PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 12(sp1): 01–06
https://doi.org/10.14719/pst.6324

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RESEARCH ARTICLE

Strategies for managing of lesion nematode (*Pratylenchus zeae*) in sugarcane fields

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OPEN ACCESS

ARTICLE HISTORY
Received: 25 November 2024
Accepted: 30 December 2024
Available online
Version 1.0 : 15 February 2025

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
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CITE THIS ARTICLE
Jayakumar J, Senthilkumar T, Ravichandran V, Ganapathy S, Malathi G. Strategies for managing of lesion nematode (*Pratylenchus zeae*) in sugarcane fields. Plant Science Today. 2025;12(sp1): 01-06. https://doi.org/10.14719/pst.6324

Abstract

Lesion nematodes cause significant yield losses in sugarcane, with global reductions ranging from 10 to 40%. Identifying effective bioagents for soil application is critical to improving sugarcane production. Misdiagnosing nematode infestations as nutrient deficiencies or soil toxicity can limit nutrient availability to plants, exacerbating the problem. To address the challenges posed by lesion nematodes in sugarcane, the present investigations was conducted in a farmer's field under the sugarcane variety CoC 25 in Vriddhachalam, Cuddalore district, Tamil Nadu, India. The study evaluated the efficacy of talc-based fungal antagonists, including *Trichoderma viride, T. asperellum, Purpureocillium lilacinum, T. harzianum, Pochonia chlamydosporia, T. reesei* and *Clonostachys frosea* as well as bacterial antagonist such as *Bacillus firmus, B. subtilis* and *Lysinibacillus fusiformis* against lesion nematode *Pratylenchus zeae*. These biocontrol agents were applied at 2.5 kg/ha and compared with the standard recommendation of carbofuran applied at 33 kg/ha. All tested fungal and bacterial antagonists significantly reduced the soil population density of lesion nematode. In the first year, sett treatment experiments demonstrated that the application of the fungal antagonist *P.lilacinum* at 2.5 kg/ha reduced nematode populations by 74.3% compared to the untreated control. This treatment also improved germination (84.26%), tillers count (166.62%), commercial cane sugar percentage (12.80%), cane yield (120.50%) and sugar yield (16.82%). Similarly, the bacterial antagonist, *Bacillus subtilis* at 2.5 kg/ha reduced nematode populations by 71.3% and improved germination (85.22%), tillers count (165.32%), commercial cane sugar content (12.30%), cane yield (118.60%) and sugar yield (16.66%). In the second year, combining *P. lilacinum* at 2.5 kg/ha with Fluensulphone 2% GR at 10 kg/ha resulted in a reduction of lesion nematode populations by 81.23% and achieved a maximum cane yield of 118.3 t/ha. The application of *P. lilacinum* proved to be highly effective in reducing nematode populations and is recommended for inclusion in an integrated nematode management module.

Keywords

Bacillus subtilis; biological control; lesion nematode; *Purpureocillium lilacinum;* sugarcane

Introduction

Sugarcane (*Saccharum officinarum* L.), a member of the Poaceae family, is a globally significant crop cultivated for sugar production, juice extraction and various byproducts. Its cultivation is predominantly concentrated in tropical and subtropical regions, where it serves as a major economic driver for domestic use and international trade. As a vital cash crop, sugarcane plays a crucial role in meeting both household and industrial demands through the production of sugar and its derivatives.

Plant-parasitic nematodes are one of the most significant biotic constraints affecting sugarcane productivity. Among agricultural crops, sugarcane experiences considerable financial losses due to nematode infestation, with significant yield reductions reported globally. In India, nematodes are estimated to cause a 10-40% reduction in sugarcane yields, posing a serious threat to the country's sugar industry (1). Globally, sugarcane is associated with 48 genera and 275 species of nematodes reported across 36 countries. In India, the major parasitic nematodes affecting sugarcane belong to 5 genera: *Hoplolaimus* spp., *Pratylenchus* spp., *Meloidogyne* spp., *Tylenchorhynchus* spp. and *Helicotylenchus* spp. (2).

In Tamil Nadu, sugarcane production is severely affected by nematodes, particularly *Pratylenchus, Meloidogyne, Tylenchorhynchus, Helicotylenchus* and *Hoplolaimus* (3). On average, nematodes are estimated to reduce sugarcane yields by 15.3% annually (4).

