Biocontrollers in the management of yam dry rot nematodes

Produtos biocontroladores no manejo de nematoides da casca-pretada-inhame

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ABSTRACT: One of the main diseases affecting yam crops (Dioscorea spp.) in Brazil is the dry rot caused by Scutellonema bradys, Pratylenchus brachyurus and P. coffeae nematodes. The use of biological control agents is an auspicious procedure which has been tested in order to reduce losses by pathogens. The objective of this work was to evaluate the nematicidal activity in vitro and in vivo of commercial biological products on yam dry rot nematodes. Products based on Trichoderma harzianum (2.0 x 10^6 conídas mL^-1) at dosages of 1.5 and 2.0 mL 200 L^-1 of water; Bacillus subtilis 20% - 1.0 x 10^8 cfu g^-1 + B. licheniformis 20% - 1.0 x 10^8 cfu g^-1, at 100 and 150 g 100 L^-1; B. subtilis 200 g kg^-1 + B. licheniformis 200 g kg^-1 at 1.50 and 200 g 100 L^-1; combination of rhizobacteria including Bacillus spp. and organic carbon at 3L and 7L 100 L^-1; and the control (distilled water), were tested in in vitro assays on S. bradys or Pratylenchus sp. In experiments performed under greenhouse conditions, healthy seed tubers were planted in sterilized soil and thirty days later the soil was infested with a suspension of 1,000 specimens of a mixed population of S. bradys and P. coffeae. Then, after 30 days products based on B. subtilis 20% + B. licheniformis 20% - 150 g 100 L^-1; T. harzianum 2 L 200 L^-1 and rhizobacteria + organic carbon 150 g 100 L^-1, at 100 mL of soil. Three months after planting, the percentage of sprouting of the seed tubers was evaluated and in the fifth month, the nematode population densities were determined. The sprouting of seed tubers was of 100% in all treatments. Bacillus subtilis 20% + B. licheniformis 20% and T. harzianum caused 89% and 61% mortality in S. bradys respectively, at the highest concentrations. In specimens of Pratylenchus sp., rhizobacteria + organic carbon exhibited 51% and 45% mortality at higher and lower concentrations, respectively. Under greenhouse conditions, B. subtilis 20% + B. licheniformis 20% and rhizobacteria + organic carbon were more effective in reducing nematode population densities, compared to the control.

KEYWORDS: Dioscora spp.; Scutellonema bradys, Pratylenchus sp.

RESUMO: Uma das principais doenças que afetam a cultura do inhame (Dioscora spp.) no Brasil é a casca-pretada causada pelos nematoides Scutellonema bradys, Pratylenchus brachyurus e P. coffeae. O uso de agentes de biocontrole é uma prática que vem sendo testada visando a redução de perdas por patógenos. O presente trabalho teve por objetivos avaliar em ensaios in vitro e in vivo o efeito nematicida de produtos biológicos comerciais sobre os nematoides causadores da casca-pretada-do-inhame. Em testes in vitro foram avaliados produtos à base de Trichoderma harzianum (2.0 x 10^6 conídas mL^-1) nas dosagens de 1.5 e 2.0 mL 200 L^-1 de água; Bacillus subtilis 20% - 1.0 x 10^8 UFC g^-1 + B. licheniformis 20% - 1.0 x 10^8 UFC g^-1, 100 e 150 g 100 L^-1; B. subtilis 200 g kg^-1 + B. licheniformis 200 g kg^-1 at 1.50 and 200 g 100 L^-1; combinação de rizobactérias incluindo Bacillus spp. e carbono orgânico 5 e 7L 100 L^-1, além da testemunha (água destilada), sobre S. bradys e Pratylenchus sp. Em ensaios conduzidos em casa de vegetação, rizóforos-sementeidos foram cultivados em solo esterilizado e aos trinta dias o solo foi infestado com uma suspensão de 1,000 espécimes de uma população mista formada por S. bradys e P. coffeae. Trinta dias após a infestação do solo foram aplicados os produtos à base de B. subtilis 20% + B. licheniformis 20% - 150 g 100 L^-1; T. harzianum - 2 L 200 L^-1; rizobactérias + carbono orgânico - 7 L 100 L^-1, no volume de 100 mL por vaso. Três meses após o plantio foram avaliados a brotação dos rizóforos e no quinto mês, a densidade populacional dos nematoides. A brotação dos rizóforos-semente foi de 100% em todos os tratamentos. Bacillus subtilis 20% + B. licheniformis 20% e T. harzianum causaram 89% e 61% de mortalidade em S. bradys, respectivamente, nas maiores concentrações avaliadas. Em espécimes de Pratylenchus sp., destacou-se o produto à base de rizobactérias + carbono orgânico, apresentando 51% e 45% de mortalidade na maior e menor concentração, respectivamente. Em condições de casa de vegetação, B. subtilis 20% + B. licheniformis 20% e rizobactérias + carbono orgânico mostraram-se mais eficazes na redução da densidade populacional dos nematoides, comparados à testemunha.

