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Bio-efficacy of *Trichoderma viride* against the root-knot nematode (*Meloidogyne incognita*) in tomato plant

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Abstract
In the present studies effectiveness of antagonistic fungi viz. *Trichoderma viride* were evaluated against *Meloidogyne incognita* on tomato. The application of *T. viride* significantly increased shoot weight and decreased root weight of tomato in a dose dependent manner. Doses of 6 gm/kg soil as soil treatment and $9x10^8$ cfu/ml as bare root dip treatment showed maximum increase in shoot weight and decrease in root weight. On the other hand, the antagonistic fungi caused significant reductions in number of galls, egg masses, eggs per egg mass and reproductive factors of *M. incognita* in a dose dependent manner. The fungi caused the maximum reductions in these parameters at two highest doses of 6 gm/kg soil as soil treatment and $9x10^8$ cfu/ml as bare root dip treatment. It is, therefore, concluded from the present evaluation that the indigenous isolates of *T. viride* have the potential to control *M. incognita* as both the treatments viz. soil application and bare root dip treatment.

Keywords: Bacterial concentration *Trichoderma viride*, biotic-induced resistance, tomato, nematode *M. incognita*, bare root dip, soil drench

Introduction
Plant parasitic nematodes constitute one of the biotic factors negatively influencing increased tomato production. The estimated losses induced by plant parasitic nematodes is worth US$ 118 billion worldwide (Atkinson *et al.*, 2012) [2]. There are over 4,100 species of plant-parasitic nematodes described. Of these, the top most economically important obligate plant parasitic genus is *Meloidogyne* spp. distributed worldwide (Jones *et al.*, 2013) [5]. The root-knot nematode consists of extremely polyphagous nematode groups because it has more than 3000 host species including vegetables, fruits, oil, fiber, grains and leguminous crops, next to weeds that are considered secondary hosts to nematodes (Khalil, 2013 and Jones *et al.*, 2013) [6, 5]. The most frequently occurring, species of root-knot nematodes include *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla*, which are responsible for high economic losses to varied crops. Root-knot nematodes (RKN) are one of the major pests of tomatoes worldwide and limit fruit production (Sikora and Fernandez, 2005; Tisserat, 2006) [16, 18]. The average crop yield losses are estimated to be in the neighborhood of 25% with damage in nematode uncontrolled individual fields even reaching up to 60% (Sasser *et al.*, 1982) [14].
Apart from the chemical management of root-knot nematode, since emphasis in recent times, have been on ecofriendly methods of pest management, use of biopesticide / bioagents that have gained more importance in recent years due to their advantages like maintenance of ecological balance. Identification of indigenous / native isolates of bioagents would be less expensive compared to the chemicals problems of resurgence by the nematode can be minimized, ecofriendly, help to achieve pollution free environment, reduces residues and health hazards, easy movability and once established remain effective over long periods (Affokpon *et al.*, 2011) [1]. Root-knot nematodes are mainly controlled by the application of nematicides and resistant cultivars. Although nematicides can effectively manage nematodes but their usage is limited due to their short-term effects, high costs, non-availability, resistance development in nematodes, health and environmental hazards, residual toxicity and adverse effects on the beneficial micro flora and fauna in the soil besides phytotoxic effects on the crop. Biological control of plant parasitic nematodes through microorganisms offers an alternative or supplemental management tool to replace chemical methods. Use of biological control agents is considered to be innocuous and economically feasible (Mukhtar *et al*., 2017b) [9]. These biocontrol agents can also be integrated with other management practices in integrated nematode management (Shahzaman *et al*., 2015; Khan *et al*., 2017; Rahoo *et al*.,

2017, 2018a, b) [15, 7, 12, 11, 13]. In recent years, several fungal and bacterial bio-agents have been tested for managing root-knot nematodes. The main criteria for successful deployment of these biocontrol agents in fields are their ability to suppress nematode populations and restrain their multiplication and enhance yields profitably in the presence of nematodes. For their suitability as nematode suppressive agents, the reductions in reproductive and developmental potentials of nematodes by these biocontrol agents must be assessed. *Trichoderma* is a ubiquitous fungus and have shown variations in aggressiveness among various isolates from different regions of the world. This necessitates that indigenous isolates of the fungus should be used for the management of root-knot nematodes. As there is little information on the effects of indigenous isolates of *T. harzianum* and *T. viride* on the reproductive potential of nematodes and growth variables of hosts, therefore, the objective of the present study was to evaluate the suppressive effects of these two fungi on the reproductivity of *M. incognita* resulting in growth variables of tomato.

