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Short Communication

Management of Root-Knot Nematode, *Meloidogyne incognita*, in Tomato with Two *Trichoderma* Species

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ABSTRACT

In the present studies comparative effectiveness of two antagonistic fungi viz. *Trichoderma harzianum* and *T. viride* were evaluated against *Meloidogyne incognita* on tomato. The application of *T. harzianum* and *T. viride* significantly increased shoot weight and decreased root weight of tomato in a dose dependent manner. Doses of 8×10^3 and 1×10^4 cfu/g of soil showed maximum increase in shoot weight and decrease in root weight. On the other hand, both 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. Both the fungi caused the maximum reductions in these parameters at two highest doses of 8×10^3 and 1×10^4 cfu/g of soil. The increases or reductions were slightly greater with *T. harzianum* than those with *T. viride*. It is, therefore, concluded from the present evaluation that the indigenous isolates of *T. harzianum* and *T. viride* have the potential to control *M. incognita*.

Tomato (*Solanum lycopersicum* L.), an important member of family solanaceae, is widely cultivated in the tropical and temperate regions of the world. In Pakistan, tomato is cultivated on 62 thousand hectares with an annual production of 599 thousand tons which is very low as compared to other tomato growing countries of the world. Many pests including plant pathogenic nematodes attack tomato and are responsible for causing severe growth retardation (Ashfaq *et al*., 2015, 2017; Riaz *et al*., 2015; Fateh *et al*., 2017; Javed *et al*., 2017a, b; Kassi *et al*., 2018; Nabeel *et al*., 2018). Among plant parasitic nematodes, root-knot nematode (*Meloidogyne incognita*), is one of the most important nematodes associated with low production of tomato in Pakistan (Kayani *et al*., 2017; Tariq-Khan *et al*., 2017). Root-knot nematodes are ranked at the top among the five major plant pathogens and the first among the ten most important genera of plant parasitic nematodes in the world (Mukhtar *et al*., 2017a; 2018). They have wide geographic distribution, large host range and high destructive potential. They have been reported to be implicated with other plant pathogens like *Ralstonia solanacearum* and result in disease complexes and aggravation of wilt diseases (Shahbaz *et al*., 2015; Aslam *et al*., 2017a, b). In Pakistan, *M. incognita* has been found one of the most dominant root-knot species and rampant in the vegetable-producing areas of Pakistan and considerably reduces growth and yield (Kayani *et al*., 2018). The worldwide distribution of this species is 47% and in Pakistan its overall occurrence is 52%. Overall yield losses of 50 to 80% have been reported to be caused by root-knot nematodes in vegetables and 24 to 38% yield losses due to root-knot nematodes have been estimated on tomato. Root-knot nematodes have become a serious threat to the profitable cultivation of tomato in the country. The yield losses by root-knot nematodes are mainly caused due to buildup of inoculum of the nematode and repeated cultivation of same cultivars in the same land every year (Hussain *et al*., 2016).

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). These biocontrol agents can also be integrated with

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other management practices in integrated nematode management (Shahzaman *et al*., 2015; Khan *et al*., 2017; Rahoo *et al*., 2017, 2018a, b). 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.

Materials and methods

A population of root-knot nematode (*Meloidogyne incognita*) initially isolated from cucumber roots, identified on the basis of perineal pattern and maintained on the highly susceptible cultivar of tomato (money maker) was used in the assessment. The nematode was mass produced on tomato cv. Money maker and second stage juveniles were extracted from the infected roots for inoculation of plants (Mukhtar et al., 2017b).

For mass production of biocontrol agents *Trichoderma harzianum and T. viride* used in the experiment were obtained from the Institute of Agricultural Sciences, University of the Punjab, Lahore. Both the biocontrol fungi were mass produced on wheat grains. Wheat grains were chopped, soaked in water for 12 h, blotted dried and 250 g each was added in 500 ml flasks. The grains in flasks were double-autoclaved at 15 psi for about 30 min. The sterilized wheat grains in flasks were inoculated separately with pure cultures of each of the antagonistic fungi and incubated at 25±1°C for 15 days. The flasks were shaken at alternating days for uniform colonization of the fungi. The concentration of spores per gram of the grains was counted using haemocytometer after making spore suspensions in distilled water.

