Research Article

Efficacy of *Metarhizium anisopliae* and *Purpureocillium lilacinum* isolates in the control of *Meloidogyne incognita* race 3 in cotton[1]

Bruno Scentinela Jacintho Paes[2], Henrique Roberto de Araújo[3],
Claudio Marcelo Gonçalves de Oliveira[2], José Eduardo Marcondes de Almeida[2]

ABSTRACT

Plant-parasitic nematodes such as *Meloidogyne incognita* cause severe damage to the cotton root system, impairing plant development and yield. The use of fungi-based nematicides presents a sustainable and promising alternative for nematode management. Two greenhouse experiments were conducted to evaluate the efficacy of isolates of the *Metarhizium anisopliae* and *Purpureocillium lilacinum* entomopathogenic fungi in controlling *M. incognita*, in TMG91WS3 cotton cultivar. The experiment 1 included 12 treatments: one inoculated control, four *M. anisopliae* isolates and seven *P. lilacinum* isolates, with four replicates. At 162 days after sowing, all treatments significantly reduced the final population of *M. incognita*, except *M. anisopliae* IBCB Ma03. The *P. lilacinum* IBCB Pl06 and *M. anisopliae* IBCB Ma04 isolates were the most effective, with reproduction factors below 1 and efficiency close to 80 %. The experiment 2 included 11 treatments: one control, four *M. anisopliae* and six *P. lilacinum* isolates, with six replicates. After 97 days, only the IBCB Pl06 isolate differed from the control in the final population variable, although all tested isolates, except *M. anisopliae* IBCB Ma03, showed positive efficacy for the *P. lilacinum* (> 90 %) and *M. anisopliae* IBCB Ma04 (85 %) isolates.

KEYWORDS: *Gossypium hirsutum*, root-knot nematode, entomopathogenic fungi.

RESUMO

Eficácia de isolados de *Metarhizium anisopliae* e *Purpureocillium lilacinum* no controle de *Meloidogyne incognita* raça 3 em algodoeiro

Fitonematoides como *Meloidogyne incognita* causam sérios danos ao sistema radicular do algodoeiro, comprometendo a sua produtividade. Nematicidas à base de fungos representam uma alternativa sustentável e promissora para o manejo de nematoides. Dois experimentos em casa-de-vegetação foram conduzidos para avaliar a eficácia de isolados dos fungos entomopatogênicos *Metarhizium anisopliae* e *Purpureocillium lilacinum* no controle de *M. incognita*, em algodoeiro cv. TMG91WS3. O experimento 1 incluiu 12 tratamentos: um controle inoculado, quatro isolados de *M. anisopliae* e sete isolados de *P. lilacinum*, com quatro repetições. Aos 162 dias após a semeadura, todos os tratamentos reduziram significativamente a população final de *M. incognita*, exceto *M. anisopliae* IBCB Ma03. Os isolados *P. lilacinum* IBCB Pl06 e *M. anisopliae* IBCB Ma04 foram os mais eficazes, com fator de reprodução abaixo de 1 e eficiência próxima de 80 %. O experimento 2 incluiu 11 tratamentos: um controle, quatro isolados de *M. anisopliae* e seis de *P. lilacinum*, com seis repetições. Após 97 dias, apenas o isolado IBCB Pl06 diferiu do controle na variável população final, embora todos os isolados testados, exceto *M. anisopliae* IBCB Ma03, tenham apresentado eficácia positiva para isolados de *P. lilacinum* (> 90 %) e *M. anisopliae* IBCB Ma04 (85 %).

PALAVRAS-CHAVE: *Gossypium hirsutum*, nematoide-das-galhas, fungos entomopatogênicos.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is a crop of strategic importance for Brazilian agribusiness. Brazil's cotton production for the 2024/2025 season is projected at 3.95 million tons, reflecting a 10.3 % increase in the planted area, when compared to the previous season (Abrapa 2025). However, despite these significant gains, several biotic constraints continue to limit yield. Among these, soil-borne pathogens, particularly plant parasitic nematodes, are persistent threats to cotton and various other crops (Perina et al. 2022).

