



Full Length Article

Assessment of Biological Products for the Control of *Meloidogyne javanica* in Sugarcane

Franciele Alves Carneiro¹, Renato Andrade Teixeira², Gabriel Silva Belo³, Eder Marques⁴ and Mara Rubia da Rocha^{5*}

¹PhD, Universidade Federal de Goiás, College of Agronomy, Phytosanitary Department, Nematology, Avenida Esperança, s/n, 74.690-900, Campus Samambaia, Goiânia, Brasil

²Professor (0000-0002-8819-9186), Instituto Federal de Mato Grosso, Campus Sorriso, Avenida dos Universitários, nº 799, Bairro Santa Clara, 78.895-150, Sorriso, MT, Brasil

³Master's student, Universidade Federal de Goiás, College of Agronomy, Phytosanitary Department, Nematology, Postgraduate Program in Agronomy, Avenida Esperança, s/n, 74.690-900, Campus Samambaia, Goiânia, Brasil

⁴Professor (0000-0003-3014-0517), Universidade Federal de Goiás, College of Agronomy, Phytosanitary Department, Plant Pathology Research Center (NPF), Postgraduate Program in Agronomy (PPGA), Avenida Esperança, s/n, 74.690-900, Campus Samambaia, Goiânia, Brasil

⁵Professor (0000-0001-8338-4107), Universidade Federal de Goiás, College of Agronomy, Phytosanitary Department, Nematology, Postgraduate Program in Agronomy (PPGA), Avenida Esperança, s/n, 74.690-900, Campus Samambaia, Goiânia, Brasil

*For correspondence: mrocha@ufg.br

Received 25 August 2025; Accepted 29 November 2025; Published online 11 February 2026

Editor: Muhammad Amjad Ali

Abstract

Root-knot nematodes (*Meloidogyne* spp.) significantly impair sugarcane production, causing annual losses of approximately 30% and reducing crop longevity. The increasing use of microbial agents in nematode management highlights their potential as key tools in integrated control strategies. This study aimed to compare the efficacy of different commercial biological products on the population density of *Meloidogyne javanica* and on the development of sugarcane (variety RB 867515). Three greenhouse experiments were conducted using six treatments: Nemix C[®] (*Bacillus subtilis* + *Bacillus licheniformis*), Nemat[®] (*Purpureocillium lilacinum*), Ecotrich[®] (*Trichoderma harzianum*), Serenade[®] (*B. subtilis*), NemOut[®] (*B. subtilis* + *B. licheniformis* + *T. longibrachiatum*), and a control. Across all experiments, the biological products significantly suppressed *M. javanica* populations, with control rates exceeding 70% in Experiment I, 80% in Experiment II, and 75% in Experiment III. Importantly, none of the treatments negatively affected plant growth, as indicated by fresh and dry shoot mass and fresh root mass. Overall, the evaluated biological products consistently reduced nematode populations while maintaining normal plant development, demonstrating their strong potential for use in sugarcane nematode management.

Keywords: *Bacillus* spp.; Microbiological management; *Purpureocillium lilacinum*; *Saccharum* spp.; *Trichoderma* spp

Introduction

Sugarcane (*Saccharum* spp.) is a semi-perennial grass of the Poaceae family, initially domesticated in New Guinea (Babu *et al.* 2022). It is recognized as one of the most important crops both in Brazil and internationally, it is primarily utilized as a raw material in the production of its two main products, sugar and ethanol, while also contributing to employment and income generation (Defante *et al.* 2020). Brazil is the world's leading producer of sugarcane (FAO 2023), with an estimated production of approximately 676.8 million tons in

the 2024/2025 harvest (CONAB 2025). The country also stands as the largest exporter and producer of sugar, accounting for 38.7 million tons, and the second largest producer of ethanol globally (CONAB 2025). Brazilian sugarcane production has more than doubled in recent decades; however, the adverse effects of this growth, particularly environmental impacts and implications for production processes, remain insufficiently understood (Bordonal *et al.* 2018). Intensive sugarcane cultivation demands knowledge, inputs, and technological advancements that can influence crop development (Raza and Yeng 2021).

To cite this paper: Carneiro FA, RA Teixeira, GS Belo, E Marques, MRD Rocha (2026). Assessment of biological products for the control of *Meloidogyne javanica* in sugarcane. *Intl J Agric Biol* 35:350303. <https://doi.org/10.17957/IJAB/15.2448>

© 2026 The Authors. International Journal of Agriculture and Biology published by Friends Science Publishers, Faisalabad, Pakistan

This is an open access article under the terms of the Creative Commons Attribution License, which permits non-commercial use, distribution and reproduction in any medium, provided the original work is properly cited

Alterations in the physical, chemical, and biological environments caused by sugarcane monoculture (Tayyab *et al.* 2021), coupled with climate change, may contribute to the emergence of pests and diseases, such as phytonematodes (Hussain *et al.* 2019; Dutta and Phani 2023).

