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BIOLOGICAL CONTROL OF THE BANANA BURROWING NEMATODE, *RADOPHOLUS SIMILIS* WITH SELECTED NEMATOPHAGOUS FUNGI

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ABSTRACT

The burrowing nematode, *Radopholus similis,* is the most important banana root pathogen in tropical and subtropical regions of the world. The efficacy of *Paecilomyces lilacinus*, *Penicillium oxalicum* and *Metarrhizium anisopliae* were evaluated on *R. similis. In vitro* assay indicated that the three fungal isolates are capable of infecting *R. similis*. High percentage mortality of 95.2%, 93.1% and 80.4% caused by *P. lilacinus, P. oxalicum* and *M. anisopliae,* respectively, was observed 12 days after inoculation. Treatments were comparable with phenamiphos (Nemacur10G) and significantly different from the untreated control (P = 0.05). Plant height, pseudostem girth, root weight, number of functional leaves obtained from banana cv. Lakatan inoculated with fungal isolates were significantly different compared to the untreated control. *P. lilacinus* was comparable to phenamiphos, with reduced percentage root necrosis whereas *P. oxalicum* and *M. anisopliae* were ineffective.

Key words: *Radopholus similis, Paecilomyces lilacinus, Penicillium oxalicum, Metarrhizium anisopliae*

INTRODUCTION

Radopholus similis is one of the most important nematode species attacking bananas in vast commercial plantations (Cavendish type) in Central and South America with potential damage on plantain and cooking bananas in the lowlands of Eastern and Central Africa and the Caribbean as well in Asia and the Pacific (Sarah *et al.*, 1996). In the Philippines, *R. similis* has become a problem when large volume of planting materials of Giant Cavendish from Central America were commercially raised by farmers in the early 1970s (Davide, 1982).

Surveys on the occurrence and distribution associated with Giant Cavendish in Davao, Mindanao, Philippines revealed *Meloidogyne* spp. and *R. similis* as the main species found widely distributed and more destructive than the other species (Davide and Gargantiel, 1974; Davide and Zarate, 1977; Boncato and Davide, 1980). Recently, Zorilla *et al.* (2004) found the same genera of nematodes, in addition to *Helicotylenchus multicinctus*, which were prevalent in Oriental Mindoro, a province in Luzon, Philippines.

R. similis caused two types of damages on banana. First, the destruction of the root and corm tissues which impairs water

and mineral uptake. This leads to a reduction of plant growth and development, thus severe losses in bunch weight can be observed which increase significantly the time period between two successive harvests. Second is more important - the uprooting or toppling over of the whole plant particularly during strong winds and heavy rains. This is attributed to poor anchorage due to severe rotting of the root (Sarah *et al.,* 1996).

Of all groups of plant parasitic nematodes, these migratory endoparasites are likely to be the most difficult to control using natural enemies. *R. similis* and *Pratylenchus* spend much of their lives in roots and tend to be found in soil only when plants are stressed, senescing or diseased. Since eggs can be laid in root tissue and juveniles can be hatched and developed to maturity without moving from roots, multiplication sometimes can proceed to several generations without nematodes being exposed to soil-borne antagonists (Stirling, 1991).

Biological control offers an alternative approach in controlling plant parasitic nematodes, considering the innate problems related to the environmental pollution and health hazards of chemical controls particularly nematicides. The most opportunistic Hypomycetes consistently associated with the pathology of plant parasitic nematodes is *Paecilomyces lilacinus*. *P. lilacinus* in particular is a soil inhabiting fungus that is capable of parasitizing nematode eggs, juveniles and adult and even reducing the populations of other plant parasitic nematodes (Jatala *et al.*, 1979; Generalao and Davide, 1986; Stirling, 1991).

However, there were few attempts that evaluate biological control agents for *R. similis*. To name a few, *Paecilomyces lilacinus*, *Penicillium anatolicum* and *Arthrobotrys cladodes* reduced *R. similis* population in the soil and roots of Philippine banana (Generalao and Davide, 1986; Tandingan and Davide, 1986). In Costa Rica, a powder formulation ABG-9008 of *Myrothecium* is a promising bionematicide against the same banana nematode (Marin *et al.*, 2000). Microbial filtrates and purified fractions of *P. oxalicum* and *P. anatolicum* and *Aspergillus niger* were found to have nemacidal effects on *Meloidogyne incognita* and *R. similis* by soil drench (Molina and Davide, 1986). However, no further studies have been done using *P. oxalicum* whereas *M. anisopliae* has not yet been tested as biocontrol agent of *R. similis*. This study was conducted to evaluate the efficacy of *P. oxalicum* and *M. anisopliae* for control of *R. similis in vitro* and *in vivo* assays in the greenhouse.

