PURPUREOCILLIUM LILACINUM AND *METARHIZIUM ANISOPLIA*E ISOLATES FOR BIOCONTROL AGAINST LESION AND ROOT-KNOT NEMATODES INFECTING SUGARCANE IN GREENHOUSE CONDITIONS

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ABSTRACT

Sugarcane is a major crop in Brazil, and its extended growth cycle favors the buildup of plant-parasitic nematodes such as *Pratylenchus zeae* and *Meloidogyne javanica*. This study evaluated the effects of different isolates of *Metarhizium anisopliae* and *Purpureocillium lilacinum* from the Biological Institute on nematode suppression and sugarcane performance. Two greenhouse experiments were conducted: Experiment I used naturally infested soil, and Experiment II used sterilized substrate inoculated with *P. zeae*. In Experiment I, isolates PI06 and Ma04 reduced *P. zeae* by 59% and 66%, respectively, while PI02 and PI06 reduced *M. javanica* by 87% and 68%. In Experiment II, Ma04 reduced *P. zeae* by 74% (Rf = 2.5), and PI06 by 64% (Rf = 3.5). Both isolates improved plant growth. These results show that Ma04 and PI06 are promising agents for nematode management in sugarcane.

Keywords: biological control, *Meloidogyne javanica*, *Pratylenchus zeae*, sustainable agriculture

RESUMO

A cana-de-açúcar é uma cultura de grande importância no Brasil, e seu ciclo prolongado favorece o acúmulo de nematoides fitoparasitas, como *Pratylenchus zeae* e *Meloidogyne javanica*. Este estudo avaliou os efeitos de diferentes isolados de *Metarhizium anisopliae* e *Purpureocillium lilacinum*, provenientes do Instituto Biológico, na supressão de nematoides e no desempenho agronômico da cana-de-açúcar. Dois experimentos em casa de vegetação foram conduzidos: o Experimento I utilizou solo naturalmente infestado, enquanto o Experimento II utilizou substrato esterilizado inoculado com *P. zeae*. No Experimento I, os isolados PI06 e Ma04 reduziram *P. zeae* em 59% e 66%, respectivamente, enquanto PI02 e PI06 reduziram *M. javanica* em 87% e 68%. No Experimento II, Ma04 reduziu *P. zeae* em 74% (FR = 2,5) e PI06 em 64% (FR = 3,5). Ambos os isolados promoveram o crescimento das plantas. Esses resultados mostram que Ma04 e PI06 são agentes promissores para o manejo de nematoides na cultura da cana-de-açúcar.

Palavras-chave: controle biológico, *Meloidogyne javanica*, *Pratylenchus zeae*, agricultura sustentável

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INTRODUCTION

The importance of sugarcane (*Saccharum* spp. L.) in Brazil dates back quite a few centuries and, in the current context, it remains a key commodity for the national economy, particularly in São Paulo State. The 2024/25 harvest reached an estimated production of 676.96 million tons (CONAB, 2025). However, this yield was achieved under adverse climatic conditions, marked by low rainfall and high temperatures, factors that directly impact crop development (FLACK-PRAIN et al., 2021). Such edaphoclimatic stressors not only limit plant growth but also intensify the damage caused by plant-parasitic nematodes, as they exacerbate symptoms typically observed in the aerial parts of the plant. Among the main pathogens affecting sugarcane are nematodes from the genera *Pratylenchus* and *Meloidogyne*, whose effects are worsened under drought stress and high temperatures, leading to significant yield losses even in managed areas (CADET & SPAULL, 2005). Given that this climatic pattern is likely to persist in future seasons, the need for effective monitoring and control strategies becomes increasingly critical, particularly in long cycle crops such as sugarcane (FLACK-PRAIN et al., 2021).

