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BIOFERTILIZER IN THE CONTROL OF THE NEMATODE *Pratylenchus brachyurus* IN SUGARCANE CROP

Grasiele Teixeira da Silva[1*], Anderli Divina Ferreira Rios[2], Rafael Matias da Silva[3], Wilian Henrique Diniz Buso[2], Denise Rodrigues Conceição[1], Rafaela Santos de Oliveira[1]

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ABSTRACT - Biofertilizers can also be used as plague and disease control agents. The commercial product Microgeo® is a biological biofertilizer rich in microorganisms. Few studies have been carried out to evaluate the interaction between biofertilizer and nematodes. The objective of this study was to evaluate the efficiency of the Microgeo® biological fertilizer to control the nematode *Pratylenchus brachyurus* in two sugarcane cultivars. The work was carried out in greenhouse conditions with a completely randomized experimental design (DIC), in a triple factorial scheme (2 x 2 x 4), being two types of sugar cane (CTC 04 and CTC 9003), two types of management of the biofertilizer (Management 1: application of the biofertilizer and inoculation of the nematode at 0 days after transplanting (DAT), management 2: application of the biofertilizer and inoculation of the nematode in the roots of the plant 30 days later) and four doses of the liquid biofertilizer Microgeo® (0 L ha^{-1}; 150 L ha^{-1}; 300 L ha^{-1}; 450 L ha^{-1}), with four replications. 800 specimens of *P. brachyurus* were inoculated in each experimental plot. The results showed that cultivating CTC 9003 during the conduct of the experiment showed greater susceptibility to the nematode compared to cultivating CTC 04. The biofertilizer Microgeo® showed no curative or preventive effect on the control of the nematode under the conditions of the experiment.
Keywords: *Saccharum officinarum*, biological control, reproduction factor, root lesion nematode.

BIOFERTILIZANTE NO CONTROLE DO NEMATOIDE Pratylenchus brachyurus NA CULTURA DA CANA-DE-AÇÚCAR

RESUMO - Os biofertilizantes também podem ser usados como agentes de controle de pragas e doenças. O produto comercial Microgeo® é um biofertilizante biológico rico em microorganismos. Poucos estudos foram realizados para avaliar a interação entre biofertilizante e nematoides. Objetivou-se avaliar a eficiência do adubo biológico Microgeo® no controle do nematoide *Pratylenchus brachyurus* em duas cultivares de cana-de-açúcar. O trabalho foi conduzido em condições de casa de vegetação com delineamento experimental inteiramente casualizado (DIC), em esquema fatorial triplo (2 x 2 x 4), sendo duas cultivares de cana-de-açúcar (CTC 04 e CTC 9003), dois tipos de manejo do biofertilizante (Manejo 1: aplicação do biofertilizante e inoculação do nematoide aos 0 dias após o transplantio (DAT), manejo 2: aplicação do biofertilizante e inoculação do nematoide nas raízes da planta 30 dias depois) e quatro doses do biofertilizante líquido Microgeo® (0 L ha^{-1}; 150 L ha^{-1}; 300 L ha^{-1}; 450 L ha^{-1}),com quatro repetições. Foram inoculados 800 espécimes de *P. brachyurus* em cada parcela experimental. Os resultados demostraram que a cultivar CTC 9003 durante a condução do experimento apresentou maior suscetibilidade ao nematoide comparado a cultivar CTC 04. O biofertilizante Microgeo® não mostrou efeito curativo nem preventivo no controle do nematoide nas condições do experimento.
Palavras-chave: *Saccharum officinarum*, controle biológico, fator de reprodução, nematoide das lesões radiculares.

INTRODUCTION

Brazil is the world's largest producer of sugarcane (*Saccharum* sp.). The production estimate for the 2019/2020 harvest will be 615.98 million tons. The Midwest region is the second largest producer of sugarcane. The state of Goiás stands out as the second largest sugarcane producer in the country. The estimated planted area is approximately 949.2 thousand hectares for 2019/2020 crop, showing an increase of 3.5% over the 2018/2019 crop and for the state of Goiás is estimated a productivity of 76,926 kg ha^{-1} (CONAB, 2019).

