The Pharma Innovation Journal 2022; 11(5): 2242-2244

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(5): 2242-2244

www.thepharmajournal.com
Received: 08-02-2022
Accepted: 19-03-2022

Sujata
Department of Nematology, CCS Haryana Agricultural University, Hisar, Haryana, India

JA Patil
Department of Nematology, CCS Haryana Agricultural University, Hisar, Haryana, India

Babita Kumari
Department of Nematology, CCS Haryana Agricultural University, Hisar, Haryana, India

Lochan Sharma
Department of Nematology, CCS Haryana Agricultural University, Hisar, Haryana, India

Saroj Yadav
Department of Nematology, CCS Haryana Agricultural University, Hisar, Haryana, India

Corresponding Author:
Sujata
Department of Nematology, CCS Haryana Agricultural University, Hisar, Haryana, India

Management of root-knot nematode, *Meloidogyne incognita* infecting okra using botanicals

Sujata, JA Patil, Babita Kumari, Lochan Sharma and Saroj Yadav

Abstract
Experiment was conducted to evaluate the efficacy of various botanicals against *Meloidogyne incognita* under pot conditions. Five indigenous botanicals *i.e Azadirachta indica, Ricinus communis, Eucalyptus globules* and *brassica* sp. (cabbage and cauliflower) were evaluated against *Meloidogyne incognita* under screen house conditions. Soil amended with leaves of botanicals enhanced plant growth parameter of okra and reduced the nematode reproduction. Among all the treatments, *A. indica* significantly reduced the nematode population as compared to untreated check. Shoot length (23.14 cm), root length (12.08 cm) and shoot weight (5.81 g) were significantly increased in soil amended with chopped leaves of *Ricinus communis* 20 g/kg soil while minimum number of galls was recorded in treatment where leaves of *A. indica* 20 g/kg soil was applied. Infestation of root-knot nematode in okra was reduced in all treated pots compared to the untreated control.

Keywords: *Meloidogyne incognita, Azadirachta indica, Ricinus communis, Eucalyptus globulus*, Botanicals

1. Introduction
Okra (*Abelmoschus esculentus*) commonly referred to as Bhindi, is a member of Malvaceae family. It is a summer growing crop that is grown in many countries. It is available almost throughout the year. Okra is grown for its immature edible pods which are consumed as a vegetable. Okra's soft young fruits are used in curries, stews and soups. This vegetable is good source of minerals, vitamins, antioxidants and fibers. It contains a sticky juice that people use to thicken sauces (Arapitsas, 2008) [2]. Many biotic and abiotic factors are responsible to limit the production of okra. Among biotic factors, plant parasitic nematodes play major role. Root-knot nematode infects a wide range of important agricultural crops and causing more damage to vegetable crops in tropical and subtropical countries (Osman *et al*., 2012; Youssef *et al*., 2012) [9, 18]. In India, root-knot nematodes are reported to cause annual losses of Rs. 2480.86 million in okra (Kumar *et al*., 2020) [8]. Root-knot nematodes cause characteristic galls on roots and these galls may be small, big and compound according to host. These galls restrict the flow of water and nutrients to the plants and infected plants appear less vigorous than healthy plants which can be yellowed, are liable to wilt in hot weather conditions and respond poorly to fertilizer application. Damaged areas usually appear as irregular patches and are frequently associated with light-textured soils (Roberts, 2008) [13]. *Meloidogyne incognita* is one of the major constraints to okra (*Abelmoschus esculentus* L.) production in vegetable growing areas of Haryana as well as other parts of the India. Several, chemical nematicides are used to manage this nematode but they are highly toxic compounds that have very low LD_{50} values. Most of them are withdrawn from the market because of their detrimental effect on human health and environment pollution. So, there's need of development of natural products with nematicidal activity such as plant extracts or utilization of various plant parts. Generally, several allelochemicals are produced during decomposition of plant leaves to suppress soil-borne diseases and pest (Berbegal *et al*., 2008) [4]. With this background a preliminary study was carried out to evaluate the various botanicals to manage the root-knot nematode, *M. incognita*.

2. Materials and Methods
Present investigations were carried out to evaluate the efficacy of different shredded leaves on root-knot nematode in okra. The experiment was conducted in the screen house of Department of Nematology, CCS Haryana Agricultural University, Hisar.

2.1 Maintenance of nematode culture
Pure culture of root-knot nematode, *M. incognita* from single egg mass was propagated on brinjal c.v. BR112 in screen houses. The culture was allowed to multiply for 2-3 generations and further sub cultured. General care and maintenance were given to plants as per practices of CCSHAU, Hisar.

2.2 Experimental procedure
Fresh leaves of various plants i.e., *Azadirachta indica, Ricinus communis, Eucalyptus globulus* and *Brassica* spp. (cabbage and cauliflower) were collected from the University field and washed under tap water to get rid of adhered soil particles or dust. Further, they were cut into small pieces and incorporated in pots containing nematode infested soil. Each of the botanicals were applied at doses *i.e.* 10 and 20 g leaves /kg soil. Ten days waiting period was given for proper decomposition of the leaves. Okra (cv. Hisar Unnat) sowing was done in pots after waiting period. Two thousand root-knot juveniles were inoculated per pot. Ten treatments with three replications were arranged in CRD on the bench of screen house. Soil samples (200 cc soil) were processed and active nematode juveniles extracted by combining Cobb's sieving and decanting technique (Cobb, 1918) [5] followed by Modified Baermann's Funnel Technique (Schindler, 1961) [14]. *Meloidogyne incognita* juveniles per ml of suspension were counted under stereo zoom microscope. The number of the juveniles per 200 cc soil was calculated. Galls were counted with the help of magnifying lens.

