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Management of Root Knot Nematode, *Meloidogyne incognita* Affecting Chickpea, *Cicer arietinum* for Sustainable Production

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Management of phytoparasitic nematodes with plant products has been supported to increase awareness of environmental hazards. A glasshouse experiment was conducted to control the root knot nematode, *Meloidogyne incognita* on chickpea, *Cicer arietinum* L. var. "Avarodhi", a highly proteinaceous crop by different concentrations of leaf extract of Persian lilac, *Melia azedarach.* Results revealed that the plants treated with higher concentrations of leaf extracts of *M. azedarach* showed the least impact of *M. incognita* as compared to the other concentrations. Plants inoculated alone with the 1000 second stage juveniles (J_2) of *M. incognita* showed highest reduction in plant growth parameters of shoot and root length (cm), fresh and dry weight (g), number of flowers and pods, total chlorophyll content and nitrate reductase activity with increased root knot index. Plants treated with lower concentrations of leaf extract also showed significant control over the untreated inoculated plants. These treatments also reduced the root knot index to a significant level. The study advocates the management of nematodes with organic amendment which will act as an asset to control environmental pollutuion.

Keywords: Root knot nematode, *Meloidogyne incognita,* Chickpea, leaf extract, *Melia azedarach*

Chickpea, *Cicer arietinum* L. (Family-Fabaceae) is the important pulse crop. This is an important source of dietary protein, vitamin, and some minerals and is extensively used as a protein adjunct to starch diets. On the global basis plant parasitic nematodes are estimated to cause 13.7% losses in yield of chickpea [1] with a total amount of 7620 Hg/Ha yield [2]. Chickpea being susceptible crop to many endoparasitic and ectoparasitic nematodes viz., *Meloidogyne incognita, M. javanica* [3], *Heterodera swarupi* [4], *Pratylenchus thornei, Helicotylenchus* spp. [5], hence their management is so far difficult. In India, the root-knot nematode is reported to reduce its yield from 17% to 60% depending on nematode inoculum density and soil types [6]. *Meloidogyne* spp., root knot nematode is one of the most harmful nematode pests in both tropical and subtropical crop production regions and cause extensive economic damage worldwide [7]. Root-knot in chickpea has been reported in various states of India [8,9]. Nematode not only suppresses the plant growth but also interferes in the nodulation, nitrogen fixation and adversely affects the overall yield. Modern way of nematode management is totally based on the nematicides as higher population growth demands increase in crop production. But on the other hand these nematicides are not only toxic to the root-knot nematodes but also accumulate in plant. These nematicides often lead to environmental pollution and even the depletion of stratospheric zone [10]. Hence, there is an urgent need for an eco-friendly substitute for nematode management. Many naturally occurring compounds are known to possess nematicidal activity [11]. Plant parts possess nematostatic as well as nematicidal property [12,13].Plant parts/products proved to be the promising alternative means and showed toxicity to pest up to a certain extent and their application offers complete economic advantage [14,15]. Soil amending with neem and its products has been reported as effective against different phyto- parasitic nematode in various plants [16, 17]. Biocontrol of nematode has been emphasized against chemical means of management, as the use of nematicides are hazardous to environment which in some cases further leads to biomagnifications [18,19]. Application of oil cake was found as organic amendment to control root-knot nematode attacking chickpea [20]. Some plant latex are also known to possess some nematicidal property [21,22].The aim of present study was to determine the nematicidal activity of leaf extract of bakain, *M. azedarach* on root knot nematode, *M. incognita* affecting chickpea, *C.arietinum.*

MATERIALS AND METHODS

A glasshouse experiment was conducted selecting chickpea, *Cicer arietinum* var. "AVARODHI" as a host plant and root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood as pathogen. Two

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hundred seeds of chickpea var. avarodhi were surface sterilized with 0.1% solution of mercury chloride ($HgCl_2$) and then washed thoroughly with double distilled water. Six seeds were then sown in each clay pots (15 cm in diameter) containing steam sterilized soil (7 clay: 2 sand: 1 farmyard manure), pH - 7.2. Each pot was then treated individually with different concentrations, *viz.*, 10ml, 20ml, 40ml, 80ml and 100 ml of leaf extract of *Melia azedarach*.

