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Formulation of bionematicides based on bacteria for the control of the lesion nematode in common bean

Formulación de bionematicidas a partir de bacterias para el control del nematodo lesionador en frijol

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ABSTRACT

Bacteria belonging to the genus Bacillus can promote plant growth and suppress plant-parasitic nematode populations. Based on this hypothesis, eight strains of the bacterium (SF 262, SF 264, SF 266, SF 267, SF 268, SF 271, SF 292 and SF 629) were formulated and applied as a seed treatment and/or sprayed on the soil surface with the aim of controlling *Pratylenchys brachyurus* in common bean under field conditions. The application of the bacterial isolates neither improved the grain yield and the root mass nor reduced the number of nematodes in the soil and in the roots of common bean. Thus none of the isolates of *Bacillus* spp. evaluated in this work are promising for the control of the lesion nematode on common bean.

Key words: Biological control, *Phaseolus vulgaris*, *Pratylenchus brachyurus*, rhizobacteria.

RESUMEN

Bacterias del género Bacillus pueden suprimir poblaciones de nematodos fitoparásitos. Sobre la base de esta hipótesis, ocho cepas de Bacillus (SF 262, SF 264, SF 266, SF 267, SF 268, SF 271, SF 292 y SF 629) fueron formuladas y aplicadas en el tratamiento de semillas y en la superficie de suelo para el control de Pratylenchus brachyurus *en frijol. La aplicación de las cepas no aumentaron la producción de granos y la masa de las raíces, ni redujeron el número de nematodos en el suelo y en las raíces. Así, ninguna de las cepas de Bacillus es promisoria para el control del nematodo lesionador en frijol.*

Palabras clave: *control biológico,* Phaseolus vulgaris*,* Pratylenchus brachyurus*, rizobacterias.*

Introduction

Plant-parasitic nematodes are one of the most important pathogens of the common bean (*Phaseolus vulgaris* L.) in Brazil, especially root-knot nematodes (*Meloidogyne* spp. Goeldi) and lesion nematodes [*Pratylenchus brachyurus* (Godfrey) Filipjev and Stekhoven]. The increase of the population of these pathogens in some of the major grain production areas in the country is favored by the continuous growing of susceptible crops (common bean, soybean and maize), moderate to high temperatures (15-30°C) and adequate soil moisture (40-60% of field capacity) during the whole year. Due to the difficulty of eradicating nematodes from infested areas, these pathogens must be managed using integrated strategies (De Waele and Elsen, 2007). In this context, bionematicides can be used as an additional method for controlling nematodes in the field. However, despite the potential of microorganisms as biological control agents of plant-parasitic nematodes, such as *Bacillus* spp., few bioproducts are available to growers in Brazil (Neves *et al*., 2009). Thus we formulated the hypothesis that bionematicides based on propagules of eight isolates of *Bacillus* spp. can control the lesion nematode (*P. brachyurus*) in common bean under field conditions.

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Material and methods

The isolates SF 262, SF 264, SF 266, SF 267, SF 268, SF 271, SF 292 and SF 629 were mass-produced in 250-mL Erlenmeyer flasks containing 50 mL MSF broth (2.5g NaCl; 2g KH_2PO_4; 2.5g K_2HPO_4; 0.25g $MgSO_4$.7H2O; 0.1g $MnSO_4$.H2O; 5 g dextrose and 4 g yeast extract per 1,000 mL of water). The flasks were kept on a rotary shaker (250 rpm) at 25 ºC for 96 h, which culminated with the formation of endospores. The broth was then centrifuged at 2,000 g for 15 min. The suspension was kept in a refrigerator (4 ºC) until use. The formulations were composed of 10-40% of the bacterial suspension (10^9 colony-forming units/mL), 1-3% xanthan gum, 1-5% of talc, 3-8% triton X-100, 5% of trealose and 35-65% of the liquid carrier - water, soybean oil and MSF broth (Burges, 1998). The pH of the formulations was adjusted to 7.0. The bionematicides were kept in 500-mL dark flasks and stored at the room temperature (25±2 ºC) or in the refrigerator (8 ºC±1 ºC). The shelf-life of the formulations was evaluated weekly by plating them on MSF broth in Petri dishes (90 mm in diameter), followed by the incubation at 25 ºC. The number of colony-forming units (CFU) was recorded after 72 h.