PALAVRAS-CHAVE: Dioscora spp., Scutellonema bradys, Pratylenchus sp.
INTRODUCTION

Yam (Dioscorea spp.) belonging to the botanical family Dioscoreaceae are found throughout the tropics, especially in Africa, Latin America, the Caribbean and Asia (Lebot, 2009). Brazil ranks the 13th position in yam production, with approximately 250 thousand tons/year produced in an area of around 26 thousand ha, with an average yield of 9.8 t/ha (FAO, 2018). In this country the most representative fields are located in the northeast region (Nobre, 2012).

In Brazil, yam production faces various constraints, among which are the dry rot disease caused by the plant-parasitic nematodes *Scutellonema bradys* (Steiner & LeHew) Andrassy, *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven and *P. coffeae* (Zimmermann) Filipjev & Schuurmans Stekhoven (Moura, 2016). Dry rot causes a marked reduction in the quality, marketability and edible traits of tubers (Coyne & Affokpon, 2018).

The management of dry rot disease in Brazil is based on nematode-free seed tubers, planted in soil free of nematodes, and crop rotation with antagonistic crops (Moura, 2016). At present, there is not a single nematicide registered to be applied on yam crops in this country (Agrofit, 2020). However, other strategies such as the use of plant extracts from garlic (*Allium sativum* L.), cassava (*Manihot esculenta* Crantz), papaya (*Carica papaya* L.), mint (*Mentha piperita* L.), gliricidia (*Gliricidia sepium* Jacq. Steud.) (Coimbra et al., 2006); *Annona* spp. and *Croton heliotropifolius* Kunth. (Lima et al., 2019); custard apple (*Annona squamosa* L.) (Magalhães, 2020), and biocontrol using metabolites produced by actinobacteria strains (Santos et al., 2016), have drawn attention.

According to Stirling (1991) more than 200 natural enemies of plant-parasitic nematodes, among them, fungi and bacteria have been tested. *Trichoderma* species produce toxic substances which can influence hatching and motility of nematodes (Meyer et al., 2000). Rhizobacteria, especially *Bacillus subtilis* (Ehrenberg) Cohn, were related as antagonistic to plant-parasitic nematodes (Ferraz et al., 2010). However, studies on the management of yam dry rot nematodes by the use of these organisms are limited. In view of this, the objectives of this work were to evaluate the in vitro nematicidal effect of biological products on *S. bradys* and *Pratylenchus* sp. and the efficiency of in vitro selected products to reduce these nematodes on yam plants.
MATERIAL AND METHODS

In vitro trials - motility and mortality of *Scutellonema bradys* and *Pratylenchus* sp.

