

EFFICIENCY OF SOME PLANT EXTRACTS IN THE CONTROL OF ROOT-KNOT NEMATODES *MELOIDOGYNE* SPP.

Sanaa A. Haroon¹, Baraa A.A. Hassan¹, Fatma M.I. Hamad¹, and Mostafa M. Rady²

¹Plant Protection Department, Faculty of Agriculture, Fayoum University, 63514 Fayoum, Egypt

²Botany Department, Faculty of Agriculture, Fayoum University, 63514 Fayoum, Egypt

ABSTRACT

Nematicidal activities of leaf or root extracts of four selected medicinal plants (*Azadirachta indica* L., *Moringa oleifera* L., *Lantana camara* L., and *Glycyrrhiza glabra* L.) were tested against the larvae of the root-knot nematode; *Meloidogyne* spp. Leaves of each of *A. indica*, *M. oleifera*, and *L. camara* and roots of *G. glabra* were air-dried, grinded then extracted by the appropriate organic solvent. The nematode juveniles were exposed to these extracts at concentrations 500, 1000, 2000, 4000 ppm for 24, 48 or 72 hs. *A. indica* extract was the most effective in increasing larval mortality, followed by *M. oleifera* extract. There was a gradual increase in larval mortality with increasing the extract concentration and the duration of exposure. Being the most effective, the crude extract of *A. indica* was analyzed for the effective ingredients by using GC/MS. The active chemical constituents were Hexane, 2, 4-dimethyl; Hexane, 2,2,5-trimethyl; Cyclohexane, 2,4-diethyl-1-methyl; Methylbicyclo[4.2.0] Octane; Methallylcyclohexane; Cyclohexane ethyl-; Heptane, 2,6-dimethyl; Cyclohexane, 1,2,4-trimethyl-, (1 α ,2 β ,4 β)-; Trans-1,2-Diethyl cyclopentane; Octane, 4- methyl-; Heptane, 2,3-dimethyl-; Benzene, 1,3-dimethyl-; Cyclohexane, 1,1,2-trimethyl-; Cyclopentane, 1-methyl-2-propyl-; 1-Ethyl-4-methylcyclohexane; p-Xylene; Nonane; Cyclohexane, 1-ethyl-4-methyl-, cis-; Benzene (1-methylethyl)-; Cyclohexane, propyl-; Octane, 2,6-dimethyl-; Benzene, propyl-; Heptane, 3-ethyl-2-methyl-; Benzene, 1-ethyl-2- methyl-; Benzene, 1,2,3-trimethyl-; Decane; Decane, 4-methyl-; Decane, 2-methyl, Undecane; Undecane, 2-methyl-; Dodecane; -; o-Xylene; Mesitylene; and Naphthalene, decahydro-, trans-.

Keywords: plant extract, *Azadirachta indica*, *Moringa oleifera*, *Lantana camara*, *Glycyrrhiza glabra*, *Meloidogyne* spp., larval mortality, GC-MS.

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are very common and the most important nematode species of greenhouse-growing plants. The indiscriminate use of chemical nematicides to control these nematodes causes great injuries to human beings, animals, vegetations and to the environment as a whole due to their non-target effect, hazardous nature besides being expensive. So, with the increasing awareness of possible deleterious effects of the chemicals, biological control of plant pathogens have received considerable attention (**Garima et al., 2005**).

The management of these nematode-parasites has little chance of success and is uneconomical because they live in the soil and feed on the internal plant tissues. Preventing the introduction of nematodes with planting material, seeds, or

soil, using rotation and mixed cropping with the poor host, using nematode resistant varieties or rootstocks, and lowering nematode populations through nematicides are some of the most frequently used strategies (Ploeg, 2008). Until recently, methyl bromide was widely used to manage nematodes and other soil-borne pathogens in high-value horticultural crops. However, concerns on its impact on environment necessitate the ban or revoke of this methyl bromide in 2005 for its gas emission and global warming. Although nematicides are effective in nematode management, it discourages users because of their high costs, non-availability at the time of need, the hazards they pose on human as well as on non-target organisms (Nagaraju et al., 2010). Other options for the management of root-knot nematodes become imperative and there is an increasing interest in non-chemical nematode management strategies (Kerry, 1990).

Certain medicinal plant extracts and their constituents were experimentally used for the control of certain nematodes (Jeyaprakash et al., 2011, Kadam et al., 2012, Azhagumurugan and Ranjan, 2014).

The current study was designed to evaluate the toxic effect of four plant extracts; lantana (*Lantana camara*), neem (*Azadirachta indica*), moringa (*Moringa oleifera*), and liquorice (*Glycyrrhiza glabra*) on larvae of the root-knot nematode (*Meloidogyne* spp.).

