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Effect of Bio Control Agents in the Management of Citrus Nematode *(T. semipenetrans)* on Khasi Mandarin Seedling

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Studies on the effect of *Purpureocillium lilacinum, Glomus fasciculatum, Trichoderma harzianum,* vermicompost and carbofuran 3G in the management of *Tylenchulus semipenetrans* on *Khasi* mandarin under pot condition showed that all thetreatments were effective in increasing plant growth parameters of Khasi mandarin. All the treatments significantly decreased the final nematode population in soil and root over control. The treatment with vermicompost @ 15g/plant+ *T. harzianum* 10g/plant was found to be most effective in plant growth parameters of Khasi mandarin. The treatment with carbofuran 3G@ 4g/plant was found to be best in suppressing the final nematode population in soil and root followed by the treatment with *G. fasciculatum*@ 150spores/plant + *P. lilacinus*@10g/plant.

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1. INTRODUCTION

"Citrus is one of the most important fruit crops, particularly in the tropical and subtropical areas of the world. It includes different citrus fruit crops like oranges, lemons, grapes and limes. In Assam lemon, Sweet lemon (Mousambi) and orange occupied an area of 16058, 385 and 12680 ha with the production of 160339, 6056 and 179168 metric tonnes respectively" [1]. In Dibrugarh district, it covers 468 ha of Assam lemon with a production of 4067 metric tonnes and oranges 75 ha with a production of 966 metric tonne [2]. "The citrus nematode, *Tylenchulus semipenetrans* (Cobb) has been recognized as one of the greatest threats throughout the world which limits the production of citrus under a wide range of environmental and edaphic conditions (Parvez et al*.,* 2003). The pest is cosmopolitan in distribution and has been reported to cause losses of up to 43.3% of *C*. *aurantium"* [3].

In the State of California, *T. semipenetrans* was found to cause 10-60% reduction on growth and 10-20% in yields of lemon and oranges [4,5,6].

Emphasis is now focused on the biological method of nematode control, as the nematicides may have a toxic effect on non-target soil microfauna and flora, resulting in ecological imbalance. Among various bio-control agents, *Purpureocillium lilacinum,Glomus fasciculatum* and*Trichoderma harzianum* play an important role in nematode management.

Among the nematode-destroying fungi, there is an important group of fungi, which are capable of rapidly colonizing nematode reproductive structures and thus destroying females, cysts and eggs. The common genera of such fungi are *Cylindrocarpan, Exophiala, Fusarium, Gliocaladium, Purpureocillium, Phoma, Torula, Verticillium,* etc. The fungus *P.lilacinus*, a common soil hyphomycete has demonstrated tremendous potential as biocontrol agent of nematode [7].

"VAM fungi and plant parasitic nematodes are commonly found inhibiting the rhizosphere of the same plant, each having a characteristic but opposite effect on plant vigour. VAM fungi may stimulate plant growth, whereas the obligate plant parasitic nematodes usually suppress plant growth. It has been observed that the plants colonized with mycorrhizal fungi grow well inspite of the presence of damaging levels of plant parasitic nematodes and this may be due to improved plant nutrition especially phosphorus" [8].

Ravichandra [9] reported that "*Trichoderma* sp. is one of the most commonly used and popular bioagents against nematodes. Mechanisms involved in antagonism of *Trichoderma* sp. against nematode management are mycoparasitism, antibiosis, competition, tolerance to biotic and abiotic stresses through an enhanced root development, solubilisation and sequestration of inorganic plant nutrients and induce resistance".

"The application of vermicompost has a positive effect on plant nutrition, photosynthesis, the chlorophyll content of the leaves and improves the nutrient content of the different plant components and improves the plant quality and act as a deterrent to pests and diseases" [10]. Domínguez et al. [11] investigated "the potentiality of vermicompost in the suppression of plant pathogens, insects, mites and plant parasitic nematodes. He reported that the addition of vermicompost to soils increases microbial diversity, providing a wider range of microorganisms that can act as biocontrol agents against different plant pathogens including nematodes".

Very little work has so far been done on the citrus crop in Assam, except work [12] on management in Khasi mandarin (*Citrus reticulata* Blanco), Deka [13] on pathogenicity and management on *Citrus jambhiri*. Therefore present investigation is an attempt to study the management of citrus nematode, *T. semipenetrans* on Khasi mandarin.

2. MATERIALS AND METHODS

The pot experiment was carried out in the net house of the Department of Nematology to study the effect of *Purpureocillium lilacinum, Glomus fasciculutum, Trichoderma harzianum*, vermicompost and carbofuran 3G in the management of citrus nematode, *Tylenchulus semipenetrans* on khasi mandarin. Khasi mandarin (*Citrus reticulata*) seedlings were planted in a 3kg capacity pot filled with sterilized soil and inoculated with *T. semipenetrans* @ 1 nematode per gram of soil. The pots were arranged in a completely randomized design (CRD) with 5 replications for each treatment. Vermicompost and carbofuran 3G were applied 15 days and 3 days before planting respectively. The formulation of *Purpureocillium lilacinum* and *Trichoderma harzianum* was applied at the time of planting according to the treatments. Infested soil of *G. fasciculatum* was applied at the time of planting.

