STUDY ON SOME SPECIES OF *Trichoderma* FOR THE MANAGEMENT OF ROOT KNOT NEMATODE (*Meloidogyne* spp.) IN TOMATO

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ABSTRACT

Pot experiment was conducted to find out the competence of some commercial product of *Trichoderma* spp. on the management of root knot nematode in tomato crop. The experiment was conducted in RCBD having seven treatments of six different *Trichoderma* spp. including one control in three replications. Each treatment had five pots. Twenty five days old seedling of Shrijana hybrid tomato was transplanted and inoculated with 1500 eggs of *Meloidogyne* spp. in soil after five days of transplanting of tomato seedlings. The spore suspensions of different *Trichoderma* spp. adjusted to 1 × 10^6 spores/ml and also inoculated per pot with 30 ml after one week of nematode inoculation. The same concentration and amount of *Trichoderma* spores solution was again inoculated after one month from the date of first application. Sixty five days after transplanting, all tomato plants were uprooted and the number of galls in root, galling index and fresh root weight were determined based on a 0-10 scale. The result showed that the treatments varied significantly in root knot index and number of galls per root system and fresh root weight per plant. The lowest gall index was observed in *T. harzianum* – Nemastin (1.6), followed by *T. harzianum* (T22) – Rootshield Plus (2.37) and *T. viride* (Ashtha TV) (3.43). Similarly, lowest numbers of galls (9.13) was observed in *T. harzianum* – Nemastin than all other treatments. There was no significant difference in fresh root weight per plant of tomato among the treatments except *T. harzianum* and *T. harzianum* (T22). Significantly highest suppressive effect on the nematode population was achieved by *T. harzianum*- Nemastin (*Rf* 0.45), followed by *T. harzianum,* (T22) (*Rf* 0.61), and *T. viride,* (Ashtha TV) (*Rf* 1.43). Different isolates of *T. harzianum* suppressed root knot disease of tomato as compare the isolates of *T. viride.* Hence, *T. harzianum* –Nemastin can be applied as a biological control agent to reduce the nematode population.

Key words : Gall Index, *Meloidogyne* spp., root knot nematode, tomato, *Trichoderma* spp.

INTRODUCTION

Nematodes are usually microscopic in size and commonly described as filliform or thread like multi-cellular invertebrates (Krueger and McSorley, 2008). At least one nematode species is reported to parasitize every cultivated plant species (Luc *et al.,* 2005). Plant parasitic nematodes (PPNs) have long been known to cause extensive crop losses through reduced yields, shortened productive life, or lowered value of produce (Fitzgerald, 1950). It destroys crops and causes economic losses equal to those of' any other pathogens. Root-knot nematodes (*Meloidogyne* spp.) pose a serious threat to crops throughout the world and are considered to be the most destructive and cause huge losses among the PPNs. Almost all the vegetables in tropical and warm areas of the temperate regions are attacked by this nematode (Hussain *et al.*, 2012). It has been estimated that in tomato fields, 30% plants are

affected by root knot nematodes and in certain areas, the tomato cultivation has been abandoned due to very poor yields on account of this disease (Agrios, 2005). Estimates of vegetable crop losses due to *Meloidogyne* species, mainly *M. incognita* and *M. javanica,* are 24 to 38% for tomato (Sasser, 1979). In many countries, due to this nematode, it is often impossible to grow important vegetables like tomato in the tropics and semi-tropics. The infestation level of root-knot nematode alone has been reported up to 80% on tomato and okra and observed losses worth of 46% of total production (Bhatti & Jain, 1977). In Hemza at Kaski district, a report revealed that root knot nematode caused upto 27-30% yield reduction on tomato production in plastic tunnel (Baidya *et al.,* 2013).

Several methods of effectively controlling nematodes are available. Plant extract or residues used in control of nematode have advantage of cheapness and availability over the conventional methods (Izougu *et al.*, 2012). Use of synthetic nematicides has proved effective except for hazardous effects of chemicals, high cost, not being eco-friendly among others (Izouga *et al.*, 2014). In other side, several problems are associated with the use of chemicals such as their poor penetration into the nematode eggs, rapid leaching and degradation and a serious threat to the environment. In other side, a large number of bio-control agents have been tested to manage root-knot nematodes with encouraging results. The biological control agents like *Trichoderma harzianum* and *T. virede* have showed promising results against the nematode (Pathak & Kumar, 2003). *Trichoderm*a has been extensively tested for controlling plant parasitic nematodes in vegetable crops (Sikora, 2008). *T. harzianum* has been reported an effective bio-control agent against root knot and other nematodes (Parveen *et al.*, 1993; Khan & Saxena, 1997). The use of bio-control agents is gaining importance in the field of nematode management and has emerged as a promising alternative to the use of synthetic pesticide (Wilson *et al.*, 1993). So, continuous effort has to be given for monitoring and identification of plant parasitic nematodes to find out the real situation of nematode variability and their population in fields and also effort in assessing the effectiveness of various species of commercial product of *Trichoderma* available in market for the management of root-knot nematode.

