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Research Article

BIOLOGICAL CONTROL OF ROOT KNOT NEMATODE *Meloidogyne incognita* IN BHENDI

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Abstract: Biological control of root knot nematode with antagonistic fungiis a promising technique which may be incorporated in integrated nematode management. Hence, the bio control based technology involving for the management of bhendi nematodes were investigated. An experiment was conducted under glass house conditions for the management of root knot nematode *Meloidogyne incognita* in bhendi with different bio-control agents. Bhendi seed (cv.co2) were planted @ five seeds/pot filled with 5 kg steam sterilized soil mixture. The pots were watered periodically and one week after planting, seedlings were thinned to one per pot and inoculated with freshly hatched second stage juveniles of *M. incognita*. Various treatments tested were T_1-Seed treatment of *Purpureocillium lilacinum*@ 10g/kg, T_2-Seed treatment of *Trichoderma viride* @ 10g/kg, T_3-Seed treatment of *Pseudomonas fluorescens*@ 10g/kg, T_4-Soil application of *P. lilacinum*@ 2.5kg/ha, T_5-Soil application of *T. viride*@ 2.5kg/ha, T_6-Soil application of *P. fluorescens*@ 2.5kg/ha, T_7-Soil application of Carbofuran 3G @ 33kg/ha and T_8-Untreated control. At 90 days after planting, the plants were uprooted with roots intact and observations of height of the shoot (cm), length of root (cm), fresh shoot weight (g), and fresh root weight(g) dry shoot weight (g), were recorded. Besides, nematode population/200 g soil, number of adult female/g root, number of eggs/egg mass and root knot index were also recorded. Among the bio control agent, soil application of *P. lilacinum*@ 2.5kg/ha recorded the maximum shoot length of 35.27 cm, fresh shoot weight of 16.45g, dry shoot weight 6.18g, fruit yield 54.34g and increase the root length of 28.22 cm, fresh root weight 12.50g and dry root weight of 5.54g was recorded. The treatment was on par with seed treatment of *P. lilacinum*@ 9g/kg. Among the different types of bio-control agents, highest reduction in root knot index (1.0), Maximum reduction in number of *M. incognita* adult females (12.42), number of egg masses/g root (5.47), number of eggs per egg mass (92.22), and soil nematode population (98.33) was recorded in soil application of *P. lilacinum*@ 2.5kg/ha followed by seed treatment of *P. lilacinum*@ 10g/kg.

Keywords: *Biocontrol agents, Root knot nematode, Bhendi*

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Introduction

Okra (*Abelmoschus esculentus* L.) is one of the commercially exploited vegetable crops and commonly known as Lady's finger or Bhindi in different parts of the world. This crop has been cultivated throughout the tropical and warm temperate regions of the world for its green tender fruits. Bhendi is enriched with carbohydrates, proteins, vitamins, antioxidants, minerals, and dietary fibre. The root knot nematode, *Meloidogyne incognita* is one of the major constraints in the production of bhendi in tropical and subtropical regions. The root-knot nematodes (Meloidogyne spp.) are sedentary endoparasites and are among the most damaging agricultural pests attacking a wide range of crops [1] in particular vegetables, causing dramatic yield losses mainly in tropical and sub-tropical agriculture [2]. The infection begins with root penetration of second-stage juveniles (J2) that hatch in soil from eggs encapsulated in egg masses produced by females on infected roots. *M. incognita*, a root knot nematode, causes 14.1% of yearly losses in bhendi in India. [2].

Estimated yield losses of up to 90% have been recorded under field conditions, depending upon initial soil nematode population densities [3,4]. Present strategies for nematode management largely depend on cultural practices such as crop rotations are widely used but not effective when adopted individually. The use of resistant varieties for commercial purpose has limited scope due to lack of resistance genes in cultivable crops.

At the moment, none of the commercial okra cultivars grown in India are *M. incognita* tolerant or resistant. Furthermore, the indiscriminate use of chemical nematicides for the management of plant parasitic nematodes has negative effects on human health, beneficial organisms, and the environment, and the phase out of front-line chemical nematicides has fueled research into safe and cost-effective alternatives. The external symptom on the aerial part of affected plant includes dwarfing, foliar discoloration, wilting and premature death of the plants. However, the external manifestation of the symptoms depends on severity of nematode infection. Even though application of nematicides for the control of root-knot nematodes has been found effective, nowadays use of bio-agents is encouraged due to economical and eco-friendly method of nematode management. Use of beneficial microorganism as talc formulation is explored extensively for the management of nematode population in various crops. Hence, an attempt was made to study the biocontrol agents against *M. incognita* in bhendi.

Materials and Methods

M. incognita inoculum isolated from naturally infected tomato plants was obtained from pure culture generated by single egg mass and maintained on roots of tomato plants (*Solanum lycopersicum* L., nematode susceptible variety) in greenhouse.

