

Original Research Article

Effect of Some Plant Methanol Extracts on Egg Hatching and Juvenile Mortality of Root-Knot Nematode *Meloidogyne incognita*

ABSTRACT

Aims: This research was conducted to find out the effect of four plant methanol extracts against the root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood in laboratory.

Place and Duration of Study: Labrotory experiments were conducted at the University of Gaziosmanpasa, Turkey.

Methodology: For this purpose, bead tree (*Melia azaderach*), hops (*Humulus lupulus*), elderberry (*Sambucus nigra*), poison hemlock (*Conium maculatum*) were collected from different parts of the Turkey. Methanol extracts from four plant parts were screened for egg hatchability and nematicidal activity against second stage juveniles (J₂) of *M. incognita*. The nematode eggs and juveniles were exposed to different concentrations of extracts during 24, 48 and 72 hrs. The concentrations as 0.5%, 1.0%, 2.5%, 5.0% and 10% were prepared by diluting stock solution with 10% acetone containing distilled water.

Results: The results show that, *Melia azaderach*'s 10,0% (v/v) concentration significantly reduced the egg hatchability (98,1%) and it was followed by *S. nigra* (94,1%). At the end of 24 hrs incubation, 5,0% and 10,0% (v/v) concentration of *M. azaderach* extract and 2,5%, 5,0% and 10,0% (v/v) concentration of *S. nigra* produced 100% mortality in juvenil stage of the nematode.

Conclusion: The results of our investigation show that four plants contain nematicidal compounds. it seems that the use of plant extracts might have increasing popularity in future as a component or ingredient of biopesticides, Therefore, Further research is necessary to found out toxic compounds released by species and carried out experiments in vivo for the control of root-knot nematode *M. incognita*.

Keywords: *Meloidogyne incognita*, juvenil mortality, egg hatching, plant extracts, root-knot nematode

1. INTRODUCTION

The root-knot nematodes (*Meloidogyne* spp.) are the major pests in many countries, and the cause of serious yield losses on a wide range of crops [20]. Root-knot nematodes cause losses up to 80% in heavily infested fields [28]. Totaly, more than 60 *Meloidogyne* species have been described in the world [15;23]. The most destructive species is *M. incognita* which cause serious problem in various agricultural crops. This species is one of the most common and economically important root-knot nematodes in vegetable growing areas in Turkey [10;11].

Management of root-knot nematodes under field conditions with chemicals is expensive and could create a potential hazard to the environment and human health. Therefore, scientists identified natural product with nematicidal activity such as plant extract, root exudates, plant volatiles etc. Plants are an important source of naturally occurring pesticides. A number of workers reported about the nematicidal properties of many plants products against *M. incognita*. [30] have also reported some plants belonged 57 families have been shown to nematicidal compounds. A wide variety of plant species, representing 57 families have been shown to nematicidal compounds [30]. These compounds are known as alkaloids, diterpenes, fatty acids, glucosinolates, isothiocyanates, phenols, polyacetylenes, sesquiterpenes and thienyls [5;7;12;18;21;29]. In recent years, there has been considerable interest in the nematicidal properties of compounds, but reports on the effect of extract on root knot nematodes are still limited in Turkey. Therefore, the aim of this study was to determine the *in vitro* effect of methanol extracts obtained from four plants on egg hatching and mortality of second stage juveniles of *M. incognita*.

2. MATERIAL AND METHODS

In this study, egg masses and second-stage juveniles (J2) of the root knot nematode *M. incognita* was used as test pathogen. Different parts of four plants used against *M. incognita* were *M. azaderach*, *H. lupulus*, *C. maculatum* and *S. nigra*.

2.1 Culture of root-knot nematode

The second-stage juveniles and egg masses of *M. incognita* used in the study was obtained from susceptible tomato (*Lycopersicon esculentum* L.) plant roots with galls from greenhouse.

2.2 Collection of Plant Material

The plants (Table 1) were collected from the various parts of Turkey during spring and summer of 2006 and 2007. *Melia azaderach* was collected from Adana and Hatay province, *H. lupulus*, *S. nigra*, and *C. maculatum* were collected from around Tokat province. Each plant species was air dried in shade at room temperature (25±1 °C) for 3 weeks and different part of the plants powdered in an electronic glinder.

