Effects of different inoculum densities of *Trichoderma harzianum* and *Trichoderma viride* against *Meloidogyne javanica* on tomato

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**KEYWORDS**

Biological control; Efficacy; Root-knot nematode; *Solanum Lycopersicum*; *Trichoderma* spp.

**Abstract** A greenhouse experiment was conducted to evaluate the effects of different inoculum densities of two Saudi isolates of *Trichoderma harzianum* and *Trichoderma viride* against *Meloidogyne javanica* on tomato. Four densities (10^4, 10^6, 10^8 and 10^10 spores/g of soil) of each fungus were used. The results indicate that all four inoculum densities of the two *Trichoderma* species suppressed the nematode reproduction and root galling; and increased the growth of tomato plants, compared to controls. Efficacy of both fungi increased as their inoculum densities increased. Generally, 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.

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1. Introduction

The free-living soil fungus *Trichoderma* spp. is a potential biological control agent of plant-parasitic nematodes (Jatala, 1986; Spiegel and Chet, 1998). Biocontrol of the root-knot nematodes (*Meloidogyne* spp.) by different species of *Trichoderma* has been reported by several scientists (Sharon et al., 2001, 2007, 2011; Affokpon et al., 2011; Mascarin et al., 2012; Nasrinseid et al., 2011; Rao et al., 1998; Spiegel et al., 2007; Al-Shammari et al., 2013).

Although *Trichoderma* species are sometimes found associated with *Meloidogyne* spp. in field soils and can penetrate their eggs and females, their successful deployment as a biocontrol agent against nematodes may depend on a thorough understanding of this fungus. Compatibility between the fungal isolate, host cultivar and soil substrate may, therefore, play an important role in the proliferation and persistence of *Trichoderma* spp. in soil. It is important that biocontrol isolates are able to compete and persist in the environment, rapidly colonize and efficiently proliferate on newly formed roots (Sariah et al., 2005) and provide continued benefits over the duration of annual crops (Harman, 2000). Several articles have been published on *Trichoderma* spp. against *Meloidogyne* spp. with good results (Sahebani and Hadavi, 2008; Affokpon et al., 2011; Mascarin et al., 2012; Jindapunnapat et al., 2013). However, some important factors that are required for proper evaluation were sometimes neglected, especially the parasitic potential of the fungus in relation to its inoculum densities.
To fully evaluate the potential of a biological control agent, a dose–response relationship between the concentration of the applied antagonist applied and the reduction of plant damage needs to be established. However, the inoculum density of the antagonist is difficult to determine in the kind and amount necessary for optimal activities. Different studies on antagonist dose–plant disease response relationships in biological control systems have been reported (Montesinos and Bonaterra, 1996; Smith et al., 1997). Some studies on the effects of different inoculum densities of Trichoderma against Meloidogyne spp. have demonstrated an increase in their efficacy at increasing inoculum density but up to certain levels (Jindapunnapat et al., 2013; Sahebani and Hadavi, 2008).

The purpose of this study was to evaluate the effects of four inoculum densities of two local (Saudi) isolates Trichoderma harzianum (isolate-27) and Trichoderma viride (isolate-08) on their biocontrol efficacy against Meloidogyne javanica on tomato.

2. Materials and methods

This study was conducted in the greenhouse (24 ± 2 °C). Thirty-day-old seedlings of tomato (cv. Sultana-7) were used, one seedling per pot (15 cm diam.). The soil of each pot (1500 g) was a mixture of sand, sandy loam and peat moss (2:1:1), which was previously steam-sterilized (15 Ps at 121 °C) with an autoclave for 30 min.

