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Effect of *Trichoderma viride* Strain NRRL 6418 and *Trichoderma harzianum* (*Hypocrea lixii* TWC1) on *Livistona rotundifolia* Root Knot Nematode *Meloidogyne incognita*

¹V. Jegathambigai, ²R.S. Wilson Wijeratnam and ³R.L.C. Wijesundera

¹Green Farms Ltd., Marawila, Sri Lanka

²Industrial Technology Institute, 363, Baudhaloka Mawatha, Colombo 07, Sri Lanka

³Department of Plant Science, University of Colombo, Sri Lanka

Corresponding Author: V. Jegathambigai, Green Farms Ltd., Marawila, Sri Lanka

ABSTRACT

Meloidogyne incognita is a causal agent of root knot disease in many plants. This nematode causing damage in foliage nurseries of *Livistona rotundifolia* cultivated countries especially in Sri Lanka. Due to importance of this disease in foliage nurseries and impossibility of using chemical control against this damaging agent in this research, two species of *Trichoderma viride* strain NRRL 6418, *Trichoderma harzianum* (*Hypocrea lixii* TWC1), as an antagonist fungi against *M. incognita* were isolated and identified from foliage nursery in Sri Lanka. The efficacy of these bio control agent against *M. incognita* were investigated *in vitro* and *in vivo* condition. Thus, this research was carried out to investigate the mechanisms of action by which *Trichoderma* sp. antagonizes *M. incognita* *in vitro* and the efficacy of application of a conidia suspension of *Trichoderma* for control of *M. incognita* root knot disease under field conditions. *In vitro*, the antagonistic behavior of *Trichoderma* sp. resulted in the overgrowth of *M. incognita* by *Trichoderma* sp., while the antifungal metabolites of *Trichoderma* sp. prevented the hatching of *M. incognita* eggs and the growth of the second juveniles. Light microscope observations showed the evidence that mycoparasitism contributed to the aggressive nature of the tested isolate of *T. viride* NRRL 6418 and *T. harzianum*(*Hypocrea lixii* TWC1) against *M. incognita* female body and egg masses. Under field conditions, drenching of a conidia suspension of *T. viride* NRRL 6418 and *T. harzianum* (*Hypocrea lixii*) at 1×10^{14} spore mL⁻¹ significantly reduced the disease incidence (DI) in the plant roots and also significantly increased the plant growth of *L. rotundifolia*.

Key words: *Trichoderma* sp., root knot nematode, bio control, *Livistona*, ornamental foliage

INTRODUCTION

L. rotundifolia (Queen Palm) is one of the major ornamental plants belongs to the family Arecaceae. *L. rotundifolia* is a round-leaf fountain palm found in Southeast Asia. It is a common landscaping plant in the region. It is a member of the genus *Livistona*. It is also called Footstool. It can grow in sub-tropical climates and humid, tropical areas. Green Farms Ltd., Marawila, Sri Lanka is a major exporter of queen palm mainly to Holland and substantial amount to Japan, China and Switzerland as potted plant and cut leaves. *L. rotundifolia* is cultivated as a potted plant with close spacing in the net house. It succumb to outbreak of several pests *viz.*, spider mites, grasshopper and scales. Added to this, since 2006, an outbreak of a root-knot nematode

M. incognita becomes a main problem in this palm. *M. incognita* juvenile stages caused severe damage in the active fresh roots of the queen palms thereby affecting its export quality (personal observation).

The root knot nematodes *M. incognita* is sedentary endoparasites and is among the most damaging agricultural pests, attacking a wide range of crops (Katooli *et al.*, 2010). The infections start with root penetration of second stage juveniles, hatched in soil from eggs stored in egg masses that have been laid by the female on the infected roots. Nematodes pass through an embryonic stage, four juvenile stages (J1 - J4) and an adult stage. Juvenile *Meloidogynes* hatch from eggs as vermiform second stage juveniles (J2), the first moult having occurred within the egg. Newly-hatched juveniles have a short free-living stage in the soil, in the rhizosphere of the host plant. They may reinvade the host plant of their parent or migrate through the soil to find a new host root. J2 larvae do not feed during the free living stage, but use lipids stored in the gut (Jonathan and Hedwig, 1991). These nematodes burrow into the soft tissues of root tips and young root and cause the nearby root cells to divide and enlarge. Root knot galls damage the vascular tissues of roots and thus interfere with the normal movement of water and nutrient through the plant.