Among the main factors that limit the high yields of this cultivation are the phytosanitary problems. Depending on the place and time of planting, the incidence of plagues and diseases can reach levels of economic damage. Among the phytosanitary problems of sugarcane crops are nematodes. More than 300 nematode species have been detected in sugarcane roots and in its rhizosphere (CADET; SPAULL, 2005). The most important species commonly found causing damage are *Meloidogyne javanica*, *M. incognita* and *Pratylenchus zeae* (FREITAS et al., 2004), but *P. brachyurus* species is also found in samples from sugarcane fields in several

[1]Faculdade Evangélica de Goianésia, Goianésia, Goiás, Brasil. E-mail: grasigrasisilva@hotmail.com *Corresponding Author.
[2]Universidade Federal de Goiás (UFG), *Campus* Samambaia, Goiânia, Goiás, Brasil.
[3]Instituto Federal Goiano – *Campus* Ceres, Ceres, Goiás, Brasil.

locations and may be more aggressive than *P. zeae* (BARBOSA et al., 2013).

The nematode from root lesions, *Pratylenchus brachyurus* is polyphagous and is mainly associated with grasses such as sugarcane, corn, sorghum and brachiaria (INOMOTO, 2011; BARBOSA et al., 2013; NEVES, 2013). Economic damage caused by nematodes of the *Pratylenchus genus* has already been reported in studies with wheat, rice, corn, beans, soybean, sorghum and sugarcane (INOMOTO, 2011; RACK et al., 2013).

Among the techniques recommended to control this nematode in sugarcane cultivations, the most used has been the chemical control through the use of nematicides (DINARDO MIRANDA et al., 2003). However, chemical control has its limitations and its indiscriminate use can reduce the production, put at risks the health of the applicators and consumers, and also select resistant microorganisms and destroy natural enemies naturally present in the soil (RITZINGER and FANCELLI, 2006). Rotation or succession with non-host cultivations, cleaning of machines and agricultural implements, use of tolerant plants and biological control are also important control methods for nematodes in general (LORDELLO, 1984). However, for *P. brachyurus* there is a wide range of hosts, as it is a polyphagous nematode and thus the use of rotation is difficult. The use of genetic resistance for *P. brachyurus* is still incipient, so biological control can be a good alternative for the control of this nematode.

Researches with the nematode biological control method is in most cases focused on the nematode of the branches (*Meloidogyne* spp.), therefore, studies on the biological control of nematodes of the genus *Pratylenchus* spp. in sugarcane culture are important. Several organisms are considered natural enemies of nematodes, for example: predatory nematodes, viruses, arthropods, mites, fungi and bacteria (LORDELLO, 1984). It is known that biofertilizers can interfere with the development of pests by controlling them or making the environment balanced. These products can work as resistance inducers, making the plants more resistant or tolerant. The biofertilizers have several microorganisms, mainly species of bacteria that interact in the soil and is easily adopted by farmers (NUNES et al., 2010).

The increased occurrence of *P. brachyurus* is a concerning cause in producing areas, due to the lack of information on the relationship between this nematode and sugarcane culture, which demonstrates the importance of obtaining more information in order to substantiate the control recommendations. Thus, studies are important to identify new control alternatives through the use of products that agriculturists are used to, such as biofertilizers. In view of the above, this study aimed to evaluate the action of Microgeo® bioferlilizer in the development of *Pratylenchus brachyurus* in sugarcane culture in greenhouse conditions.

MATERIALS AND METHODS

The experiment was carried out in a greenhouse located at Fazenda Escola da Faculdade Evangélica de Goianésia (FACEG), Experimental *Campus* of the Agricultural College in Goianésia (GO), under geographical coordinates: 15°19’22” S and 49°08’20” W. O of the site is classified as tropical savannah, hot and humid, with dry winter and rainy summer (*Aw*) (according to Köppen-Geiger classification).

The experimental delineation was entirely randomized (DIC), in triple factor (2 x 2 x 4), being two sugarcane cultivars (CTC 04 and CTC 9003), two biofertilizer management (management 1: application of the biofertilizer and nematode inoculation on the same day and management 2: application of biofertilizer and inoculation of nematode in the roots of the plant 30 days later), four doses of liquid biofertilizer Microgeo® (0, 150, 300 and 450 L ha^{-1}) and four repetitions.

First, sugarcane seedlings were produced by the pre-sprouting (pre-sprouted seedlings), being acquired by the company CTC (Centro de Tecnologia Canavieira), Pólo Base de Goiás, Goianésia (GO). The yolks were arranged in 300 mL disposable cups, filled with commercial compound substrate, remaining on a wooden bench located inside the greenhouse used in the experiment. After 60 days the seedlings were transplanted into definitive recipients (polyethylene bag containing 2 L of substrate each), filled with a mixture of soil (Red Latosol) + sand (Fine and Sifted), in the proportion of 2:1, with a sugar cane seedling for each container. Previously the use of the substrate mixture was sterilized in an autoclave, at 121ºC, for 50 min., following the methodology described by Tiburtino et al. (2015).

