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EFFECT OF *Trichoderma* ISOLATES ON EGG HATCHABILITY OF *Meloidogyne incognita*

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ABSTRACT

Numerous attempts have been made to use the fungus, *Trichoderma* for bio-control of *Meloidogyne* species. However, no information is available on the efficacy of local isolates of *Trichoderma* as bio-control agents against plant parasitic nematodes. Hence, two experiments (*in-vitro*) were carried out to investigate the effect of six isolates of *Trichoderma* and different spore concentrations of *T.koningii* on the egg hatchability of *Meloidogyne incognita* at | 25±2 °C |. Four local isolates of *Trichoderma* extracted from soils collected from vegetable growing areas of the Central regions of Sri Lanka were compared with a Japanese isolate and a commercial product of the fungus. Data revealed that locally identified *T. koningii* and the Japanese isolate significantly (P≥0.05) reduced egg hatch of *M. incognita* than other isolates. Five concentrations of the fungus ($4X10^3$, $4X10^4$, $4X10^5$, $4X10^6$, $4X10^7$ spores/ml) significantly (P≥0.05) reduced the egg hatchability of *M. incognita* as compared to the control. The local isolate of *T. koningii* at the rate of $4X10^3$ spores/ml can be used as a potential bio-control agent against *M. incognita*. The possibility of using *T. koningii* in the Integrated Nematode Management (INM) programmes is discussed.

KEYWORDS: Biological control, Egg hatch, *Meloidogyne incognita*, Spore concentration, *Trichoderma* isolates.

INTRODUCTION

In Sri Lanka, along with continuous land development, implementation of agricultural intensification projects, and the introduction of various plant materials and agricultural products into the island, nematode damage is becoming increasingly conspicuous in many areas of the country (Ekanayaka and Toida, 1997). Among plant parasitic nematodes (PPNs), root-knot nematodes (RKNs) are found to be the worst enemies of many crops (Rangaswami and Bagyaraj, 1996). In Sri Lanka they attack various tubers, fruits, vegetables and ornamental crops causing considerable loss of yield and affecting the quality of the produce (Wouts, 1979).

Biological control of nematodes is at present, gaining more attention in management of nematodes, because of its acceptance by humans and animals as well as to environment. A range of natural enemies including bacteria,

nematophagous fungi, predaceous nematodes and arthropods are known to cause damages to plant nematodes.

Among *Trichoderma* spp. cited as potential bio-control agents of *Meloidogyne* spp., *T. viridae* is a promising bio-control agent of *M. incognita* (Crop protection compendium, 1996). *T. harzianum* Rafai has been reported to be an effective bio agent for the management of the citrus nematode, *Tylenchulus semipenetrans* (Reddy *et al.*, 1996). Furthermore, *T. harzianum* had been used as a bio-control agent for the sustainable management of RKNs on some other crops (Rao *et al.*, 1998).

As no information is available on the effect of local isolates of *Trichoderma* spp. in controlling *Meloidogyne* spp., this study was conducted inorder to find out the efficacy of six *Trichoderma* isolates on egg hatchability of *M. incognita in vitro* and to evaluate the most suitable spore concentration of the effective isolate of *Trichoderma* on egg hatchability of *M. incognita.*

METERIALS AND METHODS

Two *in vitro* experiments were carried out at Horticultural crop Research and Development institute (HORDI) at Peradeniya to evaluate the effect of six isolates of *Trichoderma* and five spore concentrations of *T. koningii* on the egg hatching of *M. incognita* at room temperature [25 ± 2 ^{0}C]. Five isolates of *Trichoderma* were extracted from soil samples collected from vegetable growing areas in the central province of Sri Lanka using water dilution technique (Jansson and Jaffe, 1990). These local fungal isolates, a Japanese isolate and a commercial product of the fungus were cultured on Potato Dextrose Agar (PDA). Egg masses for investigations were obtained from a pure culture of *M. incognita* maintained on tomato plants susceptible cultivar Katugastota Wilt Resistant (variety KWR) in the plant house.

