RESEARCH/INVESTIGACIÓN

BIOCONTROL POTENTIAL OF FUNGAL FILTRATES ON THE RENIFORM NEMATODE (*ROTYLENCHULUS RENIFORMIS*) IN CORIANDER AND COWPEA

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ABSTRACT

Lira, V. L., D. V. Santos, R. N. Barbosa, A. F. Costa, L. C. Maia, and R. M. Moura. 2020. Biocontrol potential of fungal filtrates on the reniform nematode (*Rotylenchulus reniformis*) in coriander and cowpea. Nematropica 50:86-95.

This study evaluated the effect of fungal culture filtrates on the plant-parasitic nematode *Rotylenchulus reniformis in vitro* on cowpea and coriander plants in a greenhouse. Seventeen fungal isolates from seven genera obtained from the coriander rhizosphere were used, including: *Aspergillus*, *Fusarium*, *Purpureocillium*, *Penicillium*, *Talaromyces*, *Thielavia*, and *Trichoderma*. The biocontrol tests on *R. reniformis* were performed in 96-well culture plates and juvenile mortality rates evaluated after 24 and 48 hr of exposure to the filtrates and after 12 days for egg hatch tests. For the *in vivo* tests, cowpea and coriander were inoculated with 1,000 juveniles and eggs/pot, and the effects of the filtrates on the reproduction of *R. reniformis* were evaluated after 45 days. Filtrates from *Fusarium inflexum*, *Thielavia terricola*, *Trichoderma longibrachiatum*, *T. brevicompactum*, *T. harzianum*, *Penicillium citrinum,* and two new *Penicillium* species, showed promise for nematicidal activity and hatch inhibition. These fungi caused nematode mortality rates of 58 to 100% and allowed only 5 to 20% of the juveniles to hatch in the *in vitro* tests. In the *in vivo* tests with cowpea and coriander, filtrates from these isolates significantly reduced the number of egg masses and the reproductive factor (final population density/initial population density) of *R. reniformis*. These results demonstrate the potential and efficiency for biocontrol of *R. reniformis* by these fungi.

Key words: Alternative control, biological control, phytoparasitic, toxic metabolites

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RESUMO

Lira, V. L., D. V. Santos, R. N. Barbosa, A. F. Costa, L. C. Maia, and R. M. Moura. 2020. Potencialidade de filtrados fúngicos no biocontrole do nematoide reniforme (*Rotylenchulus reniformis*) em coentro e feijão-caupi. Nematropica 50:86-95.

Este estudo avaliou os efeitos de filtrados de culturas fúngicas sobre o nematoide parasito de plantas *Rotylenchulus reniformis* por testes *in vitro* e em plantas de feijão-caupi e de coentro, em casa de vegetação. Foram utilizados 17 isolados fúngicos de sete gêneros, obtidos da rizosfera de coentro, incluindo: *Aspergillus*, *Fusarium*, *Purpureocillium*, *Penicillium*, *Talaromyces*, *Thielavia* e *Trichoderma*. Os testes de

biocontrole de *R. reniformis in vitro* foram realizados em placas de Elisa e a taxa de mortalidade de juvenis foi avaliada após 24 hr e 48 hr de exposição aos filtrados e após 12 dias para os testes de eclosão. Para os testes *in vivo*, plantas de feijão-caupi e de coentro foram inoculadas com 1.000 juvenis/ovos por vaso e os efeitos dos filtrados sobre a reprodução de *R. reniformis* foram avaliados após 45 dias. Os isolados *Fusarium inflexum*, *Thielavia terricola*, *Trichoderma longibrachiatum*, *T. brevicompactum*, *T. harzianum*, *Penicillium citrinum* e duas novas espécies de *Penicillium*, mostraram-se promissores na atividade nematicida e na inibição da eclosão. Esses fungos causaram a mortalidade de 58 a 100% dos nematoides e permitiu que apenas de 5 a 20% dos juvenis eclodissem nos testes *in vitro*. Em teste *in vivo* com feijão-caupi e coentro, filtrados destes isolados reduziram significativamente o número de massas de ovos e o fator de reprodução de *R. reniformis*. Esses resultados demonstram o potencial e a eficiência do biocontrole de *R. reniformis* por esses fungos.