The genus *Meloidogyne*, commonly referred to as root-knot nematodes, comprises some of the most significant phytonematodes affecting sugarcane crops, resulting in considerable yield losses worldwide (Bellé *et al.* 2024; Dababat *et al.* 2025). The extent of these losses varies depending on the specific nematode species and the sugarcane cultivar involved (Dinardo-Miranda *et al.* 2019; Bhuiyan and Garlick 2021; Bellé *et al.* 2024). In this context, *M. javanica*, a species commonly found in warm and tropical climates, has a broad host range (CABI 2021) and is considered the most detrimental species in sugarcane, capable of causing substantial losses (Bhuiyan and Garlick 2021; Bhuiyan *et al.* 2024). Over the past few decades, nematode control in sugarcane has predominantly relied on the application of organophosphate and carbamate nematicides (Dinardo-Miranda 2018; Bhuiyan and Garlick 2021). While these chemicals were previously regarded as effective in rapidly suppressing nematode populations, their use is increasingly contested due to environmental concerns, adverse effects on human health, high costs, extended residual activity, restrictions on usage, and inconsistent efficacy under varying environmental conditions (Dinardo-Miranda 2018; Chen *et al.* 2020; Dutta and Phani 2023). Consequently, the exploration and development of alternative nematode management strategies, such as the use of biological control agents, are essential. These alternatives should be integrated with other control measures to enhance overall management effectiveness (Dinardo-Miranda 2018; Bhuiyan *et al.* 2024).

Among the measures that can be employed in the integrated management of nematodes, biological control has emerged as one of the most environmentally safe and effective approaches (Tranier *et al.* 2014). According to a meta-analysis conducted by Benedetti *et al.* (2021), approximately 659 articles focusing on this topic have been published since 2000, suggesting that the use of microorganisms has proven to be efficient in controlling *M. javanica* nematodes, although few studies have been conducted within the pathosystem of this nematode species × sugarcane. Among the most effective soil-borne fungi for nematode biocontrol, *Purpureocillium lilacinum* (Thom) Samson is one of the best-known. The mechanisms of this fungus primarily affect nematode eggs through the production of leucinotoxins, proteases, chitinases, and acetic acid, and it has also been reported to parasitize mobile nematode stages and sedentary females (El-Habashy *et al.* 2021; Xu *et al.* 2021; Saleh *et al.* 2023; Rigobelo *et al.* 2024). Certain species of the genus *Trichoderma* Pers. are also effective in controlling root-knot nematodes, acting through the parasitism of eggs and juveniles *via* the enzymatic activity of chitinase and protease, releasing toxic metabolites that prevent root penetration and parasite

multiplication, while also stimulating plant defense mechanisms (El-Habashy *et al.* 2021; Lubian *et al.* 2021; Tariqjaveed *et al.* 2021; Yao and Chen 2023). In addition to nematophagous fungi, endophytic bacteria also contribute to the suppressive activity against this group of nematodes. Among them, various *Bacillus* species stand out, synthesizing toxic metabolites that can affect the reproductive cycle of phytonematodes, particularly during oviposition and egg hatching. These bacteria are also characterized by their ability to interfere with plant-host recognition, modifying root exudates into byproducts that prevent the detection of the chemotropic stimulus by infective nematode forms, thereby inhibiting root penetration. Furthermore, similar to other biocontrol agents, these bacteria promote plant growth through the synthesis of phytohormones such as auxin, gibberellin and cytokinin (Mazzuchelli *et al.* 2020; El-Habashy *et al.* 2021; Dinardo-Miranda *et al.* 2022; Gattoni *et al.* 2023). In this context, the present study aimed to evaluate the effectiveness of various commercial biological products based on *Purpureocillium lilacinum*, *Bacillus* spp. and *Trichoderma* spp. for the management of *Meloidogyne javanica* in sugarcane cultivated under controlled greenhouse conditions.

