

## Eco-Friendly Management of Root-knot Nematode, *Meloidogyne javanica* in Okra (*Abelmoschus esculentus*) crop

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### ABSTRACT

The need for alternatives to nematicides has stimulated research focusing on sustainable and eco-friendly management of plant parasitic nematodes. All the concentrations of the aqueous extracts from fresh leaves of *Datura stramonium* (20% w/v) caused the mortality of second stage juveniles of *Meloidogyne javanica* whereas the more mortality occurred in 1:5 concentration and the least in 1:80 concentration. Juvenile mortality decreased with an increase in the dilution of all the extracts. As an increase in exposure time, juvenile mortality was also increased. Potted soil application of *Trichoderma viride* @ 20 g/kg of soil was significantly effective against *M. javanica* and to improve growth parameters of okra. This study also revealed that *Azadirachta indica* leaves @ 30 g/kg of soil in pots when incorporated, improves the plant growth and reduced the *M. javanica* infestation in okra. Moreover, *D. stramonium*, *A. indica* and *T. viride* can be used as an alternative in eco-friendly management of root-knot nematode.

**Key words:** *Meloidogyne javanica*, *Trichoderma viride*, *Datura stramonium*, *Azadirachta indica*, Eco-friendly management

### INTRODUCTION

Global crop loss caused by plant parasitic nematodes is more than \$100 billion annually and in India, the annual estimated crop losses due to major plant parasitic nematodes have been worked out to be about Rs. 242.1 billion<sup>4</sup>. Root-knot nematode, *Meloidogyne* spp., are the major plant parasitic nematodes attacking most of the crops. Nematicides are very much effective but their unsafe use may result in acute poisoning and deaths are a problem especially in developing countries. The process of de-registration of some hazardous

nematicides, increases the pressure on farmers to use non-chemical pest control methods that do not pollute the environment. The emphasis on use of botanical extracts for controlling root-knot nematode (RKN) is becoming appealing because of the growing problem of environmental pollution arising from the use of persistent pesticides.

Okra, popularly known as Bhindi or lady's finger is an important vegetable crop grown in tropical and sub tropical regions. It is rich in vitamins, minerals, proteins and carbohydrates.

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Looking to the importance of vegetables and their demand supply gap, high priority is being accorded to vegetable production in the county. Besides this, production of vegetables is still very low due to number of biotic and abiotic factors including losses caused by phytonematodes. So, biological control is an environmental friendly and economically viable alternative as it offers durable and cost effective alternatives to soil applied nematicides. The nemato-toxicity of leaf extracts of different plants have been reported by many workers<sup>2,13</sup>. *Datura stramonium* also known by its common names ‘Jimson weed’, ‘devils snare’ and ‘datura’. All parts of this plant contain dangerous levels of the tropane alkaloids atropine, hyoscyamine and scopolamine, which are classified as delirients or anticholinergins<sup>9</sup>.

*Trichoderma* is a very effective biological mean for plant disease management especially the soil borne. It is a free-living fungus which is common in soil and root ecosystems. It is highly interactive in root, soil and foliar environments. It reduces growth, survival or infections caused by pathogens by different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions, and enzyme secretion. Among this biocontrol agent, *Trichoderma viride* had been more effective in controlling RKNs<sup>12</sup>.

On the other hands, biocides or botanical pesticides are plants that contain natural substances, which exhibit pesticidal properties. Biocides are receiving increasing attention because of their environmental friendliness and compatibility with other types of pesticides. The addition of chopped neem leaves to soil for the control of plant parasitic nematodes has been extensively documented<sup>8</sup>. The nematicidal activity of neem could be due to any of the several known biologically active principles it contains, such as nimbine, nimbidine, thionemone, kemferol, azadirachtine, etc., which are nematicidal in nature<sup>5</sup>. The present study is undertaken on the use of botanicals (chopped neem leaves) as organic soil amendments and plant extracts (*D. stramonium*) in the control of RKN and reported efficacy of soil microflora (*T. viride*) against RKN.

