Diversitas Journal

ISSN 2525-5215
DOI: 10.17648/diversitas-journal-v6i1-1572

Volume 6, Número 1 (jan./mar. 2021) pp: 24-35.
https://periodicos.ifal.edu.br/diversitas_journal/

Biocontrollers in the management of yam dry rot nematodes

Produtos biocontroladores no manejo de nematoides da casca-preta-do-inhame

Cecilia Hernandez Ramirez[1]; Alverlan da Silva Araújo[2]; Gilson Moura Filho[3]; Fernando da Silva Rocha[4]; Marylia Gabriella Silva Costa[5]; Maria de Fatima Silva Muniz[6]

[1]ORCID: 0000-0002-8639-0470; Postgraduate student Universidade Federal de Alagoas (UFAL), Campus de Engenharias e Ciências Agrárias, Rio Largo – AL, Brazil, Email: cecih967@gmail.com
[2]ORCID: 0000-0001-7784-3266; Postgraduate student UFAL, Rio Largo - AL, Brazil, Email: alverlanaraujo134@gmail.com
[3]ORCID: 0000-0003-0951-959X; Prof. Dr. UFAL, Rio Largo – AL, Brazil, Email: gmf.ufal@yahoo.com.br
[4]ORCID: 0000-0002-2506-3441; Prof. Dr. Universidade Federal de Minas Gerais (UFMG), Montes Claros – MG, Brazil, Email: rochafsplant@yahoo.com.br
[5]ORCID: 0000-0002-0669-6438; Drª Postdoctoral Program/CAPES, UFAL, Rio Largo - AL, Brazil, Email: marylia_gabriella@hotmail.com
[6]ORCID: 0000-0003-1748-4569; Profª Drª UFAL, Rio Largo – AL, Brazil, Email: mf.muniz@uol.com.br

Recebido em: 07 de novembro de 2020; Aceito em: 01 de janeiro de 2021; publicado em 31 de 01 de 2021. Copyright© Autor, 2021.

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 10^9 conidia mL^{-1}) at dosages of 1.5 and 2.0 L 200 L^{-1} of water; *Bacillus subtilis* 20% - 1.0 x 10^{11} cfu g^{-1} + *B. licheniformis* 20% - 1.0 x 10^{11} cfu g^{-1} at 100 and 150 g 100 L^{-1}; *B. subtilis* 200 g kg^{-1} + *B. licheniformis* 200 g kg^{-1} at 130 and 200 g 100 L^{-1}; combination of rhizobacteria including *Bacillus* spp. and organic carbon at 5L 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. brady*s 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 7 L 100 L^{-1}, at 100 mL per pot, were applied to the 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: *Dioscorea* spp.; *Scutellonema bradys*; *Pratylenchus* sp.

RESUMO: Uma das principais doenças que afetam a cultura do inhame (*Dioscorea* spp.) no Brasil é a casca-preta 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-preta-do-inhame. Em testes *in vitro* foram avaliados produtos à base de *Trichoderma harzianum* (2,0 x 10^9 conídios mL^{-1}) nas dosagens de 1,5 e 2,0 L 200 L^{-1} de água; *Bacillus subtilis* 20% - 1,0 x 10^{11} ufc g^{-1} + *B. licheniformis* 20% - 1,0 x 10^{11} ufc g^{-1}, 100 e 150 g 100 L^{-1}; *B. subtilis* 200 g kg^{-1} + *B. licheniformis* 200 g kg^{-1} - 130 e 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-semente sadios 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 efetivos na redução da densidade populacional dos nematoides, comparados à testemunha.
PALAVRAS-CHAVE: *Dioscorea* 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) Andrássy, *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 heliotropiifolius* 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 cm^3 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= Final population (soil+roots)/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[1] (%)	Mortality (%)	Immobility[1] (%)	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} – 130 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.

