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ENVIRONMENT FRIENDLY MANAGEMENT STRATEGY FOR ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA)* ON OKRA (*ABELMOSCHUS ESCULENTUS* L. MOENCH)

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ABSTRACT

Okra (*Abelmoschus esculentus*), a member of the Malvaceae family, is an important vegetable crop cultivated globally, especially during the summer and rainy seasons. However, it serves as a major host for plant-parasitic nematodes, with the root-knot nematode (*Meloidogyne incognita*) being particularly damaging. Although chemical nematicides are effective, they pose serious environmental risks. As an alternative, certain microorganisms that act as antagonists to plant-parasitic nematodes can be used for biological control. In this study, the effectiveness of various bio-agents (*Trichoderma harzianum*, *Purpureocillium lilacinum*, *Bacillus subtilis*, *Pochonia chlamydosporia*, *Bacillus amyloliquefaciens*, *Bacillus pumilus*, *Pseudomonas fluorescens*, *Glomus fasciculatum*, and *Metarhizium anisopliae)* was evaluated against *M. incognita* in okra. These bio-agents were applied as seed treatments at 1% w/w. The crop was harvested 45 days after sowing, and observations were made on plant growth parameters (shoot length (cm), shoot weight(g), root length(cm), and root weight(g)) and nematode reproduction (number of galls per plant, number of egg masses per plant, number of eggs and larvae per egg mass, and final nematode population per 200 cc of soil). The results showed that seed treatment with *Trichoderma harzianum* was the most effective in enhancing plant growth and significantly reducing root-knot nematode reproduction in okra. This was followed by *Purpureocillium lilacinum* and *Pochonia chlamydosporia*, which also showed notable improvements in plant health and nematode reproduction reduction.

***Keywords*:** Okra, Bio-agents, Root-knot nematode, *Meloidogyne incognita.*

Introduction

Okra (*Abelmoschus esculentus* L. Moench), belonging to the family Malvaceae, is one of the most important vegetable crops worldwide. It is an economically significant biannual crop that originated in the tropical regions of Afro-Asian countries and is predominantly cultivated in hot climates (Mohammadi *et al.,* 2021). Okra is widely grown in various parts of the world, including India, and serves as an important source of nutrients such as proteins, lipids, carbohydrates, and essential minerals like calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), potassium (K), sodium (Na), and zinc (Zn). It also contains vital vitamins such as A and B (Patil *et al.,* 2020). The plant has an upright growth habit and produces hibiscus-like flowers (Smith *et al*., 2002). It is typically grown in tropical and warm temperate climates (Ijewere, 2012). Mihretu *et al.* (2014) highlighted its multi-purpose nature, noting that the fresh leaves, buds, flowers, seeds, and immature pods of okra are all of economic value.

India is the second-largest vegetable-producing country in the world, following China. Total area under okra crop cultivation in India is 548.95 ha and the

production rate is 6819 MT per ha (GOI, 2022–23). Major okra-producing states in India include Gujarat, West Bengal, Odisha, Madhya Pradesh, Bihar, Chhattisgarh, Uttar Pradesh, Andhra Pradesh, Assam, and Rajasthan. In Rajasthan, okra is cultivated over an area of 2.46 thousand hectares with a total production of 10.60 million tonnes (GOI, 2022–23). Key okra-producing districts in Rajasthan are Ajmer, Alwar, Jaipur, Kota, Bundi, Chittorgarh, Bhilwara, Rajsamand, Jhalawar, Jodhpur, Sikar, Nagaur, Dausa, Sriganganagar, Pali, Sirohi, Banswara, Pratapgarh, and Udaipur.

Okra is particularly vulnerable to root-knot nematodes (RKN), which are among the most destructive pests affecting the crop (Noling, 2012). These nematodes severely damage the root system, reducing plant vigor and significantly delaying and lowering pod production (Bolles and Johnson, 2012). Hussain *et al*. (2016) also reported the negative impact of root-knot nematode infestation on okra, highlighting substantial yield losses. Okra is attacked by more than 15 genera of plant-parasitic nematodes, with *Meloidogyne incognita*, *Hoplolaimus indicus*, *Aphelenchus avenae*, *Helicotylenchus* spp., *Rotylenchulus reniformis*, and *Tylenchorhynchus* spp. being the most significant. Among these, *Meloidogyne incognita* (root-knot nematode) is considered the most damaging (Bhosle *et al.,* 2004).