Meloidogyne incognita (Kofoid & White 1919) Chitwood 1949, also called southern root-knot

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[2] Instituto Biológico, Campinas, SP, Brazil. *E-mail/ORCID*: brunoscentinelajacinthopaes@gmail.com/0000-0002-3076-6344; marcelonematologia@gmail.com/0000-0002-1677-6853; jose.marcondes@sp.gov.br/0000-0003-2551-6313.
[3] Universidade Estadual de Campinas, Campinas, SP, Brazil. *E-mail/ORCID*: henriqueroberto58@gmail.com/0009-0006-7474-4215.
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nematode, is among the most damaging nematodes affecting cotton, being known for its broad host range, infecting over 3,000 plant species and causing substantial economic losses (Lopes & Ferraz 2016, Soares 2022).

To mitigate the damage caused by plant parasitic nematodes, various control methods have been developed, including chemical, cultural and biological approaches. In the context of biological control, several fungal agents have shown nematicidal activity, particularly *Purpureocillium lilacinum* (syn. *Paecilomyces lilacinus*), *Pochonia chlamydosporia* and *Trichoderma* spp., all of which have demonstrated efficacy against plant parasitic nematodes (Siddiqui & Mahmood 1996, Poveda et al. 2020, Stone & Bidochka 2020, Zarrin et al. 2015).

More recently, attention has turned to *Metarhizium anisopliae* (Metsch.) Sorokīn, a soil-inhabiting entomopathogenic fungus, which has potential biocontrol as agent for plant parasitic nematodes. Preliminary studies reported up to 76.9 % of reduction for *M. incognita* populations in coffee roots (*Coffea arabica* L. cv. Novo Mundo) treated with *M. anisopliae* (Oliveira et al. 2021). This fungus produces a range of secondary metabolites, such as toxins and enzymes, which possible contribute to its nematicidal activity and plant growth promotion (Poveda et al. 2020).

Additional investigations have highlighted the effectiveness of *M. anisopliae* against *Heterodera avenae* and *Meloidogyne javanica* (Ghayedi & Abdollahi 2013, Abdollahi 2018). Ghayedi & Abdollahi (2013) conducted a study on *M. anisopliae*, isolating the fungus from soil samples in Boyer-Ahmad, Iran. *In vitro* assays showed a high pathogenicity against second-stage juveniles (J2) of *H. avenae*, with parasitism rates ranging from 14.9 to 47.1 %, depending on the conidial concentration (10^3 to 10^7 conidia mL^{-1}). Abdollahi (2018) demonstrated that soil application of *M. anisopliae* spore suspension, combined with plant residues such as chopped oak stems and leaves, significantly reduced the number of galls, egg masses and eggs of *M. javanica* in tomato plants. Similarly, Devi (2018) emphasized the need to expand the use of *M. anisopliae* in sustainable agriculture systems, highlighting its dual function as both a biocontrol agent and a promoter of plant growth and yield.

Based on this context, the present research aimed to evaluate the individual effects of *M. anisopliae* and *P. lilacinum* isolates on the control of *M. incognita* in cotton, as well as on agronomic performance indicators.

MATERIAL AND METHODS

Two greenhouse experiments were conducted under controlled conditions at the Instituto Biológico, in Campinas, São Paulo state, Brazil (22º54'S, 47º00'W and altitude of 707 m).

The average temperature during the experiments was 32 ± 3 ºC. The plots consisted of 800-cm^3 plastic pots filled with 750 cm^3 of autoclaved commercial substrate Tropstrato Florestal® (pH of 6.0; composed of pine bark, vermiculite and single superphosphate), with one cotton plant per pot.

Cotton cv. 'TMG91WS3' was used in both experiments. Two untreated seeds were sown in each pot, and one seedling was maintained after emergence. The weaker seedling was removed by cutting the shoot to avoid root disturbance.

To preserve nematode virulence, the isolate was periodically inoculated on different host plants. Annual identification of *M. incognita* was confirmed using perineal pattern morphology (Kleynhans 1986, Jepson 1987) and isoenzyme electrophoresis (esterase profile), identifying the isolate as *M. incognita* race 3 (Alfenas & Brune 2006).