MATERIALS AND METHODS

The experiments were conducted at National Plant Pathology Research Centre, NARC, Khumaltar, to know the effect of various *Trichoderma* spp. on root knot nematode in tomato, hybrid "Srijana" in two growing seasons (April-June and August-October, 2021 AD). The experiment was setup in a Randomized Complete Block Design (RCBD). There were seven treatments with six different species of *Trichoderma* and one control (Table 1) with three replications. One replication of each treatment contained five pots with plant and altogether 105 pots kept in distance between two adjacent replications was 30 cm and between two adjacent treatments within each replication 10 cm. Soil collected from the field at NARC, Khumaltar, was cleaned by removing plant debris and pebbles and autoclaved for 30 min at 15 psi and 120 ^{0}C. The cooled soil was mixed well with properly decomposed compost in the ration of 4:1, i.e. 800 g soil with 200 g compost. The plastic pots of 12.5 cm diameter and 18.0 cm height were filled with 1 kg soil/pot upto 2 cm below the mouth of pots. Twenty-five days old seedlings were transplanted, one plant per pot. Irrigation was done immediately after transplanting, then an alternate day for whole experiment period.

Nematode eggs were extracted from the knotted tomato roots. The root was cleaned in tap water to remove soil, and detached from plants with a scissor. The roots were vigorously shaken in 200 ml 0.5% sodium hypochlorite (25 ml of 4% NaOCl mixed in 175 ml of distilled water) solution in conical flasks for 4 min, to dissolve the gelatinous matrix of egg sac and release the eggs. The

suspensions were filtered first through a 125 µm mesh sieve to separate debris and then through a 30 µm mesh sieve to retain eggs on the sieve. The eggs collected in 30 µm sieve were thoroughly washed with water and poured from the sieve to a beaker making final volume of 300 ml. One ml of aliquot was placed in a nematode counter with the help of pipette after stirring the suspension and the eggs were observed under stereoscope and counted. The process was repeated for three times and theaverage nematode count was maintained at around 300 eggs per ml. Five days after transplanting the seedlings, 3 cm deep three holes were made close to each plant with a stick and the plants were inoculated with 5 ml inoculum/plant using a plastic syringe. Thus, each plant was inculated with 1500 eggs of *Meloidogyne* spp. After inoculation, the holes were covered with the surrounding soil.

Commercial products of different *Trichoderma* spp. were multiplied in PDA plates (Table 1). The plates were incubated at 25 ^{0}C for two weeks. As the *Trichoderma* colony grew on PDA, it formed concentric rings and changed colour from white to green as it matured and sporulated. The plates were then used for conidia extraction. The mycelia and conidia developed on PDA were carefully scraped with a slide, and suspended in 500 ml distilled water for each species. Spores were separated from mycelia by sieving through a muslin cloth. The spore suspensions collected in a beaker were adjusted to 1×10^6 spores/ml after counting spores using a haemocytometer. One week after nematode inoculation, 3 cm deep three holes were made with a stick around each plant at rhizosphere and 10 ml spore suspension was poured/hole (30 ml/plant). The same concentration of *Trichoderma* spores solution was prepared and again inoculated in the pot after one month from the first inoculation.

Sixty days after transplanting, all tomato plants were carefully uprooted, roots were cleaned with tap water and assessed for root gall intensity caused by *Meloidogyne* spp. The galling index (root gall index) was determined of each root system based on a 0-10 scale and its explanation given by Bridge & Page (1980) (Table 2). All lateral roots were separated from tap root by cutting carefully with scissors. Galls on each lateral root and tap root of each plant of each treatment were counted with naked eyes. The same roots were used for extraction of eggs and juveniles. Mean number of galls/plant/treatment was calculated.