## MATERIAL AND METHODS

**Extraction of inoculum:** - The culture of *M. javanica* was maintained on okra plants in infested soil pots inside the screen house. Infected plants were uprooted, carefully washed in running tap water, egg- masses and J<sub>2</sub> larvae were collected in to Petri dishes containing distilled water for experimental studies.

**In vitro experiment:** - 40 gram (g) of fresh leaves from *D. stramonium* was separately mixed with 200 ml distilled water in an electric blender for 3 minutes. The mixture was allowed to stand for 2 hrs and filtered through a Whatman filter paper. The filtrate was considered as standard and assayed against juveniles. The experiment was carried out in sterilized Petri dishes. The obtained filtrate was designated as crude extract and further diluted with 5, 10, 20, 40 and 80 ml distilled water before using it for the juvenile mortality test. The Petri dishes with distilled water was taken as control. For studying the juvenile mortality, 100 J<sub>2</sub> of *M. javanica* were suspended in 10 ml of different extracts. Dead nematodes were counted every 24 hours for seven days and recorded as percentage.

**In vivo study:** The *T. viride* culture was obtained from the Department of Nematology, CCS HAU Hisar. The fungus culture was inoculated on Potato Dextrose Agar (PDA) slants and incubated at 25±2°C for 7-10 days. The fungus growing on the medium was isolated and identified using morphological characters of the mycelia and spores. The identified culture was incubated at 25±2°C for 10 days and used for experimental studies. *T. viride* culture added to the sterilized soil and then okra seeds were sown after one week. After germination, seedlings were thinned to one per pot and then inoculated with 500 second stage juveniles of *M. javanica*. The experiments were terminated 40 days after sowing and observations were made on plant growth parameters viz., shoot length, root length, shoot and root weight, number of galls, number of egg mass and final nematode population.

In another experiment, chopped leaves of neem (*Azadirachta indica*) @ 10, 20, 30 and 40 g/pot were incorporated into the soil as

per treatments. To ensure decomposition of the amendments, the pots were irrigated and one week later okra seeds were sown. After germination, seedlings were inoculated with 1000 second stage juveniles of *M. javanica*. The experiment was terminated 40 days after sowing. Then plants were uprooted, washed gently in tap water and plant growth parameters, number of galls per plant and final nematode population in soil were recorded. All the experiments were made triplicate and conducted in a completely randomized block design.

#### Data Analysis

The data obtained in the *D. stramonium* experiment was analysed statistically using sine arc transformation. The ratio of dead nematodes/number of total nematodes expressed the percentage mortality as follows:

$$\text{Percent mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae}} \times 100$$

In *T. viride* and neem leaves experiments, the data obtained was analyzed statistically by a one factor analysis of variance (ANOVA).

## RESULTS AND DISCUSSION

### Effect of *Datura stramonium* leaf extract on juvenile mortality of root-knot nematode, *Meloidogyne javanica*

Figure 1 shows the effect of different levels of concentrations of *D. stramonium* on juvenile mortality of *M. javanica*. The result shows that the 1:5 concentration recorded the highest juvenile mortality (97 %), followed by 1:10 concentration (92 %) and lowest mortality was recorded in the 1:80 concentration and control. Significant increase in mortality as compared to control was also observed at other concentrations though it was comparatively less than the 1:5 and 1:10 concentration. The present study also reveals that juvenile mortality increased with increase in concentration of the extract. The results are in agreement with Hasabo and Noweer<sup>3</sup> who found that mortality effect of an extract on nematode is concentration dependent. The effect of the different levels of the extract on juvenile mortality could be due to the presence of tannins, alkaloids and flavonoids which have been reported to killed nematodes<sup>1,15</sup>. The control recorded 0 % mortality upto 72 hours because it was only distilled water<sup>14</sup>.