For culturing nematodes, egg masses of *Meloidogyne incognita* were handpicked with sterilized forceps from the heavily infected roots of egg plant, *Solanum melongena.* These egg masses were washed in double distilled water, placed in 15 mesh sieve (8 cm in diameter) containing double layered tissue paper in petriplates in water. These were incubated at 28±2°C to obtain freshly hatched second stage juveniles (J_2) of *M. incognita*. Hatched juveniles were collected from petriplates in 100 ml beaker.

Experiment designed:

Extract preparation: Aqueous extract of chopped leaves of *Melia azedarach* was prepared and different dilutions were made viz. 10ml, 20ml, 40ml, 80ml and 100ml. Each pot was then treated with these individual dilutions and 1000 (J_2) second stage juvenile of *M.incognita.*

T1- *Melia azedarach* (100 ml) +1000 J_2
T2- *Melia azedarach* (80 ml) +1000 J_2
T3- *Melia azedarach* (40 ml) +1000 J_2
T4- *Melia azedarach (*20 ml) + 1000 J_2
T5- *Melia azedarach* (10 ml) +1000 J_2
T6 -Untreated inoculated (1000 J_2)
TC - Untreated uninoculated (control)

Each treatment was replicated four times. The plants were irrigated regularly. Mature plants were uprooted 60 days after inoculation. Roots were washed thoroughly with running tap water. Plant growth parameters length (shoot and root) in centimeter, weight (fresh and dry) in grams, number of flowers, number of pods, number of nodules and root-knot index was assessed on 0-5 scale [23]

Chlorophyll content
Chlorophyll in fresh leaf sample was extracted in 80% acetone in mg/g and estimated by the Mackinney method [24].

Nitrate reductase activity
The activity of nitrate reductase (NRA) was measured following the method adopted by Jaworski [25]. The fresh leaf samples were cut into small pieces and transferred to plastic vials containing phosphate buffer (pH 7.5) followed by the addition of potassium nitrate and isopropanol solutions. The reaction mixture was incubated at 30ºC for 2 hours, followed by the addition of N-1- naphthyletylenediamine dihydrochloride and sulphanilamide. The absorbance of the colour was read at 540nm and was compared with that of the calibration curve in $\mu molg^{-1}$ of leaves.

Data was analyzed statistically by SPSS 12.00 Software (SPSS. Inc., 1989-2006, USA) ANOVA and Significance of difference was tested by least significant digit at 5 and 1%.

RESULTS AND DISCUSSION

It was found that chickpea, *C.arietinum* var. "Avarodhi" was susceptible to the root-knot nematode *M. incognita*. All the treatments significantly reduced the intensity of root-gall disease of Chickpea (Table 1). Shoot and root length decreased in all the inoculated plants but there was non-significant reduction in plants treated with the higher concentration (100 ml) of leaf extract of *M. azedarach* T1(67.94 cm). Highest plant length was recorded in untreated uninoculated plants TC (75 cm). Plants treated with 80 ml concentration T2 (61.54 cm) and other concentrations also showed control over reduction in plant length as compared to the length of inoculated untreated T6 (37.43 cm) plants (Table-1).

In case of fresh and dry weight T6 (16.82 g & 3.72 g) plants showed the highest impact of nematode infestation. Plants TC (30.71 g & 6.74 g) were recorded to have the highest fresh and dry weight. Plants T1 (27.53 g & 6.46 g) as compared to other treated plants are least influenced by nematode impact. All the plant growth parameters were shown to have positive effect when treated with the leaf extract of *Melia azedarach* against *M. incognita*. Number of flowers and overall

yield of plants were most affected in case of untreated inoculated plants T6 (10.75 & 6.25) (Table-1).