The extent of damage caused by lesion nematodes depends on the species present, with symptoms including thickened, blackened primary roots and a lack of fine secondary or tertiary roots. Common symptoms include yellowing, chlorosis in patches, pale leaves and general stunting of the plant. Nematodes invade the cortical parenchyma of roots, causing browning and collapse of adjacent cells. Affected sugarcane roots exhibit dark, round or elongated lesions are thickened and show a scarcity of fine roots.

Previous studies have suggested that soil amendments like press mud and oil cakes are effective in managing sugarcane lesion nematodes (5, 6). Intercropping sugarcane with sunhemp, marigold or daincha, in combination with the application of press mud (25 t/ha) or neem cake (2 t/ha), has proven to be effective in controlling sugarcane lesion nematodes (7-9). Considering the eco-safe approaches, the current investigation focused on the evaluation of bacterial and fungal antagonists against sugarcane lesion nematode.

Farmers solely depend on synthetic nematicides for nematode management. Hence, the identification and promotion of biocontrol approaches is an urgent necessity. Environmentally friendly tactics, like using nematode-antagonistic biocontrol agents, are gaining prominence as viable alternatives. This study assessed the efficacy of talc-based formulations of fungal antagonists (*Trichoderma viride, T. asperellum, T. harzianum, P. lilacinum, P. chlamydosporia, T. reesei* and *Clonostachys frosea*) and bacterial antagonists (*Bacillus firmus, B. subtilis* and *Lysinibacillus fusiformis*) for managing sugarcane lesion nematodes.)

Materials and Methods

Biocontrol agents used

The fungal antagonists (*T. viride, T. asperellum, T. harzianum, P. chlamydosporia, T. reesei* and *C. frosea*) and bacterial antagonists (*B. firmus, B. subtilis* and *L. fusiformis*) were obtained from the Department of Plant Pathology, Tamil Nadu Agriculture University, Coimbatore. Additionally, the fungal antagonist *P. lilacinum* was procured from the Department of Nematology at the same institution.

Field experiments

Impact of fungal antagonists on sugarcane lesion nematode management

First-year field experiments were conducted in a nematode-infested field at a farmer's site in Vriddhachalam, Cuddalore, Tamil Nadu, India, using the sugarcane variety CoC 25. Sugarcane setts were planted in ridges and furrows spaced 90 cm apart, which were prepared manually using spades. The initial nematode population in the field, recorded before planting ranged from 414 to 464 individuals per 200 g of soil. The experimental field soil was classified as clay loam, with a pH of 7.9, available nitrogen at 280.6 kg/ha, phosphorus 30.6 kg/ha and potash 185.2 kg/ha, as recorded before planting.

The treatments consisted of the application of *T. viride*, *T. asperellum*, *P. lilacinum*, *T. harzianum*, *P. chlamydosporia*, *T. reesei*, *C. frosea*, each at 2.5 kg/ha, along with an untreated control. The trial was set up using a randomized block design with 3 replications.

Influence of bacterial antagonists for the management of lesion nematode

A separate first-year field experiment on bacterial antagonists was conducted simultaneously at the same farmer's field in Vridhachalam, Cuddalore district, Tamil Nadu, India, using the sugarcane variety CoC 25, which was naturally infested with *P. zeae*. Sugarcane setts were planted in ridges and furrows with a row spacing of 90 cm. The initial lesion nematode population in the soil ranged from 386.3 to 428.4 individuals per 200 g of soil. The soil properties were consistent with the previews experiment layout.

The treatments consisted of T_1: *B. firmus* (2.5 kg/ha), T_2: *B. subtilis* (2.5 kg/ha), T_3: *L. fusiformis* (2.5 kg/ha) and T_4: untreated control.

The trial was established using a randomized block design with 3 replications to ensure robust statistical analysis.

Integrated management of lesion nematodes in sugarcane using P. lilacinum

In the second year, a field experiment was conducted at a farmer's field in Vridhachalam, India, to evaluate the efficacy of the fungal antagonist *P. lilacinum* for the integrated management of lesion nematodes in sugarcane (variety Coc 25) naturally infested with *P. zeae*. Sugarcane setts were planted in ridges and furrows with a 90 cm spacing, which were prepared manually. The initial lesion nematode population ranged from 502.0 to 561.3

individuals per 200 g of soil. The soil properties were consistent with the previews experiment layout, recorded as clay loam, with recorded pH, available nitrogen, phosphorus and potash levels.

