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Nematode Suppressive Effect of Botanicals Against the Root – Knot Nematode *M. incognita* infesting *Solanum melongena* L.

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ABSTRACT

Application of organic soil amendments is one of the widely used potent and facile methods for controlling the root-knot nematode disease in the vegetable crops. The experiment was conducted with the aim to test the nematicidal potential of locally available weed plants *Achyranthes aspera*, *Colocasia esculenta, Monstera diliosa, Tinospora cardifolia* and *Abutilon indicum* against root-knot nematode, *Meloidogyne incognita* infecting brinjal in pot condition. Results revealed that amending the pot with chopped leaves of selected plants @50g and @100g reduced the nematode populations and root-knot indices, subsequently increased the yield and yield attributing characters of brinjal "BR- 112". Further, among all treatments, *A. aspera* was found significantly superior at both the doses i.e. @50g and @100g in reducing the nematode population and increased biochemical parameters and yield of brinjal followed by *C. esculenta, M. diliosa, T. cardifolia* and *A. indicum* with the same doses. The obtained results proved that above tested weed used as amendment through integrated approach would be more useful to reduce root-knot nematode infection in vegetable crops and will be an asset in the clean and pollution free environment.

Key words *Botanicals, Brinjal, Management, Nematicidal Activity, Root-Knot Nematode.*

Brinjal/eggplant (*Solanum melongena* L.) belongs to the Family Solanaceae. It is an important solanaceous crop of sub-tropics and tropics. In India, it is one of the most common, popular and principal vegetable crops grown throughout the country except higher altitudes. Brinjal fruit is low in calories and fats, contains mostly water, some protein, fiber and carbohydrates. Plant-parasitic nematodes are omnipresent in agricultural soils where they cause damage to a wide range of crops (Jones *et al.,* 2013). Root-knot nematode, *Meloidogyne incognita* is a most destructive species to cause serious disease in agricultural and horticultural crops. The estimated annual crop loss due to root-knot nematode is $100 billion worldwide (Oka *et al.,* 2000). Estimated overall average annual yield loss on the world's major crops due to damage by plant parasitic nematodes is 12.3% (Ravichandra, 2008). Plants have limitless ability to synthesize aromatic substances, mainly secondary metabolites such as alkaloids, tannins, saponins, flavonoids and phenolics, which play defensive role in plants and therefore they protect the plants from their invaders like fungi, bacteria, viruses and nematodes (Mithraja *et al.,* 2011). Many strategies like cultural practice, physical method, crop rotation, organic amendments etc. were used to control root-knot nematode. Synthetic/ chemical nematicide is a fastest method to control the root-knot nematode. But it is hazardous to the human being and environment. An organic amendment is a best tool for managing the root knot nematode disease. Organic amendments not only manage the nematode infection but it also improves the soil fertility and crop yield. In recent years, studies have shown the importance of natural nematicidal compounds in the plants themselves that have potential to suppress nematode populations (Pavaraj *et al.,* 2010; Moosavi, 2012; Nelaballe and Mukkara, 2013). Plant parts having many nematicidal compounds like alkaloids, flavonoids etc. which are responsible to disrupt the multiplication rate of second stage of juvenile's of *M. incognita.* The application of botanical extracts either enabled the plants to resist the nematode invasion or activated directly the defense mechanisms of plants (Mukhtar *et al.,* 2013). Certain plants kill or repel pests, disrupt their life cycle or discourage them from feeding. The main goal of this experiment is to evaluate the potentiality of botanicals against *Meloidogyne incognita* on brinjal cv. 'BR-112' in an eco-friendly manner.

MATERIALS AND METHODS

Test Plant and Pathogen

Brinjal (*Solanum melongena* L. cv. 'BR-112', Family-Solanaceae) was selected as a test plant and *Meloidogyne incognita* (Kofoid and White, 1919; Chitwood, 1949), was chosen as test pathogen to evaluate the effect of organic soil amendments on the management of *Meloidogyne incognita*.