The two species used in this study namely: T. harzianum (isolate-27) and T. viride (isolate-08) were kindly provided by Prof. Younes Yousef Molan, Department of Plant Protection King Saud University, Riyadh, Saudi Arabia. These two fungal species were, originally isolated along with other species from soil samples collected from different agricultural fields in Riyadh region, Saudi Arabia, using dilution plate method onto Trichoderma selective media (TSM) according to Elad and Chet (1983). The fungal isolate T. harzianum (isolate-27) and T. viride (isolate-08) were purified through subcultures from single spores and identified to species level based on the sequences of the Internal transcribed spacer regions 1 and 2 (ITS1 and ITS2) of the ribosomal DNA (Maymon et al., 2004; Hermosa et al., 2000).

In previous in vitro and greenhouse tests, we tested eight Saudi isolates of Trichoderma against M. javanica. Based on the results of these tests (un-published data), two promising isolates namely: T. harzianum (isolate-27) and T. viride (isolate-08) were selected for the present study. The Trichoderma isolates were first cultured on Potato Dextrose Agar (PDA) on petri plates. The plates were incubated at 24°C for 14 days. The produced conidia were collected from the culture surfaces by flooding with sterile distilled water and gently scraping the produced conidia were collected from the culture surfaces by plates. The plates were incubated at 24°C for 14 days. The results of these tests (un-published data), two promising isolates namely: T. harzianum (isolate-27) and T. viride (isolate-08) were selected for the present study. The Trichoderma isolates were first cultured on Potato Dextrose Agar (PDA) on petri plates. The plates were incubated at 24°C for 14 days. The produced conidia were collected from the culture surfaces by flooding with sterile distilled water and gently scraping the colony surface with a sterile scraper (Jansson et al., 1985).

Pure culture of M. javanica was obtained for tomato plants grown in earthen pots. For the M. javanica inoculum, eggs were extracted by the NaOCl technique (Hussey and Barker, 1973) from the roots of a pure greenhouse culture of M. javanica on tomato. The egg suspension was adjusted to 2000 eggs/ml.

Four densities of fungal spore suspension (10^4, 10^6, 10^8 and 10^10 spores/g of soil) were calculated by hemocytometer (Booth, 1971) for each fungal species. The conidial suspension of each density for each fungus was mixed thoroughly with the soil of each pot. At the same time, the suspension of 10,000 M. javanica eggs in 5 ml water was also mixed thoroughly with the potting soil. Mixing of both inocula with the soil of each pot was done thoroughly in a plastic bag. Then, thirty-day-old tomato seedlings were transplanted immediately into the infested pots (one seedling/pot). Control treatments included untreated seedlings and nematode treated seedlings. Each treatment was replicated four times. The treatments were arranged on a greenhouse bench (24 ± 2°C) in a randomized complete block design. Seedlings were irrigated and fertilized with a nutrient solution (1 g water soluble fertilizer N-P-K in 1 liter water) as needed till the end of the test.

Fifty-five days after inoculation, the test was terminated. Fresh weights of plant shoots and roots, numbers of root galls, eggs (Hussey and Barker, 1973), and egg masses were recorded. Second-stage juveniles (J2) in the soil were extracted by the modified centrifugal-floatation method (Barker, 1985), and counted. Final population densities of nematodes were determined and the reproduction factor (RF) (Oostenbrink, 1966) was calculated. Data were statistically analyzed using analysis of variance (ANOVA), and treatments means were separated by Fisher’s least significant difference (LSD) using SAS (SAS, 2013).

3. Results

As inoculum densities of both fungi were increased, improved host growth and suppression of root galling increased (P ≤ 0.05) (Table 1). However, the two highest densities (10^6 and 10^10 spore/g soil) of both fungi showed persistent and significant (P ≤ 0.05) effects. T. harzianum was relatively more effective in improving the host growth than T. viride (Tables 2 and 5).

Nematode reproduction (eggs, J2, and RF) was increasingly suppressed as the inoculum densities of both fungi were increased (Tables 3 and 4). Again, the two highest densities were the most effective in suppressing nematode reproduction (Tables 4 and 5). T. harzianum was more (P ≤ 0.05) effective in suppressing nematode reproduction than T. viride (Table 5).