Nematode inoculum consisted of eggs and second-stage juveniles (J2) extracted from infected roots using a modified Coolen & D'Herde (1972) protocol. Roots were blended in a 0.5 % NaOCl solution for 60 seconds, and the suspension was filtered through a series of sieves (60-200-500 mesh; 0.250-0.074-0.025 mm). The resulting suspension was used for inoculation.

Isolates of *M. anisopliae* and *P. lilacinum* were obtained from the fungal collection maintained at the Instituto Biológico. All isolates were freshly cultured and exhibited spore viability above 80 %. The fungal isolates were preserved through lyophilization and reactivated on Petri dishes containing BDA supplemented with an antibiotic used to prevent contamination. Once sporulation was observed, conidia were scraped from the agar surface and suspended in sterile Milli-Q water containing 0.01 % Tween 80. This suspension was then inoculated onto autoclaved rice incubated under controlled conditions (25 ºC; 12:12 h light-dark photoperiod). After sufficient sporulation

(around 7 days), conidia were recovered and spore concentrations were determined using a Neubauer hemocytometer under a microscope. The fungal suspensions were standardized to a concentration of 5×10^8 viable conidia mL^{-1}. Thirty milliliters of each fungal suspension were applied via seed furrow.

The first experiment (summer-autumn, 2024) consisted of 12 treatments: one inoculated control, four *M. anisopliae* isolates (IBCB Ma01; IBCB Ma02; IBCB Ma03; IBCB Ma04) and seven *P. lilacinum* isolates (IBCB Pl01; IBCB Pl02; IBCB Pl03; IBCB Pl05; IBCB Pl06; IBCB Pl07; IBCB Pl08), each with four replicates. Inoculation with *M. incognita* was performed at sowing by placing 2,500 eggs and J2 nematodes into two 2-cm-deep holes near the seeds. Plants were maintained in the greenhouse throughout the cotton cycle and irrigated daily.

At 162 days after sowing (DAS), the following variables were evaluated: final nematode population in the roots (Fp) and reproduction factor (Rf = Fp/initial population); nematode suppression efficiency compared to control (E%), calculated according to the Abbott's formula, using the expression: E% = [(C - T)/C] × 100, where C represents the mean value observed in the control treatment and T the mean value in the treated group; root fresh mass; gall index, determined using the following rating scale: 0 = no galls; 1 = 1 to 2; 2 = 3 to 10; 3 = 11 to 30; 4 = 31 to 100; 5 = more than 100 galls per root; and shoot dry mass. Nematodes were extracted from the roots using the same method as for inoculum preparation, whereas the nematode counts were performed under a light microscope using Peters counting slides with two 1-mL subsamples.

The second experiment was conducted under the same greenhouse conditions during summer, 2024. This experiment included 11 treatments: one inoculated control, four *M. anisopliae* isolates (IBCB Ma01; IBCB Ma02; IBCB Ma03; IBCB Ma04) and six *P. lilacinum* isolates (IBCB Pl01; IBCB Pl02; IBCB Pl03; IBCB Pl04; IBCB Pl05; IBCB Pl06), with six replicates per treatment. Inoculation was performed as in the first experiment, using 2,400 *M. incognita* specimens per replicate. Fungal applications followed the same methodology. Evaluations were carried out at 97 DAS and included the same variables: final population, reproduction factor, suppression efficiency, root fresh mass, gall index and shoot dry mass.

Both experiments were conducted in a completely randomized design. Statistical analyses were performed using the Sisvar software (Ferreira 2011) and treatment means compared by the Tukey test at 5 % of significance. When necessary, data were log-transformed [log (x + 1)] to meet the assumptions of normality, which were verified using the Shapiro-Wilk test.

RESULTS AND DISCUSSION

In the experiment 1, all fungal isolates significantly reduced the final population (Fp) of *M. incognita*, if compared to the control, except for the IBCB Ma03 isolate, which was the only one showing similarity. The untreated control showed the highest Fp (9,135) and reproduction factor (Rf) of 3.6. In contrast, fungal treatments reduced the Rf values to 0.8-2.2. The highest means in suppressing the nematode were observed for *M. anisopliae* IBCB Ma04 (Fp = 1,922; Rf = 0.8) and *P. lilacinum* IBCB Pl06 (Fp = 1,984; Rf = 0.8), with nematode suppression efficiency compared to control (E%) of 79 and 78.3 %, respectively. Other well-performing isolates included *M. anisopliae* IBCB Ma01 and *P. lilacinum* IBCB Pl03 and Pl02, with E% above 68 % and Rf values between 0.9 and 1.1 (Table 1).