Chemical nematicide is one of the primary means of control for plant-parasitic nematodes. However, the potential negative impact on the environment and ineffectiveness after prolonged use have led to a total ban or restricted use of most nematicides and an urgent need for safe and effective options. Application of microorganisms antagonistic to *Meloidogyne* spp, or compound produced by these microbes, could provide additional opportunity for managing the damage caused by root-knot nematode (Khan *et al.*, 2005).

Only a limited work has been done on this nematode pest infecting *L. rotundifolia* in Sri Lanka. Indiscriminate use of pesticides causes a great harm to human being, animals, vegetation and to environment as a whole due to their non-target effect and hazardous nature (Vyas and Patel, 2002). Therefore, biological control agents are gaining importance in the field of nematode management. Another importance of these agents is their role as plant growth promoting microorganism (Sharma and Pandey, 2009).

Many fungal and bacterial agents have been examined over a period of time for their potential as bio control agents (Sharma and Pandey, 2009). Direct pathogenicity of fungal biocontrol agents is one of the main mechanisms responsible for plant parasitic nematode control; however, secondary metabolites from fungi also contain compounds which are toxic to plant parasitic nematodes (Dababat and Sikora, 2007).

Several attempts have been made to use *Trichoderma* species to control plant parasitic nematodes. Windham *et al.* (1989) has reported the reduction of egg production by the root knot nematodes *M. incognita* following soil treatment with a *Trichoderma* conidial suspension. *Trichoderma* sp have been used as a bio control agents against plant parasitic nematodes and this fungus may also promote plant growth and have the ability to colonize root surfaces and the cortex. (Sharon *et al.*, 2001). *Trichoderma* species led to inhibition of the nematode activity and movements *in vitro* during one week exposure. *Trichoderma viride* in combination with organic amendments was also known to produce growth hormones, which were observed to have added response in boosting the plant vigour. It has been reported that *Trichoderma* has not only been proved to parasitize nematodes and inactive pathogen enzymes but also help in tolerance to stress condition by enhanced root development. It participates in solubilization of inorganic nutrients

(Sharma and Pandey, 2009). Prasad and Anes (2008) reported that ethyl acetate and methanol extracts of *T. viride* and *T. harzianum* significantly reduced the total number of galls and *M. incognita* population in Okra.

The aim of this investigation was to isolate indigenous antagonistic fungi *Trichoderma* sp from ornamental foliage nursery and to determine their efficacy against root-knot nematode *M. incognita* infecting *L. rotundifolia* and also, their potential to control *M. incognita* was compared with using the standard chemical control agent: Carbofuran.

MATERIALS AND METHODS

The antagonistic fungi *Trichoderma* species were isolated from soil samples obtained from Green Farms Ltd, Marawila, Sri Lanka using the soil dilution technique (Year, 2005, 2006). *Trichoderma* sp. were isolated from organic rich soil within a 15 cm depth by plating soil suspension after necessary serial dilution directly on PDA. Five milliliter of soil suspension was placed in 15 mL molten, cooling PDA, swirled and allowed to solidify. The set up was incubated 5-7 days at 28°C. The isolates were purified by the single spore method. The fungi were identified on the basis of their morphological and reproductive characters (Anonymous, 2006; Bissett, 1991; Lieckfeldt *et al.*, 1999; Samuels *et al.*, 1998; Watanabe, 2002) and the pure cultures of *Trichoderma* were maintained on PDA medium and stored at - 4°C. And further isolated species were identified through DNA sequencing. Identified species were *T. viride* strain NRRL 6418 and *T. harzianum* (*Hypocrea lixii* TWC1). These two species of *Trichoderma* were used to evaluate their efficacy against root-knot nematode *M. incognita* infecting *L. rotundifolia*.

