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Full Length Research Paper

Trichoderma harzianum reduces population of *Meloidogyne incognita* in cucumber plants under greenhouse conditions

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***Trichoderma harzianum* strain ESALQ-1306 was assessed for its potential biological control against *Meloidogyne incognita* race 4 under *in vitro* and greenhouse conditions. *In vitro,* we noticed 64% of conidial attachment on eggs and second-stage juvenile immobilized by the fungus. In greenhouse, cucumber plants were treated with different concentrations of conidial suspensions before and after inoculation with *M. incognita*. After 60 days, the most efficient treatment provided about 50% nematode reduction by using three sequential applications of *T. harzianum.* However, the fungus did not increase the fresh and dry root weight. We conclude that this fungus rises as a good option of biological control for the integrated management of root-knot nematode in protected cultivation of cucumbers.**

Key words: Biological control, *Cucumis sativus*, root-knot nematode, nematophagous fungus.

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp) are ubiquitous plant parasitic organisms that threaten a wide range of crops in worldwide and are difficult to be controlled by stand-alone control measures (Barker et al., 1985). Due to the harmful consequences caused by synthetic chemical pesticides in agriculture, including environmental pollution and poisoning among farmers, there is an increasing demand for alternative control tools to be developed aiming at a sustainable integrated pest management.

Biological control agents have a great impact on regulating root-knot nematode populations and have been used in combination with other strategies for the management of these pathogens. Because of the same vegetable crop is continuously cultivated on the same greenhouse area throughout the year without crop rotation, root-knot nematode population have an ideal environment to increase their number and cause severe yield losses in the absence of control measures. In Brazil, most of the growers of protected vegetable crops, like cucumbers, have fought root-knot nematodes by using resistant cultivars, non-host plants and chemical nematicides, such as the organophosphate Furadan™.

Nematophagous fungi are important biological control agents because they possess the potential to reduce the population of root-knot nematodes (Haran et al., 1996). Species of *Trichoderma*, with special attention to *Trichoderma harzianum* Rifai, 1969 (Ascomycota: Hypocreaceae), are well-known to present biocontrol activity against several plants pathogenic fungi through various mechanisms: antibiosis, competition, mycoparasitism, and enzymatic hydrolysis (Harman et al., 2007; Howell, 2003). All mechanisms, except competition, can play a role in biocontrol process against root-knot nematodes (Sharon et al., 2001). Moreover, these fungi may also promote plant growth and induce systemic resistance in plants (Inbar et al., 1994; Yedidia et al., 1999). For those reasons, *T. harzianum* shows a tremendous potential for

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the biocontrol of root-knot nematodes along with other fungal plant pathogens.

The decreasing of root-knot nematodes population has been observed in field using *T. harzianum* (Harman et al., 2007). However, as far as we are concerned, there are very few reports of bionematicide effect of *Trichoderma* in protected vegetable crops in Brazil. Therefore, this work aimed to evaluate the efficiency of *T. harzianum* on *M. incognita* (Kofoid and White) Chitwood, population in cucumber plants under greenhouse conditions.

METHODOLOGY

The greenhouse experiment was set up in the Depart-ment of Entomology and Acarology at ESALQ-USP, Piracicaba-SP, Brazil. This experiment was performed with the aim to determine whether fungal soil treatments interfere with nematode penetration through the roots along with the inhibition of nematode development within the roots. There were three preparations of *T. harzianum* strain ESALQ-1306 and two control groups. This fungal strain was originally isolated from the soil collected in Piracicaba-SP (Brazil), and it is currently available in the Brazilian market and commercialized by the company Itaforte BioProdutos Ltda. (Itapetininga, SP, Brazil). One control group was comprised by untreated nematode-infested soil (positive control), and the other contained only free-nematode plants (referred hereafter as blank control). This last control group (untreated) was used just as a standard for comparison of the root fresh and dry weights among treatments. First, cucumber seeds (*Cucu-mis sativus* L., cv. SMR 58) were treated with technical powder of *T. harzianum* aerial conidia (10 mg equivalent to ~ 4.5×10^8 viable conidia) by coating 30 g seeds with 1 g conidia. Seeds were previously wetted by adding 15 ml water for 1 kg before being treated with conidia. Fungus-treated seeds were immediately sown in Styrofoam trays containing autoclaved soil mix composed by a commer-cial organic substrate (Basaplant®, São Paulo, Brazil), clay soil (red distroferric latosol) and sand at proportion of 2:1:1 (v/v/v). This soil was collected in a non-grower's field in Piracicaba (Brazil). Prior to using this soil mix, the soil was autoclaved twice at different occasions at 120°C and 1 kgf/cm^2 for 2 h to guarantee a substrate free of other living organisms.