Nematode populations were obtained from yam tubers with dry rot symptoms from yam fields in the state of Alagoas. Tuber peels were processed according to Coolen and D’Herde (1972), and nematode identification was performed under a light inverted microscope, according to their morphological features (MAI & MULLIN, 1996).

The biological products: Presence® WS (based on *Bacillus subtilis* 20% - 1.0 x 10^{11} cfu g^{-1} + *B. licheniformis* 20% - 1.0 x 10^{11} cfu g^{-1}, FMC); Quartzo® WS (*B. subtilis* 200 g kg^{-1} + *B. licheniformis* 200 g kg^{-1}, FMC); Trichodermil® SC 1306 (*Trichoderma harzianum* 2.0 x 10^{9} viable conidia mL^{-1}, Koppert); Bio Ax®, plant bio-activator (combination of rhizobacteria including *Bacillus* spp. and organic carbon, AMK) were diluted in distilled water at dosages of 100 and 150 g 100 L^{-1}; 130 and 200 g 100 L^{-1}; 1.5 and 2.0 L 200 L^{-1}; and 5 and 7 L 100 L^{-1}, respectively and the control treatment (only distilled water).

Two experiments were accomplished in Kline slides where 200 µL of each product and concentration were added before 20 specimens of the nematodes (juveniles and adults of *S. bradys* or *Pratylenchus* sp.) were individually transferred from the extraction suspension to each concavity. Subsequently, plates were placed in plastic boxes containing paper towel moistened with distilled water, aiming to keep up humidity at 25°C in a growth chamber. After 24 h, the immobile nematodes were quantified under an inverted light microscope and immediately transferred to distilled water for 24 h before being counted. Specimens who were paralyzed after the period of incubation in water were considered dead.

The experimental design used for both assays was a completely randomized design with nine treatments and five replicates. All data were submitted to analysis of variance, and the means were compared by Scott-Knott test at 5% probability.

Greenhouse experiments – management of yam dry rot disease

Two experiments were performed from January to June, 2020, in a greenhouse located at the Research Center for Agricultural Sciences, Federal University of Alagoas (UFAL) (9°28’29.1”S; 35°49’43.6”W and 127 meters above sea level), in Rio Largo, AL,
Brazil. Mean maximum and minimum temperature were of 35ºC and 19.1º C (Laboratory of Irrigation and Agrometeorology, CECA/UFAL).

In both experiments, healthy yam (*D. cayenensis*) tuber seeds from a farm field in the state of Alagoas were used. In the first trial tuber seeds with fresh weight from 52 to 159 g were used, while in the second one the value ranged from 58 to 126 g. Tubers were planted in 3-L plastic pots containing sterilized soil.

Inoculation was achieved with naturally infected yam tubers processed according to Coolen and D’Herde (1972) method and nematodes were identified under an inverted light microscope, according to their morphological features, based on females (MAI & MULLIN, 1996). Regarding the genus *Pratylenchus*, characters such as the number of annules of the lip region, stylet length and the presence or absence of males, were considered (GONZAGA et al., 2016).

Thirty days after planting the soil was infested with 1,000 specimens of a *S. bradys* and *P. coffeae* mixed population (experiment I: 98% *S. bradys* and 2% of *P. coffeae*, and experiment II: 90% *P. coffeae* and 10% *S. bradys*). The inoculum was distributed in two roles of approximately 2 cm deep around the stem of each plant. After 30 days the treatments were applied to the soil: *Bacillus subtilis* 20% + *B. licheniformis* 20%, 150 g 100 L⁻¹; *T. harzianum*, 2 L 200 L⁻¹ and rhizobacteria + organic carbon, 7 L 100 L⁻¹, at 100 mL per pot. The control treatment (containing only the nematodes) was executed with only water. The experimental design was completely randomized, with four treatments and seven replicates.

