

Biological nematicides associated with biofertilizers in the management of *Pratylenchus zaei* in sugarcane

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ABSTRACT: The aim of the present study was to assess *Pratylenchus zaei* control in sugarcane by using biological nematicides in association, or not, with biofertilizers. Sugarcane pre-sprouted seedlings were transplanted to pots and inoculated with 2 mL of a solution containing 1000 *P. zaei* in the planting furrows, treated with the combinations based on two biological nematicides, three biofertilizers, and their respective controls, and one absolute control (without treatment or nematode). Results evidenced that biological nematicides based on *Trichoderma harzianum* and *Bacillus* spp., when applied alone, promoted reductions in the nematode population of 33.81 and 31.62%, respectively. For nematodes per gram of root, the reductions were 34.67 and 25.38%. On the other hand, biofertilizers efficiency in the nematode reproduction presented variations. However, all treatments reduced nematode penetration in the sugarcane root system, with reductions varying from 31.17 to 53.21%. The same was observed in the mortality test, where nematode mortality ranged from 38.95 to 81.10%.

Key words: biological control; plant hormones; root lesion nematodes; *Saccharum officinarum*

Nematicidas biológicos associados a biofertilizantes no manejo de *Pratylenchus zaei* em cana-de-açúcar

RESUMO: O objetivo do presente estudo foi avaliar o controle de *Pratylenchus zaei* em cana-de-açúcar utilizando nematicidas biológicos associados ou não a biofertilizantes. Mudanças pré-germinadas de cana-de-açúcar foram transplantadas para vasos e inoculadas com 2 mL de solução contendo 1000 *P. zaei* nos sulcos de plantio, tratadas com as combinações baseadas em dois nematicidas biológicos, três biofertilizantes e as respectivas testemunhas, e um controle absoluto (sem tratamento ou nematoide). Os resultados evidenciaram que os nematicidas biológicos à base de *Trichoderma harzianum* e *Bacillus* spp., quando aplicados isoladamente, promoveram reduções na população de nematoides de 33,81 e 31,62%, respectivamente. Para nematoides por grama de raiz, as reduções foram 34,67 e 25,38%. Por outro lado, a eficiência dos biofertilizantes no controle da reprodução do nematoide apresentou variações. Mas, todos os tratamentos reduziram a penetração do nematoide no sistema radicular da cana-de-açúcar, com reduções variando de 31,17 para 53,21%. O mesmo foi observado no teste de mortalidade, cuja mortalidade do nematoide variou de 38,95 a 81,10%.

Palavras-chave: controle biológico; hormônios vegetais; nematoide das lesões radiculares; *Saccharum officinarum*

Introduction

Sugarcane (*Saccharum* spp. L. hybrid) is one of the most important crops in Brazil, however, despite the economic importance of this species, its cultivation is often made in sandy-soil sites of low fertility, fact that often limits yield, due to the presence of phytonematodes (Severino et al., 2010). These plant-parasite nematodes generally infect the roots and cause physiological changes and injuries that compromise the plant development and directly affects the productivity. Root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood and *M. javanica* (Treub) Chitwood, and root lesion nematode (*Pratylenchus zeae* Graham and *P. brachyurus* (Godfrey) Filipjev & Sch. Stekhoven) are among the most damaging species to the crop. There are records in Brazil about yield-loss up to 20% in sugarcane crops attacked by *M. javanica* and *P. zeae*, and of 30 to 50% in sugarcane cultivation areas exposed to *M. incognita* (Dinardo-Miranda, 2005).

Controlling these pathogens is complex, since there are no highly resistant cultivars to the nematodes (Dinardo-Miranda et al., 2002; Santos et al., 2012). Such control is mainly based on using non-host plants during sugarcane field reform (Oliveira et al., 2008) and on chemical control, by applying nematicides in the planting furrow (Dinardo-Miranda et al., 2002; Dinardo-Miranda, 2005). Thus, the search for new alternatives capable of composing the integrated management system must be constant.

