SUPPRESSION OF ROOT-KNOT NEMATODES IN TOMATO AND CUCUMBER USING BIOLOGICAL CONTROL AGENTS

Kalele, D.N.[1], Affokpon, A.[2], Coosemans, J.[2] and Kimenju, J.W.[1]
[1]University of Nairobi, P. O. Box 30197, Nairobi
[2]Division of Crop Biotechnics, Katholieke Universiteit Leuven, De Croylaan 42, 3001 Heverlee, Belgium
Email: wkimenju@yahoo.com

ABSTRACT

Antagonistic fungi are continuously attracting a lot of attention as alternatives to chemical control of root-knot nematodes. The egg-pathogenic fungus *Paecilomyces lilacinus*, particularly strain 251 has shown promising potential as a biological control agent against various plant-parasitic nematodes. Strain 251 of *P. lilacinus* (PL251) and *Arthrobotrys conoides* (Melodogone) were tested for their efficacy against *Meloidogyne* spp. in tomato and cucumber under greenhouse conditions. The study aimed at determining the application rates and timing of application of the fungi. Both pre-planting and at planting application of PL251 were found to reduce nematode populations and root galling in both tomato and cucumber. Pre-planting soil treatment (0.4g/10 L of soil) reduced final nematode populations by 69% and 73% in the roots and soil, respectively, compared to the non-inoculated control in tomato. However, soil treatment at planting recorded reduction level of 54% and 74% in the roots and soil, respectively. Use of *A. conoides* showed lower nematode control in cucumber. Only 28% and 21% reduction levels were recorded in the roots and soil when the fungus was applied at planting, respectively. This study has demonstrated that PL251 has a promising potential that could be exploited in the management of *Meloidogyne* spp. in vegetable production systems.

Key words: *Arthrobotrys conoides*, Cucumber, *Lycopersicum esculentum*, Nematode biocontrol agents, *Paecilomyces lilacinus*

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are a major constraint to successful vegetable production all over the world, causing severe damage that leads to dramatic yield losses (Sikora and Fernandez, 2005). Control of root knot nematodes has been primarily accomplished through chemical nematicides (Widmer and Abawi, 2000). However, due to the significant drawbacks of the chemical control including threats to human health and the environment, biological control has become one of the promising alternatives (Stirling, 1991). The egg-pathogenic fungus *Paecilomyces lilacinus* is the most widely tested biological control agent used for management of plant-parasitic nematodes (Atkins et al., 2005). This study was undertaken to determine the appropriate application rate and time of biological agents for the control of root knot nematodes in tomato and cucumber.

Arthrobotrys species are trapping fungi which immobilize nematodes using non-adhesive knobs and constricting rings. They ensnare active nematodes using one or more types of mycelial traps (Viaene et al., 2006). Some *Arthrobotrys* species have been formulated and applied, but they have given mixed results (Viaene et al., 2006). *Arthrobotrys conoides* has a remarkable morphological adaptation and is not an obligate predator. It grows on complex media and can be isolated easily (Duddington, 1955; Tarjan, 1960).

A dose-response relationship between the concentration of antagonist applied and the reduction of plant damage and nematode population needs to be established to harness maximum potential of biological control agents. Dose-response models may help in understanding factors affecting biocontrol agents and also in predicting biocontrol efficiency under varying conditions (Larkin and Fravel, 1999). The objective of the present study was to determine the efficacy of *P. lilacinus* strain 251 and *A. conoides* against root knot nematodes in tomato and cucumber and to establish the appropriate application dosage and time.

MATERIAL AND METHODS

Experimental Design

The study was conducted in a greenhouse set at temperature of 23^{o}C, with *Paecilomyces lilacinus* strain 251 (PL251) and *Arthrobotrys conoides* (Melodogone) test products. The PL251 Bio Act WP was obtained from Biobest, Belgium and the *A. conoides* was obtained from De Ceuster nv, Belgium. Two experiments, laid out in a completely randomised design (CRD), with five replicates were carried out. Pots were filled with soil naturally infested with *Meloidogyne* species. The soil was obtained from an experimental field at St. Katelijne-Waver (Belgium).

The test crops were tomato (*Solanum lycopersicum* L. syn *Lycopersicum esculetum* Mill) var. Admiro F1 and cucumber, which are highly susceptible to *Meloidogyne* spp. Each experiment consisted of soil treated with variable doses of the fungi. Nematode infested soil without fungal inoculum was included as a control. Each experiment had two sets of similar treatments in which the first set the fungi were applied at planting and in the second set the fungi were applied into the soil two weeks before planting (pre-planting).