In vitro study: Root knot nematode infected fields were randomly selected in each locality and 5 media samples were taken from each bed (4×42 m) and pooled into one sample. The upper 3-5 cm surface media was removed and about 250 g media along with 10 g feeder roots samples were collected from queen palm rhizosphere to a depth of 5-10 cm (Santhosh *et al.*, 2005). The samples were placed in polythene bags, sealed labeled and then brought to the laboratory for nematode isolation. *M. incognita* female bodies and egg masses were removed aseptically from the palm, *L. rotundifolia* roots. Eggs separated individually from egg masses using NaOCl @ 0.5% were hatched in water to produce second juveniles (Sharon *et al.*, 2001). Ten petri dishes were filled with very thin layer of distilled water. Young embryonated eggs (before appearing of first juvenile stage), Second stage juveniles emerged from the eggs, females and young egg masses (including gall) were collected from *L. rotundifolia* root galls. One milliliter drop consisting approximately either 50 eggs or 50 second stage juveniles or 3 females were placed near the corner of the chamber and two such drops were placed at two opposites corners of the chamber. Each chamber contains eggs, juveniles and adult female bodies of the *M. incognita*.

T. viride NRRL 6418 and *T. harzianum* (*Hypocrea lixii*) grown on potato dextrose agar were separately introduced at the centre of the chamber as a small (4 mmØ) agar disk (Sharon *et al.*, 2001). Each life stage was examined for infection of *Trichoderma* and the experiment was replicated 10 times, repeated two times. Control experiments were kept with the nematode, *M. incognita* life stages without the antagonistic fungus. These chambers were incubated at the room temperature of 27±3°C and the interaction between the fungus and the nematodes was qualitatively monitored for one week under the microscope. Egg masses were crushed on a slide to examine possible infection of eggs by the fungus.

Field evaluation: Three field experiments were conducted in different locations, periods and for different age of the plants at Green Farms Ltd, Marawila, Sri Lanka. Experiments were carried out in the growing seasons 2007, 2008 and 2009 in soil naturally infested with *M. incognita* in a Farm located in the low country intermediate zone. These experiments were carried out in two seasons of the year. Climatic conditions of this research site are, Average Annual Rain fall (in 30 years)- 1620 mm, minimum and maximum relative humidity of 60-90%, minimum and maximum day temperature 25-32°C, minimum and maximum night temperature 20-27°C. Standard agronomic practices were followed throughout the research period. The data was subjected to Analysis of Variance (ANOVA).

***Trichoderma* biomass and formulation production**

Preparation of solid media: Paddy soaked in water for 6 h was parboiled in a pressure cooker (1.1 kg cm⁻² pressure for 45 min). After parboiling the closed container was kept in a cooler room (15±2°C) for 2 h and 5 kg of parboiled paddy was equally distributed among 50 polyethylene bags. The mouth of each bag was passed through a polyvinyl pipe of 2 cm diameter and 0.6 cm width and the mouth was thereafter plugged with a piece of sterilized, non-absorbent cotton. A piece of paper was wrapped over the cotton plug and the paper was kept intact using a rubber band. Plugs of uniform size of (4 mmØ) were obtained from a pure culture of a 7-day old *Trichoderma* isolate on PDA and used to inoculate the above media.

***Trichoderma* liquid formulation:** One kg of 7-day-old mass cultures was flooded with 2 L of tap water and was shaken well in a closed container. The resulting suspension was filtered through muslin cloth. The filtrate was diluted with tap water to obtain conidia concentration range of 10¹⁴ cfu mL⁻¹ for field application. One liter of this conidia suspension was mixed with 1 mL surfactant (wetting agent -commercial product from Lankem Ltd.) before applications.

Preparation of potting media: Potting mixture was prepared by mixing together coir dust, cow dung and compost in 7:2:1 ratio, respectively. pH level was between 4.5-5.5 and this level of pH was maintained throughout the experimental period. EC was maintained at lower than 1.8 mS cm⁻¹

Evaluation of *Trichoderma* spp against root knot nematode *M. incognita*

Trial 1: This study was conducted at Green farm Ltd., Marawila from July 2007 to June 2008. Healthy with out root knot damage *L. rotundifolia* seedlings (7 cm pot) were arranged as follows in an area previously infected with root knot nematode. Beds measuring, 3×30.6 m length and 3.6 m width accommodating 9720 numbers of potted queen palms was divided into 36 plots each plot consisting 270 potted queen palms seedlings. A randomized complete block design was used and each treatment was replicated 12 times. Plots were marked using replicate tags.

