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Effect of neem leaf extract on egg hatchability of root-knot nematode

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ABSTRACT:

Nowadays, plant extracts are extensively used as eco-friendly strategies for biological control of parasitic pests, including the root-knot nematodes, instead of using chemical pesticides. Therefore, the aim of this study was to examine leaf extract of neem plants (*Azaddirachta indica*) against the root-knot nematode; *Meloidogyne* spp. The obtained results showed that there was a gradual decrease in egg hatching with increasing the neem leaf extract concentrations and the duration of exposure. The neem extract is highly effective against egg hatching being the IC_{50} scored 397.1 ppm. Consequently, neem extract caused 75, 79, and 81% inhibition of egg hatching on root-knot nematode at the concentrations of 2000, 4000, and 6000 ppm, respectively at 72h. In contrast, the egg hatching (%) was progressively increased with increasing the exposure time from 24 to 72h. Under the application of plant extract concentrations for different periods, the lowest effective plant extract concentration was 2000 ppm, which gave the least inhibition, followed by 4000 ppm. The highest effective extract concentration was 6000 ppm, conferring the lowest egg hatching. It has been concluded from the results of the present study that the leaf extract of *A. indica* has the ability to inhibit the egg hatchability of root-knot nematode. Thus, this finding is important in the identification and development of alternative strategies in controlling the root-knot nematodes. There is, however, further work is needed to identify some of these main compounds after purification. This study confirms the presence of nematicidal compounds in petroleum ether fractions of *Azadirachta indica*, which were responsible for the prevention of egg hatching of root-knot nematodes at some concentrations, especially 6000 ppm.

KEYWORDS: Neem leaf extract *Azadirachta indica*, *Meloidogyne* spp., Egg hatchability.

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1- INTRODUCTION:

Nematodes are found in a wide variety of habitats. free-living nematodes live in the soil, in freshwater, marine, sands and muds. In soil, they are important components of nutrient turnover. Other nematodes are parasites of almost every species of animals, humans, plants and they cause enormous social and economic damage (**Perry, 2011**). The nematodes parasitize plants to seek suitable food. This food source is basically planted cell content's. These response to parasitism is the reaction to the cellular feeding of the nematode **(Ahmad *et al.*, 2010)**. Most phytoparasitic nematodes infect plant roots and some species have evolved sophisticated interactive relationships with host cells to sustain a sedentary parasitic habit **(Davis *et al.*, 2004)**. Plants carry a wide range of microorganisms in their phyllosphere and rhizosphere which not only cause a large variety of diseases but also control of pathogens. Nematodes have an important role in agro-ecosystem, causing a reduction in plant productivity and growth **(Elekcioglu *et al.*, 1994)**.

Root-knot nematodes (*Meloidogyne* spp.) are very common and the most important nematode species of greenhouse-growing plants. Indiscriminate use of chemical nematicides to control nematode causes great injuries to humans being, animal, vegetation and to the environment as a whole due to their non-target effect, hazardous nature besides they are expensive. So with the increasing awareness of possible deleterious effects of the chemicals, biological controls of plants pathogen 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).**

Extract from certain plants is used to control certain nematode because environmental consideration and costs of nematicides dictate that other methods of control may be investigated, on alternative method is the use of antagonistic plants in rotation with or interplanted with crop plants. Certain medicinal plant extracts and their constituent were experimentally used for this aim **(Jeyaprakash *et al.*, 2011; Kadam *et al.*, 2012; Azhagumurugan and Ranjan, 2014; Haroon *et al.*, 2018 & 2019).**

The current study was designed to evaluate the potential beneficial effects of neem plant extract (*Azadirachta indica*) on the control of the root-knot nematode (*Meloidogyne* spp.) through the toxic effect on egg hatchability.

2- MATERIAL AND METHODS:

Plant material used in the experiment:

As shown in Table 1, Leaves of neem were collected from mature plants grown at Demo Experiment Farm of Faculty of Agriculture, Fayoum University, Fayoum, Egypt.

Table 1: Information about the plant used in the present study.