Biological control emerges as an effective alternative for plant-parasite nematodes management, since there are many microorganisms with antagonistic potential, such as fungi belonging to the genus *Trichoderma* and bacteria of the genus *Bacillus* among them. These fungi and bacteria show high potential in reducing nematodes in many pathosystems (Affokpon et al., 2011; Cardoso & Araújo, 2011; Freitas et al., 2012; Xiang et al., 2017). Both present multiple action forms; *Trichoderma* spp., for instance, can produce toxic compounds (Sharon et al., 2001) and enzymes linked to phytonematode parasitism (Zhang et al., 2015), causing changes in root exudates and inducing plant-defense mechanisms (Kath et al., 2017). *Bacillus* spp. can produce toxins that inhibit the nematode activity, affecting the hatching, displacement and reproduction (Fernandes et al., 2014; Castaneda-Alvarez & Aballay, 2016; Zhang et al., 2016; Xiang et al., 2017), also affecting the root exudates (Araújo et al., 2002; Araújo & Marchesi, 2009) and promoting systemic resistance (Velmurugan et al., 2009; Castaneda-Alvarez & Aballay, 2016).

Biofertilizers are composed of macro and micronutrients, which can act in an indirect way, causing changes in the cell wall, making it thicker and lignified (Wang et al., 2003; Lenz et al., 2011), also having straight action in nematodes, as evidenced for root-knot nematodes (El-Nagdi & Fattah, 2011; Mattei & Dias-Arieira, 2015). Thus, the aim of the present study was to assess the efficiency of biological nematicides, and the possible interaction with biofertilizers, in controlling *P. zeae* in sugarcane crops.

Material and Methods

Pratylenchus zeae reproduction in sugarcane crops subjected to biological control and to biofertilizers

Two experiments were carried out in a greenhouse located at the geographic coordinates: 23°24'15.73" S, 51°56'28.13" W, and 517 m altitude. The study followed a completely randomized design, using a 3 x 4 factorial scheme: two biological nematicides and one control, and three biofertilizers and one control, with six replicates per treatment. An absolute control (no treatments and nematodes) was used to assess the vegetative parameters. Experiments were conducted between 03/21/2017 and 05/3/2017, with the temperature varying from 21.4 to 30.8 °C, and general temperature of 26.1 °C. While in the second period, the experiment was conducted between 09/05/2017 and 11/21/2017, with temperature averaging from 20.6 to 29.1 °C degrees, and general temperature of 24.8 °C. Pots containing 3 L of a substrate composed of soil (Dystrophic Red-yellow Oxisol) and sand (granulometry of 1:1; v:v) - previously autoclaved at 120 °C for 2 hours - were used in the experiment. All experiments were fertilized with NPK, according to the culture needs.

Soil infestation with nematodes was performed through the deposition of 1000 *P. zeae* specimens, diluted in 2 ml of water, in the plant furrows of sugarcane seedlings. Then, a soil layer (approximately 2 cm) was added in order to avoid straight contact between the products and the nematode. Subsequently, the treatments with biological control agents and biofertilizers were applied on the soil, and pre-sprouting seedlings of 45-day-old sugarcane cv. RB867515 were planted. A pure population of *P. zeae* from maize cv. Bandeirantes kept in the greenhouse was used in the experiment. Specimens were obtained based on the methodology suggested by Coolen & D'Herde (1972). The suspension was calibrated to 500 nematodes mL⁻¹, by using a Peters slide coupled to a light microscope. The seedlings came from of sugarcane with one-gem; they were deposited on trays covered with washed sand and cultivated for 45 days in the greenhouse.

