RESEARCH NOTE/NOTA DE INVESTIGACIÓN

PURPUREOCILLIUM LILACINUM (LILACEL®) IN THE TREATMENT OF BANANA PLANTLETS INFECTED BY PLANT-PARASITIC NEMATODES

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ABSTRACT

Paz Filho, E. R., M. F. S. Muniz, A.V. D. L. Almeida, N. H. M. Soares, G. Moura Filho, and F. S. Rocha. 2019. *Purpureocillium lilacinum* (Lilacel®) in the treatment of banana plantlets infected by plant-parasitic nematodes. Nematropica 49:134-139.

Among pests and diseases affecting banana crops, which ultimately lead to yield losses, the presence of plant-parasitic nematodes, such as *Radopholus similis*, *Helicotylenchus multicinctus*, *Pratylenchus coffeae,* and *Meloidogyne* spp., are to be considered. The distribution of these pathogens in the field occurs mainly via propagative material. The objective of the present study was to evaluate banana suckers cv. Comprida, naturally infected by a mixed population of *R. similis* (3.21%), *Helicotylenchus* sp. (7.91%), *Meloidogyne* sp. (1.37%), and *Pratylenchus* sp. (87.51%), under greenhouse conditions, treated with *Purpureocillium lilacinum* (Lilacel® $1x10^8$ CFU/ml). Five doses of the bionematicide were tested (0.2, 0.4, 0.6, 0.8, 1.0%), plus controls of Carbofuran (Furadan® 350 SC at 4 ml/L water) and water. Banana suckers were dipped in the treatment solutions for 60 min and planted in pots filled with sterilized soil. The experiment was performed in a completely randomized design with eight replicates. The percentage of emergence and the nematode population in the soil, rhizome, and roots, as well as the reproduction factor, were evaluated after 4 months. The regression analyses between the variables were represented by linear, quadratic, and square root models. Final nematode populations were reduced in all treatments when compared to the negative control (water).

Key words: Biological nematicide, *Musa* spp., nematophagous fungus, *Pratylenchus* spp.

RESUMO

Paz Filho, E. R., M. F. S. Muniz, A.V. D. L. Almeida, N. H. M. Soares, G. Moura Filho, e F. S. Rocha. 2019. *Purpureocillium lilacinum* (Lilacel®) no tratamento de mudas de bananeira infectadas por fitonematoides. Nematropica 49:134-139.

Entre os problemas fitossanitários que afetam a cultura da bananeira resultando em perda de produção, destaca-se a presença de nematoides, tais como, *Radopholus similis*, *Helicotylenchus multicinctus*, *Pratylenchus coffeae* e *Meloidogyne* spp. A dispersão desses patógenos se processa principalmente por meio do material propagativo. O objetivo do presente trabalho foi avaliar o efeito da aplicação de

Purpureocillium lilacinum (Lilacel® 1x10^8 UFC/ml) no tratamento de mudas de bananeira cv. Comprida naturalmente infectadas por uma população mista constituída por *R. similis* (3,21%), *Helicotylenchus* sp. (7,91%), *Meloidogyne* sp. (1,37%) e *Pratylenchus* sp. (87,51%), em condição de casa de vegetação. Foram testadas cinco dosagens do bionematicida (0,2; 0,4; 0,6; 0,8; 1,0%), além das testemunhas (Carbofurano e água). As mudas foram imersas em cada um dos tratamentos por 60 minutos e plantadas em vasos contendo solo esterilizado. O delineamento experimental foi inteiramente casualizado com oito repetições. Decorridos quatro meses de cultivo realizaram-se as avaliações das populações dos nematoides no solo, nas raízes e rizomas e o fator de reprodução. As análises de regressão entre as variáveis foram representadas pelos modelos linear, quadrático ou raiz-quadrático. Em todos os tratamentos com o bionematicida houve redução das populações finais de nematoides quando comparadas à testemunha negativa (água).

Palavras chave: Fungo nematófago, *Musa* spp., nematicida biológico, *Pratylenchus* spp.

