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Nematicidal Effect of Weeds for the Sustainable Management of Root-Knot Nematode, *Meloidogyne incognita* Parasitizing *Cicer arietinum* L.

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ABSTRACT

Chopped leaves of six weeds *viz*., *Ageratum conyzoides*, *Eichhornia crassipes, Ipomoea carnea, Nicotiana plumbaginifolia, Acalypha indica* and *Trianthema portulacastrum* were used as organic amendments to control root-knot nematode, *M. incognita* and determine its impact on growth and physiological parameters of chickpea cv. 'Avarodhi' under glasshouse conditions. All the treatments had a significant effect in reducing the infestation caused by *M. incognita* and enhanced the growth and physiological parameters of chickpea. The pots treated with *A. conyzoides* showed highest improvement in growth of chickpea and reduced the root-knot infestation caused by *M. incognita*. It was followed by *E. crassipes, I. carnea, N. plumbaginifolia* and *A. indica*. Treatment with *T. portulacastrum* was found to least effective. These results may be due to the presence of some phytochemicals release after the decomposition of organic matter which had toxic and deleterious effect on *M. incognita*.

Key words *Chickpea, Chopped leaves, Growth parameters, Meloidogyne incognita.*

Pulses form an integral part of the cropping systems of farmers all over the world. These crops occupy an important position in the human diet. They play an important role in increasing the soil fertility by fixing the atmospheric nitrogen through nodules present on the root system. Chickpea (*Cicer arietinum* L., Family-Fabaceae) ranks as the world's third most important pulse crop after bean and pea. Chickpea being susceptible to many endoparasitic and ectoparasitic nematodes *viz., Meloidogyne incognita, M. javanica* (Ali and Askary, 2001). In India, *Meloidogyne* species such as *M. incognita* and *M. javanica* have been reported to cause 19-40% and 24-61% economic losses to chickpea, respectively (Ali *et al*., 2010). The symptoms of nematode infection include formation of root galls which results in growth reduction, nutrient and water uptake reduction, increased wilting and poor yield. The potential host range of *Meloidogyne* species encompasses more than 3000 plant species (Abad *et al.*, 2003). Root-knot 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 (Natarajan *et al*., 2006). Root-knot nematode can be controlled through the use of cultural methods crop rotation, resistant varieties, biocontrol agents and organic amendments. The use of chemical nematicides has been the most effective method for root-knot nematode management, but they resulted in environmental degradation and left harmful effects on flora and fauna. Therefore, there is a need to find new, cheap, eco-friendly and harmless methods of nematode management. It has been shown that the efficacy of organic amendments against nematodes depends on their chemical and physical properties and the type of microorganisms that develop during degradation (Rodriguez-Kabana *et al*., 1987). Aqueous extracts of *Datura stramonium, Azadirachta indica, Calotropis procera* and *Crotalaria juncea* reduced the infestation of *M. incognita* (Nelaballe and Mukkara, 2013; Saeed *et al*., 2015; Abbassy *et al*., 2017). Many botanical extracts have been found to contain phytochemicals such as alkaloids, tannins, saponins, flavonoids, diterpenes, glucosinolates, acetylenes and thienyls (Chitwood 2002) which are effective against plant-parasitic nematodes (Adegbite, 2003). Hence, the present experiment was undertaken to determine the nematicidal effects of chopped leaves of some weeds *viz*., *A. conyzoides*, *E. crassipes, I. carnea, N. plumbaginifolia*, *A. indica* and *T. portulacastrum* against nematode infestation on chickpea under glasshouse conditions.

MATERIALS AND METHOD

Host plant and Pathogen

Chickpea (*Cicer arietinum* L.) cv. 'Avarodhi', Fabaceae was selected as host crop. The root-knot nematode, *Meloidogyne incognita* (Kofoid and White 1919; Chitwood 1949) was selected as test pathogen.

Management of *M. incognita* by using chopped leaves of some weeds as organic amendments against *M. incognita*.