Sugarcane favors the increase in population density of plant-parasitic nematodes (PPN) associated with its cultivation, since under favorable conditions for the pathogen, multiple successful reproductive cycles can occur (CASTILHO & VOVLAS, 2007). In tropical countries, like Brazil, *Pratylenchus zeae* Graham, 1951, *Meloidogyne javanica* (Treub, 1885; Chitwood, 1949), and *M. incognita* (Kofoid & White, 1919; Chitwood, 1949) are considered the key species of economic importance to the crop (CADET & SPAULL, 2005). These nematodes have been widely distributed throughout the country for decades, causing severe root damage and significantly compromising the agronomic performance of the plant (NOVARETTI et al., 1974; SASSER & FRECKMAN, 1987).

Several strategies have been developed to manage parasitic nematodes, including chemical, cultural, and biological approaches. In biological control, the use of fungi has gained prominence, with *Purpureocillium lilacinum* (Thom) (syn. *Paecilomyces lilacinus*), *Pochonia chlamydosporia*, and *Trichoderma* spp. demonstrating significant activity against nematodes such as *Meloidogyne* spp. and *Pratylenchus* spp. (STONE & BIDOCHKA, 2020). *Metarhizium anisopliae* (Metsch.) Sorokīn, well-known as an entomopathogenic fungus, has emerged as a potential biological agent for managing PPN (OLIVEIRA et al., 2021; ROSSI et al., 2006). The effectiveness of *M. anisopliae* has also been observed against other PPN in different countries, such as *Rotylenchulus reniformis* in Egypt, *Heterodera avenae* in Iran, and species of *Meloidogyne* in India (STONE & BIDOCHKA, 2020). Both experiments by Ghayedi and Abdollahi (2013), conducted in Iran *in vitro*, demonstrated the fungus's potential against *H. avenae*, reinforcing its candidacy for commercial development. In light of the above, the present research evaluated the individual effectiveness of different *M. anisopliae* and *P. lilacinum* isolates in suppressing *P. zeae* and *M. javanica*, as well as their effects on the agronomic performance of sugarcane in greenhouse conditions.

MATERIAL AND METHODS

Plant material

The sugarcane cv. 'CTC 3445' seedlings were provided by Centro de Tecnologia Canavieira – CTC (Piracicaba, São Paulo State, Brazil) and used in both experiments. At the time of transplanting, seedling height was standardized

at 30 cm. The experiments were conducted with one seedling per plastic pot with a total volume of 1.7 L were used. The pot layout was defined by an upper radius of 12 cm, a lower radius of 9.7 cm, and a height of 20.7 cm. The pots were filled with 1,000 cm³ of infested soil (Experiment 1) or autoclaved substrate Tropstrato Florestal® (Experiment 2) (pH of 6.0; composed of pine bark, vermiculite and single superphosphate – Autoclaved for 121ºC for 2 hours), and 20 days after transplanting, 1 g of N-P-K fertilizer (4-14-8) was applied per pot. Both experiments were conducted under greenhouse conditions, and plants were watered twice daily during periods of higher temperatures. The experiments were carried out during the summer–autumn period of 2024 and the summer period of 2025 in Campinas, Brazil. Due to greenhouse conditions, internal temperatures frequently reached 32–34 °C and night-time temperatures of approximately 23–25 °C, under a natural photoperiod.

Fungal isolates and application

Isolates of *Purpureocillium lilacinum* and *Metarhizium anisopliae* were obtained from the fungal collection maintained at the Instituto Biológico, Campinas, São Paulo State, Brazil (22°54'S, 47°00'W; altitude 707 m). The isolates were freshly cultured and exhibited conidial viability above 80%. Fungal isolates were preserved by lyophilization and subsequently reactivated on Petri dishes containing potato dextrose agar (PDA) supplemented with an antibiotic to prevent bacterial contamination. After visible sporulation, conidia were gently scraped from the agar surface and suspended in sterile Milli-Q water containing 0.01% (v/v) Tween 80 to ensure homogeneous dispersion. This suspension was then used to inoculate autoclaved rice, which was incubated under controlled conditions (25 °C, 12:12 h light–dark photoperiod) to promote mass sporulation.