Palavras chave: Controle alternativo, controle biológico, fitoparasitário, metabólitos tóxicos

INTRODUCTION

Cowpea (*Vigna unguiculata*) and coriander (*Coriandrum sativum*) are widely cultivated in Brazil, especially in the Northeast, being an important component of regional cuisine. Despite their commercial importance, these crops face serious problems with pests, among them the plant-parasitic nematode *Rotylenchulus reniformis* Linford and Oliveira (1940). Within this genus, *R. reniformis* is the most important from an economic perspective. Indeed, when compared to other plant-parasitic nematodes, *R. reniformis* is the seventh most important globally (Jones *et al.*, 2013). *Rotylenchulus reniformis* is a polyphagous species with more than 300 host plants species. This nematode causes reductions in the productivity of many crops, and economic losses may exceed millions of dollars (Robinson *et al.*, 1997; Lawrence *et al.*, 2006). In the northeast of Brazil, the production of coriander, melon, and soursop suffers the most significant losses due to *R. reniformis* (Moura *et al.*, 1997, 2002, 2005).

The use of nematicides, crop rotation, and resistant cultivars are the most common control methods used against plant-parasitic nematodes. Chemical nematicides, although still widely used have high costs, can cause public health problems, and can have strong environmental impacts (Lira *et al.*, 2018). For short cycle crops (less than three months), the use of nematicides is prohibited in the field, since the residual systemic effects persist for more than 90 days in the plant. Coriander and melon have a production cycle of only 30 and 60 days, respectively. Thus, biological control of plant diseases and pests is an alternative and promising method of practical and economic value in these crops. Biological control has additional advantages compared to chemical methods, because it leaves no residues in food, is easy to apply, favors the conservation of the environment, and does not induce the emergence of resistant species (Nunes *et al.*, 2010). In soil, there are approximately 200 natural enemies of plant-parasitic nematodes (Pimentel *et al.*, 2009). Fungi are considered to have the most potential for biological control. The main mechanisms attributed to biological control of nematodes include competition for space and nutrients, induction of plant resistance, predation, parasitism, and antibiosis (Stirling, 2014).

Studies have highlighted the presence of toxic metabolites in fungal culture filtrates with nematostatic and nematicidal action. Li and Zhang (2014) collected more than 200 compounds extracted from fungi that were toxic to more than 20 different species of nematodes. However, knowledge of metabolites with nematicidal properties against *R. reniformis* is limited. Thus, in this study we determined the effect of filtrates of fungi isolated from the coriander rhizosphere on mortality and hatching of *R. reniformis*.

MATERIALS AND METHODS

Obtaining R. reniformis *isolates*

Soil and root samples from coriander 'Verdão' were collected from commercial production areas of Vitória de Santo Antão, Pernambuco, Brazil, to obtain populations of *R. reniformis*. Samples were collected at random, placed in plastic bags, and nematodes extracted from soil using the Jenkins method (1964). Nematodes were extracted from roots by the

blender method and centrifugation (Coolen and D'Herde, 1972). Nematode populations were identified using the classification of Jatala (1991) and cultured on coriander. Plants were watered and fertilized as necessary and grown in a greenhouse at the Agronomic Institute of Pernambuco, Recife, Pernambuco, Brazil.

Isolation and production of pure fungal cultures

The soil samples collected to obtain *R. reniformis* were also used to isolate fungi. Aliquots of these samples were placed in plastic bags and stored at 5°C until processed. The soil samples were processed following the methods of Clark (1965). Briefly, 25 g of each soil sample was added to an Erlenmeyer flask containing 225 ml of sterile distilled water and manually homogenized for 5 min. Subsequently, serial dilutions were made by a factor of 10^{-4} from which aliquots of 1 ml were transferred to Petri dishes containing potato-dextrose-agar medium (PDA), supplemented with 10 mg/L of chloramphenicol. The plates remained in BOD (Biochemical Oxygen Demand) at 28ºC ± 1ºC, and the growth of colonies was observed for seven days.