Material and Methods

Isolation, identification and multiplication of indigenous biocontrol agents

Sample collection

The soil samples were collected from different sites of the JNKVV fields, as well as from formers fields from July to September, 2016.The samples were taken from 15 cm depth and collected in sterile polyethylene bags which were transported to the laboratory and stored at four degree Celsius. Isolation of *Trichoderma viride* was done employing a serial dilution technique. One milliliter of each solution was depipetted onto a Rose Bengal Agar (RBA) plate and incubated at 28 °C for one week in an incubator. The culture plates were examined daily and each colony that appeared was considered to be one colony forming unit (cfu). After enumeration of cfu, individual colonies were isolated from the same plates and each uncommon colony was reisolated onto a fresh Potato Dextrose Agar (PDA) petri plate. Distinct morphological characteristics were observed for identification and the plates were stored at four degree Celsius. Two techniques, visual observation on petri dishes and micro-morphological studies in slide culture, were adopted for identification of *Trichoderma viride*. For visual observation, the isolates were grown on PDA agar for 3-5 days. The mode of mycelial growth, color, odour and change of medium Colour for each isolate were examined every day. For micromorphological studies, a slide culture technique was used. Examination of the shape, size, arrangement and development of conidiophores or phialides provided identification of *Trichoderma viride*.

Identification

The fungus *Trichoderma viride* was identified on the basis of following microscopic characters.

Branching pattern highly variable, often consisting of a distinct central axis with many Longibrachiatum-like solitary phial ides near the tip, branches forming lower down paired or not, increasing in length with distance from the tip and rebranching in similar pattern; often no discernible central axis, fertile branches often arising from swollen nodes, often sinuous or curved, terminating in divergent to verticiliate whorls of two or three phial ides, solitary phialides common; in all cases branches tending to be widely spaced. Phialides lageniform, at most swollen in the middle, straight or often hooked, (5.0-) 6.5-11.0 (-18) µm long, (1.5-) 2.5-3.5 (-4.0) µm at the widest point, L/W (1.2) 2.0-4.5 (-7.8). Supporting cell (1.5-) 2.2-3.2 (-4.5) µm wide; ratio of length of phialide to width of supporting cell (1.4-) 2.4-4.6 (-7.5); ratio of width of phialide to width of supporting cell (0.5-) 0.9-1.4 (-2.0). Conidia subglobose, (2.7-) 3.2-4.2 (-5.0) x (3.0-) 3.5-4.5 (-5.5) µm, L/W (0.8-)1.1-1.3(-1.5), green, coarsely warted to tuberculate. Chlamydospores infrequent, terminal and intercalary.

Efficacy of *Trichodema viride* against root-knot nematode as soil treatment.

The experiment was conducted in ten cm earthen pots containing 500 g sterilized soil employing *Trichoderma viride.*

The fungus *Trichoderma viride* was multiplied on sorghum seeds. The seeds were boiled in water for half an hour and excess moisture was drained. The boiled seeds of sorghum were filled in polypropylene bags @ 500g seeds / bag and autoclaved at temp. 121.6 ^{0}C and pressure 1.05 kg/cm^2 for 20 minutes. After cooling, the bags were inoculated with pure culture of *T. viride* and incubated at 24 °C for ten days. When sufficient growth was achieved the seeds along with fungus were mixed with the pot soil @ 2, 4, 6g/kg soil. Before mixing the fungus spore, load was determined by haemocytometer.

Four repetitions for each treatment were maintained and inoculated with 1000 freshly hatched surface sterilized second stage juveniles as per the method described earlier. One uninoculated and one inoculated controls were also maintained. The pots were randomized on the glass house bench following Complete Randomized Design. The experiment was allowed to run for 45 days and irrigated with sterilized distilled water so as to obtain optimum moisture conditions. All the plant protection measures were employed to grow healthy crop. The glass house temperature ranged from 12 to 39 ^{o}C during the course of investigation.

