Saudi Journal of Biological Sciences (2016) **23**, 288–292

King Saud University

Saudi Journal of Biological Sciences

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

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

Ahmad Saad Al-Hazmi *, **Muhammad TariqJaveed**

Department of Plant Protection, King Saud University, P.O Box 2460, Riyadh 11451, Saudi Arabia

Received 17 December 2014; revised 1 April 2015; accepted 15 April 2015
Available online 22 April 2015

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.

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; Naserinasab 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.

* Corresponding author.
E-mail address: asalhazmi@ksu.edu.sa (A.S. Al-Hazmi).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2015.04.007

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 Psi 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 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 24̊C 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 scrapper (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 ($P \leqslant 0.05$) (Table 1). However, the two highest densities (10^8 and 10^{10} spore/g soil) of both fungi showed persistent and significant ($P \leqslant 0.05$) 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 ($P \leqslant 0.05$) 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*	Total plant fresh weight		No. of galls/g of root	
	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 bc
10^4	42.2 c	39.9 c	136.9 b	148.7 b
0 (Nematodes alone)	38.5 c	38.5 c	171.2 a	168.2 a
0 (seedlings alone)	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 ($P \leqslant 0.05$).
* Inoculum density: 10^4, 10^6, 10^8, 10^{10} = spore/g soil.