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Biological Control of Mulberry Root Knot Nematode *Meloidogyne incognita* by *Trichoderma harzianum*

J. Sukumar*, S. D. Padma and U. D. Bongale

Karnataka State Sericulture Research and Development Institute, Thalaghattapura, Bangalore 560 062, India.

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***Trichoderma harzianum*-THN1 parasitising the egg masses of root knot nematode *Meloidogyne incognita* was isolated from galled mulberry roots and evaluated for its potential to control root knot disease. In pot experiments root galling was reduced and leaf yield increased significantly following soil treatment with *T. harzianum*-THN1. The extracts obtained from the soils inoculated with *T. harzianum*-THN1 drastically inhibited the hatching of nematode eggs and the effect was irreversible even after the eggs were transferred to fresh water. The fungus was equally effective in controlling the disease in nematode infested mulberry garden under field conditions which was significant over the most commonly used egg parasitic fungus *Paecilomyces lilacinus*. The disease reduction recorded with *T. harzianum* was on par with the plants treated with the nematicide Carbofuran. The results suggest that *T. harzianum*-THN1 could be used as a potent eco-friendly biocontrol agent against *M. incognita* in mulberry without any residual toxicity to silkworms. *T. harzianum*-THN1 can form an important component of integrated disease management package in mulberry cultivation.**

Key words: Mulberry, Root knot, *Meloidogyne, Trichoderma*, Biocontrol

Introduction

Karnataka state accounts for over 60 percent of the total mulberry silk produced in India. Mulberry (*Morus indica* L.), the chief source of food for silkworm (*Bombyx mori* L.) is severely affected by the root knot disease caused by *Meloidogyne incognita* (Kofoid & White) Chitwood. The disease occurs in over 70 percent of mulberry gardens (Govindaiah, 1990; Sukumar and Padma, 1999) resulting in leaf yield and quality reduction of up to 22 percent (Sukumar and Padma, 1999; Sukumar and Govindaiah, 2001). The nematode forms a disease complex with the opportunistic fungal pathogen *Lasiodiplodia theobromae* causing stem canker and black root rot leading to death of plants (Sukumar and Padma, 1999). Leaves from diseased plants when fed to silkworms affect their health and reduces the cocoon yield and quality (Sukumar and Padma, 1999; Gupta, 2001). Sericulturists are reluctant to control the root knot disease using the nematicide Carbofuran due to its high residual toxicity to silkworms, prohibitive cost and adverse impact on soil micro-organisms.

Biocontrol of root knot disease is more beneficial in view of silkworm and soil health. Several reports are available on the efficacy of fungi against *Meloidogyne* species. *Paecilomyces lilaceous* has been effectively used against *M. incognita* in tomato and potato (Parvatha Reddy, 1987), Okra and mungbean (Shahzad and Ghaffar, 1989) and *M. arenaria* in soyabean (Morgan-Jones *et al.*, 1984). Reports are also available on the parasitization of nematode eggs by *Verticillium chlamydosporium* in many crop plants (De leif *et al.*, 1993; Martens and Stirling, 1993; Sharma, 1998). Several attempts have been made to use *Trichoderma* spp. to control plant parasitic nematodes. Windham *et al.* (1989) reported reduced egg production by *M. arenaria* following soil treatment with *T. harzianum* (T-12) and *T. Koningii* (T-8). Reduction of *M. javanica* infection with several isolates of *Trichoderma lignorum* and *T. harzianum* has been reported (Spiegel and Chet, 1998). It is reported that the reduced egg production is mainly due to the reduction in root penetration by the nematodes which is once again related to reduced nematode population due to egg and larval parasitization by

*To whom correspondence should be addressed.
Head, Department of Mulberry Pathology and Microbiology, Karnataka State Sericulture Research and Development Institute, Thalaghattapura, Bangalore 560 062, India. Tel: 080 23448677; E-mail: vivi10@rediffmail.com

the fungus (Parvatha Reddy *et al.*, 1996; Saifulla and Thomas, 1996; Sharon *et al.*, 2001). However, no such reports are available on the use of *T. harzianum* in the control of *M. incognita* in mulberry.

During the field evaluation of *Trichoderma* species obtained from the rhizosphere of mulberry against the black root rot pathogen *Lasiodiplodia theobromae,* an isolate of *T. harzianum*-THN1 was found to heavily parasitise the egg masses of *M. incognita.* The fungus was isolated and evaluated for its efficacy in controlling the root knot in mulberry and the results are presented in this report.

Materials and Methods

The experiments were conducted on M-5 mulberry variety of *Morus indica* L. The root knot nematode *M. incognita* was propagated on either tomato (*Lycopersicon esculentum* Mill var. *Pusa Ruby*) or on potted mulberry plants. Eggs were separated from the egg masses with sodium hypochlorite (0.5% – 1 min), hatched in water to obtain infective juveniles.