From the uprooted tomato plants, whole root system of each plant was detached with the help of scissors. The soil was washed off from all roots and fresh root weight was taken using a digital weighing balance. Replication and treatment means/plant were computed. During counting, the suspension was well shaken and an aliquot of 1 ml was taken for egg counting under a Stereoscope microscope at 40 × magnification. Number of eggs was expressed per 50 ml suspension and it was the total number of eggs/root system and mean number of eggs/plant was computed.

Juveniles of nematodes were extracted from soil following the method described by Hooper *et al.* (2005). Each sample was crumbled finely with hand. A tissue paper was spread inside a plastic sieve placed on an extraction plastic tray. 100 g of soil sample was spread over the tissue paper and clean tap water was carefully added from one side of the extraction tray until the soil layer was wet totally. The extraction tray and the sieves were labeled to identify the soil samples. The extraction sets were left as such for 24 hr to let swim the juveniles from soil to the extraction tray.

After 24 hr, the plastic sieves with soil were lifted up to drain the water into the extraction tray. The water with nematodes from the extraction tray was poured into a labeled beaker. A wash bottle was used to rinse the tray and the water was also added into the beaker total volume 300 ml as stock solution. The suspension was left as such for an hour to settle down the nematodes. Ten milliliter suspension from the stock sample was pipetted out after stirring well and kept into counting plates for

juveniles count. Total number of each type of juvenile present in 10 ml of suspension was counted, and from this number, total number of nematodes present in 300 ml stock suspension (i.e. 100 g soil) was calculated.

Final population of root knot nematode was determined by adding mean number of eggs and juveniles/plant, and mean number of juveniles/100 g soil in each treatment. Reproduction of *Meloidogyne* spp. was calculated and expressed as reproductive factor (*Rf*). A *Rf* is number of nematodes (eggs and juveniles) produced after uprooting plants from one egg inoculated at the beginning.

Reproductive factor (*Rf*) = *Pf/Pi*

where,

Pf = final population of eggs and juveniles of *Meloidogyne* spp. after uprooting
Pi = initial population of eggs of *Meloidogne* spp. at the time of inoculation (i.e. 1500 eggs/plant)

The collected data were compiled and entered as per Ms-excel program. Analysis of variance for all parameters was carried out as per the procedures given in M-STAT. Duncan's Multiple Range Test (DMRT) was done for mean separation from the reference of Gomez and Gomez (1984). Graphs and tables were constructed by using the MS-excel computer software program.

Table 1. List of different *Trichodema* spp. with their concentration per ml for inoculation

SN	Treatments	Symbol	Inoculation ml /pot	Source
1.	*Trichoderma harzianum* (Nemastin)	T_1	30 ml	Kan Biosys Pvt. Ltd, Maharashtra, India
2.	*Trichoderma harzianum* (T22) (Rootshield Plus)	T_2	30 ml	AP Biochemical, Gujarat, India
3.	*Trichoderma asperellum*	T_4	30 ml	DORA Agri Tech, Suzhou, China
4.	*Trichoderma viride* (Ashtha TV) cfu: 2×10^9/g	T_3	30 ml	Lila Agrotech Pvt. Ltd. Kolkata, West Bangal, India
5.	*Trichoderma viride* (BIOCARE-F) 1.25% W.P. (cfu: 2×10^9/g)	T_5	30 ml	Harsidhi Biotech Pvt. Ltd. Bihar, Uttar Pradesh, India
6.	*Trichoderma viride* (Prarambha Trichioderma) cfu: 1×10^7/g	T_6	30 ml	Prarambha Biotech, Pvt. Ltd. Kalanki, Kathmandu, Nepal
7.	Control	T_7	30 ml (water)	

Table 2. A scale for determining root gall index caused by *Meloidogyne* spp. in roots of vegetables

Galling index (0-10 scale)	Root system galled (%)	Explanation of rating
0	0	Complete and healthy root system, no infection
1	10	Very few small galls can only be detected upon close examination
2	20	Small galls/knot only, but clearly visible, main root clean
3	30	Some larger knot visible, main root clean
4	40	Larger knot predominate, but main root clean
5	50	50% of root infested k knotting on some main roots, reduced root system
6	60	Knotting on main roots
7	70	Majority of main roots Knotted
8	80	All main roots, including tap root, knotted and few clean root visible
9	90	All roots severely knotted and plant usually dying
10	100	All roots severely knotted, no root system and plant dead