Treatments used in this experiment were given in below:

- T1 *Trichoderma harzianum* (*Hypocrea lixii*) at 1×10¹⁴-1×10¹⁵ cfu g⁻¹
- T2 Carbofuran at 02 g pot⁻¹
- T3 Control (water) at 75 mL pot⁻¹

T. harzianum (*Hypocrea lixii*) was used from the stock cultures maintained at Green Farms Ltd. *T. harzianum* conidia suspensions were prepared according to their concentrations and drenched to the pots in two weeks intervals as a liquid formulation. Carbofuran granules were used as a standard check, applied as spot application directly in to the pots at four weeks interval as a granular application. Control was treated with field water.

A total of 270 palms were confined as a replicate from which 15 palms were randomly drawn to assess the effect of the treatments. No of galls /palm seedling were counted by visual method. Nematode infections such as formation of root galls were recorded in two weeks interval. The data was analyzed using SPSS version 10.

Study of growth parameters with different biological and chemical treatments: In monthly interval, 12 random samples were taken in different treatments of every replicates, separately. Then growth parameters such as height of plant (cm) and weight of roots and shoots (g) were measured following the methods suggested by Spiegel and Chet (1998). Then taken average measurement and also drawn the bar chart separately for weight and height of plants with different treatments.

Trial 2: The *L. rotundifolia* palm field naturally infected with *M. incognita* was selected to evaluate the efficacy of *Trichoderma* sp. 18 months old *L. rotundifolia* (12 cm) pot (750 mL) plants naturally infected with root rot nematode *M. incognita* were selected for this trial. *T. harzianum* (*Hypocrea lixii*) and *T. viride* strain NRRL 6418 were used from the stock cultures maintained at Green Farms Ltd. The conidial suspension consisting *T. viride* NRRL 6418 and *T. harzianum* (*Hypocrea lixii*) @ 10^{14} cfu mL⁻¹ was prepared by equally mixing the two cultures. Both species of *Trichoderma* together at 10^{14} cfu mL⁻¹ were drenched at fortnight intervals and the other treatment consisting of Cabofuran @2.5 g/pot (750 mL) kept as standard check. Cabofuran was applied at four weeks interval as granular application. Two treatments such as:

- [T1]- *T. viride* NRRL 6418 + *T. harzianum* (*Hypocrea lixii*) mixture at 1×10^{14} cfu mL⁻¹ as a liquid formulation and at two weeks intervals
- [T2]- 2.5 gram 3% cabofuran/pot at as a granular application four weeks interval

Field experiment was arranged in a randomized complete block design. Treatments were replicated five times. The plot size of each replicate was 3×42 m. Each plot accommodating 1500 potted plants. Nematodes surviving at pre and post treatments were counted. Number of root knot galls/plant and presence of females and eggs inside the galls were recorded. First reading was taken three weeks after first application and then continued at three weeks interval. Root knot disease incidence was measured by means of number of root gall per plant. The data was analyzed using SPSS version 10.

Trial 3: The methodology conducted in Trial 2 was repeated. Instead of chemical treatment, irrigation water was used to find the efficacy of *Trichoderma* sp. on controlling root knot nematode. 12 months old *L. rotundifolia* (12 cm) pot (750 mL) plants naturally infected with root rot nematode *M. incognita* were selected in the plantation. A Field experiment was arranged in a randomized complete block design. Treatments were replicated 5 times. The plot size of each replicate was 3 m×42 m. Each plot accommodating 1500 potted plants. Two treatments such as:

- [T1]- *T. viride* strain NRRL 6418 + *T.harzianum* (*Hypocrea lixii*) mixture at 1×10^{14} cfu mL⁻¹. two weeks intervals as a liquid formulation and
- [T2]- Untreated control was followed

Number of root knot galls/plant was recorded. The first reading was taken three weeks after first application and then continued at three weeks interval. Root knot disease incidence was measured by means of number of root gall per plant. The data was analyzed using SPSS version 10.

RESULTS AND DISCUSSION

In vitro: *T. harzianum* (*Hypocrea lixii*) and *T. viride* strain NRRL 6418 was tested for their capacity to reduce the incidence of the root-knot nematode *M. incognita* on *L. rotundifolia*. Comprehensive studies at the laboratory revealed the nematocidal potential of selected *T.viride* and *T. harzianum* for controlling *M. incognita*. Direct interaction of *T.viride* and *T. harzianum* with nematodes was observed *in vitro* under sterile condition using the method described by Sharon *et al.* (2001). Direct parasitism of *Trichoderma* strands on separated egg/egg masses/female body was observed under the microscope (Goswami *et al.*, 2006). *Trichoderma* incubated *in vitro* with the nematode gave promising results (Spiegel *et al.*, 2004). The hyphae of *Trichoderma* were penetrated and coiled the female body. Eggs were also colonized and egg masses were penetrated by fungal strands. *T. viride* and *T. harzianum* were able to colonize *M. incognita* eggs and second juveniles and female. *In vitro* studies demonstrated that both tested isolates were effective in causing nematode mortality compared with the control (Fig. 1, 2).