For the purification of fungal cultures, fragments of the colonies were transferred to PDA + chloramphenicol (10 mg/L) in Petri dishes, and their growth followed for seven days. After purification, the samples were transferred to test tubes (18 × 180 mm) containing PDA, for species identification and maintenance.

Identification of the fungi

The isolates were identified using stereo and light microscopes by observing the macroscopic (color, aspect, and diameter of colonies) and microscopic (microstructures) characteristics based on the specialized taxonomic literature (Ellis, 1971, 1976; Klich, 2002; Samson and Houbraken, 2011). For molecular analysis, the genomic DNA of a culture grown for five days was extracted using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI) according to the manufacturer's instructions. Then, PCR reactions were conducted to amplify fragments of sequences of transcribed internal spaces (ITS) using the primers ITS1 and ITS4 (White *et al.*, 1990), elongation factor 1-α (EF-1α) using the primers EF1α and EF2 (O'Donnell *et al.*, 1998), and β-tubulin (BenA) using the primers Bt2a and Bt2b (Glass and Donaldson, 1995). The protocol for PCR reactions were described by Samson *et al.* (2010). The amplified products were purified with PureLink – PCR Purification Kit (Invitrogen, Carlesbad, CA) and sequenced at the Multi User Sequencing and Gene Expression Platform of the Federal University of Pernambuco, Recife, Pernambuco, Brazil. The sequences obtained were compared using BLASTn with sequences in GenBank (http://www.ncbi.nlm.nih.gov).

Obtaining fungal filtrates

Seventeen fungal isolates were selected and inoculated into Petri dishes containing PDA medium. After seven days of incubation at 28°C ± 1°C, discs of each culture were transferred to a 250 ml Erlenmeyer's flask containing 100 ml of Czapek-Dox broth, previously sterilized at 120°C for 20 min (Costa *et al.*, 2001). After 15 days at 27°C in an orbital agitator at 160 rpm, the contents were passed through a Whatman filter paper No. 1, followed by a 0.22-µm porosity cellulose acetate membrane for the elimination of any residues and conidia of fungi. The obtained liquid phase (fungal filtrate) was used for the assays.

In vitro *biological control experiments with fungal filtrates and* R. reniformis

Egg masses from a pure culture of *R. reniformis* were extracted from roots using 0.5% sodium hypochlorite solution according to Hussey and Barker (1973). The eggs were placed in a hatching chamber at 27°C and juveniles were collected daily and stored at room temperature until the number needed for the experiment was obtained.

For the juvenile mortality experiment, a bioassay was conducted in 96-well culture plates following the methodology adapted by Costa *et al.* (2001). Each well received 100 µl of the filtrate of each fungus and 20 µl of a suspension containing 50 juveniles of *R. reniformis*. Each treatment was a fungal filtrate, with treatments replicated four times in a completely randomized design. The controls were Czapek-Dox Broth and distilled water. Then the plates were sealed with plastic wrap and placed in growth chamber at 27°C in the dark. The number of immobilized nematodes was

counted using a stereo microscope after 24 and 48 hr using the method of Chen and Dickson (2000). Nematodes that did not move after the addition of a 1.0% solution of 1 M sodium hydroxide were considered dead. For the egg hatching experiments, the same methodology as for the juvenile mortality experiment was used. In this assay, 50 eggs were added per well in 50 µl of water. Egg hatch was determined 12 days after application of the filtrates.

Data were transformed to arcsine $\sqrt{x} / 100$ and subjected to analysis of variance (ANOVA) using the software SASM - Agri (Canteri *et al.*, 2001). Means were compared by the Tukey test at 5% probability.