The experiment was terminated 45 days after inoculation and observations on plant height, root length, shoot weight (fresh and dry). Root weight (fresh and dry), number of galls, number of egg masses/gall were recorded. The data was statically analyzed using appropriate statistical procedure.

Efficacy of *Trichodema viride* against root-knot nematode as bare root dip treatment.

Seeds of tomato (*Lycopersicon esculentum* cv. Pusa Ruby) after surface sterilization in one per cent sodium hypochlorite solution (NaOCl) were washed thoroughly under running tap water and allowed to dry under a laminar flow hood. The seeds were sown and two week old seedlings were uprooted and washed in sterilized distilled water to remove soil particles. These seedlings were dipped in spore suspensions of *Trichoderma viride.* The suspension with 9x10^8 cfu/ml was considered as standard (S). The standard solution was then diluted with sterilized distilled water so as to make concentration 9 x 10^8 cfu/ml, 9 x 10^6 cfu/ml and 9x10^4 cfu/ml. The seedlings were dipped in these concentrations for 1 hour and were transplanted in 10 cm earthen pots. After setting of roots, each pot was inoculated with 1000 freshly hatched surface sterilized second stage juveniles of *M. incognita.* One uninoculated and one inoculated with root-knot nematode were maintained as control and carbofuran @ one g/ kg soil also served as one of the as treatment. Each treatment was repeated four times and randomized on the glass house bench following Complete Randomized Design and irrigated with

sterilized distilled water to maintain adequate moisture. All the plant protection measures were applied to grow disease free plant. The glass house temperature ranged from 12 to 39 ^{o}C during the course of investigation.

The experiment was terminated 45 days after inoculation and observations on plant height, root length, shoot weight (fresh and dry). Root weight (fresh and dry), number of galls, number of egg masses/gall were recorded. The data was statically analyzed using appropriate statistical procedure.

Result

Efficacy of *Trichoderma viride* isolate against root-knot nematode (*Meloidogyne incognita*) as soil treatment.

The data presented in Table 1. indicated that maximum plant height (26.33 cm) was noted in uninoculated control followed by carbofuran (25.58 cm). *Trichoderma viride* at 6, 4 and 2 gm/kg recorded 24.05, 21.05 and 19.98 cm plant height respectively. Minimum (18.78 cm) plant height was recorded in inoculated control. Similar trend was noted with root length. Significantly higher root length (19.03 cm) was noted with 6gm/kg soil and minimum (9.13 cm) in inoculated control. Carbofuran showed maximum root length (21.85 cm). Fresh weights of shoots and roots were also influenced by higher dose of *Trichoderma viride* at (6gm/kg) which recorded 6.73 and 1.26 gm weight respectively. Minimum fresh shoot and root weights (3.72 and 0.58 gm) were recorded in inoculated control and maximum (9.77 and 1.08 gm) shoot and root weights were recorded with uninoculated control. Significant increase in the weights of fresh shoots and roots was also noted with 2gm/kg (4.35 and 0.75 gm) and 4 gm/kg (5.32 and 0.89 gm) in *Trichoderma viride* incorporated pots against inoculated control. Maximum (1.61 gm) fresh weight of root was recorded in carbofuran @ 1gm/kg soil. On dry weight basis, maximum (4.45 and 0.66 gm) shoot and root weights were recorded in uninoculated control and minimum (1.48 and 0.32 gm) in inoculated control. Significant increase (3.31 and 0.90 gm) in the weight was noted in *Trichoderma viride* 6gm/kg soil followed by 4gm/kg (2.84 and 0.78 gm) and 2gm/kg (2.66 and 0.55 gm) *Trichoderma viride* incorporated pot soil. Maximum (1.06 gm) dry weight of root was recorded with carbofuran @ 1g/kg soil as against minimum in inoculated control.

Minimum number of (7.25) galls/plant were recorded in carbofuran followed by the treatment where *Trichoderma viride* is incorporated in pot soil @ 6gm/kg (15.00). Significantly reduced numbers of galls were recorded in 4gm/kg (18.25) and 2gm/kg (21.25) as against maximum number of (34.50) galls in inoculated control.