Materials and Methods

Description of treatments and experimental design

Three experiments were conducted at different times under controlled greenhouse conditions. The treatments applied in this study were as follows: (1) *Bacillus subtilis* + *B. licheniformis* (Nemix® 1.0 kg p.c./ha); (2) *Purpureocillium lilacinum* (Nemat® 600 g p.c./ha); (3) *Trichoderma harzianum* (Ecotrich® 600 g p.c./ha); (4) *B. subtilis* (Serenade® 4.0 L p.c./ha); (5) *B. subtilis* + *B. licheniformis* + *T. longibrachiatum* (Nemout™ 15 kg p.c./ha); and (6) an untreated control. The solution volumes for each microbial product were prepared in accordance with the dosage recommendations provided on the manufacturer's label. The solution products were applied to the roots of the seedlings or to the cuttings using a 5 mL micropipette at the time of transplantation into the bags. In Experiments I and II, mini-stems of the sugarcane variety RB867515 were used as the propagation material, while in Experiment III, seedlings from a commercial nursery of the same variety were employed. In Experiments I and II, mini-stems containing a single bud were used because this material is commonly adopted in commercial sugarcane propagation and allows rapid and uniform plant establishment under greenhouse conditions. In Experiment III, pre-grown seedlings were selected to ensure plants with more developed root systems at the time of inoculation, enabling reliable evaluation at later assessment dates (60 and 90 DAI). Therefore, the choice of propagation material in each experiment was based on the specific objectives and duration of the trials, aiming to minimize variability in plant growth and nematode infection dynamics.

Experiments I, II, and III were all conducted under greenhouse conditions using a completely randomized design (CRD). Experiment I was carried out from March to May, with average minimum and maximum temperatures of 19°C and 47°C, respectively. It followed a 5 × 2 factorial scheme, with five treatments and two evaluation times (30 and 60 days after inoculation – DAI), using five replicates per treatment. Experiment II took place from September to November, with average temperatures ranging from 19°C to 40°C. It consisted of six biological treatments, also with five replicates, and a single evaluation at 60 DAI. Experiment III was conducted from October to January, with temperatures between 20°C and 47°C. It followed a 6 × 2 factorial scheme, including six biological treatments and two evaluation periods (60 and 90 DAI), with five replicates.

The 60-day-old sugarcane seedlings and mini-cuttings, each containing one bud, were transferred to 3-L polyethylene bags (17 × 30 cm) filled with a substrate composed of a 1:1 (v/v) mixture of washed medium-grade sand and a loamy soil previously collected from an agricultural area free of nematicide application. The substrate was thoroughly homogenized and sterilized by autoclaving at 120°C and 1 atm for 20 min., following a double cycle of autoclaving on consecutive days to ensure complete elimination of microorganisms.

Inoculum and inoculation of *Meloidogyne javanica*

The *M. javanica* population used as inoculum originated from Rocha *et al.* (2025), where its identity was previously confirmed through esterase phenotype analysis following Carneiro and Almeida (2001). This population was originally collected from naturally infested fields and has been maintained under controlled greenhouse conditions, ensuring its stability, viability, and pathogenicity. We selected this population because it is well characterized, widely used in nematology research in Brazil, and reliably reproduces under greenhouse conditions, providing consistent inoculum for experimental assays. The inoculum was multiplied on tomato plants of the Santa Cruz Kada Gigante cultivar and maintained in a greenhouse. After 90 days, the eggs were extracted following the method of Coolen and D'Herde (1972). The egg concentration was determined using a Peters counting chamber, and the suspension was calibrated to 600 eggs per mL.

Inoculation was performed 30 days after planting the cuttings and, in the seedlings, 7 days after transplanting, by depositing 5 mL of the inoculum suspension, containing a population density of approximately 3,000 eggs + J2 (second-stage juveniles) of *M. javanica* per plant. The suspension was applied around the plants in two equidistant holes in the soil, approximately 3 cm deep.

Variables evaluated

The variables evaluated in the experiments were: fresh root

mass (FRM), fresh and dry mass of the aerial part (FMAP and DMAP) and nematode population density (PD) (eggs + J2/10 g of root). To obtain the dry mass of the aerial part, the plants were collected and had their roots washed and separated from the aerial part, and both parts were weighed to determine the fresh mass of each plant. Then, the leaves were placed in paper bags and in an oven at 65°C for 72 h or until reaching constant weight and, subsequently, their dry mass was determined. The nematodes were also extracted from the roots by the method of Coolen and D'Herde (1972). After extraction, the nematodes were counted with the aid of the Peters chamber under an optical microscope (40x). The population density (PD) of *M. javanica* was expressed as the number of eggs + J2/10 g of roots. The control index (CI) was calculated based on the relationship between the control's PD and the PD observed in treatments with biological products.

Statistical analysis

The data obtained were subjected to verification of homogeneity, and those that did not indicate normal distribution were transformed using the Box and Cox (1964) algorithm, with the Action Stat 3.7 software (2014). The analysis of variance was performed using the F test at the 1% probability level, and the comparisons of the means between the treatments were conducted using the Tukey test at the 5% probability level, with the Assistat 7.7 software (Silva and Azevedo 2016).

