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CONTROL OF ROOT KNOT NEMATODE IN LETTUCE AND WATERCRESS WITH PREDATORY FUNGI ISOLATED FROM THE SOIL OF THE CERRADO BAIANO

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Resumo

Entre as doenças que ocorrem na alface e no agrião, as causadas pelo nematoide das galhas, *Meloidogyne* spp., são responsáveis pelo baixo desenvolvimento vegetativo destas plantas. Dessa forma este trabalho teve como objetivo avaliar a eficácia de dois isolados de fungos predadores de nematoides do gênero *Arthrobotrys*, obtidos no Cerrado baiano, e aplicados de forma individualizada ou em combinação, no controle do nematoide das galhas na alface e no agrião. Os dois isolados de fúngicos foram multiplicados em placas de Petri contendo meio CMA e incubados a 25oC,em câmara de crescimento tipo BOD, por 10 dias. No interior de uma câmara de fluxo laminar, as placas foram abertas e, com o auxílio de um furador de rolha, foram obtidos 150 discos pré-colonizados com os fungos. Em seguida, os discos foram colocados no interior de um saco plástico contendo cerca de 1000g do substrato comercial tipo vermiculita, e mantidos por 7 dias em temperatura ambiente. Após esse período, o substrato infestado com os fungos predadores de nematoides foi colocado em bandejas de mudas do tipo tubetes, e a semeadura da alface e do agrião foi realizada. Quinze dias após a semeadura da alface e do agrião, foi realizado o transplante para sacos plásticos contendo substrato esterilizado, compostos por uma mistura de solo, esterco bovino e areia na proporção de (2:1:1). Quinze dias depois do transplante, foi realizada a infestação dos substratos com 3000 ovos de *M. incognita*, sendo feitos dois furos ao lado de cada muda e realizada a aplicação dos ovos com auxílio de uma pipeta automática. Trinta e cinco dias após essa infestação com ovos foi realizada a avaliação no número de galhas e ovos no sistema radicular da alface e do agrião e avaliada a altura e peso seco da parte aérea. Os dois isolados fúngicos testados reduziram significativamente o parasitismo do nematoide das galhas na cultura da alface e no agrião.

Palavras-chave: *Meloidogyne; Arthrobotrys, Lactuca sativa L., Nasturtium officinale R.Br.*

CONTROLE DO NEMATOIDE DE GALHAS NA ALFACE E NO AGRIÃO COM FUNGOS PREDADORES ISOLADOS DOS SOLOS DO CERRADO BAIANO

Abstract

Among the diseases affecting lettuce and watercress, those caused by root-knot nematodes (*Meloidogyne* spp.) are responsible for the poor vegetative development of these plants. Therefore, this study aimed to evaluate the effectiveness of two isolates of nematode-predatory fungi from the genus *Arthrobotrys*, obtained from Cerrado soil in Bahia, applied individually or in combination, in the control of root-knot nematodes in lettuce and watercress.The two fungal isolates were propagated on Petri dishes containing CMA medium and incubated at 25°C in a BOD-type growth chamber for 10 days. Inside a laminar flow chamber, the plates were opened and, using a cork borer, 150 fungal-colonized discs were obtained. These discs were then placed into a plastic bag containing approximately 1000 g of commercial vermiculite substrate and maintained at room temperature for 7 days. After this period, the substrate colonized by the nematode-predatory fungi was placed in seedling trays (tube-type cells), and lettuce and watercress were sown. Fifteen days after sowing, seedlings were transplanted into plastic bags containing a sterilized substrate composed of a mixture of soil, cattle manure, and sand in a 2:1:1 ratio.Fifteen days after transplanting, the substrates were inoculated with 3000 Meloidogyne incognita eggs by making two holes next to each seedling and applying the eggs with the aid of an automatic pipette. Thirty-five days after nematode inoculation, the number of galls and eggs in the root system of lettuce and watercress was evaluated, along with plant height and shoot dry weight. Both fungal isolates significantly reduced root-knot nematode parasitism in lettuce and watercress.