MATERIALS AND METHODS

Pure Cultures of *Radopholus similis* and Fungal Isolates

Monoxenic culture of *Radopholus similis* isolated from Davao, Mindanao, Philippines was prepared using carrot discs following the protocol of Spiejer and De Waele (1997). Nematodes were cultured for 5 wk on stock carrot disc culture using sterile distilled water and passed through 25-µm mesh sieve. Suspension containing juveniles (female and male nematodes) was surfaced sterilized with 2000 ppm streptomycin sulfate for overnight. After three series of washing with sterile distilled water, individual nematodes were aseptically inoculated on carrot disc and incubated at 28°C for 4 to 6 wk.

Fungal isolates, *P. oxalicum* and *M. anisopliae* and the *P. lilacinus* (patented strain 251), an active component of BIOACT (Los Baños, Philippines) as standard were grown on Potato Dextrose Agar (PDA) slants and incubated at room temperature for 5 to 10 days.

In vitro Bioassay

One thousand nematodes collected from carrot disc cultures were inoculated or

Immersed in sterile Petri dish with 10 ml suspension of each fungal isolate (~ 250,000 spores/ml). Plates were sealed with parafilm and incubated at room temperature. Petri dishes with sterile distilled water and 200 ppm phenamiphos (Nemacur 10G) inoculated with *R. similis* served as negative and positive control, respectively. All treatments were replicated four times and laid-out using simple Complete Randomized Design (CRD). Using the dissecting microscope (Motic), number of dead nematodes based on motility and percentage mortality were determined after 4, 8 and 12 days. Parasitized and dead nematodes were picked and mounted in clean, ringed glass slide with small amount of acid fuchsin lactophenol for photomicroscopy.

Greenhouse Test

One month-old tissue cultured banana cv. ('Lakatan' AA genome) with susceptibility to *R. similis* were grown in clay pots containing sterilized garden soil and river sand. One thousand nematodes from carrot disc cultures were inoculated following the screening procedure of Spiejer and De Waele (1997). Inoculum was applied on three equidistant holes near the plant root zone using pipette. Each fungal isolate was applied as soil drench at 200 ml/pot (~250,000 spores/ml) 10 days after nematode inoculation. Potted plants were replicated four times and arranged in Complete Randomized Design (CRD) and allowed to grow for at least 7 wk. Phenamiphos-treated banana (200 ppm) and nematodes alone were included as positive and negative controls, respectively. Seven weeks after inoculation, plants were uprooted and root system was thoroughly washed in running water. Plant parameters such as root weight, plant height, pseudostem girth, the number of functional leaves, and percentage root necrosis were evaluated using the screening procedures of Spiejer and De Waele (1997).

RESULTS

***In vitro* Bioassay**

P. lilacinus strain, *P. oxalicum* and *M. anisopliae* inhibited *R. similis* specifically juveniles. *P. lilacinus* and *P. oxalicum* were not significantly different in reducing *R. similis* mortality (P = 0.05). High mortality rates were observed at 12 days after inoculation. *P. lilacinus* showed the highest mortality rate at an average of 95.2% after 12 days after inoculation, which is comparable with the 93.1% of *P. oxalicum* (Table 1). Phenamiphos killed all nematodes *in vitro* as early as 4 days after inoculation.

The mode of action of these fungal isolates is mainly through their parasitic activity. In *P. lilacinus* and *P. oxalicum*, mycelia and spores adhered on nematode cuticle and later on penetrated inside the host tissues (Fig. 1a, Fig.1b, Fig. 2a and Fig.2b). Parasitism was observed at the head region (Fig.1c) and towards the tail region (Fig. 2c). Complete parasitism of the nematode body eventually led to intensive hyphal growth and distortion and bloating of the cuticle. *M. anisopliae* on the other hand, showed trapping mechanism due to highly branched and growing mycelia on active juveniles followed by complete distortion and bloating of the nematode body (Fig. 3a, Fig. 3b and Fig.3c).