The treatments were:

T1 =*Purpureocillium lilacinum*@ 20g/plant;
T2 = *Glomus fasciculatum*@ 300 spores/plant;
T3 = Vermicompost@ 15g/plant + *Trichoderma harzianum* 10g/plant;
T4 = *Glomus fasciculatum* @ 150 spores/plant + *Purpureocillium lilacinum*@10g/plant ;
T5=Vermicompost@ 15g/plant + *Purpureocillium lilacinum*@ 20g/plant;
T6 = Carbofuran 3G @ 4 g/plant (W/W). T7 = Control (Nematode alone).

Observations were made on the length of the shoot and root, fresh weight of the shoot and root, and final nematode population in soil and root after six months of nematode inoculation.

3. RESULTS AND DISCUSSION

In the present investigation, the result revealed that all the treatments were found to be effective in increasing the plant growth parameters of Khasi mandarin seedlings. Maximum increase in plant growth parameters *viz*., shoot length (38.46cm), fresh weight of shoot (14.30g) and root length (31.44cm) were recorded in the treatment with vermicompost @ 15g/plant +*Trichoderma harzianum*@ 10g/plant (Table 1). The results were in agreement with The unissen et al. (2010) who observed that “vermicompost contains plant nutrients including N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu and B, and the uptake of which has a positive effect on plant nutrition, photosynthesis, the chlorophyll content of the leaves and improves the nutrient content of the different plant components (roots, shoots and the fruits). High percentage of humic acids in vermicompost contributes to plant health, as it promotes the synthesis of phenolic compounds such as anthocyanins and flavonoids which may improve the plant quality and act as a deterrent to pests and diseases”. The increased growth parameter of plants observed in the present study may also be due to the use of *Trichoderma* as it can release plant growth-promoting substances during its multiplication in the rhizosphere like indole 3-acetic acid, indole 3- acetaldehyde, indole 3- ethanol which may play role in mediating plant growth promoter [14]. Weeder et al. (2008) also reported that *Trichoderma* is highly rhizosphere competent i.e., able to colonize on roots as it develops, thus promoting plant growth. Bio control agents *Pseudomonas fluorescens*, *Trichoderma viride*, *T. harzianum* and *Pochonia chlamydosporia* are effective to reduce the citrus nematode population in nine-year old citrus, *Citrus limonia* L. garden and in increasing the fruit yield of *Citrus limonia L.* [15].

The minimum final nematode population in soil (78) was recorded in the treatment with carbofuran 3G @ 4 g/plant followed by the treatment with *G. fasciculatum*@150 spores/plant+ *P. lilacinus*10g/plant (110). All the treatments were effective in decreasing nematode population in soil over control (Table 2).

In regards to the final nematode population in root, minimum nematode population in (34.00) was recorded in the treatment with carbofuran 3G @ 4 g/plant followed by the treatment with *G. fasciculatum*@150spores/plant+*P.lilacinus*10g/plant (44.00). All the treatments were effective in decreasing the nematode population in the root over control.

Khan [16] reported that the hyphal network of vam increases the absorption area of the root system and therefore aids in water uptake, absorption and translocation of nutrients, particularly phosphorus. The plants heavily colonized with AM fungi can grow well in spite of the presence of damaging levels of nematodes. The favourable effect of mycorrhizae in decreasing the disease intensity in nematode- affected plants has been demonstrated in various crops. Baghel *et al*. (1990) reported that VAM inoculation stimulated the growth of *Citrus jambhiri* seedlings, while the citrus nematode, *Tylenchulus semipenetrans* decreased growth. When the 2 organisms were inoculated simultaneously the adverse effect of the nematode was partly nullified by the fungus and limited the development of the nematode. Reddy *etal*. (1995) observed that the inoculation of endomycorrhizal fungi *G. fasciculatum* along with soil amended with neem cake had reduced the population of *T. semipenetrans* on acid lime. Esser and El-Gholl (1993) reported that vegetative hyphae of *P. lilacinus* enter the gelatinous matrix of root-knot nematode, or grow into the vulva or open cyst nematode. When it enters inside a penetration peg forms and grows in to the eggshell. Penetrated eggs swell and buckle. Moznur [12] studied the efficacy of *Paecilomyces lilacinus* against *T. semipenetrans* on khasi mandarin at different levels and showed increased plant growth as well as reduced nematode population effectively with the increase in the level of *P. lilacinus*. Walode et al. [17] observed that integration of *P. lilacinus* (4g/kg soil), *Trichoderma harzianum* (4gm/kg soil) and *Glomus fasciculatum* (500spores/kg soil) was effective in increasing plant growth parameters and in reducing citrus nematode population both in soil and roots of rough lemon. Mahanta et al.