The egg parasitic isolate *Trichoderma harzianum*-THN1 isolated from the infected egg masses of *M. incognita* on mulberry root nodules was maintained on Potato Dextrose Agar medium. The carrier based inoculum was prepared on wheat bran according to the method described by Sivan *et al.* (1984). The preparation contained 10^{10} CFU's/gm. Another egg parasitic fungus *Paecilomyces lilacinus* was used for comparison and prepared in the similar way.

The effect of *Trichoderma* inoculated soil extracts on hatching of *M. incognita* eggs was studied by incubating the eggs in the extract for four days. The method of preparing the soil extracts was as described by Sharon *et al.* (2001). Five replicates were maintained for each treatment with 1000 eggs/replicate. After four days the eggs were transferred to plain water and hatching was observed. The observations were made using a stereo-zoom microscope (Wild M-8).

Preliminary studies were conducted in pots (20 kg soil holding capacity) for one year using sandy loam soil having a pH of 7.2 and OC 0.6%. The soil was determined to be free of root knot nematodes and stored in dry for two months before use. The activities of *T. harzianum*-THN1 wheat bran preparation and wheat bran without the fungus were assessed maintaining non-treated soil as control. The preparations were mixed with soil (1% wt/wt) keeping 12 replicates per treatment. Each pot was inoculated with freshly hatched juveniles to get one juvenile/gm of soil (15,000 juveniles/pot). In post-inoculated treatments, the fungal preparation was inoculated 10 days after the plants were inoculated with juveniles. The inoculation with fungal preparation was repeated after six months. The leaf yield was estimated after 55 days of growth. Five harvests were made in a year and at the end of the fifth harvest 25 grams of roots were sampled per plant and the root knot indices were evaluated on a scale of 0 – 5 (Taylor and Sasser, 1978) as given below

0 = No galls/egg masses
1 = 1 – 2 galls/egg masses
2 = 3 – 10 galls/egg masses
3 = 11 – 30 galls/egg masses
4 = 31 – 100 galls/egg masses
5 = > 100 galls/egg masses

Field evaluation was carried out in an eight-years-old severely infested mulberry garden with sandy loam soil having two juveniles/gm, pH 6.8 and OC 0.6%. One kilogram of inoculum was multiplied for one week on 25 kg neem oil cake powder with 30% moisture and incubated in shade covered by a wet cloth. The preparation was mixed with one ton of well-decomposed farmyard manure and incubated for a week in shade, which was later, applied at the rate of 3.5 kg/micro plot (12' × 12' with 36 plants each). The plots were laid out in randomised complete block design. The fungal inoculations were made twice a year at six monthly intervals along with recommended dose of farmyard manure (20 tons/ha/year) in two split doses. Control plots received similar inputs without the fungus. Leaf harvest was made after 55 days of growth on whole plot basis excluding the border rows. The experiments were conducted for two years (2001 – 2003) with 10 leaf harvests. The root knot and egg mass indices were evaluated on 0 – 5 scale each year at the end of 5th harvest by scoring root knots and egg masses on 25 grams of roots sampled/plant, from 10 plants/replicate selected at random. *Paecilomyces lilacinus* applied similar to *T. harzianum* and Carbofuran (2.5 kg a/ha), applied either once at the beginning of the experiment or twice a year in two split doses were also evaluated for comparison. The egg mass infection by the fungus was confirmed by trypan blue staining method.

The data were subjected to one-way analysis of variance (Sunderaraj *et al.*, 1972) and the treatments were compared among themselves by following the critical difference test at 5% level.

Results

Effect of soil extracts on hatching

Soil extracts of *T. harzianum*-THN1 and *P. lilacinus* inoc-

Table 1. Effect of *T. harzianum*-THN1 inoculated soil extracts on hatching of *M. incognita* eggs (1000 eggs/replicate and average of three trials)

Treatment	No. of juveniles hatched	% Inhibition over control
T1. *T.harzianum* inoculated soil extract	380	55
T2. *P. lilacinus* inoculated soil extract	495	41
T3. Uninoculated soil extract	846	–
T4. Carbofuran solution (10 ppm)	0	100
T5. Distilled water (control)	838	–
CD at 5%	45.30	–

Table 2. Efficacy of *T. harzianum*-THN1 on root knot development and leaf yield in potted mulberry under pre and post inoculated conditions (average of five harvests)

Treatments	Pre-inoculated treatments				Post-inoculated treatments			
	Leaf yield/plant (gm)	Leaf yield increase (%)	Gall index	Reduction in root galling (%)	Leaf yield/plant (gm)	Leaf yield increase (%)	Gall index	Reduction in root galling (%)
T1. *T. harzianum* wheat bran preparation	60	40	2.1	58	52	30	3.1	38
T2. Wheat bran without fungus	45	5	5.0	–	42	5	5.0	–
T3. Control (untreated)	43	–	5.0	–	40	–	5.0	–
CD at 5%	7.2	–	1.3	–	8.8	–	1.2	–

ulated soils significantly inhibited the hatching of *M. incognita* respectively by 55 and 41 percent (Table 1). The inhibition by *T. harzianum*-THN1 inoculated soil extracts was significant over *P. lilacinus*. Total inhibition of egg hatching was recorded in the nematicide Carbofuran solution. There was no significant change in hatching percentage even after the eggs were transferred to plain water after four days indicating the ovicidal effects of the extracts.

