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The effect of *Lantana camara* leaf extract on the larvae of the nematode *Meloidogyne javanica* in Wasit Governorate

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Abstract: *This study was carried out in the laboratories of the College of Science - University of Wasit, as well as the laboratories and greenhouses affiliated with the Biological Control Center - Department of Agricultural Research - Ministry of Science and Technology from 25/10/2022 to 20/5/2023 to evaluate the effect the Lantana myna leaf extracts on the rootknot nematode Meloidogyne javanica and its density. The results of Lantana camara leaf for both aqueous and alcoholic extracts on the vitality of the second larval instar of the rootknot nematode M. javanica showed that both the aqueous and alcoholic extracts and all their concentrations used had a significant impact on the vitality of the larvae and achieved a large killing rate that was significantly higher. On the control treatment, the effect of the plant extract on nematode larvae was according to the concentration that was applied, and the proportion of larval deaths at a concentration of 1000 ppm was 30% after 24 hours of treatment, and it began to increase with increasing concentration until it reached 77.79% at a 2,000 parts per million and after the the same time frame. When it comes to the aqueous extract, the proportion of dead larvae at 1000 parts per million was 51.82% after 24 hours of treatment, and it began to increase with increasing concentration until it reached 95.96% at a density of 2000 parts per million and after the same time frame as in the instance of the alcoholic extract. From this we conclude the possibility of using the plant, Lantana camara, as a biological pesticide to prevent infection with root knot nematode.*

Keywords: *Lantana camara; root-knot nematodes; biological control*

INTRODUCTION

Root knot nematode is one of the most important economic pests in the world and causes global economic losses estimated at $118 billion (1). Losses in tomato crops in tropical and subtropical regions are estimated at 30-50% (2). The damage caused by this pest is not limited to a specific

crop or family, but rather includes a wide range of plant families, including fruit and vegetable trees (3). indicated that the total losses of grape seedlings in the world due to these caecilians range from (12.5-20)%. The losses caused by nematodes to vegetable crops annually were estimated at 10%, and 50% of these losses are due to infection with the genus *Meloidogyne*. This indicates the economic importance of this pest and the necessity of combating it by all available means (4). Although the use of chemical pesticides that kill nematodes is effective and gives good and quick results in addition to their ease of application, in recent times their use has become unfavorable and they have begun to be removed from the markets in some developed countries due to the many side effects on human health and environmental safety. Therefore, specialists in the field of pest control have taken They are thinking about alternative methods that are safer and safer for human health (5, 6).so it has become necessary to develop alternative, environmentally friendly, safe and effective methods and strategies for combating nematodes, and among these methods is biological control used to control pests. In various parts of the world (7, 8).However, the use of plant extracts as a soil amendment or agricultural by-product that provides nematode control in the agricultural system as an alternative to synthetic chemical nematicides has increased worldwide according to (9). Many plant-based compounds with anti-nematicidal activity were evaluated and found to be effective, inexpensive, and environmentally safe (10). Hence the search for acceptable alternative management strategies using plant materials as organic nematicides, reported (11). The aqueous extract of plant leaves is a rich source of terpenoids, steroids, and alkaloids, as the myna leaf extract showed antimicrobial activity and insecticidal and nematicidal activities (12).

MATERIALS AND METHODS

Samples Collection

25 random samples of soil and infected roots with root-knot nematode were collected from fields and greenhouses of nightshade and cucurbit family plants from eight agricultural sites in Wasit Governorate (Kut Center, Al-Ahrar, Al-Hay, Al-Numaniyah, Badra, Al-Kardhiyah, Sheikh Saad, Al-Dujaili) after observing the symptoms appearing on the group. Vegetative withering and yellowing of plant leaves in the autumn season for the period from 5/11/2022 to 22/12/2022, as 3-5 cm of the upper layer of soil was removed and about 1 kg of soil and 10 g of infected roots were collected at a depth of 20-30 cm (13).where the samples were taken randomly for each location in the selected fields and placed in plastic bags (polyethylene) moistened with a small amount of water with the information of each sample written on them and brought to the Mycology Laboratory at Wasit University/College of Science/Department of Life Sciences. The samples were preserved. In the refrigerator at a temperature of 5°C (14, 15).Within a period not exceeding three days until the necessary tests were carried out on them, some of them were transferred to the Integrated Control Center/Agricultural Research Department/Biological Control Department/Ministry of Science and Technology in Baghdad.

