

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.705.216>

Effect of the Introduction Time of *Trichoderma harzianum* into Soil on its Biocontrol Potential against *Meloidogyne javanica* on Tomato Plants under Greenhouse Conditions

Omeed A.M. Abdelrafaa^{1*}, Ekhlas H.M Ahmed², Nayla E. Haroun³,
Ahmed A.M. Dawabah⁴, Fahad A. Al-Yahya⁵ and Hamzeh A. Lafi⁵

¹Al Mahalliah Trading and Agriculture Company, Ltd., Riyadh, Saudi Arabia

²Department of Plant Protection, College of Agricultural Studies,
Sudan University of Science and Technology

³University of Hafr Albatin, the university college in Al- khaffi, Department of Biology,
Kingdom of Saudi Arabia

⁴Department of Nematode Diseases, Plant Pathology Research Institute, Agricultural
Research Center, 9 Gamaet El-Qahera street, Giza 12619, Egypt

⁵Department of Plant Protection, College of Food and Agricultural Sciences, King Saud
University, P. O. Box 2460, Riyadh 11451, Saudi Arabia

*Corresponding author

ABSTRACT

Keywords

Application time,
Biological control,
Trichoderma harzianum,
Meloidogyne javanica,
Solanum lycopersicum

Article Info

Accepted:
16 April 2018
Available Online:
10 May 2018

The effect of the introduction time of *Trichoderma harzianum* into soil on its biocontrol potential against *Meloidogyne javanica* on tomato plants was evaluated in a greenhouse pot experiment. Seven introduction times (inoculations) of *T. harzianum* were tested. The fungus was added simultaneously and 5, 10 and 15 days, either before or after the infestation of tomato soil with *M. javanica* eggs. Plants inoculated with *M. javanica* alone, *T. harzianum* alone and non-treated plants served as controls. Results showed that all *T. harzianum* applications suppressed ($P \leq 0.05$) *M. javanica* reproduction and the root damage of the tested tomato plants, while generally increased the tomato growth parameters. The time of the fungus application into soil was found to be very critical. The early applications of the fungus (5, 10 and 15 days before soil infestation with *M. javanica*) were the most effective.

Introduction

Tomato, *Solanum lycopersicum* L., is ranking first among the world vegetable cultivation, where it accounts for 16% of the world vegetable production (130 million metric tons/year) (FAO, 2015) unfortunately; tomato plants are attacked by many plant pathogens either in the fields or greenhouses. The most

common of these pathogens are: *Verticillium*, *Fusarium*, tobacco mosaic virus, and the root-knot nematodes, *Meloidogyne* spp. (Anastasiadis. *et al.*, 2008). Root-knot nematodes, especially *M. javanica* The root-knot nematode, *Meloidogyne incognita*, is a sedentary endoparasitic plant pathogen with a broad host range. These nematodes are of considerable economic importance,

responsible for low crop yields and annual losses in tropical countries of 22% (Sasser, 1979). These parasites are the most prevalent in the country (Hussain *et al.*, 2012; Kayani *et al.*, 2012a, 2013) and accounts for growth impairment (Hussain *et al.*, 2011a, Irshad *et al.*, 2012; Maleita *et al.*, 2012). Use of chemicals for nematode control, though very effective, cannot be adopted by the farmers in developing countries due to their high cost. In developed countries, nematicides are undesirable due to associated problems of residual toxicity, environmental pollution and public health hazards (Thomason, 1987). These factors are an impetus for the discovery of alternative methods for nematode control (Tzortzakakis and Petsas, 2003). One alternative to chemical nematicides is the use of biological control agents, either alone or integrated with other pest management strategies (Davies *et al.*, 1991; Hussain *et al.*, 2011b; Singh *et al.*, 2012). Microbial pesticides rely upon the potential biochemicals synthesized by the microbes and it requires in small quantities often decomposing rapidly (Tian *et al.*, 2007; Elyousr *et al.*, 2010; Khan and Haque, 2011). Biopesticides are less toxic than chemical pesticides and safer to the beneficial microorganism, human and environment. In a number of instances, they have been proved as effective control agents in managing plant parasitic nematodes. In general, they have specific mode of action with a narrow range of targets. Since, they are slow acting upon the target organisms, they need relative critical application time which results in suppression of populations rather than their elimination (Elyousr *et al.*, 2010). In biological control, many antagonists have shown efficacy against root-knot nematodes. Among these, the fungus *Trichoderma harzianum* has been found to be an effective bio control agent for the management of root-knot and other nematodes (Casas-Flores and Herrera-Estrella, 2007; Lòpez-Llorca *et al.*, 2008; Hallmann *et al.*, 2009; Moosavi and