Experiment 1					
Source of variation	**Df**	**PS**	**PR**	**PT**	**RF**
Treatment	3	5.0984 ^{ns}	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					
Source of variation	**Df**	**PS**	**PR**	**PT**	**RF**
Treatment	3	36.1213*	13.123*	45.6483*	0.0104*
Error	24	8.7516	3.8954	11.4349	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%; ^{ns} no significant; CV. – coefficient of variation.
PS- Nematode population in the soil (100 cm^3); 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% - 150 g 100 L^{-1}	17.1a	46.34b	63.48b	0.064b	105.1b	78.64b	183.78b	0.184b
Trichoderma harzianum - 2 L 200 L^{-1}	12.1a	47.93b	60.07b	0.060b	163.4a	139.48a	302.91a	0.303a
Rhizobacteria + organic carbon - 7 L 100 L^{-1}	17.9a	45.67b	63.53b	0.064b	107.5b	100.88b	208.38b	0.208b

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 cm^3); 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.

REFERENCES

1. AGROFIT. Sistema de agrotóxicos fitossanitários. 2020. Available at: http://www.agrofit.com.br/novoportal. Accessed on: 01 Sept 2020.
2. AL-HAZMI, A. S.; TARIQJAVEED, M. Effects of different inoculum densities of *Trichoderma harzianum* and *Trichoderma viride* against *Meloidogyne javanica* on tomato. *Saudi Journal of Biological Sciences*, v. 23, n. 2, p. 288-292, 2016.

3. ALVES, G. C. et al. Avaliação *in vitro* do efeito de rizobactérias sobre *Meloidogyne incognita*, *M. javanica* e *Pratylenchus zeae*. *Revista Arquivos do Instituto Biológico*, v. 78, n. 4, p. 557-564, 2011.
4. ARAÚJO, F. F.; MARCHESI, G. V. P. Uso de *Bacillus subtilis* no controle da meloidoginose e na promoção do crescimento do tomateiro. *Ciência Rural*, v. 39, n. 5, p. 1558-1561, 2009.
5. BRIDGE, J.; STARR, J. L. Yams (*Dioscorea* spp.). In: BRIDGE, J.; STARR, J. L. *Plant nematodes of agricultural importance* – a color handbook. London: Academic Press, 2007. p. 79-83.
6. COIMBRA, J. L. et al. Toxicidade de extratos vegetais a Scutellonema bradys. Pesquisa Agropecuária Brasileira, v. 41, n. 7, p. 1209-1211, 2006.
7. COOLEN, W. A.; D'HERDE C. J. A method for the quantitative extraction of nematodes from plant tissue. Ghent, Belgium: State Agricultural Research Centre, 1972.
8. COYNE, D. L.; AFFOKPON, A. Nematode Parasites of Tropical Root and Tuber Crops. In: SIKORA, R. A. et al. *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. 3 ed. Cambridge, UK: CABI, 2018. p. 252-289,
9. FAOSTAT - Food and Agriculture Organization of the United Nation Available at: <http://www.fao.org/faostat/en/#data/QC>. Accessed on: 20 ago. 2020.
10. FERRAZ, S. et al. Manejo sustentável de fitonematoides. 1 ed., Viçosa: Editora UFV, 2010.
11. GOLZARI, H. et al. Elucidating the parasitic capabilities of Trichoderma against Meloidogyne javanica on tomato. Insight Plant Disease, v. 1, n. 1, p. 12-19, 2011.
12. GONZAGA, V. et al. Gênero Pratylenchus. In: OLIVEIRA, C. M.G.; SANTOS, M. A.; CASTRO, L. H. S. (Org.). Diagnose de fitonematoides. Campinas, SP: Milleniuum Editora, 2016. p. 71-98.
13. IBRAHIM, D. S. S. et al. Role of Trichoderma spp. in the management of plant-parasitic nematodes infesting important crops. In: ANSARY, R. A.; RIZVI, R.; MAHMOOD, I. (Eds.). Management of phytonematodes: recent advances and future challengs. Gateway East, Singapore: Springer, 2020. p. 259-278.
14. JENKINS, W. R. A rapid centrifugal-flotation technique for separating nematode from soil. Plant Disease Reporter, v. 48, n. 9, p. 692, 1964.

