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STUDIES ON THE EFFICACY OF NEEM AND FUNGAL ISOLATES ON *MELOIDOGYNE INCOGNITA* INFESTING *SOLANUM MELONGENA* L.

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ABSTRACT

A study was conducted under pot conditions to testify the efficacy of neem seed powder; neem leaf powder and 2 fungal isolates, *Trichoderma harzianum* and *T. glaucum* to estimate pathogenicity of *M. incognita* infesting *Solanum melongena*. The freshly hatched second stage juveniles (J2) inoculated @ 2000J_2/pot and neem seed powder, neem leaves powder and fungal isolates *T. harzianum* and *T. glaucum*, were applied @ 0.2%. The observations of the study revealed that the infested brinjal plants showed maximum root gall formation and decrease in root and shoot length as well as their fresh and dry weight with the increase in levels of infestations as compared to control. It was also observed that the brinjal plants amended with neem seed powder separately and with a combination of neem seed powder and *T. harzianum* showed better growth and significant reduction in gall formation (54.19% & 49.36% respectively) over untreated inoculated plants.

Key words: *Meloidogyne incognita*, Bio-management, *Trichoderma* spp. *Solanum melangena*

INTRODUCTION

The root-knot nematodes (*Meloidogyne* spp.) are sedentary endoparasites and are among the most damaging agricultural pests, attacking a wide range of crops (Barker *et al*., 1985). In India, four species of *Meloidogyne* are commonly found viz., *M. incognita, M. javanica, M. arenaria* and *M. hapla* and almost all cultivated plants of economic importance are vulnerable to attack by one or the other species or by races of root-knot nematodes in different parts of the world (Sasser, 1980). Brinjal, member of the family Solanaceae, is a popular vegetable of the masses and native of India. *M. incognita* is a major constraint in the production of brinjal and causes severe loss. Control of root-knot nematodes has been accomplished primarily through chemical nematicide, crop rotation, resistant varieties where available (Widmer and Abawi, 2000). The soil amendment by various organic, inorganic additives and botanicals with bioinoculants has been developed as a new trend in recent researches to control *Meloidogyne* spp. (Ahmad and Khan, 2004; Brand *et al*., 2004). Barua and Bora (2008) demonstrated efficacy of *Trichoderma harzianum* against *M. incognita*.

The present investigation was undertaken to find out the potentiality of neem seed powder, neem leaves powder and two of the fungal species *viz. Trichoderma harzianum* and *T. glaucum* as soil amendment against *M. incognita* infesting brinjal plants.

MATERIALS AND METHODS

The experiment was conducted in earthen pots (15cm dia) filled with 2kg sterilized sandy loam soil. Single seedling was transplanted in each pot and inoculated, served as control. Neem leaves and seed powder was applied alone @ 0.2% and in combination with fungal isolates @ 0.2%.

Plant and Inoculation material

Brinjal seedlings cv. Pusa Purple long were raised in sterilized sandy loam soil along with sterilized compost manure. Three weeks old seedlings were transplanted in earthen pots. Infected root samples were collected from the nearby brinjal fields of District Meerut. The infected roots were washed with running tap water and egg masses were isolated in petriplates on moist double layered tissue paper and incubated for two days at 28±2^{0}C in BOD. Isolation of fungus was done by dilution method, as 1g of soil (air dried) diluted with 9ml of distilled water and further diluted up to 7 times by mixing 1ml into next 9 ml water. Thus from the prepared stock solution 1ml was poured on PDA media plates. All the petriplates were incubated in BOD at 25±2^{0}C for 5 days. The grown colonies of different fungi were separated by single spore method (Johnson and Booth, 1914) identifies with the help of taxonomic keys. The fungal strains/isolates were identified as *T. harzianum* and *T. glaucum*. Neem seed and leaves powder were prepared after drying the green seeds and green leaves in oven at 70^{0}C for one hour, grinded in mortar and sieved to make fine powder. After one week, the transplantation seedlings were inoculated with 2000J_2/pot and each level was replicated 4 times and un-inoculated kept as control. After 7 days of inoculation, the study was conducted as per experimental design:

T_1 = Un-infested Control

T_2 = Infested Control

T_3 = Infested plants treated with neem seed powder

T_4 = Infested plants treated with neem leaves powder

T_5 = Infested plants treated with *T. harzianum*

T_6 = Infested plants treated with *T. glaucum*

T_7 = Infested plants treated with neem seed powder + *T. harzianum*

T_8 = Infested plants treated with neem seed powder + *T. glaucum*

Inoculation and soil amendment

The experiment was terminated after 75 days of inoculation and plants were removed carefully. Roots were washed thoroughly in tap water for root-knots counting. Plant growth parameters and pathogenicity was recorded from all the treatments.

