JOURNAL OF PURE AND APPLIED MICROBIOLOGY, May 2015. Vol. **9**(Spl. Edn. 1), p. 535-539

Effect of *Trichoderma harzianum* on *Meloidogyne javanica* in Tomatoes as Influenced by Time of the Fungus Introduction into Soil

Muhammad TariqJaveed* and Ahmad Saad Al-Hazmi

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

(Received: 07 February 2015; accepted: 10 March 2015)

A greenhouse pot experiment was conducted to determine the effect of time of introduction of *Trichoderma harzianum* on its biocontrol efficacy against *Meloidogyne javanica* in tomatoes. The experiment consisted of all possible combinations of individual, concomitant and sequential inoculations of *T. harzianum* and *M. javanica.* Results indicated that time of the fungus introduction into the soil proved to be important. Introduction of *T. harzianum* to *M. javanica*-infested soil at or prior (one or two weeks) to nematode inoculation suppressed the nematode reproduction and increased host growth. However, such effects were increasingly greater when the fungus was introduced two or one week prior to nematode inoculation.

Key words: Antagonistic effect, Application time, Biological control, Nematophagous fungi, Root-knot nematodes, *Solanum lycopersicum*.

The free-living soil fungus *Trichoderma* spp. is an important biological control agent of plant-parasitic nematodes[1,2,3]. Control of the root-knot nematodes (*Meloidogyne* spp.) by different species of *Trichoderma* has been reported by several scientists[1,3,4,5,6,7,8,9,10].

The antagonistic organisms that are transferred with the soil must be given time to reproduce to suppressive levels[11]. *Trichoderma harzianum* (Rifai) was found to be more effective against *Meloidogyne javanica* (Treub) when both organisms were applied 18 days before transplanting of tomato seedlings than when both applied at transplanting[12]. *Paecilomyces lilacinus* (Thom) was more effective against *M. incognita* (Kofoid and White) Chit. when it was delivered into the nematode-infested soil ten days before planting of tomato[13]. However, Walia *et al.*[14] reported that application of *P. lilacinus* 10 days before or after sowing of okra seeds were equally effective against *M. javanica*. When the algae *Microcoleus vaginatus* (Vauch.) Gomont was introduced 10 days prior to the *M. incognita*, damage of tomato plants was reduced and nematode density was suppressed[15].

In previous *in vitro* and greenhouse tests (un-published data), we found that a local isolate of *T. harzianum* (isolate no. 27) was the most effective isolate, among eight tested isolates and species of *Trichoderma*, against *M. javanica*. This present study was conducted to determine the effect of time of introduction of *T. harzianum* into soil on its biocontrol efficacy against *M. javanica* on tomato.

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), a

* To whom all correspondence should be addressed.
Tel: +966509720562;
E-mail: tariqjaved22@gmail.com

mixture of sand, sandy loam and peat moss (2:1:1), was previously steam-sterilized with an autoclave for 30 minutes.

The experiment consisted of individual, concomitant and sequential inoculation of *M. javanica* and *T. harzianum*. The experiment included eight treatments (table 1) , namely: 1) *M. javanica* alone (N); 2) *T. harzianum* alone (T); 3) both organisms simultaneously (N + T); 4) nematode first and then fungus one week later ; 5) nematode first and then fungus two weeks later ; 6) fungus first and then nematode one week later ; 7) fungus first and then nematode two weeks later ; and 8) control (non-inoculated seedlings).

The inoculum of *M. javanica* consisted of eggs which were extracted by the NaOCl method (Hussey and barker, 1973[16]) from the roots of a pure greenhouse culture of *M. javanica* on tomato. The egg suspension was adjusted to contain 2000 eggs/ml.

The fungus *T. harzianum* (isolate no. 27) was obtained from the mycological unit (Prof. Younes Yousef Molan), Department of Plant Protection at King Saud University, Riyadh, Saudi Arabia. This species was originally isolated, along with other species and isolates of *Trichoderma*, 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[17]. These fungal isolates were purified through subculturing from single spores and, then, identified to Species level by Prof. Younes Molan based on sequences of the Internal transcribed spacer regions 1 and 2 (ITS1 and ITS2) of the ribosomal DNA[18,19].For inoculum preparation, *T. harzianum* (isolate no. 27) wasre-cultured on Potato Dextrose Agar (PDA) on petri plates and 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 scrapper.The suspension was, then, passed through cheese cloth in sterile distilled water[20].

At inoculations with the nematode and/ or the fungus, each seedling was inoculated with 10000 eggs of *M. javanica* and /or1 × 10^{10} conidia/ g soil of the fungus *T. harzianum*. The nematode egg inoculum, suspended in 5 ml of water, was equally distributed through three small holes made in the soil around the seedling stem. Inoculation with the fungus was made by distributing and mixing the fungal inoculum thoroughly with the soil surface of the designated pots.The eight treatments (table 1) were arranged on greenhouse bench in a complete randomized design. Seedlings were irrigated, and fertilized as needed, till the end of the test.

