 57.7, 51.2 and 28.1 % following roquette, onion, eucalyptus and aloe oils application, respectively.

The ability of plant oils to reduce the interaction damage results from *F. oxysporum* f. sp. *lycopersici* plus *M. incognita* is presented in Table 3. The results showed that all the tested plant oils reduced both the interaction damage expressed as Fusarium wilt without significant differences between the use of roquette, onion or eucalyptus oil.

Table 1: *In vitro* effect of some plant oils on egg hatching of *M. incognita* and growth of *F. oxysporum* f. sp. *Lycopersici*

Treatment	Concentration of plant oils									% of inhibition growth of *F. oxysporum* f.sp. *lycopersici*
	25%			50%			100%			
	No. of hatched juveniles	Reduction %	alive juveniles %	No. of hatched juveniles	Reduction %	% of alive juveniles	No. of hatched juveniles	% of reduction	% of alive juveniles	
Roquette oil	292.0e	87.0	28.2	237.3e-	89.4	0.0	168.7e	92.5	0.0	67.7e
Onion oil	373.7d	83.4	33.3	317.7d	85.9	0.0	185.7d	91.7	0.0	71.8d
Eucalyptus oil	526.0c	76.6	41.7	438.7c	80.5	0.0	242.7c	89.2	0.0	60.6c
Aloe oil	1054.7b	53.1	58.8	870.7b	61.3	0.0	434.3b	80.7	0.0	46.2b
Control	2248.0a	0.0	100	2248.0a	0.0	100	2248.0a	0.0	100	100a

Means followed by the same letter within the same column are not significantly different according to the Duncan's multiple range test ($p \leq 0.05$).

Table 2: Effect of some plant oils on the reduction of Fusarium wilt and root-knot nematode diseases in Supermarmande cultivar of tomato.

Treatment	Infested with *F. oxysporum* f.sp. *lycopersici* (FOL)				Infested with *M. incognita* (MI)			
	Fusarium wilt (%)	with Reduction (%)	Weight of shoot dry	Increase in dry weight (%)	No. of galls/2g roots/system	Galls reduction (%)	Weight of shoot dry	Increase in dry weight (%)
Soil infested with FOL+MI	85.7a	0.0	2.60f	0.0	351.7a	0.0	2.60e	0.0
Soil infested with FOL or MI and seedling treated with roquette oil	27.7d	67.7	4.77b	83.5	245.7c	30.1	4.3b	65.4
Soil infested with FOL or MI and seedling treated with onion oak	31.7d	63.0	4.56b	75.4	237.7c	32.4	4.1c	57.7
Soil infested with FOL or MI and seedling treated with eucalyptus oil	30.0d	65.0	4.19c	61.2	238.7c	32.1	3.93c	51.2
Soil infested with FOL or MI and seedling treated with aloe oil	51.0c	40.5	3.37d	29.6	273.3b	22.3	3.33d	28.1
Soil infested with FOL or MI only	64.3b	25.0	3.06e	17.7	282.3b	19.7	3.25d	25.0
Control (autoclaved soil and non-treated seedling)	0.0e	100	5.97a	129.6	0.0d	100	5.97a	129.6

Means followed by the same letter within the same column are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

Table 3: Effect of some plant oils on the reduction of the interaction damage results from *F. oxysporum* f.sp. *lycopersici* and *M. incognita*

Treatment	Fusarium Wilt (%)	Wilt Reduction (%)	No. of galls/ 2g root system	Galls Reduction (%)	Dry weight (g/plant)	Increase in dry weight (%)
Soil infested with FOL+MI	85.67a	0.0	351.7a	0.0	2.6f	0.0
Soil infested with FOL + MI and seedling treated with roquette oil	27.67d	67.7	224.3c	36.2	4.92b	89.2
Soil infested with FOL + MI and seedling treated with onion oil	25.0d	70.8	227.7c	35.3	3.45c	32.7
Soil infested with FOL+ MI and seedling treated with eucalyptus oil	26.67d	68.9	240.0c	31.8	3.61c	38.9
Soil infested with FOL + MI and seedling treated with aloe oil	40.0c	53.3	324.0a	7.9	2.77f	6.5
Soil infested with MI only	0.0e	100	324.0a	7.9	3.25d	25
Soil infested with FOL only	64.33b	24.9	0.0d	100	3.06e	17.7
Control (autoclaved soil and non-treated seedling)	0.0e	100	0.0d	100	5.97a	129.6

