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EVALUATION OF NATIVE ISOLATES OF *TRICHODERMA VIRIDE, METARHIZIUM ANISOPILAE* AND *FUSARIUM VERTICILLIOIDES* AGAINST ROOT KNOT NEMATODE IN TOMATO

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ABSTRACT

An investigation on evaluation of nematode antagonistic fungi against *Meloidogyne incognita* (Kofoid and White) Chitwood in tomato was carried out at Department of Nematology, College of Agriculture, Vellayani during 2018-2019. The objective was to evaluate bio-control potential of nematode antagonistic fungi isolates of *Trichoderma viride, Metarhizium anisopliae, Fusarium verticillioides, Piriformospora indica* and chemical Cartap hydrochloride against *Meloidogyne incognita* and its growth promotion in tomato. The results revealed that soil drenching of isolate of *Trichoderma viride* (1%w/v) with RKN inoculation was effective in reducing the nematode population in soil (83.00) and root (44.25) and number of galls (18.00) and number of egg masses (34.25) roots. Efficacy of isolate *Trichoderma viride* was found to be statistically on par with cartap hydrochloride 4% G (25kg/ha), and *Piriformospora indica* (1% w/v) in reducing the number of females of root knot nematode in roots. Isolate of *Trichoderma viride* with RKN inoculation was significantly superior to all other treatments in improving the growth parameters like plant height (86.05 cm), fresh shoot weight (204.88 g), fresh root weight (81.00). Without RKN inoculation also, isolate of *Trichoderma viride* recorded the highest plant height (81.23 cm), fresh shoot weight (176.38 g) and fresh root weight (80.25). Significantly superior yield was also recorded by isolate of *Trichoderma viride* both with (346.75 g/plant) and without(361.13 g/plant) RKN inoculation. Thus, the results of present study showed that the use of isolate of *Trichoderma viride and Metarhizium anisopliae* and *Fusarium* verticillioides were effective for the control of root knot nematode (*Meloidogyne incognita*) in tomato.

Keywords **:** Root knot nematode*, Meloidogyne incognita, Trichoderma viride, Metarhizium anisopliae, Fusarium verticillioides, Piriformospora indica*

Introduction

Plant-parasitic nematodes are recognized as major pathogens infecting crop plants and cause crop losses throughout the world. *Meloidogyne incognita* is probably the most economically important plant-parasitic nematode among the tropical and subtropical regions. This nematode is extremely polyphagous, attacking both monocotyledons and dicotyledons. It is estimated that they infect more than 3000 species of plants, including a large number of cultivated plants (Abad *et al*., 2003). They caused projected yield loss of 12.3 per cent ($157 billion dollars) worldwide and of which $40.3 million is reported from India (Singh *et al*., 2015). According to reports, a high annual loss has been documented in many crops in India due to root knot nematode (RKN) *Meloidogyne incognita* infection, including tomato (11–35%), okra (10–29%), and brinjal (10–42%) (Kumar *et al*., 2020).

Several management measures were employed to control RKN in infested areas. The common practices were the use of chemical nematicides and its impending negative impact on environment. Fungi may contribute up to 80 per cent of the total microbial biomass in many soils (Clark and Paul, 1970; Shields *et al.,* 1973). In nature, fungi continuously destroy nematodes in virtually all soils because of their constant association with nematodes in the rhizosphere. The fungal antagonism consists of a great variety of organisms which vary considerably in their biology and taxonomy and play a major role in recycling the

carbon, nitrogen and other important elements from the rather substantial biomass of nematodes. Introduction of antagonists in the soil micro environment has resulted in an efficient method for biological control of nematodes (Akhman *et al*., 2002). Fungal biological control is an exciting and rapidly developing research area and there is growing attention in the exploitation of fungi for the control of nematodes (Moosavi, and Zare, 2020). Hence, the start for the isolation of native strains of antagonistic fungi that can be developed as an effective bio-agent against RKN.

