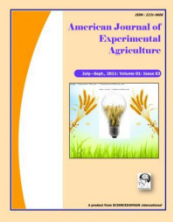

American Journal of Experimental Agriculture
4(11): 1471-1479, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

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

Faruk Akyazi[1*]

[1]*Department of Plant Protection, Faculty of Agriculture, Ordu University, 52200, Turkey.*

Author's contribution

This whole work was carried out by author FA.

Original Research Article

Received 31st March 2014
Accepted 28th May 2014
Published 30th June 2014

ABSTRACT

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 at the University of Gaziosmanpasa, Turkey. For this purpose, bead tree (*Melia azedarach*), hops (*Humulus lupulus*), elderberry (*Sambucus nigra*), poison hemlock (*Conium maculatum*) were collected from different zones of the Turkey. Methanol extracts from four plant parts were screened for egg hatchability and nematicidal activity against second stage juveniles (J_2) 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%, 2.5%, 5% and 10% were prepared by diluting stock solution with 10% acetone containing distilled water.

As a result, *M. azedarach*'s 10% (v/v) concentration significantly reduced the egg hatchability (97%) and it was followed by *S. nigra* (92.9%). At the end of 24 hrs incubation, 5% and 10% (v/v) concentration of *M. azedarach* extract and 2.5%, 5.0% and 10% (v/v) concentration of *S. nigra* produced 100% mortality in juvenile stage of the nematode.

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*.

**Corresponding author: Email: farukakyazi@hotmail.com;*

Keywords: Meloidogyne incognita; Juvenile mortality; egg hatching; plant extracts; root-knot nematodes.

1. INTRODUCTION

The root-knot nematodes (*Meloidogyne* spp.) are one of the main pests in many countries, which cause serious yield losses in certain crops [1]. Root-knot nematodes cause losses up to 80% in heavily infested fields [2]. Totaly, more than 60 *Meloidogyne* species have been described in the world [3,4]. The most destructive species is *M. incognita* which cause serious problem in various agricultural crops. Moreover, this species is one of the most common and economically important root-knot nematodes in vegetable growing areas in Turkey [5,6].

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 researchers reported about the nematicidal properties of many plants products against *M. incognita* [7]. A wide variety of plant species, representing 57 families have been shown to nematicidal compounds [7]. These compounds are known as alkoloids, diterpenes, fatty acids, glucosinolates, isothiocyanates, phenols, polyacetlenes, sesquiterpenes and thienyls [8,9,10,11,12,13]. 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. Hence, 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. MATERIALS AND METHODS

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

2.1 Culture of Root-Knot Nematode

In this study, the second-stage juveniles and egg masses of *M. incognita* were obtained from susceptible tomato (*Lycopersicon esculentum* Mill.) plant roots with galls from greenhouse.

2.2 Collection of Plant Material

The plants (Table 1) were collected from the various zones of Turkey during spring and summer of 2006 and 2007. *Melia azedarach* 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±1ºC) for 3 weeks and different part of the plants powdered in an electronic grinder.

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

Species	Family	Common name	Tissue used
Conium maculatum L.	Apiaceae	Poison hemlock	Fruit +Leaves+Stem
Melia azedarach L.	Meliaceae	Bead tree	Fruit
Humulus lupulus L.	Cannabaceae	Hops	Inflorescences
Sambucus nigra L.	Caprifoliaceae	Elderberry	Fruit + Leaves +Stem

2.3 Preparation of Methanol Extract

Plant extracts were prepared according to the procedure described by [14] and [15]. 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±1ºC). The extract was filtered through cheese cloth and solvent was evaporated in a rotary evaporator (RV 05 Basic 1B, IKA Group) at 32±2ºC. The residue was dissolved in 10% acetone containing distilled water. Test concentrations were prepared as 0.5%, 1%, 2.5%, 5% and 10% (v/v).

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 was 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 completely randomized design (CRD).