Three months after planting, the percentage of tuber sprouting was evaluated, and the nematode population densities in 100 cm³ of soil, according to Jenkins (1964), and in the root system according to Coolen and D’Herde (1972), were evaluated in the fifth month. The reproduction factor was calculated for each replication \((RF = \frac{\text{Final population (soil+roots)}}{\text{Initial population}})\). Data were transformed to \(\sqrt{(x + 1)}\) and the means were compared by Scott-Knott test at 5% probability.
RESULTS AND DISCUSSION

In vitro trials - motility and mortality of *Scutellonema bradys* and *Pratylenchus* sp.

Products based on rhizobacteria + organic carbon and *T. harzianum* inhibited the mobility and caused significant (P≤0.05) mortality to *S. bradys*, both concentrations, and *B. subtilis* 20% + *B. licheniformis* 20% caused higher percentage of immobility and mortality only in the highest dosage (Table 1). However, the product with *B. subtilis* 200 g kg⁻¹ + *B. licheniformis* 200 g kg⁻¹ (Quartzo®) did not affect the nematodes. According to Torres (2019) this product did not demonstrate efficiency to control *Meloidogyne incognita* Kofoid & White on cotton (*Gossypium hirsutum* L.), when treating seeds or soil under greenhouse conditions. Concerning *Pratylenchus* sp., the product based on rhizobacteria + organic carbon caused the highest immobility and mortality for both concentrations tested. The products based on *Bacillus subtilis* 20% + *B. licheniformis* 20% and *B. subtilis* 200 g kg⁻¹ + *B. licheniformis* 200 g kg⁻¹ showed 30% of nematode mortality at the dosages of 150 g 100 L⁻¹ and 130 g 100 L⁻¹ of water, respectively.

Among the action mechanisms used by *Trichoderma* species against plant-parasitic nematodes, direct parasitism of eggs and juveniles by the increase of chitinases and proteases activity are documented (SAHEBANI; HADAVI, 2008). High percentage (around 80%) of parasitized eggs and second-stage juveniles (J2) of *M. javanica* (Treub) was observed after 48 h of incubation with *T. harzianum* (GOLZARI et al., 2011). Mascarin; Bomfim Junior; Araujo Filho (2012) verified conidia of *T. harzianum* attached and immobilized 64% of *M. incognita* J2 race 4. However, the mechanism of action of the fungus was not investigated in the present study.

The beneficial effects of *Bacillus* on plant-parasitic nematodes may be influenced by species and lineages of the bacteria. Alves et al. (2011), observed in *in vitro* tests, that *B. subtilis* and *B. amyloliquefaciens* (Priest) affected motility of *M. javanica* J2, whereas for *P. zeae* (Graham) this effect was only observed with the utilization of *B. amyloliquefaciens*. These results are partially in accordance with the data obtained in the present study, considering the product based on *B. subtilis* 20% + *B. licheniformis* 20% caused mortality of 89% in *S. bradys* and 30% in *Pratylenchus* sp. at the highest concentration tested (Table 1).
Table 1. Immobility and mortality of juveniles and adults of Scutellonema bradys and Pratylenchus sp., after exposure for 24 hours at different dosages of biological products, followed by incubation in water for 24 hours.

| Treatment | Scutellonema bradys | Pratylenchus sp. |
|-----------|----------------------|------------------|
|           | Immobility (%) | Mortality (%) | Immobility (%) | Mortality (%) |
| Control (water) | 6.0 D | 6.0 D | 5.0 C | 5.0 C |
| Rhizobacteria + organic arbono -5 L 100L^{-1} | 39.0 C | 36.0 C | 55.0 A | 45.0 A |
| Rhizobacteria + organic arbono - 7 L 100L^{-1} | 49.0 C | 44.0 C | 54.0 A | 51.0 A |
| B. subtilis 20% + B. licheniformis 20% - 100 g 100 L^{-1} | 16.0 D | 12.0 D | 19.0 C | 19.0 B |
| B. subtilis 20% + B. licheniformis 20% - 150 g 100 L^{-1} | 93.0 A | 89.0 A | 32.0 B | 30.0 B |
| B. subtilis 200 g kg^{-1} + B. licheniformis 200 g kg^{-1} - 150 g 100 L^{-1} | 13.0 D | 7.0 D | 31.0 B | 30.0 B |
| B. subtilis 200 g kg^{-1} + B. licheniformis 200 g kg^{-1} - 200 g 100 L^{-1} | 9.0 D | 7.0 D | 13.0 C | 13.0 C |
| Trichoderma harzianum – 1.5 L 200 L^{-1} | 39.0 C | 38.0 C | 29.0 B | 22.0 B |
| Trichoderma harzianum – 2 L 200 L^{-1} | 62.0 B | 61.0 B | 27.0 B | 23.3 B |
| C. V (%) | 15.7 | 19.2 | 19.6 | 19.7 |