Aspergillus awamori, an RKN trapping fungi isolated from the rhizosphere of tomato showed 44.9 per cent control efficacy against *M. incogtina* (Cui *et al.,* 2015*)*

The nematode antagonistic fungi belonging to genera *viz, Fusarium*, *Acremonium*, *Trichoderma*, *Chaetominum*, and *Purpureocillium lilacinum*, can produce numerous metabolites under *in vitro* conditions. When *Acremonium srictum*, *A. implicatum*, *P. lilacinum*, and *Trichoderma harzianum* were grown in liquid media, their culture filtrates were toxic to J_2 of *M. incognita* (Gowswami *et al.,* 2008). *T. harzianum* was able to penetrate nematode eggs and significantly decrease the hatching of *M. javanica* eggs. The tomato plants inoculated with the fungi showed an increase in the activity of resistance related enzymes *viz*, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase (Sahebani and Hadavi, 2008). In recent decades, biocontrol technologies have gained popularity in crop plant disease management since they additionally reduce or eliminate the need for hazardous chemicals. Insecticides were also discovered to be more affordable and effective in pest control initiatives. In light of all of these factors, the current study was conducted to assess the effectiveness of few fungal isolates against the root knot nematode (*Meloidogyne incognita*).

Materials and Methods

A pot culture study was conducted to assess the effect of isolates of *Trichoderma viride, Metarhizium anisopliae, Fusarium verticillioides*, *Piriformospora indica* (1% w/v) and chemical cartap hydrochloride (4% G) against RKN. The research was conducted in glass house. Tomato seedlings (Vellayani Vijay) were raised in pro trays filled with denematised coir pith and vermicompost. Sieved field soil, sand and farm yard manure in the proportion of 2:1:1 were mixed and packed in a polypropylene cover for autoclaving at 15kPa pressure and 121°C for 20 minutes. This denematised potting mixture was used in maintaining monoxenic culture of nematodes and pot culture experiment. Fungal inoculation was done by incorporating the mycelium at the rate of 1% (w/v) into the transplanting medium (sterile vermiculite and perlite in the ratio of 3:1 (v/v)) filled in pro tray cavities (5 cm dia × 5 cm depth).

M. incognita population obtained from naturally infected tomato plant was multiplied and maintained on susceptible tomato plant variety, Vellayani Vijay, grown in grow bags filled with sterilized loamy soils. The egg masses from infected plants were extracted and placed on culture plates containing distilled water and kept for 48-72 hrs in dark at 23°C to allow hatching. 5mL of freshly hatched juveniles were inoculated in grow bag. Inoculated plants were maintained throughout the experiment for the assortment of egg masses and second stage juveniles as and when required. Egg masses were collected in sterile distilled water after surface sterilization and incubated at 28±2°C in BOD for 2 days for juvenile emergence (J_2) using Modified Baermann's funnel technique (Schindler, 1961). The bottom of Petri plates (10 cm diameter) were poured with sterile water and above that a concave wire mesh covered with double layered tissue paper was placed carefully without breaking the tissue paper. The egg masses were then spread uniformly on the tissue paper. The edges of the tissue paper spreading outside the wire mesh were bend back to keep away from the trickling of water drops from the edges as it might transmit nematodes. Petri plates were then completely filled with water and maintained a level 5 mm over the wire mesh. This plate was incubated at room temperature. After 24 to 48 hrs the wire mesh along with filter paper was removed and the extracted nematodes in the Petri plate were collected and counted under a stereo zoom microscope using hand tally counter.

Twenty one day old tomato seedlings (variety Vellayani Vijay) which were raised in pro trays filled with denematised coir pith and vermicompost were transplanted in 8 kg capacity pots with denematised potting mixture. After establishment of seedlings, freshly hatched second stage 2000 juveniles of *M. incognita* were inoculated in the root zone of transplanted seedlings. Fungal isolates were applied 48 hrs before nematode inoculation as soil drench. Tomato plants were maintained as per the POP Recommendations of Kerala Agricultural University (KAU, 2016). The experiment was carried out in completely randomized block design with six treatments and four replications. The experiment consisted of the following six treatments:T1- Isolates of *Trichoderma viride,* T2-Isolates of *Metarhizium anisopliae,* T3- Isolates of *Fusarium verticillioides,* T4

- *Piriformospora. indica* (1% w/v), T5- Cartap hydrochloride 4% G (25kg/ha) and T6-Untreated check -control).