Keyword: *Meloidogyne; Arthrobotrys*, *Lactuca sativa* L., *Nasturtium officinale R.Br.*

Introduction

Several phytosanitary problems are responsible for production losses in lettuce (*Lactuca sativa* L.) and watercress (*Nasturtium officinale* R.Br) in the field, with nematode-induced diseases being among the most significant. Among the plant-parasitic nematodes affecting lettuce and watercress, species of the genus *Meloidogyne*, commonly known as root-knot nematodes, stand out—particularly *Meloidogyne incognita* (Kofoid; White, 1919). These root galls reduce the vegetative development of plants by impairing water and nutrient absorption, and also make the plants more vulnerable to secondary infections by soilborne pathogens (Carneiro, 2000). Due to the short growing cycle of these crops, chemical control using nematicides is not recommended because of the residual effects of these products on the plants. This makes biological control a viable strategy for managing these pathogens. Among the microorganisms used in biological nematode control, predatory fungi—known for their ability to form trapping structures to capture these pathogens in the soil—have shown promising results (Araújo *et al.,* 2006). However, despite the

potential of predatory fungi in nematode management, most biological control studies involving fungi have focused almost exclusively on egg- and female-parasitic species such as *Pochonia chlamydosporia* and *Purpureocillium lilacinum*. Therefore, the aim of this study was to evaluate the potential of two isolates of the nematode-predatory fungi *Arthrobotrys*, obtained from the Cerrado biome in Bahia, applied either individually or in combination, to control root-knot nematodes in lettuce and watercress.

Materials and Methods

Isolation of Nematode-Predatory Fungi

Soil samples collected from agricultural and native areas of the Cerrado biome in the municipality of Catolândia, Bahia, were used for the isolation of nematode-predatory fungi. The samples were transported in insulated boxes and taken to the Phytopathology Laboratory at UNEB Campus IX. Fungi were isolated using the soil sprinkling method described by Barron (1977) and modified by Santos, Ferraz and Muchovej (1992), by adding a suspension containing previously homogenized soil samples to Petri dishes containing 2% water-agar medium. Subsequently, four nutrient-agar discs containing free-living nematodes were added to stimulate the formation of trapping structures by nematophagous fungi. The plates were then sealed and maintained in an incubator (BOD) at 25°C for 15 days.During this period, fungal development and the presence of nematodes captured or preyed upon by the fungi were observed using an inverted microscope. To obtain pure fungal cultures for identification, fungal structures found on nematodes were transferred to Petri dishes containing PDA (Potato Dextrose Agar) medium under sterile conditions in a laminar flow chamber. After transfer, the plates were kept in a BOD incubator set at 25°C for about 10 days until fungal development and sporulation allowed identification at the genus level.

Multiplication of *M. incognita* and Egg Extraction for Experimental Setup

The initial *M. incognita* population was obtained from roots of tomato plants (cv. Santa Cruz Kada) infected with the nematode and maintained in a greenhouse. To extract nematode eggs, roots were carefully removed from the pots, immersed in a bucket of water to wash off excess soil, and then processed according to the methodology described by Hussey and Barker (1973), as modified by Bonetti and Ferraz (1981). In this procedure, roots were blended in a 0.5% sodium hypochlorite solution for 30 seconds. The egg suspension was poured through stacked 200 and 400 mesh sieves, and the eggs retained on the 400 mesh sieve were collected. The number of eggs was quantified using a Peters counting slide under a light microscope.

Preparation of Filtrates from Nematode-Predatory Fungi

To obtain fungal filtrates, approximately 10 fungal-colonized discs, prepared as described earlier, were transferred to Erlenmeyer flasks containing liquid BD culture medium (potato broth + dextrose) inside a laminar flow chamber. The flasks were sealed and placed on an orbital shaker at 25°C for two weeks. After this period, the fungal filtrates were obtained by pouring the liquid medium through gauze into a beaker, resulting in the fungal filtrate used for treatments 3 and 4, in which Fungi 1 (Catolândia) and Fungi 2 (JCO) were combined, as described in Table 1.

Reduction of Root-Knot Nematode Parasitism in Lettuce Using Predatory Fungi from the Bahia Cerrado

The experiment was conducted in a greenhouse at the State University of Bahia (UNEB), Campus IX, located in the municipality of Barreiras. A completely randomized design was used with 10 replications and 6 treatments (Table 1).

Table 1. Description of Treatments

Treatment 1	Pre-infestation of the seedling substrate with fungal isolate from Catolândia + *M. incognita* eggs
Treatment 2	Pre-infestation of the seedling substrate with fungal isolate obtained from JCO Bioprodutos + M. incognita eggs
Treatment 3	Pre-infestation of the substrate with fungal isolate (Catolândia) + filtrate from fungal isolate obtained from JCO Bioprodutos + *M. incognita* eggs
Treatment 4	Pre-infestation of the substrate with fungal isolate from JCO Bioprodutos + filtrate from fungal isolate from Catolândia + M. incognita eggs
Treatment 5	Absolute control (no fungi, no nematodes)
Treatment 6	Inoculated control (lettuce + *M. incognita* eggs)

