The Effect of Some Plant Extracts on Root-knot Nematode *Meloidogyne incognita* Populations on Pepper and Tomatoes*

Ramazan ÇETİNTAŞ[1], Rebin A. QADIR[2]**
[1]KSU, Agricultural Faculty, Department of Plant Protection, Kahramanmaras, Turkey
[2] Bakroja Technical Agriculture Institute, Sulaimani-Iraq

Received (Geliş): **04.07.2014** **Accepted** (Kabul): **28.10.2014**

Abstract: The root-knot nematode, *Meloidogyne incognita*, is one of the major pathogen causing great losses in many crops worldwide. Nematicidal activity of five plant essential oils (onion, QL agri35, bay tree, eucalyptus, mustard) against *M. incognita* were investigated in tomato and pepper. Experiment was designed as randomized complete block design with three nematode inoculums (0, 1000 and 2000 J2 per plant) and three essential oil concentration (0, 100 and 250 µL per plant) replicated four times. There were no significant differences between nematode inoculums level and essential oil concentration used. However, all plant extract treatments restrained nematode populations in both tomatoes and pepper host plants. Among the essential oils, eucalyptus sustained the highest plant heights of 34.20±2.9 cm and 29.55±3.4 cm for tomato and pepper, respectively. Among all treatments, eucalyptus reduced the number of second-stage juvenile in the soil (Rf) significantly in both tomatoes (0.70±0.1) and pepper (0.10±0.2). Among five plant essential oils, application of a rate of 100 µL per pot could give the best results in root-knot nematode control and be an alternative to the some current control methods. Further studies are needed in the area of using plant extracts as an alternative to other nematode control tactics.
Key words: *Meloidogyne incognita***,** plant essential oils**,** nematode control, bionematicide

INTRODUCTION

Meloidogyne species are distributed worldwide; some occurring in the tropics, subtropics and others in temperate regions where they cause serious problems both to the quality and quantity of crops (Sasser, 1980). *Meloidogyne incognita* is more common in warm temperate, tropical and subtropical regions of the world (Perry *et al*., 2009) and it is considered to be the most destructive pathogen in many crops root-knot nematodes can cause great damage to the major crop losses in yield. It is complicated to guess yield suppression caused by plant parasitic nematodes due to wide spacious species (Cetintas *et al*., 2010).

Meloidogyne species are polyphagous plant parasites attacking up to 5500 different high plant species (Trudgill and Blok, 2001). These plant species include vegetables, ornamental and even weeds. Albeit most *Meloidogyne* species have a wide host range, some *Meloidogyne* species, such as *M. incognita* and *M. arenaria* can be categorized into races based on their host specificity (Taylor and Sasser., 1978).

Tomato (*Lycopersicon esculentum*) is one of the most popular and widely used vegetables in the world (Norman, 1992). Ecological and geographical condition in Turkey allows producing good quality tomatoes in lot quantities. Tomatoes production in Turkey estimated to be around 10.7 million metric tons in 2009 (Anonymous, 2009). Tomato production in the world is affected by the root-knot nematode damage causing a 30-50% yield loss (Sasser and Freckman., 1987; Jonathan *et al*., 2001; Saravanpriya and Sivakumar, 2005). Pepper (*Capsicum annuum var. menderes*) also is one of the most important vegetables in Turkey. Most common pepper varieties are susceptible to the root-knot nematode *Meloidogyne incognita*.

A number of control methods are applied to control or reduce the population of root-knot nematodes. The more effective and traditional method of nematode management is considered to be the chemical control. However, the possibility negative impacts on environment after prolonged use have led to a total ban of most chemical nematicides (Zuckerman and Esnard, 1994). Environmental hazards and high cost of chemical nematicides encourage the scientists to look for natural compounds with less toxicity and eco-friendly alternative. Essential oils extracted from plants were found to possess antimicrobial and insecticidal activity (Oka *et al.*, 2000). In this study, the effects of essential oils drived from five different plants, including onion (*Allium cepa* L.), QL Agri 35 (*Quillaja saponaria* Molina), bay tree (*Laurus nobilis* L.), Eucalyptus (Eucalyptus spp.), and mustard (*Brassica* spp.) on root knot nematode *M. incognita* were investigated on two crops, tomatoes and pepper.

