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DOSE-RESPONSE EFFECT OF *Pochonia chlamydosporia* AGAINST *Meloidogyne incognita* ON CARROT UNDER FIELD CONDITIONS[1]

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ABSTRACT - The application of a bionematicide based on chlamydospores of *Pochonia chlamydosporia* (Pc-10) can be an important strategy for reducing the damage caused by *Meloidogyne incognita* on carrot. Based on this perspective, the nematicidal effects of 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 kg ha^{-1} of Pc-10 were evaluated on carrot cv. Juliana under field conditions. Carrot yield and nematode population were influenced by increasing doses of Pc-10. The application of 3.0 kg ha^{-1} of Pc-10 increased the marketable production of carrot roots by 41.7% compared to the untreated control, whereas the production of unmarketable roots and the nematode population in the soil were reduced by 48.7% and 61.4%. The application of 3.0 kg ha^{-1} of Pc-10 reduces *M. incognita* population and improves carrot quality and yield.

Keywords: Biological control. *Daucus carota*. Nematophagous fungus. Root-knot nematode.

EFEITO DOSE-RESPOSTA DE *Pochonia chlamydosporia* SOBRE *Meloidogyne incognita* EM CENOURA EM CONDIÇÕES DE CAMPO

RESUMO – A aplicação de um bionematicida à base de clamidósporos de *Pochonia chlamydosporia* (Pc-10) pode se tornar uma importante estratégia para reduzir os danos causados por *Meloidogyne incognita* em cenoura. Baseado nessa perspectiva, o efeito nematicida de 0; 0,5; 1,0; 1,5; 2,0; 2.5 e 3,0 kg ha^{-1} de Pc-10 foi avaliado em área de produção de cenoura cv. Juliana em condições de campo. A produtividade de cenoura e a população do nematoide foram influenciados por doses crescentes de Pc-10. A aplicação de 3,0 kg ha^{-1} de Pc-10 aumentou a produção de raízes comerciais de cenoura em 41,7% comparada com aquela obtida na testemunha não tratada, enquanto que a produção de raízes não-comerciais e a população do nematoide no solo foram reduzidos em 48,7% e 61,4%, respectivamente. A aplicação de 3,0 kg ha^{-1} de Pc-10 reduz a população de *M. incognita* e aumenta a qualidade e a produtividade das raízes de cenoura.

Palavras-chave: Controle biológico. *Daucus carota*. Fungo nematófago. Nematoide de galhas.

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INTRODUCTION

Carrot (*Daucus carota* L.) is one of the most important vegetable crops in Brazil and plays an important role in the economy of many municipalities in Minas Gerais State, such as Rio Paranaíba and São Gotardo. Because carrot production is labor intensive, this crop also plays a social role by providing job in these regions.

Marketable carrot production may be reduced in fields infested with *Meloidogyne* Goeldi species (root-knot nematode, RKN). Forking and galling caused by RKN result in carrot roots being discarded (HAY; PETHBRIDGE, 2005). In Brazil, *Meloidogyne javanica*, *M. incognita*, *M. arenaria* and *M. hapla* are the species that cause most damage to carrot crops (SILVA et al., 2011). Brazilian growers use crop rotation, fallow land, chemical nematicides and biological control agents to manage nematodes on this crop, with nematicides being increasingly replaced of by biological products.

Over the last few decades, the fungus *Pochonia chlamydosporia* Zare and Gams has been studied for use as a biological control agent against RKN in many countries (DE LEIJ; KERRY; DENNEHY, 1992; STIRLING; SMITH 1998; PUERTAS et al., 2006; DALLEMOLE-GIARETTA et al., 2012; MANZANILLA-LÓPEZ et al., 2013). This fungus colonizes and infects RKN eggs and exposed females, reducing the number of infective second-stage juveniles (J_2) (MANZANILLA-LÓPEZ et al., 2013). The antagonist produces chlamydospores, which are resistant resting spores that can be cultivated *in vitro* at laboratory conditions. Some *P. chlamydosporia* isolates can colonize the roots of different plant species, thus increasing the number of chlamydospores in the soil (MANZANILLA-LÓPEZ et al., 2013).

