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Comparative efficacy of bio control agents against root knot nematode (*Meloidogyne incognita*) infecting brinjal

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Abstract
A replicated pot culture experiment was conducted in the net house of Department of Nematology Assam Agriculture University, Assam during *Rabi* season with bio formulations like *Trichoderma viride*, *Pasteuria penetrans*, FYM and *Glomus fasciculatum* to compare their effectivity against root knot nematode (*Meloidogyne incognita*), along with chemical check (Carbofuran 3G), in a poly house against a susceptible local variety of brinjal (JC1). Data on plant growth parameters and nematode infestations were recorded 8 weeks after inoculation. Treatment of soil with Carbofuran 3G @ 9 g/m^2 manifested best results of plant height (33.84cm), fresh weight of shoot (21.69 g), dry weight of shoot (5.22 g), fresh weight of root (12.84 g) and dry weight of root (1.32 g) and lowest infestation values of no. of galls (11.60), no. of eggmasses (17.20) and final nematode population (150.60/250cc soil) but soil treatment with *T. viride* conveyed significant results promoting plant growth and declining gall formation and nematode multiplication along with. The mechanism of mycoparasitism, antibiosis, and competition of *Trichoderma* has been widely studied. The chitinolytic enzyme system and cell wall degrading enzymes like gliotoxin, peptaibols plays a significant role in egg parasitism. Our result advocates wide scale application of selected metabolites like *Trichoderma sp* to induce host resistance and also represents a powerful tool for the implementation of IPM strategies to play a major role in crop protection and bio-fertilization.

Keywords: bio control agents, root knot nematode, brinjal, IPM

1. Introduction
Brinjal (*Solanum melongena* L.) also known as aubergine or eggplants, is one of the most important vegetable crops of India. Cultivated for its immature fruits, brinjal is considered as one of the most nutritive vegetable. It's a good source of calcium, phosphorous, iron, vitamin particularly 'B' group and also is a raw source of energy and carbohydrate [2]. In Assam, brinjal is cultivated both during *Kharif* and *Rabi* seasons and ranks 5th covering an area of 12,798 ha with an annual production of 167578 metric tonnes [1]. There is a wide spectrum of abiotic and biotic stress including pathogens causing different diseases in brinjal, with insects, fungi, bacteria and nematodes causing damage dominantly. Plant parasitic nematodes have a substantial impact on human welfare and economy [26]. These parasites are the most prevalent in the country. [15-17] and accounts for growth impairment [13, 20].With a growing dominance of nematodes in India, *Meloidogyne incognita* is considered as an important limiting factor in yield reduction of egg plants. Reddy [25] recorded 33.70 percent losses in yield due to attack of *Meloidogyne sp*. A crop loss of 39.17 percent due to attack of *M. incognita* on brinjal at Jorhat was recorded by Hazarika [10] in Assam. The current control of nematodes relies mainly on multiple fungicides and pesticides application that exert selection a pressure on *M. incognita* increasing the risk of nematode resistance development [12]. To add to it, (Moreover) the frequent and indecisive application of chemicals not only hinders the soil microbes but also becomes detrimental to the natural enemies and exhibits public health hazards [28]. Brinjal consumed as a raw entity may also result in unacceptable residues of pesticides due to indiscriminate use. Many biological agents have shown efficacy like *Trichoderma harzianum* has been found to be an effective biocontrol agent for the management of root-knot and other nematodes [21]. Furthermore, *Pasteuria penetrans*, an obligate parasite, has been widely investigated for its efficacy against root knot nematodes throughout the world [9]. VAM also exhibits promising growth parameters against the root knot nematode.To eliminate the probabilities of further deterioration of the environment, a comparative management practice

was imposed, which is lacking, to study the efficacy of biocontrol agents for the management of *M. incognita* on brinjal.

2. Materials and method

The experimental site was located in the net house of Department of Nematology Assam Agriculture University, Assam. Experiment was conducted during *Rabi* season of 2016-17 under pre requisite environmental conditions.

2.1 Nematode inoculum

Meloidogyne incognita, was raised from a single egg mass. For nematode reproduction, the most susceptible variety of tomato (cv. Pusa Ruby) was used as the host plant. Three week- old tomato plants were transplanted into pots containing 2.5 kg formalin sterilized sandy loam soil. One week after transplantation, the plants were each inoculated with approximately 1,000 freshly hatched second stage juveniles (J_2s) of *M. incognita* by making holes in the soil around the stem of each plant. The plants were kept in a green house at 25±2°C and watered as needed.