Treatments were composed by: *T. harzianum* + control without biofertilizer; *T. harzianum* + Biozyme TF°; *T. harzianum* + Seed+°; *T. harzianum* + Raizal°; *Bacillus* spp. + control without biofertilizers; *Bacillus* spp. + Biozyme TF°; *Bacillus* spp. + Seed+°; *Bacillus* spp. + Raizal°; plants without biological control + Biozyme TF°; without biological control + Seed+°; without biological control + Raizal°; and non-treated and non-inoculated control. The commercial name of the products, active ingredients and doses are described in Table 1.

Plants were carefully harvested 70 days after the experiment installation, and the roots and shoots were separated from each other. Roots were washed in water and deposited on absorbent paper to remove the water excess; then, they were weighed to calculate the root fresh weight. Subsequently, they were subjected to the aforementioned nematode extraction process and the obtained samples were assessed with the Peters slide coupled to a light microscope. Total number of nematodes per root system was recorded

Table 1. Commercial name, active ingredient, concentration and dose of the commercial products.

Commercial name	Active ingredient (AI)	IA Dose	100L water dose
Trichodermil®	<i>Trichoderma harzianum</i>	48 (g L ⁻¹)	300 mL
Quartzo®	<i>Bacillus subtilis</i> + <i>B. licheniformis</i>	200 g Kg ⁻¹ + 200 g Kg ⁻¹	200 g
Biozyme TF®	N, K ₂ O, B, Fe, Mn, S, Zn, C organic	18, 60, 0.96, 4.8, 12, 12, 24, 42 (g L ⁻¹)	500 mL
Raizal®	N soluble, P ₂ O ₅ soluble, K ₂ O soluble	9, 45, 11(%pp)	2 Kg
Seed+®	Mg, S, Fe, Zn.	12.3, 35.7, 22.1, 24.6 (g L ⁻¹)	600 mL

Manufacturer data: Trichodermil® and Quartzo®: FMC; Biozyme TF®, Raizal®, Seed+®: Arysta.

(juveniles and adults), and it was divided by the root weight in order to find the number of nematodes per gram of root.

Plant height (shoot) - in centimeters (cm) - was assessed at 10, 20 and 30 DAP (days after planting) and at the end of the experiment (70 DAP); the fresh and dry weight - in grams (g) - were only assessed at the end of the experiment. The shoot dry weight was calculated after the drying process was conducted in a force air circulation oven at 65 °C for 72 hours.

Penetration test

The penetration experiment was conducted in order to determine the effect of the product on pre and post-penetration. For this, products used in the reproduction experiment were applied alone, in a completely randomized experimental design, with seven treatments and four replicates. The experiment was conducted in a greenhouse between 04/14/2017 and 05/07/2017, with minimum and maximum temperatures of 20.2 and 30.0 °C respectively, and a general temperature mean of 25.1 °C.

The same methodologies were adopted to get the inoculum, to apply the treatments and to conduct the experiments. However, for this trail, 700 mL pots were used, and the plants were inoculated with 700 nematodes.

After 10, 17 and 24 days after the transplantation, the root system was collected and subjected to the acid fuchsin staining methodology by Byrd et al. (1983), after the washing and weighing processes, in order to assess the number of nematodes (juveniles and adults) that had penetrated the roots. Likewise, the total number of nematodes and the number of nematodes per gram of root were evaluated.

The effect of the products on the mortality of *Pratylenchus zeae*

Two experiments were carried out in a completely randomized design, with six treatments and six replicates. Treatments, as well as doses, were the same used in the penetration experiment, but water was used as control.

Nematodes were obtained through the methodology mentioned above. Then, the suspension was placed in Baermann funnel at room temperature; the active nematodes were collected 24 hours later. Suspensions were calibrated to 50 nematodes mL⁻¹. Test microtubes were filled with 2 mL of the suspension and with the products in the respective percentages of the sample volume: *T. harzianum* 0.8%; *B. subtilis* + *B. licheniformis* 0.5%; Biozyme TF® 1.2%; Raizal® 5.0% e Seed+® 1.5%.