Means followed by the same letter within the same column are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

DISCUSSION

The present investigation showed that all tested plants oils decreased the number of hatched juveniles and the highest reduction was obtained following roquette oil treatment, while the least one was aloe oil. Some oils and crude extract of some plants were used to suppress the hatching of juveniles (Yadav, 1970, Chatterjee, *et al.*, 1982 and Hashim, 1993) and penetration of nematodes (Hashim, 1993).

Also, the present study illustrate that all the tested oils inhibited the mycelial growth of *F. oxysporum* f. sp. *lycopersici*, where the highest inhibition was obtained by onion oil and the least one was aloe oil. These results are in parallel with other studies, where the oils of orange, peppermint, eucalyptus (Pattnaik, *et al.*, 1996), thyme, lavender, mint (Zambonelli *et al.*, 1996) coriander, cumin and fennel (Pandy and Pant, 1997) inhibited the mycelial growth of many fungi.

In the present study, all the tested oils reduced either the Fusarium wilt, number of nematode galls or the interaction damage resulted by the combination between *M. incognita* and *F. oxysporum* f. sp *lycopersici* and improved the shoot dry weights of tomato plants. The interaction damage resulted from *Meloidogyne* spp. and several formae specials of *F. oxysporum* have been studied in several crops more than other fungus-nematode combination (Castillo *et al.*, 2003).

Some plant oils such as roquette, onion, eucalyptus, and aloe could be used in tomato plants as protectant from the infection with fungus and/or nematode under greenhouse conditions instead of the application of soil fumigants (Colyer *et al.*, 1997) or nematicides (Kirkpatrick, 1994). Further examinations are needed to test these materials under field conditions and other plants.

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خفض الضرر في نباتات الطماطم نتيجة التداخل بين نيماتودا تعقد الجذور *Meloidogyne incognita* والفطر المسبب للذبول الفيوزاريومي *Fusarium oxysporum* f. sp. *lycopersici* بواسطة بعض الزيوت النباتية

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تم دراسة تأثير الفعل الإبادي ضد النيماتودا و الفطر لأربعة من الزيوت النباتية وبتركيزات قدرها 25، 50 و 100% وذلك بمعاملة جذور بادرات الطماطم بهذه الزيوت بغرض تقليل الضرر الناتج من الفعل المشترك لكل من نيماتودا تعقد الجذور *Meloidogyne incongnita* و الفطر المسبب للذبول الفيوزاريومي *Fusarium oxysporum* f. sp. *lycopersici*. أوضحت النتائج أن كل الزيوت المختبرة قد أحدثت انخفاضا معنويا لنسب فقس بيض النيماتودا وأن هذا الانخفاض يتناسب طرديا مع التركيز وكان أعلى الزيوت تأثيرا هو زيت الجرجير ثم زيت البصل ثم زيت الكافور ثم زيت الصبار. أيضا أوضحت النتائج وجود اختلافات معنوية بين أنواع الزيوت المستخدمة من حيث قدرتها التثبيطية ضد نمو هيفات الفطر وأن أعلى نسبة انخفاض لنمو هيفات الفطر تم إحداثها بفعل زيت البصل (71.8%) ثم الجرجير (67.7%) ثم الكافور (60.6%) و أخير الصبار (46.2%). من ناحية أخرى أحدثت كل الزيوت المختبرة إنخفاضا في الضرر المشترك الناتج عن فعل نيماتودا تعقد الجذور و الفطر المسبب للذبول الفيوزاريومي و كان زيت البصل هو الأكثر كفاءة لتحقيق هذا الإنخافض يليه الكافور يليه الجرجير ثم الصبار.

ويمكن القول بأنه يمكن استخدام بعض الزيوت النباتية مثل زيوت البصل و الجرجير و الكافور لمعاملة نباتات الطماطم في البيوت المحمية لمكافحة نيماتودا تعقد الجذور و الفطر المسبب للذبول الفيوزاريومي بدلاً من استخدام المبيدات التقليدية.