Results

Based on the experiments, it was observed that all microbial products were effective in reducing the population density of *Meloidogyne javanica* in sugarcane under greenhouse conditions, although they did not affect plant development (Table 1-3). The formulations Nemix (*Bacillus subtilis* + *Bacillus licheniformis*), Nemat (*Purpureocillium lilacinum*), and Ecotrich (*Trichoderma harzianum*) were the most effective in reducing the nematode population across the three evaluations. The results of each assay are presented below.

Experiment I

There was no interaction between the biological products and the evaluation periods for any of the variables analyzed. This indicates that the effect of the products did not depend on the time of evaluation (30 or 60 DAI). The vegetative variables, fresh and dry mass of the aerial part (FMAP and DMAP) and fresh root mass (FRM), were only affected by the evaluation periods (Table 1), with higher values at 60 DAI. This result was expected, as it reflects the natural growth and biomass accumulation of sugarcane plants over time. Therefore, biological agents had no effect on plant development.

Table 1: Effect of bioformulated products on fresh and dry mass of the aerial part (FMAP and DMAP), fresh root mass (FRM), and population density (PD) of *Meloidogyne javanica* (eggs + J2/10 g of roots) in sugarcane, variety RB 867515, after 30 and 60 days of inoculation

Bioformulation	Experiment I												CI ^y
	FMAP (g)			DMAP (g)			FRM (g)			PD ^x			
	30 DAI	60 DAI	Average	30 DAI	60 DAI	Average	30 DAI	60 DAI	Average	30 DAI	60 DAI	Average	
<i>P. lilacinum</i>	23.63	47.20	35.41	3.694	11.07	7.38	2.96	5.89	4.42	149	534	341 bc ^z	73.72
<i>T. harzianum</i>	28.51	33.51	31.01	4.54	8.36	6.45	2.96	4.97	4.06	453	752	603 ab	53.54
<i>B. subtilis</i>	21.55	40.78	31.16	3.35	8.36	5.85	3.15	5.79	4.47	185	438	312 c	75.96
<i>B. subtilis</i> + <i>B. licheniformis</i> + <i>T. longibrachiatum</i>	24.64	49.80	37.22	3.92	13.20	8.56	3.49	7.57	5.53	192	1.073	632 abc	51.30
Control	25.21	54.16	39.69	4.06	13.71	8.88	2.63	6.40	4.52	337	2.258	1298 a	-
Average	24.71 B	45.09 A	34.90	3.91 B	10.94 A	7.42	3.08 B	6.12 A	4.60	263 B	1.011 A	637	-
CV%	40.19			41.53			58.42			12.21			-

^xBox-Cox transformed data^yControl index (%)^zAverages followed by the same capital letter in the row and lowercase letter in the column do not differ significantly from each other (Tukey 5%)**Table 2:** Effect of bioformulated products on fresh and dry mass of the aerial part (FMAP and DMAP), fresh root mass (FRM), and population density (PD) of *Meloidogyne javanica* (PD) (eggs + J2/ 10 g of roots) in sugarcane, variety RB 867515, after 60 days of inoculation

Bioformulation	Experiment II				
	FMAP (g)	DMAP (g)	FRM ^w (g)	PD ^x	CI ^y
<i>B. subtilis</i> + <i>B. licheniformis</i>	25.97	5.62	7.09	149 b ^z	84.74
<i>P. lilacinum</i>	29.30	6.38	7.73	187 b	80.85
<i>T. harzianum</i>	23.79	5.69	6.00	149 b	84.74
<i>B. subtilis</i>	26.36	5.94	4.71	260 b	73.38
<i>B. subtilis</i> + <i>B. licheniformis</i> + <i>T. longibrachiatum</i>	26.57	5.64	6.08	280 ab	71.34
Control	25.10	5.45	5.74	977 a	-
CV%	30.42	32.56	37.83	18.01	-

^wNon-significant^xBox-Cox transformed data^yControl index (%)^zAverages followed by the same lowercase letter in the column do not differ significantly from each other (Tukey 5%)**Table 3:** Effect of bioformulated products on fresh and dry mass of aerial part (FMAP and DMAP), fresh root mass (FRM) and population density (PD) of *Meloidogyne javanica* (DP) (eggs + J2/ 10 g of roots) in sugarcane, variety RB 867515 after 60 and 90 days of inoculation

