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Efficiency of *Pochonia chlamydosporia* in *Meloidogyne incognita* control in lettuce crop (*Lactuca sativa* L.)

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Abstract

The aim of this study was to evaluate the efficiency of the isolate Pc-10 of the fungus *Pochonia chlamydosporia* in the *Meloidogyne incognita* control, in two areas of lettuce production. To this purpose, two experiments were conducted in areas naturally infested with the nematode, located in northwest Paraná, Brazil. The nematode initial population (IP) was evaluated before of the fungus application. The fungus, produced on rice grain, was applied to the soil at concentrations of 0, 10, 20 and 30 g, together with 100 g of cattle manure. Each gram of the product contained 1 x 10^8 fungal spores. After 15 days, seedlings of lettuce cv. Babá de Verão were planted and grown for 45 days. Posteriorly, the final population of the nematode in the soil ; the number of galls and eggs + second-stage juveniles (J_2) per root system and the fresh and dry weight of the aerial part were evaluated. In one of the experimental areas, the fungus was found to reduce the number of nematode eggs irrespective of the dosage, while in the other area, the treatments reduced the final population of J_2 in the soil. An increase in vegetative parameters was observed in the area of lower soil fertility when doses of 10 and 30 g of the product were used.

Key words: Antagonistic fungi, biological control, management, *Meloidogyne incognita*, *Pochonia chlamydosporia*, root-knot nematode.

Introduction

The occurrence of root-knot nematodes, *Meloidogyne* spp., in vegetable crops is very common and these parasites are often identified as yield-limiting factors. In the case of lettuce (*Lactuca sativa* L.), various authors have shown that the majority of cultivars are susceptible to root-knot nematodes [3, 14] and controlling them is very complicated, especially in organically grown crops.

Considerable efforts have been made to find alternative pest control methods, including the use of antagonistic plants, the addition of organic matter and varietal control, among others. In this context, biological control is an important option for management nematodes in vegetable crops. Among biocontrol agents, the fungus *Pochonia chlamydosporia* (Goddard) Zare and Gams (sin. *Verticillium chlamydoporium*) is notable for key characteristics, such as parasitism of eggs and females, ease of cultivation in a culture medium, rhizosphere competence and production of resistance spores (chlamydospores) [6, 7]. First report of this fungus reducing nematode number was published in 1974, when it was isolated from cysts of *Heterodera schachtii*, according to Schmidt [15]. Since then, many studies have been conducted, in particular with the aim of contributing to the control of root-knot nematodes. In Cuba, this fungus is sold commercially under the name KlamiC®, and is recommended as a new option for the integrated management of root-knot nematodes on vegetable crops [4].

In a study carried out by Coutinho *et al.* [2], the isolate Pc-10 applied at a dosage of 5,000 chlamydospores g^{-1} of soil led to a reduction in the number of galls and eggs of *Meloidogyne javanica* (Treub) Chitwood. According to Lopes *et al.* [9], the reduction in the number of eggs of this nematode ranged from 54.1% to 85.6% in different experiments using four isolates of the fungus. Despite the positive results obtained for nematode control using this fungus, few experiments have been conducted in the field. As a result, the aim of the present study was to evaluate the efficiency of the isolate Pc-10 of *P. chlamydosporia* in controlling *Meloidogyne incognita* (Kofoid and White) Chitwood, in areas of commercial lettuce production.

Materials and Methods

The study took place in two commercial lettuce-growing areas, naturally infested with *M. incognita*, located in northwest Paraná, Brazil (Area A: Chacara Pequena Gema and Area B: Chacara 29 B-3). Each experiment occupied an area of 150 m^2 and was divided into blocks, with eight replications for each treatment. Each experimental plot consisted of a bed of 32 plants distributed in four rows, with a space of 0.25 m between rows and 0.25 m between plants; the distance between the beds was 0.6 m. The plants in the two outer rows and at the end of each row represented the border plants. Mixture soil samples (500 g) taken in both areas, at a depht of 20 cm, before the initial of the experiment and subimtted to chemical analysis.

The treatments used were the control (without the treatment) and the isolate Pc-10 of fungus *P. chlamydosporia* produced on rice grain, applied at a dosage of 10, 20 and 30 g per m^2 of lettuce bed. The product included approximately 1 x 10^8 fungus spores (chlamydospores) per gram. For each 1 m^2 of lettuce bed, 100 g of

cattle manure was distributed on the surface of the soil immediately before the treatments were applied, and both were incorporated into the soil, at a depth of 10 cm, 15 days before the seedlings were transplanted.