The recorded data were subjected to analysis by using SPSS software. Regression analysis was used to determine the relationships between root knot index and total plant fresh weight and dry weight separately

RESULT AND DISCUSSION

In the present investigation, the observations were recorded on various plant growth parameters viz. length and weight (fresh and dry) of root and shoot and estimation of root knot formation in treated and un-treated plants (Table-1, 2). The highest root length was recorded in the infected plants amended with neem seed powder followed by infected plants amended with neem seed powder and *T. harzianum* (Table-2). The highest root weight (fresh and dry) was recorded in infected control plants, followed by infected plants treated with *T. glaucum.* The shoot length was recorded highest in healthy uninfected plants followed by infected plants amended with neem seed powder. The highest shoot weight (fresh and dry) was recorded in uninfested plants followed by plants treated with neem seed powder (Table-1). Observations on nematode multiplication by the formation of root knots and release of egg masses on root surface were recorded and recorded the least in infected plants amended with neem seed powder (45 and 37.75 respectively) and highest in infected untreated plants (98.25 and 100.75 respectively, Table - 2). The relationship relationship between root knot index and total plant fresh weight and dry weight were established and presented in Table - 3.

Neem seed contains high amount of nitrogen and is used as manure in field. Many workers have studied the use of neem seed and some inorganic fertilizer for the increased plant growth (Singh and Sitaramaiah, 1971; Gowda, 1972; Khan *et al*., 1974). Addition of neem seed powder considerably increased phenolic content of the soil and this is due to the presence of phenol in undecomposed seeds (Alam *et al*., 1980; Sitaramaiah and Singh, 1978). Akhtar and Malik (2000) have reported that the extract of neem seeds

Table 1. Effect of *Meloidogyne incognita* infestation on brinjal plants and efficacy at different inauculates.

Treat-ment	Shoot			Root			No. of root knot /root	% reduction in knot information	No. of egg mass/ root	% reduction in egg mass	RKI
	Length (cm)	Fresh wt. (gm)	Dry wt. wt.(gm)	Length (cm)	Fresh (gm)	Dry wt. (gm)					
T_1	32.1±0.83 (34-30)	21.68±0.22 (21.2-22.1)	5.67±0.07 (5.5-5.8)	11.4±0.59 (10.5-12.9)	1.21±0.06 (1.0-1.3)	0.64±0.03 (0.5-0.7)	NIL		NIL		NIL
T_2	18.6±0.55 (19.5-17)	4.28±0.09 (4.02-4043)	0.14±0.02 (0.1-0.2)	6.55±0.33 (6-7.5)	8.44±1.05 (8.2-8.6)	1.61±0.33 (1-2.4)	98.25±1.55 (95-100)	———-	100.75±1.18 (95-102)	—-	4
T_3	29.8±0.31 (30.5-29)	16.78±0.29 (17.4-16)	4.63±0.18 (4.1-4.9)	17.7±1.11 (15-20)	2.08±0.51 (2.0-2.1)	0.77±0.26 (0.4-1.5)	45.0±1.80 (40-45)	54.19	37.75±1.25 (33-41)	63.0	2
T_4	22.7±0.77 (24.2-21.1)	11.11±0.47 (10-11.9)	1.59±0.16 (1.1-1.8)	13.2±1.41 (10-16.5)	4.68±1.12 (4.5-4.8)	1.06±0.08 (0.8-1.1)	85.2±1.50 (82-89)	13.23	86.5±1.44 (85-91)	14.14	4
T_5	23.8±1.25 (20-29.9)	9.43±0.23 (8.9-9.9)	1.20±0.08 (1.0-1.4)	10.1±0.92 (8-12.5)	5.45±1.32 (5.2-5.7)	1.09±0.44 (0.5-2.4)	88.75±0.96 (86-92)	9.66	90.5±1.38 (90-92)	10.17	4
T_6	23.2±1.16 (20-25.5)	6.35±0.41 (5.1-6.8)	0.44±0.13 (0.2-0.7)	9.3±0.56 (8.2-10.5)	6.34±1.42 (5.9-6.9)	1.21±0.46 (0.3-2.5)	92.75±1.78 (90-97)	5.59	98.0±1.49 (95-102)	2.72	4
T_7	27.3±0.62 (28-25.5)	15.30±0.25 (14.8-15.9)	2.43±0.15 (2.1-2.8)	16.7±0.43 (16-18)	4.19±0.96 (3.2-5)	1.03±0.26 (0.3-1.4)	49.75±1.58 (45-52)	49.36	50.0±1.65 (52-60)	50.37	2
T_8	24.9±0.41 (24-26)	14.35±0.14 (14-14.6)	2.33±0.16 (2-2.7)	12.8±1.24 (10.5-15)	3.85±0.94 (3.6-4)	0.82±0.11 (0.4-0.9)	55.5±0.75 (53-57)	43.51	65.75±0.87 (65-68)	34.73	3