Preparing the inoculum of the *Meloidogyne javanica*

M. javanica juveniles were isolated from the roots of infected tomato plants, which were obtained from greenhouses in the village of Al-Bayda in Wasit Governorate, according to the method described by (16).which consists of placing 300 grams of infected roots in an electric blender and adding a sodium hypochlorate solution. NaOCl at a concentration of 1% for 30 seconds. Then the mixture was passed through a series of sieves (50, 75, 125 micrometers), and the larvae were collected from the 50-micrometer sieve. The larvae were washed with distilled water to get rid of the NaOCl solution. The larvae were collected from the inner edge of the sieve and then collected in a glass beaker. The number of larvae per ml of root knot nematode larval suspension was calculated using a nematode counting slide by filling the slide chamber with 1 ml of larval suspension and examined under compound microscope with 10X magnification. The average number of larvae in (10) squares was calculated and the result was multiplied by the slide constant. 125 to find the average number of larvae in 1 ml and adjust the concentration to 200 larvae/ml.

Preparing *Lantana camara* extraction

Lantana camara plants were obtained from the public gardens of Wasit Governorate for the purpose of using their extracts in this study. It is preferable to collect the plants at the beginning of the flowering season, as this is the stage of storing secondary compounds with physiological activity that is important for the plants themselves and for other organisms (17). Botanical samples were collected from the leaves of the plant *Lantana camara* and weighed after collection. They were cleaned and washed well to remove any dust stuck to them, After that, they were placed at room temperature (25±2) on plastic trays. in order to dry them, constantly swirling to allow air to circulate and guarantee proper drying. Then they were weighed after five days, every 24 hours, until the weight was stable for a period of four days. After they were completely dried, the samples were crushed and ground using an electric grinder to obtain the powdered leaves. They were then placed inside a nylon bag, marked with a piece of paper indicating the place and time of collection, the name of the plant, and the dried plant part. After that, they were maintained in a dry environment until the extraction procedure was completed (18).

Aqueous extract

In a 1000 ml beaker, 100 g of dry powdered Lantana camara leaves were combined with 400 ml of distilled water to create an aqueous extraction, which was then let to sit at room temperature for 24 hours. then filter the mixture using several layers of medical gauze to get rid of plankton. Then it is expelled. Centrally at a speed of (3000) rpm for 10 minutes, then filter the extract using 0.1 Whatman No. filter papers. To obtain a clear solution, dry the extract using the oven at a temperature of 37°C, then store it in the refrigerator at a temperature of 4°C. Until use (19, 20).

Alcoholic extract

To create the alcoholic extract, 100 g of dry powdered Lantana camara leaves were placed in 1000 ml beakers, and 500 ml of ethyl 70% alcohol was added. then letting it sit at room temperature for a full day. After that, the mixture was sedimented for 15 minutes at 3000 revolutions per minute using a centrifuge. and filtered using Whatman No.1 filter paper. The solution was evaporated with a rotary vacuum evaporator at a temperature of 40°C until a thick solution was obtained. It was dried in the incubator at a temperature of 37°C for (3-4) days and the resulting powder was stored. until used, keep in the refrigerator at 4°C. (21).The stock solution (20,000) ppm was prepared by dissolving (0.5) g of the aqueous and alcoholic *Lantana camara* leaf powder separately in (25) ml of water, and a series of concentrations required for the study were prepared, namely (1000, 1200, 1400, 1600, 1800, 2000) parts per million.