Table 1. Effect of different concentrations of leaf extract of *Melia azedarach* on plant growth parameters of chickpea var. Avarodhi against *Meloidogyne incognita*

Treatment	Plant length (cm)	Plant fresh weight (g)	Plant dry weight (g)	Chlorophyll (mg g^{-1})	NRA (µmol$h^{-1}g^{-1}$)	Number of pods	Number of Flowers	Number of Nodules	Root-knot index
T1	67.94	27.53	6.46	2.462	0.434	29.51	23.63	4.09	1.66
T2	61.54	25.18	5.84	2.401	0.415	27.75	21.86	3.64	2.03
T3	56.78	23.84	4.91	2.016	0.368	24.83	18.65	3.03	2.54
T4	51.56	21.56	4.72	1.878	0.249	21.88	16.84	2.63	2.95
T5	42.74	20.24	4.37	1.705	0.191	19.97	12.43	1.99	3.29
T6	37.43	16.82	3.72	1.006	0.071	10.75	6.25	1.28	4.60
TC	75	30.71	6.74	2.773	0.496	37.75	30.25	4.60	-
LSD (p=0.05)	5.11	2.14	0.476	0.187	0.031	2.28	1.76	0.286	0.246
LSD (p =0.01)	7.17	3.00	0.667	0.262	0.044	3.20	2.46	0.401	0.346

Values are mean of four replicates, T6 Control= Untreated Inoculated Control, TC Control= Untreated Uninoculated Control NRA=Nitrate Reductase Activity

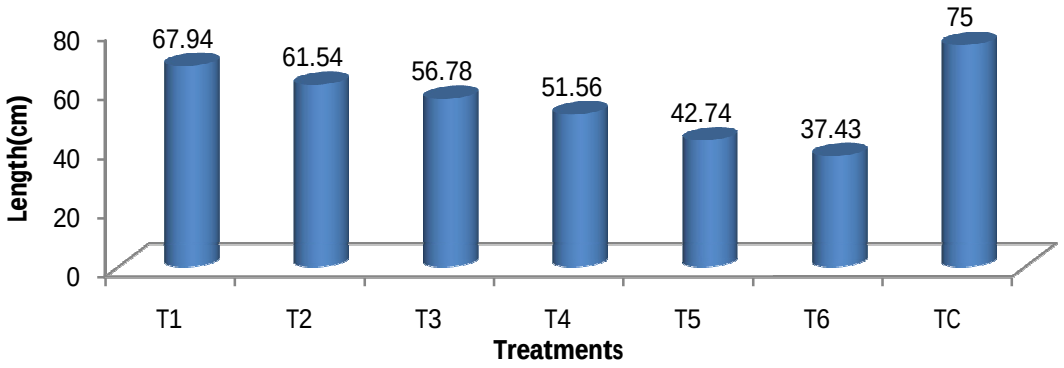

Fig. 1. Histogram showing the effect of different concentrations of leaf extracts of *Melia azedarach* on plant length against *Meloidogyne incognita* affecting chickpea var. 'Avarodhi' .

Fig. 2. Histograms showing the effect of different concentrations of *Melia azedarach* on fresh & dry weight of chickpea var. 'Avarodhi' against *Meloidogyne incognita*

Chlorophyll estimation and nitrate reductase activity (NRA) showed that the amount of total chlorophyll content and nitrate reductase activity decreased in all the inoculated plants. Highest chlorophyll content and nitrate reductase activity was shown by the untreated uninoculated plants TC (2.773 mg g^{-1} & 0.496 µmol$h^{-1}g^{-1}$**)** but plants treated with the higher concentration of *M. azedarach* T1 (2.462 mg g^{-1} & 0.434 µmol$h^{-1}g^{-1}$**)** also showed non-significant reduction in plant growth parameters. Chemical released during the decomposition of organic amendments such as phenols etc. may induce disease resistance in the roots of host plants. Transplanting of neem seedlings with chickpea plants was highly effective in reducing the impact of *M. incognita* [26]. Higher plants have yielded a large number of active compounds including alkaloids, cyanogenic glycosides, terpenoids, diterpenoids, triterpenoids, lipids, fattyacids, steroids, polyacetylenes, quassinoids, glucosinolates, isothiocyanates, flavonoids, simple and complex phenolics etc. (11,27).Therefore it was concluded that the severe infection caused by *Meloidogyne* spp. could be lowered by the plant products in view of eco-friendly environment. This has an advantage against expensive and hazardous chemical nematicides. Plant products proved as cheap and degradable source. This paves the way for the healthy and pollution free sustainable environment.

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