English Name	Scientific Name	Family	Plant part used
Neem	*Azadirachta indica* (**Taye *et al.*, 2013**)	Meliaceae	Leaves

Preparation of plant extracts:

Plant leaves were plucked from their branches and spread on polythene sheets on benches in the laboratory for ten days to air dry. The dried materials were ground to fine particles using a blender. An amount of 400 ml ethanol 95% was added to 100 g of ground plant material (*A. indica*) and shaken on a rotary in a shaker at 120 rpm for 24 hours. The solution was filtered through muslin cloths then through Whatman No. 1 filter paper and the material was vacuumed in a rotary evaporator at 40°C to obtain organic crude extracts (solvent is eliminated) **(Brauer and Devkota, 1990)**.

Extraction of nematode eggs:

Eggs were obtained from a culture of nematode infected roots of tomato; root pieces containing egg masses were cut into small pieces and placed in a container of 500 ml capacity with 200 ml of 0.5% Clorox (sodium hypochlorite) solution shaken vigorously by hand for 4 min. This was done in order to digest the gelatinous matrix encasing the eggs. The solution was then poured through two nested sizes, 200-mesh (75 µm) and 500 mesh (25 µm). Eggs in the 500 mesh sieve were washed free of clorox solution with a slow stream of cold tap water into a container previously marked to contain 1 L. The cut roots in the original container were washed twice with water to obtain additional eggs. The collected eggs were topped with water to obtain the egg-water suspension for *in vitro* studies **(Hussey and Barker, 1973)**.

Counting of root-knot nematodes eggs:

Number of eggs in aqueous suspension was determined by using a stereo microscope. One milliliters of the egg-water suspension was pipetted after bubbling air through the suspension for homogeneity and dispensed into a counting tray. Counting was done two times and the mean number of eggs/ml estimated **(Haroon *et al.*, 2019)**.

Hatchability test:

A suspension of eggs in water was prepared. One ml of egg suspension (100±10 eggs/ml) and 5 ml of extract was transferred in Petri dishes and kept at room temperature. Each treatment was 3-time replicated. The Petri dishes containing 1 ml egg suspension and 5 ml water served as control. After 24, 48, and 72 hours of exposure, the number of hatching eggs was counted under an inverted microscope **(Hussey and Barker 1973)**.

Nematicide:

Oxamyl (Vydate) 24% L. Methyl-N'N'- dimethyl-N [(methyl) carbamoyl-oxy]-1-thioxamidate was used at the rate of 1L/100L.

Statistical analysis:

Statistically, the obtained data were subjected to analysis of variance (ANOVA), followed by Duncan's multiple range Tested (DMRT) to compare means **(Duncan, 1955).**

3- RESULTS AND DISCUSSION:

Effect of exposure time and Inhibition Concentration (IC):

Regarding the effect of neem plant extract on egg hatching of root-knot nematode after 72h, data in Table 2 show that toxicity of extract [IC_{50} and IC_{90}, as well as slope value] was calculated. It shows that the neem extract is highly effective against egg hatching being the IC_{50} scored 397.1 ppm. Consequently,

neem extract caused 75, 79, and 81% inhibition of egg hatching on root-knot nematode at the concentrations of 2000, 4000, and 6000 ppm, respectively at 72h.

Table (2): Effect of neem extract on egg hatching (%) of root-knot nematode (*Meloidogyne* spp.) after 72 hours from exposure.

Neem extract	Concentration (ppm)			IC_{50} (ppm)	95% Confidence limits		IC_{90} (ppm)	Slope ± SE
	2000	4000	6000		Lower	Upper		
	75*	79	81	397.1	0.0	1220.9	63732.5	0.58 ± 0.27

*Inhibition of egg hatchability (%)

Effects of neem extract concentrations and exposure time:

The mean performance of neem extract concentrations and exposure time on egg hatching of root-knot nematode are shown in Table 3 and Figs. 1-2. Egg hatching (%) was progressively reduced with increasing the concentration of the extract from 2000 to 6000 ppm. In contrast, the egg hatching (%) was progressively increased with increasing the exposure time from 24 to 72h.

Table 3: Mean performance (± SE) of neem extract concentrations and time on egg hatching of *Meloidogyne* spp.