The soil (60% sand; 19% silt; 20% clay; 1% organic matter and pH 7.6) used in the experiment was sterilized with formalin, passed through a 3.5 mm mesh sieve to remove large stones and plant residues and added to pots.

Table I.- Comparative effects of *T. harzianum* and *T. viride* on shoot weight (SW) and root weight (RW).

Concentration (cfu/g of soil)	Increase in SW (%)		Decrease in RW (%)	
	T. h.	*T. v.*	*T. h.*	*T. v.*
2×10^3	5.2±1.6	4.4±1.7	4.8±1.3	3.1±2.1
4×10^3	10.5±1.4	8.3±1.4	7.9±1.5	7.5±1.9
6×10^3	23.9±3.5	21.4±2.1	19.2±2.9	13.6±1.1
8×10^3	40.7±1.9	34.0±2.3	28.4±2.8	25.2±3.4
1×10^4	42.5±1.2	35.6±3.9	31.5±1.5	26.8±3.7

Values are means of five replications. Means sharing common letters do not differ significantly according to Fisher's Protected Least Significant Difference test (P > 0.05). *T. h., T. harzianum; T. v., T. viride.*

Table II.- Comparative effects of *T. harzianum* and *T. viride* on shoot length (SL) and root length (RL).

Concentration (cfu/g of soil)	Increase in SL (%)		Increase in RL (%)	
	T. h.	*T. v.*	*T. h.*	*T. v.*
2×10^3	6.1±1.7	5.4±1.3	3.7±0.7	2.5±0.6
4×10^3	8.9±1.7	6.3±1.6	5.3±1.9	3.8±1.4
6×10^3	18.8±2.0	16.8±1.5	14.4±1.8	12.5±1.9
8×10^3	35.9±2.7	27.6±1.8	37.2±2.8	23.7±2.8
1×10^4	32.7±2.4	33.2±2.8	34.4±2.6	31.5±2.7

For abbreviations and statistical details, see Table I.

The comparative biocontrol potential of *T. harzianum* and *T. viride* against *M. incognita* was tested in pots. The biocontrol agents were each mixed with the formalin sterilized soil at the rates of 2×10^3, 4×10^3, 6×10^3, 8×10^3, or 1×10^4 cfu per g of soil. The treated soil (1 kg per pot) was then put in plastic pots. Three-week-old healthy seedlings of tomato cv. Money maker were transplanted individually in each pot. One week after transplantation, the plants were inoculated with approximately 2,000 freshly hatched second stage juveniles (J2s) of *M. incognita*. Nematode-inoculated plants without antagonists served as controls. There were five replications for each treatment. The pots were arranged in Completely Randomized Design in a greenhouse at 25±2°C for 7 weeks and watered as needed. After seven weeks, the plants were carefully removed from the pots. The shoots were severed from the roots. Shoot and root lengths were measured, and fresh root and shoot weights were determined. The galls and egg masses on each plant root system were counted under a stereomicroscope at 40×. For estimation of total nematode populations, eggs were extracted from the roots of individual plants and juveniles were extracted from the soil from each pot (Mukhtar et al., 2017b). The total number of eggs and nematodes in soil formed the total population. The reproductive factor was calculated by dividing the final population by the initial one. Percent

reductions or increases in plant growth parameters and nematode infestations were calculated over controls as described by Kayani *et al*. (2018).

Completely randomized design was used in the experiment. All the data were subjected to analysis of variance using GenStat Package 2009 (12th edition) version 12.1.0.3278 (www.vsni.co.uk). Means were compared by Fisher's Protected Least Significant Difference Test at 5%.

Results and discussion

The application of *T. harzianum* and *T. viride* increased shoot weight and length and decreased root weight and length of tomato in a dose dependent manner (Tables I, II). On the other hand, both 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 (Tables III, IV). It was also observed that the reductions were slightly greater with *T. harzianum* than those with *T. viride*.