The gall index ranged from 0.25 to 2, with no significant differences among the treatments, except for the IBCB Ma04 and Pl06 isolates, which showed the lowest indices (0.25), indicating fewer root galls. The root fresh mass ranged from 27.3 to 55.3 g. Although significant differences were observed between the IBCB Ma03 and IBCB Pl02 isolates, none of the treatments differed statistically from the control. IBCB Ma03 produced the highest root fresh mass (55.3 g), although it was not among the most effective in nematode control. Shoot dry mass was the highest for the IBCB Ma02 (52.1 g) and IBCB Ma04 (55.1 g) isolates, but not surpassing the control (38.5 g) (Table 1).

In the experiment 2, most of the treatments didn't differ from the untreated control (Fp = 32,185; Rf = 13.4) on the reproduction of *M. incognita*. The *P. lilacinum* IBCB Pl04 isolate reduced these values, with *P. lilacinum* IBCB Pl04 (Fp = 690; Rf = 0.3) achieving E% of 97.9 %. Although no significant differences were observed among the other treatments, *M. anisopliae* IBCB Ma04 showed a high efficiency value (E% = 84.7 %; Rf = 2.1).

Conversely, IBCB Ma03 resulted in the highest nematode population (Fp = 122,705; Rf = 51.1), suggesting a possible stimulatory effect on pathogen reproduction (Table 2).

The gall index ranged from 2.00 to 4.66, with the lowest values observed in treatments with *P. lilacinum* IBCB Pl04 (2.00), Pl03 (2.33) and Pl02 (2.75) isolates (Figure 1). Regarding agronomic variables, no significant differences were observed for root fresh mass, which ranged from 6.28 to 8.15 g, or shoot dry mass (7.4 to 9.2 g) (Table 2).

The use of entomopathogenic fungi to control plant parasitic nematodes has gained increasing attention as a sustainable alternative to chemical nematicides, particularly for *M. incognita* (Fontes & Valadares-Inglis 2020, Oliveira et al. 2021). While *P. lilacinum* is widely recognized and commercially used in the biological control of this nematodes (Isaac et al. 2023, Khan & Tanaka 2023), the present study highlights, in a novel and significant way, the high potential of some *M. anisopliae* isolates in managing *M. incognita* in cotton.

The earliest documented use of *M. anisopliae* in this context was reported by Rossi et al. (2006), who applied the fungus at 30 kg ha^{-1} of fungal powder to the soil of a sugarcane field using a backpack sprayer, diluted in 100 L ha^{-1} of water. The treatment with *Metarhizium anisopliae* (30 kg ha^{-1}) significantly reduced the population of *Meloidogyne* spp. in roots, with a mean of 157.5 individuals per 10 g of root,

Table 1. Final population (Fp) and reproduction factor (Rf) of *Meloidogyne incognita*, and gall index (GI), root fresh mass (RFM) and shoot dry mass (SDM) of cotton treated with *Metarhizium anisopliae* and *Purpureocillium lilacinum* isolates in the experiment 1.