The effect of *Lantana camara* extract on nematode

The effect of the alcoholic and aqueous extract of the *Lantana camara* plant on the larvae of the nematode *M. javanica* was tested by preparing several concentrations (1000, 1200, 1400, 1600, 1800, 2000) ppm, where 5 ml of each concentration of the extract was added in petri dishes with a diameter of 5 A container containing (1.5) ml of nematode suspension (300) larvae. The dishes were incubated at a temperature of (25±2)°C. Three replicates were allocated for each treatment according to a completely randomized design. The percentage of larval deaths was calculated under a dissecting microscope after 24 hours and 48 hours (22, 23). and the immobile larvae were considered dead. While the larvae that did not die are mobile and active.

RESULTS AND DISCUSSION

The effect of *Lantana camara* leaf extract on nematode larvae

The results of aqueous and alcoholic *Lantana camara* leaf extracts effects on the vitality of the second larval stage of the root knot nematode *M. javanica* (Table 1) showed that both aqueous and alcoholic extracts, with all their concentrations used in the experiment, affected the vitality of the larvae and reached the large killing rate that was significantly superior to the comparison concentrations. The results also showed that the death percentage of larvae treated with the aqueous extract and the alcoholic extract was 47.09% and 65.42%, respectively, after 24 hours of treatment. The alcoholic extract was significantly superior to the aqueous extract in terms of effect, while the average percentage of death of larvae treated with the aqueous extract and the alcoholic extract was Alcoholic 65.42% and 68.11% respectively after 48 hours of treatment and without significant differences between the two extracts in terms of effect. As for the concentrations of the extract, the concentration exceeded 2000 ppm, recording the highest inhibition rate of 86.88% after 24 hours, with a significant difference from all the lower concentrations, while the two concentrations (2000 and 1800) ppm recorded the highest rate of inhibition after 48 hours, amounting to 95.25% and 96.8%,

respectively, and without a difference. Significant between them, However, after (24 and 48) hours, the concentration of 1000 ppm showed the lowest inhibition rate, at 40.91% and 52.35%, respectively. The results indicate that the effect on the larvae is proportional to the concentration used, as the effect or percentage of larval deaths increases with increasing concentration, as the percentage of larval deaths at a concentration of 1000 ppm was 30% after 24 hours of treatment, and it began to increase with increasing concentration until it reached 77.79% at 2000 parts per million and, in the case of the aqueous extract, after the same amount of time. The percentage of larval deaths at the concentration of 1000 ppm was 51.82% after 24 hours of treatment, and it began to increase with increasing concentration until it reached 95.96% at a density of 2000 parts per million and after the same time period in the case of Alcoholic extract. The results also showed that the percentage of larvae killed at a concentration of 1000 ppm was 52.38% after 48 hours of treatment, and it began to increase with increasing concentration until it reached 96.76% at a concentration of 2000 parts per million and, in the case of the aqueous extract, following the same time frame. The percentage of larvae killed was at concentration of 1000 ppm, it was 52.94% after 48 hours of treatment, and it began to increase with increasing concentration until it reached 97.56% at a concentration of 2000 parts per million and, in the case of the alcoholic extract, following the same time frame.

Table 1. The effect of *Lantana camara* leaf extract on the percentage of mortality of *M. Javanica* larvae.

The average	Larvae die after 48 hours		The average	Larvae die after 24 hours		Concentrations (ppm)
	Extract type			Extract type		
	Alcoholic	Aqueous		Alcoholic	Aqueous	
0	0	0	0	0	0	Control
52.35	52.94	52.38	40.91	51.82	30	1000
65.9	66.61	65.93	52.71	65.24	40.17	1200
72.45	78.31	76.45	64.41	74.59	54.23	1400
83.6	85.5	83.59	71.41	81.67	61.14	1600
92.25	95.84	92.27	77.48	88.69	66.27	1800
96.8	97.56	96.76	86.88	95.96	77.79	2000