The mortality test was evaluated after 48 hours of incubation (BOD at 27 ± 1 °C, in the dark); counting the number

of dead and living nematodes separately. Nematodes were marked as dead when they stood still after the application of 10% sodium hydroxide (0.1N). Mortality percentage was calculated through the equation: dead nematodes (%) = (dead nematodes x 100) / (dead nematodes + living nematodes).

Statistical analysis

In the first experiment, for the nematological variables the data were submitted to a factorial analysis, comparing the treatments with the use of biological organisms by the Tukey test, at 5% probability. As for the vegetative variable, they were submitted to analysis of variance and Tukey's test, at 5% probability. Scott-Knott was used in comparisons conducted in the second and third experiments, at 5% probability. The analyzes were performed in the statistical software Sisvar (Ferreira, 2011).

Results and Discussion

Nematode reproduction

Regarding the nematological parameters, there was interaction between the biological control and biofertilizers factors in both parameters. Both biological control products used in experiment 1, in the absence of biofertilizer, reduced the total *P. zeae* population. Biological control with *T. harzianum*, when applied alone, reduced nematode reproduction, but it did not show any different results when it was used with the biofertilizers Biozyme TF® and Seed+® (Table 2). *Trichoderma* spp. efficiency in controlling nematodes in sugarcane plants was observed by Freitas et al. (2012) through the significant phytopathogen reproduction decrease. They found its efficiency in promoting the death of juveniles, as well as the parasitism capacity of the eggs by the *Trichoderma* isolates. The efficiency of these fungi, including *T. harzianum*, for the biological control of nematodes has been previously demonstrated in different patosystems (Affokpon et al., 2011; Freitas et al., 2012; Al-Hazmi & Tariq Javeed, 2016; Kath et al., 2017).

Trichoderma spp. are characterized by the different action mechanisms to nematode control, including changes in root exudates and plant defense-mechanism induction; therefore, it can increase the enzyme activity involved in synthesizing resistance components (Hwang & Benson, 2002; Kath et al., 2017).

The product based on *Bacillus* spp. promoted nematode population reduction when it was used alone; however, its application in association with biofertilizers promoted a population increase (Table 2). Similarly, there was reduction

Table 2. Total number of *Pratylenchus zeae* and *Pratylenchus zeae* per gram of root in sugarcane plants 70 days after inoculation with 500 nematode specimens, subjected to different biological control (BC) treatments in two experiments conducted in different periods.

Treatments	<i>P. zeae</i> total			<i>P. zeae</i> g ⁻¹ root		
	Without CB	<i>T. harzianum</i>	<i>Bacillus</i> spp.	Without CB	<i>T. harzianum</i>	<i>Bacillus</i> spp.
Experiment 1						
Control - without bioestimulant	1506 aA	774 bB	776 bB	75 aA	46 bB	48 cB
Biozyme TF [®]	905 bB	1033 abAB	1573 aA	46 bB	49 bB	107 aA
Seed+ [®]	1414 aA	1145 abA	1656 aA	62 abAB	51 bB	77 bA
Raizal [®]	1481 aA	1785 aA	1328 aA	82 aA	81 aA	64 bcA
CV (%)	19.39			20.40		
Experiment 2						
Control - without bioestimulant	1984 aA	1607 abB	1752 abB	61 aA	43 abB	52 aAB
Biozyme TF [®]	1068 abC	1731 aB	2120 aA	36 bA	51 aA	52 aA
Seed+ [®]	844 bB	1269 abA	1393 abA	23 bB	34 bcAB	41 abA
Raizal [®]	719 bA	691 bA	865 bA	28 bA	21 cA	33 bA
CV (%)	23.64			24.53		

Means followed by the same lowercase letter in the column and uppercase letter on the row did not differ from each other in the Tukey test, at 5% probability level.