1 Data transformed to $\sqrt{(x + 1)}$. Means followed by the same letter do not differ by Scott-Knott at 5% probability.

Identification of Pratylenchus species

The morphometric characters observed for populations of Pratylenchus spp. (n = 20) in the experiments I and II were: body length = 557 (474 - 711) µm, 583 (481 - 656) µm; stylet length = 16 (15-17) µm, 16 (14-18) µm; vulva position = 82 (79-84)% 83 (75-87)%, respectively; labial region with two annules; female tail end predominantly truncate. Other observation included the presence of males. These data are in accordance with the description of Gonzaga et al. (2016) for P. coffeae.
Greenhouse experiments - management of yam dry rot disease

Statistical significance was observed for nematode population densities and reproduction factor regarding the treatments, except for the nematode population in the soil in the first experiment (Table 2). In general, the application of products based on *B. subtilis* 20%+ *B. licheniformis* 20%, *T. harzianum* and rhizobacteria + organic carbon on the soil, significantly reduced (P≤0.05) nematode populations in the experiment 1, however, in the experiment 2, only *B. subtilis* 20%+ *B. licheniformis* 20% and rhizobacteria + organic carbon showed efficiency (Table 3). Tuber sprouting was of 100% in all treatments. The difference observed between experiments is attributed to the different mixed nematode populations considering that in the first experiment *S. bradys* was the dominant species whereas in the second one *P. coffeae* was the prevalent species.

In both experiments low RF values were observed in the control plants (RF<1) (Table 3). This likely occurred because nematode population was not assessed on tubers, due to their poor development during the experimental evaluation. According to Bridge and Starr (2007) severe symptoms of the disease are more evident in older and mature tubers.

The reduction of nematode populations by the use of *Trichoderma* has been reported by some authors, mainly with *Meloidogyne* spp. Jindapunnapat; Chinnasri; Kwankuae (2013), using a commercial formulation based on *T. harzianum* to control *M. enterolobii* (Rammah & Hirschmann) in guava (*Psidium guajava* L.), observed reduction in the number of J2 in the soil and roots. Positive result was also noticed in the reduction of *M. javanica* reproduction in tomato (*Solanum lycopersicum* L.) using *Trichoderma* species (AL-HAZMI & TARIQJAVEED, 2016).

Mechanism of action such as the induction of resistance, antibiosis, competition, direct parasitism and enzimatic hydrolysis have been attributed to the effective management of plant diseases by the use of *Trichoderma* species (IBRAHIM et al., 2020).

Other microorganisms such as *Bacillus* spp. are used as biocontrol agents in several crops, however, Wepukhul et al. (2016) observed that some isolates of *B. subtilis* significantly increased the number of free-living nematodes, and also *Helicotylenchus* and *Scutellonema* populations in common bean (*Phaseolus vulgaris* L.). On the other hand,
Machado and Costa (2017) observed that *B. subtilis* reduced *P. brachyurus* population in soybean (*Glycine max* (L.) Merril) roots and soil.