The treatments consisted of T_1:*P. lilacinum* (2.5 kg/ha), T_2:T_1+ Press mud (15 t/ha), T_3: T_1+FarmYard Manure (12.5 t/ha), T_4:T_1+ Intercropping with marigold, T_5: T_1+ Intercropping with Sunhemp, T_6: T_1+ Neem cake (1 t/ha), T_7: T_1+ *Calotropis* leaves (2.5 t/ha), T_8: T_1+ Vermicompost (1 t/ha), T_9: T_1+ Fluensulphone 2% GR (10 kg/ha), T_{10}: T_1+ Carbofuran (33 kg/ha), T_{11}: Carbofuran (33 kg/ha), T_{12}: Untreated control.

The trial was designed using a randomized block design with 3 replications to minimize experimental error.

Nematode population assessment

Soil samples were collected at 3 months intervals up to 360 days after planting. The decanting and sieving method (10) was used to extract nematodes from the soil samples. A modified version of Baermann's funnel method (11) was then used to extract male vermiform stages and juvenile second stages nematode.

Assessment of plant growth and yield parameters

The cane yield and quality parameters, including sugar yield and commercial cane sugar percentage (CCS%), were estimated (12) at the time of termination of experiment. The sett germination percentage was assessed and recorded 35 days after planting. The tiller counts were recorded 90 days after planting and the yield parameters were recorded at the time of harvest.

Data analysis

All the data collected were subjected to analysis of variance (ANOVA). Statistical analysis was performed using IRRISTAT, version 92, developed by the Biometric Unit of the International Rice Research Institute (IRRI), Philippines. Data means were separated using Duncan's multiple range test (13).

Results and Discussion

Influence of fungal antagonist on sugarcane nematodes

The application of *P. lilacinum* at 2.5 kg/ha significantly reduced nematode populations in treated plots compared to the initial nematode population. Post-treatment nematode populations, recorded at 90-days interval throughout the crop duration, showed a consistent reduction trend. The *P. lilacinum* treatment resulted in a significant decline of 74.30% in nematode population over the untreated control (Table 1), followed by a 67.60% reduction observed in *T. viride*. The treatment with *P. lilacinum* at 2.5 kg/ha significantly improved germination percentage (84.26%), increased the number of tillers per ha (166.62), commercial cane sugar percentage (12.80%), cane yield (120.50 t/ha) and sugar yield (16.82 t/ha) compared to the untreated control (Table 2).

Table 1. Influence of fungal antagonists for the management of sugarcane lesion nematode

Treatments	Initial nematode Population (Nos/200 g of soil)	Nematode Population*				Per cent reduction over untread control
		90 DAP	180 DAP	270 DAP	360 DAP	
T_1- *Trichoderma viride* 2.5 kg/ha	432.3	156.3	171.6	195.2	216.3	67.6
T_2- *Trichoderma asperellum* 2.5 kg/ha	414.2	170.3	177.2	200.3	222.4	64.3
T_3- *Purpureocillium lilacinum* 2.5 kg/ha	460.6	138.2	159.3	187.6	199.2	74.3
T_4- *Trichoderma harzianum* 2.5 kg/ha	426.3	175.5	181.3	206.5	230.2	62.7
T_5- *Pochonia chlamydosporia* 2.5 kg/ha	435.2	164.2	185.2	212.3	236.3	61.2
T_6- *Trichoderma reesei* 2.5 kg/ha	440.3	180.3	192.3	219.2	242.6	60.3
T_7- *Clonostachys frosea* 2.5 kg/ha	464.4	184.2	202.4	222.3	247.3	58.7
T_8- Untreatd control	422.2	519.4	575.3	589.2	643.6	-
SEM	--	7.18	3.44	5.25	2.22	-
CD (P=0.05)	--	21.62	9.25	16.22	7.16	-