Field evaluation: Experiments were carried out to study the effect of two antagonistic fungal bio agents along with cow dung and cabofuran against root knot nematode *M. incognita* infecting *L. rotundifolia* under net house condition. Bio-agents viz., *T. harzianum* (*Hypocrea lixii*) and *T. viride* strain NRRL 6418 in combination with cow dung promoted plant growth, reduced number of galls/plant, female body and egg masses/root system. The *Trichoderma* sp. along with cow dung showed least nematodes reproduction factor as compared to untreated infested plants (Table 2)

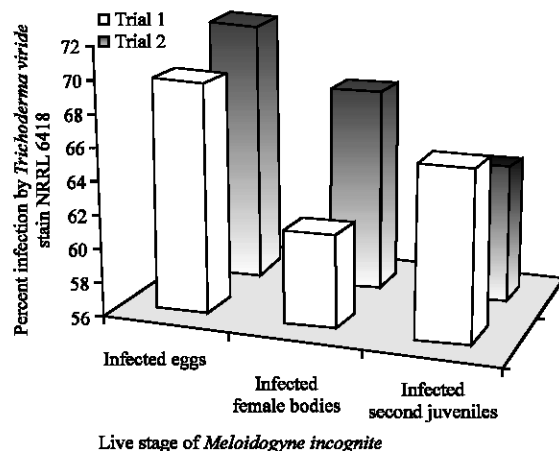


Fig. 1: Infection rate of *Trichoderma viride* strain NRRL 6418 on different live stages of *Meloidogyne incognita* under *in vitro* condition

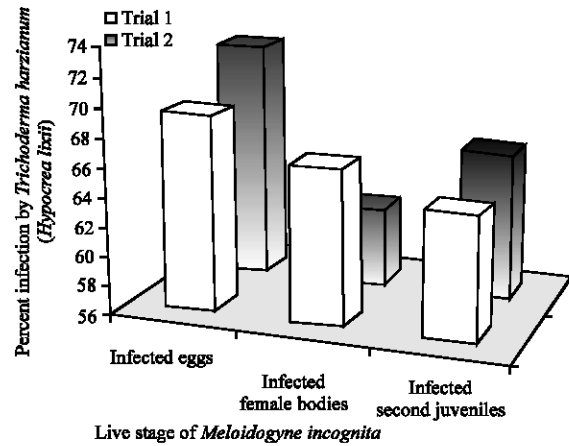


Fig. 2: Infection rate of *Trichoderma harzianum* (*Hypocrea lixii*) on different live stages of *Meloidogyne incognita* under *in vitro* condition

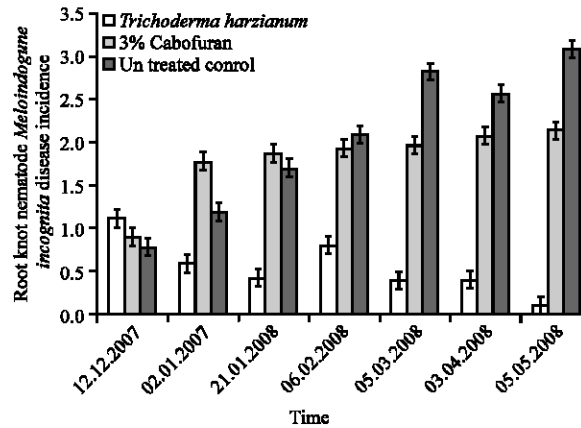


Fig. 3: Comparison of effect of *Trichoderma harzianum* and Cabofuran applications on the number *Meloidogyne incognita* root knot galls formation

Table 1: Comparison of efficacy of *Trichoderma* treatment and Cabofuran applications on the root knot galls disease incidence in *L. rotundifolia*