Root-knot nematodes (*Meloidogyne* spp.) represent the most prevalent group of plant-parasitic nematodes, affecting a wide range of economically valuable crops worldwide (Shakeel, 2020). Over 100 species of root-knot nematodes have been identified globally (Hunt and Handoo, 2009). Crop losses due to *Meloidogyne* spp. are estimated to range from 5% to 43% in vegetables (Gautam *et al*., 2014). Sikora and Fernandez (2005) reported that severe infestations by *Meloidogyne* spp. can lead to yield losses of up to 27% in okra.

In recent years, the global use of conventional nematicides for managing plant-parasitic nematodes has decreased significantly due to concerns over the toxicity of many synthetic chemicals to non-target organisms and their environmental persistence. This has created an urgent need for safer and more sustainable alternatives. Biological control methods, particularly the application of bio-agents, have emerged as promising options because they provide effective, environmentally friendly, and long-lasting protection against nematode pests (Anita and Samiyappan, 2012). Considering these factors and the considerable economic damage caused by root-knot nematodes, the present research was conducted to explore and evaluate the potential of bio-agents in nematode management, aiming to fill existing gaps in knowledge.

Materials and Methods

The experiment was conducted in the Department of Nematology at Rajasthan College of Agriculture, Udaipur, during the 2023 and 2024 Kharif growing seasons. This pot experiment aimed to evaluate the efficacy of various bio-agents (*Purpureocillium lilacinum*, *Pochonia chlamydosporia*, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Bacillus pumilus*, *Pseudomonas fluorescens*, *Glomus fasciculatum*, *Metarhizium anisopliae*, and *Trichoderma harzianum)* in managing root-knot nematode (*Meloidogyne incognita*) in okra. These bio-agents were applied as seed dressings at a concentration of 1% w/w. An untreated control was maintained for comparison. To apply the bio-agents, the required quantity of okra seeds was weighed and placed in a beaker. The appropriate amount of each bio-agent was then added and thoroughly mixed to achieve an even and smooth coating over the seeds. The experiment was laid out in a Completely Randomized Design with three replications. After 45 days of sowing, the plants were carefully uprooted from each pot, and data were recorded on plant growth parameters, including shoot length (cm), shoot weight (g), root length (cm), and root weight (g). Nematode infection parameters were also assessed, including the number of galls per plant, number of egg masses per plant, number of eggs and larvae per egg mass, and the final nematode population per 200 cc of soil.

Disinfection and filling of pots:

Earthen pots were thoroughly washed, cleaned, and disinfected prior to use by rinsing them with a 4% formalin solution. The pots were then left to allow the formalin to evaporate completely before being used in the experiment. For this trials, uniform-sized earthen clay pots were selected and each was filled with 2.5 kg of nematode-infested soil. To ensure proper drainage, each pot had a hole at the bottom, which was covered with a piece of broken clay pot before filling with soil. An equal amount of soil was added to each pot, leaving sufficient space at the top for irrigation. Five seeds of the susceptible okra variety ‘Deepika’ were sown per pot. Additionally, each pot was filled infested soil with a nematode population density of two larvae per gram of soil.

Collection and processing of samples

Soil samples were carefully collected from each experimental pot, properly labelled, and transported to the laboratory, where they were stored under

refrigeration until further analysis. Nematodes were extracted from the soil using the Decanting and Sieving Method as described by Cobb (1918), followed by the Baermann Funnel Technique (Christie and Perry, 1951). The extracted samples were then examined under a microscope to determine the population of the target nematode species.

Counting of galls and egg masses per plant and eggs and larvae per egg mass

After harvest, root samples were collected from each experimental pot, properly labelled, and brought to the laboratory for analysis. For estimating nematode presence in roots, root samples were first soaked in water and gently washed to eliminate any remaining soil particles. The cleaned roots were then stained with a 0.1% acid fuchsin solution in lactophenol at 80°C for 2-3 minutes, following the procedure outlined by McBeth *et al.* (1941). After staining, the samples were stored in clear lactophenol for at least 24 hours before microscopic examination. To assess the reproductive potential of the nematodes, egg masses were randomly selected and carefully removed from the stained roots. Each egg mass was placed in a drop of clear lactophenol on a glass slide, covered with a coverslip, and gently pressed to spread the contents evenly. The number of eggs and larvae per egg mass was then counted using a stereoscopic binocular microscope with the aid of a telecounter.

Identification of root-knot nematode

Root-knot nematode-infested roots were thoroughly washed and stained with 0.1% acid fuchsin in lactophenol at 80°C for 2–3 minutes (McBeth *et al*., 1941). After gently rinsing with tap water, the roots were placed in clear lactophenol for at least 24 hours and then examined under a stereoscopic binocular microscope. Following staining, the females were teased out from the roots and perineal patterns were prepared (Taylor and Netscher, 1974). Observations of several patterns were recorded, and the nematode species was identified as *Meloidogyne incognita* (Eisenback *et al*., 1981).