MATERIALS AND METHODS

Plant Materials Used:

Leaves of moringa and neem were collected from mature plants grown in Demo Experimental Farm of Faculty of Agriculture, Fayoum University, Fayoum, Egypt. Lantana leaves were collected from the gardens of the said University.

However, liquorice roots were collected from private fields located in Abshwai district, at Fayoum also.

Preparation of Extracts:

Plant leaves were air-dried in the laboratory for ten days. The dried materials were finely ground. An amount for *L. camara* and *G. glabra* ethanol (95%) and for *A. indica* and *M. oleifera* petroleum ether were added at 400 ml solvent to 100 g of ground plant then shaken by a rotary shaker at 120 rpm for 24 hours. The solution was filtered through muslin cloths and through Whatman No. 1 filter paper and vacuumed in a rotary evaporator at 40 °C to obtain the crude extracts (solvent is eliminated) (Brauer and Devkota, 1990). Extracts were used at 4000, 2000, 1000 and 500 ppm concentrations; dilution with distilled water.

Extraction and Counting of Nematode Juveniles:

From root: Juveniles of the root-knot nematode were extracted from infested roots of tomato, using modified Baermann tray method (Whitehead and Hemming, 1965). The roots were chopped with a pair of scissors and 10g and placed each 10g in a plastic sieve lined with a two-ply tissue paper placed in a plastic plate. Tap water was poured carefully into these plastic plates until the tissue became moist and left for 48h then poured separately into beakers and allowed 24h more for the juveniles to settle at the bottom. The volume of each

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suspension was standardised to 50ml. Aliquots of 1ml of each suspension was taken with a pipette into a counting tray. Counting was done using an inverted microscope. Each suspension was homogenized by blowing air through with a pipette.

From soil: The soil for each course was mixed very well and placed in 1000 ml beaker, sufficient water was added to each sample to the volume of 600 ml, stirred for 20 seconds and allowed to settle for 30 sec, and decanted onto a 35-mesh sieve nested over a 400-mesh sieve. Sieves were held at an angle of 35°-40° during decanting to minimize the chance of small nematodes passing directly through the sieve. Using a wash bottle, the 35-mesh sieve was rinsed while still over the 400-mesh sieve (excessive rinsing washes small nematodes through both sieves). The debris and nematodes are washed from the 400-mesh sieve into a 150 ml beaker, then poured into 50ml centrifuge tubes and centrifuged at 3000 rpm for 4 min, then water was decanted (nematodes are in the soil pellet in the bottom of the tubes). Sucrose solution was then used to refill the tubes with a motorized stirring rod. After mixing these tubes were centrifuged for 30-60 sec. at 3000 rpm again. The nematodes will remain suspended in the sucrose solution. This suspension was decanted onto a 500-mesh sieve, slowly recovering small nematodes. A final rinse was made for the residue and nematodes from the 500-mesh sieve into a 1500 ml beaker (about 20 ml of water is suitable for making counts).

Mortality Test of Nematode Juvenile:

Eggs were placed in water and incubated at 28°±2°C. After hatching, the second stage juveniles were collected and a suspension of juveniles in water was prepared. 1 ml of egg suspension (100±10 juveniles/ml) and 5 ml of leaf or root extract for each plant were transferred into different Petri dishes and kept at room temperature. Each treatment was replicated three time. The Petri dishes containing 5 ml water served as control. Percentage mortality was calculated after 24, 48, and 72 hours of exposure, the number of killed juveniles was counted under an inverted microscope. The toxicity of root extract was assessed based on the mean percentage of the dead nematodes. Nematodes were considered dead if they did not move when probed with a fine needle (Abbasi *et al.*, 2008). Mortality of larvae was calculated as a percent of total larvae suspended (Ahmad *et al.*, 2004) and LC₅₀ and LC₉₀ values were determined by using probit analysis (Finney, 1971).

3.8. Gas Chromatography-Mass Spectrometry (GC/MS) Analysis:

The GC column was a 30 m (0.25 mm i.d., film thickness 0.25 µm) HP-5MS (5% diphenyl) dimethylpolysiloxane capillary column. The GC conditions were as follows: injector temperature, 240°C; column temperature, isothermal at 50°C for 2 min, then programmed to 280°C at 6°C/min and held at this temperature for 2 min; ion source temperature, 200°C; detector temperature, 300°C. Helium was used as the carrier gas at the rate of 1 ml/min. The effluent of the GC column was introduced directly into the ion source of the MS. Spectra were obtained in the EI mode with 70 eV ionization energy. The sector mass

analyzer was set to scan from 40 to 400 amu for 5 s. These data were obtained from the Environmental and Food Pollutants Laboratory at the Faculty of Agriculture, Fayoum University.