Zare, 2012). This present study was conducted to determine the effects of the introduction time of *T. harzianum* into soil on their biocontrol potential against *M. javanica* on tomato under greenhouse conditions

Materials and Methods

Sample collection

Fungal collection, isolation and maintenance

Soil samples were collected from seven different greenhouses located on farms in Riyadh, al muzahimiyah area the soil samples were taken from greenhouses which haven't been treated with any of the fungicide within the last 7 days. About 25 grams of top soil (after removal of 2 cm of soil surface) were collected in sterile cups, labeled with date and source of collection. The samples were transported to the laboratory and processed within 2 hours of collection (Babu *et al.*, 2000).

For isolation from soil, serial dilution and pure plate technique was used. Dilution at 10^{-1} , 10^{-2} and 10^{-3} , were subsequently spread onto Semi-selective Medium plates and incubated at $25 \pm 1^\circ\text{C}$ for 14 days (Gaugler and Bilgrami, 2004). The fourteen days old of fungal culture was cut into small plug of mycelium using sterile borer and transferred to a new plate of Potato Dextrose Agar (P.D.A.). Sub-culturing was repeated several times in order to get a pure culture. The pure cultures were maintained on P.D.A. plates at 4°C .

Fungal identification

Morphological identification

Purified cultures (from seven days old fungal culture) of the isolated fungi were examined macroscopically and microscopically in order

to identify the strain on the basis of their morphological traits and cultural characteristics of the fungi such as mycelium growth, colony texture, spores production and other characteristics (Andrews, 2006). Microscopic examination for the purpose of identification was carried out by transferring a portion of the mycelium to clean microscopic glass slides. For better analysis of morphological characteristics, the slides were stained with Lactophenol blue solution to enhance contrast. The prepared slides were examined microscopically at 40× magnification.

Preparation of spore suspension of *T. harzianum*

Fifteen days before the experiment, the fungal was cultivated on P.D.A. media plates in order to produce viable spores in sufficient numbers to allow a range of tests to be conducted on spores of the same age and life history. Spore suspension was obtained by washing the ascospores that formed on the surface of plates with 11ml of 0.01% sterile Tween 20. The suspension was collected in a sterile 100 ml Erlenmeyer flask and loosened by shaking with sterile glass beads for 2 hr. The density of spore suspension was adjusted with 0.01% sterile Tween 20 to correspond to a final concentration of approximately 5×10^7 spore/mL. The number of spores was quantified using a Neubauer counting chamber and a compound microscope (400×) (Chandra, 2006). The suspension is then filtered through gauze and stored in refrigerator at a temperature of 4 ° C until use.

Collection of the nematode *Meloidogyne javanica*

Egg collection

Nematode eggs were extracted from heavily galled tomato roots originally obtained from a

greenhouse cultured with tomato according to procedure described by Reimann *et al.*, (2008). Galled roots were washed free from soil under tap water, cut into 1 cm pieces and macerated in a Warring blender at high speed for 20 s and collected in a glass bottle. Sodium hypochlorite (NaOCl) was added to a final concentration of 1.5% active chlorine to separate eggs from their surrounding gelatinous matrix (Figure 1). The bottle was shaken vigorously for 3 min and the suspension was thoroughly washed with tap water through a sieve combination (250, 100, 45 and 25 µm) to remove the NaOCl. Eggs were collected on the 25 µm sieve and washed with tap water into a beaker and used directly for assay after an aliquot was removed to estimate the number of eggs per milliliter.

Nematode juvenile collection

Eggs were surface disinfested with sodium hypochlorite as described above and washed with sterile filtered 1% sodium Thiosulfate for 3 minutes followed by sterile water for 5 minutes the eggs were then transferred for hatching onto nylon screens (30-µm-pore size) in sterile water. J2 that passed through the filter within 72 hrs were collected and used immediately for assays.