Plant-parasitic nematodes are amongst the constraints to banana (*Musa* spp.) production in Brazil. Many nematode species are associated with the crop, however, only *Radopholus similis* (Cobb) Thorne, *Helicotylenchus multicinctus* (Cobb) Golden, *Pratylenchus coffeae* (Zimmermann) Filipjev and Schuurmans Stekhoven, and *Meloidogyne* spp. are considered economically important pests (Cordeiro *et al*., 2016). These plant-parasitic nematodes are involved in the destruction of the primary roots, disrupting the anchorage system and resulting in toppling of the plants (Sikora *et al*., 2018). According to Ritzinger *et al*. (2007), some species may cause crop yield losses up to 100%, depending on the environmental conditions, level of nematode inoculum, and the banana cultivar. On plantain (*Musa* spp.), in a field naturally infested with *R. similis*, yield reduction of about 50% was reported over the first two crop cycles (Fogain, 2000). Furthermore, in a field trial with plantain involving the plant-parasitic nematodes *R. similis*, *P. coffeae*, *H. multicinctus,* or *Meloidogyne* spp., the mean bunch weight of harvested plants was lower for all nematode treatments than controls. Reductions were between 33.3% and 50.8%, even for first cycle mother plants, and lower across crop cycles (Coyne *et al*., 2013).

In Brazil, the methods recommended for the management of plant-parasitic nematodes on banana are based on the use of micro-propagated clean plants, crop rotation, and chemical nematicides (Salomão and Siqueira, 2015; Cordeiro *et al*., 2016). Alternative management approaches, for instance the use of biological control agents such as the fungus *Purpureocillium lilacinum* (Thom) Samson (formerly *Paecilomyces lilacinus*) applied to the soil, have been reported (Kiewnick *et al*., 2004; Mendoza and Sikora, 2009; Silva *et al*., 2017). In addition, *P. lilacinum* has been used in crops such as soybean [*Glycine max* (L.) Merr.] for seed treatment against plant-parasitic nematodes (Nunes *et al*., 2010; Almeida *et al*., 2016). However, information about the application of this fungus to treat banana planting material has not been recorded. The opportunity for preventing the spread of plant-parasitic nematodes by investing in better quality planting material should be considered by farmers. In view of this, the objective of the present study was to evaluate doses of the biological product Lilacel® containing *P. lilacinum* in the treatment of banana suckers naturally infected with plant-parasitic nematodes.

The experiment was performed at the Center for Agricultural Sciences, Federal University of Alagoas, Rio Largo, state of Alagoas, Brazil, from July to November 2017, with an average monthly temperature ranging from 20 to 31ºC. Planting material consisted of suckers of banana cv. Comprida (cooking banana) obtained from a field with a history of infection by nematodes. Before the application of the treatments, the initial population (Pi) of nematodes was estimated in each individual sucker by extraction of nematodes from 10 g of rhizome tissues using a maceration and centrifugal-flotation method (Coolen and D'Herde, 1972).

The experiment was conducted in a completely randomized design, with seven treatments and eight replicates, where each experimental unit was considered one plant per pot. The treatments consisted of five doses of the biological product Lilacel® (1x10^8 colony forming units – CFU/ml) at 0.2%, 0.4%, 0.6%, 0.8%, and 1.0% plus water (negative control) and the nematicide Carbofuran (Furadan® 350 SC at 4 ml/L water) as a positive control. After an immersion period of 60 min, the plantlets were transferred to 8-L plastic pots containing sterilized soil and maintained under greenhouse conditions.

After 4 months, the percentage of emergence of suckers and the nematode population in the soil, rhizome, and roots were evaluated. The nematodes were extracted from 100 cm^3 of soil and 10 g of plant tissue (roots and rhizome) according to Jenkins (1964) and Coolen and D'Herde (1972), respectively. After extraction, nematodes were fixed in a hot 4% formaldehyde solution. The identification and quantification of the nematodes were based on counting 1 ml suspensions in a Peters' slide using an inverted light microscope according to Mai and Mullin (1996) and Mekete *et al*. (2012). The nematode reproduction factor (Rf) (Rf = final population (root+rhizome+soil)/initial population from the rhizome) for each sample was calculated according to Oostenbrink (1966).