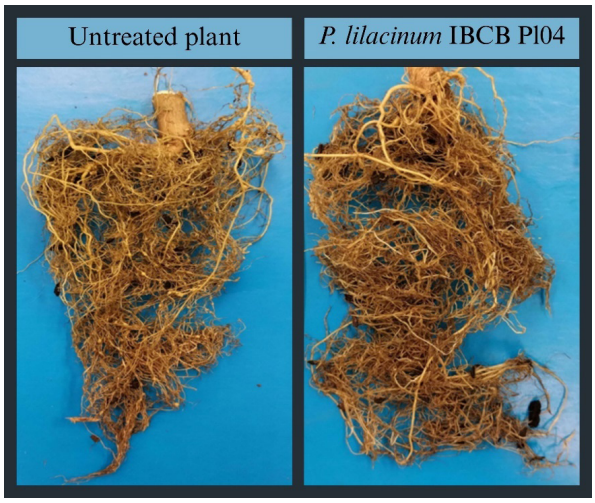
Treatments	Fp	E%	Rf	RFM (g)	GI	SDM (g)
Control	9,135 a*	-	3.6 a	36.9 ab	2.0 bc	38.5 ab
M. anisopliae IBCB Ma01	2,167 b	76.3 a	0.9 b	45.8 ab	1.6 abc	41.7 ab
M. anisopliae IBCB Ma02	4,762 b	47.9 c	1.9 b	29.6 ab	2.7 bc	52.1 a
M. anisopliae IBCB Ma03	5,633 ab	38.3 c	2.2 ab	55.3 a	2.2 bc	42.1 ab
M. anisopliae IBCB Ma04	1,922 b	79.0 a	0.8 b	28.3 ab	0.2 a	55.1 a
P. lilacinum IBCB Pl01	3,666 b	59.9 b	1.5 b	41.5 ab	0.7 ab	35.2 b
P. lilacinum IBCB Pl02	2,910 b	68.1ab	1.1 b	27.3 b	2.0 bc	43.7 ab
P. lilacinum IBCB Pl03	2,180 b	76.1 a	0.9 b	29.6 ab	1.5 abc	40.9 ab
P. lilacinum IBCB Pl05	2,691 b	70.5 ab	1.0 b	49.5 ab	2.0 bc	29.9 b
P. lilacinum IBCB Pl06	1,984 b	78.3 a	0.8 b	28.9 ab	0.2 a	33.2 b
P. lilacinum IBCB Pl07	3,600 b	60.6 b	1.4 b	31.3 ab	1.7 abc	32.5 b
P. lilacinum IBCB Pl08	2,867 b	68.6 ab	1.11 b	36.4 ab	2.0 bc	39.1 ab

* Means of four replicates. Means followed by the same letter in the column did not differ according to the Tukey test (0.05). E%: nematode suppression efficiency compared to control.

Table 2. Final population (Fp) and reproduction factor (Rf) of *Meloidogyne incognita*, and gall index (GI), root fresh mass (RFM) and shoot dry mass (SDM) of cotton treated with *Metarhizium anisopliae* and *Purpureocillium lilacinum* isolates in the experiment 2.

Treatments	Fp	E%	Rf	RFM (g)	GI	SDM (g)
Control	32,185 a*	-	13.4 b	8.1 a	4.7 a	7.6 a
M. anisopliae IBCB Ma01	24,733 ab	23.2 c	10.3 b	8.0 a	4.0 a	7.4 a
M. anisopliae IBCB Ma02	10,810 ab	66.4 b	4.5 c	7.0 a	4.0 a	8.1 a
M. anisopliae IBCB Ma03	122,705 a	-281.2 d	51.1 a	7.3 a	4.2 a	8.5 a
M. anisopliae IBCB Ma04	4,927 ab	84.7 a	2.1 c	7.6 a	4.2 a	8.4 a
P. lilacinum IBCB Pl01	8,430 ab	73.8 ab	3.5 c	7.1 a	2.7 a	7.8 a
P. lilacinum IBCB Pl02	4,685 ab	85.4 a	2.0 c	6.9 a	2.7 a	8.0 a
P. lilacinum IBCB Pl03	1,016 ab	96.8 a	0.4 c	6.3 a	2.3 a	9.0 a
P. lilacinum IBCB Pl04	690 b	97.9 a	0.3 c	7.6 a	2.0 a	7.9 a
P. lilacinum IBCB Pl05	3,212 ab	90.0 a	1.3 c	7.6 a	3.5 a	9.2 a
P. lilacinum IBCB Pl06	5,210 ab	83.8 a	2.2 c	6.7 a	3.0 a	8.9 a

* Means of six replicates. Means followed by the same letter in the column did not differ according to the Tukey test (0.05). E%: nematode suppression efficiency compared to control.