Table 1. Plant species used against egg and larvae *Meloidogyne incognita*

Species	Family	Common Name	Tissue Used
<i>Conium maculatum</i> L.	Apiaceae	Poison hemlock	Fruit +Leaves+Stem
<i>Melia azaderach</i> L.	Meliaceae	Bead tree	Fruit
<i>Humulus lupulus</i> L.	Cannabaceae	Hops	Inflorescences
<i>Sambucus nigra</i> L.	Caprifoliaceae	Elderberry	Fruit + Leaves +Stem

2.3 Preparation of Methanol Extract

Plant extracts were prepared according to the procedure described by [13] and [14]. The powdered material (100 g) was soaked in 600 ml of methanol in 1000 ml erlanmayer flask. The flasks placed on a horizontal shaker (HS 260 Basic, IKA Group) and shaken (120 oscillations/min for 24 hrs) at room temperature (25±1 °C). The extract was filtered through cheese cloth and solvent was evaporated in a rotary evaporator (RV 05 Basic 1B, IKA Group) at 32 ± 2 °C. This was called stock solution. Concentrations as 0.5%, 1.0%, 2.5%, 5.0% and 10% (v/v) were prepared by diluting stock solution with 10% acetone containing distilled water.

2.4 Egg hatch activity of the plant extract

To study the effect of the plant extracts on egg hatching of *M. incognita*, one medium sized egg masses were handpicked from the galled tomato roots and transferred 2 ml of the extract of each plant species into watch cavity glass slide. The egg masses placed in sterile distilled water and %10 acetone containing distilled water as control. The number of hatched juveniles were counted after 24, 48 and 72 hrs. After the last counting, the egg masses were transferred into distilled water at 24, 48 and 72 hrs for reversibility test. Each treatment was replicated three times and the cavity glass slides were arranged in randomized complete block design.

2.5 Nematicidal activity of the plant extracts

To study the effect of methanol extract of each plant species on mortality of *M. incognita*, 2 ml of each concentration of extract was poured in a glass cavity slide and 20 freshly hatched J₂ of *M. incognita* placed in each glass slide. Juveniles kept in sterile distilled water and %10 acetone containing distilled water as control. Treatments were replicated three times and dead and alive nematodes in each cavity slide were counted with stereo microscope (Olympus 40X) after 24, 48 and 72 hrs. Percentage of nematode mortality was calculated according to the Abbott's formula;

3. RESULTS AND DISCUSSION

In the present study the efficacy of four methanol extracts was evaluated against hatching of *M. incognita* eggs and mortality of second-stage juveniles.

3.1 Inhibition effect of extracts on egg hatching

The results of the effect of four plant methanol extracts on egg hatching were presented in table 2. All plant species extracts importantly inhibited the nematode egg hatching as compared to the control and distilled water. Although the highest concentration at level 10% (v/v) of the extracts of *M. azadirach*, *S. nigra*, *C. maculatum* caused the highest egg hatching inhibitions as 97%, 92.9%, 43.3% respectively, *H. lupulus* showed the highest egg hatching inhibition at level 2.5 % (v/v) as 76.3 %. Furthermore, inhibition percentages of the mediums used as controls, the distilled water and control containing 10 % acetone were approximately similar at level 10% (v/v) but control containing acetone was higher than distilled water at the other concentration levels. This suggested that acetone containing medium is more promising to add extra support to the inhibition of the eggs.

3.2 Juvenile mortality

The mortality results of the extracts on juveniles were presented in table 3. In the juvenile mortality assay, all plant methanol extracts at different concentrations caused 100 % juvenile mortality. Exposure time also affected the mortality rate positively that increased with time at all concentration levels. The highest mortality (100%) except *C. maculatum* (55%) was generally provided by all plant extracts at level 10% (v/v) in 24 hrs. At the end of 24 hrs, the most effective extract belonged *S. nigra* which provided the faster effectiveness than the others at 2.5 %, concentration. After 24 hrs incubation, fruit extracts of *M. azadirach* (at 5 % and 10 % concentration), extracts from various parts of *S. nigra* (at 2.5 %, 5 % and 10 % concentration), and inflorescences extracts of *H. lupulus* (at 10 % concentration) caused 100% second stage juveniles mortality. However, *C. maculatum* extracts (at 5 % and 10 % concentration) showed 100 % juvenile mortality after 48 hrs incubation.

Our investigation has successfully revealed the effectiveness of the extracts obtained from the plants. Hatching inhibition of the eggs generally increased with time and extract concentrations but when eggs were transferred into the distilled water and control containing

126 acetone, the inhibition decreased and was reversible. The mortality rate of J2 significantly
 127 increased with the extract concentrations ($P \leq 0.05$, Duncan) and the mortality rates also
 128 increased with time. However a number of studies had been published before about plant
 129 extracts effecting *Meloidogyne* genus [1;3;8;9;22;24;27;31;32], many of studies were about
 130 *M. azedarach* extracts. [19] reported that *M. azedarach* leaf and seed extracts inhibited
 131 hatching of *M. incognita* eggs (associated with a delay in embryonic development), reduced
 132 nematode motility and killed the larvae. [17] reported *M. azedarach* extract caused 96 %
 133 mortality on *M. incognita* juveniles at 1:10 dilution in vitro after 24 hrs. Joymati (2009)
 134 reported 1 ppm concentration of *M. azedarach* extract caused mortality more than 60 % on
 135 juveniles of *M. incognita* within 24 hrs. [26] pointed out that the effect of the melia methanol
 136 extract obtained from *M. azedarach* fruit provided 100% nematode control at doses higher
 137 than 2.5% w/w with EC_{50} value of 0.916% w/w. [6] reported that *M. azedarach* fruits
 138 containing aldehydes, carboxylic acids and alcohols were used to control *M. incognita* on
 139 cucumber. Crushed fruits of *M. azedarach* were tested in the soil at the rates of 30 and 60 g
 140 kg^{-1} and they exhibited nematocidal activity similar to the one of fenamiphos (0.02 g a.i.
 141 kg^{-1}) in terms of nematode population in roots and soil as well as reproduction rate. The
 142 extracts acted directly as nematocidal and also reduced the activity of the antioxidant
 143 enzymes and trigger the host defense. [4] reported different concentrations of aqueous
 144 extracts (0%, 1%, 2% and 4%) in vitro of dried leaves, seed, seed kernels and seed coats of
 145 *M. azedarach* L. were tested for their effects on second stage juveniles of *M. incognita*. The
 146 extracts of all plant parts and at every concentration immobilized 100% of J2s. Maximum J2
 147 mortalities (100%) were given by concentrations of 2% and 4% of seed kernel and 4% of
 148 whole seed.

149 Our search revealed the effective results. Especially the effectiveness of *M. azedarach* was
 150 compatible with the previous investigations and seemed promising to be used nematode
 151 control. The extracts of *C. maculatum* L., *H. lupulus* L.; *S. nigra* L. were also effective in
 152 relatively varying rates but need more search of them as plant extracts for nematode control.
 153 In addition those, investigation has been conducted by now about the nematocidal effects of
 154 *M. azedarach* in Turkey.

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 156 The approach and results obtained here can be used to manage nematode problems. Many
 157 reports also confirm the success of plant extracts on nematodes. The results of our
 158 investigation are supported with the previous studies which are the evidence of the success
 159 on the nematodes. Therefore, it seems that the use of plant extracts might have increasing
 160 popularity in future as a component or ingredient of biopesticides, but still need to be found
 161 out toxic compounds released by species and carried out experiments in vivo for the control
 162 of root-knot nematode *M. incognita*.

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166 **Table 2.** Effect of methanol extracts on *Meloidogyne incognita* egg hatching after 24, 48 and
 167 72 hrs incubation

Treatments		Number of eggs hatched						Total hatched eggs	Inhibition % over distilled water	Inhibition % over control
		Extract			Distilled water*					
		24h	48h	72h	24h	48h	72h			
<i>Conium maculatum</i>	Distilled water	23,3*	18,0	14,3	83,3	122,3	82,7	344,0	-	-
	Control	14,3	8,0	4,3	90,3	95,0	81,7	293,7	14,6	-
	0,5%	14,3	8,0	4,3	36,3	73,0	102,3	238,3	30,7	18,8