In vivo *test on cowpea and coriander plants with fungal filtrates and* R. reniformis

The fungal filtrates that showed the best results in *R. reniformis in vitro* juvenile mortality and egg hatching experiments (mortality of juveniles over 60% and hatching rate less than 50%) were evaluated in the greenhouse on cowpea 'BR-IPA 206' and coriander 'Verdão'. The plants were grown in plastic bags containing 500 g of autoclaved soil with one plant per container. Fifteen days after planting, 10 ml of a suspension containing 1,000 *R. reniformis* juveniles and eggs was inoculated at the base of each plant. Three days after inoculation, 50 ml of each fungal filtrate was added around the base of the plant according to Ali (1990). The experimental design was completely randomized, with eight fungal filtrate treatments and two controls (inoculated with *R. reniformis* and not inoculated) for each type of plant (coriander and cowpea). Treatments were replicated four times.

After 45 days of inoculation, the plants were removed from the substrate and the soil and roots processed using the Jenkins (1964) and Coolen and D'Herde (1972) methods, respectively, for nematode extraction. For each plant the length of the shoots and roots, biomass of dry shoots and fresh roots, number of egg masses, and reproduction factor were evaluated. The total *R. reniformis* population density in soil (PS) and in roots (PR) was determined. The reproduction factor (RF) was calculated as the total final nematode population (Pf) divided by the initial nematode population (Pi) (Oostenbrink, 1966). Normality was determined by the Shapiro-Wilk test and a homogeneity of variances by Bartlett. All results were transformed into $\sqrt{(x + 1)}$ and submitted to ANOVA. Means were compared by the Tukey test at 5% probability.

RESULTS

Identification of the fungi

According to morphological and molecular analyses, the 17 isolates were identified as: *Aspergillus fumigatus*, *A. parasiticus*, *A. terreus*, *Curvularia lunata*, *Fusarium inflexum*, *F. solani*, *Purpureocillium lilacinus*, *Penicillium* A25, *Penicillium* TA14, *Penicillium* 20.2, *Penicillium* 19.3, *P. citrinum*, *Talaromyces* TM 4, *Thielavia terricola*, *Trichoderma brevicompactum*, *Trichoderma harzianum*, and *T. longibrachiatum*. The isolates of *Penicillium* A25, TA14, 20.2, 19.3, and *Talaromyces* TM 4 are probably new species and will be described in the future.

Effect of fungal filtrates on mortality and hatching of juveniles and eggs of R. reniformis in vitro *tests*

The fungal filtrates of the 17 isolates had nematicidal activity against the juveniles of *R. reniformis*, with the results being proportional to the exposure time to the filtrates (Table 1). *Penicillium* A25, *Penicillium* TA14, *P. citrinum*, *T. terricola*, *T. harzianum* and *F. inflexum* caused 100% mortality of juveniles after 48 hr immersion. The filtrates of *T. longibrachiatum* and *T. brevicompactum* caused 97.0 and 88.5% mortality of *R. reniformis* juveniles, respectively. The filtrate of *A. fumigatus* was also effective, with 55.2% mortality of *R. reniformis* juveniles. The filtrates from *Penicillium* 19.3, *Talaromyces* TM4, *A. terreus* and *A. parasiticus* had a low toxic effect on *R. renifomis* juveniles, with mortality rates ranging from 34.9 to 48.5%, but still significantly different from the controls. The filtrate from *A. parasiticus*, *C. lunata*, *F. solani*, *Penicillium* 20.2, and *P. lilacinus* had similar nematicidal activity compared to the controls with mortality rates less than 18%. The pH of the fungal filtrates ranged from 3.7 to 9.4 (Table 1).

All of the fungal filtrates that had nematicidal effects against *R. reniformis* juveniles also caused reductions in egg hatching. *Aspergillus fumigatus*, *A. terreus*, *C. lunata*, *Penicillium* A25, *Penicillium* TA14, *P. citrinum*, *Penicillium* 19.3, *T. terricola*, *T. brevicompactum*, *T. harzianum*, *T.*

Table 1. Effect of filtrates of fungal cultures on mortality of juveniles and hatching of eggs of *Rotylenchulus reniformis.*