in the number of nematodes g⁻¹ of root in the treatment without biofertilizer, in both biological control products (Table 2). *Bacillus* spp. are bacteria characterized by their capacity to produce toxins and by having different action modes over nematodes. Such toxins can directly influence the nematode reproduction, even more efficiently in the oviposition stage, changing the juvenile hatching rate (Castaneda-Alvarez & Aballay, 2016; Zhang et al., 2016; Xiang et al., 2017). Furthermore, as it was already reported for *Trichoderma*, *Bacillus* spp. produces enzymes, such as chitinases, glucanases and peroxidases, stimulating the plants and increasing the defense-enzyme production (Tian et al., 2007; Velmurugan et al., 2009; Castaneda-Alvarez & Aballay, 2016). There is also reports about the nematode reduction in sugarcane plants due to *B. subtilis* application in the planting furrow that corroborate with the present study (Cardoso & Araújo, 2011).

When analyzing the biofertilizer effects associated to biological control, it was possible to observe that the Biozyme TF[®] reduced the total number of nematodes in comparison to the control in the absence of biological control. The same trend was observed in the number of nematodes g⁻¹ of root, where Biozyme TF[®] - applied without biological control - reduced the number of nematodes when compared to the control (Table 2). Overall, the biofertilizer Raizal[®] did not present any control effect on the *P. zeae* population. On the other hand, all biofertilizers increased the total nematode population in the combined application with *Bacillus* spp.

Results of the second experiment corroborated with the first about the biological control efficiency, which, albeit presenting lower reduction, was statistically different from the control in the total number of nematodes (Table 2). In contrast, some biofertilizers presented the best results for reducing the total numbers of nematodes, compared to the control. Seed+[®] and Raizal[®] reduced the number of nematodes when applied alone, for both total and nematode g⁻¹ root parameters. However, Seed+[®] did not differ from control when it was applied with biological control for both parameters, while Raizal[®] promoted a reduction of the

nematodes when applied with biological controls, but only in the nematode number.g⁻¹ root parameter. The best results regarding number of nematodes g⁻¹ of root reduction were obtained by *T. harzianum* and *Bacillus* spp. applied without biofertilizers, while in the treatment without biological control all biofertilizers reduced this parameter (Table 2).

Research about the effect of biofertilizers on nematodes are scarce in the literature, but these products are composed of different micro and macronutrients. These nutrients can be directly related to plant growth promotion and to vegetal resistance increase. Such resistance can be achieved by anatomy changes, cell wall thickness increases and/or by the lignification of cell wall in the epidermis, i.e., it reinforces the mechanical barriers (Schnug et al., 1995; Wang et al., 2003; Lenz et al., 2011). It is possible to observe changes in the biochemical properties due to the production of inhibitory and/or repellent compounds (synthesis of toxic composites); fact that impairs the penetration of some plagues (Wang et al., 2003).

It is important pointing out that the association with biofertilizers usually impairs the efficiency of biological control agents, probably due to changes in the root exudates of plants treated with biofertilizers, being able to compromise the attraction and colonization by fungi and bacteria (De Pascale et al., 2017). Therefore, it is necessary to conduct new studies to prove this hypothesis.

With regard to the vegetative parameter in experiment 1, there was no significant effect for the factors, as well as for the interaction among them, for plant height, regardless of the assessment data, and for shoot fresh weight 70 days after inoculation (data not presented). On the other hand, the biofertilizer factor changed the shoot dry weight and the root fresh weight; all the biofertilizers increased the shoot dry weight, compared to the control. Raizal[®] showed the highest root fresh weight in comparison to the other products (Table 3). Experiment 2 also did not show any interaction between the factors, with only the biological control significant to the height of plants assessed 20 days after sowing. Only the

Table 3. Shoot dry weight (SDW) and root fresh weight (RFW) of sugarcane plants 70 days after planting (DAP) in experiment 1; plant height at 20 DAP and root fresh weight (RFW) in experiment 2, subjected to treatments with different biofertilizers and biological control products, and inoculated with 500 *Pratylenchus zeae* specimens.