Pre and post treatment counting	Mean value of root galls (number plant ⁻¹) disease incidence under <i>Trichoderma</i> treatment	Mean value of root galls (number plant ⁻¹) disease incidence under Cabofuran treatment	p-value
Pre-trt count	9.00	8.40	0.531
Post-trt count 1	7.600	5.200	0.332
Post-trt count 2	4.20	8.20	0.057
Post-trt count 3	2.000	4.200	0.043
Post-trt count 4	2.600	4.000	0.297
Post-trt count 5	3.200	4.200	0.452
Post-trt count 6	3.20	5.00	0.378
Post-trt count 7	3.800	4.400	0.758
Post-trt count 8	0.1600	3.400	0.0010
Post-trt count 9	0.1800	3.400	0.0023
Post-trt count 10	0.2600	4.600	0.0034
Post-trt count 11	0.200	1.140	0.000
Post-trt count 12	0.2000	0.8400	0.001

Table 2: Comparison of efficacy of *Trichoderma* applications with Un-treated control on the root knot galls disease incidence in *L. rotundifolia*

Pre and post treatment counting	Mean value of root galls (number plant ⁻¹) disease incidence under <i>Trichoderma</i> sp. treatment	Mean value of root galls (number plant ⁻¹) disease incidence under Un-treated control	p-value
Pre-trt count	5.152	2.224	0.000
Post-trt count 1	3.664	4.488	0.006
Post-trt count 2	1.152	4.448	0.000
Post-trt count 3	0.536	2.912	0.000
Post-trt count 4	0.904	9.024	0.000
Post-trt count 5	0.648	7.360	0.000
Post-trt count 6	0.736	6.688	0.000
Post-trt count 7	0.720	7.376	0.000
Post-trt count 8	0.224	9.472	0.000

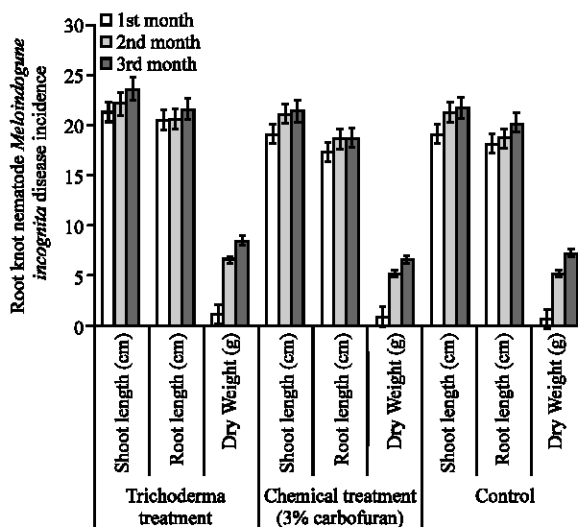


Fig. 4: Comparison of effect of *Trichoderma* and Cabofuran applications on the growth parameters of *Livistona rotundifolia*

These results showed that the antagonistic fungus, *T. viride* NRRL 6418 and *T. harzianum* (*Hypocrea lixii*) excelled in suppressing the galls formation in the roots of the palm as well as significantly reduced the number of nematodes (Fig. 3, Table 1, 2).

DISCUSSION

In vitro: Results showed that these *Trichoderma* species with different mechanism such as lysis of cell wall, inhibited growths of the juveniles of *M. incognita* and parasitized the eggs/female body of *M. incognita* and thus showed its antagonistic effects against causal agent of root knot seedlings. *T. viride* NRRL 6418 and *T. harzianum* (*Hypocrea lixii*) after seven days destructed and lysis the eggs and females bodies. Identified *Trichoderma* species caused parasitize (to hyphal contact method) eggs and female bodies (Fig. 1, 2). *Trichoderma* had proved to infect on the eggs and adult females of the root knot nematode, *M. incognita*. Since the female adult was sedentary in its movement, the antagonistic fungus readily infected the nematode. But infection of *Trichoderma* on eggs was prudent it is best opted at the beginning of the live stage of the nematode to get

controlled. Sharma and Saxena (1992) reported that culture filtrate of *T. viride* adversely influenced hatching of *M. incognita* larvae with highest inhibition of hatching occurring in the standard concentration of the filtrate. When culture filtrates of the two fungi were mixed together, the relative toxicity of the separate filtrates was unaffected. Sahebani and Hadavi (2008); reported that direct parasitism of *Meloidogyne* eggs through increase in extracellular chitinase activity, which would be indicator of egg infection capability and inducing plant defense mechanisms leading to systemic resistance are two main suppression mechanisms used by *T. harzianum* against nematodes. *T. harzianum* must be able to produce extracellular chitinase and proteinase because of the proteinaceous and chitinase nature of nematode egg shell. Other extracellular protein nature has been induced by colloidal chitin which may be involved in nematode egg penetration.