Nematode identification

Nematode identification was performed by experts from the Laboratory of Department of Plant Protection, King Saud University. Based on morphological and morphometrical characteristics, specimens were identified as the root-knot nematode, *Meloidogyne javanica*.

Study site

The laboratory work was carried out in department of Plant Protection, King Saud University. The experiment was carried out

during the period from October 2017 to January 2018.

Plant's temperature conditions in greenhouse experiments

The experimental plants were kept in the green house where the temperature was ranged from $30 \pm 2^\circ \text{C}$ during the day to $22 \pm 2^\circ \text{C}$ during the night.

Type of tomato used

Tomato of Newton coated seeds type medium hybrid was purchased from the local Agricultural Company in Riyadh city.

Soil mixture preparation

Soil, sand and compost were collected from agronomy farm at Riyadh city composited and mixed well in a ratio of 6:2:1 respectively (Johnson and Curl, 1972). The mixture was autoclaved at 121°C for 15 minutes at 15 psi. The sterilized soil was allowed to cool at room temperature and used for filling the plastic tray and pots used for seedlings.

Preparations of pots

Plastic pots of 1000 cm^3 were cleaned, washed, dried up properly and sterilized with 70% ethanol. Each pot was filled with 1000 g of sterilized soil mixture for later use. Data were statistically analyzed using analysis of variance (ANOVA), and treatments means were separated by protected Fischer's least significant difference (LSD) using SAS.

Results and Discussion

Shoot and root fresh weight

Trichoderma harzianum increased significantly ($P < 0.05$) root and shoot fresh weight, whether introduced after or prior (5,

10 and 15 days) to nematode inoculation, compared to inoculation with nematode alone (Table 1 and Figure 1). However, effects of the fungus introduction after (5 and 10 days) to nematode inoculation were not different ($P \leq 0.05$). Similarly, introduction of *T. harzianum* at all times reduced the number of galls/plant (Table 2). However, such reduction of galls was greater when the fungus was introduced 15 days before to nematode inoculation. Reduction number of egg mass/plant of *M. javanica* was greater when the fungus was introduced 15 days before to nematode inoculation. However, such reduction number of galls/plant and number of egg mass/plant was obtained ($P \leq 0.05$) by *T. harzianum* at all times of the fungus introduction compared to nematode alone (Table 2 and Figure 2).

The introduction of *T. harzianum* to *M. javanica* - infested soil at or prior to nematode inoculation increased host growth and suppressed nematode reproduction compared to controls. Our results support previous reports on the efficacy of different species and isolates of *Trichoderma* against *Meloidogyne* spp. (Mascarin *et al.*, 2012; Pandey *et al.*, 2003; Siddique *et al.*, 2001). The suppressive effects of *T. harzianum*, reported in this study, on nematode number of galls/plant and number of egg mass/plant are strong evidence that considerable parasitism was occurring. Our results indicate that time of the fungus introduction into the soil proved to be very important. Greater control effects were achieved when *T. harzianum* was introduced 15 days after to soil infestation with the nematode. Similar evidence was previously reported. The extra time given to *T. harzianum* was very advantageous to have an established fungal population. A similar conclusion was suggested by Al-Hazmi *et al.*, (2008) working with the nematode-trapping fungus of *Arthrobotrys conoides* against *M. incognita* on corn.

Table.1 Effect of the inoculation time with *Trichoderma harzianum* on the root and shoot fresh weights of tomato plants infected with *Meloidogyne javanica*, 60 days after inoculation

Treatment	Root fresh weight (g)	Shoot fresh weight (g)
<i>T. harzianum</i> + <i>M. javanica</i> at the same time	25.84 b	32.50 bc
<i>T. harzianum</i> , 5 days before <i>M. javanica</i>	22.65 cd	31.94 bc
<i>T. harzianum</i> , 10 days before <i>M. javanica</i>	24.55 bc	32.45 bc
<i>T. harzianum</i> , 15 days before <i>M. javanica</i>	29.26 a	38.10 a
<i>T. harzianum</i> , 5 days after <i>M. javanica</i>	22.02 de	29.98 c
<i>T. harzianum</i> , 10 days after <i>M. javanica</i>	21.65 de	23.94 d
<i>T. harzianum</i> , 15 days after <i>M. javanica</i>	20.00 fg	23.16 d
<i>T. harzianum</i> alone (control).	25.22 b	32.86 b
<i>M. javanica</i> alone (control).	19.06 g	22.34 d
Non-inoculated plants (free control).	29.90 a	35.98 a