2.3 Observations
Observations on plant growth parameters (shoot height, root length and fresh shoot weight) and nematode parameters (galls per plant, soil population) were taken.

2.4 Statistical Analysis
Statistical analysis for screen-house experiment was done using OPSTAT software available on-line at CCS HAU website (www.hau.ernet.in).

3. Results and Discussion
Application of the chopped leaves as soil amendment caused significant increase in plant growth parameters and reduced the nematode reproduction in okra. All pots treated with botanicals *viz., Azadirachta indica, Ricinus communis, Eucalyptus globulus* and *brassica* spp. (cabbage and cauliflower) showed nematicidal properties against nematodes. Among the treatments *R. communis* was found most effective to enhance the plant growth parameters *viz.,* shoot length (23.14 cm), root length (12.08 cm) and fresh shoot weight (5.81 g) while *A. indica* 20 g/kg soil significantly reduced the galls (32) and soil nematode population (443.2 per 200 cc soil). Chopped leaves of *R. communis* and *A. indica* 10g/kg soil were the most effective in improving plant growth parameters and statistically at par with other treatments. In case of *A. indica* 10 g/kg soil significantly reduced the galls and final nematode population.
Shoot length was statistically at par with treatment of cabbage and cauliflower 10g/kg soil, and cauliflower 20g/kg soil. Although plant growth was enhanced in presence of *R. communis* leaves but maximum reduction in nematode population was observed in *A. indica*. Data (Table 1) indicated that there was significant reduction (50.4 per cent) in final nematode population under *A. indica* 20 g per kg of soil as compared to untreated check and other treatments. Galls were significantly reduced in both doses of *A. indica i.e.* 10 and 20g/kg soil but statistically at par with each other whereas significantly differ from untreated check.
A lot of work has been done on different botanicals to manage root-knot nematodes (*Meloidogyne* spp.). They showed that the incorporation of various phytotherapeutic substances into soil may be efficient for managing nematodes. Mechanisms to manage nematodes are partially attributed to the chemical breakdown of products glucosinolates (GLS) present in *Brassica* spp., ricinin in *R. communis*, azadirachtin in *A. indica* and nematicidal compound in *E. globulus* (Djian-Caporalino *et al.*, 2005; Patil *et al*., 2020) [6, 11]. Soil amendment with *A. indica* was the foremost useful means for nematode management (Alam, 1993) [1]. *Azadirachta indica* constituents like Nimbin, Solanin, Thionemone, azadirachtin and a variety of flavonoids have nematicidal action (Thakur *et al*., 1981) [15]. The nematicidal activity of *A. indica* may be due to the occurrence of active substances like azadirachtin, present in neem, that might be absorbed by the plant roots which altered the chemical composition of plants and such roots excretes some influence on pathogenesis of *Meloidogyne* spp (Patil *et al.*, 2018, Askary, 2021) [12, 3]. Parveen and Bhat (2011) [10] reported nematicidal activities of *A. indica* bark powder in pot experiment against *Rotylenchulus reniformis* and *M. incognita* on *Ricinus communis*. Hatipoglu and Kaskavalci (2007) found maximum plant height and reduced nematode population with *R. communis* leaves. Increase in growth and decreased in nematodes parasitism was also recorded in plants amended with *E. globulus*. *Eucalyptus globulus* might be highly toxic to nematode juveniles. Leaves of *E. globulus* are reported to manage plant parasitic nematodes (Elbadri *et al.*, 2008) [7]. However, it's been generally assumed that they are supported the assembly of toxic volatile compounds during breakdown of leaves which are harmful for nematode survival (Wiggins and Kinkel, 2005) [16]. These mechanisms need to be further explored specifically with the target to develop plant based nematicide, which can be effective against various soil borne nematodes as well as root pathogens without abating the soil environment.

Table 1: Effects of various botanicals on plant growth parameters of okra infected with root-knot nematode

Treatments (Chopped leaves of plants)	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	No. of galls	Percent increase over untreated check	Final nematode population (FNP)	Percent increase over untreated check
Azadirachta indica 10 g/kg soil	18.45	8.08	2.6	24	64.70	487.9	45.40
Azadirachta indica 20 g/kg soil	21.40	9.48	3.98	23	66.17	443.2	50.40
Ricinus communis 10 g/kg soil	21.97	10.44	3.89	29	57.35	623.9	30.18
Ricinus communis 20 g/kg soil	23.14	12.08	5.81	32	52.94	604.0	32.41
Eucalyptus globulus 10 g/kg soil	16.90	7.39	2.2	38	44.11	698.3	21.86

Eucalyptus globulus 20 g/kg soil	15.33	8.30	1.8	32	52.94	701.3	21.52
Cabbage 10 g/kg soil	14.60	7.70	2.21	45	33.82	748.4	16.25
Cabbage 20 g/kg soil	16.03	7.87	1.84	44	35.29	733.7	17.90
Cauliflower 10 g/kg soil	13.86	7.43	1.70	43	36.76	751.8	15.87
Cauliflower 20 g/kg soil	14.64	7.73	1.72	47	30.88	754.9	15.53
Untreated check	13.27	3.97	1.1	68	-	893.7	-
C.D. (P=0.5%)	1.2	0.8	0.4	2	-	9.7	-

Note: Data are average value of three replications
Initial inoculum level: 2 larvae/g soil

4. Conclusion

These findings suggested that wasteland plants have the potential of nematicidal activity and implying that adding botanicals to soil could lead to the development of new nematicides for the management of *M. incognita*. For the creation of novel nematicidal chemicals, more research is needed, and plant secondary metabolites can help uncover promising compounds for chemical synthesis. A plant metabolomic approach, which allows researchers to look at plant metabolites as end products of cellular activities, could be a useful tool in this context.

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