[18] found that the application of *P. lilacinus* infected grain cause a significant reduction in nematode population in soil and root over control. The minimum nematode population was recorded in the treatment with *P.lilacinus* infected grain@30gm/tree basin. Sreenivasan et al. (2021) observed the individual and combined effect of neem seed kernel extract (NSKE) and liquid *Purpureocillium lilacinum* formulation delivered through a drip irrigation system on acid lime trees naturally infested with *Tylenchulus semipenetrans*.

Both bio products were applied at 4l/ha, but NSKE alone delivered twice at 30 days interval and their effect was compared with carbofuran 3G at 100g/tree as spot application. Results revealed that both NSKE and *P. lilacinum* tested, significantly supressed *T. semepenetrans* density in soil and acid lime roots. This combined treatment reduced *T. semipenetrans* density in soil by 70.0-88.8% and in roots by 73.6-79.2%. The integrated approach caused 26.3-33.6% more fruit yield with higher (1:3:2-1:4:2) cost benefit ratio.

Kumar [19] reported that “various microbes and soil-dwelling predatory mites (Acari: Mesostigmata) have been evaluated as augmentative biological control agents against citrus parasitic nematodes.They include fungi (*Trichoderma* spp., *Purpureocillium lilacinum*, *Pochonia chlamydosporia*, and mycorrhizae, *Glomus* spp.) and bacteria (Bacillus spp., *Pseudomonas fluorescens, Streptomyces avermitilis*, and *Pasteuria* spp.), that have been applied against parasitic nematodes in different citrus cultivars. Integration of bionematicides with other management tools as well as the conservation and enhancement of indigenous antagonists, will further enhance the management of citrus parasitic nematodes”.

“Non-chemical practices which could substantially help in preventing and controlling *Tylenchulus semipenetrans.* This approach employs preventive techniques such as include eradication of infected plants, crop husbandry, sanitation, soil disinfection and solarization, incorporation of organic soil amendments, and crop rotation and scions, and unfavorable horticultural features” [20-23].

Table 1. Effect of bio control agents on the growth parameters of Khasi mandarin

Sl No.	Treatments	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Fresh root weight (g)
1	T1	28.32^{d}	24.90^{c}	9.80 ^{d}	3.54c
2	T2	33.56 ^{c}	27.78^{b}	11.02^{bcd}	5.04^{b}
3	T3	38.46 ^{a}	31.44^{a}	14.30 ^{a}	5.42^{a}
4	T4	35.16^{bc}	27.32^{b}	11.92^{bc}	5.28^{b}
5	T5	37.16^{ab}	30.36^{a}	12.36^{b}	5.36^{a}
6	T6	30.14 ^{d}	23.64^{c}	10.26^{cd}	2.98^{c}
7	T7	18.18 ^{e}	16.14^{d}	5.68^{e}	2.00^{d}
	S.Ed(±)	1.14	0.99	0.79	0.25
	CD at 5%	2.34	2.03	1.63	0.52

Note: Values of shoot and root length, fresh weight of shoot and root within parentheses are square root ($\sqrt{x}$+ 0.5) transformed data Mean followed by the same letter in the superscript(s) are not significantly different

Table 2. Effect of Bio control agents on development of *T. semipenetrans* on Khasi mandarin

Sl No.	Treatments	Soil nematode population (200 cc)	%Decrease over control	Total Nematode population per root system (2g)	%Decrease over control
1	T1	138 (12.21)[bc]	63.87	58 (8.07)[bc]	60.8
2	T2	130 (11.87)[bc]	65.96	56 (7.95)[bc]	62.16
3	T3	146 (12.55)[b]	61.78	60 (8.19)[b]	59.45
4	T4	110 (10.94)[c]	71.2	44 (7.13)[cd]	70.27
5	T5	128 (11.76)[bc]	66.49	52 (7.68)[bc]	64.86
6	T6	78 (9.28)[c]	80	34 (6.31)[d]	77.02
7	T7	382 (20.01)[a]	-	148 (12.64)[a]	-
	S.Ed(±)	0.63		0.44	
	CD at 5%	1.30		0.91	

Note: Values of final nematode population within parentheses are square root ($\sqrt{x}$+ 0.5) transformed data
Mean followed by the same letter in the superscript(s) are not significantly different

4. CONCLUSION

In the present investigation the treatment with with vermicompost @ 15g/plant + *Trichoderma harzianum*@ 10g/plant was found to be best in increasing plant height, root length, fresh weight of shoot and root. All the treatments were found to be effective in increasing the plant growth parameters and reducing the nematode population in soil and root over control. Among the biocontrol treatments *G. fasciculatum*@ 150 spores/plant + *P. lilacinus*@ 10g/plant was found to be effective in suppressing the nematode population in soil as well as in root.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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