Conc. (ppm)	Means ± SE	Time (h)	Means ± SE
0^-	52.44 ± 6.0 a	24	13.53 ± 6.2 c
0^+	5.89 ± 0.2 e	48	21.87 ± 4.2 b
2000	19.56 ± 1.7 b	72	28.80 ± 2.6 a
4000	16.00 ± 1.7 c	-	-
6000	13.11 ± 1.9 d	-	-

Negative control (0^-; nematode + water) and possative (0^+; nematode + nematicide)

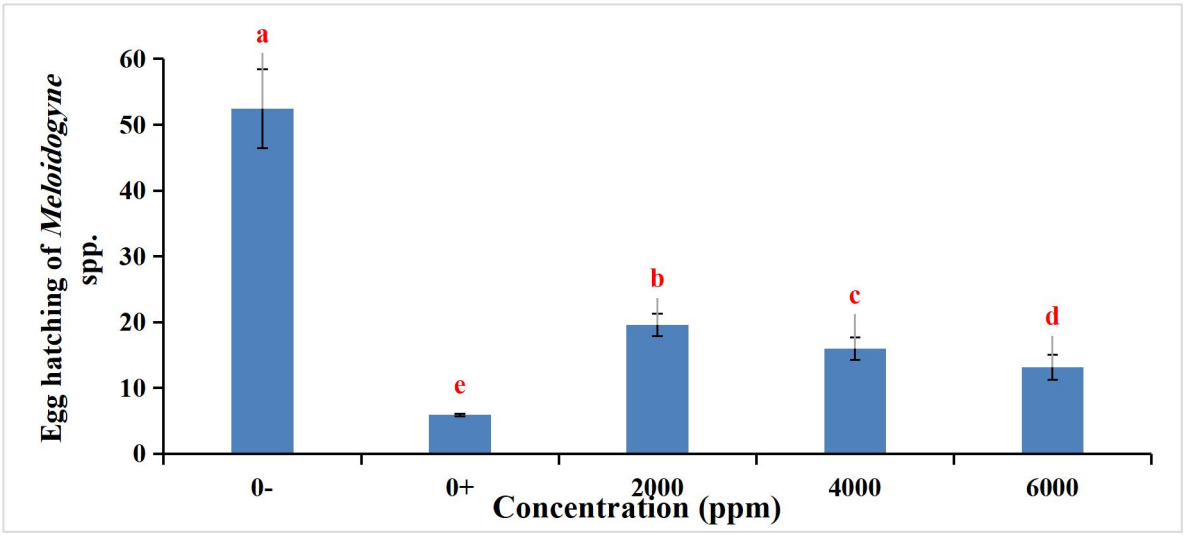

Fig. 1: Mean performance (± SE) of the effects of neem extract concentrations on egg hatching of *Meloidogyne* spp.

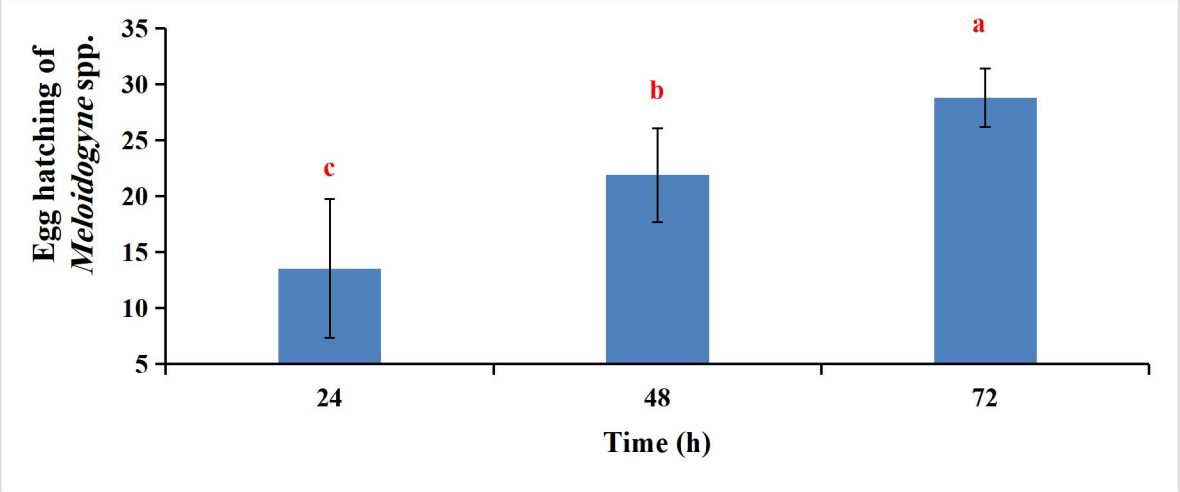

Fig. 2: Mean performance (± SE) of exposure time of neem extract on egg hatching of *Meloidogyne* spp.