Data are means of four replicates. Means, in each column, followed by the same letter(s) are not significantly different according to Fischer's protected least significant difference (LSD) test ($P \leq 0.05$)

Table.2 Effect of the inoculation time with *Trichoderma harzianum* on the number of galls and egg masses developed by *Meloidogyne javanica* on tomato roots, 60 days after inoculation

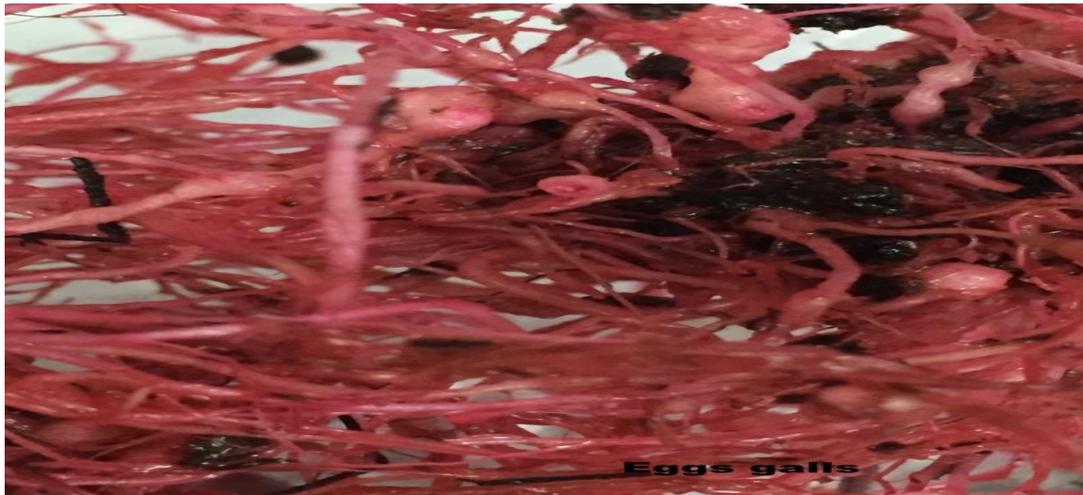
Treatment	Number of galls/plant	Number of egg masses/plant
<i>T. harzianum</i> + <i>M. javanica</i> at the same time	50.25 de	14.00 ef
<i>T. harzianum</i> , 5 days before <i>M. javanica</i>	52.25 de	16.20 e
<i>T. harzianum</i> , 10 days before <i>M. javanica</i>	55.00 d	18.00 e
<i>T. harzianum</i> , 15 days before <i>M. javanica</i>	21.00 e	5.00 f
<i>T. harzianum</i> , 5 days after <i>M. javanica</i>	73.25 d	28.20 d
<i>T. harzianum</i> , 10 days after <i>M. javanica</i>	109.75 c	38.4 c
<i>T. harzianum</i> , 15 days after <i>M. javanica</i>	171.25 b	58.20 b
<i>T. harzianum</i> , alone (control).	-	-
<i>M. javanica</i> alone (control).	511.75 a	208.00 a
Non-inoculated plants (free control).	-	-

Data are means of four replicates. Means, in each column, followed by the same letter(s) are not significantly different according to Fischer's protected least significant difference (LSD) test ($P \leq 0.05$)

Fig.1 Green house experiments



Fig.2 Egg galls



Although *M. javanica* caused considerable crop damage to tomatoes in Saudi.

Our results conclude that time can be an important factor in success of fungal bio control agent. Results showed that when *T. harzianum* isolate are introduced into soil at or prior to nematode inoculation they can be more effective in managing *M. javanica* because they need certain time to be established in soil.

All *T. harzianum* treatments significantly decreased the number of galls and egg amasses developed by *M. javanica* on tomato plants.

All *T. harzianum* treatments significantly increased the root and shoot fresh weights of tomato plants infected with *M. javanica*.

Infestation of soil with *T. harzianum*, 15 days before the inoculation with *M. javanica* was

the best treatment in controlling *M. javanica* on tomato and increasing plant growth.