Similarly, there was significant decrease (12.00) in number of egg masses/gall in carbofuran followed by the treatment where *Trichoderma viride* was inoculated @ 6gm/kg soil (41.25). Significantly decreased number of egg masses/gall was also recorded in 4gm/kg (55.75) and 2gm/kg (61.25) as against maximum number of (79.25) egg masses/gall in inoculated control.

Efficacy of *Trichoderma viride* isolate agaist root-knot nematode (*Meloidogyne incognita*) as bare root dip treatment.

The data presented in Table 2. indicated that maximum plant height (31.48 cm) was noted in carbofuran followed by *Trichoderma viride* at $9x10^8$ cfu/ml, (28.68 cm). *Trichoderma viride* at $9x10^6$, and $9x10^4$ cfu/ml, recorded 26.73 and 25.40 cm and uninoculated control (22.50 cm) plant height respectively. Minimum plant height (17.65 cm) was recorded in inoculated control. Similar trend was noted with root length. Significantly higher root length (21.83 cm) was noted with *Trichoderma viride* at $9x10^8$ cfu/ml, and minimum (9.15 cm) with inoculated control. Carbofuran showed maximum root length (22.60 cm).

Fresh weight of shoots and roots were also influenced by the higher dose of *Trichoderma viride* at $9x10^8$ cfu/ml, which recorded (8.90 and 2.12 gm) weights respectively. Minimum fresh shoot and root weights (3.62 and 0.79 gm) were recorded in inoculated control and maximum (9.67 and 2.85 gm) in carbofuran. Significant increase in fresh shoot and root weights were also noted in *Trichoderma viride* at $9x10^6$, (8.81 and 1.43 gm) and $9x10^4$ cfu/ml, (7.75 and 1.11 gm) root treated plant as against inoculated control.

On dry weight basis, maximum (5.02 and 0.93 gm) shoot and root weights were recorded in carbofuran and minimum (1.47 and 0.45 gm) in inoculated control. Significant increase (4.76 and 0.89 gm) in weights was noted in *Trichoderma viride* at $9x10^8$ cfu/ml followed by *Trichoderma viride* at $9x10^6$, (4.38 and 0.83 gm) and $9x10^4$ cfu/ml, (3.97 and 0.61 gm) *Trichoderma viride* root treated plants. Minimum number of (9.75) galls/plant were recorded in carbofuran followed by the treatment where roots were dipped in *Trichoderma viride* at $9x10^8$ cfu/ml, (28.75). Significantly reduced numbers of galls were recorded in *Trichoderma viride* at $9x10^6$ (46.50) and $9x10^4$ cfu/ml, (60.75) as against maximum number of (70.25) galls in inoculated control.

Similarly, there was significant decrease (12.75) in number of egg masses/gall in carbofuran followed by the treatment where roots dipped in *Trichoderma viride* at $9x10^8$ cfu/ml, (38.50). Significantly less number of egg masses/gall was also recorded in *Trichoderma viride* at $9x10^6$ (51.25) and $9x10^4$ cfu/ml (73.25) as against maximum number of (83.50) egg masses/gall in inoculated control.

Plate 1: Effect of *Trichoderma viride* against root knot nematode (*Meloidogyne incognita*) as bare root dip treatment in tomato plant

Plate 2: Effect of *Trichoderma viride* against root knot nematode (*Meloidogyne incognita*) as soil treatment in tomato plant

Table 1: Efficacy of *Trichoderma* viride agaist root-knot nematode (*Meloidogyne incognita*) as soil treatment

