

CONTROL OF *Meloidogyne incognita* INFESTATION IN TOMATO BY *Rauvolfia tetraphylla***TAPAN KUMAR MANDAL^a AND B. NANDI^{b1}**^{a,b}Department of Zoology, Malda College, Malda, West Bengal, India**ABSTRACT**

Ethanol extract of root, leaf and fruit of *Rauvolfia tetraphylla* were assayed against *Meloidogyne incognita* juveniles (J_2). The juveniles were exposed in root, leaf and fruit extract at 2 mg/ml concentration for 6 hours in the laboratory. All the three extracts of *Rauvolfia tetraphylla* exhibited highly promising mortality (60-95%) after 6 hours exposure. Ethanol extract of root and fruit, dissolved in distilled water when applied by foliar spray at the concentration of 2mg/ml on tomato, inoculated with *Meloidogyne incognita* juveniles (J_2), reduced nematode infestation and promoted plant growth. Root and fruit extract treatment on tomato has increased significant resistance to *Meloidogyne incognita* infestation in terms of reduced root galling and increased biomass. Leaf extract though caused 60% mortality of *Meloidogyne incognita* juveniles in *in vitro* test had no significance effect in controlling *Meloidogyne incognita* infestation in tomato plant.

KEYWORDS : *Rauvolfia tetraphylla*, *Meloidogyne incognita*, Tomato, Phytochemical

The fast growing population in our country has necessitated increased food production and this in turn has put tremendous pressure on our environment. Nematodes comprise a large phylum of animals that includes plant and animal parasitic nematodes as well as many free living species. Plant parasitic nematodes are obligate parasites, obtaining nutrition only from the cytoplasm of living plant cells. *Meloidogyne incognita* is a major plant parasitic nematodes, is extremely polyphagous and attacks both monocotyledons and dicotyledons and thereby affecting quality and quantity of the crop production in many annual and perennial crops. Infected plants shows typical symptoms including root galling, stunting and nutrient deficiency, particularly nitrogen deficiency. (Davis and May, 2005) reported that the yield loss of cotton production caused by *Meloidogyne incognita* in 2002 was estimated to be between 18.0-47.3%. Root-knot nematodes (*Meloidogyne*) are among the most destructive nematodes in agriculture, causing an estimated yearly crop loss of \$ 100 billion worldwide (Oka et al., 2000). Nematodes are difficult to control because of their wide host range and high rate of reproduction, with females capable of producing up to thousand eggs. At present, the major control method of nematode is based on the use of chemical nematicides. Chemical nematicides, though effective in reducing root-knot nematode infection are not always cost effective and is economically viable only for high value crop. Chemical nematicides are often phytotoxic, cause environmental pollution endangering the life of many animals including fish (Landu and Tucker, 1984) and even contaminate

ground water and leave undesirable residues in edible parts of plants (Lue et al., 1984). Because of these inconveniences scientists identified natural products with nematicidal activity such as root exudates, plant volatile compounds (Linford et al., 1938), endophytic bacteria (Vetrivelkalai et al., 2010) and plant extracts (Pavaraj et al., 2010). A wide variety of plant species, representing 57 families have been shown to nematicidal activity (Sukul, 1992). A few nematicidal compounds from the plants of compositae family were isolated and identified by (Gommers, 1973). Nematicidal phytochemicals are generally safe for the environment (Chitwood, 2002). These compounds include repellents, attractants, hatching stimulants or inhibitors and nematotoxins, either constitutive or formed in response to nematode presence (Chitwood, 2002). The aim of the present study was to evaluate the nematicidal activity of *Rauvolfia tetraphylla* against root-knot nematode *Meloidogyne incognita*.

MATERIALS AND METHODS**Preparation of Plant Extract**

Leaves, fruits and roots of *Rauvolfia tetraphylla* L were collected, air dried, ground and soaked separately in ethanol for one week at room temperature. After one week, ethanol extracts were filtered through a cotton plug and then the ethanol was allowed to evaporate and the residues were dried in a dessicator over anhydrous calcium chloride. The residues obtained were termed the crude extracts. These crude extracts were used to study the nematicidal activity.

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In Vitro Test

Crude extract of leaves, fruits and roots were dissolved in distilled water at 2 mg/ml concentration. Egg masses of *Meloidogyne incognita* were collected from tomato (*Lycopersicon esculentum* L) and kept in distilled water for hatching. Active *Meloidogyne incognita* juveniles (J_2), obtained from the egg masses, were kept in sterile distilled water in cavity blocks, each containing $100 \pm 10 J_2$. Water was pipette out and immediately replaced by 2 ml of test solution. One cavity block containing sterile tap water served as the control. Observation was made at room temperature. ($30 \pm 2^\circ\text{C}$) every hour for 6 hours.