Results and Discussion

The present study demonstrated the significant role of bio-agents in managing root-knot nematode (*Meloidogyne incognita*) infestation in okra. Seed treatments with various bio-agents, including *Purpureocillium lilacinum*, *Pochonia chlamydosporia*, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Bacillus pumilus*, *Pseudomonas fluorescens*, *Glomus fasciculatum*, *Metarhizium anisopliae*, and *Trichoderma harzianum* at 1% w/w, showed significant improvements in plant growth and reductions in nematode reproduction compared to the untreated control. Among these, *Trichoderma harzianum* exhibited the most pronounced effect on enhancing shoot and root growth while effectively suppressing nematode parameters such as gall formation and egg mass production. This was closely followed by *Purpureocillium lilacinum* and *Pochonia chlamydosporia* treatments, which also significantly reduced nematode populations and improved plant vigor.

These results align with earlier findings where *Trichoderma* species have been reported to control nematodes through multiple mechanisms, including antibiosis, competition, mycoparasitism, and enzymatic degradation of nematode structures (Gonzalez *et al*., 2012; Sharon *et al*., 2001). The production of antinematode metabolites by *Trichoderma* likely immobilizes juvenile nematodes, reducing root penetration and infection. Similarly, *P. lilacinum* has been documented as an effective bio-agent against root-knot nematodes, particularly in reducing root galling and nematode reproduction (Hanawi, 2014; Meghwal and Baheti, 2017).

The antagonistic fungi *Metarhizium anisopliae* also showed nematode suppression, consistent with reports of its root colonization ability and the production of sticky conidia that adhere to nematode cuticles, facilitating parasitism (Jahanbazian *et al.,* 2015; Wang and leger 2007). Additionally, bacterial bio-agents like *Bacillus subtilis* contributed to nematode management by producing antimicrobial compounds such as subtillin and bacitracin, which may disrupt nematode physiology (Killani *et al*., 2011).

Table 1 : Effect of different bio-agents on plant growth and nematode reproduction parameter of okra infected with *Meloidogyne incognita* under cage house conditions.

Treat-ment	Shoot length (cm)			Shoot weight (g)			Root length (cm)			Root weight (g)			No. of galls per plant			No. of egg mass per plant			No. of eggs and larvae per egg mass			Final nematode Pop. 200cc soil		
	2023	2024	Pooled	2023	2024	Pooled	2023	2024	Pooled	2023	2024	Pooled	2023	2024	Pooled	2023	2024	Pooled	2023	2024	Pooled	2023	2024	Pooled
T1	47.66	48.66	48.16	43.00	44.00	43.50	37.33	38.00	37.66	28.33	28.66	28.50	30.66	30.00	30.33	20.00	22.00	21.00	50.33	52.33	51.33	261.00	268.00	264.50
T2	44.00	45.00	44.50	40.00	39.33	39.66	33.00	32.33	32.66	24.00	23.00	23.50	38.00	37.00	37.50	28.00	27.33	27.66	64.67	66.67	65.67	304.00	310.33	307.16
T3	32.00	32.33	32.16	26.33	25.00	25.66	26.33	27.00	26.66	15.33	16.66	16.00	54.66	55.00	54.83	45.00	43.33	44.16	121.33	128.00	124.67	467.00	484.00	475.50

T4	41.00	42.33	41.66	37.66	36.33	37.00	31.66	30.00	30.83	21.66	20.00	20.83	42.00	43.00	42.50	32.00	31.66	31.83	82.00	78.67	80.33	363.66	372.66	368.16
T5	29.33	29.00	29.16	23.00	22.66	22.83	20.66	20.66	20.66	13.66	14.33	14.00	58.66	58.00	58.33	51.33	51.00	51.16	158.00	151.00	154.50	594.66	605.00	599.83
T6	27.66	26.66	27.16	20.33	20.66	20.50	18.33	18.66	18.50	11.66	12.66	12.16	61.00	61.33	61.16	54.00	55.00	54.50	164.33	162.67	163.50	498.00	525.00	511.50
T7	38.33	40.66	39.50	35.00	34.00	34.50	28.33	28.66	28.50	19.33	21.00	20.16	46.33	47.66	47.00	35.33	36.33	35.83	87.67	86.00	86.83	389.00	392.33	390.66
T8	25.00	24.66	24.83	19.00	18.33	18.66	16.33	15.00	15.66	9.66	10.33	10.00	63.33	63.66	63.50	57.33	58.66	58.00	196.67	194.33	195.50	653.66	675.33	664.50
T9	35.00	35.66	35.33	28.66	30.33	29.50	24.33	24.66	24.50	17.33	18.66	18.00	49.00	52.00	50.50	43.66	43.00	43.33	106.67	110.67	108.67	423.66	433.33	428.50
T10	18.33	18.00	18.16	12.33	13.00	12.66	10.00	9.33	9.66	5.33	6.00	5.66	70.33	72.33	71.33	67.33	68.66	68.00	225.67	228.33	227.00	702.66	711.66	707.16
SE(m)±	0.59	0.55	0.49	0.53	0.54	0.51	0.47	0.54	0.41	0.40	0.44	0.53	0.57	0.71	0.63	0.65	0.65	0.59	1.41	1.89	2.02	4.66	3.74	3.73
C.D.at 5%	1.77	1.65	1.59	1.59	1.62	1.65	1.40	1.62	1.33	1.21	1.32	1.69	1.71	2.12	2.02	1.93	1.95	1.88	4.19	5.59	6.45	13.84	11.12	11.94