The application with the biofertilizer was performed throughout the experiment on the same day and directed to the soil around the plants at ten days after transplanting the seedlings. The population of *P. brachyurus* was obtained from naturally infested soil in the municipality of Campinorte (GO) and multiplied in corn plants (hybrid AG 1051), sown in greenhouse. The inoculum was obtained from the corn plants, submitted to nematode extraction at 70 days after sowing. The nematode was inoculated in two seasons, depositing 6 mL of the suspension containing 800 specimens of *P. brachyurus* per plot, in orifices of 2 cm depth and close to the seedlings stem. The counts of the individuals were performed by multiple sampling until the exact quantification of the number of specimens.

Daily, from the date of transplanting the seedlings, watering was done according to the necessity of supplying the soil, taking care not to soak the substrate and this was the only culture treatment performed in the experiment. Two days before finishing the experiment, the irrigation was suspended to facilitate the removal and separation of the roots from the soil.

The evaluation of the agronomic characters and the extraction of the nematodes in the roots was done 100 days after the transplanting of the pre-sprouted seedlings. The aerial part of the plant was evaluated by the number of tillers, which were counted in each plot and then discarded. The roots were packed in plastic bags and then taken to the Soil Laboratory. The roots were washed in running water to eliminate soil particles and left on paper towels to eliminate the excess of water. Later they were weighed on a digital scale and cut into pieces approximately 2 cm long. The roots were taken to the blender, added 250 mL of water and crushed for 30 sec. The suspension obtained was poured on a sieve with 100 mesh opening superimposed on the other with 400 mesh opening. The residues retained in the 100 mesh sieve were discarded and the nematodes retained in the 400 mesh sieve were transferred to 50 mL of plastic recipients.

The quantification of nematodes was performed with the aid of an optical microscope using a Peters camera. The Reproduction Factor (RF) of the nematodes in each treatment was calculated by the ratio between the final population (pf) and the initial population (pi) according to Oostenbrink (1966). The initial population considered was the inoculum concentration of 800 specimens per vessel, and the final population the total nematode volume obtained at 90 DAI (days after inoculation) for management 1 and 60 DAI for management 2.

The analysis of variance was performed and the Tukey test was applied, there is 5% error probability for the reproduction factor (RF). The statistical analyses were performed with the assistance of the Assistat software (SILVA and AZEVEDO, 2002).

RESULTS AND DISCUSSION

The interaction between sugarcane cultivars and the two biofertilizer managements was significant (Table 1), where the two cultivars were host to *Pratylenchus brachyurus* nematode, taking into consideration, however, the degrees of susceptibility in the assessment of management 1. The RF of nematode in management 1 was lower in the CTC 04 cultivar, when compared to the nematode in the CTC 9003 cultivar. In management 2, no difference was observed between the cultivars (P>0.05).

TABLE 1 - Reproduction factor (RF) of *Pratylenchus brachyurus* in sugarcane cultivars and Microgeo® handling.

Sugarcane cultivars	Nematode reproduction factor	
	Management 1	Management 2
CTC 04	2,3 bA*	2,9 aA
CTC 9003	4,5 aA	2,7 aB

*Averages followed by the same capital letter in the row and lowercase in the column don’t differ statistically from each other by Tukey’s test, at 5% error probability.

In Figure 1 the number of individuals of *Pratylenchus brachyurus* is observed, as a function of the cultivars and the management of the biofertilizer, confirming a significant difference between the CTC 9003 and CTC 04 cultivars submitted to management 1 and the difference between the CTC9003 cultivar submitted to management 1 and 2.

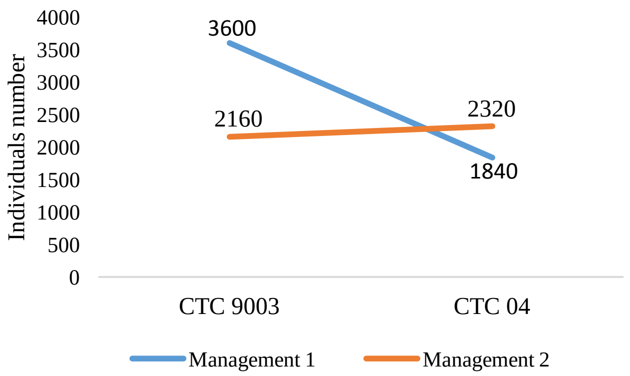

FIGURE 1 - Number of individuals of *Pratylenchus brachyurus* according to the types of management (1 and 2) and sugarcane cultivars.

