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In vitro efficacy of Microbial Consortia against Tomato Root Knot Nematode [*Meloidogyne incognita* (Kofoid and White)]

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ABSTRACT: Tomato (*Solanum lycopersicum* L.) is known as the poor man's orange and it is extensively grown worldwide. It is affected by various diseases like fungal, bacterial, viral and nematodes. Among these, root knot nematodes (RKN) which is the most important plant parasitic pathogen that causes significant crop loss alone or in combination with fungal and bacterial wilt pathogens. Unscientific usage of pesticides aggravates densely virulent strains, apart from polluting the environment and causing human health hazards. The use of different combinations of biocontrol agents with respect to consortia is effectively managed with multicidal action against the target pathogen. In this present investigation, the study was carried out to explore the activities of microbial consortia against root-knot nematode of tomato under *in vitro* conditions. The experiment was carried out at the Department of Plant Pathology, KRCCH, Arabhavi. The 10 treatments were studied with different combinations of biocontrol agents. The results of our investigation show that among the tested bioagents T_9 (*Trichoderma harzianum* + *Metarhizium anisophile* + *Purpureocillium lilacinum* + *Beauveria bassiana* + *Bacillus subtilis* + *Pseudomonas fluorescens*) exhibited the minimum egg hatching per cent of 55.65 at 96 hours of exposure period when compared to T_{10} (untreated control) recorded 11.85 per cent of egg hatching.

Keywords: Bio-control agents, egg hatching, microbial consortia, root knot nematode.

INTRODUCTION

Tomato (*Solanum lycopersicum* L. 2n=24) is one of the most important solanaceous vegetable crop. Its good adaptability has led to widespread distribution around the global growth. It is also rich in phyto-nutrients including lycopene, which functions as an antioxidant and also excellent sources of Vitamin A, B and C. There are many biotic and abiotic factor cause economic damage. At present days plant parasitic nematode in tomato crop causing major yield reduction and also interact with other pathogens cause severe complex wilt diseases. Nematodes cause 21.3 per cent yield losses amounting to Rs. 102,039.79 million (1.58 billion USD) annually (Vinod *et al.,* 2020). Root knot nematodes (*Meloidogyne incognita*) infestation poses a considerable constraint on tomato production. The application of chemical nematicides will become prohibited due to not only the increase of resistance in the target pathogen but also caused the environmental hazard. Biological control agents (BCAs) function as inducers of resistance against nematodes. They possess the capability to directly mitigate the harm inflicted by plant-parasitic nematodes through mechanisms such as parasitism, antibiosis, paralysis and the production of lytic enzymes. To reduce such condition, the use of bioagents are found increase in attention and use of such bioagents offer an effective, safe, persistent and natural durable protection against *Meloidogyne incognita*. While chemical nematicides are highly effective in managing root knot nematode, due to their cost effective and adverse impact on beneficial soil microorganisms, potential carcinogenicity in humans necessitate the exploration of alternative strategies. The current investigation was aimed to explore eco-friendly management practices for management of root knot nematode infestation in tomatoes. This was achieved by utilizing a mixture of diverse biocontrol agents/microbial consortia having multisite mode of action against damaging root knot nematode reduction in sustainable way.

MATERIAL AND METHODS

The infected tomato root samples were collected from Kittur Rani Channamma college campus, Arabhavi. The experiment was laid out in a completely randomized design with three replications and ten treatments**.** The bioagents *viz.*, *Trichoderma harzianum, Metarhizium anisophile, Purpureocillium lilacinum, Beauveria bassiana, Bacillus subtilis* and *Pseudomonas fluroscence* which were obtained from Department of Plant Pathology KRCCH, Arabhavi and other campuses of UHS Bagalkot were used for consortia preparation and evaluation against root knot nematode under *in vitro* condition. The effectiveness of different consortia was evaluated on egg hatching under *in vitro* conditions by following standard protocol. Data were recorded at 24, 48, 72 and 96 hours after inoculation.

A. Collection of eggs

The infected tomato roots having galls developed by *Meloidogyne incognita* were collected. The roots of the uprooted plants were washed under running tap water. The galls were separated from the root. After that, the galls were dissected with a sterilized dissecting needle and egg were hand picked up from the galled root with help of dropper. The picked eggs were kept in sterilized cavity block containing sterilized water.

B. Effect on egg hatching

To investigate the impact of bio consortia on egg hatching, 10 ml of each bio consortia solution (1%) was introduced into 5 cm diameter petri dishes. Three replicates were maintained for each treatment, while control petri plates were kept by adding 10 ml of sterilized distilled water. In each petri dish, 1 ml of suspension containing 20 eggs of *M. incognita* was dispensed using a dropper. The petri plates were then incubated at room temperature, and observations regarding egg hatching were recorded at 24, 48, 72 and 96 hours post-inoculation. Egg hatching inhibition was specifically noted at the 96 hour mark. The percentage of eggs hatched and the level of inhibition were calculated using the formula outlined Ravichandra (2010).

$$\%\text{ Egg Hatching} = \frac{\text{Number of hatched juveniles}}{\text{Total number of eggs inoculated}} \times 100$$

$$\text{Hatch inhibition of eggs (\%)} = \frac{\text{Total number of eggs} - \text{hatched number of eggs}}{\text{Total number of eggs inoculated}} \times 100$$