After approximately seven days of incubation, when abundant sporulation was observed, conidia were recovered from the rice substrate by a dry separation process. The sporulated rice was placed in a 20-mesh sieve (0.25 mm aperture) and manually agitated for approximately five minutes. During agitation, conidia were detached from the rice grains and, by gravitational force, passed through the sieve openings. The detached conidia were collected in a clean plastic bag positioned directly beneath the sieve. This method allowed efficient separation of dry conidia from the substrate without mechanical damage. Subsequently, conidial concentration was determined using a Neubauer hemocytometer under an optical microscope (Leica DMLS). The fungal suspension was standardized to a concentration of 1×10^8 viable conidia mL^{-1}. Thirty milliliters of each suspension were applied on soil close the seedlings.

Experiment I - Reaction of fungal isolates in naturally infested field soil

The first experiment was conducted under greenhouse conditions during the summer–autumn period (January to May, 2024) and consisted of seven treatments: one inoculated control, four *P. lilacinum* isolates (IBCB Pl 01; 02; 06 and 07), and two *M. anisopliae* isolates (IBCB Ma03 and 04), each with five replicates (totaling 35 experimental units). For the experiment, soil samples were collected from sugarcane fields in Ribeirão Preto, State of São Paulo, naturally infested with *Meloidogyne javanica* and *Pratylenchus zeae* (275 *M. javanica* and 200 *P. zeae* per 1,000 cm³ of soil), using a shovel to a depth of 0–20 cm, and nematodes were extracted from soil samples using the Jenkins (1964) centrifugal flotation and sieving method. The soil was subjected to physicochemical characterization and classified as sandy clay, presenting a

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slightly acidic pH (6.2). Mineral fertilization was standardized and adjusted according to the nutritional requirements of sugarcane, ensuring uniform nutrient availability across all experimental units.

At 131 days after transplanting (DAT), the following parameters were evaluated: fresh root mass (g); shoot dry mass (g); shoot height (cm), final nematode population in the roots (Fp) to *M. javanica* and *P. zeae*; 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. Nematodes were extracted from the roots using a modified protocol by Coolen and D'Herde (1972). To standardize the extraction process, roots were cut into small pieces using scissors, and only 20 grams of root tissue per plant were processed. To estimate the variable Fp, the values obtained were adjusted based on the total fresh root mass of each plant. Nematode counts were performed under a light microscope (Leica DMLS) using Peters counting slides with two 1 mL subsamples.

Experiment II - Evaluation of fungal isolates on *Pratylenchus zeae* reproduction under artificially inoculated substrate

The experiment was conducted under greenhouse conditions during the summer–autumn period (January to April 2025). The *P. zeae* isolates used in the second experiment were obtained from specimens recovered at the end of the first experiment and subsequently maintained in the Nematology Laboratory, Biological Institute, Campinas, State of São Paulo, Brazil (22°54' S, 47°00' W; 707 m a.s.l.). To preserve nematode aggressiveness, populations were periodically rotated on maize and sugarcane plants, until the natural senescence of the plants. Nematode inoculum was extracted from infected roots using the same protocol by Coolen and D'Herde (1972). The resulting suspension, containing eggs and motile forms, was used for inoculation. A total of 2,000 *P. zeae* specimens (eggs and motile forms) per pot were inoculated into the soil through two holes made diagonally to the seedling roots.

This experiment included five treatments: one inoculated control, two *M. anisopliae* isolates (IBCB Ma03 and 04), and two *P. lilacinum* isolates (IBCB Pl06 and 07), with four replicates per treatment, totaling 20 experimental units. Both the treatment application and nematode inoculation were conducted on the same day as seedling transplantation. Fungal applications followed the same methodology as Experiment I, and the evaluations were carried out at 109 DAT or days after the inoculation (DAI). In this experiment, the same variables were evaluated in Experiment I, with the addition of the reproduction factor [Rf = Fp / initial population inoculated (2,000)].