6.791	N.S		7.483	10.582	L.S.D 0.05
	68.11	65.42		65.42 47.09	The average
	N.S			4	L.S.D 0.05

* NS means no significant difference

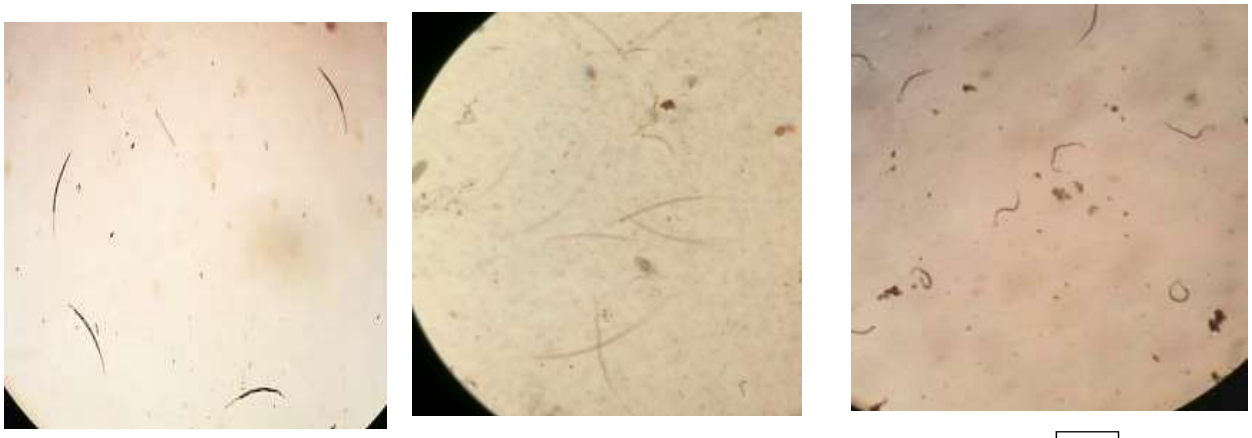

Fig. 1. The second larval stage of the nematode *M. javanica*.

Treatment of alcoholic extract =A Treatment of aqueous extract =B Control Treatment= C