Experiment 1

Twenty uniform egg masses (about 8000 eggs) of *M. incognita* were placed on each of forty-two microsieves (75 µm aperture, 20 mm dia.). They were enclosed in petri dishes partially filled with distilled water (Control) or fungal suspension of each isolate of *Trichoderma* (*T. koningii*, *T. harzianum*, *Trichoderma* ii, *Trichoderma* sp. (J), *Trichoderma* sp. (C) and *T. flavus*) sufficient to cover the egg masses. The dishes with egg masses were maintained at 25 ± 2 ^{0}C. There were six replicates for each treatment and dishes were arranged in a completely

randomized block design on a bench in the laboratory. At 24, 48 and 72 hours after inoculation of fungal treatment, the number of emerged juveniles was counted and fresh changes of distilled water and fungal suspensions were made. At the end of the experiment, the egg masses were treated with 1% Sodium hypochlorite to dissolve the gelatinous matrix and the unhatched eggs were counted. Egg hatch was expressed as a percentage of the total initial egg number of 20 egg masses and the mean percentage egg hatch was calculated. The data were subjected to the analysis of variance and treatments means were compared using Least Significant Difference (LSD) test at $p \geq 0.05$.

Experiment II

In this experiment, five spore concentrations ($4X10^3$, $4X10^4$, $4X10^5$, $4X10^6$ and $4X10^7$ spores/ml) of *T. koningii* were prepared and evaluated as per method described in Experiment 1. The percentage cumulative egg hatch in each concentration and the control treatment (distilled water) was calculated and the data were subjected to the analysis of variance and the treatments means were compared using LSD test at $p \geq 0.05$.

RESULTS AND DISCUSSION

Experiment 1

Table 1 and figure 1 show the effect of six *Trichoderma* isolates on egg hatch of *M. incognita* at 24, 48 and 72 hours after inoculation (HAI) of fungal treatments.

Table 1. Effect of six different isolates of *Trichoderma* on egg hatch of *M. incognita* at 24, 48 and 72 hours after inoculation of fungal treatments.

		% mean cumulative egg hatch		
Treatment	*Origin*	*24 HAI*	*48 HAI*	*72 HAI*
T1 – Control		15.45 a	29.86 a	44.62 a
T2 – *T. koningii*	Sri Lanka	5.47 b	12.13 b	16.93 b
T3 – *T. harzianum*	Sri Lanka	8.58 b	18.44 ab	35.1 ab
T4 – *Trichoderma* ii	Sri Lanka	7.83 ab	23.69 ab	31.42 ab
T5 – *Trichoderma* sp. (J)	Japan	8.74 ab	16.67 ab	22.63 b
T6 - *Trichoderma* sp. (C)	Japan	6.16 b	31.21 a	44.86 a
T7 – *T. flavus*	Sri Lanka	8.51 ab	19.02 ab	25.86 ab

Means followed by the same latter in each column are not significantly different at $p \geq 0.05$ in LSD test.

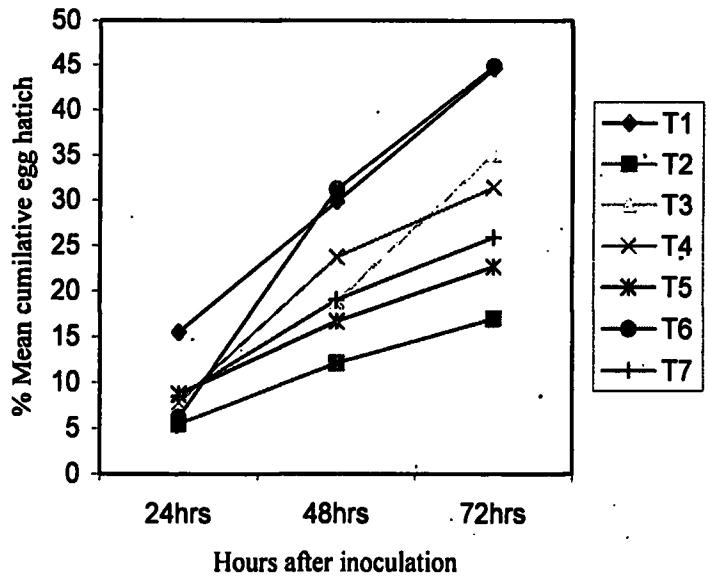

Figure 1. Effect of six isolates of *Trichoderma* on egg hatch of *Meloidogyne incognita.*

At 24 hours, egg hatch of *M. incognita* was low ($p \geq 0.05$) in all the fungal treatments than control that of in. Differences in egg hatch among fungal treatments were however not significant, the lowest egg hatch of 5.47% was observed with *T. koningii.*

At 48 hours, *T. koningi* has given the lowest egg hatch of 12.1% than in all other treatments. No significant differences were observed among four *Trichoderma* isolates; *T. harzianum*, *Trichoderma* ii, *Trichoderma* sp. (J) and *T. flavus* at 48 hours after inoculation (table 1). Egg hatch between control treatment and *Trichoderma* sp. (C) was not significant. At 72 hours, the lowest egg hatch of 16.93% was observed in *T. koningii* and it was not significant ($p \geq 0.05$) from *Trichoderma* sp. (J). Egg hatch in *T. koningii* and *Trichoderma* sp. (J) were significantly ($p \geq 0.05$) lower than the control and other four fungal treatments.