In Brazil, the isolate Pc-10 of *P. chlamydosporia* var. *chlamydosporia* was screened and used to control *Meloidogyne javanica* in tomato (DALLEMOLE-GIARETTA et al., 2012). A bionematicide based on chlamydospores of Pc-10 was formulated and applied to manage *M. javanica* in cucumber (VIGGIANO et al., 2014), lettuce and carrot (DALLEMOLE-GIARETTA et al., 2013), as well to control *Meloidogyne incognita* in lettuce (DIAS-ARIEIRA et al., 2011) and carrot (BONTEMPO et al., 2014). According to Bontempo et al. (2014), the bionematicide based on *P. chlamydosporia* controlled nematodes on carrot when applied at a dose of 3 kg ha^{-1}. It is hypothesized that doses lower than 3 kg ha^{-1} may also control the pathogen and therefore reduce nematode management costs. Therefore, the effect of Pc-10 doses from 0.5 to 3 kg ha^{-1} on *M. incognita* was evaluated on carrots in field conditions.

MATERIAL AND METHODS

A bionematicide based on the isolate Pc-10 (Rizotec®, wettable powder formulation, Rizoflora Biotecnologia S.A., Viçosa, Minas Gerais, Brazil) was applied at doses of 0 (untreated control), 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 kg ha^{-1} to control *M. incognita* in a commercial carrot field in Rio Paranaíba, Minas Gerais, Brazil (19°18'S; 46°09'W; 1,160 m). The nematode was identified by electrophoresis as Est I1 (CARNEIRO; ALMEIDA, 2001). The experimental area had previously been cultivated for three years with a pasture of *Brachiaria brizantha* (Hochst.) Stapf. The experiment was carried out from November 2011 to March 2012.

The biological product had 3×10^8 viable chlamydospores g^{-1} and was compared with a standard bionematicide used on the farm, based on the mix of nematophagous fungi and *Bacillus* sp. (5 kg ha^{-1}, Profix Max®, wettable powder formulation, Agrivalle Biotecnologia Agrícola, Pouso Alegre, Minas Gerais, Brazil). Biological treatments using a bionematicide based on the isolate Pc-10 and the standard treatment described above are hereafter referred to as Pc-10 and NFB. The soil in the experimental area had a pH of 6.05; 3.7% of organic matter; 41.5% of clay; 35% of sand; 23.5% of silt; 17.24 mg dm^{-3} of P; 1.6 cmolc dm^{-3} of Ca^{+2}; 0.92 cmolc dm^{-3} of Mg^{+2}. Prior to sowing, lime (1,730 kg ha^{-1}) and a 02-24-12 N-P-K formulation (2,200 kg ha^{-1}) were applied to the soil. Urea (80 kg ha^{-1}) and potassium chloride (150 kg ha^{-1}) were applied at 45 days after sowing (DAS) as top-dressing fertilization, and a 25-00-25 N-P-K formulation (180 kg ha^{-1}) was applied at 65 DAS.

Raised seed beds were mechanically prepared (1.80 m wide and 0.4 m between seed beds), and seeds of carrot cv. Juliana were sown at the rate of 26 seeds m^{-1}. Each experimental plot was 3 m long and 1.80 m wide, comprised of four double lines of carrot (12 cm between the double line and 14 cm between plants on the line). The plants were irrigated every two days by a center pivot system.

To determine the initial population (Pi) of *M. incognita* in the soil (number of J_2/100 cm^3 $soil^{-1}$) before bionematicide application, three cores were taken to a depth of 20 cm using a soil auger to form a composite soil sample from each experimental plot. Second-stage juveniles (J_2) were extracted from soil samples according to Jenkins (1964).

After sowing the seeds and sampling the soil for nematodes, the Pc-10 and NFB bionematicides were diluted in water and applied to the surface of the seed beds in the experimental plots (each 3 m long) with the aid of a backpack sprayer pressurized with CO_2 (Herbicat, Catanduva, São Paulo State, Brazil), equipped with a bar and two fan-type nozzles 11002, spaced 0.5 m apart, with spray volume adjusted to 300 L ha^{-1}. The plants were

thinned at 10 DAS, leaving 13 plants m^{-1}, for a final density of 750,000 plants ha^{-1}. The plots were harvested at 104 DAS by digging up the plants from each of two central double lines and then discarding 50 cm at each end. Foliage was discarded, and the taproot mass was evaluated (kg $plot^{-1}$) and categorized as either marketable, unmarketable without visible galls or unmarketable with visible galls (BONTEMPO et al., 2014; WALKER, 2004). Soil samples were also collected from each plot after harvesting carrots to determine the final population of J_2 in the soil (Pf).