Treatment	Experiment 1 (70 DAP)	
	SDW (g)	RFW (g)
Without bioestimulant	5.05 c	18.95 b
Biozyme TF [®]	5.43 b	18.99 b
Seed+ [®]	5.63 b	20.25 ab
Raizal [®]	6.43 a	22.20 a
CV (%)	8.32	7.36

Treatment	Experiment 2 (20 DAP)	Experiment 2 (70 DAP)
	Height (cm)	FRW (g)
Without biological control	11.91 b	30.49 b
<i>T. harzianum</i>	12.66 ab	41.28 a
<i>Bacillus</i> spp.	13.87 a	35.61 ab
CV (%)	19.77	31.34

Means followed by the same letter in the column did not differ from each other in the Tukey test, at 5% probability level. CV = coefficient of variation.

treatment with *Bacillus* spp. was better than the control, without the biological control. *Bacillus* spp. was better than the control in root fresh weight results 70 days after sowing (Table 3).

The best results of the biofertilizers on root weight are due, possibly, the plant nutrition; with it also being the main factor responsible for the observed gain. In addition, these products often have vegetal hormones that contributes to the plant development. On the other hand, it is worth highlighting that the resistance-induction process demands higher energetic cost (Dietrich et al., 2005), and it can explain the lack of results for the vegetative parameters in some trials.

Nematode penetration in the root system

Root weight on the treatments *T. harzianum*, *Bacillus* spp. and Biozyme TF[®] at 10 DAI was higher than that found in the control, but there was no difference between the treatments at 17 DAI. Only Biozyme TF[®] presented root weight higher than the control at 24 DAI (Table 4).

Overall, all the treatments reduced the nematode penetration in the root system of sugarcane plants. The total number of nematodes was smaller at 10 DAI on treatments with *Bacillus* spp. and Biozyme TF[®], indicating the fast action of the product within the first 10 days, differently from the other products, which presented reduction, but took longer to control the pathogen. All treatments reduced the total *P. zeae* penetration in the roots at 17 and 24 DAI (Table 4).

Trichoderma harzianum, *Bacillus* spp. and Biozyme TF[®] reduced the number of nematodes g⁻¹ of root at 10 DAI compared to the control (Table 4). All treatments reduced the number of nematodes g⁻¹ of root compared to the control at 17 and 24 DAI, with the highest reductions observed on the treatments Biozyme TF[®], Seed+[®] and Raizal[®] at 24 DAI (Table 4).

Results of the penetration experiment corroborate with the presented hypothesis, since both products based on the

Table 4. Root weight and *Pratylenchus zeae* penetration (total and per gram of root) in sugarcane plants subjected to different treatments in the sowing furrow 10, 17 and 24 days after inoculation (DAI) with 500 nematode specimens.

Treatment	10 DAI	17 DAI	24 DAI
Root weight (g)			
Control	1.09 b	1.35 ^{ns}	2.09 b
<i>T. harzianum</i>	1.73 a	1.16	2.59 b
<i>Bacillus</i> spp.	1.65 a	1.95	2.96 b
BiozymeTF [®]	1.95 a	1.89	3.87 a
Seed+ [®]	1.35 b	1.68	2.09 b
Raizal [®]	1.25 b	1.64	2.73 b
CV (%)	24.02	19.55	22.54
Total nematodes			
Control	68 a	129 a	249 a
<i>T. harzianum</i>	64 a	54 c	94 b
<i>Bacillus</i> spp.	52 b	90 b	150 b
Biozyme TF [®]	35 b	104 b	136 b
Seed+ [®]	86 a	61 c	72 b
Raizal [®]	60 a	45 c	43 b
CV (%)	21.13	27.29	28.30
Nematode g ⁻¹ of root			
Control	66 a	97 a	129 a
<i>T. harzianum</i>	40 b	48 b	46 b
<i>Bacillus</i> spp.	35 b	51 b	64 b
Biozyme TF [®]	18 b	62 b	37 c
Seed+ [®]	62 a	38 b	35 c
Raizal [®]	50 a	29 b	16 c
CV (%)	22.15	29.37	31.12