Table III.- Comparative effects of *T. harzianum* and *T. viride* on nematode infestations.

Concentration (cfu/g of soil)	Decrease in No. of galls (%)		Decrease in No. of egg masses (%)	
	T. h.	*T. v.*	*T. h.*	*T. v.*
2×10^3	7.1±1.7	6.4±1.8	9.7±3.3	7.5±1.8
4×10^3	14.9±1.8	13.3±1.3	15.2±2.7	10.8±2.2
6×10^3	23.8±3.7	18.8±2.3	19.4±2.6	12.5±2.9
8×10^3	32.9±4.3	25.7±3.0	36.6±3.5	33.8±3.7
1×10^4	35.7±4.0	32.8±3.4	33.9±3.2	30.2±3.4

For abbreviations and statistical details, see Table I.

Table IV.- Comparative effects of *T. harzianum* and *T. viride* on nematode reproduction.

Concentration (cfu/g of soil)	Decrease in No. of eggs/egg mass (%)		Decrease in reproductive factor (%)	
	T. h.	*T. v.*	*T. h.*	*T. v.*
2×10^3	4.1±0.9	3.4±0.6	12.7±1.3	9.5±1.1
4×10^3	7.2±1.1	6.3±0.8	13.8±1.7	9.8±1.2
6×10^3	13.8±1.5	10.8±1.2	18.4±2.1	11.5±1.3
8×10^3	15.9±1.9	12.7±1.5	27.2±1.5	23.7±1.7
1×10^4	22.7±2.3	20.1±1.9	44.4±3.1	31.5±1.9

For abbreviations and statistical details, see Table I.

Trichoderma is a ubiquitous soil fungus which colonizes root surfaces and root cortices (Sharon *et al*., 2009). Several species of *Trichoderma* including *T. harzianum, T. viride*, *T. atroviride*, and *T. asperellum*, have provided excellent control of root-knot nematodes in previous studies (Sharon *et al*., 2007). Application of *Trichoderma* species resulted in reduced nematode galling and improved plant growth and tolerance. The highly branched conidiophores of *Trichoderma* produce conidia that can attach to different nematode stages. Conidial attachment and parasitism varies among fungal species and strains (Sharon *et al*., 2007). This process was often associated with the formation of fungal coiling and appressorium-like structures. *T. harzianum* colonizes isolated eggs and J2s of *M. javanica* (Sharon *et al*., 2007). Successful parasitism of the nematode by *Trichoderma* requires mechanisms to facilitate penetration of the nematode cuticles or eggshells. The involvement of lytic enzymes has long been suggested and demonstrated in *Meloidogyne* parasitism (Spiegel *et al*., 2005). Besides direct antagonism, other mechanisms involved in *Meloidogyne* control by *Trichoderma* spp. include production of fungal metabolites and induced resistance (Freitas *et al*., 1995; Goswami *et al*., 2008). In general, *Trichoderma* should be applied before planting to achieve maximum nematode control as good establishment of the fungus in plant rhizospheres seems to be important for nematode control.

The reason for increased plant growth, yield and other parameters observed here could be attributed to the release of growth promoting substances by bio-agents or by producing toxic metabolites which inhibit nematodes and exclude other deleterious microorganisms. The result obtained in current investigation uphold the results observed by Goswami *et al*. (2008) who observed increased growth and yield of tomato, soybean, tobacco and capsicum in pot and field experiments by the inoculation of *Trichoderma* spp. Reduction in nematode galls and egg masses might be due to high rhizosphere competency of bio-agents as they can easily colonize roots and may reduce feeding sites for nematodes. The reduction of root gall number may be due to the failure of majority of the juveniles to penetrate the host root. In the present study, the effectiveness of *T. harzianum* was slightly better than that of *T. viride*. This might be due to variations in genetics, pathogenic potential and the origin of the isolates.

Conclusion

It is concluded from the present evaluation that the indigenous isolates of *T. harzianum* and *T. viride* have the potential to control *M. incognita* and other species of root-knot nematode infecting vegetables.

Statement of conflict of interest

Authors have declared no conflict of interest.

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