Bioformulation	Experiment III												CI ^y
	FMAP (g)			DMAP (g)			FRM (g)			PD ^x			
	60 DAI	90 DAI	Average	60 DAI	90 DAI	Average	60 DAI	90 DAI	Average	60 DAI	90 DAI	Average	
<i>B. subtilis</i> + <i>B. licheniformis</i>	30.23	34.16	32.19	6.33	8.7	7.52	8.52	11.29	9.90	637	2.454	1.545 b ^z	80.33
<i>P. lilacinum</i>	21.01	39.95	30.48	4.67	10.69	7.68	8.28	14.16	11.22	600	5.364	2.982 b	62.03
<i>T. harzianum</i>	24.51	44.53	34.52	4.98	12.29	8.64	8.32	15.23	11.77	396	3.293	1.845 b	76.51
<i>B. subtilis</i>	21.93	38.7	30.31	4.59	10.9	7.75	8.28	11.32	9.80	817	5.393	3.105 ab	60.46
<i>B. subtilis</i> + <i>B. licheniformis</i> + <i>T. longibrachiatum</i>	20.71	41.37	31.04	4.23	10.69	7.46	7.91	13.51	10.71	1.418	3.334	2.376 b	69.74
Control	23.54	37.71	30.62	4.95	10.41	7.68	7.82	13.54	10.68	2.828	12.879	7.853 a	-
Average	23.65 B	39.4 A	31.53	4.96 B	8.7 A	7.52	8.52 B	11.29 A	9.90	637 B	2.454 A	1.545	-
CV%	26.15			29.19			13.13			13.08			-

^xBox-Cox transformed data^yControl index (%)^zAverages followed by the same capital letter in the row and lowercase letter in the column do not differ significantly from each other (Tukey 5%)

The population density of *M. javanica* was influenced by the products used and the evaluation periods independently (Table 1). As expected, nematode population density increased at 60 DAI, following plant development and indicating ongoing nematode reproduction in the root system. The effect of the biological products was evident on the population density of *M. javanica*, with emphasis on the treatments with *B. subtilis* and *P. lilacinum*, which showed the highest control rates (75% and 73%, respectively) and differed significantly from the control. However, although

they did not differ from the control, the treatments with *T. harzianum* and *B. subtilis* + *B. licheniformis* + *T. longibrachiatum* still achieved control levels above 50%.

Experiment II

In this trial, evaluated only at 60 DAI, all bioformulated products significantly reduced the population density of *M. javanica* compared with the untreated control (Table 2). The treatments showed control indices ranging from 71% to

84%, with the formulations containing *B. subtilis* + *B. licheniformis*, *P. lilacinum* and *T. harzianum* presenting the lowest nematode densities and not differing statistically from each other. In contrast, the formulation composed of *B. subtilis* + *B. licheniformis* + *T. longibrachiatum* displayed an intermediate effect, differing from the control but showing higher population density than the most effective treatments.

Regarding plant growth variables (FMAP, DMAP and FRM), statistical comparisons were conducted as requested; however, no significant differences were detected among treatments (Table 2), indicating that under the conditions of this experiment, particularly at 60 DAI, the reductions in nematode population were not yet reflected in measurable improvements in biomass accumulation. Although numerical variation among treatments was observed, the high coefficients of variation (30–38%) and the lack of statistical significance suggest that the vegetative parameters were more variable and less sensitive than nematode population density at this early evaluation stage.

Experiment III

In this bioassay, there was no interaction between the evaluation times and biological control agents for the variables accessed. The vegetative variables FMAP, DMAP and FRM were only affected by the evaluation times, with higher values observed in the evaluation at 90 DAI, which is explained by the growth of the plants over time. Therefore, the products did not influence the development of the plants (Table 3). The effect of the biological products on the population density of *M. javanica* was significant, presenting a control index that varied from 60 to 80%, with emphasis on the treatment of commercial products containing *B. subtilis* + *B. licheniformis* and *T. harzianum* in their formulations, which presented the greatest reductions in the number of nematodes in relation to the control.

Discussion

This study evaluated biological products formulated with *Bacillus* spp. and *Trichoderma* spp. for the control of root-knot nematodes (*M. javanica*) in sugarcane under greenhouse conditions. It is known that the use of chemical nematicides has not been considered a good control measure due to environmental damage, human health risks, high costs, residual effects, and lack of consistency in control levels (Dinardo-Miranda 2018; Chen *et al.* 2020; Dutta and Phani 2023). The results observed here showed the efficiency of the tested biological products, ranging from 51.30% to 84.74%, although they did not affect plant development. This result is impressive, since Dinardo-Miranda *et al.* (2022) described a control efficiency of this nematode by cabosulfan in sugarcane, in the field, of up to 51%. In general, Nemix (*B. subtilis* + *B. licheniformis*), Nemat (*P. lilacinum*), and Ecotrich (*T. harzianum*) were the biological products that showed the greatest reductions in *M.*

javanica population density when compared to the control and other treatments.