**Table 1: Effect of leaf extract on larval mortality and exposure time (days) on *Meloidogyne javanica***

Exposure time (days)	Mean Juvenile Mortality (%) at different concentration					
	1:5	1:10	1:20	1:40	1:80	Control
1 day	56.78(48.81)	45.87(42.49)	25.83(30.42)	7.23(15.69)	0.00(1.00)	0.00(1.00)
2 days	67.81(55.73)	54.33(47.46)	37.77(37.84)	13.19(21.11)	0.00(1.00)	0.00(1.00)
3 days	75.92(60.19)	61.12(51.53)	45.67(42.49)	20.21(26.79)	0.47(1.91)	0.00(1.00)
4 days	81.17(64.37)	73.37(58.88)	52.34(46.31)	26.71(31.07)	2.59(9.35)	0.36(1.91)
5 days	87.60(69.41)	80.34(63.65)	59.54(49.97)	31.59(34.23)	5.25(13.33)	1.17(6.53)
6 days	93.33(75.01)	88.67(70.30)	63.37(52.71)	35.44(36.45)	6.98(15.33)	3.76(10.49)
7 days	97.01(80.08)	91.89(73.19)	69.78(56.55)	41.13(39.99)	7.87(15.69)	5.65(13.75)

\*Figures in parentheses are arc sine transformed values

#### C.D. (5%)

Concentration	: 0.61
Days	: 0.57
Interaction (Concentration x Days)	: 1.49

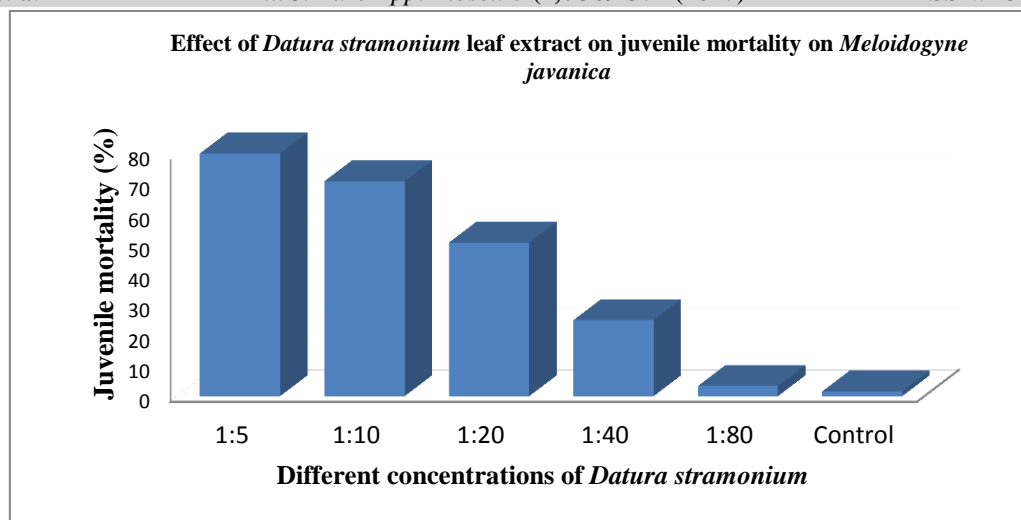


Fig. 1: Effect of *Datura stramonium* leaf extract on juvenile mortality on *Meloidogyne javanica*

In the juvenile mortality assay, *D. stramonium* leaf extract 1:5 concentration was found to be most toxic to the juveniles (Table 1). The results show that the plant has got some nematicidal potential against root-knot nematodes. It may be more effective for the management of root knot nematodes using the leaf extract of *D. stramonium*. Similar findings were reported by Muhammad *et al.*<sup>6</sup>, who founded that the leaves of *D. stramonium* was the most effective in the management of root-knot nematode. After one day of exposure of juveniles to different dilutions, maximum mortality was observed at 5 and 10 ml concentration. As the exposure period increased, juvenile mortality also increased. This observation corroborates the findings of Nimbalkar and Rajurkar<sup>7</sup> who observed that increase in exposure time increases juvenile mortality.