RESULTS

Efficiency of Plant Extracts and Lethal Concentration (LC):

Data in Table (1) and Figs. (1–4) show the toxic effect of some plant extracts on larvae of root-knot nematode. According to the LC₅₀ values, the plant extracts are arranged for their efficiency in an descending order as follows: neem, moringa, lantana and liquorice with values 2393.7, 2819.1, 3361.8 and 3668.7 ppm, respectively at 48 h.

Table (1): Mortality percentages of root-knot nematode (*Meloidogyne* spp.) treated with plant extracts.

Extract	Exposure period (h)	Concentration (ppm)				LC ₅₀ (ppm)	95% Confidence limits		LC ₉₀ (ppm)	Slope ± SE
		500	1000	2000	4000		Lower	Upper		
Neem	24	9*	14	23	37	8271	4981	24105	109935	1.14±0.23
	48	24	33	45	61	2394	1814	3614	35754	1.09±0.20
	72	61	70	82	94	348	177	504	3255	1.32±0.22
Moringa	24	7	12	21	36	8029	5062	20715	83719	1.26±0.24
	48	19	29	42	58	2819	2148	4287	33131	1.20±0.20
	72	47	61	76	90	602	414	773	4537	1.46±0.21
Lantana	24	5	10	20	34	9682	5635	31016	117687	1.18±0.24
	48	13	24	38	54	3362	2574	5094	29725	1.35±0.21
	72	31	51	69	83	993	792	1200	6249	1.60±0.21
Liquorice	24	2	8	17	31	7862	5297	16387	48014	1.63±0.28
	48	6	20	34	51	3669	2893	5220	21911	1.65±0.22
	72	15	41	62	78	1473	1257	1730	6635	1.96±0.21

*Mortality (%)

The descending arrangement of the tested plant extracts according to their LC₅₀ values was; neem, moringa, lantana and liquorice with LC₅₀ of 347.5, 602.3, 993.3 and 1472.5 ppm, respectively after 72 h. Neem extract caused 61, 70, 82 and 94% mortality at the concentrations of 500, 1000, 2000 and 4000 ppm, respectively at 72h followed by moringa extract causing 47, 61, 76 and 90% at the some concentrations, respectively, meanwhile the liquorice extract concentrations caused 15, 41, 62 and 78% mortality at some concentrations, respectively.

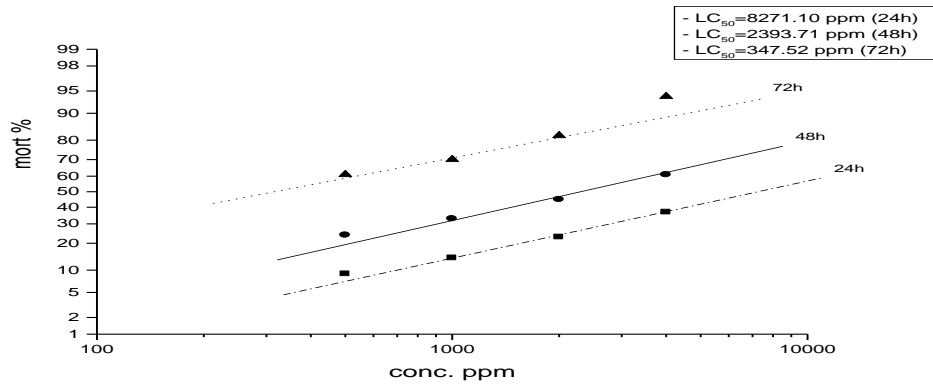


Fig. (1): LC₅₀ values for neem extract against larvae of root-knot nematode (*Meloidogyne* spp.) at different periods.

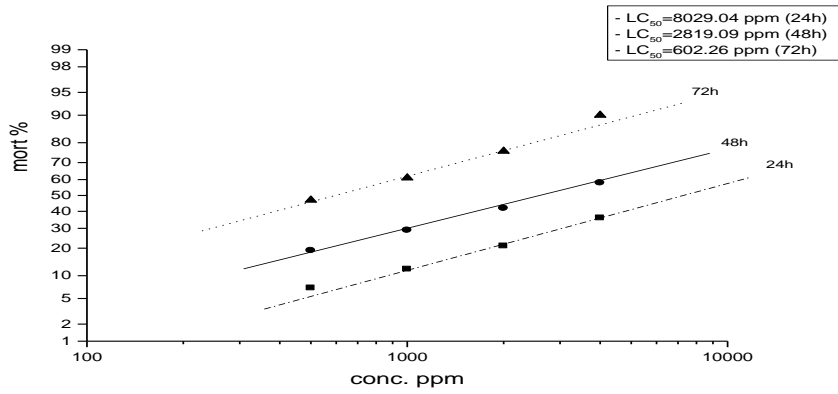


Fig. (2): LC₅₀ values for moringa extract against larvae of root-knot nematode (*Meloidogyne* spp.) at different periods.