For evaluating the nematicidal potential of chopped leaves of weeds as organic amendment, pots filled with 1kg sterilized field soil mixed with farmyard manure in the ratio of 4:1 were autoclaved at 20 pound pressure at 121°C for twenty minutes. The pots were then treated individually with two different doses (50g and 100g) of chopped leaves of six weeds *viz., Ageratum conyzoides* (Family-Asteraceae), *Eichhornia crassipes (*Family-*Pontederiaceae), Ipomoea carnea* (Family-Convolvuaceae), *Nicotiana plumbaginifolia* (Family-Solanaceae), *Acalypha indica* (Family-Euphorbiaceae) and *Trianthema portulacastrum* (Family-Aizoaceae). Each treatment including untreated inoculated and untreated

uninoculated (control) was replicated four times. The pots were watered after treatment for proper decomposition of the organic additives. After a waiting period of fifteen days, 5-7 presoaked seeds of chickpea cv.'Avarodhi' were sown in each pots. After two weeks of germination thinning of these plants as done and only one plant was left in each pot. Inoculation was done after the 15 days of germination of the seeds. Three to five holes of 3-5 cm depth were accomplished in the rhizosphere (1-5 cm) around the plant root. A total of 1500 second stage juveniles (J2) of *M. incognita* were added into the holes using a sterilized pipette per pot. The holes were filled gently with sterilized soil. The untreated inoculated and untreated uninoculated pots served as control. The experiment was terminated 60 days after inoculation and the growth parameters, physiological parameters and pathological parameters were determined.

Experimental design

The fourteen treatments including the untreated uninoculted and untreated inoculated control with four replicates were kept in a greenhouse.

T1- *Ageratum conyzoides* (50g) +1500 J2

T2-*Ageratum conyzoides* (100g) +1500 J2

T3- *Eichhornia crassipes* (50g) +1500 J2

T4-*Eichhornia crassipes* (100g) +1500 J2

T5- *Ipomoea carnea* (50g) +1500 J2

T6-*Ipomoea carnea* (100g) +1500 J2

T7- *Nicotiana plumbaginifolia* (50g) +1500 J2

T8-*Nicotiana plumbaginifolia* (100g) +1500 J2

T9- *Acalypha indica* (50g) +1500 J2

T10-*Acalypha indica* (100g) +1500 J2

T11-*Trianthema portulacastrum* (50g) +1500J2

T12-*Trianthema portulacastrum* (100g) +1500 J2

T13 -Untreated inoculated (1500 J2)

T14 - Untreated uninoculated (Control)

Observation

Pollen fertility

Estimation of pollen fertility was done following the method of Stanley and Linskens (1974).

Pollen fertility was estimated by staining the pollen grains with 2% acetocarmine solution.

$$\text{Pollen fertility (\%)} = \frac{\text{Number of fertile pollen} \times 100}{\text{Total number of pollen}}$$

Chlorophyll estimation

The chlorophyll content in the fresh leaves was estimated following the method of Mackinney (1941). One gram of finely cut fresh leaves was ground to a fine pulp using a mortar and pestle after adding 20 ml of 80% acetone. The mixture was centrifuged at 5000 rpm for 5 minutes and collected in a 100 ml volumetric flask. The absorbance was read at the wavelength of 645nm and 663nm against blank (80% acetone) on a spectrophotometer. The chlorophyll content present in the extract (mg g^{-1}tissue) was calculated using the following equation:

$$\text{mg total chlorophyll } g^{-1} \text{ tissue} = 20.2\,(A645) + 8.02\,(A663) \times \frac{V}{1000 \times W}$$

Nitrate Reductase Activity (NRA)

The nitrate reductase activity in fresh leaves was estimated by the following method of Jaworski (1971). The leaves were cut into small pieces (1-2 cm). 0.2g of these chopped leaves were weighed and transferred to plastic vials. To each vial, 2.5 ml of phosphate buffer pH 7.5 and 0.5ml of potassium nitrate solution was added followed by the addition of 2.5 ml of 5% of isopropanol. These vials were incubated in BOD incubator for 2 hours at 30±2°C in dark. Incubated mixture of 0.4 ml was taken in a test tube to which 0.3 ml each of sulphanilamide solution and NED-HCl were added. The test tubes were left for 20 minutes for maximum colour development. The mixture was diluted to 5 ml Double Distilled Water (DDW). The absorbance was read at 540 nm using spectrophotometer.

Carotenoid estimation

The carotenoid content in the fresh leaves were estimated following the method of MacLachlan and Zalik (1963). For carotenoid estimation, the procedure for the preparation of extract from the fresh leaves are same that of chlorophyll estimation. However the absorbance of extract (supernatant) was read at the wavelength 480 and 510 nm for carotenoid estimation against blank (80%) acetone on Spectrophotometer. The carotenoid content present in the extract was calculated by following formula.

$$\text{Carotenoid content} = 7.6\,(\text{O.D. } 480) - 1.49\,(\text{O.D. } 510) \times \frac{V}{DXWx1000}$$

O.D. = Optical density of extract (leaf sample) at given wavelength (480 and 510 nm)

V= Final volume

W=Fresh weight

D= Length of path of light

Pathological parameters

Number of eggmasses

The eggmasses were counted following the procedure of Daykin and Hussey (1985). The roots were dipped in Phloxine B solution (0.015%) for 20 min and were then washed with running tap water to remove the residual Phloxine B. The eggmasses take a pink-red colour where as the roots remain colourless or stain lightly.

Root-knot index

The degree of root-knot nematode infection was recorded according to rating degree given by Taylor and Sasser (1978) as under:

Root Knot Index	Number of galls/root system
0	0
1	1-2
2	3-10
3	11-30
4	31-100
5	>100

Statistical Analysis

The data of the experiments were analyzed statistically using the Statistical Package for the Social Sciences SPSS 12.00 Software (SPSS Inc., Chicago, IL, USA) for analysis of variances (ANOVA). All the values were presented as the mean which were compared according to Least Significant Differences/Critical Differences (C.D) at p=0.05 and p=0.01 level. Duncan's Multiple Range Test was employed to test for significant difference between the treatments.

RESULTS AND DISCUSSION

The results of the present investigation showed that chopped leaves of *A. conyzoides*, *E. crassipes, I. carnea*, *N. plumbaginifolia, A. indica* and *T. portulacastrum* applied @ 50g and 100g per pot as organic amendments showed the significance enhancement in growth and growth yielding attributes of chickpea and reduced root-knot infestation as well as multiplication caused by *M. incognita* under glasshouse conditions. Among all the treatments, maximum increase in plant growth parameters *viz*., shoot and root length (cm), fresh and dry weight of shoot and root (g) were recorded with the application of *A. conyzoides* @100g per pot. It was followed by *E. crassipes, I. carnea*, *N. plumbaginifolia, A. indica* and least was observed in *T. portulacastrum* by similar dose (Table-1). It may be due to the presence of compounds release after the decomposition of chopped leaves of weed which had toxic effect on nematode survival. The nematicidal effect of chopped leaves also has contributed towards the increase in number of flower, pod and nodules, highest being found in the treatment of *A. conyzoides* @ 100g/pot and least was in *T. portulacastrum* @ 50g/pot. Other weeds *viz*., *E. crassipes, I. carnea*, *N. plumbaginifolia* and *A. indica* applied @50g and 100g per pot also significantly improved number of flower, pods and nodules as compare to untreated

Table 1. Effect of fresh chopped leaves of some selected weeds on plant growth of chickpea cv. 'Avarodhi' against root-knot development caused by *Meloidogyne incognita* in pots.

Treatment	Dose (g)	Length (cm)		Total	Fresh weight (g)		Total	Dry weight (g)		Total	Number of flowers	Number of pods
		Shoot	Root		Shoot	Root		Shoot	Root			
Acalypha indica	50	32.6bc	11.0bc	43.6bc	43.50bc	9.61bc	53.11cd	9.0b	2.17b	11.17b	33de	31de
	100	32.8bc	11.5bc	44.3bc	45.41bc	9.80bc	55.21bc	9.38b	2.21b	11.59b	35cd	33cd
Ageratum conyzoides	50	33.9bc	12.0bc	45.9bc	46.76bc	10.34bc	57.04bc	9.49b	2.27b	11.76b	37bc	35bc
	100	35.2b	13.0b	48.2b	48.44b	11.38b	59.82b	10.00b	2.32b	12.32b	39b	37b
Eichhornia crassipes	50	33.6bc	11.8bc	45.8bc	44.75bc	10.18bc	54.93	9.38b	2.24b	11.62b	35cd	33cd
	100	33.8bc	12.6bc	46.4bc	46.25bc	10.30bc	56.55bc	9.82b	2.27b	12.09b	37bc	35bc
Ipomea carnea	50	33.2bc	11.6bc	44.8bc	44.25bc	10.02bc	54.27bc	9.27b	2.21b	11.48b	35cd	33cd
	100	33.5bc	12.2bc	45.7bc	46.09bc	10.18bc	56.27bc	9.71b	2.25b	11.96b	37bc	35bc
Nicotiana plumbaginifolia	50	32.9bc	11.4bc	44.3bc	43.96bc	8.81bc	52.77cd	9.16b	2.18b	11.34b	35cd	33cd
	100	33.1bc	11.9bc	45bc	45.87bc	10.01bc	55.88bc	9.52b	2.23b	11.75b	37de	35de
Trianthema portulacastrum	50	30.8cd	10.7bc	41.5cd	41.76cd	9.05bc	50.81de	8.80b	2.15b	10.95b	33de	31de
	100	32.5bc	11.1bc	43.6bc	43.89bc	9.51bc	53.4cd	9.27b	2.19b	11.46b	35cd	33cd
UIC		26.7e	8.0d	34.7e	33.71e	7.43d	41.14f	6.92c	1.84c	8.76c	29f	27f
UUC		57.2a	18.5a	75.7a	72.62a	17.42a	90.04a	15.45a	3.87a	19.32a	59a	55a

Values are mean of four replicates, UIC=Untreated Inoculated Control, UUC= Untreated Uninoculated Control.
Means in each column followed by the same letter are not significantly different according to Duncan Multiple Range Test (p d"0.05).

Table 2. Effect of fresh chopped leaves of some selected plant species on morphological, physiological and pathological parameters of chickpea cv. 'Avarodhi' in relation to root-knot development and multiplication of *Meloidogyne incognita* in pot.

Treatment	Dose (g)	Chlorophyll (mg/g)	Carotenoid (mg/g)	NRA (µm $g^{-1}h^{-1}$)	Pollen fertility (%)	Number of nodules	Eggmasses/plant	Eggs/ Eggmass	Nematode population/250g of soil	Root Knot Index
Acalypha indica	50	1.40cd	0.347de	0.264cd	59.05b	38cd	108cd	128bc	1119cd	4.3bc
	100	1.43cd	0.358de	0.276cd	59.23b	40cd	98de	117bc	1131bc	4.2bc
Ageratum conyzoides	50	1.54bc	0.379bc	0.298bc	61.01b	43cd	85ef	102cd	892gh	4.0cd
	100	1.60b	0.392b	0.312b	61.21b	47b	77fg	91ef	846hi	3.8de
Eichhornia crassipes	50	1.50bc	0.372cd	0.290bc	59.72b	42cd	88ef	106cd	977fg	4.0cd
	100	1.56bc	0.380bc	0.301bc	61.00b	45bc	80ef	95de	905gh	3.8de
Ipomea carnea	50	1.47bc	0.360de	0.281bc	59.54b	41cd	92ef	110cd	1042ef	4.2bc
	100	1.51bc	0.372cd	0.296bc	59.85b	43cd	83ef	97de	988fg	4.0cd
Nicotiana plumbaginifolia	50	1.44cd	0.356de	0.273cd	59.21b	39cd	100de	121bc	1092de	4.2bc
	100	1.48cd	0.365de	0.284bc	59.58b	42cd	93ef	108cd	1117cd	4.0cd
Trianthema portulacastrum	50	1.37cd	0.340ef	0.258cd	58.88b	36de	115bc	135b	1167b	4.5b
	100	1.40cd	0.352de	0.270cd	59.00b	38cd	104b	123bc	1144bc	4.2bc
UIC		1.19d	0.295g	0.220e	50.65c	33f	156a	270a	1621a	5.0a
UUC		2.50a	0.597a	0.445a	90.14a	70a	0h	0g	0j	0f

Values are mean of four replicates, UIC=Untreated Inoculated Control, UUC= Untreated Uninoculated Control, NRA= Nitrate Reductase Activity.