2.5 Nematicidal Activity of the Plant Extract

To study the effect of methanol extract of each plant species on mortality of *M. incognita,* pure cultures of *M. incognita* were maintained on tomato (*Lycopersicon esculentum* Mill.) roots in pots in the greenhouse. Second-stage juveniles were obtained from hatched eggs by incubating handpicked egg masses in sterile distilled water at 28ºC. Then 2 ml of each concentration of extract was poured in a glass cavitiy slide and 20 freshly hatched J_2 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 microscop (Olympus 40X) after 24, 48 and 72 hrs. Parcentage of nematode mortality was calculated according to the Abbott's formula [16].

3. RESULTS AND DISCUSSION

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 extracts recorded inhibition in the nematod egg hatching as compared to the control and distilled water. Although the highest concentration at level 10% (v/v) of the extracts of *M. azedarach and S. nigra* caused the highest egg hatching inhibitions as 97%,

92.9%, *H. lupulus* showed the highest egg hatching inhibition at level 2.5% (v/v) as 76.3% over control (Table 2). Furthermore, the medium used as control containing 10% acetone were prevented egg hatching over the distilled water at different rates.

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 belongs to *S. nigra* which provided the faster effectiveness than the others at 2.5%, concentration. After 24 hrs incubation, fruit extracts of *M. azedarach* (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 succesfully revealed the effectiveness of the obtained extracts. Hatching inhibition of the eggs generally increased with exposure time and extract concentrations but when eggs were transferred into the distilled water and control containing acetone, the inhibition decreased and was reversible which mean that plant extracts have nematistatic effects. The mortality rate of J2 significantly increased with the extract concentrations (P≤ 0.05, Duncan) and the mortality rates also increased with exposure time. However a number of studies had been published before about plant extracts effecting *Meloidogyne* genus [17,18,19,20,21,22,23,24,25], many of studies were about *M. azedarach* extracts. [26] reported that *M. azedarach* leaf and seed extracts inhibited hatching of *M. incognita* eggs (associated with a delay in embryonic development), reduced nematode motility and killed the larvae. [27] reported *M. azedarach* extract caused 96% mortality on *M. incognita* juveniles at 1:10 dilution in vitro after 24 hrs. [28] reported 1 ppm concentration of *M. azedarach* extract caused mortality more than 60% on juveniles *of M. incognita* within 24 hrs. [29] pointed out that the effect of the melia methanol extract obtained from *M. azedarach* fruit provided 100% nematode control at doses higher than 2.5% w/w with EC_{50} value of 0.9% w/w. [30] reported that *M. azedarach* fruits containing aldehydes, carboxylic acids and alcohols were used to control *M. incognita* on cucumber. Crushed fruits of *M. azedarach* were tested in the soil at the rates of 30 and 60 g kg^{-1} and they exhibited nematicidal activity similar to fenamiphos (0.02 g a.i. kg^{-1}) in terms of nematode population in roots and soil as well as reproduction rate. The extracts acted directly as nematicidal and also reduced the activity of the antioxidant enzymes and trigger the host defense. [31] reported different concentrations of aqueous extracts (0%, 1%, 2% and 4%) *in vitro* of dried leaves, seed, seed kernels and seed coats of *M. azedarach* L. were tested for their effects on second stage juveniles of *M. incognita*. The extracts of all plant parts and at every concentration immobilized 100% of J_2s. Maximum J_2 mortalities (100%) were given by concentrations of 2% and 4% of seed kernel and 4% of whole seed.

Our search revealed that the effectiveness of *M. azedarach* was compatible with the previous investigations and seemed promising to be used nematode control. The extracts of *C. maculatum*, *H. lupulus*, and *S. nigra* were also effective in relatively varying rates but need more search for nematode control.