Collection of Infected Root

Survey of infected field was conducted on Agra road, Aligarh, U.P (India). Infected roots of the plants gently removed from the soil and kept in polythene bags and then labeled. Further all these roots were brought to the laboratory for the examination. Juveniles of *Meloidogyne*

Table 1. Effect of soil amendments with fresh chopped leaves of some selected plant species on the root-knot development caused by *Meloidogyne incognita* and plant growth of Brinjal/eggplant cv. 'BR-112' in pots

Treatment	Dose per pot (g)	Plant length (cm)			Fresh weight (g)			Dry weight (g)			Root-knot index
		Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total	
Abutilon indicum	50	26.8^{cde}	16.6^{ef}	43.4^{g}	22.8^{fg}	17.3^{de}	40.1^{fg}	6.5^{fg}	3.4^{efg}	9.9^{efg}	4.2^{bcd}
	100	28.3^{bcd}	17.5^{de}	45.8^{def}	23.7^{ef}	18.2^{cde}	41.9^{ef}	6.7^{efg}	3.5^{def}	10.2^{def}	4.1^{bcde}
Achyranthes aspera	50	31.6^{b}	21.0^{c}	52.6^{bc}	26.8^{bc}	21.2^{bc}	48.0^{c}	7.4^{bc}	4.3^{bc}	11.7^{bc}	3.8^{efg}
	100	32.3^{b}	22.0^{b}	54.3^{b}	28.9^{b}	22.3^{b}	51.2^{b}	7.6^{b}	4.6^{b}	12.2^{b}	3.6^{g}
Colocasia esculenta	50	30.8^{bc}	19.4^{cd}	50.2^{bcd}	25.3^{bcde}	20.4^{bcd}	45.7^{cd}	7.2^{bcd}	4.2^{bcd}	11.4^{cd}	3.9^{defg}
	100	31.3^{b}	21.0^{c}	52.3^{bc}	26.6^{bcd}	21.1^{bc}	47.7^{c}	7.4^{bc}	4.4^{bc}	11.8^{bc}	3.7^{fg}
Monstera deliciosa	50	29.6^{bc}	18.0^{cde}	47.6^{cde}	24.6^{def}	19.6^{bcde}	44.6^{d}	7.0^{cde}	3.9^{cde}	10.9^{cde}	4.0^{cdef}
	100	30.3^{bc}	19.0^{cd}	49.3^{bcd}	25.7^{bcd}	20.3^{bcd}	46.0^{cd}	7.3^{bc}	4.1^{bcd}	11.4^{cd}	3.8^{defg}
Tinospora cardifolia	50	28.5^{bcd}	17.8d^{e}	46.3^{def}	23.7^{ef}	18.4^{cde}	42.1^{ef}	6.8^{def}	3.6^{def}	10.4^{def}	4.1^{bcde}
	100	29.2^{bc}	18.0^{cde}	47.2^{cde}	24.4^{def}	19.4^{bcde}	43.8^{de}	7.0^{cde}	3.8^{cde}	10.8^{cde}	4.0^{cdef}
UIC	_	24.8^{f}	14.8^{g}	39.6^{h}	17.9^{i}	13.5^{f}	31.4^{i}	4.2^{h}	3.0^{i}	7.2^{i}	5.0^{a}
UUC	_	50.7^{a}	30.3^{a}	81.0^{a}	39.7^{a}	26.1^{a}	65.8^{a}	9.8^{a}	6.2^{a}	16.0^{a}	0^{h}

Each value is the mean of four replicates.

Initial inoculums 1500 (J2) of *Meloidogyne incognita* per pot.