The reduction of nematode populations by the use of rhizobacteria has been attributed to mechanisms such as, inhibition of hatching, increase of immobility and mortality of *Meloidogyne* spp. J2 in tomato (ARAÚJO & MARCHESI, 2009), rice (*Oryza sativa* L.) (SOUZA JÚNIOR et al., 2010), and banana crops (*Musa* spp.) (RIBEIRO et al., 2012).

Studies related to the dry rot management using *Trichoderma* or *Bacillus* spp. were not found in the literature, which difficult a comparison between results. Further research is needed to test the efficiency of these products on yam fields infested by *S. bradys* and/or *Pratylenchus* sp.

**Table 2. Synthesis of analyses of variance for the variables evaluated in yam plants after inoculation with Scutellonema bradys and Pratylenchus coffeae, using different biological products.**

| Source of variation | Df | PS        | PR        | PT         | RF         |
|---------------------|----|-----------|-----------|------------|------------|
| **Experiment 1**    |    |           |           |            |            |
| Treatment           | 3  | 5.0984*** | 11.2268* | 15.08*     | 0.0011**   |
| Error               | 24 | 3.3244    | 2.8237    | 3.70018    | 0.0002     |
| Means               |    | 4.09      | 7.33      | 8.47       | 1.037      |
| CV (%)              |    | 44.6      | 22.9      | 22.7       | 1.5        |
| **Experiment 2**    |    |           |           |            |            |
| Treatment           | 3  | 36.123*   | 13.123*   | 45.6483*   | 0.0104*    |
| Error               | 24 | 8.7516    | 3.8954    | 11.4449    | 0.0264     |
| Means               |    | 12.04     | 10.56     | 16.03      | 1.126      |
| CV (%)              |    | 24.6      | 18.7      | 21.1       | 4.6        |

**Significant at 1% probability by the F test; *significant at 5%; nm no significant; CV. – coefficient of variation.**

PS- Nematode population in the soil (100 cm³); PR –nematode population in roots (1 g); PT – Total nematode population (soil+root); RF – Reproduction factor.
Table 3. Scutellonema bradys and Pratylenchus coffeae populations in the soil, roots, total nematode population and reproduction factor after application of different biological products in the soil.

| Treatment                              | Experiment 1 |          |          |          | Experiment 2 |          |          |          |
|----------------------------------------|--------------|----------|----------|----------|--------------|----------|----------|----------|
|                                        | PS     | PR    | PT     | RF      | PS     | PR    | PT     | RF      |
| Control                                | 29.3a  | 85.74a| 115.03a| 0.115a  | 238.4a | 140.71a| 379.13a| 0.379a  |
| B. subtilis 20% + B. licheniformis 20% | 17.1a  | 46.34b| 63.48b | 0.064b  | 105.1b | 78.64b| 183.78b| 0.184b  |
| Trichoderma harzianum - 2 L 200        | 12.1a  | 47.93b| 60.07b | 0.060b  | 163.4a | 139.48a| 302.91a| 0.303a  |
| L⁻¹                                      |         |        |         |         |         |        |         |         |
| Rhizobacteria + organic carbon - 7 L   | 17.9a  | 45.67b| 63.53b | 0.064b  | 107.5b | 100.88b| 208.38b| 0.208b  |
| 100 L⁻¹                                 |         |        |         |         |         |        |         |         |

Data transformed to $\sqrt{x} + 1$. Means followed by the same letter do not differ by Scott-Knott test at 5% probability.

PS- Nematode population in the soil (100 cm³); PR – Nematode population in roots (1 g); PT – Total nematode population (soil +root); RF – reproduction factor.

CONCLUSIONS

The product based on rhizobacteria + organic carbon exhibited good potential to reduce motility and cause mortality of both S. bradys and Pratylenchus sp. in vitro.

The application of rhizobacteria + organic carbon and B. subtilis 20% + B. licheniformis 20% on soil reduced the population density of yam dry rot nematodes in greenhouse.

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