The Lilliefors test of homogeneity of variances and normality of the data was applied and only log (x+1) achieved normally distributed data. After transformation, the results were subjected to analysis of variance (F-test) and the averages were grouped by Scott-Knott test at 5% significance. In addition, regression analyses between nematode population densities in soil, roots, and rhizome, total nematode populations, and Rf versus doses of Lilacel® were conducted with log (x+1) transformed data. The statistical models were chosen based on the significance of the regression coefficient by F-test, considering the values of t-test and the mean square error of regression and also the analysis of variance of the data set. When more than one model was significant, the model with the highest determination coefficient (R^2 adjusted) was used. The statistical analyses were performed using the softwares SAEG 5.0 and Fcalc 1.2 (Moura Filho and Cruz, 2000).

The Pi was a mixed nematode population consisting of *R. similis* (3.21%), *Helicotylenchus* sp. (7.91%), *Meloidogyne* sp. (1.37%), and *Pratylenchus* sp. (87.51%). There was no significant difference ($P \leq 0.05$) by the Scott-Knott test to Pi, showing a uniform infection level of plant-parasitic nematodes among treatments (Table 1). The percentage of sucker emergence in all treatments was 100%.

Nematode population densities and Rf were reduced by the doses of Lilacel® and also by the use of the nematicide compared to the untreated control (Table 1, Fig. 1). A similar effect was reported by Mendoza and Sikora (2009) who demonstrated that

Table 1. Initial nematode population density (Pi) in 10 g banana rhizome; nematode population densities in 100 cm^3 soil (NPS), 10 g roots (NPR) or rhizome (NPRH), total number of nematodes (TNN), and reproduction factor (Rf) assessed 4 months after the application of Lilacel®, a commercial formulation of *Purpureocillium lilacinum*, at different rates on banana plantlets. The nematode population was a mixture of *Pratylenchus* sp., *Helicotylenchus* sp., *Radopholus similis*, and *Meloidogyne* sp. Water and Carbofuran (Furadan® 350 SC at 4 ml/L water) served as controls.

Treatments	Pi	NPS	NPR	NPRH	TNN	Rf
Water	91 a	815 a	1253 a	635 a	2703 a	38.82 a
0.2 %	70 a	458 b	386 b	353 b	1196 b	17.5 b
0.4 %	75 a	338 c	353 b	358 b	1048 b	15.2 b
0.6 %	63 a	310 c	418 b	213 c	940 c	17.3 b
0.8 %	64 a	308 c	295 b	243 c	845 c	13.8 b
1.0 %	59 a	253 c	190 c	250 c	693 d	14.0 b
Carbofuran	69 a	258 c	173 c	124 d	554 d	8.4 b
MSR	0.0298^{ns}	0.0188^{**}	0.0299^{**}	0.0243^{**}	0.0075^{**}	0.0388^{**}
DF	49	49	49	49	49	49
Mean	70	391	438	311	1140	18
CV (%)	9.5	5.4	6.9	6.5	2.9	16.4

Analysis of variance with the data converted into log (x+1). Means followed by the same letter within a column do not significantly differ by the Scott-Knott test ($P<0.05$). MSR: Mean-square residue. DF: Degrees of Freedom. ^{ns}Not significant at 5% probability by F-Test. **Significant at 1% probability by F-Test. CV: coefficient of variation.