Means in each column followed by same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

UIC- Untreated Inoculated Control; UUC- Untreated Uninoculated Control.

incognita (Kofoid and White, 1919; Chitwood, 1949) were prepared from a pure culture that was previously cultured by eggmasses and propagated on eggplant (*Solanum melongena* L.) in the glasshouse of Section of Plant Pathology and Plant Nematology, Department of Botany, Aligarh Muslim University, Aligarh, India. Surfacely attached eggmasses were detached by using sterilized forceps from the infected roots. These eggmasses were placed in 15 mesh sieves (8 cm in diameter) having crossed layer of tissue paper. These were kept in petridishes full of water so that eggmasses may remain in contact with water. These petridishes were then incubated at 28 ±2°C for hatching and for freshly hatched second stage juveniles (J2) of *Meloidogyne incognita*.

Maintenance of Seedlings

The seeds of brinjal cv. 'BR-112' were surface sterilized in 0.01% $HgCl_2$ for three minutes and then rinsed with Double Distilled Water (DDW) three times. For nursery preparation seeds were sown in autoclaved clay pots (30 cm diameter) along with the soil. Three weeks after germination of proper seedlings of brinjal cv. 'BR-112' were transplanted to each 15 cm diameter clay pots filled with 1 kg autoclaved soil. These pots were treated with fresh chopped leaves of different plants *viz., Achyranthes aspera* L. (Family- Amaranthaceae), *Colocasia esculenta* L. (Family- Araceae), *Monstera deliciosa* Liebm. (Family- Araceae), *Tinospora cardifolia* (Thunb.) Miers (Family- Menispermaceae) and *Abutilon indicum* (Link) Sweet (Family- Malvaceae) applied two different doses @50g/pot and @100g/pot. The pots were watered regularly for proper decomposition of the organic additives for two weeks. As the seedlings get established each of them was inoculated with 1500 hatched second stage juveniles (J2) of *Meloidogyne incognita*. Each treatment was replicated four times.

Inoculation was done by making 3 holes in the pots soil nearby to the roots at the same distance in the manner so that root don't get damage. Then requisite amount of suspension having necessary number of second stage juveniles was procured into the holes and then covered them with the soil.

Table 2. Effect of soil amendments with fresh chopped leaves of some selected plant species on the root-knot development caused by *Meloidogyne incognita* and plant growth of Brinjal/eggplant cv. 'BR-112' in pots

Treatment	Dose per pot (g)	Chlorophyll content (mg/g)	Carotenoid content (mg/g)	Eggmasses / Root	Egg / Eggmasses	Nematode population/ 250g soil	Yield / Plant (g)
Abutilon indicum	50	1.42^{fg}	0.368^{g}	154.0^{d}	162.0^{c}	1004.0^{c}	162.7^{i}
	100	1.45^{ef}	0.371^{def}	146.0^{e}	153.0^{d}	998.0^{d}	174.0^{g}
Achyranthes aspera	50	1.58^{b}	0.379^{c}	122.0^{i}	141.0^{fg}	972.0^{h}	198.0^{c}
	100	1.60^{b}	0.383^{b}	116.0^{j}	130.0^{h}	962.0^{i}	206.0^{b}
Colocasia esculenta	50	1.50^{d}	0.376^{cd}	130.0^{h}	146.0^{e}	980.0^{fg}	183.6^{e}
	100	1.54^{c}	0.380^{c}	123.0^{i}	138.0^{g}	970.0^{h}	196.0^{c}
Monstera deliciosa	50	1.47^{de}	0.373^{cde}	136.0^{g}	159.0^{cd}	984.0^{f}	178.3^{f}
	100	1.50^{d}	0.377^{cd}	131.0^{h}	142.0^{f}	978.0^{g}	189.8^{d}
Tinospora cardifolia	50	1.44^{ef}	0.370^{def}	148.0^{e}	152.0^{d}	992.0^{e}	172.5^{g}
	100	1.48^{de}	0.374^{de}	140.0^{f}	148.0^{e}	990.0^{e}	181.4^{ef}
UIC	_	1.28^{h}	0.267^{h}	178.0^{a}	268.0^{a}	1598.0^{a}	143.6^{k}
UUC	_	2.57^{a}	0.582^{a}	0^{k}	0^{i}	0^{j}	304.0^{a}

Each value is the mean of four replicates.