	1,0%	15,7	3,7	2,7	68,7	55,7	95,3	241,7	29,8	17,7
	2,5%	10,0	5,0	2,7	72,0	89,7	95,7	275,0	20,1	6,4
	5,0%	4,0	4,7	1,3	14,7	98,3	135,7	258,7	24,8	11,9
	10,0%	0,0	0,0	0,3	1,0	32,7	132,7	166,6	51,6	43,3
<i>Melia azaderach</i>	Distilled water	60,7	50,0	63,7	29,7	54,0	83,3	341,3	-	-
	Control	44,7	26,3	36,7	24,7	31,0	56,3	219,7	35,6	-
	0,5%	23,7	7,3	11,7	12,3	14,3	14,0	83,3	75,6	62,1
	1,0%	20,0	12,0	11,0	16,0	19,7	36,3	115,0	66,3	47,7
	2,5%	24,7	4,7	8,7	39,0	42,7	40,3	160,0	53,1	27,2
	5,0%	14,7	0,0	0,0	41,3	42,3	18,3	116,7	65,8	46,9
	10,0%	5,7	0,0	0,0	0,0	1,0	0,0	6,7	98,1	97,0
<i>Sambucus nigra</i>	Distilled water	25,7	57,0	42,3	43,0	30,3	43,7	242,0	-	-
	Control	17,7	45,0	38,7	34,7	29,3	36,3	201,7	16,7	-
	0,5%	10,7	35,7	38,0	9,0	12,0	11,0	116,3	51,9	42,3
	1,0%	11,0	14,7	51,7	49,0	40,3	34,7	201,3	16,8	0,2
	2,5%	11,7	3,3	6,0	65,0	24,0	22,7	132,7	45,2	34,2
	5,0%	5,7	1,3	2,7	25,0	15,0	21,3	71,0	70,7	64,8
	10,0%	0,0	0,0	0,3	1,7	7,0	5,3	14,3	94,1	92,9
<i>Humulus lupulus</i>	Distilled water	49,0	76,3	45,3	51,3	37,0	24,7	283,7	-	-
	Control	48,7	53,7	39,0	23,3	27,3	16,3	208,3	26,7	-
	0,5%	25,0	8,3	33,0	16,7	17,7	11,0	111,7	60,6	46,4
	1,0%	37,3	55,3	37,7	66,7	27,7	30,0	254,7	10,2	N.A*
	2,5%	9,0	10,0	0,0	7,3	18,3	4,7	49,3	82,6	76,3
	5,0%	24,0	0,0	0,0	60,0	83,7	4,7	172,3	39,3	17,3
	10,0%	10,0	0,0	0,0	6,7	72,7	27,0	116,3	59,0	44,2

168 *After a 72 hrs exposure to plant extract, the egg masses were transferred to sterile distilled
 169 water

170 *Average of 3 replications.

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175 **Table 3.** Effect of methanol extract on *Meloidogyne incognita* second-stage juveniles %
 176 mortality after 24, 48 and 72 hrs incubation (Mean± Standart Error).

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Treatments	n	Mean mortality rates		
		24 h	48 h	72 h
		Mean±SE	Mean±SE	Mean±SE
Distilled water	20	0,00±0,00c	0,00±0,00d	5,00±0,00c
Control	20	0,00±0,00c	3,33±1,67d	5,00±0,00c

<i>Conium maculatum</i>	0,5%	20	6,67±1,67bc	6,67±1,67cd	11,67±1,67c
	1,0%	20	5,00±2,89bc	25,00±12,58c	38,33±10,93b
	2,5%	20	23,33±12,02b	58,33±19,22b	96,67±3,33a
	5,0%	20	30,00±18,03ab	100,00±0,00a	-
	10,0%	20	55,00±10,41a	100,00±0,00a	-
<i>Melia azaderach</i>	Distilled water	20	0,00±0,00c*	0,00±0,00d	5,00±0,00c
	Control	20	0,00±0,00c	6,67±6,67d	16,67±3,33b
	0,5%	20	15,00±2,89b	16,67±3,33c	16,67±3,33b
	1,0%	20	16,67±6,67b	43,33±3,33b	53,33±6,67a
	2,5%	20	20,00±0,00b	100,00±0,00a	-
	5,0%	20	100,00±0,00a	-	-
	10,0%	20	100,00±0,00a	-	-
<i>Sambucus nigra</i>	Distilled water	20	0,00±0,00b*	0,00±0,00b	3,33±3,33c
	Control	20	0,00±0,00b	0,00±0,00b	1,67±1,67c
	0,5%	20	0,00±0,00b	3,33±1,67ab	36,67±8,82b
	1,0%	20	0,00±0,00b	6,67±3,33a	71,67±6,01a
	2,5%	20	100,00±0,00a	-	-
	5,0%	20	100,00±0,00a	-	-
	10,0%	20	100,00±0,00a	-	-
<i>Humulus lupulus</i>	Distilled water	20	0,00±0,00c*	0,00±0,00d	5,00±0,00c
	Control	20	0,00±0,00c	3,33±3,33d	8,33±1,67c
	0,5%	20	6,67±6,67c	6,67±6,67d	23,33±3,33b
	1,0%	20	5,00±2,89c	31,67±10,14c	36,67±8,82b
	2,5%	20	6,67±3,33c	61,67±24,89b	76,67±23,33a
	5,0%	20	21,67±1,67b	100,00±0,00a	-
	10,0%	20	100,00±0,00a	-	-

*Means within each column with different letters are significantly different at $P \leq 0.05$ according to Duncan's multiple range test after arcsine square root transformation.

4. CONCLUSION

The results presented in this paper lead to the conclusion that plant spices exhibited some nematicidal compound which cause reduction in egg hatching and death of second stage juveniles of *M. incognita*. There is therefore need to find out toxic compounds released by plant spices and carry out experiments *In vivo* for the control of root knot disease by *M. incognita*

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