15. JINDAPUNNAPAT, K.; CHINNASRI, B.; KWANKUAE, S. Biological control of root-knot nematodes (*Meloidogyne enterolobii*) in guava by the fungus *Trichoderma harzianum*. *Journal of Developments in Sustainable Agriculture*, v. 8, n. 2, p. 110-118, 2013.
16. LEBOT, V. *Tropical root and tuber crops*: cassava, sweet potato, yams, and aroids. Wallingford, UK: CABI, 2009.
17. LIMA, R. S. et al. Extratos aquosos de *Annona* spp. e *Croton heliotropiifolius* sobre *Scutellonema bradys* e prospecção química dos compostos. *Summa Phytopathologica*, v. 45, n. 2, p. 223-224, 2019.
18. MACHADO, A. P.; COSTA, M. J. N. Biocontrole do fitonematoide *Pratylenchus brachyurus in vitro* e na soja em casa de vegetação por *Bacillus subtilis*. *Revista Biociências*, v. 23, n. 1, p. 83-94, 2017.
19. MAGALHÃES. I. C. S. *Extrato aquoso de folhas de pinheira no manejo da casca-preta-do-inhame*. 2020. Dissertação (Mestrado em Proteção de Plantas) – Centro de Ciências Agrárias, Universidade Federal de Alagoas, Rio Largo, 2020.
20. MAI, W. F.; MULLIN, P. G. *Plant-parasitic nematodes*: a pictorial key to genera. 5th ed. New York: Cornell University, 1996.
21. 21.MASCARIN, G. M.; BOMFIM JUNIOR, M. F.; ARAÚJO FILHO, J. V. *Trichoderma harzianum* reduces population of *Meloidogyne incognita*, in cucumber plants under greenhouse conditions. *Journal Entomology and Nematology*, v. 4, n. 6, p. 54-57, 2012.
22. MEYER, S. L. F. et al. Evaluation of *Trichoderma virens* and *Bulkholderia cepacia* for antagonistic activity against root-knot nematode, *Meloidogyne incognita*. *Nematology*, v. 2, n. 8, p. 871-879, 2000.
23. MOURA, R. M. Doenças do inhame-da-costa. In: AMORIM, L.; REZENDE, J. A. M.; BERGAMIM FILHO, A.; CAMARGO, L.E.A.(Ed.). Manual de Fitopatologia – doenças das plantas cultivadas. 5.ed. Ouro Fino, MG: Agronômica Ceres, 2016. v. 2, p. 477-483.
24. NOBRE, S. A força da cultura do inhame em Alagoas. 2012. Available at: <http://www.sebrae.com.br/uf/alagoas/areas-de-atuacao/agronegocios/cultura-doinhame/integra_bia/ident_unico/4140>. Accessed on: 15 Sept. 2020.

25. RIBEIRO, R. C. F. et al. Rizobactérias no controle de *Meloidogyne javanica* e mal do Panamá em bananeira. *Nematropica*, v. 42, n. 2, p. 218-226, 2012.

26. SAHEBANI, H.; HADAVI, N. Biological control the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum. Soil Biology and Biochemistry*, v. 40, n. 8, p. 2016-2020, 2008.

27. SANTOS, J. F. et al. Actinobacteria and organic fertilizers for management of the nematode Scutellonema bradys in yam plants. Revista Caatinga, v. 29, n. 3, p. 548-588, 2016.

28. SOUZA JUNIOR, I. T. et al. Biocontrole da queima-das-bainhas e do nematoide-das-galhas e promoção de crescimento de plantas de arroz por rizobactérias. *Pesquisa Agropecuária Brasileira*, v. 45, n. 11, p. 1259-1267, 2010.

29. STIRLING, G. R. *Biological control of plant parasitic nematodes*: Progress, problems and prospects. Wallinford, UK: CAB International, 1991.

30. TORRES, C. A. R. *Seleção de estirpes de Bacillus spp. e produtos comerciais para o biocontrole de Meloidogyne incognita em algodoeiro*. 2019. Dissertação (Mestrado em Fitopatologia) – Departamento de Fitopatologia do Instituto de Ciências Biológicas, Universidade de Brasília, Brasília, 2019.

31. WEPUKHULU, M. et al. Effect of *Bacillus subtilis* on bean nematodes in Kenya : A laboratory and green house experiment. *International Journal of Academic Research and Development*, v. 1, n. 8, p. 26-31, 2016.