Means in each column followed by the same letter are not significantly different according to Duncan Multiple Range Test (p d"0.05).

inoculated (Table-1). The chopped leaves of weeds also brought significant increase in physiological parameters like chlorophyll content (mg g^{-1}), carotenoid content (mg g^{-1}), nitrate reductase activity (µm g-1h-1) and pollen fertility (%) as compared to untreated inoculated control. Highest found in treatment with *A. conyzoides* @ 100g/pot followed by similar doses of *E. crassipes, I. carnea, N. plumbaginifolia*, *A. indica* and least was observed in *T. portulacastrum* (Table-2). The root-knot nematode, *M. incognita* was found highly pathogenic on chickpea in untreated inoculated control pots where the growth and physiological parameters were recorded minimum and pathological parameters like number of eggmasses, number of eggs, nematode population and root-knot index were found highest. In respect of number of eggmasses, number of eggs, nematode population and root knot index, a significant difference was observed in the treated plants compared to untreated control, where minimum number of eggmasses, number of eggs, nematode population and root-knot index was recorded in *A. conyzoides* while maximum in untreated control. However, other treatments *viz*., *E. crassipes, I. carnea*, *N. plumbaginifolia, A. indica* and *T. portulacastrum* also showed reduction in number eggmasses, number of eggs, nematode population and root-knot index (Table-2). From the above results it was found that among the tested weeds *A. conyzoides* at both the doses @50g and 100g per pot proved most effective and gave consistently better results for enhancing the growth and physiological parameters of chickpea and reduced the pathological parameters. It was followed by *E. crassipes, I. carnea*, *N. plumbaginifolia, A. indica* and *T. portulacastrum* (Table 1, 2).

The results of the present study showed that amending the pots using chopped leaves of weeds *viz*., *A. conyzoides*, *E. crassipes, I. carnea*, *N. plumbaginifolia, A. indica* and *T. portulacastrum* reduced the root-knot infestation and enhanced the plant growth parameters of chickpea. Our results are in conformity with several

researchers (Mohilal and Dhanachand, 2003; Tariq and Siddiqui, 2005; Asif *et al*., 2016). The observed results might be due to the fact that addition of compost to the soil increases soil nutrient status, changes the physical and trophic structure of soil which might affect the plant growth and yield performances (Pandey, 2000). The mechanisms of plant extracts action may include denaturing and degrading of proteins, inhibition of enzymes and interfering with the electron flow in respiratory chain or with ADP phosphorylation (Konstantopoulou *et al.,* 1994). Chitwood (2002) suggested that the nematicidal properties of plant species vary considerably with plant species and cultivar, plant tissue used, plant growth stage, application method and the nematode species tested. The chopped leaves of weeds reduced the formation of galls caused by root-knot nematode and number of eggmasses in the root system. This could be due to the chemicals present in the chopped leaves that may possess nematicidal properties. The galls on the root system might disturb important root functions like uptake and transport of water and nutrients (Abdi, 1996). Increases in plant growth might be due to the increase in nutrients supply to the soil, resulting from the addition of chopped leaves of weeds. The addition of weeds as organic amendments creates a better environment for the growth of the roots in soil environment. Soil amendments with leaves of many plants may be shown to reduce the rhizospheric population of *Meloidogyne incognita*. *Brassica* green manures are known for limiting reproduction of nematodes, because once chopped and incorporated into the soil they produce glucosinolates, a process called biofumigation (Ploeg, 2007). Qamar *et al.* (2005) observed that isolated chemical constituents such as lantanoside, lantanone, camaric acid and oleanolic acid from aerial parts of *L. camara*, possessing nematicidal activity against root-knot nematode, *M. incognita*. The increase in chlorophyll contents in leaves in the presence of decomposed organic wastes (Siddiqui and Akhtar, 2008) due to increase in N uptake by the addition of organic compounds resulted in increased photosynthetic efficiency, translocation of nutrients and other metabolites toward formation of fruits. Some leguminous plants are known to contain lectins, such as concanavalin A from *Canavalia ensiformis*, which may disrupt nematode behaviors, such as host finding (Marban-Mendoza *et al*., 1987). Ricin is a highly toxic lectin from castor bean (*Ricinus communis*), which was found to inhibit the mobility of *M. incognita* (Rich *et al.*, 1989). Therefore, it was concluded that the severe infection caused by *Meloidogyne incognita* could be lowered by the use of chopped leaves of weeds as organic amendments in view of eco-friendly environment. This has an advantage against expensive and hazardous chemical nematicides. Plant products proved as cheap and degradable source and pave the way for the healthy and pollution free sustainable environment. Other area of further research may include the use of such promising botanical extracts in integrated pest management strategy and evaluation of their effects on other soil borne plant diseases.

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