MATERIAL and METHODS

Source of Nematodes and Essential Oils

Nematode inoculum was taken from galled roots from tomatoes from nematode infested vegetable farms of Kahramanmaras, Turkey. Species identification was assured by two major methods. First, morphological observations and perineal patterns images derived from a single egg mass of the particular culture. Twenty females were extracted from roots of tomato grown in a growth chamber. Perineal patterns were prepared following procedures of Hartman and Sasser (1985). Second, 66 single females of nematode isolates were

*Part of MSc thesis of the second author.
**Corresponding author: Çetintaş, R., cetintas@ksu.edu.tr

subjected to PAGE (polyacrylamide gel electrophoresis), using a Bio-Rad mini-PROTEIN II (Bio-RAD, Singapore) kit. Two females of *M. javanica* per gel were used as standards. Electrophoresis was carried out in a refrigerated discontinuous buffer system with 8% acrylamide running gel, pH 8.8, and 4% acrylamide stacking gel, pH 6.8 (Cetintas *et al.*, 2007). Following electrophoresis, the gels were removed and placed in a staining solution. After staining, the gels were visualized for esterase phenotype bands followed the method of Harris and Hopkinson (1976).

Five essential oils derived from five different plants sources used in this study were onion (*A. cepa*), QL Agri 35 (*Q. saponaria*), Bay tree (*L. nobilis*), eucalyptus (*Eucalyptus* spp.), and mustard (*Brassica* spp.). Source of plants, plant parts and extraction method of oils are shown in (Table 1).

Experiments

The experiments were conducted in a glass house and a growth chamber located in the Agriculture Faculty of Kahramanmaraş Sütçü Imam University, Kahramanmaraş, Turkey. Two plant types used in this study were a commonly grown a tomato cultivar and pepper.

Trial One

After the preparation of needed seedlings, and nematode inoculums, the soil for the pots was arranged and the experiment was set up on the 3th of April 2013. Soil for pots experiments were arranged with a ratio of 60% sand, 25% clay and 15% organic matter. Twelve weeks old root-knot nematode infested seedlings of tomato and pepper plants were cleaned of debris gently by washing with stream water. The roots were cut into 2 cm small pieces and shaken manually for 2-3 minutes in a one liter size beaker, containing 500 ml of 0.5% sodium hypochlorite (NaOCl) solution (Hussey and Barker, 1973). One week after transplanting the four weeks old tomatoes and pepper seedlings into the soil filled pots, they were inoculated with three levels of root-knot nematodes, *M. incognita*. The levels were consisted of control (0 J2/eggs soil plant), low (1000 J2/eggs soil plant) and high (2000 J2/eggs per plant/pot). Four holes were formed in a square shape approximately 2 cm distended from the roots. One week after inoculations of nematodes, the planted pots were treated by five different essential oils with three concentrations each consisting control (0 µL\plant), low (100 µL\plant) and high (250 µL\plant). Plants were watered daily and fertilized weekly with (Fulvix %5 (%50 organic matter, %2.5 N, %0.5 organic N, %4 K_2O). Every 10 to 12 days, the plants were applied soap solution to manage pest (mostly white fly) populations. The glass house experiment was classified as a randomized complete block design (RCBD) with five treatments replicated four times.

Table 1. The source and extraction methods of plant essential oils used in the study

Source of plant	Scientific name	Plant parts	Extraction method
Onion	*Allium cepa*	Bulb	Steam distillation
Bay tree	*Laurus nobilis*	Leaves	Steam distillation
Eucalyptus	*Eucalyptus* sp.	Leaves	Steam distillation
Mustard	*Brassica* sp.	Seeds	Steam distillation
QL Agri 35	*Quillaja saponaria*	Root	Press-Maceration

The test plants were harvested (60±2) days after the nematode inoculation. The plants were cut off at the ground level discard and the green parts were put in paper bags individually. The soil was removed from the roots by gently shaking the plants and stored in a refrigerator at 4 ˚C for further analyses. Plants heights were recorded biweekly from transplanting date of seedlings to the harvest. Root systems were washed individually, root galling indices were recorded using the scale of 0-5; where 0=no galls, 1=1-2 galls, 2=3-10 galls, 3=11-30 galls, 4=31-100 galls, and 5= >100 galls per root system (Taylor and Sasser, 1978). Root systems were stained with food coloring (red) (Thies *et al*., 2002), and egg mass indices were recorded using a 0-5 scale, where 0=no egg masses, 1=1-2 egg masses, 2=3-10 egg masses, 3=11-30 egg masses, 4=31-100 egg masses, and 5= >100 egg masses per root system (Taylor and Sasser, 1978).Fresh and dry weight of the plant tissue and root systems were determined. An 80 cm^3 soil sample from each pot was assayed to determine the number of second stage juveniles (J2) of *M. incognita* using modified Baermann technique (Whitehead and Hemming, 1965). After (13±1) days, the extracted samples were taken and sieved (25µm openings), placed in a counting dish and counted under stereomicroscope with appropriate magnification. The reproduction factor (RF) was determined as followed: The reproduction factor (RF) = final population (Pf) / initial population (Pi) (good host RF≥1, poor host 0.1 <RF> 1, non-host RF ≤ 0.1) (Sasser *et al*., 1984).

Statistical Analysis

Data from nematode EMI (egg mass index), GI (galling index) and Rf (final nematode population from pots), plant height, plant fresh weight, plant dry weight, root fresh weight, and root dry weight were subjected to ANOVA using (SPSS Statistics version 20.0.0), and treatment means were separated by dependent variable and the experiments were compared using t-test.

Trial Two

The experiment was repeated and tomato and pepper seedlings were transplanted at 23th August, 2013. Site preparation, nematode inoculation levels, treatment applies, and installation experimental methodology were the same as first trial. After (60±2) days, tomato and pepper plants were harvested, plant height, green part fresh weight, green part dry weight, root fresh weight and root dry weight, galling indices, egg mass indices, and reproduction factor (Rf) were determined as described in the first trial.

RESULT and DISCUSSION

The observation of the morphology of perennial pattern taken from single females from tomato showed that the morphological character is typically matches with *M. incognita* based on (Eisenback *et al.,* 1985). *Meloidogyne incognita* perennial pattern was oval to rounded, typically with high, squared, dorsal arch, striae usually wavy, lateral field absent or weakly demarcated by forked striae.

Results of PAGE also ensured that the nematode species used in the experiment revealed unique esterase isozyme bands of *M. incognita* compared to the standard *M. javanica*.

There was a significantly effect of host plant varieties on all parameters (*P≤0.05*). There was a significant effect of treatments on plant height (PH) and green part dry weights (GDW). Also, different nematode levels influenced the root-galling (GI) significantly. However, there was not any significant differences between the interactions of nematode level (N) x treatment (T), nematode level (N) x treatment level and nematode level (N) x treatment (T) x treatment level (L) on plant height, green part fresh weight (GFW), green part dry weight (GDW) and root galling (GI) (*P≤0.05*) (Table 2).

Analysis of variance showed that egg mass indices (EMI) were affected significantly by plant varieties and nematode levels. On the other hand, among all variables, root fresh weight (RFW) was differed by only plant varieties. Additionally, treatments levels and plant varieties affected the root dry weight (RDW) significantly. Reproduction factor (Rf) was affected significantly by all variables except for treatment level (L) and Nematode level (N) x treatment Level (L) interaction (*P≤0.05*) (Table 3).

Pooled data of two trails are shown in Table 4. Excluding non treated control group, the greatest plant height (PH) was recorded in Eucalyptus treatment for both tomatoes (34.20cm) and pepper (29.55cm). The greatest weight of green fresh plant part (GFW) was obtained in Eucalyptus (37.90g) on tomato host plant. On the other hand, the greatest weight of green dry part (GDW) was observed in Eucalyptus for tomatoes and pepper, as 7.17g and 2.60g, respectively. Galling indices per root system (GI) were recorded the lowest in Eucalyptus (2) on pepper. Among all treatments, the greatest fresh root weight (RFW) was reported on Eucalyptus (1.12g) on pepper. However, the greatest dry root weight (RDW) was recorded in Eucalyptus, 2.75g and 1.05g, on tomatoes and pepper, respectively. Finally reproduction factor was the lowest in Eucalyptus treatment for both tomatoes (0.70) and pepper (0.10) in the high nematode inoculums level (Table 4).

Our results showed that the mustard essential oil affected both tomatoes and pepper growth negatively by causing a great phytotoxicity and killing a few number of host plants in both treatment levels.

Table 2. Analysis of variance for the effects of five essential oils treatments and their three application rates to three inoculums levels of *Meloidogyne incognita* and their interaction on the plant height, green part fresh weight, green part dry weight, root galling of tomato and pepper

Source	df	PH (cm)	F-value	GFW (g)	F-value	GDW (g)	F-value	GI	F-value
Host Plant (P)	1	0.00*	25.78	0.00*	112.29	0.00*	260.12	0.00*	199.33
Treatment levels (L)	2	0.00*	7.49	0.22	1.50	0.02*	3.74	0.95	0.05
Nematode levels (N)	2	0.93	0.06	0.12	2.11	0.48	0.73	0.00*	275.02
Treatment (T)	3	0.00*	5.36	0.87	0.23	0.57	0.66	0.89	0.19
NxT	6	0.97	0.21	0.99	0.12	0.86	0.42	0.89	0.38
NxL	2	0.91	0.08	0.85	0.15	0.91	0.09	0.40	0.92
TxL	6	0.43	0.98	0.14	1.61	0.68	0.65	1.00	0.00
NxTxL	6	0.86	0.41	0.75	0.57	0.93	0.29	0.07	1.96

Root galling: 0-5 scale, where, 0: No gall, 1: 1-2 galls, 2: 3-10 galls, 3: 11-30 galls, 4: 31-100 galls, 5: >100 galls (Taylor and Sasser., 1978) (P≤0.05)
* significant at (*P≤0.05*)

Sangwan *et al*. (1990) reported that few essential oils extracted from plants have been evaluated for their nematicidal effects. Another study by Gong *et al*. (2013) showed that the garlic straw delayed the development of *M. incognita* from juvenile to adult, which could have reduced the incidence of *M. incognita* by extending the nematode life cycle and reducing the reproduction rate.

The mode of action of azadirachtin (a neem refined product) is like alkali halides because hyperactivities were observed in nematodes exposed to low

concentration of neem products (Javedet *al.*, 2007). These products are absorbed by the plants and when nematodes come in contact with them for feeding so they inhibit or delay their development.

Nematicidal activity of essential oils extracted from 27 plant species were evaluated in laboratory and pot experiment tested against root-knot nematode, found to be highly promising in controlling of nematodes. Essential oils of *Carum carvi*, *Foeniculum vulgare*, *Mentha rotundifolia*, and *Mentha spicata* showed the highest nematicidal activity among the in vitro tested oils. These oils from *Origanum vulgare*, *O. syriacum*, and *Coridothymus capitatus* mixed in sandy soil reduced the root galling of cucumber seedlings in pot experiments (Oka *et al*., 2000).

Table 3. Analysis of variance for the effects five essential oils treatments and their three application rates to three inoculums level of *Meloidogyne incognita* and their interaction on the root fresh weight, root dry weight, reproduction factor (Rf) and egg masses of tomato and pepper

Source	df	EMI	F-value	RFW (g)	F-value	RDW (g)	F-value	Rf	F-value
Host Plant (P)	1	0.00*	7.29	0.00*	109.34	0.00*	187.06	0.00*	87.52
Treatment levels (L)	2	0.78	0.24	0.98	0.01	0.00*	5.15	0.27	1.29
Nematode levels (N)	2	0.00*	918.34	0.52	0.64	0.39	0.93	0.00*	28.83
Treatment (T)	3	0.79	0.33	0.87	0.22	0.46	0.85	0.02*	3.38
NxT	6	0.36	1.10	0.94	0.27	0.98	0.17	0.00*	4.32
NxL	2	0.73	0.30	0.54	0.61	0.19	1.66	0.49	0.70
TxL	6	0.29	1.24	0.76	0.55	0.85	0.44	0.00*	3.28
NxTxL	6	0.46	0.94	0.91	0.34	0.85	0.43	0.00*	3.69

Egg masses: 0-5 scale, where, 0: No egg mass, 1: 1-2 egg masses, 2: 3-10 egg masses, 3: 11-30 egg masses, 4: 31-100 egg masses, 5: >100 egg masses (Taylor and Sasser, 1978). (P≤0.05). Rf =Pf/Pi, good host (Rf≥1), poor host (0.1<Rf>1), non-host (Rf≤0.1) (Sasser et al.. 1984)
* Significant at (*P≤0.05*)

Table 4. Means of plants height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, galling indices, egg mass and Rf in tomatoes and pepper (Mean±SD)

Treatments	PH (cm)	GFW (g)	GDW (g)	GI	EMI	RFW (g)	RDW (g)	Rf
Tomato host plant								
Onion	29.60±3.4	34.15±14.2	5.52±1.3	4.50±0.6	5.00±0.0	4.30±2.5	1.95±0.5	1.10±0.0
Bay tree	32.20±1.8	33.27±10.9	6.62±1.2	5.00±0.0	5.00±0.0	2.80±0.9	2.07±0.8	2.00±1.6
Eucalyptus	34.20±2.9	37.90±10	7.17±0.7	4.75±0.5	5.00±0.0	3.75±1.3	2.75±0.6	0.70±0.1
QL Agri 35	32.10±2.9	29.70±8.3	6.00±1.7	5.00±0.0	5.00±0.0	4.42±0.8	2.67±0.5	2.00±1.4
Control	38.90±10.7	21.93±15.7	2.95±2.5	0.0±0.0	0.0±0.0	0.74±0.6	0.48±0.3	0.0±0.0
Pepper host plant								
Onion	27.00±3.8	14.72±2.2	1.82±0.2	2.25±0.5	5.00±0.0	0.67±0.1	0.65±0.1	0.25±0.1
Bay tree	29.10±1.5	14.02±3.8	2.05±0.4	2.75±0.5	4.75±0.5	0.70±0.2	0.65±0.2	0.30±0.3
Eucalyptus	29.55±3.4	15.92±2.6	2.60±0.6	2.00±0.0	4.75±0.5	1.12±0.4	1.05±0.3	0.10±0.1
QL Agri 35	28.65±2.6	18.92±6.0	2.50±0.3	3.00±0.8	4.00±0.8	0.90±0.3	0.82±0.2	0.15±0.2
Control	31.26±2.9	29.75±10.1	4.25±1.3	0.0±0.0	0.0±0.0	3.47±1.8	1.00±0.4	0.0±0.0

*Data are means of four replications (pooled data of two trails) where used to compare between plant essential oils. Plant height, green part fresh weight, green part dry weight, root fresh weight, root dry weight, root galling : 0-5 scale, where, 0: No galls, 1: 1-2 galls, 2: 3-10 galls, 3: 11-30 galls, 4: 31-100 galls, 5: >100 galls. Egg masses: 0-5 scale, where, 0: No egg mass, 1: 1-2 egg masses, 2: 3-10 egg masses, 3: 11-30 egg masses, 4: 31-100 egg masses, 5: >100 egg masses (Taylor and Sasser, 1978).
Reproduction factor (Rf) =Pf/Pi, good host (Rf≥1), poor host (0.1<Rf>1), non-host (Rf≤0.1) (Sasser *et al*., 1984).

CONCLUSION

This study revealed some differences in nematicidal effect of plant essential oils used on root-knot nematode, *M. incognita* populations on tomatoes and pepper. Firstly, mustard oil showed a great phytotoxicity to all plants for all levels indicating that it was not good choice in nematode management. Whereas, date from this study demonstrated that eucalyptus was more effective in reducing number of J2 in soil in both plants and reducing root galling in pepper than other plant essential oils tested. Also, eucalyptus oil increased the plant height, green fresh weight, green dry weight and root dry weight in tomatoes. It was evident that eucalyptus essential oil deserves a serious consideration for inclusion into the nematode management tactics.

Further field or greenhouse studies in different conditions and locations need to be conducted to see the possible implementation opportunities of these essential oils finding to farmers in vegetable growing areas.

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