- Dose of all bio-agents at 1% w/w
- Initial nematode population- 2 J_2/200cc soil
- Replication-3

T1- *Trichoderma harzianum*, T2- *Purpureocillium lilacinum*, T3- *Bacillus subtilis*, T4- *Pochonia chlamydosporia*, T5- *Bacillus amyloliquefaciens*, T6- *Bacillus pumilus,* T7- *Pseudomonas fluorescens,* T8- *Glomus fasciculatum,* T9- *Metarhizium anisopliae,* T10- Control

Table 2 : Per change in plant growth parameter and nematode reproduction through application of bio-agents against root-knot nematode, *Meloidogyne incognita* infecting okra under cage house conditions.

Treat-ment	Shoot length (cm)			Shoot weight (g)			Root length (cm)			Root weight (g)			No. of galls per plant			No. of egg mass per plant			No. of eggs and larvae per egg mass			Final nematode Pop. 200cc soil		
	2023	2024	Pooled	2023	2024	Pooled	2023	2024	Pooled	2023	2024	Pooled	2023	2024	Pooled	2023	2024	Pooled	2023	2024	Pooled	2023	2024	Pooled
T1	160.01	170.33	165.20	248.74	238.46	243.60	273.30	307.29	289.86	431.52	377.67	403.53	56.41	58.52	57.48	70.30	67.96	69.12	77.70	77.08	77.53	62.86	62.34	62.60
T2	140.04	150.00	145.04	224.41	202.54	213.27	230.00	246.52	238.10	350.28	283.33	315.19	45.97	48.85	47.43	58.41	60.20	59.32	71.35	70.81	71.07	56.74	56.39	56.56
T3	74.58	79.61	77.09	113.54	92.31	102.69	163.30	189.39	175.98	187.62	177.67	182.69	22.28	23.96	23.13	33.17	36.89	35.06	46.23	43.94	45.08	33.54	31.99	32.76
T4	123.68	135.17	129.41	205.43	179.46	192.26	216.60	221.54	219.15	306.38	233.33	268.02	40.28	40.55	40.42	52.47	53.89	53.19	63.66	65.55	64.61	48.25	47.64	47.94
T5	60.01	61.11	60.57	86.54	74.31	80.33	106.60	121.44	113.87	156.29	138.83	147.35	16.59	19.81	18.23	23.76	25.72	24.76	27.18	29.49	28.34	15.37	14.99	15.18
T6	50.90	48.11	49.56	64.88	58.92	61.93	83.30	100.00	91.51	118.76	111.00	114.84	13.27	15.21	14.26	19.80	19.90	19.85	29.98	35.18	32.60	29.13	26.23	27.67
T7	109.11	125.89	117.51	183.86	161.54	172.51	183.30	207.18	195.03	262.66	250.00	256.18	34.12	34.11	34.11	47.53	47.09	47.31	61.15	62.34	61.75	44.64	44.87	44.76
T8	36.39	37.00	36.73	54.10	41.00	47.39	63.30	60.77	62.11	81.24	72.17	76.68	9.95	11.99	10.98	14.85	14.56	14.71	12.85	14.89	13.88	6.97	5.10	6.03
T9	90.94	98.11	94.55	132.44	133.31	133.02	143.30	164.31	153.62	225.14	211.00	218.02	30.33	28.11	29.20	35.16	37.37	36.28	52.73	51.54	52.13	39.71	39.11	39.41
T10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