The first experiment studied the effect of normal doses of PL251 against *Meloidogyne* species in tomato. The fungus was tested at three rates (0.1, 0.2 and 0.4 g/10 L of soil) based on the most common doses as recommended by manufacturers and a control. The second experiment compared the effect of PL251 and *A. conoides* against *Meloidogyne* spp. in cucumber. Fungal inoculum density was as described in the first experiment.

Estimation of Nematode Population and Damage

The initial population (Pi) of second stage *Meloidogyne* juveniles (J_2) in the soil was determined at the start of each experiment. From the homogeneous bulk soil sample used in each experiment, 500 g was taken and J_2 were extracted from 100 cm^3. Final nematode population density at harvest (Pf) from both soil and roots was evaluated. A bulk soil and root samples, approximately 500 g and 20 g, respectively, were taken from each treatment.

For the soil samples, the same procedure as for the determination of Pi was followed. For the roots, the bulk sample was gently cleaned to remove all soil, chopped into segments of about 1 cm and 10 g taken for use in extracting nematodes. Nematodes were extracted from soil and roots using the modified Baermann technique (Hooper, 2005). Nematode multiplication rate for each crop was calculated using the relationship Pf/Pi based on soil counts of J_2 populations (Verdejo et al., 2003).

Total number of galls was estimated for both crops. In tomato, estimation was done by tracing a 3cm x 3cm (area of 9 cm^2) squared paper at four points on the root surface and counting the number of galls within that area. The total number of galls was expressed as per the total surface area of the root system. In the cucumber experiment three plants from each treatment were randomly selected, their roots mixed thoroughly and the sample weight recorded. A 2 g sample was taken, number of galls counted and the total number of galls per root system expressed as the number of galls per the total weight of the root system.

All the data collected were tested for homogeneity of variance and subjected to one-way analysis of variance, after log (x+1) transformation. If the overall F test was significant, means were separated using the Tukey Honest Significance test at $P = 0.05$.

RESULTS

Suppression of *Meloidogyne* spp. on Tomato by PL251

Results showed that different application rates of PL251 had a significant effect on parameters describing plant damage by *Meloidogyne* spp. PL251 was effective in significantly reducing J_2 populations in both the soil and root s when fungal inoculation was done at planting (Table1). The highest reduction level was recorded from application dose of 0.4 g/ 10 L of soil with about 54% and 74% reduction level in roots and soil, respectively, as compared to the non-inoculated control. However, no significant differences (P>0.05) were recorded between 0.2 g and 0.4 g concentrations with respect to J_2 populations in the root, as well as J_2 populations in the soil for all doses.

The fungal strain also reduced root galling and Meloidogyne spp. multiplication rate. Although there was no significant difference (P>0.05) between the application doses for the two variables, the number of galls per root system and multiplication rate were significantly lower (P<0.05) in the fungi-inoculated pots than in the non-inoculated pots.

Table 1. Effect of varying pre- and at–planting PL251 application rates on *Meloidogyne* spp. populations, multiplication rate (Pf/Pi) and root galling in tomato

	Rate	J_2/ g root	J_2/ 100 cm^3 soil	Galls/root system	Pf/Pi
At planting	Control	316.3 a	299.3 a	1919.0 a	1.33 a
	0.1 g	196.0 b	125.7 b	1239.7 ab	0.55 b
	0.2 g	153.7 bc	94.3 b	1115.7 b	0.41 b
	0.4 g	144.7 c	76.7 b	993.3 b	0.34 b
Pre-planting	Control	324.0 a	310.3 a	1755.3 a	1.37a
	0.1 g	127.0 b	99.3 b	1020.5 b	0.44 b
	0.2 g	95.3 b	94.7 b	1147.5 ab	0.42 b
	0.4 g	99.7 b	83.0 b	943.4 b	0.37b

Each mean is an average of five replicates. Within a column means compare effect of application rate on nematode population, damage and multiplication rate. For a given application time, means within a column followed by different letters are significantly different at P=0.05.

The effect of PL251 on *Meloidogyne* spp. soil populations, and nematode multiplication rate when fungal inoculation was done at pre-planting were similar to the results obtained when the fungi was inoculated at planting (Table 1). While for J_2 populations in the root and root galling different results were obtained. There were no significant differences (P≥0.05) in J_2 populations in the root for the various PL251 doses. For root galling, 0.2 g dose did not differ significantly from the non-treated control as well as from the other doses, although 0.1 g and 0.4 g doses differed significantly (P≤0.05) from the non-treated control.

Effect of PL251 and *A. conoides* on *Meloidogyne* spp. when Applied at Planting in Cucumber

In the second biocontrol experiment, PL251 was more effective in reducing *Meloidogyne* J_2 population in cucumber roots than A. *conoides*. Application of PL251 at dosages higher than 0.1 g reduced J_2 numbers significantly compared to similar dosages of *A. conoides* (Table 1). Only 28% (at 0.4 g/10 L of soil) and 21% (at 0.2 g/10 L of soil) reduction level was recorded in J_2 populations in roots and soil, respectively, when *A. conoides* was applied in comparison to 72% and 59% with PL251. Nematode populations in the roots remained very high, although 0.4 g dose differed significantly from the non-inoculated control.