Two isolates of nematode-predatory fungi from the genus *Arthrobotrys* spp.—one obtained from Catolândia as described in section 2.1, and another provided by the company JCO Bioprodutos, which conducts bioprospecting of beneficial fungi in soils of the Bahian Cerrado—were propagated in Petri dishes containing CMA medium and incubated at 25°C in a BOD-type growth chamber for 10 days. After this period, in a laminar flow chamber, the plates were opened and 150 fungal-colonized discs were obtained using a cork borer. These discs were then placed into a plastic bag containing approximately 1000 g of commercial vermiculite substrate and kept at room temperature for 7 days. After incubation, the substrate colonized by the nematode-predatory fungi

was transferred to seedling trays (tube-type cells), and lettuce seeds were sown. Fifteen days after sowing, the lettuce seedlings were transplanted from the trays into plastic bags filled with sterilized substrate composed of a 2:1:1 mixture of soil, cattle manure, and sand. Fifteen days after transplanting, the substrates were infested with 3000 *M. incognita* eggs, which were applied using an automatic pipette into two holes made beside each lettuce plant. To assemble the treatments involving fungal filtrate combinations (Table 1), approximately 30 mL of fungal filtrate—obtained after growing the nematode-predatory fungi in liquid BD medium (potato broth + dextrose), as described in section 2.2—was applied, following a 15-day interval after the nematode infestation. Thirty-five days after nematode inoculation, the lettuce plants were removed from the substrate, and the roots were washed in standing water to remove all adhering soil. The roots were immersed in phloxine B for twenty minutes, and the number of galls was quantified using a stereomicroscope and a cell counter. Subsequently, nematode eggs were extracted using the Hussey and Barker (1973) technique, modified by Boneti and Ferraz (1981), as previously described, and the number of eggs per root system was determined using a light microscope. The shoot height was measured using a graduated ruler. The data obtained were tabulated and submitted to analysis of variance (ANOVA) using the F-test, and means were compared using Tukey's test at a 5% significance level, with the ASSISTAT statistical software, version 7.7 (Silva; Azevedo, 2009).

Reduction of Root-Knot Nematode Parasitism in Watercress Using Nematode-Predatory Fungi from the Bahia Cerrado

The experiment was conducted in a greenhouse at the State University of Bahia – UNEB, Campus IX. The experimental design was completely randomized, with twelve replications and three treatments. The same two isolates of nematode-predatory fungi from the genus *Arthrobotrys* spp. used in the lettuce experiment were employed. These fungi were cultured for 30 days on PDA medium at 25°C. After this period, the plates containing fungal colonies were opened, and 30 colonized discs were obtained using a cork borer. These were used to inoculate 600 g of commercial vermiculite substrate. After inoculation with the fungi, the substrate was placed into seedling trays (tube-type cells), and one watercress seed was sown per cell. Thirty days after sowing, seedlings were transplanted into plastic bags containing sterilized substrate composed of a 2:1:1 mixture of soil, cattle manure, and sand. Five days after transplanting, the substrate was infested with 3000 *M. incognita* eggs, which were inoculated into two holes beside each seedling. Thirty-five days after egg infestation, each plant was carefully removed from the bags for evaluation of the number of root galls and eggs in the root system. The shoots were placed into paper bags and dried in a forced-air oven at 65°C for 72 hours to determine dry mass. Egg extraction followed the methodology of Hussey and Barker (1973), modified by Boneti and Ferraz

(1981), and counts were performed using a light microscope. The data were tabulated and submitted to analysis of variance using the F-test, and means were compared using Tukey's test at a 5% significance level, with the ASSISTAT statistical software, version 7.7 (Silva; Azevedo, 2009).

Results and Discussion

The two isolates of nematode-trapping fungi (Catolândia and JCO), applied either in combination or individually, significantly reduced the number of galls and eggs of *M. incognita* in the lettuce root system when compared to the control (Table 2). When applied individually, the JCO fungus was more effective than the Catolândia isolate in reducing the number of galls and eggs in the lettuce root system (Table 2). Supporting these results, the same fungal isolates also proved effective in reducing root-knot nematode parasitism in tomato crops (Santos; Coimbra, 2022).

The rapid colonization of the lettuce rhizosphere, promoted by applying the fungus to the seedling substrate prior to lettuce sowing, may have enabled the formation of trapping structures, thus allowing for the control of root-knot nematodes. Previous studies have shown the importance of this pre-colonization for successful biological control mediated by predatory fungi. According to Lopes *et al.* (2007), despite the sophisticated predation mechanisms of nematode-trapping fungi, they rely on rapid soil colonization to be effective in biological control.

Table 2. Effect of two isolates of nematode-trapping fungi, obtained from the Brazilian Cerrado, on shoot height, number of galls, and eggs per gram of root in lettuce inoculated with *Meloidogyne incognita*.