After 90 days of the growth, the plants were uprooted, thoroughly washed and then the plant height (cm), fresh shoot weight (g), fresh root weight (g), and grain yield per plot, population of nematodes in soil (200cc) and in 5 g roots, number of galls in root, gall index, number of females and number of egg masses in 5 g root were observed. The soil population of *M. incognita* was determined using Cobb's sieving and decanting technique (Cobb, 1918) and modified Baermann's method (Schindler, 1961) and the root knot index was recorded based on 0-5 rating scale according to the number of galls per root system in which 0=No galls, 1=1-25 galls/root system, 2=26-50 galls/root system, 3=51-75 galls/root system 4=76-100galls/root system and 5=>100 galls/root system (Heald *et al*., 1989). Root samples were cut into 2-3 cm length and stained by differential staining method using acid fuschsin-lactophenol mixture. The processed roots were squeezed between glass slides, teased with a clean needle and females were count using microscope. Egg mass number in roots was calculated following the method of Southey (1986).

Result and Discussion

All treatments could significantly reduce the population of nematodes over control both in soil and roots at harvest. Fungal inoculation of *Trichoderma viride* at the rate of 1% (w/v) with RKN inoculation recorded the lowest nematode population in (200 cc) soil (83.00) (table 1) giving 77.71 per cent reduction over the control and (44.25) in root giving 75.91 per cent reduction over the control (fig.1). Application of artap hydrochloride 4% G (25kg/ha) with RKN inoculation and soil drenching with *Piriformospora indica* (1% w/v) with RKN inoculation gave 98.25 and 99.75 *M. incognita* juveniles in soil respectively, while in roots, soil drenching with *Piriformospora indica* (1% w/v) with RKN inoculation recorded 52.50 and it was comparable with the application of Cartap hydrochloride 4% G (25kg/ha) with RKN inoculation (54.00). The efficacies of fungal formulations in managing nematodes have been reported earlier by several scientists. Villanueva and Davide (1984) reported that *Paecilomyces lilacinum* successfully controlled nematode *M. incognita* in tomato. Dube and Smart Jr (1987) observed that soil applications of *Paecilomyces lilacinus* (Thom) Samson and *Pasteuria penetrans* (Thorne) Sayre and Starr resulted in higher control levels of *M. incognita* (Kofoid and White) Chitwood population, when compared to control treatments or to the antagonist alone. Reduced nematode population with *Paecilomyces lilacinus* application was reported earlier by Pathak and Saikia (1999), Jonathan and Rajendran (2000), Khan and Verma (2004) and Narayana *et al.,* (2017). Siddiqui and Haque (2000) also observed reduction in *M. javanica* (Treub) Chitwood when the biological control agent *P. chlamydosporia* was used.

The mean number of galls in 5 g root ranged from 18.00 to 27.00 in treated plants as against 87.75 in control plants. The lowest mean gall number (18.00) was observed in plants inoculated with *Trichoderma viride* @ of 1% (w/v) giving 79.49 per cent reduction over the control (Fig. 1) followed by treatment *P. indica* (1% w/v) with RKN inoculation (21.75). Among treatments maximum number of galls was observed from Cartap hydrochloride 4% G (25kg/ha) with RKN inoculation (27.00) followed by isolates of *Fusarium verticillioides* (1% w/v) (26.00) and isolates of *Metarhizium anisopliae* (1% w/v) (23.25). Gall index of 2 was recorded in treatments with isolates of *Trichoderma viride* @ 1% (w/v), isolates of *Metarhizium anisopliae* (1% w/v), isolates of *Fusarium verticillioides*(1% w/v) and *P. indica* (1% w/v) with RKN inoculation as against four in control plants. Kiewnick and Sikora (2006) also observed that a preplanting soil treatment of *P. lilacinus* strain 251 (PL251), reduced root galling by 66 per cent in tomato. Khan *et al.* (2012) observed suppression of galls by the biocontrol agents like *P. chlamydosporia, P. lilacinus* and *T. harzianum*.