J_2 numbers in soil treated with *A. conoides* and PL251 were significantly lower ($P<0.05$) than in the non-treated soil. The different doses of PL251 showed significant reduction in J_2 populations both in the roots as well as in the soil. There were no significant differences ($P>0.05$) in root galling between the PL251 treated and the non-treated soil.

Table 2. Effect of *Arthrobotrys conoides* and *Paecilomyces lilacinus* strain 251 on *Meloidogyne* spp. populations, multiplication rate and root galling in cucumber treated at planting

Bioagent	Treatments	J_2/g root	J_2/100 cm^3	Galls/root
A. conoides	Control	984.3 a	407.7 a	640.3 a
	0.1 g	948.0 a	357.0 b	737.0 a
	0.2 g	813.0 ab	320.6 b	645.3 a
	0.4 g	703.3 b	323.0 b	550.3 a
P. lilacinus	Control	984.3 a	407.7 a	640.3 a
	0.1 g	336.7 bc	209.0 b	639.0 a
	0.2 g	418.3 b	165.7 b	612.0 a
	0.4 g	269.6 c	189.3 b	515.7 a

Each mean is an average of five replicates. Within a column means compare the effect of application doses for each fungus on nematode populations, damage parameter and multiplication rate. Means with different case(s) are significantly different ($P\leq0.05$)

Biocontrol Efficacy of *A. conoides* and PL251 on *Meloidogyne* spp. in Cucumber Treated before Planting

Results obtained when both fungi were applied into the soil before planting of cucumber are shown in Figures 1 and 2. *A. conoides* showed no significant differences ($P>0.05$) in the reduction of J_2 populations in soil as well as in root galling when fungal application was done before planting. J_2 populations in cucumber roots were significantly lower in plants that were treated with 0.2 g and 0.4 g of *A. conoides*. On the other hand, PL251 showed significant effect in the reduction of soil and root J_2 populations as well as reducing root galling when compared with the non-inoculated control. All PL251 doses differed significantly ($P \leq 0.05$) from the control in reducing J_2 populations in cucumber roots. However for J_2 populations in the soil only the highest dose (0.4 g/10 L of soil) differed significantly from the non-inoculated control. J_2 population reduction level of 75% and 51% in roots and soil, respectivel, was recorded with the use PL251.

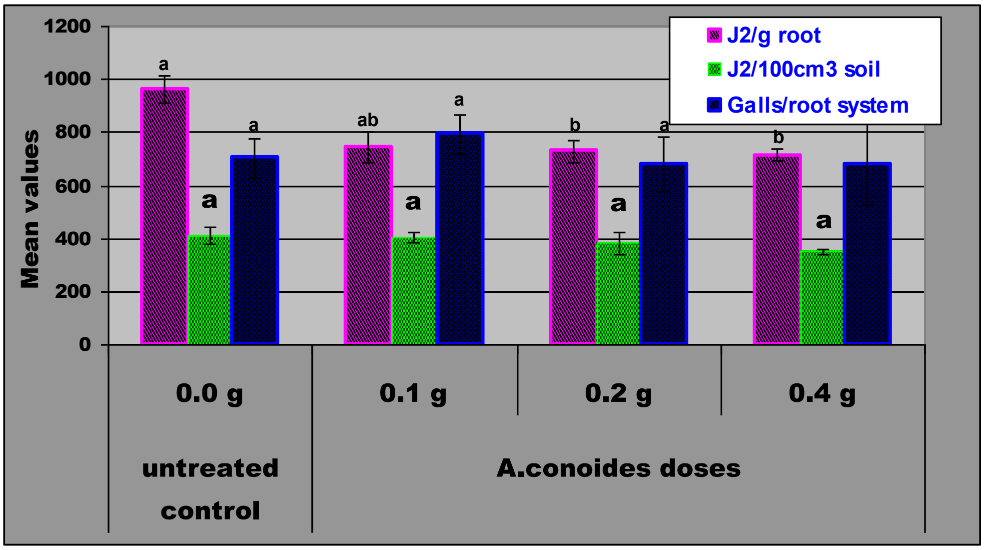

Figure 1. Effects of pre-planting application of *Arthrobotrys conoides* on *Meloidogyne* species populations and root galling in cucumber. Bars headed by different letters are significantly different. Error bars represent standard error. Doses are expressed as per 10 L of soil. Mean value is the number of nematodes per 100 cm^3 soil and root galls per root system.