Photos: Bruno Scentinela Jacintho Paes

Figure 1. Comparison between cotton roots of plants untreated and treated with *Purpureocillium lilacinum* IBCB P104. Treated roots show a visibly lower number of galls, when compared to the control, indicating the effectiveness of the treatment.

when compared to 1,470 in the untreated control. This result ranked it among the most effective treatments to control *Meloidogyne*. For *Pratylenchus* spp., although no statistical difference was observed, if compared to the control (590.0), the treatment with *M. anisopliae* showed the lowest mean population (172.5), indicating a promising trend in reducing this nematode as well.

More recently, Oliveira et al. (2021) evaluated the response of banana and coffee plants to *M. incognita* infection and assessed the biological control potential of *M. anisopliae* and *P. lilacinum*. Four experiments were conducted using banana cv. Prata Anã and coffee cvs. Arara and Mundo Novo. Across all trials, fungal treatments effectively reduced nematode densities. The Rf values were 4.2 in banana treated with 4.8 kg ha^{-1} of fungal powder of *M. anisopliae*, 2.3 in coffee cv. Arara treated with 70 g ha^{-1}, and 1.2-1.3 in cv. Mundo Novo treated with 2.4 kg ha^{-1}. Notably, *M. anisopliae* achieved up to 76.9 % of nematode reduction, with results statistically similar to those of *P. lilacinum*. However, when both fungi were combined, Rf values were unexpectedly higher, suggesting possible antagonistic effects.

In contrast, the current research reports even more promising outcomes for *M. anisopliae*, particularly for the IBCB Ma04 isolate. In the experiment 1, Ma04 achieved an Rf of 0.8 and a control efficiency of 79 %. In the experiment 2, this isolate maintained a high effectiveness, reaching a control efficiency of 84.7 %, with an Rf of 2.1. These results not only surpass those obtained by Oliveira et al. (2021), but also demonstrate the potential of this isolate in cotton, an application not previously explored. The consistent performance of Ma04 across both experiments underscores its robustness and reinforces its suitability for integration into nematode management programs. Moreover, these findings suggest that *M. anisopliae*, when using well-characterized and effective isolates, may deliver nematode suppression equal to or better than that provided by *P. lilacinum*.

In the experiment 1, all treatments were statistically similar to the control for shoot dry mass. However, it is worth noting that *M. anisopliae* IBCB Ma02 and Ma04 promoted an increase in shoot dry mass, suggesting potential plant growth benefits, probably associated with induced systemic resistance or growth promotion (Ghayedi & Abdollahi 2013, Moosavi & Zare 2020).

Although *P. lilacinum* remains the most studied and commercially adopted fungi-based biocontrol agent for nematode management (Fontes & Valadares-Inglis 2020, Isaac et al. 2023, Khan & Tanaka 2023), some *M. anisopliae* isolates tested in this study demonstrated a comparable and, in some cases, superior efficacy. This expands the range of available biocontrol options, contributing to the diversification of agents used in sustainable nematode management programs.

Ghayedi & Abdollahi (2013) suggest that, although the mode of action of *M. anisopliae* in nematodes is not yet fully understood, it likely follows a mechanism similar to that of other fungi with adhesive spores, which are capable of attaching to the nematode cuticle, germinating, penetrating directly and developing infective hyphae within the body cavity. In pathogenicity tests, the authors reported juveniles of *H. avenae* parasitized by *M. anisopliae*. Furthermore, entomopathogenic fungi have been observed to colonize plant roots asymptomatically as endophytes. *M. anisopliae* is recognized both as an entomopathogen and a soil-dwelling endophyte, capable of colonizing root tissues and contributing to plant development and enhanced resistance to pests and diseases (Sasan & Bidochka 2012, Altinok et al. 2019). Despite these findings, further studies are required to elucidate the mechanisms by which

Brazilian isolates of *M. anisopliae* promote plant growth and suppress nematode populations.

Finally, the evaluated agronomic parameters suggest that the *M. anisopliae* application does not negatively affect plant development. Altogether, these results position *M. anisopliae* as a promising candidate for inclusion in integrated nematode management strategies.

CONCLUSIONS

1. Both *Purpureocillium lilacinum* and *Metarhizium anisopliae* showed potential for the biological control of *Meloidogyne incognita* in cotton crops;
2. The IBCB Ma04 isolate of *M. anisopliae* demonstrated equal or superior efficacy to *P. lilacinum* in reducing the nematode population.

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