Table-1 Effect of bio-control agents with nematicide on plant growth characters of tomato inoculated with Meloidogyne incognita

Treatments	Shoot length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fruit yield (g/plant)	Root length (cm)	Fresh root weight (g)	Dry root weight (g)
T_1-Seed treatment of *Purpureocillium lilacinum*@ 10g/kg	35.27	16.45	6.18	54.34	28.22	12.50	5.54
T_2-Seed treatment of *Trichoderma viride* @ 10g/kg	26.41	14.37	5.48	48.17	23.15	7.42	3.88
T_3-Seed treatment of *Pseudomonas fluorescens*@ 10g/kg	24.36	12.42	5.08	46.31	20.11	7.17	3.41
T_4-Soil application of *P. lilacinum*@ 2.5kg/ha	32.28	15.20	5.56	50.42	25.39	9.86	4.10
T_5-Soil application of *T. viride*@ 2.5kg/ha	23.05	11.64	4.77	42.52	21.11	7.30	3.12
T_6-Soil application of *P. fluorescens*@ 2.5kg/ha	21.86	13.42	4.55	40.91	19.32	7.09	3.09
T_7-Soil application of Carbofuran 3G @ 33kg/ha	29.52	14.27	5.01	42.42	22.17	8.17	3.61
T_8- Untreated control	12.46	8.88	3.42	32.17	11.46	4.19	2.58
CD (P=0.05)	3.30	0.88	0.48	14.45	2.21	0.68	0.21

Table-2 Effect of bio-control agents with nematicide on nematode population

Treatments	No. of females per g of root	No.of egg masses per g of root	No. of eggs / egg mass	Nematode population in 200g soil	Root Knot Index
T_1-Seed treatment of *P. lilacinum*@ 10g/kg	12.42	5.42	92.22	110.26	1.00
T_2-Seed treatment of *T. viride* @ 10g/kg	21.32	12.62	121.32	128.42	2.22
T_3-Seed treatment of *P.fluorescens*@ 10g/kg	25.35	18.48	138.40	143.23	3.17
T_4-Soil application of *P. lilacinum*@ 2.5kg/ha	15.68	7.64	109.18	121.32	1.42
T_5-Soil application of *T. viride*@ 2.5kg/ha	24.16	14.18	128.13	132.52	2.56
T_6-Soil application of *P. fluorescens*@ 2.5kg/ha	26.15	16.52	131.52	137.63	2.29
T_7-Soil application of Carbofuran 3G @ 33kg/ha	19.36	11.23	125.03	120.82	1.89
T_8- Untreated control	56.40	35.84	292.18	309.14	6.42
CD (P=0.05)	2.72	1.85	5.80	6.32	0.38

The experiment was conducted in the greenhouse of Department of Nematology, Anbil Dharmalingam Agricultural College and Research Institute, Navalurkuttapattu, Trichy during 2021-2022 following Complete Randomized Design (CRD) with eight treatments and each replicated three times. Seeds of bhendi var. CO_4 were planted @ three seeds /pot filled with 5 kg steam sterilized soil mixture containing 2 parts red soil, 1 part sand and 1 part well decomposed farm yard manure. One week after planting, seedlings were thinned to one number per pot. The pots were watered periodically and after seven days 5000 newly hatched juveniles from egg masses of *M. incognita* (extracted from tomato plants) were inoculated into 7.5 cm depth around the root zone of the plant in each pot. The talc based formulation of *P. lilacinum* was obtained from the Department of Nematology and *T. viride* and *P. fluorescens* were obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India. The treatments were imposed as T_1-Seed treatment of *Purpureocillium lilacinum* @ 10g/kg of seeds, T_2-Seed treatment of *Trichoderma viride* @ 10g/kg of seeds, T_3-Seed treatment of *Pseudomonas fluorescens* @ 10g/kg of seeds, T_4-Soil application of *P. lilacinum* @ 50mg/ pot with 5kg capacity, T_5-Soil application of *T. viride* @ 50mg/ pot with 5kg capacity, T_6-Soil application of *P. fluorescens* @ 50mg/ pot with 5kg capacity, T_7-Soil application of Carbofuran 3G @ 300mg/ pot with 5kg capacity and T_8- Untreated control.

The effect of the treatments on plant growth parameters of bhendi and population buildup of nematode were recorded by taking biometric measurements of shoot length (cm), fresh shoot weight (g), Dry shoot weight (g), Fruit yield (g), root length (cm), fresh root weight (g), and dry root weight(g). Nematode population/200 g soil was estimated by Cobb's sieving and decanting method [5] and Modified Baermann funnel technique [6] Number of adult female/g root, number of egg mass/ root, number of eggs/egg mass and root knot index were also recorded. The data were statistically analyzed [7] as per the analysis of variance test of completely randomized block design.