Fungal filtrates	pH	Mortality of juveniles (%) 24 hr	48 hr	Hatching (%)
Aspergillus fumigatus	5.2	20.6 def[z]	55.2 b	29.0 abcde
Aspergillus parasiticus	7.9	4.3 fg	9.5 e	62.1 fgh
Aspergillus terreus	9.4	22.8 de	34.9 cd	41.0 def
Curvularia lunata	8.7	6.4 efg	17.4 de	32.4 bcde
Fusarium solani	6.4	8.5 efg	18.2 de	67.9 gh
Penicillium A25	7.2	100.0 a	100.0 a	11.0 abc
Penicillium TA14	5.4	100.0 a	100.0 a	14.2 abc
Penicillium 20.2	8.1	4.2 fg	12.0 e	53.6 efg
Penicillium citrinum	3.7	60.9 b	100.0 a	4.6 a
Thielavia terricola	8.0	100.0 a	100.0 a	4.9 a
Trichoderma brevicompactum	7.9	60.7 b	88.5 a	8.3 abc
Trichoderma harzianum	8.0	100.0 a	100.0 a	8.7 abc
Trichoderma longibrachiatum	8.6	58.9 b	97.0 a	6.6 ab
Penicillium 19.3	4.1	32.1 cd	42.1 bc	41.0 def
Fusarium inflexum	8.0	100.0 a	100.0 a	19.5 abcd
Talaromyces TM 4	5.6	27.5 cd	48.5 bc	69.7 gh
Purpureocillium lilacinus	8.8	1.4 g	2.8 e	34.1 cde
Control A (Czapek broth)	7.2	0.5 g	2.8 e	86.6 hi
Control B (Distilled water)	7.0	0.2 g	0.7 e	99.0 i

[z]Means followed by the same letter do not differ from each other by Tukey test ($p \leq 0.05$). Values arcsine $\sqrt{x}$ / 100 transformed prior to analysis.

longibrachiatum, *F. inflexum*, and *P. lilacinus* reduced egg hatching ranging from 4.6 - 41.0% of the controls. These hatching inhibition rates were greater than those observed for *A. parasiticus*, *F. solani*, *Penicillium* 20.2 and *Talaromyces* TM4 (53.6 to 69.7 %), demonstrating low ovicidal impacts of these filtrates on *R. renifomis*.

Effect of fungal filtrates on the biocontrol of R. reniformis *on cowpea and coriander*

The evaluated fungal filtrates reduced the number of egg masses (> 83.6%) and RF (> 95%) of *R. reniformis* on cowpea compared to the control (Table 2). *Fusarium inflexum* was less effective when compared to the other treatments, but the result still differed statistically from the control. Filtrates of fungal cultures did not affect cowpea development (Table 3). The treatments with *Penicillium* A25, *P. citrinum*, *T. brevicompactum*, and *F. inflexum* produced plants with higher dry shoot biomass, differing significantly from the inoculated control with *R. reniformis* (Table 3). The filtrate of *F. inflexum* increased plant growth by 20% compared to the control. However, plants treated with filtrates of *Penicillium* TA14, *P. citrinum* and *T. longibrachiatum* had lower plant biomass compared to the other treatments and did not differ from controls. The other treatments also did not differ from the controls. Root length and biomass were not affected by the fungal filtrates (Table 3).

On coriander, the fungal filtrates reduced *R. reniformis* reproduction compared to the control with a reduction in the number of egg masses (> 95%) and RF (> 95%) (Table 4). Plant growth was greater with filtrates from *P. citrinum* and *T. brevicompactum* differing from the control with *R. reniformis* (Table 5). For the other fungal filtrate treatments, there were no significant differences with the inoculated and uninoculated controls. The plants that received filtrates of *P. citrinum* also had higher dry shoot biomass compared to the control with *R. reniformis* and *T. longibrachiatum*. Fresh root biomass and root length were not affected by the fungal filtrates (Table 5).

DISCUSSION

The demonstrated antagonistic effects against *R. reniformis* of the filtrates of *Penicillium* A25, *Penicillium* TA14, *P. citrinum*, *T. terricola*, *T. harzianum* and *F. inflexum* indicate that these fungi

have the potential to be used for nematode control. In addition to killing *R. reniformis* juveniles and reducing egg hatching, these filtrates also reduced egg mass production and, consequently, the number of eggs per root system. The filtrates were non-toxic to the plants. This is of importance for short-production cycle plants such as coriander, which only stays in the field for 30 days, making chemical control not possible.