The formulations were used for seed treatment (ST) of common bean 'Pérola' and/or sprayed on the surface of the soil (SS) in a field naturally infested by *P. brachyurus*, in São Gonçalo do Abaeté-Minas Gerais, Brazil. A split-plot randomized design with four replicates was used in the experiment. The effect of the bionematicides or water was studied as the main factor in the plots and the method of application (ST or ST+SS) was evaluated in the sub-plots as second factor. Each experimental plot had three rows of 3 m long, spaced 0.5 m between rows. The center row was used as useful plot, ignoring the 0.5 m border at the edges. Half of the seeds of each row (18 seeds) were treated with the formulations (2 $mL.kg^{-1}$, 1 x 10^9 $cfu.mL^{-1}$) or water, and then were sown. The bionematicides or water at the dose of 2 L ha^{-1} were applied on the surface of the soil of the plots with the aid of a backpack sprayer pressurized with CO_2 at 3.02 $kgf.cm^{-2}$, equipped with a bar and two fan type nozzles 11002, spaced 0.5 m apart, with spray volume adjusted to 200 $L.ha^{-1}$. Soil samples were collected from each subplot for determination of the initial population (Pi) of *P. brachyurus* before sowing. Composite soil samples consisted of three cores taken to a depth of 200 mm with a soil auger. Vermiform stages of the lesion nematode were extracted from soil samples by the centrifugal-flotation technique (Jenkins, 1964). The initial population of *P. brachyurus* in the experimental area was 21.15 ± 13.14 nematodes 100 cm^{-3} of soil.

The experiment was evaluated 90 days after sowing (DAS). The average minimum and maximum air temperatures during the experiment were 26.0 ºC and 35.0 ºC, respectively. Soil samples were collected from each sub-plot to determine the final population of nematodes in the soil and in the roots (Pf). The ratio between Pf and Pi was used to calculate the reproduction factor (R) of the nematode (Oostenbrink, 1966). Besides the yield of grains, the mass of the roots and the number of *P. brachyurus* in the roots (Coolen and D'Herde, 1972) were recorded in each sub-plot. All data were tested for normality of the error (Kolmogorov-Smirnov test), homogeneity of variances (Bartlett test) and subjected to analysis of variance (P = 0.05). Reproduction factor and number of nematodes g^{-1} root data were transformed to $\log_{10}$ (x+0.5) to achieve a normal distribution before ANOVA.

Results and Discussion

The original concentration of bacterial cells in the formulations in closed flasks, 1 x 10^9 $cfu.mL^{-1}$, was maintained up to three and six months at room temperature (25 °C±2 °C) or in the refrigerator (8 °C±1 °C), respectively. However, the viability of the formulations reduced drastically after opening the flasks due to the presence of contaminants (data not shown).

The bionematicides based on the isolates of *Bacillus* spp. (SF 262, SF 264, SF 266, SF 267, SF 268, SF 271, SF 292 and SF 629) neither increased the production of grains and the mass of the roots (Table 1) nor controlled *P. brachyurus* in common bean (Table 2), regardless the method of the application of the formulations. The yield of the crop varied from 2,524 to 4,371 $kg.ha^{-1}$ in the sub-plots, with average of 3,591 $kg.ha^{-1}$. The reproduction factor of the nematode in the soil was 6.0 on the average, with the maximum of 13.0; while an average of 171.0 nematodes were found per g of roots.