T1- *Trichoderma harzianum*, T2- *Purpureocillium lilacinum*, T3- *Bacillus subtilis*, T4- *Pochonia chlamydosporia*, T5- *Bacillus amyloliquefaciens*, T6- *Bacillus pumilus,* T7- *Pseudomonas fluorescens,* T8- *Glomus fasciculatum,* T9- *Metarhizium anisopliae,* T10- Control

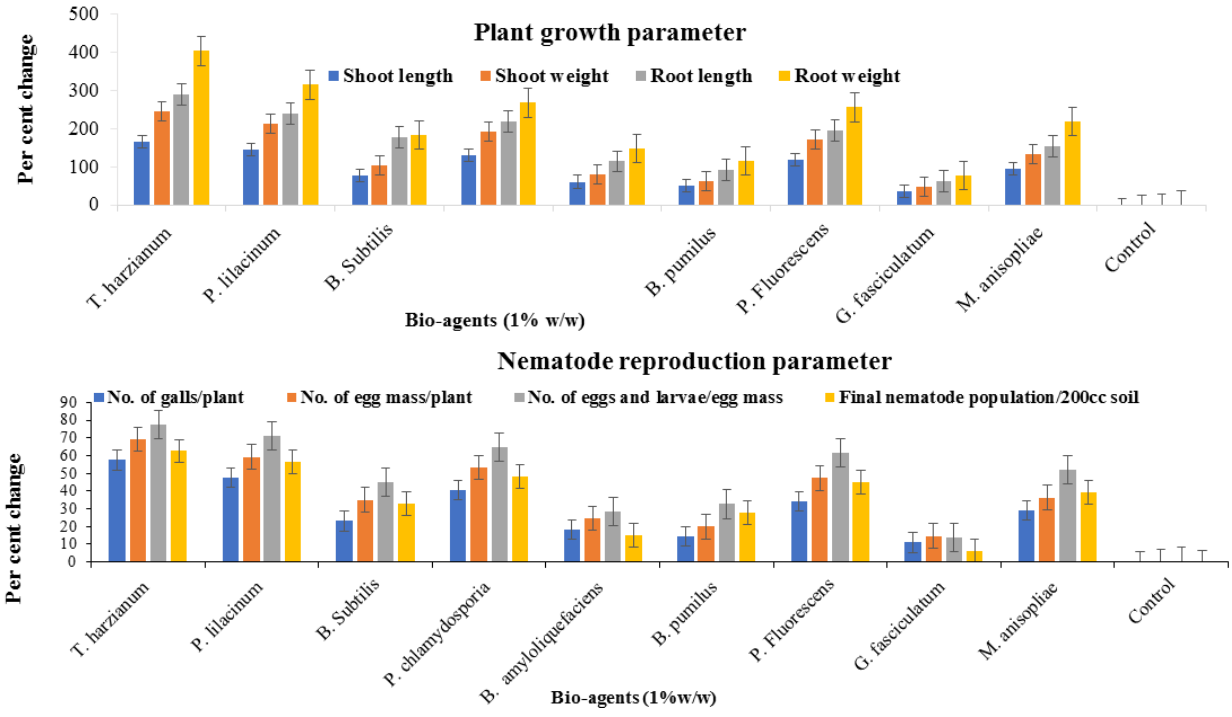

Fig. 1 : Per cent change in plant growth parameter and nematode reproduction parameter through application of bio-agents against root-knot nematode, *Meloidogyne incognita* infecting okra.

Furthermore, the enhancement of plant growth observed in bio-agent treated plants may be attributed to both direct suppression of nematodes and improved nutrient uptake facilitated by root colonization. For instance, *Pochonia chlamydosporia* is known to induce systemic resistance and promote plant growth, partly by releasing lytic enzymes such as chitinases and proteases that degrade nematode eggshells (Annapurna *et al.,* 2018). Nama and Sharma (2017) evaluated those seeds of okra cv. A-4, treated with *T. harzianum, T. viride, Pochonia chlamydosporia, P. lilacinus* and *P. fluorescens* at 20 g/kg seed, reduced root-knot nematode, *M. incognita, T. harzianum* at 10 g/kg seed followed by T. viride at 10 g/kg seed and *P. fluorescens* at 10 g/kg seed over the untreated check. Kamau 2010, showed that treatment with *T. asperellum* reduced the galling in French beans significantly under greenhouse condition

Overall, the findings confirm that seed treatment with selected bio-agents can effectively reduce nematode infestation and enhance growth parameters in okra. The bio-control mechanisms involve parasitism of nematode eggs and juveniles, competition for resources, and production of toxic metabolites, leading to suppression of nematode populations and improved crop yield. Thus, these bio-agents offer a promising eco-friendly alternative for sustainable nematode management in okra cultivation.

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