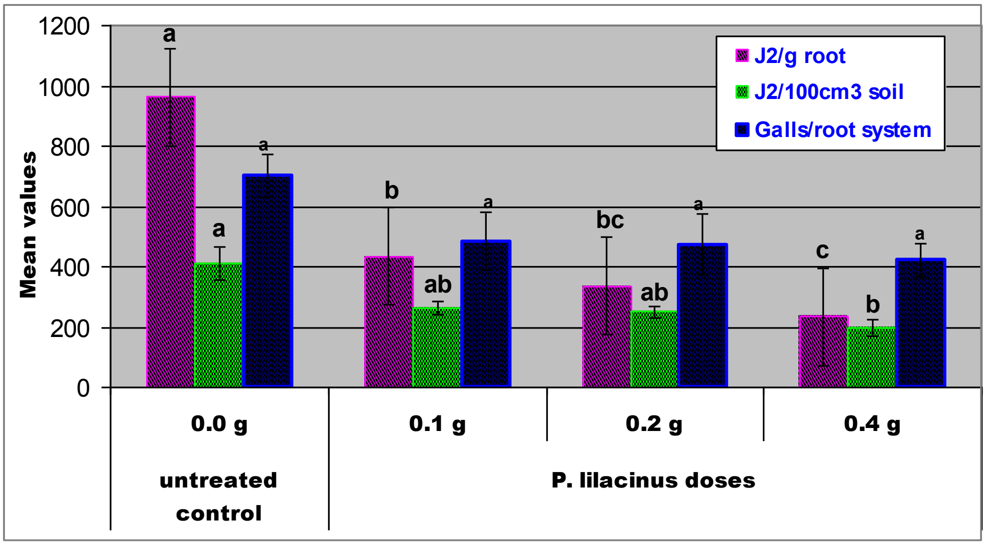

Figure 2. Effects of pre-planting application of *Paecilomyces lilacinus* on *Meloidogyne* species populations and root galling in cucumber. Bars headed by different letters are significantly different. Error bars represent standard error. Doses are expressed as per 10 L of soil. Mean value is the number of nematodes per 100 cm^3 soil and root galls per root system.

DISCUSSION

The results obtained in this study showed PL251 to have some suppressive effect as a nematode biocontrol agent. This was explained by the reduced *Meloidogyne* spp. population densities both in the soil and roots in both tomato and cucumber. In addition, reduction of nematode damage parameter i.e. root galling intensity as well as nematode reproduction rate (Pf/Pi) was attained when the application was done at planting as well as at pre-planting. *P. lilacinus* strain 251 showed promising results as a biological control agent for root knot nematodes. After 10 weeks, there was reduction of 54% to 74% of J_2 in both soil and tomato roots, respectively. The findings confirmed the results of Lara et al. (1996), who reported that *P. lilacinus* significantly reduced soil and root population of *M. incognita* and increased yield of tomato. In growth chamber experiments using tomato, Kiewnick and Sikora (2005) found that PL251 reduced between 58% and 74% the galling and population of *M. incognita*.

The pre-planting application of this biocontrol agent resulted in a higher level of reduction of J_2 population in roots. This confirmed previous finding on a Peruvian isolate of *P. lilacinus* where the greatest level of biocontrol was achieved with soil treatments at 10 days (Cabanillas and Barker, 1989) or 3 month before transplanting tomatoes (Carneiro and Cayrol, 1991).

The use of *A. conoides* (Melodogone product) as a biocontrol agent against *Meloidogyne* spp. in cucumber did not produce better results as compared to PL251. This is in contrast to results obtained by Al-Hazmi et al. (1982), who found that addition of *A. conoides* to *M. incognita* infested soil suppressed juvenile numbers and root galls development. Lack of significant nematode biocontrol by *A. conoides* could be explained by the poor germination and growth of fungal colonies as observed in the in vitro fungal viability test which showed some fungal growth initially and a decrease in fungal viability after five weeks. This could suggest that the fungi did not posses the ability to establish and colonise well in the soil and rhizosphere during the entire experimental duration. The fact that it could not be re-isolated from the soil and rhizosphere may imply that the fungus did not survive in the soil in quantities enough to enhance nematode control. This also confirms findings by Viaene et al. (2006) that showed lack of consistent results for some *Arthrobotrys* spp., formulated and applied under specific conditions.

CONCLUSION

PL251 has proved to be a reliable tool for use in suppression of root knot nematode populations in both tomato and cucumber. An application dose of 0.4 g per 10 L of soil was found to be appropriate. *A. conoides* was less effective compared to *P. lilacinus* in nematode control. More work is required on product development and improvement of the *A. conoides* in order to improve its biocontrol efficiency and thus provide farmers with a better and reliable product towards nematode management.

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