The experiment consisted of 32 plots (eight treatments and four randomized blocks). The average minimum and maximum air temperatures during the experiment were 25.2 °C and 34.0 °C. The normality and homoscedasticity of the data were confirmed by the Kolmogorov–Smirnov test and the Bartlett test. Linear models were used to evaluate the effect of Pc-10 doses on the carrot yield and the ratio Pf/Pi of J_2 in the soil (P = 0.05). The Pc-10 doses and the NFB bionematicide (standard treatment) were compared using Dunnett's test (P = 0.05). Statistical analyses were done using the R software (R DEVELOPMENT CORE TEAM, 2014).

RESULTS AND DISCUSSION

Increasing doses of Pc-10, up to 3.0 kg ha^{-1}, increased the marketable yield of carrot and reduced unmarketable roots, according to linear models ($P \leq 0.02$; $R^2 \geq 0.85$). Soil treatment with 2.5 kg ha^{-1} of Pc-10 resulted in the maximum yield of marketable roots (7.61 kg $plot^{-1}$), with increments ranging from 50.1% to 54.7% compared to the controls, dose 0 (4.92 kg $plot^{-1}$) and the standard NFB treatment (5.07 kg $plot^{-1}$). The highest dose of Pc-10 (3.0 kg ha^{-1}) had similar effects to those of the NFB (Table 1). The production of unmarketable roots was reduced by 40% to 50% when Pc-10 was applied at the highest doses (2.5 and 3.0 kg ha^{-1}) compared to the controls (Table 1). Galls induced by *M. incognita* were observed in 30% and 15.2% of the discarded roots in the untreated control and the NFB (Table 1). The number of galled roots was reduced by more than 90% after the highest doses of Pc-10 were applied.

The J_2 soil population of *M. incognita* was similar across all treatments, both at the beginning and at the end of the experiment (Table 2). The J_2 Pf/Pi ratio however was linearly reduced with increased doses of Pc-10 (Table 2). The application of 3 kg ha^{-1} of Pc-10 reduced Pf/Pi ratio by 61.4% and 55.3% compared to the untreated control and the standard treatment NFB (Table 2).

Table 1. Carrot (*Daucus carota* cv. Juliana) yield in plots infested with *Meloidogyne incognita* and treated with different doses of *Pochonia chlamydosporia*-based bionematicide (Pc-10) and a bionematicide based on a mix of nematophagous fungi + *Bacillus* spp. (NFB).

Dose	Carrot yield (kg $plot^{-1}$)		
	Marketable roots	Unmarketable roots	Unmarketable roots with galls
0 kg ha^{-1} Pc-10	4.92	3.00	0.89
0.5 kg ha^{-1} Pc-10	4.88	2.88	0.77
1.0 kg ha^{-1} Pc-10	5.28	2.62	0.61
1.5 kg ha^{-1} Pc-10	6.47	2.40	0.46
2.0 kg ha^{-1} Pc-10	6.80	2.30	0.25
2.5 kg ha^{-1} Pc-10	7.61 *	1.81 *	0.04 *
3.0 kg ha^{-1} Pc-10	6.97	1.54 *	0.02 *
5.0 kg ha^{-1} NFB	5.07 $^{+}$	3.06 $^{+}$	0.47 $^{+}$
Effect of doses of Pc-10	Y = 4.726 + 0.938.x R^2 = 0.85	Y = 3.097 - 0.489.x R^2 = 0.96	Y = 0.909 - 0.317.x R^2 = 0.98
CV (%)	17.46	26.05	38.49

*Pc-10 doses are different from the standard treatment ($^{+}$), a bionematicide based on a mix of nematophagous fungi + *Bacillus* spp. (NFB), by Dunnett's test (P < 0.05). CV (%) = Coefficient of variation.

The application of a bionematicide based on chlamydospores from the isolate Pc-10 of *P. chlamydosporia* var. *chlamydosporia* at the dose of 3.0 kg ha^{-1} (9 x 10^{11} chlamydospores ha^{-1}) suppressed *M. incognita* and improved carrot quality and yield. Several studies have been performed worldwide using *P. chlamydosporia* as a potential biological control agent against plant-parasitic nematodes (MANZANILLA-LÓPEZ et al., 2013). Most of these investigations were performed under controlled conditions and used sterilized soils, particularly for the isolate Pc-10 in Brazil (COUTINHO et al., 2009; PODESTÁ et al., 2009; DALLEMOLE-GIARETTA et al., 2011; DALLEMOLE-GIARETTA et al., 2012). In one of the few previous studies examining the effects of Pc-10 in field trials, the fungus controlled *M. incognita* (DIAS-ARIEIRA et al., 2011) and *M. javanica* (DALLEMOLE-GIARETTA et al., 2013) in lettuce. In another study, the nematicidal effect of Pc-10 on controlling *M. incognita* on carrot has been reported at using 3 kg ha^{-1} at field conditions (BONTEMPO et al., 2014). Further studies, however, would be necessary to evaluate whether

