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Management of root Knot Nematode (*Meloidogyne Incognita) using* Neem *(Azadiractha Indica)*

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Abstract: *In India, several species of Meloidogyne are reported on a variety of vegetables, cereals, pulses and ornamental plants. Root knot disease caused by M.incognita is a matter of great concern because this species affects several economically important crop plants. Okra is a very important crop by nutrition and health wise. In the present study the application of various parts of neem (seeds, leaves) altered the physiology of host plant and developed the strong defensive mechanism of the root against nematodes.so the use of neem products stimulated and change the physiology of plant cells and tissue to repel the nematode parasite. Extracts of fresh greens leaves, showed max. Reduction in egg hatching and cause great mortality of juveniles and followed by fresh green seed and dry seed extracts. In pot conditions the powder of fresh seed showed highest plant growth and reduction in root knot formation and followed by fresh leaves and dry seed powder*.
Key Words: *Meloidogyne incognita, Azadiractha indica, Abelmoschus esculents, plant-parasitic nematodes.*

I. Introduction

Environmental pollution is a world-wide problem. Nematodes, especially the plant-parasitic are the most abundantly evolved and diversified metazoans on the planet Earth. Nematodes which infest the plant Kingdom are known as plant parasitic nematodes, phytonema or phytonematodes which damage more than eighty billion dollars of crop loss in the world each year.

On the basis of world-wide survey the ten most important genera are reported to cause severe crop losses (Meloidogyne, Radopholus, RotylenchusTylenchorinchus, Xiphinema, Ditylenchus, Globodera, Helicotylenchus and Heterodera). Several hundred ectoparasitic nematodes might feed on a plant without seriously affecting production while other group only single endo-parasitic nematodes can kill a plant or reduce its productivity (Ingham, 1996). Endo-parasitic nematodes especially the root-knot nematodes (Meloidongynespp.) have been found one of the major constraints in the production of vegetables and other crops.

The highly dangerous endo-parasiticnematodeicMeloidogyneis resented by 400 species all over the World.Neem components have attracted global attention for their insecticidal, fungicidal, bactericidal and nematicidal properties. Crude neem extracts have been used at a local and small-farm level for some time in countries where neem grows. In the major countries such as USA, Canada and Europe, the commercial neem insecticides have reached the markets.

The effective, inexpensive and environmental friendly control of this major pest is a serious problem. Although the chemical control is effective but their residues remain undegradablein soil for long time. Therefore most of the chemical nematicides are banned now a day.Various workers contributed significantly to the management of root knot nematode. Some of the significant contributors areWani (2006); Ram etal (2009);Sharma et al (2007); Khan (2012);Mohnta et al (2012). To overcome this problem various soil amendments by green and compost Goswami&Neetu Singh (2012).manures, organic, inorganic fertilizers, botanicals, bioagents like nematophagous fungi, soil bacteria are found to be more satisfactory to reduce the nematode population and increased crop yield.

II. Materials And Methods:

In vitro study: in vitro study was conducted to test the ovicidal and larvicidal activity of neem extracts on M. incognita eggs and second stage juveniles. The extracts of fresh green leaves, fresh green and dry seeds were made by chopping and crushing of leaves and seeds @ 15g/50ml in distilled water (w/w) in soxlet apparatus at 70 ± 5^0C for 48 hours. All the extracts were filtered through what man filter paper and reduced on hot plate at 250c and stored in sterilized vials. The standard extracts (10%) were made by {1g extracts dissolving in 9ml distilled water (w/w)]. Further dilutions were made as 1% (1ml of standard extracts dissolved in 9ml distilled water). 5ml of all the dilutions were poured in glass petri plates and surface sterilized single egg mass was placed in all the plates and replicated thrice. All the plates were incubated in BOD at 28 ± 2^0C for 5 days. After 5 days of incubation hatching of eggs and subsequent mortality of juveniles was recorded under stereoscopic

binocular microscope. The recorded data are presented in tabular form and statistical tools were applied to find out the level of significance (Table-2).

In vivo study: Pot experiment was conducted on okra plants to root knot nematode, to observe the efficacy of neem leaves, fresh and dry seed powder, after the establishment of infection by second stage IJ_2. The study was carried out in following steps:

(1) Sowing of seed: The okra seeds were procured from the NSC, New Delhi. Seeds were raised in the sterilized soil along with the compost manure @ 100g/kg soil, at Nematology Laboratory, Department of Zoology, C.C.S. University, and Meerut.Earthen pots were filled with 1kg sterilized soil and four seeds were sowed in each pot. After 15 days of germination three seedlings was maintained in each pot.