Seedlings of lettuce cv. Baba de Verão were produced in commercial substrate Plantmax® on polystyrene trays, and transplanted to the lettuce beds 18 days after germination. In Area A, a top dressing of NPK 4-14-8 fertilizer was applied at a dosage of 182.5 g per plot. In Area B, in accordance with the rules of the institute that owns the land controlling the production of organic vegetables, foliar fertilization was carried out twice - immediately after planting and 15 days after planting - using biofertilizer Super Magro 5%, until it dripped off the leaves. The plants were drip-irrigated daily. During the experimental period, December 2009 to March 2010, temperature was monitored and data collected at the IAPAR Experimental Station in Umuarama County, Parana state.

The experiments were evaluated at the end of the crop cycle, removing four plants from the lettuce beds, separating the aerial part to determine the fresh weight (FW) and dry weight (DW) of the aerial part, and the root system to evaluate the number of galls. After the galls were counted, the nematodes were extracted from the root systems using the methodology proposed by Collen and D'Herde [1]. The eggs were counted using an optic microscope in Petri plates.

The reproduction factor (RF) was calculated according to the ratio between the final population (Pf) and initial population (Pi) of nematodes per plot. The Pi were estimated based on analysis of samples composed of 100 cm^3 of homogenized soil per plot collected from three distinct points in the locations where the seedlings were transplanted, at a depth of 20 cm, before the treatments were applied. The Pf were also estimated from samples of 100 cm^3 of soil, collected from three distinct points, when the lettuce was harvested, approximately 45 days after transplantation. Second-stage juveniles (J_2) were extracted from the soil samples by the method proposed by Jenkins [5].

The data obtained were submitted to variance analysis and the means compared using Tukey test at 5% probability, using SPSS Statistics program. To meet analysis of variance needs, the original data were transformed into $\sqrt{(x+1)}$.

Results and Discussion

In Area A, it was found that the application of the product at concentrations of 10 and 30 g reduced the nematode soil population by 40% and 81%, respectively. In the case of the control (0 g), the reduction was 0.3% and for the treatment using the 20 g dosage, there was an increase of 19%. In this area, the number of galls was significantly higher when 20 g of the product was applied than in the control, while the effects of the other treatments did not differ between themselves (Table 1). However, all dosages of the product caused reductions in the eggs + J_2 number. Lopes *et al.* [9] observed that the isolates I-28 and I-30 of *P. chlamydosporia* reduced the population of *M. javanica* in tomato plants by between 75.3% and 85.6%, although there was also variation between the results of different experiments. In the work undertaken by Coutinho *et al.* [2], the isolate Pc-10 reduced the number of galls and eggs of *M. javanica* by 36% and 60%, respectively, under controlled conditions.

In Area B, the initial population was considered to be zero because only eggs were recovered in the soil. A final population in the soil lower than that of the control was observed for all dosages of the product (Table 1). However, the treatments did not result in differences in the galls and egg+J_2 number in the radicular systems. A hypothesis to explain these results is the high temperatures during the experimental period, with minimum and maximum means of 22.2 and 30.8°C, respectively. According to Lopes *et al.* [9], the efficiency of fungi in parasitizing eggs in the soil is dependent on the temperature and the nematode's stage of embryonic development. In this context, Kerry and Bourne [6] found that when the temperature is high, fungal parasitism can be less efficient, because the J_2 hatch before the egg masses or eggs are colonized, and so the mobile forms of the nematode are not parasitized.

The reduced egg + J_2 number after the lettuce cultivation occurred, is possibly due to the short cycle of the crop, that allowed to nematode completes only one cycle of life. It was not possible to establish a relationship between the product dosage and the reduction in nematode numbers in the two areas evaluated, since in Area A there was a reduction in the number of eggs in the root system, but there was no difference in the number of nematodes in the soil, while in Area B a reduction was only observed in the

Table 1. Initial population (IP) and final population (FP) of *Meloidogyne incognita* in the soil, the number of galls and egg+J_2 per root system,weight (Fw) and dry weight (Dw) of the aerial part of lettuce submitted to different doses of Pc-10 of *Pochonia chlamydosporia*, on two agricultural properties (Area A and Area B) naturally infested with the nematode in Umuarama County, Parana state, Brazil.