Pot Test

Aseptically germinated seeds of tomato (*Lycopersicon esculentum* L. Pusa, Ruby) were sown one seed/pot (32 cm diameter) containing a mixture of clay soil and composted manure (2:1 v/v). The soil filled pots were treated with boiling water thrice to denematized the soil. The pots were divided into five groups, each of ten pots. The groups were uninoculated untreated, inoculated untreated, inoculated and treated with root extract, inoculated and treated with fruit extract and inoculated and treated with leaf extract. When the plants were at 6-leaf stage, the inoculated untreated, inoculated and treated with root extract,

inoculated and treated with fruit extract and inoculated and treated with leaf extract groups were inoculated with *Meloidogyne incognita* juveniles (J_2) at the rate of 2000 ± 100 juveniles/plant. Crude extracts were dissolved in distilled water at the concentration of 2 mg/ml and applied by foliar spray 2 days after inoculation. Treatment was repeated 4 days after the first treatment. The experiment was conducted outdoor at ambient atmospheric temperature $35 \pm 20^\circ\text{C}$ and humidity $85 \pm 5\%$. All the plants were uprooted 40 days after inoculation and their shoot lengths, shoot weights, root lengths and root weights were measured. The root galls on each plant were counted and the nematode population in 2g root and 200 g soil was estimated by the modified Baerman method. Three samples of roots from each group of plants were taken at random and total protein concentration in each sample was estimated by Lowry's method (Lowry et al., 1951). Data were analysed by ANOVA.

RESULTS AND DISCUSSION**In vitro Test**

95 % mortality of nematodes were recorded in root extract, whereas 82% and 60 % mortality of nematodes were recorded in fruit extract and leaf extract respectively

Table 1: Increase in Growth and Decrease of *Meloidogyne incognita* Infestation in Tomato Following Treatment by Ethanolic Root and Fruit Extract of *Rauvolfia tetraphylla*

Treatments*	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Root gall no.	$J_2/2\text{g root}$	$J_2/200\text{g soil}$	Root protein (mg/g)
Uninoculated untreated	60.8 ± 1.3 a	204.4 ± 8.4 a	36.6 ± 1.6 a	23.0 ± 1.2 d	-	-	-	3.4 ± 0.28 c
Inoculated untreated	45.6 ± 1.2 c	152.8 ± 5.6 c	24.7 ± 1.8 c	33.9 ± 1.7 a	262.0 ± 14.2 a	178.4 ± 12.5 a	248.7 ± 12.4 a	7.6 ± 0.36 a
Inoculated and treated with root extract	61.3 ± 1.4 a	205.3 ± 9.2 a	35.8 ± 1.9 a	25.7 ± 1.5 cd	114.2 ± 6.5 c	96.2 ± 8.4 c	126.3 ± 6.2 c	4.2 ± 0.24 c
Inoculated and treated with fruit extract	52.5 ± 1.4 b	166.8 ± 7.5 b	29.6 ± 1.5 b	28.1 ± 1.8 bc	208.3 ± 12.4 b	122.5 ± 10.2 b	192.1 ± 9.8 b	5.8 ± 0.26 b
Inoculated and treated with leaf extract	48.1 ± 1.2 bc	159.9 ± 6.4 bc	27.1 ± 1.3 bc	30.5 ± 1.4 ab	251.4 ± 15.6 a	170.6 ± 13.2 a	237.6 ± 12.8 a	6.9 ± 0.33 ab

*values are means of 10 plants with SE. a,b,c,d = different small letters in a column indicate significant difference by ANOVA at 5 % level. Dashes (-) indicate no nematodes in treatment.

after 6 hours of exposure.

Pot Test

Root and fruit extract produced increased plant growth in terms of root length, shoot weight and root length as compared to the inoculated untreated and inoculated and treated with leaf extract groups (Table,1). Though root and fruit extract both produced increased plant growth, root extract is significantly more effective than fruit extract. Root weight in inoculated untreated group as well as leaf extract treated groups were significantly increased with respect of un inoculated untreated group, root extract treated group and fruit extract treated group. This is due to the production of higher number of root galls in these two groups. Root gall number, J_2 in root and soil were significantly reduced in root and fruit extract treated groups as compared to those in the inoculated untreated and inoculated and treated with leaf extract groups. Root protein content was significantly reduced in un inoculated untreated, root extract treated and fruit extract treated groups as compared to the inoculated untreated and leaf extract treated groups. (Table,1)

The use of botanical extracts for controlling *Meloidogyne* is appealing because of the growing problem of environmental pollution arising from the use of persistent pesticidized like chlorinated hydrocarbons such as chloropicrin. Extracts of many plants with antihelminthic and antimicrobial properties have been proven effective in controlling plant parasitic nematodes (Ferris and Zheng, 1999). Many plant species produce different allelochemicals which have tremendous nematicidal potential (Sukul, 1992). Efficacy of various plant extracts in nematode control has been established. Water extracts of Indian plants, *Fleurya interrupta*, *Peritrophe bicalyculata* and *Andrographis paniculata* were nematicidal and resulted in 100% mortality of root-knot larvae within 40 minutes (Mukherjee and Sukul, 1978). Nematicidal properties of *Embllica officinalis* and *Carrissa curandas* against root-knot larvae have been reported (Haseeb et al., 1980). Nematicidal properties of ten plant species were established by (Pavaraj et al., 2012). The compounds occurring in the plants with nematicidal activity comprise a wide variety of phytochemicals, eg. Polythienyls, acetylenes, alkaloids,

fatty acids and derivatives, phenolics, terpenoids (Chitwood, 1992). In our study we observed that ethanolic root and fruit extracts of *Rauvolfia tetraphylla* reduce *Meloidogyne incognita* infestation in tomato plant. Ethanolic root and fruit extracts of *Rauvolfia tetraphylla* may contain phytochemical which is responsible for controlling root knot nematode. Further research in this area is necessary to establish the specific plant component responsible for the nematicidal activity.

ACKNOWLEDGEMENTS

The authors are thankful to Professor Nirmal Chandra Sukul of Department of Zoology, Visva Bharati University for his kind suggestion to perform this research work.

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