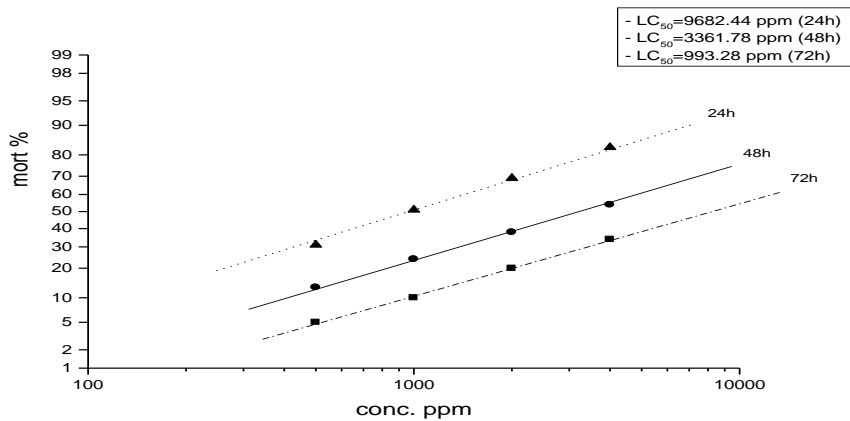


Fig. (3): LC₅₀ values for lantana extract against larvae of root-knot nematode (*Meloidogyne* spp.) at different periods.

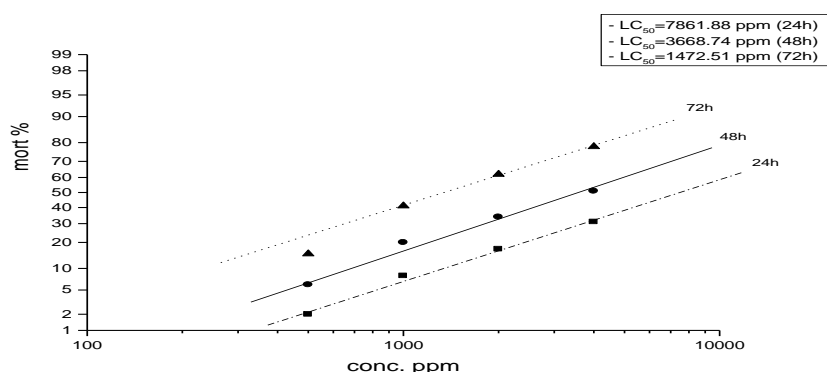


Fig. (4): LC₅₀ values for liquorice extract against larvae of root-knot nematode (*Meloidogyne* spp.) at different periods.

Effect of Plant Extract, Concentration and Exposure Time:

Regarding mean performance of plant extract, concentration and time on larvae mortality of root-knot nematode, data in Table (2) show the descending order of larvae mortality % was as followed: *A. indica*, *M. oleifera*, *L. camara* and *G. glabra*. The ascending order in regard to the larvae mortality was the extract concentration of 500, 1000, 2000 and 4000 ppm. The descending order for the larvae mortality was the treatment time of 72, 48 and 24 h.

Table (2): Mean performance (\pm SE) of plant extract, concentration and exposure time of mortality of *Meloidogyne* spp.

Plant	Means \pm SE	Conc. (ppm)	Means \pm SE	Time (h)	Means \pm SE
<i>A. indica</i>	37.0 \pm 3.2 a	0	00.0 \pm 0.0 e	24	14.4 \pm 1.1 c
<i>M. oleifera</i>	33.2 \pm 3.0 b	500	19.9 \pm 2.2 d	48	27.5 \pm 1.8 b
<i>L. camara</i>	28.8 \pm 2.7 c	1000	31.2 \pm 2.4 c	72	50.6 \pm 2.9 a
<i>G. glabra</i>	24.4 \pm 2.6 d	2000	44.1 \pm 2.7 b	-	-
	-	4000	59.0 \pm 2.6 a	-	-

Interactive Effect of Plant Extract and Its Concentration:

The interactive effect of plant extract and its concentration on larvae mortality of root-knot nematode is presented in Table (3) and Fig. (5). Neem extract significantly increased larvae mortality by 31.6, 39.2, 50.1 and 63.9% at the concentrations of 500, 1000, 2000 and 4000 ppm, respectively. In addition, larvae mortality was significantly increased by moringa extract application and the increases were 24.2, 34.1, 46.3 and 61.3% with the concentrations of 500, 1000, 2000 and 4000 ppm, respectively. Lantana extract significantly increased larvae mortality of root-knot nematode by 16.1, 28.4, 42.4 and 57.1% under the concentrations of 500, 1000, 2000 and 4000 ppm, respectively. Also, larvae mortality was significantly increased by liquorice extract application and the increases were 7.8, 23.0, 37.6 and 53.6% at the concentrations of 500, 1000, 2000 and 4000 ppm, respectively.

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Table (3): Statistical analysis for the interaction between plant extract, concentration and mortality of *Meloidogyne* spp.

EExtract Concentrate	M Means ± SE			
	<i>A. indica</i>	<i>M.oleifera</i>	<i>L. camara</i>	<i>G. glabra</i>
500	31.6 ± 5.5 j	24.2 ± 4.1 l	16.1 ± 2.7 m	7.8 ± 2.0 n
1000	39.2 ± 5.8 h	34.1 ± 5.0 i	28.4 ± 4.2 k	23.0 ± 3.5 l
2000	50.1 ± 6.0 e	46.3 ± 5.5 f	42.4 ± 5.0 g	37.6 ± 4.5 h
4000	63.9 ± 5.7 a	61.3 ± 5.4 b	57.1 ± 5.0 c	53.6 ± 4.7 d

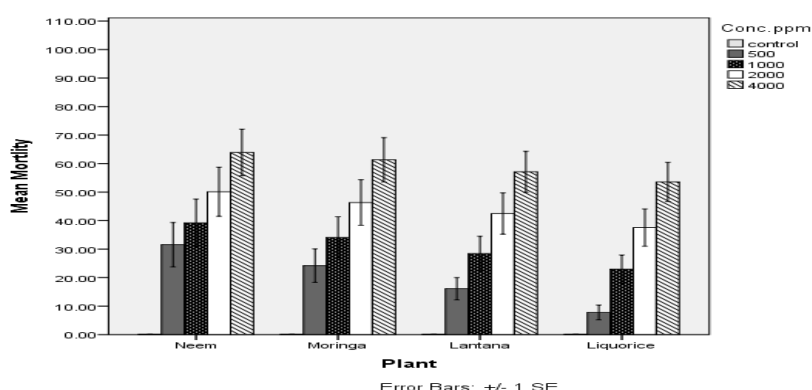


Fig. (5): Mean performance of interaction between plant extract, concentration and mortality of *Meloidogyne* spp.

Interaction Between Plant Extract and Exposure Time:

Table (4) and Fig. (6) illustrate the interactive effect of plant extract and treatment time on larvae mortality of root-knot nematode. The most effective extract is *A. indica* followed by *M. oleifera*, and the lowest effective extract is *G. glabra*.

Table (4): Statistical analysis for the interaction between plant extract, exposure time and mortality of *Meloidogyne* spp.

Plant	Time (h)	Means ± SE
<i>A. indica</i>	24	16.7 ± 2.4 i
	48	32.7 ± 3.8 e
	72	61.4 ± 6.2 a
<i>M. oleifera</i>	24	15.4 ± 2.3 i
	48	29.5 ± 3.7 f
	72	54.7 ± 5.8 b
<i>L. camara</i>	24	13.6 ± 2.2 j
	48	25.9 ± 3.6 g
	72	47.0 ± 5.5 c
<i>G. glabra</i>	24	11.9 ± 2.2 k
	48	22.1 ± 3.5 h
	72	39.2 ± 5.4 d

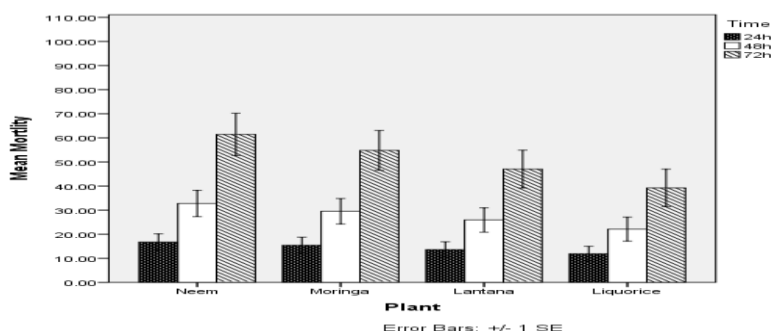


Fig. (6): Mean performance of interaction between plant extract, exposure time and mortality of *Meloidogyne* spp.

Interaction Between Plant Extract Concentration and Exposure Time:

Table (5) and Fig. (7) reveal the interactive effect of plant extract concentration and treatment time on larvae of root-knot nematode, under the application of plant extract concentration for different periods. The lowest effective concentration was 500 ppm, followed by 1000 ppm. The highest effective was 4000 ppm, which caused the highest larvae mortality.

Table (5): Statistical analysis for the interaction between plant extract concentration, exposure time and mortality of *Meloidogyne* spp.

Concentration (ppm)	Time (h)	Means ± SE
0	24	00.0 ± 0.0 k
	48	00.0 ± 0.0 k
	72	00.0 ± 0.0 k
500	24	5.9 ± 0.6 j
	48	15.4 ± 1.5 h
	72	38.4 ± 3.7 d
1000	24	11.2 ± 0.5 i
	48	26.4 ± 1.1 f
	72	56.0 ± 2.4 c
2000	24	20.3 ± 0.5 g
	48	39.8 ± 1.0 d
	72	72.3 ± 1.7 b
4000	24	34.6 ± 0.5 e
	48	56.1 ± 0.8 c
	72	86.3 ± 1.3 a

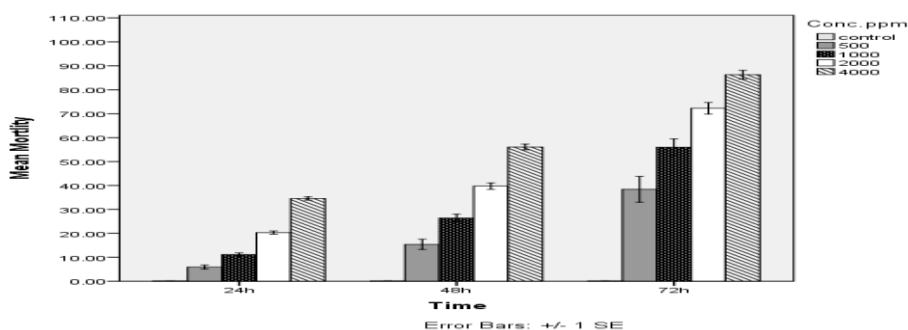


Fig. (7): Mean performance of interaction between plant extract concentration, exposure time and mortality of *Meloidogyne* spp.

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Effect of Plant Extract Concentration Over Time:

Table (6) show the effect of different concentrations of the plant extracts on larval mortality over time. Neem extract is effective in causing larval mortality with 4000 ppm concentration being more efficacious followed by moringa extract, the less effective plant extract in mortality of larvae was liquorice extract. The numbers of active and motionless juveniles were counted after 24, 48 and 72 hours exposure using an inverted microscope. The juvenile mortality was observed to increase with the increase of exposure time. Nematodes that remained motionless when touched with a needle were considered as dead. The study revealed that juvenile mortality increased with the increase in the extract concentration. The consequent percent mortality means of both plant extracts ranged by 2.3 to 37.3% at 24 h exposure, ranged by 6.0 to 60.7% at 48 h exposure and ranged by 15 to 93% at 72 h. The control treatment recorded the low mortality.

Table (6): Effect of some plant extracts on mortality of *Meloidogyne* spp. larvae in different periods.

Treatment	Conc. (ppm)	Mortality (%)		
		24h	48h	72h
<i>A. indica</i>	500	9.3 ± 0.3 t_w	24.3 ± 0.8 n	61.0 ± 2.1 f
	1000	14.0 ± 0.3 rs	33.3 ± 0.6 kl	70.3 ± 1.4 e
	2000	23.0 ± 0.3 no	45.3 ± 0.6 h	82.0 ± 1.1 c
	4000	37.3 ± 0.3 j	60.7 ± 0.5 f	93.7 ± 0.8 a
<i>M. oleifera</i>	500	7.3 ± 0.2 u_x	18.7 ± 0.5 pq	46.7 ± 1.5 h
	1000	12.3 ± 0.2 st	28.7 ± 0.5 m	61.3 ± 0.9 f
	2000	21.3 ± 0.2 n_p	41.7 ± 0.5 i	76.0 ± 0.7 d
	4000	36.0 ± 0.0 jk	58.3 ± 0.2 f	89.7 ± 0.5 b
<i>L. camara</i>	500	4.7 ± 0.2 xy	12.7 ± 0.2 st	31.0 ± 0.5 lm
	1000	10.0 ± 0.0 tu	24.0 ± 0.0 n	51.0 ± 0.3 g
	2000	19.7 ± 0.2 o_q	38.3 ± 0.3 ij	69.3 ± 0.3 e
	4000	33.7 ± 0.2 kl	54.3 ± 0.3 g	83.3 ± 0.3 c
<i>G. glabra</i>	500	2.3 ± 0.6 yz	6.0 ± 1.5 wx	15.0 ± 3.8 rs
	1000	8.3 ± 0.6 uw	19.7 ± 1.2 o_q	41.0 ± 2.6 i
	2000	17.3 ± 0.6 qr	33.7 ± 1.0 kl	61.7 ± 1.8 f
	4000	31.3 ± 0.6 lm	51.0 ± 0.8 g	78.3 ± 1.4 d
Control	0	00.0 ± 0.0 z	00.0 ± 0.0 z	00.0 ± 0.0 z

Data are means ± SE different letters in a column indicate significant differences between the treatments at $P \leq 0.05$.

Chemical Compounds of Neem Leaf Extract:

Neem as the most effective plant extract mortality of nematode larvae found in this study against *Meloidogyne* spp. was subject to GC/MS analysis, Fig. 8, which showed the following compounds: Hexane, 2, 4-dimethyl; Hexane, 2,2,5-trimethyl; Cyclohexane, 2,4-diethyl-1-methyl; Methylbicyclo[4.2.0] Octane; Methallylcyclohexane; Cyclohexane ethyl-; Heptane, 2,6-dimethyl; Cyclohexane, 1,2,4-trimethyl-, (1 α ,2 β ,4 β)-; Trans-1,2-Diethyl cyclopentane; Octane, 4- methyl-; Heptane, 2,3-dimethyl-; Benzene, 1,3-dimethyl-; Cyclohexane, 1,1,2-trimethyl-; Cyclopentane, 1-methyl-2-propyl-; 1-Ethyl-4-methylcyclohexane; p-Xylene; Nonane; Cyclohexane, 1-ethyl-4-methyl-, cis-; Benzene (1-methylethyl)-; Cyclohexane, propyl-; Octane, 2,6-dimethyl-; Benzene, propyl-; Heptane, 3-ethyl-2-methyl-; Benzene, 1-ethyl-2- methyl-; Benzene, 1,2,3-trimethyl-; Decane; Decane, 4-methyl-; Decane, 2-methyl, Undecane; Undecane, 2-methyl-; Dodecane; -, o-Xylene; Mesitylene; and Naphthalene, decahydro-, trans-.

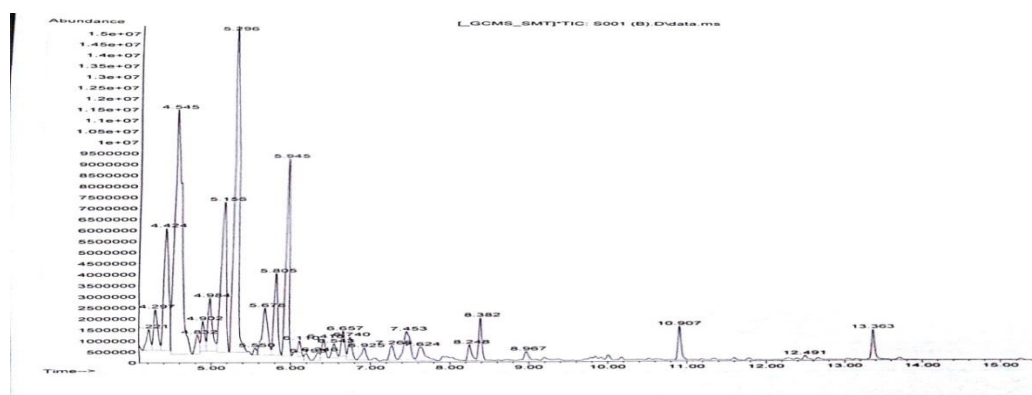


Fig. (8): GC-MS Chromatogram for the extract of *A. indica* leaves.