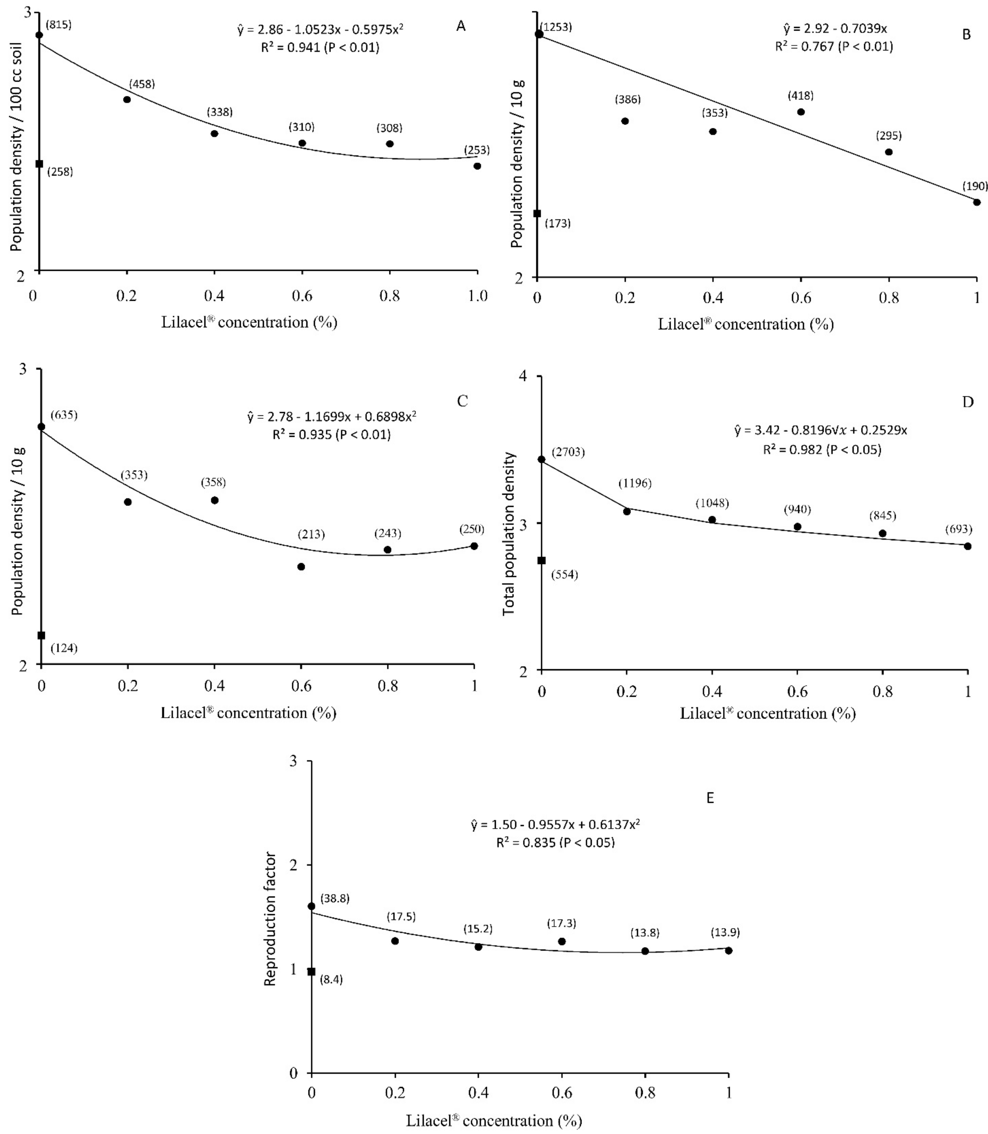

Figure 1. Nematode population densities and reproduction factor in log (x+1) scale in banana plantlets cv. Comprida in response to different concentrations of *Purpureocillium lilacinum* (Lilacel®) and nematicide Carbofuran®. A) Soil, B) Root, C) Rhizome, D) Total nematode population, E) Reproduction factor. Numbers in parentheses represent the average of the treatments with untransformed data.

P. lilacinum was an effective biocontrol agent against *R. similis* in banana when applied into the soil. Another commercial formulation Nemout®, based on *P. lilacinum,* was also effective against *Pratylenchus* spp. on sugarcane (Oliveira *et al.*, 2011).

Purpureocillium lilacinum is known for its parasitic behavior on exposed eggs and females of sedentary nematodes (Ferraz *et al.*, 2010). The *P. lilacinum* protease and chitinase enzymes, either individually or in combination, reduced hatch of *M. javanica* (Khan *et al.*, 2004). Moreover, it was also observed that occasionally, this fungus penetrated the mobile stages (juveniles and adults) of the migratory nematode *R. similis* (Khan *et al.*, 2006).

Despite the positive findings obtained in the present study, which provides another tool for the treatment of planting material infected with plant-parasitic nematodes, field experiments are needed in order to determine the efficiency of the commercial formulation Lilacel® in banana production and to confirm greenhouse results.

ACKNOWLEDGMENTS

This study was financed in part by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Finance Code 001. The authors thank Empresa Caxiense de Controle Biológico Ltda (ECCB) for providing the biological product.

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Received: *12/11/2019*

Accepted for publication: *31/V/2019*

Recibido:

Aceptado para publicación: