EFFICACY OF *Paecilomyces lilacinus* ISOLATES FOR THE CONTROL OF ROOT-KNOT NEMATODE (*Meloidogyne incognita* [Kofoid and White] Chitwood) IN SWEETPOTATO

C.D. Galano, R.M. Gapasin and J.L. Lim

Department of Plant Protection, Visayas State College of Agriculture Baybay, Leyte, Philippines.

ABSTRACT

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The application of different *Paecilomyces lilacinus* isolates grown in water hyacinth substrate, 50 days after *Meloidogyne incognita* egg inoculation increased fresh vine and fibrous root weights by 32.6-36.2% and 29.6-35.1%, respectively over the untreated control. On the other hand, chicken manure and ethoprop (Mocap)-treated plants had an increase of 37.9-43.0% and 18.6-29.9%, respectively.

The mean number of root galls, egg masses produced and nematode population in fibrous roots and soil in plants applied with *P. lilacinus* isolates ranged from 27.8-42.0, 40.9-58.1, 8.4-11.5 and 392.0-800.8, respectively. The isolates evaluated for their efficacy gave comparable effects since no significant differences in effect were observed among them. Chicken manure-treated plants had a mean number of root galls of 28.8 and egg masses of 40.3 showing an egg mass reduction of 55.7%. Ethoprop gave the lowest number of galls (4.2) and egg masses (6.2) among the treatments. Percent reduction based on egg masses produced was 93.2 relative to the untreated control.

The overall results of the study showed that the efficacy of *P. lilacinus* in reducing root-knot galls, egg masses and nematode population was comparable to chicken manure but not with ethoprop.

KEY WORDS: Chicken manure. Egg masses. Ethoprop. Galls. *Ipomoea batatas*. *Meloidogyne incognita*. *Paecilomyces lilacinus*. Root-knot nematode. Sweetpotato.

INTRODUCTION

Sweetpotato (*Ipomoea batatas* L.) is considered one of the most important root crops grown in the Philippines. The crop is primarily grown for its large edible storage roots which can be utilized as food, livestock feeds and raw material for industrial purposes notably starch and alcohol. Moreover, it is rich in vitamins A and C, carbohydrates and minerals such as calcium and iron (Edmond and Ammerman, 1971).

Sweetpotato production is beset by several problems which include its susceptibility to many pests. It is attacked by many soil-borne pathogens one of which is the root-knot nematode, *Meloidogyne incognita*. This nematode causes galling on the feeder roots, roughening and frequent cracking of tubers, and generalized decay of the entire fibrous root system, thus reducing the yield and quality of the tubers. Above-ground symptoms include stunting or poor growth, yellowing of leaves, loss of vigor and eventually death of the plants (Davide, 1972).

Infestation by this organism may reduce crop yield by more than 50% (Davide, 1972; Sasser et al, 1983) and adversely affect the quality of the produce (Radewald, 1978). In southern Arkansas nematodes aroused the interest of some pathologists due to the unusual yield loss they caused in sweetpotato (Elliot, 1918). Gapasin and Valdez (1979) found that *M. incognita* and *M. javanica* are responsible for reducing the yield of BNAS-51 variety of sweetpotato by as much as 50%. An initial population of 20,000 eggs of *M. incognita* inoculated to sweetpotato could reduce yield by 47.7% four months after inoculation. They pointed out that cracks on sweetpotato roots were not physiological manifestations of the crop as reported by some investigators but were caused by *M. incognita*.

Control of the root-knot nematode is necessary to minimize yield loss. Among the control strategies employed are cultural and biological methods, planting of antagonistic plants, use of resistant varieties, organic manure application and chemical control. Chemicals are often used by farmers because of their effectiveness and ease in application compared to the other methods of control. However, chemicals are environmental hazards because of their high residual toxicity. Likewise, chemicals are expensive and may not be practical and profitable for small-scale farmers. The growing concern for

preserving the environment from the danger of pesticide pollution has led to the search for possible alternatives in nematode control.

The control of root-knot nematode by known species of fungi such as *Arthrobytrys* sp., *Paecilomyces lilacinus* (Thom) Samson and *Metarhizium anisopliae* (Metsch) Sorokin may provide a cheap and economical alternative (Davide, 1972; Domsch, 1980). In the Philippines, effectiveness of *P. lilacinus* isolated from soil was compared with *P. lilacinus* from Peru in a series of studies conducted under greenhouse and field conditions. The study showed that *P. lilacinus* isolated in the Philippines was generally as effective as the Peru isolate in controlling root-knot nematode as well as the potato cyst nematode, *Globodera rostochiensis* (Davide and Zorilla, 1983; Villanueva and Davide, 1983). It is also documented that *P. lilacinus* is effective not only against root-knot nematodes and the potato cyst nematode but also against citrus nematode (*Tylenchus semipenetrans*), lesion nematode (*Pratylenchus* spp.), and the banana nematode *(Radopholus similis)* (Tandingan and Davide, 1986; Generalao and Davide, 1986; Gapasin, 1986).

The potential of *P. lilacinus* for controlling *M. incognita* on potatoes was also assessed in field experiments (Jatala et al, 1980; Jatala et al, 1981). Potato plants grown in plots inoculated with the fungus had significantly lower root galling index than those grown in plots to which organic matter and nematicide have been applied. About 68% of the egg masses from plants grown in fungus-treated soil were infected with *P. lilacinus* and over half of the eggs were destroyed (Jatala et al, 1980). On potato roots, eggs of *M. incognita* (Kofoid and White) Chitwood var. acrita Chitwood were found by Jatala et al (1979) to be heavily infected by *P. lilacinus*. Following inoculation into nematode-infected potato plants, this fungus was found to be capable of invading both females and egg masses of *Meloidogyne* and also cysts of *G. pallida* (Stone) Mulvey and Stone. On the other hand, Dunn et al (1982) have shown *P. lilacinus* to be capable of colonizing eggs of *M. incognita* in vitro.

The applicability of these biological control agents could lessen the dependence of sweetpotato growers, especially the small-scale farmers, on pesticides. However, the efficacy of *P. lilacinus* isolates has not yet been tested against the root-knot nematode, *M. incognita* in sweetpotato. This

study was conducted using potted sweetpotato plants inside the DPP screenhouse to evaluate the efficacy of six *P. lilacimus* isolates for root-knot control in sweetpotato; and compare the effectiveness of these isolates with ethoprop (Mocap) and chicken manure for root-knot nematode control.

MATERIALS AND METHODS

Preparation of isolates

P. lilacimus isolates used in this study were taken from previous collections of the DPP and were mass produced in water hyacinth (*Eichhornia crassipes*) substrate. The substrate was chopped and placed in heat-resistant plastic bags and sterilized by autoclaving for 1 h at 15 psi (1055 g/cm^2), then allowed to cool. After cooling, *P. lilacimus* isolates previously grown in petri plates with oat meal agar (OMA) medium were aseptically seeded separately into the substrate.

Soil sterilization, inoculation, application of treatments and planting of sweetpotato

Soil taken from the field was heat-sterilized for 2-4 h in a vat. The sterilized soil was placed in 10-in (254-cm) diameter clay pots. After placing the soil in the pots, a suspension of 5,000 eggs of *M. incognita* which served as initial inoculum was poured onto the surface and later covered with a layer of soil. After one day, the six isolates of *P. lilacimus* grown in the substrate were applied by thoroughly mixing 300 g of the substrate with *P. lilacimus* into the inoculated soil. Air-dried chicken manure at 20 tons/ha and a nematicide at the rate of 2 kg a.i./ha were applied for comparison.

One week after application of the treatments 20-25 cm sweetpotato (VSP-2) cuttings were planted in each of the 254-cm diameter clay pots. There were 45 potted plants arranged in a completely randomized design (CRD) in the experimental area with five pots per row and one plant per pot. A space of 150 cm between rows and 100 cm between pots within a row was provided. The experiment was conducted in two trials with five replicates per treatment. There were nine treatments as follows: T_1–Nonoc isolate; T_2–Peru isolate; T_3–Matalom isolate; T_4–ViSCA isolate; T_5–UPLB isolate;

T_6–Abuyog isolate; T_7–Ethoprop (Mocap, 2 kg a.i /ha); T_8–Chicken manure; T_9–Inoculated soil alone (untreated control).

Care and maintenance of plants

Sweetpotato plants were applied with 3 g/pot of complete fertilizer (14-14-14) to induce early development of the roots. They were kept relatively free from insect pests by hand picking. The plants were watered whenever necessary.

Data gathered

Fresh vine and fibrous root weight

After 50 days, the plants were uprooted and the root system was separated from the aboveground parts of the plants. The fresh vine and fibrous roots of the plants were separately weighed.

Number of galls and egg masses

After weighing the root system, the roots were examined for the presence of galls and egg masses. Gall and egg masses were examined under a stereomicroscope and counted with the aid of a hand tally counter.

Nematode density in the roots

One gram root sample per plant was taken and stained with acid fuchsin lactophenol to facilitate easy examination of the root for the presence of nematodes. Staining of roots was done by boiling the prepared chemical solution and dipping the roots in the solution for about 5 min. The roots were dissected carefully and the nematodes were counted under the stereomicroscope with the aid of a tally counter.

Nematode density in the soil

A composite of 300-g soil sample was taken after harvest from each treatment, and processed in the Nematology Laboratory using the modified Baermann funnel technique. Nematode suspension was collected 48 h after and the nematodes were killed, fixed and counted. Nematode density in the soil was expressed as the number of larvae/300 g soil/pot.

RESULTS AND DISCUSSION

Vine and fibrous root weights

The fresh vine weight of sweetpotato inoculated with different isolates of *P. lilacinus* in water hyacinth substrate increased by 32–36% compared to plants grown in inoculated soil alone (untreated control). Plants applied with chicken manure and ethoprop showed an increase of 43% and 30% in vine weight, respectively (Table 1). Likewise, differences in the mean fibrous root weight of sweetpotato were observed after application of the different treatments. Plants applied with *P. lilacinus* isolates had a mean weight range of 29–35 g. The mean fibrous root weight in chicken manure and ethoprop-treated plants were 36.7 and 27.9 g, respectively. There was an observed increase in mean weight of fibrous roots. Plants treated with *P. lilacinus*

Table 1. Mean weight of vines and fibrous roots of sweetpotato (VSP-2) 50 days after inoculation with *Meloidogyne incognita* eggs.[1]

Treatments	Fresh Weight[2]	
	Vine	Fibrous Roots
P. lilacinus Isolates		
Nonoc	116.6 (34.4)	33.0 (31.0)
Peru	113.6 (32.6)	30.5 (25.4)
Matalom	113.8 (32.7)	32.4 (29.7)
ViSCA	118.6 (35.4)	31.8 (23.2)
UPLB	114.8 (33.3)	29.6 (28.3)
Abuyog	120.1 (36.2)	35.1 (35.1)
Ethoprop (Mocap)	109.3 (29.9)	27.9 (18.6)
Chicken manure	134.4 (43.0)	36.7 (37.9)
Inoculated soil alone (Untreated Control)	76.6 —	22.8 —

[1]Means of 2 trials with 5 replicates per treatment.
[2]Numbers in parentheses denote percent increase which was based on inoculated soil alone (untreated control).

Table 2. Mean number of galls and egg masses per root system and nematode population in the soil and fibrous roots and soil of sweetpotato (VSP-2) 50 days after inoculation with *Meloidogyne incognita* eggs.[1]

Treatments	Root Galls	Egg Masses[2]	Nematode Population[3]	
			Fibrous Roots	Soil
P. lilacinus isolates				
Nonoc	31.5 b	47.1 b (48.2)	10.6 ab	548.4 b
Peru	38.7 b	54.4 b (40.2)	11.5 ab	800.8 a
Matalom	27.8 b	40.9 b (55.0)	10.4 ab	550.0 b
ViSCA	38.2 b	50.8 b (44.1)	8.4 ab	392.0 bc
UPLB	42.0 b	58.1 b (36.1)	8.7 ab	424.7 bc
Abuyog	41.2 b	50.5 b (44.4)	11.1 ab	419.1 bc
Ethoprop (Mocap)	4.2 c	6.2 c (93.2)	1.5 c	88.6 d
Chicken manure	28.8 b	40.3 b (55.7)	7.9 b	219.6 cd
Inoculated soil alone (Untreated Control)	64.5 a	90.9 a —	12.9 a	1021.0 a

[1]Means in a column having the same letters are not significantly different at 5% level based on Duncan's Multiple Range Test (DMRT); means of 2 trials with 5 replicates per treatment.

[2]Numbers in parentheses denote percent reduction based on inoculated soil alone (untreated control).

[3]Based on one g root sample per plant and 300 g soil sample per pot.

isolates showed an increase that ranged from 23-35% over that of the inoculated soil alone (untreated control). Plants treated with chicken manure exhibited the highest increase in fibrous root weight (37.9%). An increase of 18.6% was obtained when plants were applied with ethoprop. Although there were increases in vine and fibrous root weights, statistical analysis showed no significant differences among treatments.