Statistical analysis

The experiments were conducted in a completely randomized design. Statistical analyses were performed using Sisvar software (FERREIRA, 2011), and treatment means were compared using the least significant difference (LSD) test at a 5% significance level. When necessary, data were log-transformed [log (x + 1)] to meet the assumptions of normality, which were assessed using the Shapiro–Wilk test.

RESULTS AND DISCUSSION

Experiment I:

The evaluation of the effects of different isolates of *P. lilacinum* and *M. anisopliae* on sugarcane, conducted 131 days after transplanting (Table 1), revealed significant differences among the analyzed variables. The *P. lilacinum* isolate IBCB Pl06 stood out by promoting greater shoot height (205 cm), higher shoot dry mass (34 g), and greater fresh root mass (158 g), demonstrating its effectiveness both in promoting plant development and in reducing nematode populations. In contrast, the untreated control plants, exhibited the lowest values for these variables, with an average height of 150 cm, 17 g of shoot dry mass, and 110 g of fresh root mass, reflecting the negative impact of *M. javanica* and *P. zeae* infestation in the absence of treatment.

About the final population of *P. zeae* (Fp), the isolates IBCB Pl06 (6,840 specimens) and *M. anisopliae* IBCB Ma04 (5,940 specimens) were the most effective in reducing nematode density, with suppression rates (E%) of 59% and 66%, respectively, compared to the control (16,984 specimens). Conversely, *P. lilacinum* isolates IBCB Pl01 (26,768 specimens) and IBCB Pl07 (23,120 specimens) exhibited final populations higher than the control, indicating a possible lack of effect or even a stimulatory effect on the nematode. Regarding *M. javanica*, the lowest Fp values were observed with *P. lilacinum* isolates IBCB Pl02 (130 specimens) and IBCB Pl06 (324 specimens), reflecting high suppression rates of 87% and 68%, respectively.

Table 1. Evaluation of different fungal isolates of *Purpureocillium lilacinum* (Pl) and *Metarhizium anisopliae* (Ma) on sugarcane plants 131 days after transplanting under greenhouse conditions using infested soil from commercial sugarcane plantations. The following parameters were assessed: shoot height (SH), shoot dry mass (SDM), fresh root mass (FRM), final population (Fp) in 20 g of roots (*Meloidogyne javanica* – *M.j.* and *Pratylenchus zeae* – *P.z.*), nematode suppression efficiency compared to the control (E%).

Treatment	SH (cm)	SDM (g)	FRM (g)	Fp		E (%)	
				P.z.	M.j.	P.z.	M.j.
IBCB Pl06	205 a	34 a	158 a	6,840 c	324 b	59	68
IBCB Ma04	188 ab	27 ab	121 ab	5,940 c	420 b	66	59
IBCB Pl07	188 ab	33 a	126 ab	23,120 a	200 b	0	80
IBCB Pl02	186 ab	29 ab	99 b	14,590 b	130 b	14	87
IBCB Ma03	172 ab	23 bc	106 b	14,544 b	1,319 a	14	0
IBCB Pl01	161 b	22 bc	94 b	26,768 a	210 b	0	79
Control	150 b	17 c	110 b	16,984 b	1,028 a	--	--

*Means of five replicates. Means followed by the same letter in column did not differ according to LSD test (0.05).

Figure 1. Shoot height (SH) of sugarcane seedlings cv. CTC 3445 treated with different fungal isolates of *Purpureocillium lilacinum* (Pl) and *Metarhizium anisopliae* (Ma) at 131 days after transplanting in soil infested with *Meloidogyne javanica* and *Pratylenchus zeae* – Experiment 1.

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Experiment II:

In the evaluation conducted 109 days after inoculation with *P. zeae* (Table 2), all tested fungal isolates significantly reduced the nematode population and the reproduction factor (Rf) compared to the control. The control plants showed the highest values of Fp (20,062 specimens) and Rf = 10.0, while *M. anisopliae* IBCB Ma04 showed the lowest values (Fp = 5,102 specimens; Rf = 2.5), followed by *P. lilacinum* IBCB Pl06 (Fp = 7,102 specimens; Rf = 3.5). These isolates also promoted the highest values among the agronomic variables, particularly shoot height, with *M. anisopliae* IBCB Ma03 reaching 147 cm and IBCB Pl06 reaching 140 cm (Figure 1).