2.2 Extraction of *Trichoderma viride*

For mass production of the inoculum of *T. harzianum*, chopped wheat grains were immersed in water for about 10–12 h, surface dried using a paper towel, and 250 g was added to each of 500 ml capacity flasks. These were autoclaved at 15 psi for about 50 min. The sterilized wheat grains in flasks were inoculated separately with pure cultures of each of the antagonistic fungi and incubated at 25±1 °C for 15 days. The flasks were shaken on alternate days for uniform colonization of the fungus. The number of spores per gram of the grains were counted using haemocytometer after making spore suspensions in distilled water.

2.3. Preparation of *P. penetrans* endospore suspension and endospore count

P. penetrans-infected *Meloidogyne* females were dissected out from plant roots under a stereomicroscope in distilled water and kept in glass vials in a refrigerator till further use. Whenever required, the infected females were transferred to an Eppendorf tube (2 ml) in a small amount of distilled water just enough to submerge the nematodes. The females were crushed mechanically by using a plastic micro-pestle to release the endospores in water. The suspension was passed through a 500 mesh sieve and collected in a flask. The endospore count was done using a haemacytometer under a compound microscope (400 x). A stock of $4x10^6$ endospores per ml suspension was prepared for experimental purpose and preserved in a refrigerator till use.

VAM maintained on maize plants in green house was extracted by Gerdeman and Nicolson method and FYM obtained locally. A local susceptible variety of Brinjal (JC 1) which is grown popularly among farmers was subjected to exposure of nematodes and various treatments. Earthen pots of 20 cm diameter were cleaned and surface sterilized in 1% formalin. Each pots were filled with 2kg autoclaved and pulverized soil: sand: FYM mixture. The experiment was laid out in a Completely Randomized Design with six treatments each replicated five times. The various treatments were: T1: Vermicompost @ 1kg/m^2,T2: *Trichoderma viride* @ 2.5 kg/ha, T3: *Glomus fasciculatum* @ 600 spores/m^2, T4: *Pasteuria penetrans*@ $1*10^9$ spores/m^2, T5: Carbofuran @ 9gm/m^2, T6: *Meloidogyne incognita* @ 500 J_2/pot (Check).

The seeds were sown in the pot directly, with various recommended treatments 15 days prior to inoculation of seed, for proper establishment of biocontrol agents. Standardization of nematode in stock solution was done and IJ_2 were inoculated @ 1 J_2/ gm of soil.After 60 days of sowing**,** experiment was terminated and plants were uprooted for various morphometric observations like shoot length, fresh and dry shoot weight, root length fresh and dry root weight. Brinjal roots were also subjected to observations like number of galls /plant, number of egg masses/plant and root knot population in soil. Final nematode population was determined from 250gms of soil sample by Cobb's Sieving and Decanting technique [5] and modified Baermann's technique [30].

Statistical analysis was done according to Fisher's methods of analysis of variance at 5 % level of significance. Data of number of root galls and final nematode population were analyzed after square root and log transformation avoiding mean error.

3. Results

The chemical check carbofuran 3G imparted maximum shoot length non significantly followed by *Trichoderma viride* treatment with soil. (Table 1). The morphometric observations like fresh shoot weight and dry shoot weight (Table 1) had almost the same impact with chemicals and specifc biocontrol agents like (*T. viride*). The soil treatment with *T. viride* had effective results on root parameters lagging indistinctly behind chemical check carbofuran. *Trichoderma* increased the growth of roots and there was a distinct increase in the dry weight of roots. It was concluded that there was significant reduction in number of galls per plant in all treatments over check in which distinct results were obtained by chemical check closely followed by *T. viride* as biocontrol check.

Table 1: Effect of bioagents and chemicals on the plant growth parameters of brinjal

Treatments	Plant height (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)
T_1: *Vermicompost* @ 1kg/m^2	28.80	17.24	3.04	9.86	0.66
T_2: *Trichoderma harzianum* @ 2.5 kg/ha	31.45	20.04	4.31	11.29	0.86
T_3: *Glomus fasciculatum* @ 600 spores/m^2	31.38	19.83	3.88	11.04	0.77
T_4: *Pasteuria penetrans*@ $1*10^9$ spores/m^2	30.90	19.55	3.79	10.81	0.70
T_5: Carbofuran @ 9gm/m^2	33.84	21.69	5.22	12.84	1.32
T_6: *Meloidogyne incognita* @ 500 J_2/pot (Check - uninoculated)	18.92	12.46	1.49	5.02	0.33
S.Ed.(±)	1.59	1.10	0.61	0.80	0.15
$CD_{0.05}$	3.27	2.27	1.25	1.65	0.31

Means followed by the same letter in the superscript(s) are not significantly different

Table 2: Effect of bioagents and chemicals on host infection and nematode multiplication