Treatments	IP soil	FP soil	Galls	Eggs+J_2	Fw (g)	Dw (g)
			Area A			
Control	56.83^{ns}	56.67^{ns}	80.83 a	176.50 a	78.21^{ns}	6.95^{ns}
10 g	64.67	38.50	71.17 a	129.67 b	53.03	5.46
20 g	49.67	59.16	131.00 b	126.00 b	54.89	5.65
30 g	268.5	51.00	87.17 ab	125.00 b	72.47	6.44
CV (%)	42.1	21.8	18.8	17.5	12.5	10.2
			Area B			
Control	*	145.83 b	24.17 ^{ns}	96.3 ^{ns}	8.83 b	0.83 a
10 g		126.83 a	15.67	121.67	11.67 c	1.50 c
20 g		124.83 a	18.50	93.00	6.33 a	0.83 a
30 g		128.50 a	25.83	85.83	9.00 b	1.00 b
CV (%)		25.7	28.8	19.5	12.2	13.5

*IP considered to be zero, due to absence of juveniles from soil samples.
Original data transformed by $\sqrt{(x+1)}$ for analysis. In each area, means followed by the same letter in the columns do not differ between themselves by the Tukey test at 5%, ns=not significant.

Table 2. Results of chemical analyses at Chácara Pequena Gema and Chácara 29 B-3 properties in Umuarama Country, Parana state, Brazil.

Variable	pH	Al^{3+}	$H^{+}+Al^{3+}$	Ca^{2+}	Mg^{2+}	K^{+}	CTC	P	C	V
	$CaCl_2$	$cmol_c.dm^{-3}$						$mg.dm^{-3}$	$g.dm^{-3}$	%
Area A	6.6	0.00	2.03	4.66	3.09	0.55	10.33	233.90	21.12	80.35
Area B	5.6	0.00	2.74	2.13	1.00	0.69	6.56	89.20	9.35	58.21

Source: Soil Analysis Laboratory – UEM/Maringá, 2009. Ca, Mg, Al – extracted with 1M KCl; P, K – extracted with Mehlich 1; H+Al – SMP method; C Walkley & Black method.

nematode number in the soil (Table 1). One factor that may contribute to variations in results is the fact that different soils have differing levels of receptiveness to antagonistic fungi, given that they are not homogenous in terms of their chemical and physical characteristics [12]. In two experiments conducted by Verdejo-Lucas *et al.* [13], using *P. chlamydosporia* to control *M. javanica* in lettuce and tomato plants (*Solanum lycopersicum* L.), it was observed that control efficiency varied between the different experimental areas and different crops, with better results for the tomato control.

In the Area A, the treatments did not result in differences for vegetative parameters (Table 1). It is possible that greater soil fertility in this area (Table 2) contributed to better plant responses to attacks by nematodes. In other work, the isolate Pc-10 did not influence significantly the development of tomato crop, although reduced the galls and eggs number of *M. javanica* [2]. However, in the Area B, an increase in fresh weight of the aerial part was observed when 10 g of the product was applied, and increases in dry weight were observed when doses of 10 g and 30 g were applied.

Despite the variation between results in the different areas, the fungus was found to be efficient at reducing the nematode population, supporting the results obtained in previous experiments [2, 9]. According to Rodríguez *et al.*[11], when *P. chlamydosporia* or any other biological control agent is used, results as fast as those obtained via chemical control methods cannot be expected. The authors note that biological control only becomes effective over a period of time and needs to be part of an integrated management program. It is important to emphasize that the effectiveness of this fungus increases over time, given that it needs to establish itself in the soil in order to biologically regulate the soil [10]. Khan *et al.* [8] observed that the population of *P. chlamydosporia* reached higher levels after three months of inoculation, and the rise was dependent on the nematode population. In a study carried out by Verdejo-Lucas *et al.* [13], it was also found that the fungus survived in the soil for periods of eight to nine months and was compatible with intensive agricultural practices used in protected cultivation, including the chemical control of nematodes.

The desirable characteristics presented by the fungus *P. chlamydosporia* and the results obtained so far show the importance of this biological control agent in controlling phytonematodes and the need for further field experiments, in order to optimize their use by farmers.

Conclusions

The fungus reduced the nematode eggs number in one of the experimental areas. In the other area, the treatments reduced the final population of J_2 in the soil. An increase in vegetative parameters was observed in the area of lower soil fertility when doses of 10 and 30 g of the product were used.

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