DISCUSSION

The plant extracts tested in this study are found to have a high nematicidal activity against root-knot nematode due to natural nematotoxic constituents that are able to kill the nematode juveniles. In a study conducted by **Hussaini et al. (1996)**, it has been reported that leaf extracts of 11 plant species (other than the plants used in the current study) caused 90% larval mortality in *M. incognita*, *M. javanica*, and *M. arenaria*. Our results are in parallel line with these results. In addition, results of the current study are in agreement with the results of **Nandal and Bhatti (1983)** that some of the plant extracts showed significant nematicidal properties and that percent mortality of juveniles was increased with increasing the exposure time to extracts of *Annona squamosa* (L.), *Cocculus pendulus* (Forsk.) Diel, *Datura fastuosa* (L.), and *Solanum surattense* Burm. f. which caused 100% mortality of juveniles after 72 h. The same results were obtained by **Khurmar et al. (1997)**, **Nagesh et al. (1997)** and **Pandey and Dwivedi (2000)**.

According to **Khan (1990)**, many wild and cultivated medicinal plants have been shown to possess nematicidal properties against several plant-parasitic nematodes. Neem extract had a toxic effect on the root-knot nematode in vitro by increasing juvenile mortality at different concentrations of the extract. This may be due to the active chemicals found in the extract containing alkaloids, flavonoids, saponins, amides including benzamide and ketones that negatively affect the nematode juveniles. Our results have also shown that the tested extracts (i.e., *Azadirachta indica*, *Moringa oleifera*, *Lantana camara*, and *Glycyrrhiza glabra*) were also found to have a killing effect on root-knot nematode juveniles.

The present study also revealed that juvenile mortality was increased with increase in the concentration of the extract. This is in agreement with **Hasabo and Noweer (2005)** who found that the mortality effect of an extract on nematode juveniles is a concentration dependent. Furthermore, **Ameer-Zareen et al. (2003)** and **Joymati et al. (1998)** whilst working with aqueous extract of *Zingiber officinale* and *Jatropha curcas* also recorded similar results.

Our results have also shown that juvenile mortality was found to increase with an increase in the exposure time. This observation also agrees with the results of **Lashien (2002)** and **El-Nagdi and Mansour (2003)**.

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In conclusions the results of the present study, revealed that neem extract was the most effective regarding the increase in juvenile mortality compared to moringa, lantana, and liquorice extracts. The GC/MS analysis for this extract identified the active chemical compounds found as alkaloids, flavonoids, saponins, amides including benzamide and ketones.

There is, however, a need for further studies in identifying new classes of bio-pesticides from natural plants to replace the synthetic dangerous and expensive chemicals used at present.

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كفاءة بعض المستخلصات النباتية في مكافحة نيماتودا تعقد الجذور

تم في هذه الدراسة إختبار المستخلصات الطبيعية لأوراق أو جذور أربعة نباتات طبية و هي (النيم، المورينجا، اللانتانا كمارا و العرقسوس) لدراسة فاعليتها ضد نيماتودا تعقد الجذور. أوراق النيم و المورينجا و اللانتانا كمارا و كذلك جذور العرقسوس تم تجفيفها هوائياً و طحنها ثم إستخلاصها بالمذيبات العضوية. تم معاملة يرقات النيماتودا بالمستخلصات سالفة الذكر بالتركيزات المختلفة (٥٠٠، ١٠٠٠، ٢٠٠٠ و ٤٠٠٠ جزء في المليون) لمدد زمنية مختلفة (٢٤، ٤٨ و ٧٢ ساعة) و قد أظهرت النتائج تفوق مستخلص أوراق النيم على مستخلص أوراق اللانتانا كمارا و جذور العرقسوس حيث كان أكثر فاعلية في زيادة موت اليرقات يليه مستخلص أوراق المورينجا من حيث كفاءة التأثير. كذلك أشارت النتائج إلى حدوث زيادة تدريجية في معدل موت اليرقات بزيادة التركيز المستخدم و كذلك زيادة فترة المعاملة بالمستخلص. تم كذلك في هذه الدراسة فصل المركبات الفعالة في نبات النيم بصفته أفضل النباتات الأربعة في مكافحة النيماتودا بإستخدام جهاز GC/MS (و التي تتضمن القلويدات و الفلافونيدات و الفينولات وغيرها).

نتخلص من الدراسة أن مستخلص أوراق النيم قادر على زيادة معدل موت يرقات نيماتودا تعقد الجذور. و بالتالي فإن هذه الدراسة ترجع أهميتها إلى تحديد و تطوير الإستراتيجيات البديلة كإستخدام طرق طبيعية آمنة لمكافحة نيماتودا تعقد الجذور. غير أن هناك الحاجة إلى مزيد من الدراسات لتحديد أنواع جديدة من المبيدات الطبيعية التي تستخلص من النباتات لتحل محل المبيدات الصناعية الخطيرة باهظة التكاليف و ذلك لإستخدامها في مكافحة الأفات.