In general, the fungus, *T. koningii* has significantly ($p \geq 0.05$) influenced the egg hatch of *M. incognita* among the four local isolates of *Trichoderma* (at 24, 48 and 72 hours after fungal inoculation). Though there was no significant effect between *T. koningii* and the Japanese *Trichoderma* sp. (J) on egg hatch of *M. incognita, T. koningii* treated egg masses exhibited lowest egg hatch. According to Rao *et al.*, (1998), *T. harzianum* had been used as a bio control agent for effective management of root-knot nematodes on some vegetable crops.

However, its effectiveness was significantly lower ($p \geq 0.05$) than *T. koningii* and *Trichoderma* sp. (J) on egg hatch of *M. incognita* in this study.

Experiment II

Table 2 and figure 2 indicate the effect of five different spore concentrations of *T. koningii* on egg hatch of *M. incognita.*

Table 2. Effect of five different spore concentrations of *Trichoderma koningii* on the egg hatch of *Meloidogyne incognita* at 24,48 and 72 hours after the fungal inoculation.

Treatment	*% Mean cumulative egg hatch*		
	24 HAI	*48 HAI*	*72 HAI*
T 1 – Control (distilled water)	40.62 a	58.20 a	69.93 a
T2 - *T. koningii* (4×10^3)	10.44 c	24.86 c	42.25 bc
T3 - *T. koningii* (4×10^4)	11.80 bc	25.65 c	43.69 b
T4 - *T. koningii* (4×10^5)	12.87 bc	6.78 bc	42.17 c
T5 - *T. koningii* (4×10^6)	15.89 bc	31.71 bc	53.27 c
T6 - *T. koningii* (4×10^7)	17.01 b	33.66 c	47.01 c

Means followed by the same letter in each column are not significantly different at $p \geq 0.05$ in LSD test.

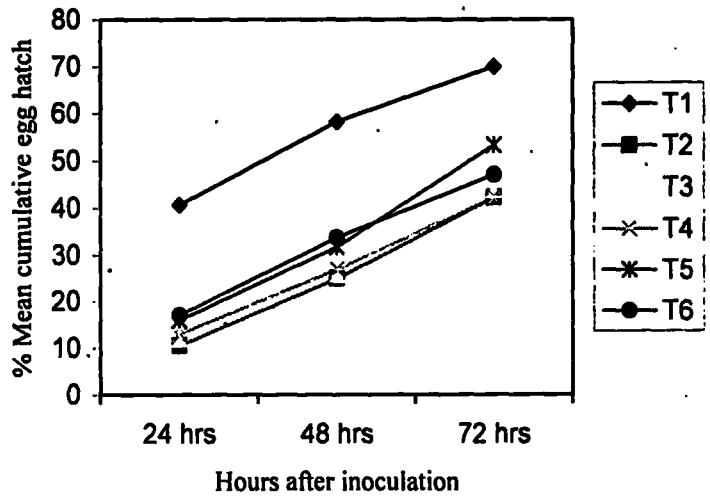

Figure 2. Effect of spore concentrations of *Trichoderma koningii* on the egg hatch of *Meloidogyne incognita.*

The egg hatch of the nematode in all the concentrations of *T. koningii* were significantly ($p \geq 0.05$) lower than the control treatment at 24, 48 and 72

hours after inoculation. No significant ($p \geq 0.05$) differences in egg hatches were observed among all the five spore concentrations of the fungus used in this experiment. This reveals that the lowest spore concentration of *T. koningii* ($4X10^3$ spores/ml) could be used to control *M. incognita* effectively.
(NB. Plates should be included.)

CONCLUSION

Results of these studies indicate that, of the six isolates used in this study, *T. koningii* and its spore concentration of $4X10^3$ spores/ml can be effectively used to control *M. incognita* on tomato. Since this fungus is a potential bio control agent of *Meloidogyne* spp., it can be included as an important component of an INM programme for controlling root- knot nematodes in vegetable growing areas. Further research for selecting suitable and locally available, cost effective culture media for the mass production of *T. koningii* would help better application of this method at farmer level in the future.

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