RESULT AND DISCUSSION

A. Effect of bioconsortia on egg hatching

The results revealed (Table 1) that different bioconsortia treatments were evaluated for their inhibitory effect on egg hatching. Observations recorded at 24, 48, 72 and 96 hours after incubation. All the tested treatments significantly reduced the egg hatching under *in vitro* condition over control. Among the different treatment tested, T_9 (*T. harzianum* + *B. subtilis* + *P. lilacinum* + *B. bassiana* + *M. anisophile* + *P. fluroscence*) recorded the lowest egg hatching per cent with 10.35, 29.35, 39.85 and 55.65 at different time intervals followed by T_7 (*T. harzianum* + *P. fluroscence* + *P. lilacinum* + *B. bassiana* + *M. anisophile*) recorded the 16.15, 36.00, 44.50, 60.50 per cent. While, maximum egg hatching was noticed in T_{10} (untreated control) with 45.00, 64.85, 75.00 and 96.50 per cent at 24, 48, 72 and 96 hours, respectively. In unison with present results, various studies have reported on inhibition of egg. Liu *et al*. (2013) suggested that release of unknown compounds, plantazolicin, which is structurally similar to telomerase inhibitor telomestatin, might be responsible for nematicidal activity of bioagent *Bacillus.* Similarly efficacy of various bacterial formulations against root knot nematode were more or less in conformity with the findings of Mane and Mhase (2017) who reported inhibition of egg hatching might be due to nematicidal effects of volatile bacterial metabolites. El-Sherif *et al*. (1994) also reported inhibitory effect of *Pseudomonas* and *Bacillus* on egg hatching. Reports given by Huang *et al.* (2010); Kavitha *et al.* (2012) on *B. subtilis* produces various antibiotic compounds, nematicidal volatiles and lipopolypeptides which were antagonistic towards the egg hatching of *Meloidogyne* sp. The antibiotic compound, fengycin produced by *B. subtilis* showed a strong lethal activity against the nematodes.

Inhibitory activity of fungal bioagent *Purpureocillium lilacinus* on egg hatching was reported by Khan *et al*. (2006) it is due to production of chitinases, leucinotoxin, proteases, and acetic acid has been associated to the infection process, which ultimately resulted in aborted embryonic development via a cascade of physiological disorders. The effectiveness of *Beauveria* may be attributed to its ability to produce beauvericin and oosporin, with beauvericin exhibiting activity against *M. incognita* eggs was reported by several previous worker (Hamill *et al*., 1969; Suzuki *et al.*, 1997; Anke *et al.*, 1995). The impact on nematodes may be linked to the mechanism of action exhibited by *Metarhizium anisopliae* conidial spores, which involve their attachment to the nematode cuticle, germination, parasitization, direct penetration and subsequent production of infective hyphae inside the nematode body was also reported by Ghayedi and Abdollahi (2013). The extensively branched conidiophores of *Trichoderma* give rise to conidia capable of adhering to various nematode developmental stages. The extent of conidial attachment and parasitic behavior varies across different fungal species and strains, as reported by Sharon *et al.* (2007).

Table 1: Effect of bioconsortia on egg hatching of *M. incognita* under *in vitro* condition.

Treatments	24 hrs		48 hrs		72 hrs		96 hrs		Inhibition % over control
	No. of eggs hatched	Egg hatching %	No. of eggs hatched	Egg hatching %	No. of eggs hatched	Egg hatching %	No. of eggs hatched	Egg hatching %	
T_1: *T. harzianum + P. fluroscence + B. bassiana + M. anisophile*	8.53	42.65	11.20	56.00	13.60	68.00	17.00	85.00	15.00
T_2: *T. harzianum + P. fluroscence + P. lilacinum+ M. anisophile*	4.40	22.00	10.80	54.00	13.03	65.15	16.17	80.85	19.15
T_3: *T. harzianum + P. fluroscence + P. lilacinum + B. bassiana*	5.17	25.85	7.63	38.15	11.07	55.35	14.07	70.35	29.65
T_4: *T. harzianum + B. subtilis+ B. bassiana + M. anisophile*	8.10	40.50	7.67	38.35	10.52	52.60	13.80	69.00	31.00
T_5: *T. harzianum + B. subtilis+ P. lilacinum + M. anisophile*	7.10	35.50	8.47	42.35	11.41	57.05	15.17	75.85	24.15
T_6: *T. harzianum + B. subtilis+ B. bassiana + P. lilacinum*	4.17	20.85	8.00	40.00	9.83	49.15	13.17	65.85	34.15
T_7: *T. harzianum + P. fluroscence + P. lilacinum + B. bassiana+ M. anisophile*	3.23	16.15	7.20	36.00	8.90	44.50	12.10	60.50	39.50
T_8: *T. harzianum + B. subtilis + P. lilacinum + B. bassiana + M. anisophile*	6.00	30.00	10.00	50.00	12.63	63.15	16.00	80.00	20.00
T_9: *T. harzianum + B. subtilis + P. lilacinum+ B. bassiana+ M. anisophile + P. fluroscence*	2.07	10.35	5.87	29.35	7.97	39.85	11.13	55.65	44.35
T_{10}: Control with inoculum	8.80	45.00	12.97	64.85	15.00	75.00	19.30	96.50	
Mean	5.76		8.98		11.40		14.79		
S. Em±	0.09		0.08		0.07		0.08		
CD @ 1%	0.37		0.33		0.30		0.35		

CONCLUSIONS

The present findings have demonstrated that the different bioconsortia treatments showed the inhibitory effect on root knot nematode egg hatching under *in vitro* condition. All tested consortia treatments recorded minimum egg hatching per cent (55.50-85.00) over untreated control (97.50) at 96 hours after incubation. Among the treatments, minimum egg hatching per cent was recorded inT_9 (*T. harzianum + B. subtilis + P. lilacinum + B. bassiana + M. anisophile + P. fluroscence*) with 55.50 per cent over T_{10} (untreated control) with 97.50 per cent.

FUTURE SCOPE

The efficacy of bioconsortia should be evaluated further under field condition and also should be focused on formulation of bioconsortia.

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Conflict of Interest. None.

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