Interactive effects of neem extract concentrations and exposure time:

Table 4 and Figs. 3-6 reveal the interactive effect of plant extract concentrations and exposure time on nematode egg hatching. Under the application of plant extract concentrations for different periods, the lowest effective plant extract concentration was 2000 ppm, which gave the least inhibition, followed by 4000 ppm. The highest effective extract concentration was 6000 ppm, conferring the lowest egg hatching.

Table 4: Mean performance (± SE) of interaction between concentration and time on egg hatching of *Meloidogyne* spp.

Concentration (ppm)	Time (h)	Means ± SE
0^{-}	24	32.00* ± 1.7 c
	48	52.00 ± 2.1 b
	72	73.33 ± 1.3 a
0^{+}	24	5.33 ± 0.3 h
	48	6.00 ± 0.0 h
	72	6.33 ± 0.3 gh
2000	24	14.00 ± 1.0 f
	48	19.67 ± 1.3 e
	72	25.00 ± 1.5 d
4000	24	10.00 ± 1.0 g
	48	17.33 ± 1.3 ef
	72	20.67 ± 1.3 e

6000	24	6.33 ± 0.9 gh
	48	14.33 ± 1.2 f
	72	18.67 ± 0.7 e

Data are means ± S.E. different lower or upper letters in a column indicate significant differences between the treatments at $P \leq 0.05$. Negative control (0^-; nematode + water) and possative (0^+; nematode + nematicide)

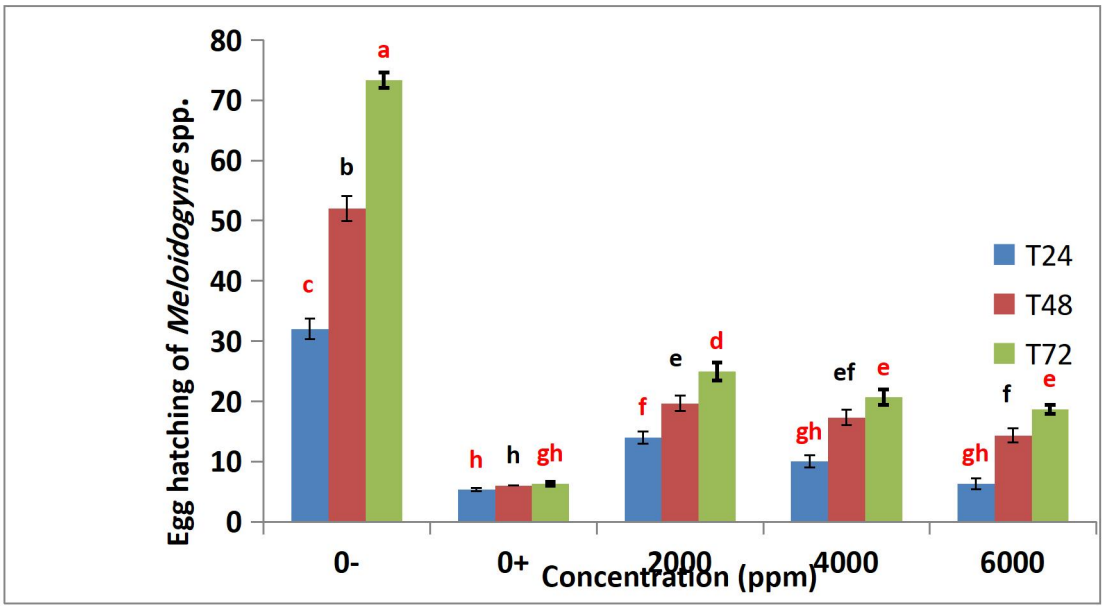

Fig. 3: Mean performance (± SE) of interaction between concentration and time on egg hatching of *Meloidogyne* spp.