Fifty five days after last inoculation, the test was terminated. Fresh weight of plant shoots and rootswere recorded. Numbers of root galls, egg masses and eggs were counted. Second-stage juveniles (J2) in the soil were extracted by the modified centrifugal floatation method[21]. Final population densities of nematodes were determined and the reproduction factor (Rf)[22] was calculated. Data were statistically analyzed using analysis of variance (ANOVA), and treatments means were separated by protected Fischer's least significant difference (LSD) using SAS[23].

RESULTS

Trichoderma harzianum increased ($P < 0.05$) shoot and total plant weights, whether introduced at or prior (1 and 2 weeks) to nematode inoculation, compared to inoculation with nematode alone (table 1). However, effects of the fungus introduction at/and prior to nematode inoculation were not different ($P \leq 0.05$). Similarly, introduction of *T. harzianum* at all times reduced the number of root galls (table 1). However, such reduction of galls was greater when the fungus was introduced two or one weeks prior to nematode inoculation.

Reproduction of *M. javanica* was suppressed ($P \leq 0.05$) by *T. harzianum* at all times of the fungus introduction (table 2). However, the greatest suppression of the nematode reproduction was obtained when *T. harzianum* was introduced twoor one weeks prior to nematode inoculation. The least reproduction was achieved when the fungus was introduced two weeks before nematode inoculation (table 2).

DISCUSSION

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. [4,7,24,25,26,27].The suppressive effects of *T. harzianum*, reported in this study, on nematode population and disease severity 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 two or one week prior to soil infestation with the nematode. Similar evidence was previously reported[12]. 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.*[28] working with the nematode-trapping fungus of *Arthrobotrys conoides* against *M. incognita* on corn.

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

Table 1. Effect of time of introduction of *Trichoderma harzianum* (T) on host response of tomato inoculated with *Meloidogyne javanica* (N).

Treatment	Root weight	Shoot weight (g)	Total plant weight (g)	Gall/root system	Gall/g root
Healthy seedlings (control)	5.01 a	38.20 bc	43.21 bc	-	-
M. javanica alone (N)	5.18 a	35.05 c	40.23 c	816.00 a	157.45 a
T. harzianum alone (T)	5.21 a	41.20 ab	46.41 ab	-	-
N + T	5.80 a	41.10 ab	46.90 ab	492.25 c	86.86 b
N →(1 wk)→ T	5.33 a	37.91 bc	43.24 bc	539.50 bc	103.30 b
N →(2 wk)→ T	5.81 a	38.67 abc	44.48 abc	600.75 b	103.96 b
T →(1 wk)→ N	6.02 a	41.79 ab	47.80 ab	380.00 d	63.46 c
T →(2 wk)→ N	5.25 a	43.15 a	48.40 a	308.50 d	61.96 c

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 ($P \leq 0.05$).
N→T= *M. javanica* followed by *T. harzianum*, T→N= *T. harzianum* followed by *M.Javanica*,wk = week (s).

Table 2. Effects of time of introduction with *Trichoderma harzianum* (T) on reproduction of *Meloidogyne javanica* (N) on tomato

Treatments	Egg mass/g	Eggs/g(X1000)	J2/100g soil	Rf*
Healthy seedling (control)	- - -	- - -	- - -	- - -
M. javanica alone (N)	100.44 a	21.79 a	201.3 a	11.6 a
T. harzianum alone (T)	- - -	- - -	- - -	- - -
N+T	21.061 d	07.74 d	125.8 bcd	04.7 d
N →(1 wk)→ T	34.595 c	12.70 c	132.3 bc	06.9 c
N →(2 wk)→ T	55.752 b	17.86 b	138.8 b	10.5 b
T →(1 wk)→ N	19.509 d	05.81 d	122.0 cd	03.6 e
T →(2 wk)→ N	20.208 d	05.77 d	116.8 d	03.1 e

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 ($P \leq 0.05$).
*Rf: Reproduction factor = Final nematode density (Pf)/Initial inoculum density (Pi).
N→T= *M. javanica* followed by *T. harzianum*, T→N= *T. harzianum* followed by *M.Javanica*,wk = week (s).

Arabia, no accurate yield losses were reported. *Meloidogyne javanica* is widespread in our agricultural soils[29] may our native *T. harzianum* isolates negate some of the damage on tomatoes caused by *M. javanica.*

CONCLUSION

Our results conclude that time can be an important factor in success of fungal biocontrol 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.

ACKNOWLEDGEMENTS

The authors are very thankful to Prof. Y. Yousef Molan for providing the pure culture of *T. harzianum*, and Dr. Mahmood El-Komi for assistance in the laboratory. The authors also gratefully acknowledge their appreciation to the Agriculture Research Center at the College of Food and agricultural Sciences and Deanship of Scientific Research,King Saud University for supporting this research work.

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