The lowest number of females (22.50) in 5g root was recorded with fungal inoculation of isolate of *Trichoderma viride* @ 1% (w/v) and it was on par with *Piriformospora. indica* (1% w/v) with RKN inoculation (21.50) and also chemical treatment with cartap hydrochloride @ 1kg a.i ha^{-1} (19.75). The lowest number of egg masses (34.25) in 5g root was recorded with fungal inoculation of isolate of *Trichoderma viride* @ 1% (w/v) followed by the application of *Piriformospora indica* (1% w/v) with RKN inoculation (40.75) and also chemical treatment with cartap hydrochloride 4% G (25kg/ha) (36.25). This is in line with findings of Jonathan and Rajendran (2000). Kiewnick and Sikora (2006) also observed reduction in root galling, egg mass and final nematode population when treated with *P. lilacinus.*

Results of this study clearly highlight the efficacy of the fungal isolates in improving the plant growth parameters (table 2). All treatments showed higher growth characters compared to control both with and without RKN inoculation in root zone. Among the treatments, isolate of *Trichoderma viride* recorded higher plant height of 81.23 cm and 86.05 cm both

with and without RKN inoculation and maximum fresh root weight of 176.38 g and 204.88g both with and without RKN inoculation. The fresh shoot weight was also the highest for isolate of *Trichoderma viride* with (80.25g) and without RKN inoculation and all treatments had higher fresh weight compared to control. Regarding the yield, isolate of *Trichoderma viride* recorded the highest yield (346.75g) with RKN inoculation and (361.13 g) without RKN inoculation and these were comparable with chemical treatment with cartap hydrochloride 4% G (25kg/ha) with (331.50 g) and (339.38g) without RKN inoculation respectively. John *et al.,* (2004) reported that Amaranthus plants treated with *G. monosporum*, *G. etunicatum* and *G. mosseae* resulted in a significant increase in fresh weight of plants and reduction in nematode population in root and soil. Similar findings of RKN management and increased growth parameters in tomato with *P. lilacinus* were reported by Ahmed and Monjil (2019).

Conclusion

The results indicated that soil drenching with *Trichoderma viride* was highly effective in reducing nematode populations in both soil and roots, as well as the number of galls and egg masses. Its efficacy was comparable to cartap hydrochloride and *Piriformospora indica*. *Trichoderma viride* also significantly improved plant growth parameters and yield, both with and without nematode inoculation. The study also found that the use of *Metarhizium anisopliae* and *Fusarium verticillioides* was effective in controlling the root knot nematode. The research highlights the potential of these fungal isolates as bio-agents for managing *Meloidogyne incognita* in tomato production, offering an alternative to chemical nematicides and contributing to sustainable agricultural practices.

Table 1 : Effect of different treatments on population of Meloidogyne incognita in tomato under pot culture condition with RKN inoculation

Treatments	Population of nematodes		No. of galls (5g root)	Gall Index	Number of female (5g root)	Number of egg masses (5 g root)
	Soil (200cc)	Root (5g)				
Trichoderma viride (1% w/v)	83.00(9.16)^{d}	44.25(6.73)^{e}	18.00(4.36)^{d}	2	22.50(4.85)^{c}	34.25(5.93)^{e}
Metarhizium anisopilae (1% w/v)	160(12.66)^{b}	94.75(9.78)^{b}	23.25(4.92)^{b}	2	34.25(5.93)^{b}	45.75(6.83)^{c}
Fusarium verticillioides (1% w/v)	147.5 (12.18)^{b}	61.25(7.88)^{c}	26.00(5.19)^{b}	2	33.00(5.83)^{b}	54.25(7.43)^{b}
Piriformospora indica (1% w/v)	99.75(10.31)^{c}	52.50(7.31)^{d}	21.75 (4.77)^{c}	2	21.50(4.74)^{c}	40.75(6.46)^{cd}
Cartap hydrochloride 4% G (25kg/ha)	98.25(9.96)^{c}	54.00(7.42)^{cd}	27.00(5.29)^{b}	1	19.75(4.55)^{c}	36.25(6.10)^{d}
Control	372.25(19.31)^{a}	183.75(13.59)^{a}	87.75 (9.42)^{a}	4	64.50(8.09)^{a}	146.00(12.12)^{a}
CD (0.05)	(2.985)	(0.534)	(0.378)	-	(0.346)	(0.408)
SE+ m	(0.996)	(0.178)	(0.126)	-	(0.115)	0.136)