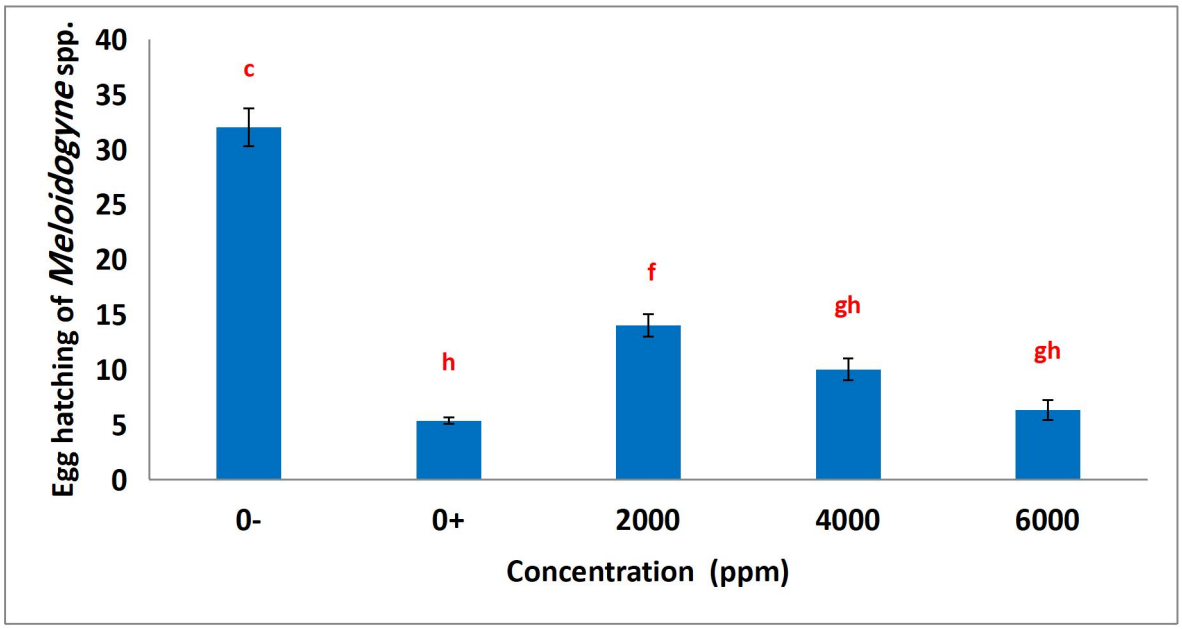

Fig. 4: Effect of neem extract on egg hatching of root-knot nematode *Meloidogyne* spp. after 24h.