Results and Discussion

All the data were analyzed and tabulated. From [Table-1] it was observed that all the biocontrol agents showed efficacy on plant growth parameters and nematode population reduction in soil as well as root when compared to untreated control. Observations showed that application of *P. lilacinum*, *T. viride* and *P. fluorescens* increased shoot length, shoot weight, dry shoot weight, fruit yield, root length, root weight and dry root weight of tomato over control in a treatment dependent manner. Among the bio control agent, soil application of *P. lilacinum*@ 2.5kg/ha recorded the maximum shoot length of 35.27 cm, fresh shoot weight of 16.45g, dry shoot weight 6.18g, fruit yield 54.34g and increase the root length of 28.22 cm, fresh root weight 12.50g and dry root weight of 5.54g was recorded. Maximum plant growth parameters were recorded in soil application of *P. lilacinum* and seed treatment of *P. lilacinum* followed by soil application of carbofuran. Application of *P. lilacinum* in soil found to be the most effective among all treatments. Highest reduction of root knot nematode adult females (12.42), No. of egg masses per g of root (5.42), No. of eggs/egg mass (92.22) and nematode population in soil (110.26) were found by application of *P. lilacinum* as seed treatment among all the treatments. Minimum number of nematode population was also recorded as 121.32, 120.82 in soil application of *P. lilacinum* and soil application of carbofuran respectively. Lowest root knot index (1.0) was recorded in seed treatment of *P. lilacinum* followed by soil application of *P. lilacinum* (1.42) and soil application of carbofuran (1.89). Root knot index was on bar with each other in case of seed treatment of *T. viride* and soil application of *T. viride* [Table-2].

As observed in the present study, [8] showed that the effect of bio control potential on root knot nematode management using commercially available *Pseudomonas fluorescens, Paecilomyces lilacinus, Verticillium chlamydosporium, Bacillus subtilis* and *Trichoderma viride* was observed that the maximum shoot length and shoot weight of bhendi was recorded 60.79% and 54.87% respectively in *P. fluorescens* (2ml/L) treated plants followed by *V. chlamydosporium* (2ml/L) compared with untreated control. Regarding yield of bhendi, *P. fluorescens* (2ml/L) treated plants recorded maximum of 0.19 kg/plant. From this experiment it is observed that the bio-control agents were effectively reduced the *M. incognita* population in soil as well as root. Suppression of root knot nematode population by *P. fluorescens* was due to its nematicidal action [9]. Using fungal/ bacterial antagonist to manage nematode problem in crop plants is a sustainable and eco friendly approach. It is important to note that biological control methods may need to be combined or integrated with each other, as well as with other management practices, to achieve optimal results. Regular monitoring and assessment of nematode populations are crucial for implementing appropriate control measures. By employing biological control strategies, growers can effectively manage the root knot nematode, *M. incognita* in bhendi crops, reducing yield losses and minimizing the need for chemical nematicides. Egg parasitism is the main mode of action of *P. lilacinum* against parasitic nematodes [10]. *P. lilacinum* is capable of colonizing the gelatinous matrix [11]. Eggs in earlier embryonic stages are reported to be more successfully infected by nematophagus fungi [12]. *T. viride* is a ubiquitous soil fungus which colonizes root surfaces and root cortices and provided excellent control of root-knot nematodes [13]. Trichoderma's highly branching conidiophores produce conidia that can adhere to various worm stages. Application of Trichoderma species resulted in reduced nematode galling and improved plant growth and tolerance. *P. fluorescens* improve the plant growth promotion of tomato because of it increase the phosphorus content of the soil or produced more indol acetic acid (IAA) as compared to untreated control [14].

In the present study, soil application of *P. lilacinum* has effectively suppressed root knot nematode infestation in bhendi than that seed treatment of *P. lilacinum*. This might be due to good establishment of the biocontrol agent in plant rhizosphere. The reason for increased plant growth, yield and other parameters observed here could be attributed to the release of growth promoting substances by bio-agents or by producing toxic metabolites which inhibit nematodes and exclude other deleterious microorganisms. The reduction in nematode galls and egg masses may be attributable to bio-agents' high rhizosphere competency, as they may easily colonize roots and may diminish nematode feeding locations. The reduction of root gall number may be due to failure of majority of the juveniles to penetrate the host root. Furthermore, the use of fungal antagonist, such as *Purpureocilium* and *Trichoderma* has shown promising for the management of *M. incognita* in bhendi.

Conclusion

The present study demonstrates that treatment of bhendi plants with different fungal/ bacterial antagonist agents against root knot nematode, *M. incognita* revealed that soil application of *P. lilacinum* has effectively suppress root knot nematode infestation in bhendi. This method not only protects the bhendi crop but also promote the overall health and resilience of the agro ecosystem. Therefore, our results concluded that application of *P. lilacinum* could be a sustainable and practical approach for managing root knot nematode in bhendi.

Application of research: Study of biological control of root knot nematode

Research Category: Vegetable crop

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Study area / Sample Collection: Krishi Vigyan Kendra, Vridhachalam, 606001

Cultivar / Variety / Breed name: Okra (*Abelmoschus esculentus* L.)

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