Greenhouse Experiment

In greenhouse test, 7 wk after inoculation, banana plants were uprooted and plant height, pseudostem girth, root weight, number of functional leaves, and the percentage root necrosis were taken. Using the plant parameters, fungi-treated plants were found significantly different for all plant parameters compared with the control plants but comparable to nematicide-treated plants (Table 2). The effect of *P. lilacinus* and phenamiphos in reducing root necrosis was not significantly different (P = 0.05). The effect of *P. oxalicum* and *M. anisopliae* was similar to the untreated control.

DISCUSSION

Parasitic action of *P. lilacinus, P. oxalicum* and *M. anisopliae* increased mortality of *R. similis*. Spore suspension of 250,000 per ml inoculated to 1,000 *R. similis* parasitized nematodes 12 days after inoculation. However, nematodes were not directly parasitized but were found immobile. Cuticle disintegration can be attributed to toxic metabolites detrimental to nematodes. *P. lilacinus, P. oxalicum* and *M. anisopliae* are capable of producing toxins lilacinin, oxalic acid and destruxins, respectively (Arai *et al.*, 1973; Friis *et al.,* 1969; Kaijiang and Roberts, 1986). Other enzymes may be expressed during the course of infection; however, these were not evaluated on this study. The effectiveness of these nematophagous fungi specifically *P. lilacinus* were already evaluated both *in vitro* and *in vivo* using *Meloidogyne incognita* in tomato and *Globodera rostochiensis* (potato cyst) and on *R. similis* in 'Cavendish' banana, respectively. Comparative studies showed that *P. lilacinus* can parasitize eggs and vermiform nematodes resulting in reduced number of nematodes by 13.1% as compared with the nematicide which gave 12.1% (Generalao and Davide, 1986; Zorilla, 1990). Other fungi evaluated are *Arthrobotrys cladodes* and *Penicillium anatolicum* immobilizing and killing *R. similis* larvae.

Phenamiphos completely immobilized and killed nematodes as early as 4 days upon nematode immersion giving 100% mortality. Nemacur is an organophosphate nematicide available as water-soluble liquids, have low volatility, can be applied on or before planting having specific effect on nematodes. *P. lilacinus* applied as soil drench were able to reduce the degree of infection of *R. similis* on 'Lakatan' which is comparable to phenamiphos. Similar studies by Generalao and Davide (1986) also showed that both substrate soil incorporation and soil drench application of *P. lilacinus, P. anatolicum* and *A. cladodes* can significantly reduced the number and size of root lesions due to *R. similis.* However, *P. oxalicum* and *M. anisopliae* did not reduce root necrosis of banana. In contrast, use of purified extracts of *P. oxalicum* provided 69 to 85% control based on banana root lesions (Molina and Davide, 1986). This could be attributed to different strains used in the study. Once again, *P. lilacinus* has proven itself effective in controlling *R. similis* using another banana cultivar 'Lakatan'.

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Table 1. Mean percentage mortality of *Radopholus similis* at 4, 8 and 12 days after inoculation with different fungal isolates

TREATMENT	MORTALITY (%)		
	4 days after inoculation	8 days after inoculation	12 days after inoculation
Paecilomyces lilacinus	4.4a	13.6a	95.2a
Paecilomyces oxalicum	4.8a	13.5a	93.1a
Metarrhizium anisopliae	1.8b	7.4b	80.4b
Phenamiphos	100c	-	-
Nematode alone	0 d	0c	1.8c

Means in rows with same letters are not significantly different among treatment means using Duncan's Multiple Range Test at 5% level of significance.

Table 2. Mean plant height (cm), pseudostem girth (cm), root weight (g) and number of functional leaves and percent root necrosis of banana cv Lakatan 7 weeks after inoculation

TREATMENT	PLANT HEIGHT (cm)	PSEUDOSTEM GIRTH (cm)	ROOT WEIGHT (g)	NUMBER OF FUNCTIONAL LEAVES	ROOT NECROSIS (%)
Paecilomyces lilacinus	22.2a	1.3a	7.8a	7	21.8a
Paecilomyces oxalicum	22.2a	1.4a	8.0a	7	37.2b
Metarrhizium anisopliae	24.1a	1.6a	9.8a	6	39.5b
Phenamiphos	22.6a	1.5a	9.6a	6	21.5a
Nematode alone	19.2b	1.1b	6.9b	5	31.5b

Means in row with same letters are not significantly different among treatment means using Duncan's Multiple Range Test at 5% level of significance.

