XXVI International Horticultural Congress: Managing Soil-Borne Pathogens: A Sound Rhizosphere to Improve Productivity in Intensive Horticultural Systems

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Biological Control of Root Knot Nematodes (*Meloidogyne* spp.) on Tissue Culture Banana (Dwarf Cavendish var. Basarai)

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Keywords: Active ingredients, toxic metabolites, *Solanum melongena*, juveniles, mortality

Abstract

Biocontrol powder Phule *Trichoderma* has been successfully used on a number of horticultural crops. The present investigation was carried out to explore the nematicidal properties of Phule *Trichoderma* against the root-knot nematode (*Meloidogyne* spp.) infesting the tissue culture banana (Dwarf Cavendish - var. Basarai). In vitro tests showed that the various concentrations of Phule *Trichoderma* prevented nematode egg hatching and also resulted in 100% mortality of nematode juveniles. Tissue culture banana plants were also dipped into various concentrations of Phule *Trichoderma* before planting out into plastic bags. Plant were inoculated with 250 nematode juveniles and after ten days, the roots were stained with cotton blue lactophenol and nematodes were counted under a dissecting microscope. The results indicated that the higher the concentration of Phule *Trichoderma* in banana plants, the lower the ability of the nematode to penetrate the roots. Furthermore, the nematode juveniles that penetrated the treated roots were found dead. This could have resulted from the toxic metabolites produced from Phule *Trichoderma*. Also, root zone treatment of plants treated with Phule *Trichoderma* prevented the development of giant cells and roots knots in treated plants while the development of giant cells and root knots were observed in untreated plants exposed to nematode infestation.

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) occur on banana and plantains worldwide and they are often the most abundant nematode species of these crops in the Asian Countries (De Waele and Davide, 1998).Numerous field experiments have shown the effectiveness of various biological control agents against root-knot nematodes. Purified extracts of several *Penicillium* species and *Aspergillus niger* showed high nematicidal activity on giant Cavendish banana (De Waele and Davide, 1998).

Phule *Trichoderma* was developed at the Biocontrol Unit of Mahatma Phule Agricultural University Rahuri, India. This biocontrol powder has been successfully used on a number of pathogens and the present investigation was carried out to explore the nematicidal properties of Phule *Trichoderma* against the root-knot nematode *Meloidogyne* spp. on dwarf cavendish banana.

MATERIALS AND METHODS

In-vitro Tests: Effects of Phule *Trichoderma* on Nematode Eggs and Juveniles

A stock solution of Phule *Trichoderma* was prepared by dissolving one gram (1g) of the biocontrol powder in one liter of distilled water. This was further diluted into the required experimental concentrations.

Eggs and nematode juveniles were extracted from a clone established on *Solanum melongena* by a modification of the centrifugal floating methods described by Whitehead and Hemming (1965). Ten nematode eggs were then incubated in 5mls of the various concentrations in glass blocks and each treatment was replicated five times while eggs incubated in distilled water served as control. Fresh solution were substituted every other

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day to avoid contamination. At the end of the 12th days the numbers of hatched eggs were determined and the hatching reactivation was also tested by dipping the unhatched eggs in distilled water.

Newly hatched nematode juveniles were placed in 10cm diameter petri-dishes filled with 10mls of the various concentrations of Phule *Trichoderma*. Dishes filled with distilled water served as control and each treatment was replicated five times. Dead and living juveniles were counted at 24, 48 and 72 hours incubation.

In-vivo Tests: Root Dipping and Soil Drenching

Roots of banana plants were first washed in distilled water and dipped into the various concentrations of Phule *Trichoderma* for 30, 60 and 90 minutes. The plants were then planted in plastic bags filled with soils and cultivated in a greenhouse. Each plant was then inoculated with 250 nematode juveniles in 50mls of water. After a period of 10 days, plants were uprooted and roots stained with cotton blue lactophenol (De Gurain, 1967). Juveniles that penetrated plant roots were counted with the aid of a dissecting microscope. In order to test the effects of soil drenching on nematode control, healthy banana plants in plastic pots were soil drenched with the various concentrations of Phule *Trichoderma* while pots drenched with distilled water served as control. Two days after the soil drenching each plants was inoculated with 250 nematode juveniles. The plant roots were later examined for the development of root-knots, necrosis and giant cell formation on the exposed roots.

RESULTS

As little as 1.0 mg a.i./liter of Phule *Trichoderma* was able to suppress the hatching of nematode eggs (Table 1). For the control experiment, the percentage egg hatch was 85% and there was no hatching reactivation in all the treatments.

The effects of the various concentration of Phule *Trichoderma* on newly hatched juveniles are indicated in Table 2. After a period of 24 hours, more than 90% of the larvae were found dead in all the concentration and 100% mortality of nematode juveniles was recorded in all the concentrations at the end of the experiment. The control experiment showed a mean percentage mortality of 0%, 6% and 10% respectively at 24, 48 and 72 hours.

The results obtained from the dipping of banana plants into concentration of Phule *Trichoderma* showed that nematode penetration was reduced with increasing concentration of Phule *Trichoderma*. Moreover nematodes that penetrated the treated plants were found dead when roots were examined. Plants which were dipped in the *Trichoderma* suspension for 60 or 90 minutes were better protected than those which were dipped only for 30 minutes. (Table 3).

For the inoculated plants which were treated with Phule *Trichoderma* applied as soil drench, galls and giants cells were not observed but some necrotic lesions were observed on inoculated untreated plant roots.

DISCUSSION

Sikora (1979) reported that infestation of banana plants by *Meloidogyne* spp. weaken the plants and make them more susceptible to root rot fungi. The necrotic lesions reported on roots might have resulted from secondary infections from fungal pathogens.

The results of nematode egg hatch showed that egg hatch was inhibited by the various concentration of Phule *Trichoderma*. The existence of fungi, parasitic to eggs of *M. incognita* has been reported by Jatala (1982), where the fungus *Paecilomyces lilacinus* penetrates the eggs of the nematode and destroyed the embryo.

Trichoderma species have been reported to produce toxic metabolites (Papavizas, 1985) and the 100% mortality of nematode juveniles exposed to concentration of Phule *Trichoderma* could have been caused by these toxic metabolites.

Conventionally, *Trichoderma* species have been extensively used in the biological control of pathogens, because of their high rate of proliferation and the inability of the

nematodes to penetrate the treated plants might have resulted from stimulated growth of *Trichoderma* around the plant roots resulting in production of toxic metabolites for the nematode.

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Tables

Table 1. Effects of various concentrations of Phule *Trichoderma* on nematode egg hatching.

Active ingredient (mg/l)	Percentage egg hatching
0.0	85
0.5	0.25
1.0	0
1.5	0

Table 2. Effects of Phule *Trichoderma* on % mortality of nematode juveniles (in % compared to control), as influenced by contact time.

Active ingredient (mg/l	24 hours	48 hours	72 hours
0.0	0	6 ± 0	10 ± 2.5
0.5	90 ± 0.72	96 ± 0.75	100 ± 0
1.0	100 ± 0	100 ± 0	100 ± 0
1.5	100 ± 0	100 ± 0	100 ± 0

Table 3. Penetration of banana plant roots by nematode juveniles after treatment with Phule *Trichoderma,* as influenced by contact time.

Active ingredient (mg/l)	30 minutes	60 minutes	90 minutes
0.0	+++	+++	+++
0.5	++	+	--
1.0	++	++	--
1.5	++	+	--

Key:
+++ High penetration
++ Moderate penetration
+ Low penetration
-- No penetration