	Treatments	Shoot height (cm)	Galls/g of root	Eggs/g of root
1	**Fungal isolate (Catolândia)**	27,20 a	32,00 b	1340,00 b
2	**Fungal isolate (JCO)**	25,20 b	19,00 c	984,00 c
3	**Isolates Catolândia + JCO (Filtrate)**	27,60 a	13,80 d	700,00 d
4	**JCO + Catolândia Isolate filtrates**	25,40 b	18,40 c	910,00 c
5	**Absolute control**	20,00 c	-	-
6	**Inoculated control**	10,60 d	64,40 a	2100,00 a
	CV %	3,01	9,14	6,92

* Means followed by the same letter in the column do not differ statistically from each other by Tukey's test at 5% probability.

Infestation of the lettuce seedling substrate with the fungal isolate (JCO) combined with the fungal isolate (Catolândia) resulted in the greatest reduction in the number of galls and *M. incognita* eggs when compared to the control (Table 2). However, the combination of JCO fungus using substrate infestation with Catolândia isolate applied in filtrate form did not achieve the same level of control, showing no statistical difference from the application of the JCO isolate alone (Table 1). The presence of metabolites with possible nematicidal activity in the JCO fungal isolate may have contributed to the greater reduction of the nematode population in lettuce. Several studies have already demonstrated the nematicidal effects of fungal filtrates on root-knot nematodes (Costa *et al.*, 2001). The release of proteases produced by nematode-trapping fungi has also been confirmed to have nematicidal action (Tavela, 2013). Regarding lettuce development, both fungal isolates, whether combined or not, significantly increased shoot height compared to both the absolute and inoculated controls (Table 1). The Catolândia isolate, when applied alone in the substrate with or without the addition of JCO fungal filtrate, resulted in the greatest lettuce growth (Table 2). Soares (2006) observed a significant increase in the shoot weight of lettuce, compared to the control, when nematode-trapping fungi of the genus Arthrobotrys were applied to the soil. The observed increase in growth, provided by the Catolândia isolate (Table 2), can be attributed the greater availability of nutrients in the rhizosphere, such as phosphorus, resulting from the fungus's activity, and the stimulation of plant root growth due to the production of growth-promoting substances, notably indoleacetic acid (IAA). This capacity is corroborated by Saranya and Patel (2022), who demonstrated the competence of the genus *Arthrobotrys* in solubilizing phosphate and secreting IAA, thereby promoting plant development.

Reduction of Root-Knot Nematode Parasitism in Watercress Using Nematode-Trapping Fungi from the Cerrado of Bahia

The two tested isolates of nematode-trapping fungi significantly inhibited the number of galls and eggs per system in the root system of watercress when compared to the control (Table 3). However, although there was a reduction in nematode parasitism in the roots of watercress, this did not result in an increase in the dry matter of the plants compared to the control (Table 3). Santos and Coimbra (2022) conducted tests using the same fungal isolates to control M. incognita in tomato plants, where they found a significant reduction in nematode infection, with control rates ranging from 66.78% to 70.38% compared to the control. This highlights the potential of both fungal isolates as biological control agents against *M. incognita.*

Table 3. Effect of nematode-trapping fungi on the control of *Meloidogyne incognita* in watercress.

	Treatments	Dry matter (g)	Galls/g of root raiz	Eggs/g of root
1	**Control**	1,132 a	24a	3000a
2	**Fungal isolate (JCO)**	1,084 a	9,4b	1092b
3	**Fungal isolate (Catolândia)**	1,49 a	8,4b	968b
	CV %	24,31	28,65	6,05

* Means followed by the same letter in the column do not differ statistically from each other according to Tukey's test at 5% probability.

Despite not having influenced the greatest vegetative growth of the watercress, the prior infestation of substrates used for watercress seedling production with nematode-trapping fungi proved effective in reducing root-knot nematode parasitism (Table 3). The application of these nematophagous fungi during the seedling stage likely facilitated better adaptation to the plant rhizosphere, ensuring more effective colonization of this environment. In the context of leafy vegetable crops such as arugula and lettuce, this approach of applying fungi to substrates is more cost-effective compared to field application, since the fungus is already present in the seedling substrate. The use of nematode-trapping fungal isolates obtained from the Cerrado soil in Bahia showed potential for nematode control in lettuce and arugula; however, further studies are needed to evaluate their control capacity against root-knot nematodes under field conditions.

Conclusion

The two isolates of nematode-trapping fungi obtained from the soil of the Bahia Cerrado significantly reduced root-knot nematode parasitism in the root systems of lettuce and watercress, highlighting the potential of these fungi for nematode control through application in seedling production substrates.

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