Figures in the parenthesis are angular transformed values

Fig.1. Effect of different treatments on population of *M.incognita* in tomato

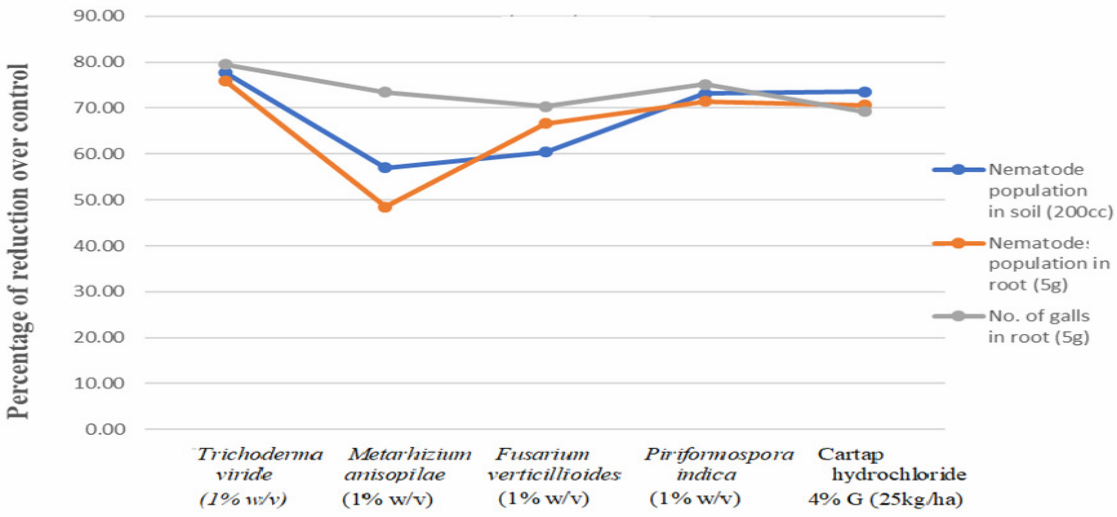

Table 2 : Effect of different treatments on biometric characters and yield of tomato under pot

Treatments	Plant height (cm)	Fresh shoot weight (g)	Fresh root weight (g)	Yield (g)	Plant height (cm)	Fresh shoot weight(g)	Fresh root weight (g)	Yield (g)
	with RKN inoculation				without RKN inoculation			
Trichoderma viride (1% w/v)	81.23[a]	176.38[a]	80.25 [a]	346.75 [a]	86.05[a]	204.88[a]	1.00[a]	61.13[a]
Metarhizium anisopilae (1% w/v)	72.32[cd]	165.22[a]	63.00[cd]	310.60[bc]	75.34[cd]	165.22[bc]	65.50[b]	324.50[bc]
Fusarium verticillioides (1% w/v)	70.25[ed]	154.75[a]	66.25[bc]	303.00[c]	71.50[d]	154.75[cd]	61.25[c]	311.50[c]
Piriformospora indica (1% w/v)	76.40[bc]	152.80[a]	59.00[d]	313.63[bc]	78.32[cb]	152.80	62.50[bc]	320.30[c]
Cartap hydrochloride 4% G (25kg/ha)	77.35[ab]	167.20[a]	68.50[bb]	331.50[ab]	80.25[b]	167.20[b]	66.00[b]	339.38[ab]
Control	57.50[e]	80.00[b]	48.50[e]	59.50[d]	59.50[e]	109.25[e]	48.50[d]	88.75[d]
CD (0.05)	4.314	39.127	4.228	25.083	4.241	32.789	10.779	2.785
SE+ m	1.441	13.068	1.412	8.388	1.417	10.951	3.600	7.610

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