Table 1. Effect of the application of formulations based on *Bacillus* spp. isolates (SF 266 to SF 629) on the surface of the soil and to the seeds on the yield of grains and mass of roots of common bean (*Phaseolus vulgaris*) cultivated in soil naturally infested by *Pratylenchus brachyurus* in Brazil.

Soil application	Yield of grains (kg.ha^{-1})*			Mass of roots (g)		
	NTS	TS	Mean	NTS	TS	Mean
SF 266	3.995	2.855	3.425ns	6.2	6.7	6.5ns
SF 268	3.657	3.066	3.361	5.3	5.4	5.4
SF 292	4.371	4.307	4.339	8.0	6.5	7.3
SF 264	4.062	3.650	3.856	7.1	7.6	7.3
SF 271	3.765	3.354	3.560	5.6	5.4	5.5
SF 267	3.701	3.477	3.589	7.6	5.9	6.8
SF 262	3.733	2.524	3.128	7.7	7.7	7.7
SF 629	2.979	3.260	3.120	7.3	8.9	8.1
Water	4.072	3.805	3.939	7.5	6.5	7.0
Mean	3.815 ns	3.367	3.591	6.9ns	6.7	6.8
CV (%)		35.4			31.5	

Data are expressed as mean of four replicates in each treatment. *Calculated from five plants in the sub-plots. Ns = non-significant according to F test (P > 0.05). NTS: non-treated seeds. TS: treated seeds with the formulations.

Table 2. Effect of the application of formulations based on *Bacillus* spp. isolates (SF 266 to SF 629) on the surface of the soil and to the seeds on the reproduction factor and the number of *Pratylenchus brachyurus* in the roots of common bean (*Phaseolus vulgaris*) cultivated in soil naturally infested by the nematode in Brazil.

Soil application	Reproduction factor			Number of nematodes g^{-1} roots *		
	NTS	TS	Mean	NTS	TS	Mean
SF 266	7.4	7.3	7.4 ns	147.5	167.5	157.5 ns
SF 268	4.5	7.9	6.2	172.5	167.5	170.0
SF 292	4.1	1.4	2.8	182.5	157.5	170.0
SF 264	12.9	6.7	9.8	190.0	190.0	190.0
SF 271	4.2	5.6	4.9	187.5	110.0	148.8
SF 267	4.9	6.0	5.5	155.0	225.0	190.0
SF 262	3.8	4.4	4.1	220.0	175.0	197.5
SF 629	5.2	3.9	4.6	157.5	135.0	146.3
Water	4.6	7.6	6.1	192.5	140.0	166.3
Mean	5.7 ns	5.6	5.7	178.3 ns	163.1	170.7
CV (%)		35.79			13.34	

Data are expressed as mean of four replicates in each treatment. *Calculated from five plants in the sub-plots. Ns = non-significant according to F test (P > 0.05). NTS: non-treated seeds. TS: treated seeds with the formulations. Reproduction factor = Final population in the soil and in the roots/Initial population in the soil (Oostenbrink, 1966).

Although experiments carried out either in laboratory or greenhouse are important to screen promising isolates, the real potential of the biological control agents is expressed under field conditions (Neves *et al*., 2009). In this case, the first challenge of the antagonist of the nematodes is to establish itself in the soil, overcoming the microbiostasis and the fluctuations of the environment conditions, before controlling the pathogen (Burkett-Cadena *et al*., 2008). The seed treatment was used in this experiment to offer competitive advantage to the isolates on the colonization of the roots and to improve the control of the nematode, such as reported by Khan *et al*. (2007). However, this ability of colonization depends on the interaction between the bacterium and the host plant. It is likely that none of our isolates are efficient colonizers of the rhizosphere

of common bean. Since the process of formulation reported in this work is simple and assures the viability of *Bacillus* spp., a further step needed is to formulate rhizocompetent isolates and evaluate them on the control of *P. brachyurus* in common bean or other crops.

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