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

	Treatments	Number of eggs hatched			Extract distilled water*			Total hatched eggs	Inhibition % over distilled water	Inhibition % over control
		24h	48h	72h	24h	48h	72h			
Conium maculatum	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
Melia azedarach	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
Sambucus nigra	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
Humulus lupulus	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

**After a 72 hrs exposure to plant extract, the egg masses were transferred to sterile distilled water. All data average of 3 replications.*

**N.A= Not Applicable*

Table 3. Effect of methanol extracts on *Meloidogyne incognita* second-stage juveniles % mortality after 24, 48 and 72 hrs incubations (Mean±Standard Error)

	Treatments	n	Mean mortality rates		
			24 h	48 h	72 h
			Mean±SE	Mean±SE	Mean±SE
Conium maculatum	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
	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	-
Melia azedarach	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	-	-
Sambucus nigra	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	-	-
Humulus lupulus	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≤ 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 species exhibited some nematicidal compound which cause reduction in egg hatching and death of second stage therefore, we need to discover new sources like plants with toxic substances could be use to manage plant nematodes.

ACKNOWLEDGMENT

This research was part of the Ph.D. thesis and funded by Gaziosmanpasa University (the scientific and research council).

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Luc M, Sikora RA, Bridge J. Plant parasitic nematodes in subtropical and tropical agriculture. 2nd ed., CABI Publishing; 2005.
2. Sikora RA, Fernandez E. Nematode parasites of vegetables. In: Luc M, Sikora, RA, Bridge J, editors. Plant parasitic nematodes in subtropical and tropical agriculture. 2nd ed. CABI Publishing; 2005.
3. Hunt D, Handoo Z. Taxonomy, identification and principal species. In: Perry RN, Moens M, Starr JL. Editors. Root- knot nematodes, 1. London: CABI; 2009.
4. Moens M. Perry R. Starr J. *Meloidogyne* species- a diverse group of novel and important plant parasites. In: R. N. Perry, M. Moens and J. L. Starr, editors. Root-knot nematodes, 1. Wallingford, UK; 2009.
5. Elekçioğlu IH, Ohnesorge B, Lung G, Uygun N. Plant parasitic nematodes in the Mediterranean region of Turkey. Nematologia Mediterranea. 1994;22:59–63.
6. Elekçioğlu IH, Uygun N. Occurrence and distribution of plant parasitic nematodes in cash crop in Eastern Mediterranean Region of Turkey. In: Proceedings of the 9th Congres of the Mediterranean Phytopathological Union, September 18–24, Kuşadası, Aydın, Turkey; 1994.
7. Sukul NC. Plant antagonistic to plant-parasitic nematodes. Ind. Rev. Life Sci. 1992;12:23-52.
8. Bruneton J. Pharmacognosy, phtochemistry, medical plants. Lavoisier Publishing, Paris, France; 1999.
9. Chitwood DJ. Phytochemical based strategies for nematode control. Annual Review of Phytopathology. 2002;40:221-249.
10. Gommers FJ. Biochemical interactions between nematodes and plants and their relevance to control. Helminthol. Abstr. Ser. B. Plant Nematol. 1981;50:9-24.
11. Latrasse A, Semon E, Quere JLL. Composition and major odorous compounds of the essential oil of Bifora radians, analdehyde-producing weed. J High Resolut Chrom, 1991;14:549-553.

12. Ma YT, Huang MC, Hsu FL, Chang HF. Hiazinedione from *Xanthium strumarium*. Phytochemistry. 1998;48:1083-1085.
13. Stevens JF, Ivancic M, Hsu VL, Deinzer ML. Prenylflavonoids from *Humulus lupulus* Phytochemistry. 1997;44:1575-1585.
14. Gokce A, Stelenski LL, Whalon ME. Behavioral and electrophysiological responses of leafroller moths to selected plant extracts. Environ Entomol. 2005;34:1426–1432.
15. Gökçe A, Whalon ME, Cam H, Yanar Y, Demirtas I, Goren N. Contact and residual toxicities of thirty plant extracts to Colorado potato beetle larvae. Arch Phytopathol. Plant Prot. 2006;40:441-450.
16. Abbott WS. A method of computing the effectiveness of an insecticide. J Econ Entomol. 1925;18:265-267.
17. Abbas S, Dawar S, Tariq M, Zaki MJ. Nematicidal activity of spices against *Meloidogyne javanica* (Treub) Chitwood. Pak J Bot. 2009;41:2625-2632.
18. Albuquerque MRJR, Costa SMO, Bandeira PN, Santiago GMP, Andrade-Neto M, Silveira ER. Nematicidal and larvicidal activities of the EOs from aerial parts of *Pectis oligocephala* and *Pectis apodocephala* Baker. An Acad. Bras. Ceinc. 2007;79(2):209-213.
19. Cetintas R, Yarba MM. Nematicidal effects of five plant essential oils on the Southern root-knot nematode, *Meloidogyne incognita* race 2. Journal of Animal and Veterinary Advances. 2010;9(2):222-225.
20. Echeverrigaray S, Zacaria J, Beltrao R. Nematicidal activity of monoterpenoids against the root-knot nematode *Meloidogyne incognita*. Phytopathology, 2010; 100: 199-203.
21. Meyer SLF, Lakshman DK, Zasada IA, Vinyard BT, Chitwood DJ. Dose-response effects of clove oil from Syzyg-ium aromaticum on the root-knot nematode *Meloidogyne incognita*. Pest Manag Sci. 2008;64:223–229
22. Oka Y, Nacar S, Putievsky E, Ravid U, Yaniv Z, Spiegel Y. Nematicidal activity of essential oils and their components against the root-knot nematode. Phytopathology. 2000;90:710–715.
23. Perez MP, Navas-Cortes JA, Pascual-Villalobos MJ, Castillo P. Nematicidal activity of essential oils and organic amendments from Asteraceae against root-knot nematodes. Plant Pathology. 2003;52:395-401.
24. Walker JT, Melin JB. *Mentha x piperita*, *Mentha spicata* and effects of their essential oils on *Meloidogyne* in soil. Journal of Nematology. 1996;24:629-635.
25. Wiratno D, Taniwiryono H, Van den Berg JAG, Riksen IMCM, Rietjens SR, Djiwanti JE, Kammenga Murk, AJ. Nematicidal activity of plant extracts against the root-knot nematode, *Meloidogyne incognita*. The Open Natural Products Journal. 2009;2:77-85.
26. Lee MJ. The effect of extract of *Melia azedarach* on *Meloidogyne incognita.* Quarterly Journal of Chinese Forestry. 1987;20(4):1-7.
27. Khurma UR, Singh A. Nematicidal potential of seed extracts: in vitro effects on juvenile mortality and egg hatch of *Meloidogyne incognita* and *M. javanica*. Nematologia Mediterranea. 1997;25(1):49-54.
28. Joymati L. Essential oil products of some medicinal plants as bio control agents against egg hatching and larval mortality of *Meloidogyne incognita.* J. Appl. Sci. Environ. Manage. 2009;13(4):91-93.

29. Ntalli NG, Menkissoglu-Spiroudi U, Giannakou I. Nematicidal activity of powder and extracts of *Melia azedarach* fruits against *Meloidogyne incognita*. Annals of Applied Biology. 2010;156:309-317.
30. Cavoski I, Chami ZAI, Bouzebboudja F, Sasanelli N, Simeone V, Mondelli D, Miano T, Sarais G, Ntalli NG, Caboni P. *Melia azedarach* controls *Meloidogyne incognita* and triggers plant defence mechanisms on cucumber. Crop Protection. 2012;35:85-90.
31. Ardakani AS. Effects of *Melia azedarach* on *Meloidogyne incognita In Vitro* and *In Vivo* Conditions. Nematol. Medit. 2012;40:55-60.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=539&id=2&aid=5114