$\mathbf{T_1}$ = Un-infested Control; $\mathbf{T_2}$ = Infested Control; $\mathbf{T_3}$ = Infested plants treated with neem seed powder; $\mathbf{T_4}$ = Infested plants treated with neem leaves powder; $\mathbf{T_5}$ = Infested plants treated with *Trichoderma harzianum*; $\mathbf{T_6}$ = Infested plants treated with *Trichoderma glaucum*; $\mathbf{T_7}$ = Infested plants treated with neem seed powder + *T. harzianum*; $\mathbf{T_8}$ = Infested plants treated with neem seed powder + *T. glaucum*; **RKI**= Root Knot Index

Table - 2. Efficacy of various treatments on different growth parameters of *Solanum melongena* infested with *Meloidogyne incognita*.

S. No.	Plant Growth and Disease Parameters	Level of Various Treatments
1.	Shoot Length	$T_1 > T_3 > T_7 > T_8 > T_5 > T_6 > T_4 > T_2$
2.	Shoot Weight	$T_1 > T_3 > T_7 > T_8 > T_4 > T_5 > T_6 > T_2$
3.	Root Length	$T_3 > T_7 > T_4 > T_8 > T_1 > T_5 > T_6 > T_2$
4.	Root Weight	$T_2 > T_6 > T_5 > T_4 > T_7 > T_8 > T_3 > T_1$
5.	Number of root knot/root	$T_2 > T_6 > T_5 > T_4 > T_8 > T_7 > T_3$
6.	Number of egg masse/root	$T_2 > T_6 > T_5 > T_4 > T_8 > T_7 > T_3$

T_1 = Un-infested Control; **T_2** = Infested Control; **T_3** = Infested plants treated with neem seed powder; **T_4** = Infested plants treated with neem leaves powder; **T_5** = Infested plants treated with *Trichoderma harzianum*; **T_6** = Infested plants treated with *Trichoderma glaucum*; **T_7** = Infested plants treated with neem seed powder + *T. harzianum*; **T_8** = Infested plants treated with neem seed powder + *T. glaucum*.

Table - 3. Regression depicting correlation coefficient between plant weight (fresh & dry) and root knot index.

Source of Variation	Degree of freedom	Sum of Squares	Mean Square	F	Significance
Coefficient Correlation with Fresh Plant Weight					
Regression	1	47.632	47.632		
Residual	6	40.984	6.831	6.973	0.039
Total	7	88.616			
Y = 6.2350 – 0.0507X, P > 0.01					
Coefficient Correlation with Dry Plant Weight					
Regression	1	12.180	12.180		
Residual	6	7.897	6.831	9.254	0.023
Total	7	20.077			
Y = 6.2350 – 0.5590X, P > 0.05					

contain phenols and tannins which are toxic to nematode hatching and mortality.

Neem seed is full of several chemical constituents, among which the limonoids are the compounds belonging to B-furano-triterpenoids alone which have been found to be nematotoxic. The presence of phenolics causes the retardation of giant cell formation and poor nutrition of root-knot larvae (Sitaramaiah and Singh, 1978). *T. harzianum* contain enzymatic secretions viz. chitinolytin, proteases and proteinases etc., which are antinemic attributes. Proteinases are involved in nematophagous fungal activity on nematode eggs (Dackman *et al*., 1989). The J_2 cuticle is mainly composed of proteins (Blaxter and Robertson, 1998); therefore it may be assumed that improved proteolytic activity of the antagonist would lead to increased bio-control ability of neem seed.

