



Effect of leaf extract of *Ageratum conyzoides* on the biochemical profile of blackgram *Vigna mungo* infected by root-knot nematode, *Meloidogyne incognita*

M. Pavaraj, K. Karthikairaj and M. K. Rajan

ABSTRACT

Meloidogyne incognita is highly pathogenic to crops causing severe damage and reduction in yield. During parasitism, nematodes exert detrimental influence on the normal physiology, growth and development of host plant and these conditions have been attributed to the effect of either direct or indirect response of the host to mechanical or biochemical activities of the nematode. Hence the present study has been made to evaluate the efficacy of a bio-nematicide, the leaf extract of goat weed plant, *Ageratum conyzoides* against the root knot nematode *Meloidogyne incognita* infecting black gram, *Vigna mungo*. In the present work, the biochemical characteristics such as total protein, lipid and carbohydrate content present in the leaves of control, inoculated control and the experimental plants treated with different concentrations (2 to 10 ppm) of *A. conyzoides* after 40 days treatment were analyzed and root gall index were also studied to estimate the nematode population density. Since this extract has a telling effect on the pathogenicity of nematode, it is recommended to be used as a bio-nematicide in the control of root knot nematode in future.

Key words: Bionematicide, Goat weed plant, Root-knot nematode, black gram, biochemical characteristics.

INTRODUCTION

Nematodes are round worms, and those that attack plants are microscopic. They cause damage to plants which is often subtle and is easily confused with nutrient problems. Although hundreds of different kinds of nematode may infect plants, less than a dozen are economically serious root feeding pathogens, and only one genus causes significant damage by feeding on foliage. If the numbers of harmful nematodes are large, plant growth is adversely affected (Desai, 2007). The root-knot nematode, *Meloidogyne incognita* affects a wide range of plants. Among them black gram is one which finds an important place in relay cropping pattern being recommended under rice follow conditions in Tamil Nadu (Kumar and Vadivelu, 1993). *M. incognita* is highly pathogenic to crops causing severe damage and reduction in yield. During parasitism, nematodes exert detrimental influence on the normal physiology, growth and development of host plant and these conditions have been attributed to the effect of both direct and indirect response of the host to mechanical and biochemical activities of the nematode (Bhargava *et al.*, 2007). Chemical nematicides are known to affect soil biosphere and create pollution hazards. Uses of botanicals for the management of nematodes are easy for application, free from environmental pollution (Sundarabau 2000).

Hence the present study has been made to evaluate the efficacy of bionematicide, the leaf extract of goat weed plant *Ageratum conyzoides* against the root-knot nematode *Meloidogyne incognita* infecting black gram *Vigna mungo*.

MATERIALS AND METHODS

Surface sterilized *Vigna mungo* seeds were sown in plastic pots of one litre capacity containing autoclaved sterilized river soil, garden soil and red soil (2:1:1). The egg masses of root-knot nematode, *M. incognita* were collected from the root galls infected plants of *Acalypha indica* and kept in separate embryo cups with 5 and 10 egg masses. The experimental plants were inoculated with 5 and 10 egg masses of the nematode by pouring into four holes and were closed with top soil. Distilled water was poured for three days after inoculation. Thereafter the nutrient solution prescribed by Arnon and Hoagland, 1940 (Table 1) and plant extract were added in alternate days. Air dried *A. conyzoides* leaves were prepared by extracting 25g of plant material in 200 ml acetone (55°C) in soxhlet apparatus (Peach and Tracey, 1956). Different concentrations of plant extract such as, 2, 4, 6, 8 and 10 ppm were prepared from stock solution using distilled water. After 40 days of treatment, the biochemical characteristics, such as total

Table 1. Preparation of normal nutrient solution for one litre of distilled water.

COMPONENTS	gm/litre
Potassium nitrate	1.020
Calcium nitrate	0.492
Ammonium di hydrogen orthophosphate	0.230
Magnesium sulphate	0.490
0.5% Ferrous sulphate	
0.4% Tartaric acid	0.6 mg
Boric acid	2.86 mg
Manganous chloride	1.86 mg
Copper sulphate	0.86 mg
Zinc sulphate	0.22 mg
Molybdc acid	0.09 mg

protein content of leaves (Lowry *et al.*, 1951), lipid content of leaves (Bragdon, 1951) and carbohydrate content of leaves (Jayaraman, 1981) were estimated. For all control, inoculated control and experimental groups three replicates were maintained in plastic troughs.

RESULTS AND DISCUSSION

In the present study, the total protein content in the leaves the control plants was found to be 33.88 ± 1.5 mg/g. The inoculated control plants have low protein content 14.253 ± 0.3 mg/g (5 egg mass inoculum) and 15.367 ± 1.6 mg/g (10 egg mass inoculum). There is an increasing trend of protein content in the leaves of treated and inoculated (5 egg masses) plants with increasing concentrations of leaf extract, from 14.330 ± 1.3 mg/g (2ppm) to 27.59 ± 1.4 mg/g (10ppm). The same trend was observed in 10 egg masses inoculum level (Table 2). Similar results were obtained by Vijay (2000), in *Vigna mungo* plants affected by *M. incognita*.