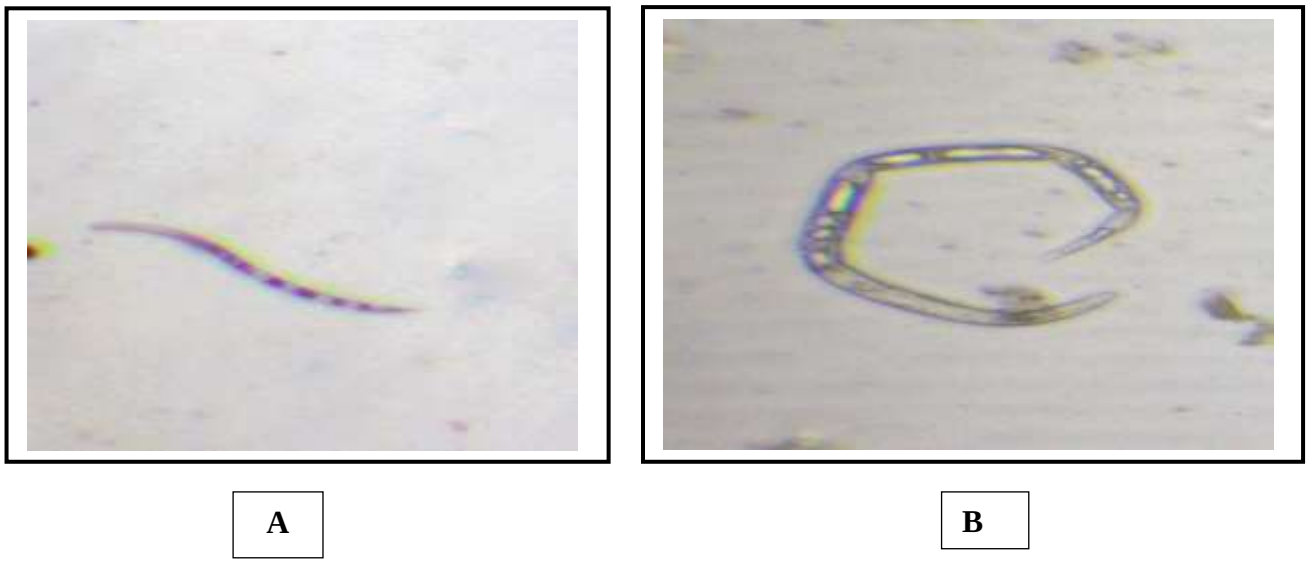

Fig. 2. The second larval stage of the nematode *M. javanica*.
Active larvae = A Dead larvae = B

It is clear from these results that the effect increases with increasing concentration of the *Lantana camara* leaf extract, and this may be due to an increase in the amount of the active substance present in the extract, which then leads to an increase in the killing rate of larvae (25).This scientific fact has been proven in many studies, as indicated by (26).The percentage of death of nematode larvae *M. javanica* treated with *Moringa oleifera* leaf extract increases with increasing concentration. The highest percentage of killing of larvae was recorded at a concentration of 100%, which was 30%, and the lowest percentage of killing was recorded at a concentration of 25%, which was 16%. It was also mentioned that the percentage of killing of eggs was also It increased with increasing concentration. The results were consistent with what was indicated by (27).Which suggested that the extract of the roots of the *Tagetes lucida* plant achieved good results in inhibiting the hatching of eggs of the nematode *M. incognita*, and that the inhibition increased with an increase in the concentration of the extract, as the 100% concentration recorded an inhibition rate of 86.6%, while the 50% concentration recorded a rate of 74.8%. A similar study in which (28). indicated that the percentage of killing larvae of the root knot nematode *Meloidogyne incognita* varies depending on the type of extract of the *Tagetes erecta* plant, as the alcoholic (ethanolic) extract has a higher killing percentage than the aqueous extract. It also indicated that the percentage of killing of larvae increases with increasing concentration of the *Tagetes erecta* plant's alcoholic and aqueous extracts. The results were also consistent with the findings of (29).It was reported that the alcoholic extract showed superiority over the aqueous extract of the *Tagetes patula* plant in the percentage of killing nematode larvae *Ditylenchus dipsaci* after 24 and 48 hours of incubation. Another similar study indicated that the population density of the root knot nematode *M. incognita* was reduced to a greater extent in soil treated with *Tagetes minuta* leaf extract at a concentration of 5% compared to soil treated with a concentration of 3%. It also indicated that tomato production increased with an increase in the concentration used of leaf extract. (30). The results also indicated that the alcoholic extract had a superior effect on the aqueous extract after 24 hours of treatment, while no significant difference appeared between them in terms of the effect after 48 hours of treatment. This may be attributed to a larger amount of active substances present in the alcoholic extract that caused the effect. While the long duration of the effect in the aqueous extract gave an increase in the killing rate, this may be due to the components of the extract differing depending on the type of solvent used in the extraction, as mentioned by (31). *Lantana camara* leaf extract differs in its killing effectiveness for nematodes. This is due to the type of solvent used in preparing the type of extract. These differences are due to the difference in the chemical nature, composition and concentration of the toxic compounds. *Lantana* species contain biocidal compounds, and these compounds belong to the Thiophene group as non-polar products. It is a substance of secondary metabolism (32, 33). Some biochemical studies have indicated that the components of the *Lantana camara* leaf extract differ depending on the type of solvent used for extraction, as indicated by (34). The acetone extract and ethanol extract contain a very high percentage of the flavonoid compound compared to the aqueous extract,

which had a very low percentage of the compound.

CONCLUSION

This study indicates that the aqueous and alcoholic extract of *Lantana camara* leaves has significant effects against *Meloidogyne javanica* and that it can be successfully used as a biopesticide.

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