Treatments	No. of galls	No. of eggmasses	Final nematode population/250cc soil
T_1: *Vermicompost* @ 1kg/m^2	27.2 (5.25)	30 (5.51)	253.73 (15.94)
T_2: *Trichoderma harzianum* @ 2.5 kg/ *ha*	18.20 (4.31)	18.00 (4..29)	175.06 (13.24)
T_3: *Glomus fasciculatum* @ 600 spores/m^2	21.0 (4.62)	20.80 (4.55)	185.40 (13.63)
T_4: *Pasteuria penetrans*@ 1*10^9 spores/m^2	23.20 (4.86)	26.40 (5.15)	235.49 (15.36)
T_5: Carbofuran @ 9gm/m^2	11.60 (3.47)	17.20 (4.20)	150.60 (12.29)
T_6: *Meloidogyne incognita* @ 500 J_2/pot (Check)	46.95 (6.86)	47.20 (6.89)	509.21 (22.57)
S.Ed.(±)	0.22	0.32	0.14
$CD_{0.05}$	0.45	0.66	0.29

Values of number of galls, eggmasses and final nematode population within parentheses are square root of transformed data

Observation of the data on eggmasses revealed that *Trichoderma viride* (T_2) registered maximum reduction in eggmasses following (T_5) (Table 2). The severity of disease and population of nematodes in soil (250gm) was significantly decreased in chemical check and pre soil treatment with *T. viride* when compared with chemicals.

4. Discussion

The chemical check carbofuran exhibited an increase in all morphometric observations and decrease in root galling and nematode population of root-knot nematode. Patil *et al*., [23] also had reported the maximum reduction in nematode population with carbosulfan application. Chemical nematicide (Carbosulfan) is most effective in increasing growth, reduction in galling [19] and also the reproduction of root-knot nematodes was lowest compared to bioagents.

Despite the propensity of nematicides to be a lethal to a broad range of soil organisms and nematodes, they also accelerated the development of resistant strains among nematodes. The chemical nematicides are outnumbered in disadvantages against biological agents. So attempts are been made to replace pesticides with less environment threating options [3]. In the experiment the next best treatments after chemical check Carbofuran 3G was soil treatment will *T. viride*. *Trichoderma* species have been long recognized as potential BCA of foliar [6] and soil borne diseases [22] The mechanisms of mycoparasitism, antibiosis and competition of *Trichoderma* spp. causing activation of plant defence system by producing enzymes like glucanases have been widely studied [12, 29]. The soil treatment with *T. viride* gave positive and conformatory results over check and resulted in increase in physical observable parameters of plant. Rao *et al.* [24] and Faruk *et al.* [7] confirmed *Trichoderma* strains to reduce nematode infestations and increase plant growth over control. Hasseb *et al.* [11] reported the interaction of *T. harzianum* and *Fusarium solani* to be detrimental against *M. incognita*. The reduction in galling might be due to the competitive ability of the *Trichoderma* species for the rhizosphere of root and its inoculation in to soil prior to 15 days before transplanting could have caused the reduction in galling percent.

Kumar and Khana [18] had highlighted that *T. viride* was efficient when eggmasses were inoculated prior to nematodes in pot culture. The reduction of egg mass of *M. incognita* may be due to parasitization of *Trichoderma* on eggmasses. *Trichoderma* excrete several lytic enzymes (glucanases, chitinases, proteases and lipases) to degrade cell wall components of other microbes [4]. Nematode eggs contain chitin and *Trichoderma* has ability to produce chitinolytic enzymes. Through direct parasitism of eggs, by increase in chitinolytic and protease activity, is introspected to be the explanation of reduction of nematode infestation in plants. [27]. The reduction of nematode eggs could have ultimately resulted in the reduction in final population of the nematodes in soil and also *Trichoderma* s*p* has specialized pressing organ appressoria that produce holes and thereby hyphae entering into the lumen of the target. Yang *et al.* [31] used *Trichoderma* in his studies as the agent to successfully suppress the final population of nematode. The application of *T. viride* to soil can effect in active colonization producing secondary metabolites including antibiotics and also solubilizing rock phosphate, metallic zinc, nitrogen use efficiency etc., so it can be suggested as a fruitful entity for enhanced crop growth and reducing *M. incognita* reproduction simultaneously maintaining eco-systems equilibrium alongside.

5. Conclusion

The control of pathogens by living organisms has an inclination towards it. The experiment conducted and the result analyzed advocates the high potential ability of bio control agents to control plant parasitic nematodes with minimal environmental defragmentation. *Trichoderma* with high diversification in soil and being a natural enemy for wide species of parasitic nematodes can be included in integrated nematode management technique. The results obtained are encouraging and further trails can be conducted in the field conditions to study the efficacy of *Trichoderma* alongside other biological agents in controlling the targeted nematode pest.

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