(2) Culture of (IJ2): The infected okra plant samples were collected from the agricultural fields of Meerut district. The plants were carefully uprooted and after removing the soil, the roots were placed in well labelled polyethylene bags. The collected roots were washed with tap water &eggs masses were isolated in petri plates. The isolated egg masses were incubated for five days at 28 ± 2^0C in BOD. Freshly, hatched IJ_2 were collected in water in 250ml beaker. The content was further reduced to small quantity to count the no. of nematodes. Homogeneous aliquot was placed in counting dish and the no. of IJ_2 in 1ml was under the stereoscopic microscope.

(3) Experimental Design: After 3weeks of seed sowing, okra seedlings were inoculated with freshly hatched M. incognita infective @ 1000 IJ_2. After 7days of inoculation plants were treated with the fresh green leaves, dry and fresh seed powder of neem @ 8% powder/ soil (w/w). All the experimental pots were replicated thrice and irrigated regulatory as needed.

(4) Termination of experiment: plants were uprooted at 65 days after inoculation. After washing, data were recorded on various plant growth parameters viz. - length of root and shoot, weight of fresh and dry shoot and root and also on disease parameters (root knot index) on the basis of no. root knots/plant. The scale (0-5) given by Taylor & Sasser (1978), was applied to find out root knot index as follows: 1, 0= no galling, 2, 1= light galling (0-25%), 3, 2= moderating galling (25-50%), 4, 3= severe galling (51-75%), 5, 4= very severe galling (76-100%).

III. Result and discussion:

An investigation was carried out to study the pathogenicity of root knot nematode Meloidogyne incognita on okra and its management through organic amendments. The present investigation was conducted to okra plants in pot conditions. The results revealed determine the efficacy of neem leaves and seeds (fresh & dry) powder against M .incognita infesting okra plants in pot conditions. The result revealed that the inoculated plants treated with FSP showed max. Plant growth parameters viz, length (shoot & root) and (fresh & dry) weight (shoot & root) and the plant disease parameters viz, no. Of root galls/root; no. Of egg mass/root; root gall index followed by FGP &DSP. The data calculated on % reduction in root knot formation in all the treatments showed highest reduction (64.76%) in FSP alone and followed by combined FSP+FLP (63.75%); DSP+FLP (53.93%); FLP (46.16%) & DSP alone (27.49%). The estimation of egg masses reduction was maximum in combined treatment of FSP+FLP (53.06%) and slightly low (52.56%) in FLP alone followed by DSP+FLP (42.15%); FLP (40.77%) and DSP alone (36.17%) (Table-1, 2). The work of Tariq &Siddiqui, 2005; Rather & Siddiqui, 2007; they also found same result in tomato also.

Azadirachtin is the main active content of neem and is reported very effective and target specific to controlling insects and nematodes pests of the various crops. The findings of the present study can be correlated with of Egumnjobi&Afolami (1976) where they have reported the successful use of neem leaves extracts in nematode control. Mishra (1999) states that the neem formulations are most effective control of root knot nematode Meloidogyne sp. as compared to other botanicals. Akhtar and Malik (2000), Siddiqui&Alam (2001), they have reported that phenols, amino acids, aldehydes and fatty acids are release from neem which is antagonistic to rootknot nematodes. Our results are supported by the study of Siddiqui &Alam, (2001); Satyandra et al (2007); Ravishankar&sharma (2005); Ganai,et al (2014); Satyandra et al (2011);**Babu**& Rana (2012)Archana& Prasad (2014) states that organic amendments of soil using dried poultry litter, municipal refuse, oil cakes of ground nut, neem mustard &neem products (which are commercial available in market) have been found effective in the control of Meloidogyne incognita.

In present study thus it may be concluded that changes in protein after infection are related to defence action, because abnormal metabolites are produced in adjacent non-infected tissues. Such metabolites accumulated in infected tissues and are toxic to parasites and inhibit their growth and penetration. The metabolites released from the chemical constituents of neem (Azadirachtin, salannin, limonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones) stimulated the plant cells to release abnormal metabolites which repel the nematodes from the uninfected cells of plant. However, the green leaves are rich in azadirachtin, salannin, meliantrol and nimbin (Jacobson, 1990; National Research Council, 1992). So the use of neem products stimulated and changes the physiology of plant cells and tissue to repel the nematode parasites.