RESULTS AND DISCUSSION

The treatments varied significantly in root knot index and number of galls per root system, but not in fresh root weight per plant. The lowest gall index was observed in *T. harzianum*-Nemastin (1.6), followed by *T. harzianum* (T22) (2.37) and *T. viride* (Ashtha TV) (3.43), which were, however, not significantly different to one another. Control plants showed considerably highest root gall index (6.08) which was significantly higher than others. Considerably, lowest numbers of galls (9.13) was observed in *T. harzianum* – Nemastin than all other treatments (Table 3). The number of galls in *T. harzianum,* (T22) (42.61) and *T. viride,* (BIOCARE-F) (71.43) inoculated plants had also significantly difference with control (132.33).

There was no significant difference in fresh root weight per plant of tomato with control except *T. harzianum* – Nemastin and *T. harzianum* (T22). However, fresh root weight appeared highest in *T. harzianum,* (T22) (11.3 g), followed by *T. harzianum*– Nemastin (8.37 g) where in control (3.75 g) showed the lowest weight.

The treatments varied considerably in number of eggs and juveniles (J2)/plant, J2/100g soil and reproductive factor (*Rf*). Significantly highest suppressive effect on the nematode population was achieved by *T. harzianum*- Nemastin (*Rf* 0.45), followed by *T. harzianum,* (T22) (*Rf* 0.61), *T. viride,* (Ashtha TV) (*Rf* 1.43) and *T. viride,* (Prarambha Trichoderma) (*Rf* 1.51) (Table 4). *T. asperellum* (*Rf* 2.10) and *T. viride,* (Prarambha Trichoderma) (*Rf 1.51)* did not show any significant difference from each other. Similarly, *T. viride* (BIOCARE-F) and control had also no significantly differ each other.

In roots, population of eggs and juveniles was recorded lower number in *T. harzianum* followed by *T. harzianum* (T22) and *T. varide* (Ashtha TV), whereas, in *T. viride* (BIOCARE-F), the nematode population (eggs and juveniles) did not show any different with control.

Table 3. Mean value of treatments effect on root gall index and fresh root weight of tomato in screen house at NPPRC, Khumaltar in two seasons (April-June and August-October), 2021

SN	Treatments	Root gall index (GI) (0-10 scale)#	No. of galls/root system#	Fresh root weight/plant (g)#
1	*Trichoderma harzianum* (Nemastin)	1.6cd	9.13c	8.37b
2	*Trichoderma harzianum* (T22) Root shield Plus	2.37c	42.61bc	11.38b
3	Trichoderma viride (Ashtha TV)	3.43bc	88.86ab	5.51a
4	*Trichoderma asperellum*	3.91b	102.05a	4.39a
5	*Trichoderma viride* (BIOCARE-F)	3.87b	71.43b	5.07a
6	*Trichoderma viride* (Prarambha Trichoderma)	3.63b	101.60a	7.23a
7	Control	6.08a	132.33a	3.65a
	Mean	3.55	78.28	6.51
	CV%	11.18	15.97	3.75
	LSD	1.07	46.31	4.4
	(P<0.05)	*	*	*

Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance, *significant at 5% level, CV = Coefficient of variation, LSD = Least Significant difference.

Mean data transferred in root square value for analysis

Table 4. Mean value of treatments effect on reproduction of *Meloidogyne* spp. on tomato in screen house at NARC, Khumaltar, during two seasons (April-June and August- Oct), 2021

SN	Treatments	*Pi* (No.)	Final population (No.)			*Pf* (No.)	*Rf*
			Eggs /root system#	J_2/ root system#	J_2 /100g soil#		
1	*Trichoderma harzianum* (Nemastin)	2000	812d	17bc	62d	891	0.45d
2	*T. harzianum* (T22) Rootshield Plus	2000	1114d	6de	97cd	1217	0.61d
3	*Trichoderma viride* (Ashtha TV)	2000	2717c	11d	137bc	2865	1.43c
4	*Trichoderma asperellum*	2000	3841b	6de	180ab	4027	2.10b
5	*T. viride* (BIOCARE-F)	2000	5242a	19b	217a	5478	2.74a
6	*T. viride* (Prarambha *Trichoderma*)	2000	3181bc	17bc	124c	3022	1.51bc
7	Control	2000	5694a	33a	201a	5928	2.96a
	Mean	2000	3186	16	145		1.227
	CV%		11.75	8.91	6.54		14.42
	LSD		671.12	5.22	43.6		0.6
	P<0.05		*	*	*		*

Mean data transferred in square root value for statistical analysis

Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance, *Significant at 5% level, CV = Coefficient of variation, LSD = Least Significant difference.