The mechanism of action for each fungus - *R. reniformis* relationship was not identified in these experiments. However, a hypothesis can be considered. According to Ali (1990), filtrates from *Penicillium* spp. can cause physiological changes in the roots of plants, affecting the plant-nematode interaction. Similar results have been obtained by Bokhari (2009) when evaluating the effect of filtrates of *Trichoderma* spp. on the control of *R. reniformis*. Filtrates of *T. hamatum* and *T. harzianum* were the most effective, resulting in decreased *R. reniformis* juvenile movement of 57 and 93%, respectively.

In the greenhouse, the fungal filtrates effectiveness was probably due to the production of toxic metabolites capable of causing immobilization and death of the nematodes before they were able to penetrate the roots. These effects may affect one or more specific life stages of the plant-parasitic nematodes (Costa *et al.*, 2001). For example, some chitinolytic species of *Trichoderma* and *Thielavia* can penetrate the egg masses through the action of chitinases and proteases preventing the hatch of juveniles and inducing plant defense mechanisms involved in the release of peroxidases, polyphenol oxidases and phenylalanine ammonia lyases, contributing further to the control of the nematode population (Sahebani and Hadavi, 2008; Siddaiah *et al.*, 2017).

In this study, in addition to causing mortality

Table 2. Effect of fungal filtrates on the reproduction of *Rotylenchulus reniformis* on cowpea.

Treatments	Eggs mass	Reproduction factor (PF/PI)
Control	45.70 a[z]	18.62 a
Penicillium A25	5.25 b	0.23 b
Penicillium TA14	3.50 b	0.10 b
Penicillium citrinum	3.25 b	0.27 b
Thielavia terricola	2.50 b	0.26 b
Trichoderma harzianum	4.50 b	0.37 b
Trichoderma brevicompactum	1.75 b	0.38 b
Trichoderma longibrachiatum	1.00 b	0.28 b
Fusarium inflexum	7.50 b	0.93 b
CV (%)	39.29	20.54

[z]Means followed by the same letters in the column do not differ from each other by the Tukey test ($p \leq 0.05$). Transformed values for $\sqrt{(x + 1)}$. *PF/PI: final population/initial population.

Table 3. Effect of fungal culture filtrates on development of cowpea infested by *Rotylenchulus reniformis.*

Treatments	Plant length (cm)	Dry shoot biomass (g)	Fresh root biomass (g)	Root length (cm)
Uninoculated control	125.50 ab[z]	1.52 a	3.00 a	12.50 a
Control with *R. reniformis*	79.50 c	0.64 b	1.00 b	13.50 a
Penicillium A25	123.70 abc	1.45 a	2.00 ab	20.50 a
Penicillium TA14	86.70 bc	1.00 ab	1.25 b	19.00 a
Penicillium citrinum	94.50 bc	1.47 a	2.00 ab	13.50 a
Thielavia terricola	116.50 abc	1.26 ab	1.50 ab	14.00 a
Trichoderma harzianum	115.50 abc	1.31 ab	1.75 ab	19.50 a
Trichoderma brevicompactum	114.00 abc	1.43 a	2.50 ab	17.20 a
Trichoderma longibrachiatum	83.20 bc	1.26 ab	2.25 ab	16.50 a
Fusarium inflexum	140.00 a	1.46 a	1.75 ab	15.00 a
CV (%)	13.53	8.94	6.80	12.80

[z]Means followed by the same letter do not differ from each other by the Tukey test ($p \leq 0.05$). Transformed values for $\sqrt{(x + 1)}$.

Table 4. Effect of fungal filtrates on the reproduction of *Rotylenchulus reniformis* in coriander plants.