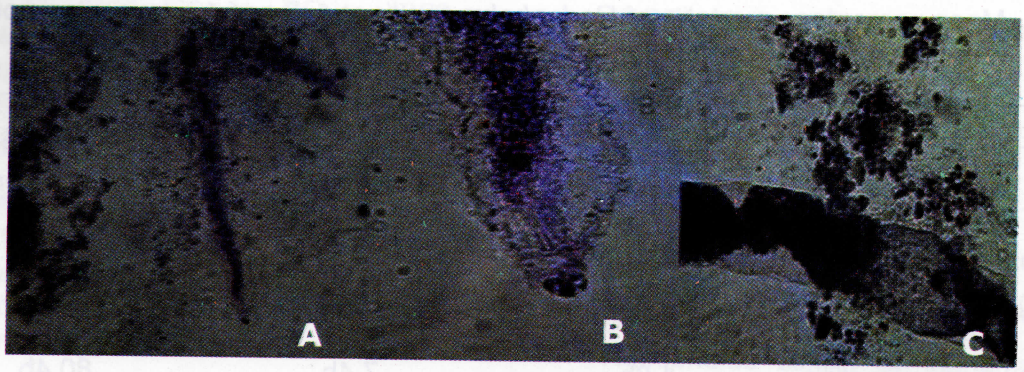

Figure 1. A. Hyphal penetration of *Paecilomyces lilacinus,* B. Complete parasitism of the nematode body and C. Infected head region.

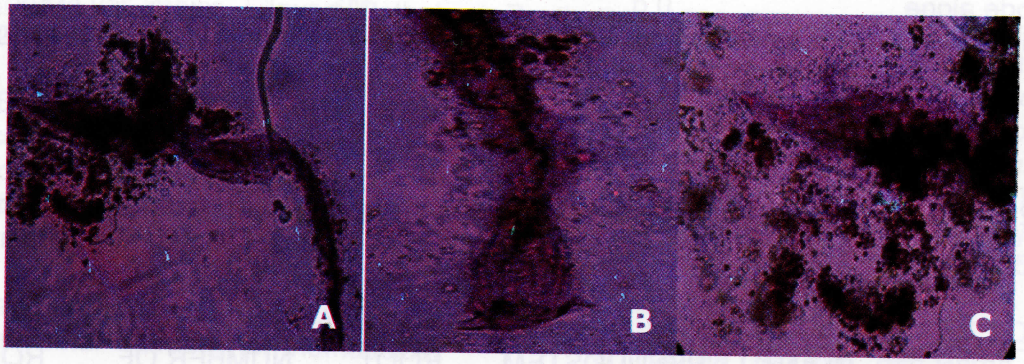

Figure 2. A. *Penicillium oxalicum* causing disintegration of the nematode body, B. Completely disintegrated head region, and C. Infected tail with spores and mycelia.

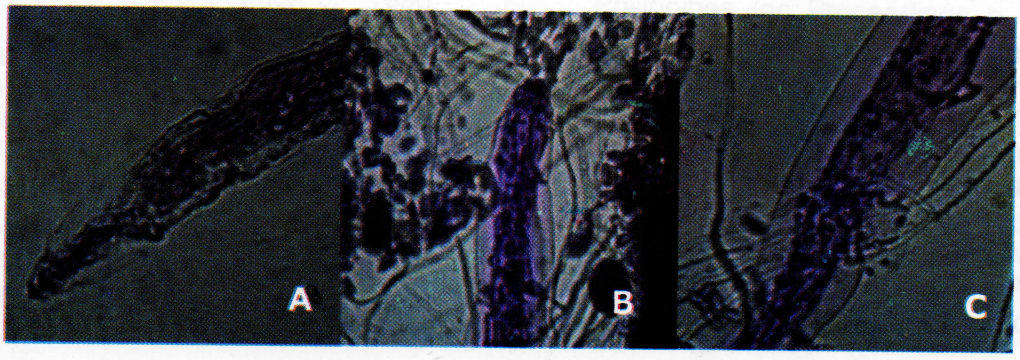

Figure 3. A. *Metarrhizium anisopliae* causing disintegrated head region, B. Trapped nematode in hyphae and C. Hyphae penetrating and trapping tail region.