4. Discussions

This study was conducted to assess, for the first time, the efficacy of two local (Saudi) isolate of T. harzianum and T. viride at different densities against M. javanica on tomato.

| Table 1 Effects of different densities T. harzianum and T. viride on host response of tomato inoculated with Meloidogyne javanica. |
|---------------------------------------------------------------|
| Fungal inoculum density | T. harzianum | T. viride | T. harzianum | T. viride |
|------------------------|-------------|---------|-------------|---------|
| 10^10                  | 60.0 a      | 51.5 a  | 74.3 c      | 78.4 d  |
| 10^8                   | 56.8 a      | 48.0 b  | 89.3 c      | 103.7 c |
| 10^6                   | 48.8 b      | 41.0 c  | 116.9 b     | 133.7 c |
| 10^4                   | 42.2 c      | 39.9 e  | 136.9 b     | 148.7 b |
| 10^2                   | 38.5 c      | 38.5 d  | 171.2 a     | 168.2 a |
| 0 (Nematodes alone)    | 39.7 c      | 39.7 e  | –           | –       |
| 0 (seedlings alone)    | 39.7 c      | 39.7 e  | –           | –       |

Data are means of four replicates. Means, in each column, followed by the same letter are not significantly different (P ≤ 0.05).

* Inoculum density: 10^2, 10^4, 10^6, 10^10 = spore/g soil.
Our results indicated that application of two native (Saudi) isolates of *T. harzianum* and *T. viride* reduced significantly root galling, egg production, and soil juveniles (J2); and increased host growth. The result support previous reports on the efficacy of *T. harzianum* or *T. viride* against some *Meloidogyne* spp. on several vegetable crops including tomato (Mascarin et al., 2012; Siddique et al., 2001; Dababat and Sikora, 2006; Windham et al., 1989; Spiegel and Chet, 1998; Sharon et al., 2001; Pandey et al., 2003). Our results suggest that both *Trichoderma* species caused direct and indirect effects on reproduction of both fungi increased, supporting similar previous studies (Jindapunnapat et al., 2013; Sahebani and Hadavi, 2008; Montesinos and Bonaterra, 1996; Smith et al., 1997). However, maximum activity of control was recorded at densities of 10⁶ and 10⁸ spore/g of soil with no significant difference between these two densities. Therefore increasing densities of both fungi from 10⁴ to 10¹⁰ did not show significant increase of activity and does not justify the use of the higher density inoculum density (10¹⁰), reproduction factor (RF) could not be reduced below 5.1. It appears that although host response and root galling were significantly improved, reproduction factor RF = Pf/Pi or reproductive potential of activity and does not justify the use of the higher density inoculum density (10¹⁰), reproduction factor (RF) could not be reduced below 5.1. It appears that although host response and root galling were significantly improved, reproduction factor RF = Pf/Pi or reproductive potential of plant-parasitic nematodes (Meloidogyne spp.) cannot be effectively achieved over this experimentally-short time, and this is true especially when the

**Table 2** Comparative effects of *Trichoderma harzianum* and *Trichoderma viride* (at increasing densities) on host response of tomato inoculated with *Meloidogyne javanica*.

| Treatment               | Total plant fresh weight (g)  | No. of galls/g root |
|-------------------------|-------------------------------|---------------------|
|                         | 10⁴   | 10⁶   | 10⁸   | 10¹⁰  | 10⁴   | 10⁶   | 10⁸   | 10¹⁰  |
| *T. harzianum*          | 42.2 a | 48.8 a | 56.8 a | 60.0 a | 116.9 c | 133.7 c | 89.3 c | 74.3 b |
| *T. viride*             | 39.9 a | 41.0 b | 48.0 b | 51.5 b | 136.9 b | 148.7 b | 103.7 b | 78.4 b |
| Nematode alone          | 38.5 a | 38.5 b | 38.5 c | 38.5 c | 170.9 a | 168.4 a | 176.9 a | 168.7 a |
| Healthy seedlings       | 39.7 a | 39.7 b | 39.7 c | 39.7 c | –       | –       | –       | –       |

Data are means of four replicates. Means, in each column, followed by the same letter are not significantly different (*P* ≤ 0.05). * Inoculum density: 10⁴, 10⁶, 10⁸, 10¹⁰ = spore/g soil.