Pi = Initial population of eggs of *Meloidogyne* spp. inoculated into a plant
Pf = Final population of eggs and Juveniles in a plant and soil of a pot
Rf = Reproductive factor
J2 = Second stage juveniles of *Meloidogyne* spp.

Interaction of Gall Index (*GI*) with Reproductive Factor (*Rf*) of *Meloidogyne* spp.

There was a direct relationship between gall index and reproductive factor of *Meloidogyne* spp. Generally, as root gall index increased reproduction of nematodes also increased and vice versa (Fig. 1). Similar trend was also reported by Baidya *et al.* (2017). Initially, there was slow or gradual increase in gall index due to suppressive effect of the treatments, and later there was abrupt rise due to sharp decline in suppression of nematode multiplication (Fig. 1).

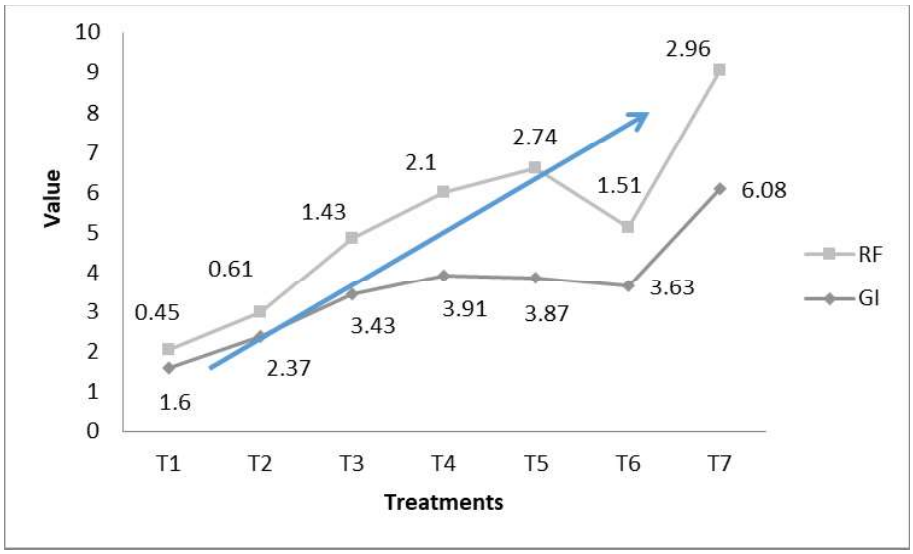

Fig. 1. Interaction of gall index with reproductive factor of *Meloidogyne* spp. in tomato in screen house at NARC, Khumaltar, during 2021.

The screen house experiment results indicated that the commercial product of *T. harzianum* - Power significantly reducing the gall formation on tomato roots caused by *Meloidogyne* spp. among the treatments at $P<0.05$. The phytopathogenecity of *Meloidogyne* was lower in the application of *T. harzianum*- Nemasin, followed by *T. harzianum* (T22) - Rootshield Plus and *T. viride* (Ashtha TV) compared to the control. The similar results were obtained by Sharon *et al.* (2001), who reported that *T. harzianum* reduced galling of root-knot nematode, *M. javanica,* on tomato plants. Furthermore, Dababat and Sikora (2007) used two species of *Trichoderma* (*T. viride*) and *T. harzianum*) and found a significant reduction in tomato root galling infested with *M. incognita*. Four densities inoculums densities of two Saudi isolates of *T. harzianum* and *T. viride* against *M. javanica* on tomato (10^4, 10^6,10^8 and 10^{10} spores/g of soil) were used and results indicate that the efficacy of *T. harzianum* was better than that of *T. viride*, especially at the highest used density (10^{10} spore/g soil) which resulted in the best control according to Al-Hazmi and TariqJaveed, 2015. In our study also showed the two

different product of *T. harzianum* was found more effective against *Meloidogyne* spp. than the product of *T. viride*.

The experimental result indicated that the application of *Trichoderma* species also increased the root weight of tomato plants with significant differences when compared with control. Application of difference species of *T. viride,* there was no significant differences among the treatments. This result agreed with the results of Dababat and Sikora (2007), which showed there was no significant variation between weights and heights of plants inoculated with different *Trichoderma* spp. Other similar findings by Ozbay and Newman (2004) showed *T. harzianum* T22 and T95 had no effect on root fresh and dry weights. Naserinasab *et al.* (2011) had also reported that inoculating the tomato seedling with *T. harzianum* didnot have consistent positive effect on fresh root weight.