The increase in vine and fibrous root weights of sweetpotato in *P. lilacinus* and chicken manure-treated plants may be attributed to a decrease in nematode population thereby reducing the number of galls and egg masses produced (Table 2) or to the fertility provided by chicken manure and the substrate (water hyacinth) where the fungus was grown. Watson (1944)

reported similar results where mulches of decaying organic matter improved the growth of the plant and in some cases actually reduced the damage caused by nematodes. Likewise, Smith and Batista (1942) pointed to an improved fertility which permitted good growth of plants brought about by some metabolic by-products in the decomposition of organic matter. In a similar study, Gapasin (1981) reported significant increases in roots, tubers and tops of sweetpotato and cassava after the application of chicken manure.

Root galls, egg masses and nematode population

Plants inoculated with *M. incognita* eggs and then applied with *P. lilacinus* isolates, chicken manure and ethoprop had significantly lower number of galls, egg masses and nematode population in soil compared to plants in inoculated soil alone (untreated control) (Table 2).

The number of root galls and egg masses produced per root applied with *P. lilacinus* isolates was lower which ranged from 27.8 to 42.0 compared to the untreated control (64.5). However, statistical analysis showed no significant differences among isolates (Table 2).

The result of the experiment clearly indicated that the application of each of the *P. lilacinus* isolates could reduce the number of galls and egg masses produced by *M. incognita*. Similar results were reported by several workers on the efficacy of *P. lilacinus* in controlling *Meloidogyne* sp. and other parasitic nematodes (Davide and Zorilla, 1983; Villanueva and Davide, 1983; Generalao and Davide, 1986; Tandingan and Davide, 1986; Gapasin, 1986). Davide and Zorilla (1986) reported that *P. lilacinus* applied either as soil drench or mixed with substrate, controlled root-knot nematode population attacking okra by 66-77% resulting in slight gall formation on the roots. Reductions in number of galls caused by *M. arenaria* on roots of *Cucurbita pepo* in greenhouse tests following addition of oats colonized by two isolates of *Gliocladium roseum* and one each of *G. catenulatum*, *P. lilacinus*, *Verticillium chlamydosporium* and *V. lamellicola* were reported by Rodriguez-Kabana et al. (1984).

According to Morgan-Jones et al. (1984) the main types of destructive activity of *P. lilacinus* are thought to be enzymatic disruption of nematode structural elements such as egg shells and larval cuticles and physiological

disturbances brought about by biosynthesis of diffusible toxic metabolites. Furthermore, hyphae of *P. lilacimus* readily penetrate egg shells of *M. arenaria* through small pores dissolved in the vitelline layer. Invaded eggs become swollen as a result of a change in shell permeability. The overall effect seems to involve disruption of embryonic development and death of larvae resulting in a reduction of nematode population (Morgan-Jones and Rodriguez-Kabana, 1985). These observations might actually have operated in the reduction of egg masses produced in this experiment.

The application of chicken manure also reduced the nematode population in the soil compared to those of some isolates of *P. lilacinus* (Nonoc, Peru and Matalom isolates) (Table 2). The effectiveness of chicken manure could be attributed to the presence of nematophagous fungi that attack root-knot nematodes. Cortado and Davide (1968) isolated nematophagous fungi e.g. *Arthrobotrys sp.* and *Dactyllela sp.* from chicken manure and straw compost. Likewise, Reyes and Davide (1975) isolated 7 genera of nematophagous fungi from animal manure and farm soils. These were *A. oligospora*, *Tricothecium musiformis*, *Caternaria anguillulae*, *Dactylaria brochopaga*, *Harposporium anguillulae*, *Acrostalagmus obovatus* and *Stylopage* sp. Results of greenhouse experiments showed that these nematophagous fungi could control root-knot infection on tomato by 38.3-70.8%. *A. oligospora* was the most effective among them. Although no isolation work was done in this experiment, it is highly probable that nematophagous fungi were present in the chicken manure. According to Davide and Quebral (1970), relatively low population densities of plant parasitic nematodes in La Trinidad Valley and Atok, Benguet may be due to application of chicken manure which harbor a number of nematode trapping fungi.

It was also possible that the changes in the physical and chemical condition of the soil might have altered the host-nematode relationship thereby resulting in the host being more resistant to the development of the nematode within its roots (Laan, 1956).

The application of ethoprop gave the lowest number of galls and egg masses and nematode population in the roots and soil compared with other treatments. Percent reduction of egg masses was 93.2. Ethoprop has been

found effective in reducing the population of parasitic nematodes including *M. incognita* in cabbage infested field. This resulted in highly significant yield increase of cabbage ranging from 25-26% (Davide and Zorilla, 1980).

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