Initial inoculum 1500 (J2) of *Meloidogyne incognita* per pot.

Means in each column followed by same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

UIC- Untreated Inoculated Control; UUC- Untreated Uninoculated Control.

Experimental design

The twelve treatments including the untreated inoculated and untreated uninoculated control with four replicates were kept in a glasshouse. Following combinations were used during the experimentation;

T1- *Achyranthes aspera* (50g) +1500 J2

T2- *Achyranthes aspera* (100g) +1500 J2

T3- *Colocasia esculenta* (50g) +1500 J2

T4- *Colocasia esculenta* (100g) +1500 J2

T5- *Monstera deliciosa* (50g) +1500 J2

T6- *Monstera deliciosa* (100g) +1500 J2

T7- *Tinospora cardifolia* (50g) +1500 J2

T8- *Tinospora cardifolia* (100g) +1500 J2

T9- *Abutilon indicum* (50g) +1500 J2

T10- *Abutilon indicum* (100g) +1500 J2

T11- Untreated inoculated (1500 J2)

T12- Untreated uninoculated (Control)

Observation

After three months of the inoculation, roots of Brinjal cv. 'BR-112' were uprooted carefully from the pots and were washed in running tap water to wash off the soil particles adhered with the root is removed. The water present in the plants was eliminated by pressing them in between the blotting sheets. The plant growth parameters, *viz.,* shoot and root length (cm), fresh and dry weight (g) of shoot and root and yield (g), biochemical parameters viz., chlorophyll and carotenoid content and pathological parameters *viz*., number of eggmasses/ root system, nematode population/ 250g of soil and Root-knot index (RKI) were examined respectively. The number of plants with galled root system and the root-knot index were evaluated on a 0-5 scale (0 = no galling; 1 = 1-2 galling; 2 = 3-10 galling; 3 = 11-30 galling; 4 = 31-100 galling; and 5 = more than 100 galling per root system according to Taylor and Sasser, 1978). The nematode populations present in the soil was estimated by Cobb's sieving and decanting technique followed by modified Baermann's funnel technique by processing 250g of soil sample.

Statistical Analysis

All statistical data analyses were performed using R version 2.14.2 software to test for significant differences between the treatment means (R Core Team, 2015).