Plastic pots were filled with 2 L of the same soil mix and each one received a single 15-day-old cucumber seedling originated either from fungus-coated seeds or untreated seeds showing the first true leaf completely expanded. Second fungal treatment consisted of a second *T. harzianum* inoculation with a suspension of 4×10^8 conidia per plant (10 ml/plant) as soon after seedlings were planted with the potting mix. This fungal suspension was prepared with a formulated emulsifiable oil of aerial conidia (Trichodermil® SC, Itaforte BioProdutos) diluted in deionized water.

After six hours of planting, 800 second-stage juveniles (J2) of *M. incognita* race 4 were added to the pots in three 5 cm depth holes around the plant base. The nema-tode inoculums was previously multiplied in cotton roots (*Gossypium hirsutum* L., cv. Delta Opal) under green-house conditions, and afterwards nematodes were extracted following the method of Coolen and D'Herde (1972) to produce the inoculum for the experiment.

Finally, the third fungal treatment consisted of the two previous mentioned fungus-treatments plus another application of 4×10^8 conidia per plant at six days after nematode inoculation by dispensing 10 ml of this conidial suspension over three 5-cm-depth holes around the plant base. Nematode-infested soil and untreated soil did not receive any fungal treatment since the beginning. Each treatment included seven independent replicates. The experiment followed a complete randomized design.

Temperature ranged from 20.6 to 33.4°C during the experimental timeframe. Plants were irrigated with plain tap water as needed over the period of experimentation. Evaluations were carried out two months after nematode inoculation by recording fresh and dry root weights and the final nematode population extracted from the root system. Nematodes were extracted from roots by using the method described in Coolen and D'Herde (1972). The reproduction factor (RF) for *M. incognita* was calculated through the formula $RF = P_f/P_i$, where P_f was the final population found in roots and P_i the initial nematode inoculum (800 J2/plant).

In vitro bioassay was performed adding 2000 fresh eggs + J2 of *M. incognita*, originally collected from infested tomato roots, into Erlenmeyer flasks containing 100 ml of sterile deionized water. After that, a suspension based on a technical powder of aerial conidia of *T. harzianum* (ESALQ-1306) was delivered at a final con-centration of 1×10^6 conidia/ml through inoculation of 1 ml of 1×10^8 conidia/mL into the flasks prepared with water + 0.01% Tween 80 solution. The untreated control received only the nematodes (eggs+J2). The flasks were placed on a rotary shaker (100 rpm) at room temperature (29±2°C) with 12 h photophase. Each treatment was repeated four times and the entire experiment was repeated twice in different occasions. One week later, 1 ml aliquot was taken from the center of each treatment for recording the contaminated eggs (scored as conidia attached to the eggs) and motionless J2, by using a Peters counting slide.

The RF response variable obtained from greenhouse experiment was transformed by 1/X to match the norma-lity assumptions, while the original data sets from root fresh and dry weight were used. Data sets were subjected to one-way analysis of variance (ANOVA) (PROC GLM), and significant differences between means were separated by the Fisher´s LSD test (Fisher´s pro-tected least significant difference) at α=0.10. The statis-tical analysis was done in the software SAS 9.2 (SAS Institute, 2008).

RESULTS AND DISCUSSION

None of the treatments of T. harzianum increased fresh and dry root weights in relation to the plants infested with nematodes ($F_{4, 30} = 0.79$; $P = 0.5398$; $F_{4, 30} = 1.7\ 9$; $P = 0.1766$, respectively).

Interestingly, all doses of the fungus reduced in some extent the RF (Table 1). The most efficient treatment was that the one with three sequential applications of *T. harzianum*, which significantly reduced the mean value of RF by causing 48.9% of nematode suppression ($F_{3, 24} = 2.54$; $P = 0.08$). Nevertheless, the high values of RF in nematode infested-plants proved that the inoculum and the environmental conditions were favorable to nematode multiplication.