The total lipid content of the control plants showed 0.702 ± 0.1 mg/g, while in inoculated control plants it was found to be decreasing to 0.680 ± 0.1 mg/g (5 egg mass inoculum) and 0.622 ± 0.04 mg/g (10 egg mass inoculum). At different concentration of *A. conyzoides* the lipid content was found to be increasing with increasing concentration of leaf extract from 0.333 ± 0.02 mg/g (2ppm), 0.414 ± 0.03 mg/g (4 ppm), 0.465 ± 0.1 mg/g (6ppm), 0.555 ± 0.1 mg/g (8ppm) to 0.612 ± 0.1 mg/g (10 ppm) at 5 egg mass inoculum level. The same trend was observed in 10 egg mass inoculum level also where the lipid content was 0.277 ± 0.1 mg/g (2ppm) to 0.568 ± 0.1 mg/g (10 ppm) (Table 2). Vaitheeswaran *et al.*, 2005 reported that the lipid content is increased in the infected untreated plants than the control plants. The low levels of sugar might be due to the possible consumption by the nematode for its sustenance or mobilization pool for synthesis of other metabolites like phenol, protein and lipid *etc.* as suggested by Owens and Specht (1966) and Kannan (1977).

Table 2. Effect of the root-knot nematode, *Meloidogyne incognita* and the leaf extract of *Ageratum conyzoides* on total protein content (mg/gm), total lipid content (mg/g), total carbohydrate content (mg/g) in the leaf of black gram, *Vigna mungo*

Inoculum / plant Egg masses	40 days of treatment						
	Control	Inoculated control	2ppm	4ppm	6ppm	8ppm	10ppm
	Total Protein content (mg/g)						
5	33.88±1.5	14.253±0.3	14.330±1.3	18.88±1.1	20.74±1.8	25.56±0.5	27.59±1.4
10		15.367±1.6	16.293±1.3	17.96±1.7	19.813±1.4	22.59±1.6	23.33±1.7
	Total Lipid content (mg/g)						
5	0.702±0.1	0.680±0.1	0.333±0.02	0.414±0.03	0.465±0.1	0.555±0.1	0.612±0.1
10		0.622±0.04	0.277±0.1	0.366±0.1	0.452±0.1	0.518±0.045	0.568±0.1
	Total carbohydrate content (mg/g)						
5	32.773±0.6	8.053±0.6	13.321±0.9	22.234 ±0.3	22.425±0.3	23.431±0.9	26.863±0.6
10		6.804±0.5	12.746±0.8	16.675±0.05	17.921±0.7	19.071±0.4	20.509±0.3

Table 3. Effect of root-knot nematode *Meloidogyne incognita* and leaf extract of *Ageratum conyzoides* on the root gall index of black gram *Vigna mungo*

Inoculum egg masses / plant	Root gall index						
	Control	Inoculated control	2ppm	4ppm	6ppm	8ppm	10ppm
5	0	3.333±1.5	3±1	2.667±0.6	2.333±0.5	1.333±0.6	0.667±0.2
10		5.333±1.5	4±1	3.667±0.5	3.333±1.5	1.667±0.6	1±0

In the experimental plants the carbohydrate content was found to be low 8.053 ± 0.6 mg/g (5 egg mass inoculum) 6.804 ± 0.5 mg/g (10 egg mass inoculum), when compared with control plants that had 32.773 ± 0.6 mg/g. There is an increasing trend of carbohydrate content in the leaves of treated plants with increasing concentrations of leaf extract, that is in 5 egg mass the carbohydrate content has been found to be 13.321 ± 0.9 mg/g (2ppm) to 26.863 ± 0.6 mg/g (10 ppm). In 10 egg masses the carbohydrate content has been found to be 12.746 ± 0.8 mg/g (2ppm) to 20.509 ± 0.3 mg/g (10 ppm) Table 2. Musabyimana and Saxena (1999) reported that the carbohydrates are quick energy source compounds obtained from the vegetable plants or crops. Due to the infection of various species of root-knot nematodes, the carbohydrate content has been decreased in banana. In the use of neem seed derivations showed better improvement in carbohydrate content than this inoculum levels.