References

- Anastasiadis, I.A., I.O. Giannakou, D.A. Prophetou-Athanasidou and S.R. Gowen. 2008. The combined effect of the application of a biocontrol agent *Paecilomyces lilacinus*, with various practices for the control of root-knot nematodes. *Crop Prot.* 27: 352-361
- Andrews, S. S. (2006). Crop residue removal for biomass energy production: Effects on soils and recommendations. *US Department of Agriculture-Natural Resource Conservation Service*, http://soils.usda.gov/sqi/files/AgForum_Residue_White_Paper.pdf (15 November 2012)
- Babu, R., Nageswari Poornima, K., and Suguna, N. (2000). Biological control potential of *Glomus fasciculatum* against *Meloidogyne incognita* on tomato and Okra. *Proc. First Nat. Sympo. Pest Management in Horticultural Crops. Environmental implications and thrusts, Bangalore, India pp-348.*
- Casas-Flores S. and A. Herrera-Estrella, 2007. Antagonism of plant parasitic nematodes by fungi. In: *The Mycota, Vol. VI: Environmental and Microbial Relationships*, 2nd edn. (C.P. Kubicek, I.S. Druzhinina, ed.), Springer-Verlag, Berlin, Pp. 147–157.
- Chandra, V. (2006). *Technology, adaptation, and exports: How some developing countries got it right*: World Bank Publicatio.
- Davies K.G., V. Laird and B.R. Kerry, 1991. The motility, development and infection of *Meloidogyne incognita* encumbered with spores of the obligate hyperparasite *Pasteuria penetrans*. *Revue de Nematologie* 14, 611–618.
- Elyousr, K.A.A., Khan, Z., Award, E.M.M and Moneim, M.F.A. 2010. Evaluation of plant extracts and *Pseudomonas* spp. for control of root-knot nematode, *Meloidogyne incognita* on tomato. *Nematropica*. 40: 289-299.
- Food Agricultural Organization (FAO). 2015. *FAO Statistical Pocketbook*. <http://www.fao.org/3/a-i4691e.pdf>
- Gaugler, R., and Bilgrami, A. (2004). Feeding behaviour. *Nematode Behaviour*. Bilgrami AL & Gaugler R, eds, 91-126.
- Hallmann J., K.G. Davies and R.A. Sikora, 2009. Biological control using microbial pathogens, endophytes and antagonists. In: *Root-Knot Nematodes* (R.N. Perry, M. Moens, J.L. Starr, ed.), CABI Publishing, Wallingford, UK, Pp. 380–411.
- Hussain M.A., T. Mukhtar and M.Z. Kayani, 2011a. Assessment of the damage caused by *Meloidogyne incognita* on okra. *Journal of Animal and Plant Sciences* 21, 857–861.
- Hussain M.A., T. Mukhtar and M.Z. Kayani, 2011b. Efficacy evaluation of *Azadirachta indica*, *Calotropis procera*, *Datura stramonium* and *Tagetes erecta* against root-knot nematodes *Meloidogyne incognita*. *Pakistan Journal of Botany* 43, 197– 204 (Special Issue).
- Hussain M.A., T. Mukhtar, M.Z. Kayani, M.N. Aslam and M.I Haque, 2012. A survey of okra (*Abelmoschus esculentus*) in the Punjab province of Pakistan for the determination of prevalence, incidence and severity of root-knot disease caused by *Meloidogyne* spp. *Pakistan Journal of Botany* 44, 2071–2075.
- Irshad U., T. Mukhtar, M. Ashfaq, M.Z. Kayani, S.B. Kayani, M. Hanif and S. Aslam, 2012. Pathogenicity of citrus nematode (*Tylenchulus semipenetrans*) on *Citrus jambhiri*. *Journal of Animal and Plant Sciences* 22, 1014–1018.
- Kayani M.Z., T. Mukhtar and M.A. Hussain, 2012a. Association of root-knot nematodes (*Meloidogyne* spp.) with cucumber in the Pothowar region of the Punjab province of Pakistan. *International Journal of Biology and Biotechnology* 9, 23–29.
- Kayani M.Z., T. Mukhtar, M.A. Hussain and M.I. Haque, 2013. Infestation assessment of root-knot nematodes (*Meloidogyne* spp.) associated with cucumber in the Pothowar region of Pakistan. *Crop Protection* 47, 49–54.
- Khan, M.R. and Haque, Z. 2011. Soil application of *Pseudomonas fluorescens* and