The outcome information of our study is in agreement with Windham et al. (1986). These authors observed a reduction in egg production by *Meloidogyne arenaria* after soil treatments with *T. harzianum* and *Trichoderma koningii*. Under greenhouse conditions, Siddiqui et al. (2001) showed that *T. harzianum* reduced the *Meloi-dogyne javanica* population in the soil (27 and 37%) and in the roots (36 and 42%), in infested soil with 2000 J2, inoculated one week after inoculation of 7×10^8 conidia to 350 g of soil with 7×10^8 conidia. However, these fungi

Table 1. Effect of *Trichoderma harzianum* ESALQ-1306 against *Meloidogyne incognita* on cucumber plants under greenhouse conditions.

Treatment	Fresh root weight (Gram per plant)[1]	Dry root weight (Gram per plant)	Reproduction factor (RF)[1]	Reduction (%)[2]
Untreated plants (blank control)	34.4 ±4.9[a]	2.5±0.8[a]	-	-
Nematode infested-plants	33.6±3.6[a]	2.0±0.3[a]	39.58±11.0[a]	-
Seed treatment with T.h[3]	34.9±4.2[a]	2.7±0.6[a]	29.5±3.2[ab]	25.4
Seed + seedling treatment with T.h.	29.2±3.7[a]	1.7±0.3[a]	28.5±6.5[b]	27.9
Seed + seedling + soil treatment with T.h.	27.0±3.1[a]	1.2±0.2[a]	20.2±2.2[b]	48.9
One-way ANOVA	F = 0.79, df = 4, 30; P = 0.5398	F = 1.7, df = 4, 30; P = 0.1766	F = 2.54, df = 3, 4, 30; P = 0.08	

[1]Means (± SE) followed by the same letters are significantly different by the Fisher's LSD test (α = 0.10), [2]Reduction in nematode population was given by the following formula: (1 – RF in treatment/RF in infested plants) × 100. [3]T.h. = *T. harzianum* applied to seeds or soil as conidia powder or conidial suspensions, respectively.

Table 2. Effect of conidial suspension of *Trichoderma harzianum* ESALQ-1306 on J2 mobility and attachment to eggs of *Meloidogyne incognita* in the *in vitro* bioassay.

Treatment	Contaminated eggs + J2 immobility (%)	
	Bioassay I	Bioassay II
Control (only nematode)	0.0 ± 0.0	0.0 ± 0.0
T. harzianum[1]	68.7 ± 11.2	59.4 ± 15.4

[1]*T. harzianum* was inoculated at 1 × 10^8 conidia/ml into 250 ml flasks in 100 ml of sterile water containing 2000 (eggs + J2) nematodes.

were not able to prevent nematode infection in roots, as we also observed in our results.

Cucumber is an excellent host for multiplication of *M. incognita* but the chemical control of this horticultural crop under protected cultivation has increased its production costs. The reduction of this nematode population through applications of *T. harzianum* ESALQ-1306 was relevant in this study, although the nematode was able to infect and multiply in cucumber roots.

T. harzianum was able to adhere and immobilize of 68.7 and 59.4% of eggs and J2 of *M. incognita* in bioassays 1 and 2, respectively (Table 2). Sharon et al. (2007) showed that eggs adhered with *Trichoderma conidia* became non-viable, thus decreasing the eclosion rate. Furthermore, Sharon et al. (2001) carrying out *in vitro* bioassays, verified that extracts of *T. harzianum* released in soil were capable to immobilize J2 of *M. javanica* and reduce egg viability. These results suggest that there are both mechanisms of fungal parasitism and lethal activity of secondary metabolites on nematodes.

T. harzianum is an ubiquitous free living mycoparasite that can be applied direct to the soil or on seedlings, associated or not to organic compounds, aiming at the control of soil-borne fungal diseases in protected crops. Moreover, the increase in crop yield provided by *T. harzianum* and its nematicidal effect add advantages to its use. Based on the results found in this study, we conclude that *T. harzianum* (ESALQ-1306) can be used as an environmentally friendly tool in the integrated pest management of *M. incognita* in protected horticultural crops, but not as a stand-alone control measure.

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