Treatments	Eggs mass	Reproduction factor (PF/PI[y])
Control with *R. reniformis*	28.75 a[z]	14.07 a
Penicillium A25	1.25 b	0.18 b
Penicillium TA14	0.25 b	0.23 b
Penicillium citrinum	0.50 b	0.38 b
Thielavia terricola	0.75 b	0.35 b
Trichoderma harzianum	1.25 b	0.36 b
Trichoderma brevicompactum	1.00 b	0.27 b
Trichoderma longibrachiatum	0.75 b	0.19 b
Fusarium inflexum	0.50 b	0.70 b
CV (%)	26.56	15.96

[y]PF/PI: final population/initial population.
[z]Means followed by the same letters in the column do not differ from each other by the Tukey test ($p \leq 0.05$). Transformed values for $\sqrt{(x + 1)}$.

Table 5. Effects of fungal culture filtrates on development of coriander infested by *Rotylenchulus reniformis.*

Treatments	Shoot length (cm)	Dry shoot biomass (g)	Fresh root biomass (g)	Root length (cm)
Uninoculated control	21.20 ab	0.44 ab	0.25 a	11.50 a
Control with *R. reniformis*	14.20 b	0.14 b	0.00 a	9.90 a
Penicillium A25	20.20 ab	0.36 ab	0.50 a	16.50 a
Penicillium TA14	20.20 ab	0.19 ab	0.75 a	13.00 a
Penicillium citrinum	22.20 a	0.47 a	0.75 a	13.00 a
Thielavia terricola	20.00 ab	0.22 ab	0.75 a	15.50 a
Trichoderma harzianum	20.50 ab	0.16 ab	0.50 a	13.50 a
Trichoderma brevicompactum	22.70 a	0.33 ab	0.75 a	11.50 a
Trichoderma longibrachiatum	17.50 ab	0.13 b	0.25 a	12.20 a
Fusarium inflexum	21.20 ab	0.17 ab	0.75 a	12.50 a
CV (%)	8.13	5.20	1.08	13.55

[z]Means followed by the same letter do not differ from each other by the Tukey test ($p \leq 0.05$). Transformed values for $\sqrt{(x + 1)}$.

of *R. reniformis* juveniles in the *in vitro* tests and inhibiting nematode reproduction *in vivo*, *F. inflexum* promoted a significant increase in the growth of cowpea plants. *Fusarium inflexum* belongs to the *F. oxysporum* species complex. Members of this complex frequently reside in the rhizosphere of several plants, even those that are not considered hosts and are able to infect and cause diseases in several botanical species. However, in many cases, this colonization can be beneficial, favoring plant development and even increasing tolerance to abiotic stress (Di *et al.*, 2016). These fungi produce volatile organic compounds that induce the growth of plants by transport and phytohormone signaling (Bitas *et al.*, 2015). This is the first report of *F. inflexum* being used in the control of plant-parasitic nematodes.

The effects of filtrates from *A. parasiticus*, *C. lunata*, *F. solani*, *Penicillium* 20.2, and *P. lilacinus* did not differ in nematode suppression from the controls. This demonstrates that a longer period of exposure of the nematodes to the filtrates of these fungi may be required in order for them to be effective. Similar results were obtained by Singh and Mathur (2010) when analyzing the antagonistic effect of *F. solani* against *Meloidogyne incognita*, with only 8.3 and 18.5% of mortality of second-stage juveniles after 24 and 72 hr of exposure to the fungal filtrate, respectively. In the case of *C. lunata* and *P. lilacinus*, the results obtained here were different from those of Khan and Husain (1989) who recorded 100% mortality of *R. reniformis* after 48 hr. This variation in the effect of fungal isolates on mortality may be due to metabolic diversity and to the degree of toxicity to the nematode under study. In the *in vivo* tests, root length did not differ significantly from the control. According to Schomarker and Been (2013), nematodes only

affect root development when present in the soil at high densities before planting, inducing the excessive formation of lateral roots to compensate for the absorption of water and nutrients. It may also be that nematode-infected plants had the same weight as those not infected because, starting from a given time, the plants acquired constant weight due to crop maturity.

Regardless of the mechanism of action involved in the antagonism of these fungal filtrates towards *R. reniformis*, more research is necessary for the elucidation of specific questions. Studies on the parasite-host interaction and nematode biology are required to advance this technique for the control of plant-parasitic nematodes.

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