T. harzianum significantly reduced egg number and juveniles. The production of antibiotics and extracellular lytic enzymes (Elad *et al,* 1982) by *Trichoderma* spp. are known to be involved in the antagonism. *T. harzianum* has also been found as an eggs parasite of *M. incognita* racs-3 killing 53% of eggs in vitro (Dos Santos *et al,* 1992).

T. harzianum parasitizes eggs and juvenile cuticle layer by dissolving the chitin layer through enzymatic activity. They proliferate within the organism and produce toxic metabolites into the medium in which they grow (Dos Santos *et al.*, 1992; Bandyopadhyay & Cardwell, 2003). *T. harzianum* was able to grow on the egg surface and penetrated the egg shell (Saifullah and Thomas, 1996). The present study may indicate that *T. harzianum* is an egg parasite of root knot nematode. Different species of *Trichoderma* have different modes of penetration (Dumas and Boyonowski, 1992). The variation in egg infection by the *T. harzianum* isolates can be related to genetic variability among isolates yielding difference in infectivity (Naserinasab *et al.*, 2011). Thus, the enzymes produced by *Trichoderma* spp., such as chitinase, glucanases and proteases seem to play an important role in parasitism (Haran *et al.*, 1996). The research by Sharon *et al.*, (2007) showed that *T. asperellum* parasitizedon egg masses, their derived eggs and the second stage juveniles (J2s). When the egg masses were destroyed, the number of the infective juveniles reduced as well as the overall number of nematodes. Thus, this explained the reason for the reduction in the number of J2 nematodes that were observed from the soils treated with *T. asperellum* compared to the soil in the control. Dos Santos *et al.*, (1992) reported *T. harzianum* as an effective egg parasite of *M. incognita*. *T. harzianum* was able to grow on the egg surface and penetrated the egg shell (Saifullah & Thomas, 1996).

The results suggest that *T. harzianum* produced chemical compounds detrimental to nematodes in soil or that the fungus stimulated some defensive mechanisms in tomato to inhibit nematode infection of the roots or to delay the development of nematodes that entered the tomato roots. Systemic acquired resistance or induced systemic resistance may have been induced in tomato in response to *T. harzianum* infection, leading to suppression of nematode infection and development (Jindapunnapat *et al.*, 2013). Exudation of organic acids such as gluconic acid, citric acid and fumaric acid by *Trichoderma* species reduces pH of soil and finally increase solubility and absorb important micronutrients required for growth of plant such as iron, manganese, magnesium, mineral cations and phosphats Benitez *et al.*, 2004. Liu *et al.* (2007) showed that *Trichoderma* species self-propagated and survived remarkably in the soil and plant rhizosphere therefore, mightremained in soil for a long period and it had very high nematicidal action to the nematodes, such as root-knot nematodes. Antagonistic fungi possessed larvicidal and oviposidal properties against root knot nematodes. It was

assessed from the present investigation that the reproductive factor of *Meloidogyne* was significantly reduced by *T. harzianum* - Nemastin, followed by *T. harzianum* (T22) – Rootshield Plus and *T. viride* (Ashtha TV) in comparision to other treatments and control. Differences in performance of various strains of *Trichoderma* species in this test could also possibly be attributed to differences in the secretion of biochemical substances (Murslain *et al.*, 2014). *Trichoderma* has not only been proved to parasitize nematodes also inactivate pathogen enzymes and help in tolerance to stress condition by enhanced root development.

CONCLUSIONS

Some commercial product of *T. harzianum* appear better than the product of *T. viride* to suppress root knot disease of tomato and multiplication of *Meloidogyne* spp. in roots and soil of tomato in screen house. In addition, the bio-control agents also reduce the number of gall formation and increase the root system. Therefore, the market available product of *T. harzianum* namely, *T. harzianum* (Nemastin) and *T. harzainum* (T22) – Root shield Plus should be suggested to tomato growers to apply as organic management tool in nematode infested fields to reduce the nematode population and increase yield of tomato. Also further investigation on identification of more effective isolates of *Trichoderma* species has to be carried out for the management of different species of root knot nematode in tomato and other vegetable crops.

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