Means followed by the same letter in the column did not differ from each other in the Scott-Knott test, at 5% probability level. CV = coefficient of variation.

microorganisms were efficient in protecting the roots and in reducing *P. zeae* penetration - mainly 25 days after inoculation. In addition to the effects already discussed, microorganisms can promote the nematode disorientation, due to the effect on the root exudates, fact that changes the chemotropic stimuli produced by the plants on the nematodes (Araújo et al., 2002).

Moreover, all biofertilizers were efficient in reducing nematode penetration. Some elements found in the biofertilizers, mainly in Biozyme TF[®], have been reported as important for plant protection against the attack of pathogens. They may have direct or indirect action on such pathogens (Shaukat & Siddiqui, 2003; Rumiani et al., 2016).

Zinc, for instance, is directly related to the membrane integrity, and increases the plant resistance against the pathogens attack to the root system (Falloon et al., 1996). Couto et al. (2016) observed stronger resistance of tomato plants to *M. incognita* when the plants were treated with boron and zinc. The organic carbon found in Biozyme TF[®] can also help to control the nematodes (El-Nagdi & Fattah, 2011), since it can influence the activity of these organisms in the soil or change the microbial population (Feng et al., 2003). Miamoto et al. (2017) conducted a research with nutrient-rich products and found similar results about the control of *M. javanica* and *P. brachyurus* in soybean, mainly when they were associated with the biological control performed with *Trichoderma* or *Bacillus*.

Mortality of *P. zeae* exposed to different treatments

Except for the treatment Raizal®, all the others promoted higher *P. zeae* mortality rates than the control in experiment 1, and the best results were obtained with the treatments *T. harzianum* and *Bacillus* spp. (Table 5). Data from the experiment 2 corroborated to the first experiment, showing increased mortality in all treatments, with emphasis to *T. harzianum* and *Bacillus* spp., whose rates were 83.9 and 81.6%, respectively, while on the control it was 29.1% (Table 5).

Some *Trichoderma* and *Bacillus* isolates can produce toxic compounds involved in nematode paralysis and death (Sharon et al., 2001; Zhang et al., 2015). Therefore, it is possible to have a direct effect about egg and juvenile, since both the fungus and bacteria produces proteases and chitinases (Zhang et al., 2015; Zhang et al., 2016; Xiang et al., 2017). Although not evaluated, changes in the pH and the osmotic pressure of the medium may be responsible for the results observed for the biofertilizers, and new studies are needed to elucidate these products mode of action.

Table 5. Mortality percentage of *Pratylenchus zeae* juveniles subjected to 48-hour exposure to different treatments.

	Experiment 1	Experiment 2
Control	48.8 c	29.1 d
<i>T. harzianum</i>	75.2 a	83.9 a
<i>Bacillus</i> spp.	80.6 a	81.6 a
Biozyme TF®	63.6 b	35.7 c
Seed+®	64.5 b	42.0 b
Raizal®	54.1 d	39.1 b
CV (%)	8.03	7.44

Means followed by the same letter in the column did not differ from each other in the Scott-Knott test, at 5% probability level. CV = coefficient of variation.

Conclusions

Both treatments with biological control reduced the *P. zeae* reproduction 70 days after inoculation, mainly when they were applied in absence of biofertilizers. The best control in the product association was observed on *Bacillus* spp. + Biozyme TF®, since it was the biofertilizer promoting the total nematode reduction without biological control. All treatments reduced nematode penetration at 17 and 24 days after inoculation and caused nematode mortality *in vitro*.

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