The effect of root-knot nematode, *M. incognita* and leaf extract of *Ageratum conyzoides* on the root gall index to measure the nematode population density of black gram, *V. mungo* was also recorded and presented in table 3. With reference to root gall index the inoculated control plants showed 3.333 ± 1.5 (5 egg mass inoculum) and 5.333 ± 1.5 (10 egg mass inoculum). The root gall index has been decreased gradually from 3 ± 1 (2ppm), 2.667 ± 0.6 (4ppm) 2.333 ± 0.5 (6 ppm) 1.333 ± 0.6 (8 ppm) and 0.667 ± 0.2 (10 ppm) at 5egg mass inoculum level. The similar observation was observed in 10 egg mass inoculum level also where the root gall index was 4 ± 1 (2ppm) 3.667 ± 0.5 (4ppm) 3.333 ± 1.5 (6ppm), 1.667 ± 0.6 (8ppm) and 1 ± 0 (10 ppm). Egunjobi and Onayemi, (1981) reported that the infection of *M. incognita* leads to the formation of galls in the root system of host plants. When treated with different concentration of leaf extract of *Azadirachta indica*. Prakesh *et al* (2008) proposed Intertrap crops for the management of this Nematode, very recently Ntalli *et al.* (2010) proposed Melia azedarach (Meliaceae) for *M. incognita* management. Since the leaf extract of *A. conyzoides* has a remarkable nematicidal property on *M. incognita*, further studies have been recommended to isolate and characterize nematicidal chemical of *A. conyzoides* by sophisticated techniques, there by it can be used in the control of plant root-knot

nematodes instead of hazardous organic synthetic nematicides in future.

ACKNOWLEDGEMENT

The authors express their profound thanks to the Management, Principal and Head of the department of Zoology, Ayya Nadar Janaki Ammal College (Autonomous); Sivakasi for providing facilities to carry out this work. The first-mentioned author whole heartedly thanks the authorities of UGC, New Delhi for providing financial assistance to carry out this project work under Rajiv Gandhi National Fellowship programme.

REFERENCES

- Arnon, D. I. and Hoagland, D. R. 1940. Crop production in artificial culture solutions and in soils with special reference to factors influencing yields and absorption of inorganic nutrition. *Soil Science*, **50**: 463.
- Bhargava, S., Sharma, M. K. and Dhasora, P. K. 2007. Histopathological and Biochemical changes induced by Root-knot nematode, *Meloidogyne incognita* on resistance and susceptible cultivars of cowpea. *Journal of Mycology and Plant Pathology*, **37**(1) : 112-116.
- Bragdon, 1951. Estimation of total fat. *Journal of Biological Chemistry*, **190**: 140-153.
- Desai, L. 2007. Molecular Plant Pathology. Paragon International publishers New Delhi: 117-133.
- Egunjobi, O.A and S.O. Onayemi, 1981. The efficacy of water extract of neem (*Azadirachta indica*) leaves as a systemic nematicide. *Nigerian Journal of Plant Protection*, **5**: 70-74.
- Jayaraman, J. 1981. Laboratory Manual in Biochemistry, Wiley Eastern Ltd., Madras. 1-65.
- Kannan, S, 1977. Studies on the hypersensitive reaction in the roots of the bean (*Dolichos lablab*) infected with the root-knot nematode. *Journal of Madurai University*. **1**: 56-62.
- Kumar, S. and Vadivelu, S. 1993. Inter-relationships between *Meloidogyne incognita* and *Rhizobium* sp. on Black gram, *Vigna mungo* (L) Hopper. *Indian Journal of Nematology*, **23** (1) : 75-81
- Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randale, R. J. 1951. Protein measurement with folin phenol

- reagent. *Journal of Biological Chemistry*, **193**: 265-275.
- Musabyimana, T. and Saxena, R. C. 1999. Efficacy of neem seed derivatives against nematodes affecting banana. *Phyto parasaitica*, **27** (1) : 43-49
- Ntail. N. G., Menkissoglu-spiroudi, U. and Giannakou. I. 2010. Nematicidal activity of powder and extracts of *Melia azedarch* fruits against *Meloidogyne incongnita*, *Annals of Applied Biology*, **156**(2). 309-317.
- Owens, R. G and Specht, H. N. 1966. Biochemical alterations induced in host tissues by root-knot nematode. *Contrib. Boyce Thompson Inst*, **23**: 181-198.
- Peach, K. and Tracey, M. V. 1956. Modern methods of plant analysis, Springer verlag, Berlin **33**.
- Prakash. A., Jagadiswari Rao and Nandagopal. V. 2008. Future of Botanical pesticides in rice, wheat, pulses and vegetables pest management. *Journal of Biopesticides*, **1**(2)-154-169.
- Sundarababu, R. 2000. Nematode problem in TamilNadu – Aspects and prospects. In: Plant Diseases Ed. P.C. Trivedi pointer publishers, Jaipur (India). 365-392.
- Vaitheeswaran, M., S.M. Ibrahim and K. Senthikumar 2005. Carbohydrate metabolism in a host plant, *Hibiscus cannabinus* infected by *Meloidogyne incognita*. *Indian Journal of Nematology*, **35** (2) : 205-206.
- Vijay, K. 2000. An ecofriendly approach to control root-knot nematode *Meloidogyne incognita* affecting black gram, *Vigna mungo* using the Thesis, Dissertation, submitted to A.N.J.A College (Autonomous), Sivakasi.

M. Pavaraj, K. Karthikairaj and M. K. Rajan

Post-graduate and Research Department of Zoology, Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi – 626 124, Tamil Nadu, India, E.mail: pavarajphd@yahoo.in