Although high control rates were observed, the performance of some products, particularly *B. subtilis* applied alone, varied across experiments. Such variation is expected in biological control because the activity of microbial antagonists depends heavily on environmental conditions, especially temperature, moisture, and substrate characteristics. Experiments I–III were conducted at different times and used different propagation materials (mini-stems *vs.* seedlings), which may have influenced root exudation patterns and microbial colonization, ultimately affecting the consistency of biocontrol. In addition, distinct physiological stages of the plants and natural fluctuations in greenhouse temperature could have altered microbial survival, competition in the rhizosphere and secondary metabolite production, contributing to differential efficacy across experiments.

The activity of *T. harzianum* against root-knot nematodes has already been demonstrated in other studies, although there is limited information on the mechanisms involved. What is known is that the fungus acts through direct parasitism of adults and eggs; through the synthesis of lytic proteins, such as chitinase and protease, capable of degrading chitin, the main polymer that constitutes the egg, which can affect the normal embryonic development of the nematode, thus favoring the entry and colonization of eggs by the fungus. In addition, the production of toxic metabolites, which affect penetration and development, may also be related to the suppression of the number of nematodes (Tariqjaveed *et al.* 2021; Yao and Chen 2023). Few biocontrol studies specifically addressing *M. javanica* in sugarcane were found in the literature, with most available reports focusing on other crops. In pepper, according to El-Habashy *et al.* (2021), the treatment with a mixture of biocontrol agents (*Paecilomyces* + *Bacillus* + *Trichoderma*) was the most effective in reducing nematode variables. Similarly, an isolate of *T. koningiopsis* was able to reduce egg mass production of *M. javanica* in beans (Lubian *et al.* 2021).

Like *Trichoderma*, the *P. lilacinum* species showed high control rates of *M. javanica* in sugarcane. The effectiveness of this fungus can be attributed to the preferential parasitism of eggs, through the production of enzymes, such as protease and chitinase, which facilitate the entry and colonization of the fungus by degrading the egg wall, causing the death of the embryos (Xu *et al.* 2021; Rigobelo *et al.* 2024), which probably resulted in a lower number of infective juveniles and, consequently, a lower population density of the nematode. Similar to what was observed here, El-Habashy *et al.* (2021) reported that one of the best control rates of *M. javanica* in pepper was the treatment with *Paecilomyces* sp. Studies in eggplant indicated that five isolates of *P. lilacinum* were able to reduce the number of galls and the gall index of this nematode in a greenhouse (Saleh *et al.* 2023).

The application of *B. subtilis* in all experiments provided effective control over the number of nematodes when compared to the control. Furthermore, the combined use of two species (*B. subtilis* + *B. licheniformis*) was more efficient in suppressing the population of *M. javanica*, resulting in a control rate above 80%. As presented in the introductory part, antagonism is the main form of control used by this rhizobacterium, which can have a direct or indirect action on this phytoparasite. Direct antagonism involves antibiosis mechanisms, such as the synthesis of toxic substances, which inhibit or prevent the development and reproduction of the nematode. Indirect antagonism is provided by the process of induced systemic resistance and interference in plant recognition, causing the mortality of infective forms (Gattoni et al. 2023). A study conducted in a naturally infested sugarcane area showed that a strain of *B. subtilis* was able to provide effective control of *Meloidogyne* spp. (Mazzuchelli et al. 2020). According to El-Habashy et al. (2021), *Bacillus* sp. applied alone moderately reduced *M. javanica* in pepper, when compared to the treatment associated with *Trichoderma* and *Paecilomyces*. On the other hand, in field evaluations, the combined application of *Bacillus subtilis* + *B. licheniformis* reduced the *M. javanica* population in sugarcane crops (Dinardo-Miranda et al. 2022).

Another factor to be considered regarding the efficiency of rhizobacteria-based products is the concentration of endospores, the dosage of the product used, and the antagonistic potential of the different *Bacillus* strains. Nemix C (*B. subtilis* + *B. licheniformis*) presented better results than the other products, which could be associated with the higher dosages, containing a higher concentration of endospores per gram of the commercial product. However, according to the study by Dinardo-Miranda et al. (2022), there was no difference in the *M. javanica* population in sugarcane using different doses of the combination of *B. subtilis* + *B. licheniformis*. In the present study, the superior and more consistent performance of the combined formulation compared to *B. subtilis* alone may reflect synergistic interactions between strains, greater diversity of antimicrobial metabolites, and improved ability to colonize the rhizosphere under fluctuating environmental conditions.