The nematode RF for the CTC 04 cultivar showed no statistical difference (P>0.05) when two managements of the biofertilizer were compared. The longer time for the evaluation of the root system didn't influence the increase of the amount of nematodes. For the CTC 9003 cultivar, the RF differed statistically when the two managements were compared, as demonstrated in Figure 1, with a difference of 1440 individuals from management 1 to management 2, being lower in the latter.

As in this study, other authors also confirm the susceptibility of sugarcane to *P. brachyurus*. Oliveira et al. (2019), conducted an experiment in a protected environment evaluating the resistance of 14 sugarcane cultivars, among them the CTC 04 and CTC 9003 cultivars to the nematode *P. brachyurus*, at the end of the experiment it was observed that all sugarcane cultivars showed susceptibility to this nematode, thus proving that the cultivars studied in the present study are in fact his hosts.

Santos et al. (2012) conducted an experiment in a protected environment, in which they analyzed the resistance of 30 sugarcane cultivars to *Pratylenchus brachyurus* and *P. zeae*, performing inoculation of 500 nematode specimens. After 60 days, the authors concluded that all the evaluated cultivars presented susceptibility to the nematodes studied.

However, we can point out that the levels of susceptibility to nematode of this genus may vary according to the sugarcane cultivars, and may be moderately or highly susceptible to nematode species, with the possibility of using some of them for planting in places infested by these nematodes.

The interaction between sugarcane cultivars and doses of biofertilizer was significant (Table 2). For the CTC 04 cultivar there was no statistically significant difference between the doses applied. For the CTC 9003 cultivar, the RF was lower when Microgeo® was not applied or with 300 L ha^{-1}. In comparison between the evaluated cultivars, a statistical difference was found only in the doses 150 and 450 L ha^{-1}, because CTC 9003 obtained an RF=5.1 while CTC04 varied from 1.8 to 3.4, in 150 and 450 L ha^{-1}, respectively.

In the present study it is possible to verify the effect of the doses of biofertilizer, with RF reduction, however not differing statistically from the results observed by Silva and Venzke Filho (2014a), who observed the interaction between phytonematoids and Microgeo® in soybean crop, in an area naturally infested by *Pratylenchus brachyurus*, *Meloidogyne* sp., and *Helicotylenchus* sp. The application of biofertilizer was performed three days after the culture emergence and evaluations at 30 and 60 days after application. The same authors verified that the application of the biofertilizer interfered in the reduction of the number of nematodes in the cultivated area. The biological fertilization may have contributed to the reduction of the nematodes, probably by increasing the number of microorganisms, which must have acted on the eggs and adults of the nematodes.

TABLE 2 - Reproduction factor (RF) of *Pratylenchus brachyurus* in sugarcane cultivars and doses of Microgeo®.

Sugarcane cultivars	Nematode reproduction fator			
	Doses of Microgeo®			
	0 L ha^{-1}	150 L ha^{-1}	300 L ha^{-1}	450 L ha^{-1}
CTC 04	3,5 aA*	1,8 bA	1,7 aA	3,4 bA
CTC 9003	2,2 aB	5,1 aA	2,2 aB	5,1 aA

*Averages followed by the same capital letter in the row and lowercase in the column don't differ statistically from each other by Tukey's test, at 5% error probability.

In the study of Silva and Venzke Filho (2017b), evaluating the action of Microgeo® in the development of nematode species (*H. glycines* and *P. brachyurus*) in soybean cultivation, no nematicide effect was observed, but when the biofertilizer was used in a preventive manner, less nematode penetration in the roots was verified.

The interaction between application management and biofertilizer doses was significant (Table 3), where in management 1, the dose of 300 L ha^{-1} provided the greatest reduction in nematode and in management 2, the smallest reductions in nematode occurred in the dose of 150 L ha^{-1}. Studies using microorganisms to control nematoids are being conducted in important crops, but the results are contradictory. Oliveira et al. (2011) in studies with sugarcane (cultivar RB 92579), evaluated the efficiency of a natural inoculant based on the fungus *Paecilomyces lilacinus* (Nemout®), isolated or interacting with insecticides, in an experiment under controlled growing conditions. At 60 days after inoculation of 2000 specimens of *Pratylenchus* spp., they observed that the biological treatment (Nemout®) decreased the population density of nematoid and the RF was less than 1.