doses lower than 3 kg ha^{-1} could also be effective in controlling the root-knot nematode and reducing carrot production costs. Therefore, this study corroborated that Pc-10 can be used to manage *M. incognita* on carrot at 3.0 kg ha^{-1}. Additional studies are needed to confirm whether Pc-10 can reduce the population of other *Meloidogyne* species on different carrot cultivars.

Table 2. Second-stage juvenile population (J_2) of *Meloidogyne incognita* in the soil at the beginning (initial population – Pi) and end of the experiment (final population - Pf) and Pf/Pi ratio of second-stage juveniles (J_2) in plots treated with different doses of *Pochonia chlamydosporia*-based bionematicide (Pc-10) and a bionematicide based on a mix of nematophagous fungi + *Bacillus* spp. (NFB).

Dose	Number of J_2 100 cm^{-3} of soil		
	Pi	Pf	Pf/Pi ratio
0 kg ha^{-1} Pc-10	17.00	24.32	1.40
0.5 kg ha^{-1} Pc-10	15.00	19.22	1.25
1.0 kg ha^{-1} Pc-10	20.00	18.28	0.92
1.5 kg ha^{-1} Pc-10	17.00	12.93	0.75
2.0 kg ha^{-1} Pc-10	19.00	13.13	0.71
2.5 kg ha^{-1} Pc-10	16.00	10.97	0.67
3.0 kg ha^{-1} Pc-10	22.00	12.08	0.54 *
5.0 kg ha^{-1} NFB	26.00 $^{+}$	31.75 $^{+}$	1.21 $^{+}$
Effect of Pc-10 doses	Non-significant	Non-significant	Y = 1.315 - 0.282.x R^2 = 0.90
CV (%)	51.90	26.12	22.13

* Pc-10 doses are different from the standard treatment ($^{+}$), a bionematicide based on a mix of nematophagous fungi + *Bacillus* spp. (NFB), by Dunnett's test (P < 0.05). CV (%) = Coefficient of variation.

The increase in marketable carrot production and the reduction of the *M. incognita* population most likely occurred because of the rapid colonization of the nematode eggs by Pc-10, thus preventing the embryo from fully developing into a J_2 (MANZANILLA-LÓPEZ et al., 2013). As a result, both the number of roots with defects and the reproductive rate of the nematode were reduced. In this study, *Pochonia chlamydosporia* could not be recovered from the soil and carrot roots to confirm the presence of the antagonist in the plots before and after the bionematicide application. However, soil fungus population increased after bionematicide application based on the difference between the control and the Pc-10 treatments on controlling the nematode. Further studies are necessary to confirm these results and to assess other methods of detecting *P. chlamydosporia* in soil.

Dose-response studies are important and can provide technical information on bionematicide development. *Pochonia chlamydosporia* is currently being used at 5,000 chlamydospores g^{-1} of soil to manage root-knot nematodes (DE LEIJ et al., 1992; STIRLING; SMITH, 1998; VIANENE; ABAWI, 2000; DALLEMOLE-GIARETTA et al., 2012), that is, 1 x 10^{13} chlamydospores ha^{-1} (1 ha: 100 m long x 100 m wide x 0.20 m depth). However, this amount of fungus may be unfeasible for field applications. Considering the concentration of the bionematicide equal to 3 × 10^{11} chlamydospores kg^{-1}, the application of 33.33 kg ha^{-1} of Pc-10 is required. The Pc-10 bionematicide at a dose of 3.0 kg ha^{-1} can be used to manage *M. incognita* on commercial carrot production, even when the amount of fungus inoculum is approximately 10 times less than those used in previous studies. It is plausible that Pc-10 doses higher than 3.0 kg ha^{-1} may be even more effective for managing the root-knot nematode on carrot. However, increased bionematicide doses may be cost prohibitive for field applications. As such, a cost-benefit analysis of using this strategy must be assessed in further studies. Therefore, Pc-10 should be integrated with other control methods to maximize *M. incognita* management.

CONCLUSION

The application of 3.0 kg ha^{-1} of Pc-10 reduces *M. incognita* population and improves carrot quality and yield.

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