Table no. 1-- (In vivo study)

Effect of neemextracts on plant growth of okra infected with M. incognita

Treat-ment	Shoot			Root			No. Of Root knot/root	%red In knot formation	No. Of Eggmasses/root	%red. In egg mass formation	Root knot Index
	Length (cm)	Fresh Wt. (gm.)	Dry wt. (gm.)	Length (cm)	Fresh Wt. (gm.)	Dry wt. (gm.)					
T0	62.33 ±1.45 (60-65)	33.33 ±1.45 (31-36)	9.33 ±0.88 (8-11)	10.33 ±0.88 (9-12)	6.67 ±0.67 (6-8)	1.67 ±0.33 (1-2)	NIL	0	NIL	0	NIL
T1	27.67 ±1.20 (26-30)	12.33 ±1.45 (10-1)	2.67 ±0.33 (2-3)	7.33 ±0.67 (6-8)	2.33 ±0.67 (1-3)	1.14 ±0.14	87.33 ±0.88 (87-89)	0	43.33 ±1.45 (41-46)	0	4
T2	29.67 ±2.03 (26-33)	13.67 ±1.20 (12-16)	3.33 ±0.88 (2-5)	10.33 ±1.20 (8-12)	2.67 ±0.88 (6-9)	1.30 ±0.21	63.33 ±0.88 (62-65)	27.49	27.67 ±1.20 (26-67)	36.17	3
T3	52.67 ±0.88 (51-54)	22.33 ±1.45 (20-25)	6.33 ±0.88 (5-8)	12.67 ±1.20 (11-15)	7.67 ±0.88 (6-9)	2.98 ±0.38 (1-3)	31.33 ±0.67 (30-32)	64.76	20.33 ±0.88 (19-26)	52.56	2
T4	52.33 ±0.88 (51-54)	20.67 ±1.201 9-23)	5.33 ±0.33 (5-6)	12.33 ±1.45 (10-15)	7.67 ±1.20 (6-9)	3.00 ±0.45 (2-4)	47.33 ±0.88 (2-4)	46.16	25.67 ±0.33 (24-26)	40.77	3
T5	43.67 ±1.20 (42-46)	19.67 ±1.20 (18-22)	4.33 ±0.33 (3-5)	10.33 ±1.45 (8-13)	7.67 ±1.45 (6-10)	2.95 ±0.55 (1-2)	40.33 ±0.88 (39-42)	53.93	25.33 ±0.88 (24-27)	42.15	3
T6	43.33 ±1.45 (41-46)	17.33± 0.88 (16-19)	4.33 ±0.88 (3-6)	10.67 ±1.20 (9-13)	4.67± 0.33 (4-5)	1.73 ±0.09 (32-41)	31.67± 0.67 (31-33)	63.75	20.33 ±0.33 (19-22)	53.06	2

T0 =control, T1= nematode alone, T2= nematode + DSP (dry seed powder), T3= nematode + FSP (fresh seed powder), T4= nematode + FLP (fresh leaves powder), T5 = nematode + DSP +FLP, T6 = nematode + FSP + FLP.

Table no. 2 -- (In vitro study)

Effect of neem leaves and seed extract on egg hatching and juvenile mortality of M. incognita										
Parameters	Control	Fresh leaves extract			Fresh seed extract			Dry seed extract		
		SE	1:10	1:100	SE	1:10	1:100	SE	1:10	1:100
Hatching	98	2.33±0.3 (2-3)	11.67± 2.03 (8-15)	27.33±3 .18 (21-31)	3.67± 0.88 (2-5)	16.33± 1.76 (13-19)	36.33± 3.28 (30-41)	7.00± 1.15 (5-9)	29.33± 4.81 (20-36)	37.33±2 .91 (32-42)
Reduction in hatching over control (%)	Nil	95.633	86.099	71.366	94.177	81.333	61.933	91.191	68.077	59.566
Mortality	Nil	1.33±0.8 (0-3)	6.67± 0.88 (5-8)	14.33± 1.45 (12-17)	1.67± 0.33 (1-2)	6.67± 1.20 (5-9)	11.33± 1.86 (9-15)	2.33± 0.33 (2-3)	6.00± 1.73 (3-9)	8.33± 1.20 (6-10)
Mortality over total hatching (%)	Nil	53.64	53.33	49.98	44.14	40.78	32.03	34.78	23.49	21.82

SE= standard extract.

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