S. No.	Treatment	Plant height (cm)	Root length (cm)	Fresh weight (gm)		Dry weight (gm)		No. of galls/plant	No. of egg masses/gall
				Shoot	Root	Shoot	Root		
1	Control (Uninoculated)	26.33*****	18.00	9.77	1.08	4.45	0.66	00	00
2	Control (Inoculated)	18.78	9.13	3.72	0.58	1.48	0.32	34.50 (6.04)******	79.25 (8.96)******
3	2 gm/kg soil	19.98	15.95	4.35	0.75	2.66	0.55	21.25 (4.72)	61.25 (7.89)
4	4 gm/kg soil	21.05	17.68	5.32	0.89	2.84	0.78	18.25 (4.39)	55.75 (7.53)
5	6 gm/kg soil	24.05	19.03	6.73	1.26	3.31	0.90	15.00 (4.00)	41.25 (6.50)
6	Carbofuron	25.58	21.85	9.52	1.61	3.87	1.06	7.25 (2.87)	12.00 (3.61)
	S.E(m)±	0.22	0.12	0.14	0.02	0.12	0.03	1.05 (1.43)	2.87 (1.97)
	CD at 5%	0.67	0.36	0.42	0.04	0.35	0.08	3.11 (2.03)	8.53 (2.61)

***** Mean of four repetations.

****** Figures in parantheses are $\sqrt{(n+1)}$ transformed values.

Table 2: Efficacy of *Trichoderma* isolate agaist root-knot nematode (*Meloidogyne incognita*) as bare root dip treatment

S. No.	Treatment	Plant height (cm)	Root length (cm)	Fresh weight (gm)		Dry weight (gm)		No. of galls/plant	No. of egg masses/gall
				Shoot	Root	Shoot	Root		
1	Control (Uninoculated)	22.5*****	15.45	7.29	1.02	2.41	0.56	00	00
2	Control (Inoculated)	17.65	9.15	3.62	0.79	1.47	0.45	70.25 (8.44)**	83.50 (9.19)**
3	$9x10^4$ cfu/ml	25.40	10.53	7.75	1.11	3.97	0.61	60.75 (7.86)	73.25 (8.62)
4	$9x10^6$ cfu/ml	26.73	15.63	8.81	1.43	4.38	0.83	46.50 (6.89)	51.25 (7.23)
5	$9x10^8$ cfu/ml	28.68	21.83	8.90	2.12	4.76	0.89	28.75 (5.45)	38.50 (6.28)
6	Carbofuron	31.48	22.6	9.67	2.85	5.02	0.93	9.75 (3.28)	12.75 (3.71)
	S.E(m)±	0.18	0.53	0.14	0.12	0.04	0.04	1.69 (1.64)	1.89 (1.70)
	CD at 5%	0.53	1.56	0.41	0.37	0.12	0.11	5.01 (2.45)	5.63 (2.58)

***** Mean of four repetations.

** Figures in parantheses are $\sqrt{(n+1)}$ transformed values.

Discussion

Effect of *Trichoderma viride* on root-knot nematode and plant growth parameters as soil treatment.

All the growth parameters increased as there was an increase in concentration of *Trichoderma viride* with increased in root-knot infested soil as depicted in Tables. The data indicated that all the growth parameters were at their maximum in soil where Trichoderma viride was added @ 6 gm/kg soil followed by 4 and 2 gm/kg soil. The fungus adversely affected reproduction of M. incognita in tomato. Number of galls/plant and egg masses/gall were decreased with increase in the inoculum level of Trichoderma viride. However, minimum numbers of galls/plant and egg masses/gall were recorded in Carbofuran (1 gm/kg soil).

The results are in accord with the findings of Mascarin *et al*. (2012) [8], Hazmi and Javeed (2016) [4]. Debabat and Sikora (2006) [3], who observed significantly reduced root galling, egg production and soil juveniles and increased plant growth due to native isolate of T. viride in tomato.

Effect *Trichoderma viride* on root-knot nematode and plant growth parameters as bare root dip treatment.

The root system of tomato plants, when exposed to various concentrations of bioagent showed better growth as against inoculated and uninoculated controls. The root system when dipped barely in aqueous spore suspension of *Trichodema viride* at $9x10^8$ concentration showed better plant growth and nematode reproduction followed by other two concentrations i.e, $9x10^6$ and $9x10^4$. Similar results have also been recorded by Nitu *et al*. (2016). The increase in biomass production may be due to the production of plant growth promoters that are thought to have indirect stimulation of nutrient uptake and by producing siderophore and antibiotics to protect plant from deletarious rhizosphere organisms. (Sundermurthy and Balabaskar, 2013) [17].

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