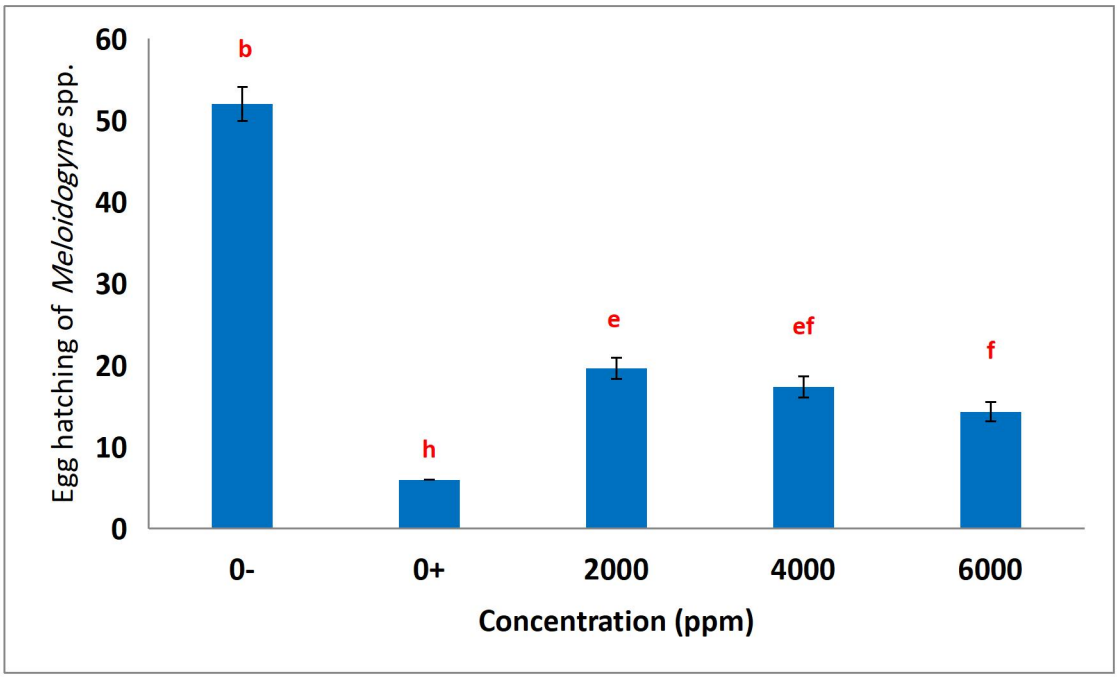

Fig. 5: Effect of neem extract on egg hatching of root-knot nematode *Meloidogyne* spp. after 48h.

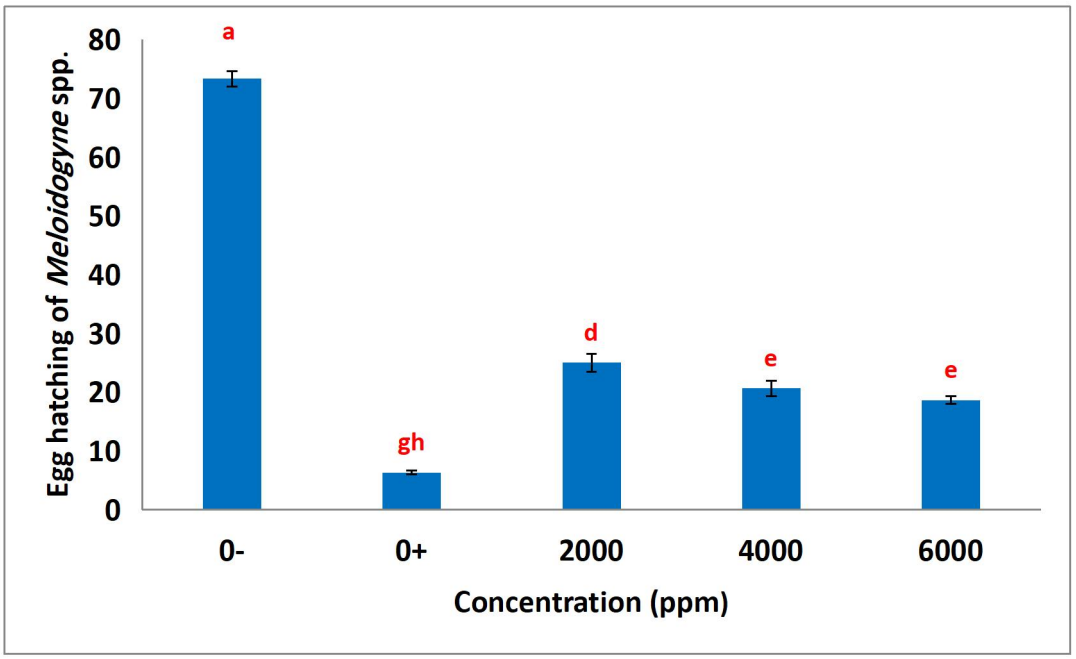

Fig. 6: Effect of neem extract on egg hatching of root-knot nematode *Meloidogyne* spp. after 72h.

The results show a gradual decrease in egg hatching with increasing the extract concentration. The increase in exposure period and an increase of the concentration also decrease the egg hatching. After 24 h application of the extracts on root-knot nematode eggs, the mean egg hatching ranged from 5.33 to 32.00%. At 48 h, mean egg hatching was ranged between 6.00 and 52.00%. The highest egg hatching was found in the control (water) (0^{-}), whilst the least hatch was in the control (nematicide) (0^{+}) followed by neem extract. In addition, at 72 h mean egg hatching was ranged between 6.33 to 73.33 %.

The recent approach in nematode control is direct method towards the possibility of reducing populations of plant-parasitic nematodes in soil by using natural substances extracted from some plants. Such methods don't lead to the disturbance of the biological balance of nature. Utilization of antagonistic plants or their byproducts is of common use all-over the world for avoiding hazards of the traditional chemical nematicides. The use of certain plant extracts for controlling plant-parasitic nematodes has been increased in the recent years (**Dias *et al.*, 2000; Pandey and Dwivedi 2000**; **Insunza *et al.*, 2001**; **Rakesh *et al.*, 2001**).