Table 2. Evaluation of different fungal isolates of *Purpureocillium lilacinum* (Pl) and *Metarhizium anisopliae* (Ma) on sugarcane plants 109 days after inoculation. The following parameters were assessed: shoot height (SH), shoot dry mass (SDM), fresh root mass (FRM), final population (FP) of *Pratylenchus zeae*, nematode suppression efficiency compared to control (E%), and reproduction factor [Rf = Fp/ initial population (2,000)].

Treatment	SH (cm)	SDM (g)	FRM (g)	Fp	E (%)	Rf
IBCB Pl06	140 a	35 a	73 a	7,102 b	64	3.5 b
IBCB Ma04	115 ab	22 a	51 abc	5,102 b	74	2.5 b
IBCB Pl07	119 ab	19 a	36 c	7,787 b	61	3.9 b
IBCB Ma03	147 a	34 a	65 ab	10,503 b	47	5.2 b
Control	80 b	15 a	45 bc	20,062 a	--	10.0 a

*Means of four replicates. Means followed by the same letter in column did not differ according to LSD test (0.05).

The role of fungi is crucial in the rhizosphere through their interactions with nematodes, contributing to nematode suppression across various soil types and geographic regions (SIDDIQUI & MAHMOOD, 1996). These interactions may occur via direct parasitism of nematodes (CHEN & CHEN, 2003; FATEMY et al., 2005) or through the secretion of enzymes and nematicidal metabolites that impair nematode viability (CHEN et al., 2000).

Entomopathogenic fungi have gained prominence in PPN management, with *P. lilacinum* already well established in commercial nematicidal formulations (FONTES & VALADARES-INGLIS, 2020; OLIVEIRA et al., 2021). Recent studies confirm *P. lilacinum* as an effective biocontrol agent against sedentary nematodes in eggplant (*Solanum melongena* L.) and tomato (*Solanum lycopersicum* L.) (ISAAC et al., 2023; KHAN & TANAKA, 2023). Therefore, the results presented in this work demonstrated a high potential for novel and unmarketed *P. lilacinum* isolates in suppressing *P. zeae* and *M. javanica* in sugarcane crops.

The results obtained agree with Jayakumar et al. (2023), who demonstrated the high efficacy of *P. lilacinum* in the management of *P. zeae* in sugarcane. In the field trial conducted by the authors, the application of the fungus at a dose of 2.5 kg/ha resulted in a 75.9% reduction in nematode population, with a prolonged effect observed up to harvest (360 days). Similarly, the IBCB Pl06 isolate was responsible for a significant suppression of *P. zeae* (59% in experiment I and 64% in experiment II). In addition, IBCB Pl06 also demonstrated a high suppression rate against *M. javanica*, with a reduction of 68% in the final population compared to the untreated control. Moreover, both studies reported positive agronomic impacts. Jayakumar et al. (2023) reported significant increases in the number of stalks per hectare, cane yield, and sugar content. The IBCB Pl06 isolate promoted significant increases in plant height, shoot dry mass, and fresh root mass compared to the untreated control.

Even more innovative are the findings regarding *M. anisopliae* isolates, expanding the known role of this species beyond insect management. Among fungi with nematicidal activity, *M. anisopliae* has attracted significant interest. Although its precise mode of action against nematodes is not fully elucidated, Ghayedi and Abdollahi (2013) proposed a mechanism similar to that other fungi with adhesive spores

that attach to the nematode cuticle, germinate, penetrate directly, and develop infective hyphae inside the host. Pathogenicity tests demonstrated *M. anisopliae* parasitizing *H. avenae* juveniles. Moreover, entomopathogenic fungi can colonize plant roots asymptomatically as endophytes, promoting plant growth and enhancing resistance to pests and diseases (ALTINOK et al., 2019; SASAN & BIDOCHKA, 2012). Despite these findings, further studies are needed to elucidate the mechanisms by which Brazilian isolates of *M. anisopliae* enhance plant growth and suppress nematode populations, a fact observed in the present research, as the plants increased shoot height and dry mass when treated with *M. anisopliae*, alongside reduced nematode population densities.