#### Effect of different treatments of biocontrol agent *T. viride* on *M. javanica* in okra

The data revealed that soil application with *T. viride* @ 15 and 20 g/kg of soil significantly reducing nematode population and increase the plant growth parameters. The shoot length and root length was observed significantly maximum and shoot weight and root weight were maximum at these doses over the treatments (Table 2). Increasing the plant growth parameters could be due to fungus colonizes near plant roots and grows on roots, provide a physical barrier for nematodes to contact, and also enhance the plant's root growth and nutrient absorption<sup>16</sup>. The significant reduction in number of galls and number of egg mass per plant was recorded in T3 and T4 among other treatments. The results are familiar with Sharon *et al.*<sup>12</sup> who suggests that reduction in nematode population, number of galls and number of egg mass per plant may be due to the increase in chitinase and protease activity and nematode egg parasitisation.

Table 2: Effect of biocontrol agent *T. viride* on *M. javanica* and okra growth parameters

Treatments	Shoot length	Shoot weight	Root length	Root weight	Number of galls	Number of egg mass /plant	Final population
T1 (5g/kg)	8.82	1.87	11.83	0.53	16.67	11.67	391.67 (19.8)
T2 (10g/kg)	12.43	2.03	13.50	0.73	13.67	8.67	346.67 (18.6)
T3 (15g/kg)	13.87	2.33	14.70	0.87	10.33	4.67	258.33 (16.1)
T4 (20g/kg)	16.53	3.13	16.40	1.10	9.67	4.33	223.33 (14.9)
T5 (nematode alone)	8.10	1.07	8.47	0.27	21.33	15.33	463.33 (21.5)
T6 (check)	12.17	2.13	13.03	0.33	-	-	-
C.D. (5%)	2.22	1.05	-	0.24	5.32	3.75	(1.8)

\*Figures in parentheses are  $\sqrt{n}$  transformed values

### Management of root-knot nematode, *Meloidogyne javanica* on okra by soil amendment with different doses of neem leaves.

Persual of data presented in Table 3 revealed that significant increase in plant growth parameters was observed when neem leaves were applied @ 30 and 40 g/kg soil as compared to other doses. The chopped neem leaves improved plant growth parameters of okra over control. Plant growth parameters of okra were improved significantly by all the different doses. These results are in combination with Ramkrishnan *et al.*<sup>10</sup>, who reported increase in plant growth characters through botanicals as soil amendments.

The addition of these organic amendments (chopped leaves) was very effective in reducing number of galls (Table 1). The highest reduction in number of galls/plant and nematode population was noted in T3 and T4, whereas the lowest reduction was observed in T1. Reduction in nematode population density by application of chopped plant leaves might have resulted from a toxic effect on the nematodes<sup>5</sup>. The present findings are in collaboration with the conclusions of Rao and Reddy<sup>4</sup> who also observed reduced root-knot nematode population as effect of soil amendments.

**Table 3: Effect of different doses of neem leaves on *Meloidogyne javanica* and okra plant growth**

Treatments	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Number of galls/plant	Final nematode population in soil (J <sub>2</sub> )/kg soil
T1 (10 g/kg soil)	15.60	6.60	1.67	50.67	750.00 (27.26)
T2 (20 g/kg soil)	17.43	8.20	1.97	40.00	526.67 (22.93)
T3 (30 g/kg soil)	21.37	10.70	2.80	26.67	456.67 (21.36)
T4 (40 g/kg soil)	24.53	12.53	3.50	21.67	323.33 (18.99)
T5 (Control)	13.63	4.10	1.33	65.67	893.33 (29.90)
C.D. (5%)	2.35	1.89	0.99	16.13	(3.59)

\*Figures in parentheses are  $\sqrt{n}$  transformed values

### CONCLUSIONS

These findings provides valuable data on wasteland plants provide a wide support for nematicidal activity and suggest that the addition of botanicals to soil has potential for development as novel nematicides for the control of the root-knot nematodes. *T. viride* incorporated to soil can be recommended as an alternative to nematicides. This knowledge will help in better developing the use of organic material in the control of plant-parasitic nematodes so that this promising idea would not be aborted on flimsy grounds. The idea should be given time to incubate and bring forth an attractive solution. It could even be a better and effective alternative to synthetic nematicides on the long run. The employment of organic materials as extracts

look promising as a model biological alternative to synthetic nematicides if properly developed so that would be cheap for the resource-poor subsistence farmer. However, more field trials need to be carried out on these materials to test their efficacy under natural conditions.

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