The plant extract which tested in this study found in most cases to have an antagonistic action and a higher nematicidal activity against root-knot nematode. So, they undoubtedly contain natural nematotoxic constituents that able to inhibit the nematode egg hatching. In a study conducted by **Hussaini *et al.* (1996)**, it has been reported that leaf extracts of 11 plant species inhibited egg hatching and caused 90% larval mortality in *M. incognita*, *M. javanica,* and *M. arenaria*, our results are agreement with the above results. In addition, results of the current study are in agreement with the results of **Nandal and Bhatti (1983)** who have reported that some of the plant extracts showed significant nematicidal properties.

Neem contains various compounds that are toxic to many groups of insects, arthropods, as well as nematodes. Among these toxic compounds, azadirachtin, a triterpenoid of the limonoid class, is the most active compound for the inhibition of nematode development **(Mordue *et al.*, 2005)**.

According to **Khan (1990)**, many wild and cultivated medicinal plants have been shown to possess nematicidal properties against several plant-parasitic nematodes. The results of this study showed that neem extract had a toxic effect on the root-knot nematode in vitro by inhibiting the egg hatching at different concentrations of the extract. It was also observed that inhibition of egg hatching increased with increasing the concentration of the extract with the highest score that was recorded with the extract concentration of 6000 ppm. This observation agrees with the findings of **Adegbite and Adesiyan (2005)** working with root extracts of *Azadirachta indica*, *Chromolaena odorata*, *Ricinus communis* and *Jatropha curcas* and recorded the gradual increase in inhibition of egg hatching with increasing the concentration of the extract. A similar finding was reported by **Ameer-Zareen *et al.* (2003)** on root-knot nematode eggs *in vitro* when they have used the aqueous extract of

ginger (*Zingiber officinale*). This study also agrees with the results of **Barker (2003)** that nematode egg hatching was influenced by the exudates from its environment. In addition, egg hatching inhibition was increased with increase in exposure time, and this result also agrees with the results of **Joymatti *et al.* (1998)**.

The Effectiveness of plant extracts depends on their concentration and the duration of exposure of the nematode to the extract **(Mahmood *et al.*, 1979; Kali and Gupta, 1980)**. The concentration of active ingredients in neem seed and leaf extracts may vary depending on the environmental condition, year of collection and geographical region of neem trees **(Zongo *et al.*, 1993)**. However, their extracts appear to contain some nematicidal compounds which tend to inhibit the hatching of egg mass and are directly toxic to *M. incognita* larvae.

The inhibitory effect of plant extracts on egg hatching of nematode according to **Adegbite and Adesiyan (2005)**, might be due to the properties of the chemical compounds present in the extract that possess ovicidal properties. It was also suggested that botanicals with nematicidal properties affect the embryonic development or kill the eggs. Presumably, these properties found to increase with an increase in time, hence, the inhibition of egg hatching tend to increase with increasing the exposure period to the extract.

These active chemicals either affect the embryonic development or kill the eggs or even dissolve the egg masses. It has been reported by **Adegbite (2003)**, **Goswami and Vijayalakshmi (1986); Hackney and Dickerson (1975)** that extracts (i.e., Siam weed; *Chromolaena odorata* L., Neem; *Azadirachta indica* A. Jass, Castor bean; *Ricinus communis*, and Lemon grass; *Cymbopogon citratrus* DC.) that contained alkaloids, flavonoids, saponins, amides including benzamide and ketones in a single form or in a combination inhibited nematode egg hatching.

From the results of the present study, it has been found that neem extract was recorded the best results regarding inhibition of egg hatching.

It has been concluded from the results of the present study that the leaf extract of *A. indica* has the ability to inhibit the egg hatchability of root-knot nematode. Thus, this finding is important in the identification and development of alternative strategies in controlling the root-knot nematodes. There is, however, further work is needed to identify some of these main compounds after purification. This study confirms the presence of nematicidal compounds in petroleum ether fractions of *Azadirachta indica*, which were responsible for the prevention of egg hatching of root-knot nematodes at some concentrations, especially 6000 ppm.

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