The nematicidal efficacy of *M. anisopliae* against *Rotylenchulus reniformis*, *H. avenae*, and *Meloidogyne* spp. has been documented in Egypt, Iran, and India (GHAYEDI & ABDOLLAHI, 2013; STONE & BIDOCHKA, 2020; YOUSSEF et al., 2020). Despite these reports, studies on nematode management using *M. anisopliae* remain scarce, especially in tropical crops like sugarcane, underscoring the relevance of this Brazilian study in providing novel and context-specific data.

The efficacy of *M. anisopliae* in the control of PPN in Brazil was previously documented by Rossi et al. (2006), who treated sugarcane fields with 30 kg/ha of the fungus and observed significant reductions in *Pratylenchus* spp. and *Meloidogyne* spp. populations. In that work, *M. anisopliae* showed statistically comparable performance to treatments with neem oil (3%) and Biopirol (3% and 5%), emphasizing the potential of this entomopathogenic fungus as a promising alternative for nematode management.

The results obtained in the present study reinforce that *M. anisopliae* is an effective option, especially the IBCB Ma04 isolate, which showed the highest suppression efficiency of *P. zeae* (74%) in the second experiment (Table 2), along with a reproduction factor (Rf = 2.5) and final population (Fp = 5,102 specimens), being statistically lower than that observed in the control plants. Similarly, the IBCB Ma03 isolate promoted an increase in shoot height (147 cm), demonstrating that, in addition to its nematicidal action, this fungal species can enhance crop agronomic performance. These findings support those reported by Rossi et al. (2006) regarding the ability of *M. anisopliae* to reduce nematode densities. A noteworthy aspect is that, unlike Rossi's study, the present work details the nematicidal effect at the species level rather than just the genus level, providing even more valuable information for integrated nematode management, especially since *P. zeae* is more aggressive toward sugarcane than other *Pratylenchus* species common in Brazil, such as *P. brachyurus* (CADET & SPAULL, 2005).

Recently, Oliveira et al. (2021) evaluated the response of banana and coffee plants to *M. incognita* infection and assessed the potential of *M. anisopliae* and *P. lilacinum*. Notably, *M. anisopliae* achieved up to 76.9% nematode reduction, with results statistically similar to those of *P. lilacinum*. In a study conducted on cotton, Paes et al. (2025) demonstrated that isolates of *M. anisopliae* and *P. lilacinum* were effective in reducing the population of *M. incognita* race 3 under greenhouse conditions. Both fungi significantly decreased nematode reproduction parameters compared with the inoculated control, confirming their antagonistic potential against root-knot nematodes in an annual crop system. The consistency of these results with those observed in the present study reinforces the broad-spectrum nematicidal activity of these fungal species across different host plants.

Although sugarcane, the crop used in the present study, is not classified as perennial, its

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semi-perennial nature and long cycle resemble the nematode management practices used in perennial crops like coffee and banana. A common feature among these crops is that growers must deal with nematode infestations over multiple consecutive seasons, which increases the need for long-term management strategies (NICKLE, 1991). In this context, *M. anisopliae* isolate IBCB Ma04 showed even more promising outcomes and agreed with findings of Oliveira et al. (2021) and Paes et al. (2025). In Experiment 1, Ma04 reduced *P. zeae* by 66%, and in Experiment 2 by 74%. These results reinforce that *M. anisopliae*, when properly selected, may equal or outperform *P. lilacinum* in nematode management programs.

CONCLUSION

The *M. anisopliae* and *P. lilacinum* isolates proved effective biocontrol against lesion and root-knot nematodes infecting sugarcane in greenhouse conditions, then improving sugarcane growth.

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