TABLE 3 - Reproduction factor (RF) of *Pratylenchus brachyurus* in sugarcane cultivars, managements and doses of Microgeo®.

Managements of the Microgeo®	Nematode reproduction fator			
	Doses of Microgeo®			
	0 L ha^{-1}	150 L ha^{-1}	300 L ha^{-1}	450 L ha^{-1}
Management 1	3,5 aAB*	4,9 aA	1,5 aB	3,7 aA
Management 2	2,2 aB	1,9 bB	2,4 aB	4,8 aA

*Averages followed by the same capital letter in the row and lowercase in the column don't differ statistically from each other by Tukey's test, at 5% error probability.

In Figure 2 it is possible to verify the number of individuals of *P. brachyurus*, according to the types of management and doses of Microgeo®. Sousa et al. (2010) evaluated the potential of arbuscular mycorrhizal fungi biocontrol to reduce the multiplication capacity of *M. incognita* species in tomato seedlings. The same authors used treatments with the presence of *Glomus clarum*, *Gigaspora albida* and *Acaulospora scrobiculata* fungus species. The results showed that *G. clarum* caused a significant reduction in the number of eggs and the rate of nematoid galls, while *Acaulospora scrobiculata* showed a high degree of infestation, not being efficient in the control of nematode.

Alves et al. (2011) studied the reaction of 20 rhizobacterial isolates on the outbreak and mobility of second stage juveniles of *Meloidogyne incognita* and *M. javanica* and on the mobility of *P. zeae in vitro*. They observed that none of the evaluated isolates influenced the outbreak of *M. incognita*, however for *M. javanica* the isolates of *Bacillus amyloliquefaciens* and *B. subtillis* presented potential for the control of *M. javanica*. For *P. zeae* nematode, isolates of *B. amyloliquefaciens* showed positive effects for the biological control of the nematode species studied.

In Brazil, few studies with alternative methods such as biological control have been conducted for sugarcane, mainly in Cerrado regions. Thus, the need to continue the studies to refine the results has increased, improving these studies by conducting experiments with longer evaluation time, verifying the production of cultivars, where nematode tolerant sugarcane cultivars can be identified or not.

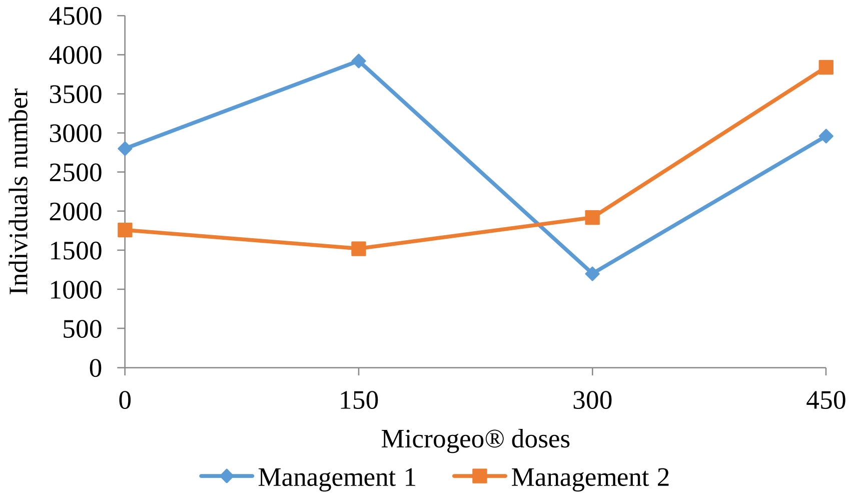

FIGURE 2 - Number of individuals of *P. brachyurus* according to the types of management (1 and 2) and doses of Microgeo®.

It is also important, the adoption of these works in naturally infested field conditions to detect the effects of liquid biofertilizer Microgeo®, if curative and/or preventive, in the field, we have natural soil microorganisms and if they can interact better with these microorganisms, creating a balanced environment. In works performed under controlled conditions such as this study, the soil was previously autoclaved and may have caused the scarcity of these natural soil microorganisms, hampering their effects.

CONCLUSIONS

The CTC 9003 cultivar during the conduct of the experiment showed a higher susceptibility to nematode than the CTC 04 cultivar.

Microgeo® biofertilizer showed no curative or preventive effect on the control of nematode in the experiment conditions.

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