Plant Pathol. J. 25(2) : 189-192 (2009)

The Plant Pathology Journal

Note

Management of Tomato Root-knot Nematode *Meloidogyne incognita* by Plant Extracts and Essential Oils

Kamal A. M. Abo-Elyousr[1]*, Magd El-Morsi Awad[2] and M. A. Abdel Gaid[3]

[1]*Plant Pathology Department, Faculty of Agriculture, Assiut University, Assiut, Egypt*

[2]*Plant Pathology Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt*

[3]*Horticulture Research Institute, Agricultural Research Center, Giza, Egypt*

(Received on January 12, 2009; Accepted on May 16, 2009)

The effect of plant extracts of eucalyptus (*Eucalyptus chamadulonsis*), garlic (*Allium sativium*), marigold (*Tagetes erecta*) and neem (*Azadirachta indica*) and essential oils were tested on the suppression of root-knot nematode *Meloidogyne incognita* under greenhouse and field conditions. *In vitro* study, all tested treatments had nematicidal effect on nematode juveniles after 24 and 48 hours from exposures. The highest percentage of nematode mortality was achieved by application of neem extract (65.4%), essential oils (64.4%) and marigold extract (60.5%), followed by garlic and eucalyptus extracts (38.7-39.5%). Under greenhouse and field conditions, neem extract and essential oils treatments were more effective in reducing population numbers of the *M. incognita* in soil and root gall index compared to other treatments. In field experiments, the maximum protection of tomato plant against root-knot nematode was obtained by application of neem and essential oil treatments, 44.2 and 32.6%, respectively.

Keywords : Eucalyptus, essential oils, garlic, marigold, *Meloidogyne*, neem, Tomato

Root-knot disease of tomato caused by *Meloidogyne incognita* is an important disease of tomato in Egypt (El-Sherif et al., 1999). Root- knot nematodes *Meloidogyne* spp. cause high levels of economic loss in many of agricultural crops worldwide. They are capable of severally damaging a wide range of crops, causing dramatic yield losses (Kiewnick and Sikora, 2006).

Many investigators had managed root-knot nematodes by using some plant extracts of certain ornamental plants (Natarajan et al., 2006 and Javed et al., 2007). Korayem et al. (1993) stated that exposure of *M. incognita* juveniles to standard water extract solution of *Artemesia obsinthium*, *Thymus vulgaris* shoot powder and *Punica granatum* fruit powder for 72 hr reduced the number of active nematodes by 100%. Some plant extracts were evaluated for their nematicidal potentials in controlling *M. incognita* infesting tomato (Javed et al., 2007). The tested plant extracts have significantly inhibited the total number of nematode juveniles, numbers of galls and egg-masses, as well as the total number of root-knot nematodes in soil.

The aims of this study were to evaluate the efficacy of certain Egyptian plant extracts and essential oils for controlling root-knot nematode *M. incognita* and yield components of tomato crops in greenhouse and field conditions.

Nematode identification. Galled roots were collected from naturally infected tomato plants. Species of *Meloidogyne* were identified on the basis of the perineal pattern of mature females according to the keys of Seinhorst (1966).

Preparation of plant extracts and essential oils. Ten grams of whole fresh leaves of eucalyptus (*Eucalyptus chamadulonsis*), garlic (*Allium sativium*), marigold (*Tagetes erecta* L) and neem (*Azadirachta indica*) were separately mixed in 100 ml distilled water in an electric blender for 10 min, and then left for 72 hr before filtration through Whatman filter paper No.1. Each filtrate was considered as a standard solution of 100% concentration and then kept in a freezer until using.

Essential oils were used at a concentration of 0.05% (diluted with tap water) by added at transplanting and after two weeks from planting. They were provided by, Dept. of Research Self-pollination of Vegetable, Horticulture Research Institute, Dokki, Giza, Egypt. Essential oils are composed oils of nigella (*Nigella sativa*), spearmint (*Mentha spicata*), eucalyptus (*Eucalyptus chamadulonsis*), cumin (*Cuminum cyminum*), thyme (*Thymbra spicata*), onion (*Allium cepa*) and lemon (*Citrus lemon*).

Effect of certain plant extracts and essential oils on mortality of nematode *in vitro*. Nematicidal effects of plant extracts and essential oils were evaluated against *M. incognita* under laboratory conditions. About 300 juveniles of *M. incognita* juveniles were transferred to the concent-

*Corresponding author.
Phone) +20103821743, FAX) +20882331384
E-mail) kaaboelyousr@yahoo.com

ration of plant extracts and essential oil in sterilized Petri dishes while tap water served as a control (Alam, 1985). Five replicates were used. Separate sets of Petri dishes were maintained for each period of observation (24 and 48 h). Mortality of nematodes was confirmed by touching the juvenile with fine needle.

Greenhouse experiment. Tomato seedlings cv. Super Strain B about 45 days old were planted in clay pots 20 cm filled with steam-sterilized sandy loam soil (two seedling/pot), five pots were used for each treatment. Each treatment was replicated two times and pots were arranged in a completely randomized design in greenhouse. A total of 500 juveniles were transferred to a glass beaker containing100 ml water and poured evenly around the stem of each tomato seedling 3-day after transplanting. One hundred ml of each treatment (plant extracts and essential oils) was applied to the soil around the plant stem after 10 and 30 days after nematode infestation. The nematicide, Vydate (Oxamyl) 10% G, (Methyl N',N'-dimethyl-N-[(methyl carbamoyl)oxy]-1-thiooxamimidate) obtained from Dwbon De-Nymwrz, USA, (0.5 g/pot) was sprinkled over the soil around the plants stems, raked and watered (100 ml) into the soil. Pots with nematode treatment alone were used as control. Each treatment had five replications and experiment was repeated twice. After 40 days from the last treatments, soil samples (250 g) were collected from rhizosphere pots and nematode populations were extracted and counted (Goodey, 1963).

Two months after nematode inoculation, plants were uprooted and washed by water. Root gall severity was assessed on a 0-5 rating scale according to the percentage of galled tissue, in which 0=0-10%; 1=11-20%; 2=21-50%; 3 =51-80%; 4=81-90%; and 5=91-100% (Barker, 1985).

Field experiment. Plant extracts and essential oils added as soil irrigation were evaluated under field conditions; each in four replicates (rows). Field experiments were divided to plots of 10.5 m^2 which included 2 rows of 1.2 m width. The tomato seedlings were transplanted during the first week of October 2006 and 2007 growing seasons. Traditional agricultural practices were carried out according to technical recommendations in tomato cultivation. The tested materials were added three times starting from October 15, 2006 and end of October. The experiments were carried out at New Valley Governorate in naturally heavily infested soil with root-knot nematodes *M. incognita*. The experiments were repeated twice in 2006/2007 growing seasons. Untreated plants served as control. All treatments were arranged in a split plots design in four replicates for each treatment. Soil and root samples were collected after 40 days from the last treatments for nematode analysis.

Percentages of reduction in the second juveniles (J2) counts were estimated according to Anderson and Tilton formula (Puntener, 1981) as follows:

$$\text{Reduction (\%)} = \frac{A - B}{B} \times 100$$

Whereas: A=Population of the treated plots after application, B=Population of the check plots before application.

Total tomato yield (tons/hectar), fruit weight (gm) and number of fruits per kg were recorded.

All obtained data were subjected to statistical analysis by using F. test and the means were compared according to L.S.D. Test (Gomez and Gomez, 1984).

***In vitro* effect of certain plant extracts and essential oils on root-knot nematode.** In general, all plant extract treatments significantly reduced the number of viable live juveniles. Data in Table 1 showed that after 24 h the greatest percentages of nematode mortality (65.3 and 64.2%) were achieved by neem and essential oils, respectively, followed by marigold (60.5%). The lowest inhibition was caused by eucalyptus and garlic extracts (38.7 and 31.5%). After 48 h, all treatments significantly reduced the number of viable nematode juvenile compared to control treatment. The highest reduction was achieved by neem extract 60%, while the lowest reduction was caused by eucalyptus extract 19.4%. The nematicidal mechanism of plant extract against plant parasitic nematode was suggested as a direct effect of second stages juveniles in particular the egg stage (Kiewnick and Sikora, 2006).

Effect of certain plant extracts and essential oils under greenhouse condition. Results in Table 1 showed that the highest percentage of nematode reduction was achieved by Vydate followed by neem extract. The lowest nematode reduction occurred in case of eucalyptus extract. Root gall index was reduced by all treatments especially when treated with Vydate, neem and essential oils.

These results are in agreement with those obtained by El-Nagdi and Mansour (2003). The inhibition of *M. incognita* population in this investigation may be due to the accumulation of toxic by-products of decomposition and/or to increase phenolic contents resulting in host resistance. Sivapalan (1972) mentioned that, the nematicidal compounds in marigold have been identified as α-terthiemyl and its analogues, which kill nematodes that enter the root. Also, Konstantopoulou et al. (1994) mentioned that the mechanisms of plant extracts action may include denaturing and degrading of proteins, inhibition of enzymes and interfering with the electron flow n respiratory chain or with ADP phosphorylation.

Table 1. Effect of some plant extracts and essential oils treatments on mortality of nematodes in laboratory test and on root galling, nematode population on tomato plants under greenhouse condition.

Treatments	(%) Nematode mortality				Greenhouse condition			
	[a]Viable juveniles after 24 h	% Reduction	Viable Juveniles after 48 h	% Reduction	[b]Root galls index	% Reduction	Nematode Population (250 c soil)	% Reduction
Eucalyptus	155 b	38.7	141 b	19.4	3.1 b	36.7	1252 b	35.9
Marigold	100 c	60.5	99 d	43.4	2.4 c	51.1	825 c	57.8
Garlic	153 b	39.5	136 c	22.3	2.1 c	57.1	805 c	58.8
Neem	88 d	65.4	70 e	60.0	1.5 d	69.4	620 d	68.2
Essential oils	90 d	64.4	89 d	49.1	1.9 d	61.2	680 d	65.2
Nematocide, Vydate	–	–	–	–	1.3 d	73.5	529 e	72.9
Control	253 a		175 a		4.9 a	–	1952 a	–

[a]Three hundred juveniles of *M. incognita* were transferred to the concentration of plant extracts and essential oil in sterilized Petri dishes and tap water served as a control. Five replicates were used. Separate sets of Petri dishes were maintained for each period of observation (24 and 48 h). Mortality of nematodes was confirmed by touching the juvenile with fine needle.

[b]Root galls index was recorded after two months from nematode inoculation, plants were uprooted and washed by water. Root gall severity was assessed according to (Barker, 1985).

Values in the column followed by different letters indicate significant differences among treatments according to least significant difference test (P=0.05).

Table 2. Effect of some plant extracts and essential oils treatments on root galling, nematode population, yield components and quality character of tomato crops under field condition

Treatments	Under field conditions				Yield of tomato crops.			Quality character of tomato crops.		
	[a]Root galls index	[b]% Reduction	[c]Nematode population (250 c soil)	% Reduction	[d]Yield ton/hectar	Number of fruits/ kg	Fruit weight (gm)	[e]Firmness	[f]T.S.S	[g]Thickness
Eucalyptus	3.5 b	18.9	1720 b	35.2	52.6 b	8.7 a	115 b	1.7	8.3	0.40
Marigold	3.1 b	27.9	1502 c	43.4	40.5 d	10.2 a	98 d	1.5	8.0	0.34
Garlic	3.1 b	27.9	1420 c	46.5	33.8 e	11.0 a	91 e	1.3	7.6	0.32
Neem	2.4 dc	44.2	1210 d	54.4	45.2 c	9.5 a	105 c	1.7	8.3	0.37
Essential oils	2.9 dc	32.6	1301 e	50.9	60.2 a	8.2 a	122 a	1.8	8.6	0.40
Nematocide, Vydate	1.5 e	65.1	1056 f	60.2	32.4 e	11.6 a	86 f	0.9	6.7	0.32
Control	4.3 a	–	2653 a	–	16.3 f	12.7 a	80 g	0.8	6.5	0.30

[a]Root galls index was recorded after two months from nematode inoculation, plants were uprooted and washed by water. Root gall severity was assessed according to (Barker, 1985).

[b]Percentage of reduction in the second juveniles (J2) counts was estimated according to Anderson and Tilton formula (Puntener, 1981).

[c]Soil and root samples were collected after 40 days from the last treatments for nematode analysis

[d]Total tomato yield (tons/ hectar), fruit weight (gm) and number of fruits per kg were recorded.

[e]Firmness measured by penetration tester apparatus (kg So cm^2).

[f]Fruit flesh thickness (cm).

[g]Total soluble solids (T.S.S) measured by Refractometer.

Values in the column followed by different letters indicate significant differences among treatments according to least significant difference test (P=0.05).

Effect of certain plant extracts and essential oils under field conditions. Under field conditions, the highest reduction percentage of nematode was achieved by the nematicide, Vydate followed by neem and essential oils while the lowest percentage was resulted by eucalyptus comparing to the control plants (Table 2). In case of disease index Vydate and essential oils were caused the highest reduction in root gall index and nematode population followed by others treatments. The results of present investigation agree with those reported by El-Zawahry (1994) who reported that plant extracts can reduce the number of nematode and disease severity in host plants.

Effect of some plant extracts and essential oils on yield and quality character of tomato crops. As shown in Table 2 the highest yield (ton/hectar) was obtained from the treatment by essential oils (60.2), followed by eucalyptus (52.6). Meanwhile, the lowest yield (16.3) was found

when the tomato plants planted without any treatments (control). Tomato plants cultivated without treatment recorded the highest number of fruits/kg (12.7) followed by the nematicide, Vydate. The treatment by using essential oils recorded the lowest rate (8.2). Also, the highest fruit weight (gm) was recorded with the treatment by using essential oils (122), followed by the treatment with eucalyptus (115), while, the lowest fruit weight (80) was observed when tomato plants without any treatment (control).

These data also indicated that using essential oils recorded the highest level of firmness, T.S.S, and thickness (1.8, 8.6, and 0.40, respectively) of tomato fruits. The lowest level of firmness, T.S.S, and thickness was recorded without treatment (0.8, 6.5, and 0.30, respectively) of tomato fruits.

The greatest increase in fruit weight was achieved by essential oils, eucalyptus and Vydate and neem followed by marigold extracts. They may due to decrease the diseases severity in tomato plants. These findings agree with Natarajan et al. (2006) who mentioned that fruit yield from tomato plants treated with *T. erecta* extracts was significantly better than untreated checks and comparable with the nematicide carbofuran-treated plants.

References

Allam, M. M. 1985. A single method for *in vitro* screening for nematoxicity. *Int. Nematol. Medit.* 22:127-129.

Barker, K. R. 1985. Nematode extractions and bioassays. In: Barker, K. R., Carter, C. C., Sasser, J. N. (Eds.), An Advanced Treatise on *Meloidogyne*, Methodology, pp. 19-35, Vol. II. North Carolina State University Graphics, Raleigh.

El-Nagdi, W. M. A. and Mansour, A. F. A. 2003. Management of the root-knot nematode, *Meloidogyne incognita* infecting sugar-beet by certain medicinal plant soil products. *Egypt. J. Agric. Res.* 28:361-367.

El-Sherif, M. A., Ali, A. H. and Barakat, M. I. C. 1999. Suppressive bacteria associated with plant parasitic nematodes in Egyptian agriculture. *Jap. J. Nematol.* 24:55-59.

El-Zawahry, Aida M. 1994. Effect of interculture of marigold, *Tagetes erecta* nematodes associated with certain fruit trees. *Inter. Nemat. Net. News lett.* 5:22-23.

Gomez, K. A. and Gomez, A. A. 1984. Statistical Procedures for Agriculture Research, 2nd ed. John Willey. New York, 680 p.

Goodey, J. B. 1963. Laboratory methods for work with plant and soil nematodes. HMSO, London, Bulletin No. 2, Ministry of Agriculture, Fisheries and Food, 47 pp.

Javed, N., Gowen, S. R., Inamul-Haq, M. and Anwar, S. A. 2007. Protective and curative effect of neem (*Azadirachta indica*) formulations on the development of root-knot nematode *Meloidogyne javanica* in roots of tomato plants. *Crop Protec.* 26:530-534.

Kiewnick, S. and Sikora, R. A. 2006. Biological control of the root-knot nematode *Meloidogyne incognita* by *Paecilomyces lilacinus* strain 251. *Biolog. Control* 38:179-187.

Konstantopoulou, I., Vassilopoulou, L., Mawogantisi, P., Pidou and Scouras, G. 1994. Insecticidal effect of essential oils, A study of essential oils extracted from eleven Greek aromatic plants on *Drosophila auroria*. *Experientia* 48:616-619.

Korayem, A. M., Hasabo, S. A. and Ameen, H. H. 1993. Effects and mode of action of some plant extracts on certain parasitic nematodes. *Nz. Pflanzenschutz, Schadlingskunde Umweltschtz*, 66:32-36.

Natarajan, N., Cork, A., Boomathi, N., Pandi, R., Velavan, S. and Dhakshnamoorthy, G. 2006. Cold aqueous extracts of African marigold, *Tagetes erecta* for control tomato root knot nematode, *Meloidogyne incognita*. *Crop Protec.* 25:1210-1213.

Puntener, W. 1981. "Manual for Field Trials in Plant Protection". Agricultural Division, Ciba Geigy Limited, Basle, Switzerland, 205p.

Seinhorst, W. 1966. Killing nematodes for taxonomic study with hot F.A.A. *Nematologica* 12:178.

Sivapalan, P. 1972. Nematode pests of tea. In: Economic Nematology, 285-311. (ed. J. M. Webster). New York: Academic Press.