Effect of pre and post inoculation of *T. harzianum*-THN1 on root knot and leaf yield

Inoculation of *T. harzianum*-THN1 before and after nematode inoculation exhibited significant reduction in root galling and increase in leaf yield (Table 2). Pre-inoculation with *T. harzianum*-THN1 recorded 40 percent recovery in leaf yield while in post-inoculated treatments the recovery was 30 percent. Similarly, the pre inoculated treatments recorded 58 percent reduction in root galling as compared to post-inoculated treatments with 38 percent reduction. Significant differences were not observed between control and those receiving wheat bran without the fungus.

Field evaluation

The evaluation was carried out for two years and ten leaf harvests were made during this period. The treatments T1, T2 and T4 were significantly effective in reducing the number of root knots and egg masses and increasing the leaf yield as compared to the control (Table 3). In the case of plants receiving a single application of the nematicide Carbofuran at the beginning of the experiment, the two years average data on root knot and egg mass indices and the leaf yield were on par with the control. *T. harzianum*-THN1 treated plants recorded 46 and 52 percent reduction in root knot and egg mass indices and a 23 percent increase in leaf yield which was on par with the plants receiving Carbofuran twice a year, recording 52 and 56 percent reduction in root knot and egg mass indices and 28 percent increase in leaf yield respectively. Both the antagonistic fungi were effective in reducing the nematode infestation and increased the leaf yield. However, *T. harzianum*-THN1 was significantly better than *P. lilacinus*, which recorded 38 and 46 percent reduction in root knot and egg mass indices respectively, and an increase of 15 percent in leaf yield over control.

Discussion

The results obtained have revealed significant biocontrol activity of *T. harzianum*-THN1 against the mulberry root knot nematode *M. incognita*. The reduction in root knot and egg mass indices by *T. harzianum*-THN1 was signif-

Table 3. Comparative efficacy of *T. harzianum-THN1* with other control agents on *M. incognita* under field conditions (Data are averages of 10 harvests)

Treatments	Root knot index	% Reduction	Egg mass index	% Reduction	Leaf yield/ plant (gm)	% Increase
T1. *T.harzianum* (Yearly twice)	2.6	46	2.4	52	218	23
T2. *P. lilacinus* (Yearly twice)	3.0	38	2.7	46	224	15
T3. Carbofuran (Once)	4.6	4	3.8	24	182	2
T4. Carbofuran (yearly twice)	2.3	52	2.2	56	227	28
T5. Control (untreated)	4.8	–	5.0	–	178	–
CD at 5%	0.45	–	0.34	–	13	–

icantly high as compared to the most commonly recommended biocontrol fungus *P. lilacinus*. The present studies have also revealed that *T. harzianum*-THN1 is effective under field conditions with disease reduction on par with the nematicide Carbofuran. The drastic reduction in the hatching of nematode eggs by the autoclaved extracts of *T. harzianum* inoculated soil could be due to the heat resistant metabolites produced by the fungus in the soil. Our results are in conformity with Sharon *et al.* (2001) on tomato, who reported reduced egg hatching in autoclaved soil extracts obtained from *Trichoderma harzianum* inoculated soils. This activity of the fungus along with its ability to colonize and kill the eggs of the nematode is responsible for the reduced disease indices and increased leaf yield. The marginal increase in the leaf yield in plants receiving wheat bran without the fungus may be due to the additional nutrition supplied by the material. The cuticle of nematode larvae is mainly composed of proteins (Blaxter and Robertson, 1998) and the proteolytic activity of the biocontrol agent results in the death of larvae either in the egg or immediately after hatching. *T. harzianum* is known to immobilize juveniles by way of proteolytic activity on their proteinaceous cuticle (Sharon *et al.*, 2001). Similar activity has also been observed with *Verticillium suchlasporium* (Lopez and Robertson, 1992), *V. chlamydosporium* (Segers *et al.*, 1996) and *Paecilomyces lilacinus* (Bonants *et al.*, 1995).

Root knot disease is the most serious perennial problem in mulberry, which is difficult to eradicate. Though the Carbofuran application twice a year is effective, the use by the sericulturists is negligible due to its prohibitive cost and high residual toxicity to silkworms which extends up to 45 days with weekly irrigations but prolongs if irrigation is irregular. Sub lethal doses of Carbofuran are also known to induce resistance in *M. incognita* (Yamashita and Viglierchio, 1987). Sericulturists generally harvest five to seven cocoon crops a year. By the use of Carbofuran one or two rearings may be missed due to the residual toxicity of the chemical. Hence, biocontrol of the nematode is the most promising proposition.

Egg parasites are more dramatic in reducing the nematode population (Sasser, 1989). The results of the present study have clearly revealed the feasibility of utilizing *T. harzianum*-THN1 for biocontrol of mulberry root knot disease in place of Carbofuran. The fungus being self-perpetuating, economical and free from residual effects can become an effective component of integrated disease management package and provide a long-term control of the disease in an eco-friendly manner.

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