- Trichoderma harzianum* reduces root-knot nematode, *Meloidogyne incognita*, on tobacco. *Phytopathol.Mediterr.*50: 257–266.
- López-Llorca L.V., J.G. Macia-Vicente and H.-B. Jansson, 2008. Mode of action and interactions of nematophagous fungi. In: Integrated management and biocontrol of vegetable and grain crops nematodes (A. Ciancio, K.G. Mukerji, ed.), Springer, Dordrecht, The Netherlands, 51–76.
- Maleita C.M.N., R.H.C. Curtis, S.J. Powers and I.M.de O. Abrantes, 2012. Inoculum levels of *Meloidogyne hispanica* and *M. javanica* affect nematode reproduction, and growth of tomato genotypes. *Phytopathologia Mediterranea* 51, 566–576.
- Mascarin, G.M., Junior, M.F.B., Filho, J.V. de A. *Trichoderma harzianum* reduces population of *Meloidogyne incognita* in cucumber plants under greenhouse conditions. *J. Entomol. Nematol.*2012; 4: 54-57.
- Moosavi M.R. and R. Zare, 2012. Fungi as biological control agents of plant-parasitic nematodes. In: Plant Defence: Biological Control (J.M. Merillon, K.G. Ramawat, ed.), Springer Science + Business Media, Dordrecht, 67–107
- Pandey, G., Pandey, R.K., Pant, H. Efficacy of different levels of Trichoderma viride against root-knot nematode in chickpea (*Cicerarietinum* L.). *Annals Plant Protection Sci.*, 2003; 11: 96-98.
- Reimann, S., Hauschild, R., Hildebrandt, U., and Sikora, R. (2008). Interrelationships between *Rhizobium etli* G12 and *Glomus intraradices* and multitrophic effects in the biological control of the root-knot nematode *Meloidogyne incognita* on tomato. *Journal of Plant Diseases and Protection*, 115(3), 108-113.
- Sasser J.N., 1979. Economic importance of *Meloidogyne* in tropical countries. In: Root-knot nematodes (*Meloidogyne* spp): Systematics, Biology and Control (F. Lamberti, C.E. Taylor, ed.), Academic Press, NY, USA, 359–374.
- Siddique, I.A., Amer-Zareen, Zaki, M.J., Shaukat, S.S. Use of *Trichoderma* species in the control of *Meloidogyne javanica*, root-knot nematode of in okra and mungbean. *Pak. J. Biol. Sci.*, 2001; 4: 846-848.
- Singh V., R. Mawar and S. Lodha, 2012. Combined effects of biocontrol agents and soil amendments on soil microbial populations, plant growth and incidence of charcoal rot of cowpea and wilt of cumin. *Phytopathologia Mediterranea* 51, 307–316.
- Thomason I.J., 1987. Challenges facing nematology: environmental risk with nematicides and the need for new approaches. In: *Vistas on Nematology* (J.A. Veech, D.W. Dickson, ed.), Hyattsville, MD, Society of Nematologists, USA, 469–479.
- Tian, B., Yang, J. and Zhang, K.Q. 2007. Bacteria used in the biological control of plant parasitic nematodes: populations, mechanisms of action and future prospects. *FEMS Microbiol. Ecol.* 61: 197–213.
- Tzortzakakis E.A. and S.E. Petsas, 2003. Investigation of alternatives to methyl bromide for management of *Meloidogyne javanica* on greenhouse grown tomato. *Pest Management Science* 59, 1311–1320.

How to cite this article:

Omeed A.M. Abdelrafaa, Ekhlal H.M. Ahmed, Nayla E. Haroun, Ahmed A.M. Dawabah, Fahad A. Al-Yahya and Hamzeh A. Lafi. 2018. Effect of the Introduction Time of *Trichoderma harzianum* into Soil on its Biocontrol Potential against *Meloidogyne javanica* on Tomato Plants under Greenhouse Conditions. *Int.J.Curr.Microbiol.App.Sci.* 7(05): 1836-1843.
doi: <https://doi.org/10.20546/ijcmas.2018.705.216>