Finally, microorganisms are known to have the ability to promote the growth of some plants, although this is not a general rule. Despite this ability as biostimulants in several crops, the treatments with microorganisms used in this study did not provide significant gains in the vegetative growth of the sugarcane variety RB867515. A similar observation was described by Mazzuchelli et al. (2020), where the application of *B. subtilis* did not result in productivity gains in sugarcane in the presence of *M. javanica*. In contrast, the combined treatment of *B. subtilis* + *B. licheniformis* resulted in a 5% gain in the productivity of sugarcane parasitized by *Meloidogyne* spp. (Dinardo-Miranda et al. 2022). The absence of a growth-promoting effect in the present study reinforces that the main contribution of these

microorganisms was nematode suppression rather than plant stimulation and suggests that growth promotion by these agents may depend strongly on environmental conditions, plant developmental stage, and crop management practices.

Conclusion

All evaluated biological products were effective in reducing the population density of *M. javanica* in sugarcane, with control rates ranging from 51% to 84%. The tested bioformulations did not affect plant development. The findings of this study highlight the promising potential of biological control agents, particularly those based on *B. subtilis*, *P. lilacinum*, and *T. harzianum*, as sustainable and effective alternatives for the management of *M. javanica*. These agents contributed significantly to the reduction of nematode populations without adversely affecting the vegetative development of sugarcane plants, thereby supporting the reduction in the use of chemical nematicides in agricultural systems. For sugarcane growers, these results reinforce the feasibility of integrating biological products into nematode management programs, potentially lowering production costs and environmental risks. Future research should include field-scale evaluations and long-term monitoring to validate the efficacy of these bioformulations under commercial production conditions and to optimize their integration into a pest management program.

Acknowledgements

This study was supported by Brazilian National Research Council CNPq with a grant for the first author.

Author Contributions

MRR and FAC planned and conducted the investigation. FAC, RAT and MRR performed the formal analysis. FAC, RAT, GSB, EM and MRR curated the data. FAC, GSB, EM and MRR contributed to the manuscript writing.

Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval

Not applicable to this paper.