Results showed significant suppression of *M. incognita* by *T. harzianum* and good plant health (Table-2, 3). The possible mechanism involved in *Trichoderma* antagonism had been studied intensively in terms of antibiotic and enzyme production as hyphal interactions (Dennis and Webster, 1971; Elad *et al*., 1982). Production of chitinases may have direct significance in the parasitism of *Trichoderma* on *M. incognita* as these enzymes function by breaking down the polysaccharides, chitin and â-glucan that are responsible for the rigidity of fungal cell walls thereby destroying the outer most body covering of the nematode. *T. harzianum* solubilizes the inorganic elements in the soil and provide to the plants. *M. incognita* may also be killed while feeding on root tissues by the systemic action of these nematicides when they are absorbed by the plant roots and Translocated in the plant system. *Trichoderma harzianum* colonize and penetrate plant root tissues and initiate a series of morphological and biochemical changes in the plant considered to be a part of plant defense response, which in the end leads to induced systemic resistance (ISR) in the entire plant.

Significant suppression of nematode multiplication by the neem seed and *T. harzianum* was due to its capability of altering root exudates, which could alter nematode behavior and suppress nematode population in root system

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REFERENCES

Ahmad, S.F. and Khan, T.A. (2004). Management of root knot nematode, *Meloidogyne incognita*, by integration of *Paecilomyces lilacinus* with organic materials in chilli. *Arch. Phytopathol. Plant Protec.,* **37 (1):**35-40.

Akhtar, M. and Malik, A. (2000). Roles of organic amendments and soil organisms in the biological control of plant parasitic nematode: a review. *Bioresource Technol.,* **74:** 35-47.

Alam, M.M., Ahmad, M. and Khan, A.M. (1980). Effect of organic amendments on the growth and chemical composition of tomato, eggplant and chilli and their susceptibility to attack by *Meloidogyne incognita* . *Plant & Soil*, **57:** 231-236.

Barker, K.R., Carter, C.C. and Sasser, J.N. (1985). *An Advanced treatise on Meloidogyne. Vol. 1. Biology and Control.* North Carolina State University, Raleigh.

Barua, L. and Bora, B.C. (2008). Comparative efficacy of *Trichoderma harzianum* and *Pseudomonas fluorescens* against *Meloidogyne incognita* and *Palastonia solanacearum* complex in brinjal. *Indian J. Nematol.* **38(1):** 86-89.

Blaxter, M.L. and Robertson, W.M. (1998). The cuticle. In: Free Living and Plant Parasitic Nematodes (RN Perry & DJ Wright, Ed) p25-48. CAB International, Wallingford, UK

Dackman, C., Chet, I. and Nordbring-Hertz, B. (1989). Fungal parasitism of the cyst nematode *Heterodera schachtii*: Infection and enzymatic activity. *Microbiol. Ecol.*, **62:** 201-208.

Debora, B., Sevastianos R., Pandey A., Zilioli, P.C., Pohl, J. and Soccol, C.R. (2004). Development of bionematicide with *Paecilomyces lilacinus* to control *Meloidogyne incognita*. *Appl. Biochem. Biotech*., **118 (1-3):** 81-88.

Dennis C, Webster J. Antagonistic properties of species-groups of *Trichoderma* III. Hyphal interaction. *Transactions of British Mycological Society.* 1971; **57**:363–369.

Elad Y, Chet I, Henis Y. Degradation of plant pathogenic fungi by *Trichoderma harzianum* . *Canad. J. Microbiol.,* 1982;**28**:719–725.

Gowda, D.N. (1972). Studies on comparative efficacy of oil cakes on control of root knot nematode, *M. incognita* on tomato. *Mysore J. agric. Sci*., **6:** 524-525.

Khan, A.M., Mashkoor, M. and Ahmad, R., (1974). Mechanism of control of plant parasitic nematodes as a result of application of oil cakes to the soil. *Indian J. Nematol.,* **4:** 93-96.

Sasser, J.N. (1980). Root knot nematodes: a global menace to crop production. *Plant Dis.,* **64:** 36-41.

Singh, R.S. and Sitaramaiah, K. (1971). Control of root knot through organic and inorganic amendment of soil. Effect of oil-cakes and sawdust. *Indian J. Mycol. And Pl. Pathol.,* **1:** 20-29.

Sitaramaiah, K. and Singh, R.S. (1978). Effect of organic amendment on phenolic content of soil and plant and response of *Meloidogyne javanica* and its host related compounds. *Plant & Soil*, **50:** 671-679.

Widmer, T.L. and Abawi, G.S. (2000). Mechanism of suppression of *Meloidogyne hapla* and its damage by a green manure of sudan grass. *Plant Disease*, **84:** 562-568.