**Table 3** Effect of different densities of *Trichoderma harzianum* and *Trichoderma viride* on reproduction of *Meloidogyne javanica* on tomato.

| Fungal inoculum density* | Egg/g root (×1000) | J2/100 g of soil | Reproduction factor** |
|--------------------------|-------------------|-----------------|----------------------|
|                          | *T. harzianum*    | *T. viride*     | *T. harzianum*       | *T. viride* |
| 0 (Nematode alone)       | 24.1 a            | 24.1 a          | 227.5 a              | 227.5 a    |
| 10⁴                      | 13.2 b            | 19.5 b          | 205.3 b              | 218.0 a    |
| 10⁶                      | 10.8 bc           | 15.2 c          | 198.8 bc             | 181.8 b    |
| 10⁸                      | 9.0 cd            | 12.0 cd         | 131.5 c              | 156.0 c    |
| 10¹⁰                     | 7.5 d             | 11.7 d          | 128.0 c              | 144.3 c    |

Data are means of four replicates. Means, in each column, followed by the same letter are not significantly different (*P* ≤ 0.05). * Inoculum density: 10⁴, 10⁶, 10⁸, 10¹⁰ = spore/g soil. ** Reproduction factor RF = Pf/Pi.

**Table 4** Comparative effects of *Trichoderma harzianum* and *Trichoderma viride* (at increasing densities) on the reproduction of *Meloidogyne javanica* on tomato.

| Treatment               | Eggs/g root (×1000) | J2/100 g of soil | RF** |
|-------------------------|---------------------|-----------------|------|
|                         | 10⁴    | 10⁶    | 10⁸    | 10¹⁰  | 10⁴    | 10⁶    | 10⁸    | 10¹⁰  |
| *T. harzianum*          | 13.2 c | 10.7 c | 9.0 b  | 7.6 b | 205.3 b | 198.8 c | 131.5 c | 128.0 c | 7.3 b | 6.9 c | 5.0 c | 5.1 c |
| *T. viride*             | 19.5 b | 15.2 b | 11.9 b | 9.4 b | 218.0 a | 181.8 b | 156.0 b | 144.3 b | 12.05 b | 8.5 b | 6.4 b | 6.6 b |
| Nematode alone          | 24.6 a | 24.1 a | 24.0 a | 24.0 a | 223.0 a | 233.0 a | 214.0 a | 240.0 a | 13.0 a | 12.9 a | 12.4 a |

Data are means of four replicates. Means, in each column, followed by the same letter are not significantly different (*P* ≤ 0.05). * Inoculum density: 10⁴, 10⁶, 10⁸, 10¹⁰ = spore/g soil.

**Figure 1** Comparative effects of *Trichoderma harzianum* and *Trichoderma viride* (at increasing densities) on host response of tomato inoculated with *Meloidogyne javanica*.
host is highly susceptible. Long-term management of root-knot nematodes should involve a combination of some suitable methods in integrated management systems. Assessment of combined species or isolates of *Trichoderma* against root-knot nematodes on different vegetable crops is needed.

Generally speaking, *T. harzianum* was more effective than *T. viride*, under our experimental conditions. The variability between these two species may be due to some reasons, among which are the genetic variability, the pathogenic capabilities, and the origin of the isolate.

Our results were obtained under the greenhouse conditions and in an autoclaved soil. Therefore, they cannot be extrapolated integrally to the field conditions where natural soil plays a very important role in the efficacy of any biocontrol agent of nematodes. However, our results are very encouraging to observe the suppressiveness effects of these two local isolates, for the first attempt, on *M. javanica* reproduction and its damages on tomato. These results provide strong support for exploring further the use of such promising indigenous isolates, and this is what we have been conducting at present time.

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