References

- Action Stat (2014). Version 3.7. EstatCamp Group. São Paulo, Brazil. Available at: <https://www.estatcamp.com/> (Accessed: 02 July 2025)
- Babu KSD, V Janakiraman, H Palaniswamy, L Kasirajan, R Gomathi, TR Ramkumar (2022). A short review on sugarcane: Its domestication, molecular manipulations and future perspectives. *Genet Resour Crop Evol* 69:2623–2643
- Bellé C, R Moccellini, M Haubert, SDAE Silva, CB Gomes (2024). Impact of different *Meloidogyne* species on the development of sugarcane plants. *Anais Acad Bras Ciênc* 96:1-9
- Benedetti T, J Huzar-Novakowski, E Sord, IR Carvalho, EC Bortoluzzi (2021). Microorganisms in the biological control of root-knot nematode: A metanalytical study. *Res Soc Dev* 10:1-19
- Bhuiyan SA, K Garlick (2021). Evaluation of root-knot nematode resistance assays for sugarcane accession lines in Australia. *J Nematol* 53:1-11
- Bhuiyan SA, K Sherring, J Eglinton (2024). Parasitic nematodes of sugarcane - A major productivity impediment and grand challenges in management. *Plant Dis* 108:2945–2957
- Bordonal RDO, JLN Carvalho, R Lal, EBF Figueiredo, BG Oliveira, NLS Jr (2018). Sustainability of sugarcane production in Brazil. A review. *Agron Sustain Dev* 38:1-23
- Box GEP, DR Cox (1964). An analysis of transformations. *J Roy Stat Soc* 26:211–252
- CABI (2021). *Meloidogyne javanica* (Sugarcane eelworm). CABI Compendium. Available at: <https://doi.org/10.1079/cabicompendium.33246> (Accessed: 02 July 2025)
- Cameiro RMD, MRA Almeida (2001). Técnica de eletroforese usada no estudo de enzimas dos nematoides de galhas para identificação de espécie. *Nematol Bras* 25:35–44
- Chen J, QX Li, B Song (2020). Chemical nematicides: Recent research progress and outlook. *J Agric Food Chem* 68:12175–12188
- CONAB (2025). Monitoring the Brazilian Sugarcane Harvest. Available at: <https://www.gov.br/conab/pt-br/atuacao/informacoes-agropecuarias/safras/safradecana-de-acucar/arquivos-boletins/4o-levantamento-safrade2024-25/boletim-cana-de-acucar-4o-levantamento-2024-25> (Accessed: 02 January 2025)
- Coolen WA, CJ D'Herde (1972). *State Nematology and Entomology Research Station*. Merelbeke, Belgium
- Dababat AA, T Paulitz, SE Laasli, R Lahlali, H Li, F Mokrini, S Dreisigacker (2025). From genes to fields: Marker-assisted selection for nematode resistance in crops. *Integr Plant Biotechnol* 3:1–18
- Defante LR, OF Vilpoux, L Sauer (2020). Importance of the sugarcane industry in the formal employment in the state of Mato Grosso do Sul during the period of 2008 to 2014. *Rev Econ Soc Rur* 58:1-20
- Dinardo-Miranda LL (2018). *Nematoides e pragas da cana-de-açúcar*, 2nd edn. Instituto Agronômico Campinas (IAC), Brasil
- Dinardo-Miranda LL, ID Miranda, HDS Silva, JV Fracasso (2022). Biological control of phytoparasitic nematodes in sugarcane fields. *Pesq Agropec Trop* 52:1-7
- Dinardo-Miranda LL, JV Fracasso, ID Miranda (2019). Damage caused by *Meloidogyne javanica* and *Pratylenchus zaei* to sugarcane cultivars. *Summa Phytopathol* 45:146–156
- Dutta TK, V Phani (2023). The pervasive impact of global climate change on plant-nematode interaction continuum. *Front Plant Sci* 14:1-14
- El-Habashy D, G Amer, A Eid (2021). Efficacy of *Paecilomyces*, *Bacillus* and *Trichoderma* as biocontrol agents against *M. javanica* on pepper under greenhouse conditions. *Assiut J Agric Sci* 52:9–9
- FAO (2023). Countries by commodity: Sugar cane. Available at: https://www.fao.org/faostat/en/#rankings/countries_by_commodity (Accessed: 02 January 2025)
- Gattoni KM, SW Park, KS Lawrence (2023). Evaluation of the mechanism of action of *Bacillus* spp. to manage *Meloidogyne incognita* with split root assay, RT-qPCR and qPCR. *Front Plant Sci* 13:1-13
- Hussain S, A Khaliq, U Mehmood, T Qadir, M Saqib, MA Iqbal, S Hussain (2019). *Sugarcane Production under Changing Climate: Effects of Environmental Vulnerabilities on Sugarcane Diseases, Insects and Weeds*. IntechOpen, London, United Kingdom
- Lubian C, OJ Kuhn, RL Portz, AM Agustinha, JR Stangarlin (2021). Biological control of *Meloidogyne javanica* in bean plants by *Hohenbuehelia* spp. and *Trichoderma koningiopsis*. *Arq Inst Biol* 88:1-9
- Mazzuchelli RCL, EHL Mazzuchelli, FF Araujo (2020). Efficiency of *Bacillus subtilis* for root-knot and lesion nematodes management in sugarcane. *Biol Contr* 143:104185
- Raza QUA, Y Geng (2021). Sugarcane industrial byproducts as challenges to environmental safety and their remedies: A Review. *Water* 13:1-19
- Rigobelo EC, D Nicodemo, OO Babalola, N Desoignies (2024). *Purpureocillium lilacinum* as an agent of nematode control and plant growth-promoting fungi. *Agronomy* 14:1-12
- Rocha MR, E Marques, DR Faria, MCC Filippi, FG Araújo, RA Teixeira (2025). Acibenzolar-s-methyl and neem oil in the management of nematodes in sugarcane. *Sugar Tech* 27:357–366
- Saleh HM, LMA Ayyash, F Shafeeq (2023). Efficiency of isolates of the fungus, *Paecilomyces lilacinus* to control disease root-knot nematode, *Meloidogyne javanica* on eggplant. In: *IOP Conference Series: Earth and Environmental Science*, Vol. 1252, pp:1-7. IOP Publishing
- Silva FAS, CAV Azevedo (2016). The assistat software version 7.7 and its use in the analysis of experimental data. *Afr J Agric Res* 11:3733–3740
- Tariqjaveed M, T Farooq, AS Al-Hazmi, MD Hussain, AU Rehman (2021). Role of *Trichoderma* as a biocontrol agent (BCA) of phytoparasitic nematodes and plant growth inducer. *J Invertebr Pathol* 183:107626
- Tayyab M, Z Yang, C Zhang, W Islam, W Lin, H Zhang (2021). Sugarcane monoculture drives microbial community composition, activity and abundance of agricultural-related microorganisms. *Environ Sci Pollut Res* 28:48080–48096
- Tranier MS, RDC Quiroz, CNA González, T Mateille, S Roussos (2014). Commercial biological control agents targeted against plant-parasitic root-knot nematodes. *Braz Arch Biol Technol* 57:831–841
- Xu WF, JL Yang, XK Meng, ZG Gu, QL Zhang, LB Lin (2021). Understanding the transcriptional changes during infection of *Meloidogyne incognita* eggs by the egg-parasitic fungus *Purpureocillium lilacinum*. *Front Microbiol* 12:1-13
- Yao X, Chen J (2023). *Trichoderma* and its role in biological control of plant fungal and nematode disease. *Front Microbiol* 14:1-15