RESULTS AND DISCUSSION

The results of present experiment showed that efficacy of chopped leaves of different *plants A. aspera, C. esculenta, M. deliciosa, T. cardifolia* and *A. indicum* were applied @50g/pot and @100g/pot as soil amendments significantly enhance the growth parameters of brinjal and reduced the pathological parameters under glasshouse conditions. Among all the treatments, *A. aspera* @100g was found highly effective to suppress the nematode infection and improved growth parameters *viz*., plant length, fresh and dry weight and yield were 54.3cm, 51.2g, 12.2g and 206.0g respectively. It was followed by *C. esculenta, M. deliciosa, T. cardifolia* and *A. indicum* were 52.3cm, 47.7g, 11.8g and 196.0, 49.3cm, 46.0g, 11.4g and 189.8g, 47.2cm, 43.8g, 10.8g and 181.4g, 45.8cm, 41.9g, 10.2g and 174.0g respectively with the same dose as compare to untreated inoculated control 39.6cm, 31.4g, 7.2g and 143.6g respectively (Table 1). The chopped leaves of plant as soil amendments also brought a significant improvement in biochemical parameters *viz*., chlorophyll (mg/g) and carotenoid content (mg/g). Highest chlorophyll content was observed in *A. aspera* applied @100g/pot 1.60 mg/g followed by *C. esculenta, M. deliciosa, T. cardifolia* and *A. indicum* at the same dose were 1.54 mg/g, 1.50 mg/g, 1.48 mg/g and 1.45 mg/g respectively, the highest carotenoid content was observed in *A. aspera* @100g/pot 0.383mg/g followed by similar dose of *C. esculenta, M. deliciosa, T. cardifolia* and *A. indicum* were 0.380mg/g, 0.377mg/g, 0.374mg/g and 0.371mg/g respectively (Table 2). Least growth parameters *viz*., length, fresh weight, dry weight and yield observed in treatment of *Abutilon indicum* @50g/pot 43.4cm, 40.1g, 9.9g, 162.7g and biochemical parameters *viz*., chlorophyll and carotenoid content 1.42mg/g and 0.368mg/g respectively at the same dose. The highest root knot nematode infestation was found in the untreated inoculated control. The growth parameters and biochemical parameters were found minimum and the pathological parameters were found maximum. The results also showed that all the botanicals significantly reduced the number of eggmasses per root system as compare to the untreated inoculated control. Maximum number of eggmasses/root system (178.0), egg/eggmasses (268.0), nematode population (1598.0) and root knot index (5.0) observed in untreated inoculated control. However, *A. aspera* @100g/pot observed best result in reduction of pathological parameters *viz*., eggmasses/root (116.0), egg/eggmasses (130.0), nematode population (962.0) and root knot index (3.6) followed by *C. esculenta (*123.0, 138.0, 970.0 and 3.7), *M. deliciosa* (131.0, 142.0, 978.0 and 3.8), *T. cardifolia* (140.0, 148.0, 990.0 and 4.0) and *A. indicum* (146.0, 153.0, 998.0 and 4.1) respectively (Table 2). From above result all the treatments significantly reduced the nematode infestation. But *A. aspera* have a great potential to disrupt the multiplication of nematode, and most effective to enhance the growth parameters, biochemical parameters and great reduction in pathological parameters.

The present study showed great potentiality of botanicals against root knot nematode *Meloidogyne incognita*. Application of different plant species as soil amendments was effective to increase the plant growth characters *viz*., plant length, weight and yield as well as biochemical parameters like chlorophyll and carotenoid content. It is also effective to reduce the pathological parameters *viz*., eggmasses/root system and great reduction in nematode population. The addition of soil amendments provides the soil with nutrients and improves soil physical properties (Giotis *et al.,* 2009). Organic amendment is a best tool to manage the root knot nematode infestation, non-hazardous to environment and growing safe and healthy food in sustainable manner without any disturbance in flora and fauna. Many plant residues and other amendments can release nitrogen compounds, organic acids, or other compounds that may have adverse effects on nematodes (Oka, 2010). Phytochemical analysis also revealed that plant is rich in alkaloids, phenols, terpenoids, and flavonoids etc have high rate of nematicidal activity (Pavela, 2004).The use of plants as nematicidal or nematostatic products has been regarded as effective, economical and eco-friendly by numerous researchers (Chitwood, 2002). Combined application of wild spinach powder and plant chopped leaves very effective against root knot nematode *M. incognita* (Asif *et al.,* 2016). Plants have nematicidal compound which inhibited the reproduction of root knot nematode (Ansari *et al.,* 2016). Hussain *et al.* (2011) confirmed the nematicidal effect of neem is attributed to naturally occurring chemicals *viz*., azadirachtin, nimbin, salannin, nimbidin, kaempferol, thionemone, quercetin etc. Our results are corroborating with Asif *et al*. (2014), Parihar *et al*. (2012) and Ahmad *et al.* (2010).

In the present investigation, effect of soil amendments on root knot nematodes *M. incognita* was confirmed that the nematicidal potential of botanicals for nematode management in brinjal. It helps to improve the crop production as well as improve the fertility and physical property of the soil. It may go long way to manage the root-knot disease which may be pollution free and non-hazardous in nature.

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