Rao *et al.* (2000) reported that *Trichoderma* sp. alone or in combination with either neem or caster cake, was most effective in parasitizing the egg masses of the *M. incognita* and significantly reduced root galling, nematode population and fecundity of the nematode. *T. harzianum* parasitizes eggs and larvae of *M. incognita*. The hyphae penetrates the eggs and larval cuticle by dissolving the chitin layer through enzymatic activity. They proliferate within the organism and produce toxic metabolites (Dos-Santos *et al.*, 1992).

Dos-Santos *et al.* (1992) reported that *T. harzianum* was a good egg parasite of *M. incognita* killing 53% eggs in *in vitro* conditions.

In vivo: The efficacy of the potential bio control agent of *Trichoderma* sp. in the management root knot nematode was achieved as the reduction in root gall formation and also the reduction in the number of female *M. incognita* and egg masses per root system of *L. rotundifolia*.

Trichoderma treatment was more effective than chemical treatment in reducing the gall formation in *L. rotundifolia*. Palms treated with *Trichoderma* sp. was significantly reduced the number of galls/plant compared with standard chemical treatment (Fig. 3 and Table 1). Further application of *Trichoderma* was able to infect the eggs, adult female and reduced the number of galls/plant. Since, Cabofuran was used very frequently to control the root knots nematodes, their usage came to standstill due to the development of resistance against these inorganic chemicals. The response of application of Cabofuran was ineffective and their persistence may pose ecological problem. Therefore, bio control is suggested to be safer solution (Sharma and Pandey, 2009). *Trichoderma* has good scope due to its effective mode of action. *Trichoderma* parasitizes the nematodes and control them easily. In addition, the eco-friendly method of control of nematodes is prioritized among the management tactics to protect the environment from polluting with chemicals. In addition the consumers of these palms prefer organic products from any countries and as such the Green Farm Ltd. at Marawila, Sri Lanka, apply organic method of controlling insects and diseases to satisfy the requirements of their clients.

The *Trichoderma* treatment also increased the fresh weight and growth rate of the *L. rotundifolia* (Fig. 4). The plant height and root weight were significantly improved compared to Cabofuran applications. These results are also supported by Spiegel and Chet (1998), Sharma and Pandey (2009).

Combination of isolated *T. viride* strain NRRL 6418 and *T. harzianum* (*Hypocrea lixii*) @ 10^{14} cfu mL⁻¹ proved that they are capable to control *M. incognita* and showed good bio control activity at the field level (Zhang and Zhang 2009). Root galling was reduced and the number of new infection had been reduced. *T. harzianum* (*Hypocrea lixii*) alone also significantly reduced gall formation and improved palm growth. 3% carbofuran treatment showed a smaller reduction in

galling formation compare with *T. harzianum* (*Hypocrea lixii*) (Fig. 3) (Sharon *et al.*, 2001; Siddiqui and Shaukat, 2004). Both *T. viride* strain NRRL 6418 and *T. harzianum* (*Hypocrea lixii*) when added together with compost + cow dung showed significant suppression of the egg masses per root system as compared to the control (Goswami *et al.*, 2006). Composts, on the other hand, can serve as an ideal food base for biocontrol agents and offer an opportunity to introduce and establish specific biocontrol agents into soils, which in turn leads to sustained biological control based on the activities of microbial communities (Hoitink and Boehm, 1999). Sharma and Pandey (2009) reported that the *Trichoderma* has not only been proved to parasitize nematodes but also help in tolerance to stress conditions by enhanced root development. It participates in solubilization of inorganic nutrients.

CONCLUSION

This study identified antagonistic fungal isolates of *T. viride* strain NRRL 6418 and *T. harzianum* (*Hypocrea lixii* TWC1) capable of producing compounds active against root knot nematode *M. incognita* infecting *L. rotundifolia*.

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