*Nematode population - Mean of 3 replications; DAP- Days After Planting

Table 2. Influence of fungal antagonists for plant growth characters and yield

Treatments	Germination percentage	Number of tillers/ha	Commercial cane sugar %	Cane yield t/ha	Sugar yield t/ha
T_1- *Trichoderma viride* 2.5 kg/ha	73.03	163.23	12.70	112.20	15.80
T_2- *Trichoderma asperellum* 2.5 kg/ha	72.16	161.22	12.62	110.30	15.22
T_3- *Purpureocillium lilacinum* 2.5 kg/ha	84.26	166.62	12.80	120.50	16.82
T_4- *Trichoderma harzianum* 2.5 kg/ha	71.20	160.16	12.60	108.26	14.32
T_5- *Pochonia chlamydosporia* 2.5 kg/ha	69.33	159.29	12.40	107.20	14.08
T_6- *Trichoderma reesei* 2.5 kg/ha	67.22	157.32	12.35	105.32	13.25
T_7- *Clonostachys frosea* 2.5 kg/ha	65.32	155.23	11.66	103.22	12.75
T_8- Untreated control	59.09	150.09	11.08	70.10	8.90
SEM	3.24	0.77	0.09	2.02	0.34
CD (P=0.05)	11.22	2.42	0.27	5.92	1.06

Influence of bacterial antagonists against sugarcane nematodes

The bacterial antagonists *B. firmus, B. subtilis* and *L. fusiformis* were evaluated for their efficacy in reducing nematode populations. Among the three bacterial antagonists, soil application of *B. subtilis* at 2.5 kg/ha resulted in a significant reduction in nematode populations (71.3%). Soil nematodes populations were significantly reduced in periodical sampling at 90 days interval after planting, compared to the initial nematode population as well as untreated control (Table 3). Soil application of *B. subtilis* also improves germination (85.22%), tiller count (165.32/ha), commercial cane sugar percentage (12.3%), cane yield (118.6 t/ha) and sugar yield (16.66) (Table 4). *B. firmus* and *L. fusiformis* also significantly reduced nematode populations, achieving reductions of 65.2% and 67.3% respectively, compared to the untreated control.

Efficacy of P. lilacinum with integrated strategies on nematodes

The fungal antagonist *P. lilacinum* was selected based on its performance in a previous field experiment, which demonstrated its efficacy in reducing nematode populations and enhancing plant growth parameters compared to bacterial antagonist. In this study, the efficacy of identified fungal antagonist *P. lilacinum* against sugarcane nematodes was evaluated. Various combinations of *P.lilacinum* neem cake, press mud, farmyard manure, vermicompost, *Calotrophis* leaves and intercropping with marigold and sunhemp were tested. The effects of these combinations were compared with standard commercial nematicides, fluensulphone and carbofuran, to assess their effectiveness against nematodes.

The results showed a significant reduction in nematode populations following the treatments. Among the tested treatments, the combination of *P. lilacinum* at 2.5 kg/ha with fluensulphone 2% GR at 10 kg/ha was the most effective in reducing nematode populations. This treatment resulted in the lowest nematode population, with post-treatment counts at 114.0, 122.2, 138.0 and 159.2 individuals per 200 g of soil at 90, 180, 270 and 360 days after planting respectively. Compared to the initial nematode population, this treatment achieved an 81.23% reduction (Table 5).

Furthermore, the combined treatment of *P. lilacinum* (2.5 kg/ha) with fluensulphone 2% GR (10 kg/ha) significantly improved several plant growth and yield parameters. These included germination percentage (80.12%), tiller count (164.14/ha), commercial cane sugar percentage (12.76%), cane yield (122.3 t/ha) and sugar yield (16.52 t/ha) (Table 6).

The significant reduction in soil nematode populations and the corresponding increase in sugarcane yield parameters observed in this study highlight the potential of eco-friendly natural supplements, including fungal antagonists combined with fluensulphone, for nematode management in sugarcane. Previous studies (14, 15) have shown that the application of neem cake at 2 t/ha and press mud at 25 t/ha effectively reduced populations of *Meloidogyne incognita*, *Pratylenchus coffeae* and *Helicotylenchus dihystera*. The combined application of organic amendments and biocontrol agents observed in this study aligns with the previous findings of (16), where the use of press mud (1 t/ha), farmyard manure (12.5 t/ha), poultry manure (1 t/ha) and *T. viride* (1.25 kg/ha) and *Pseudomonas fluorescens* (1.25 kg/ha) significantly reduced lesion nematode populations and enhanced sugarcane yield.

The addition of organic amendments, such as press mud, oil cakes, green manure and farmyard manure, promotes the growth of bacteria antagonistic to nematodes, thereby inhibiting the proliferation of plant-parasitic nematodes. The application of soil organic amendments and intercropping with marigold or sun hemp in sugarcane field has also proven its effectiveness in reducing nematode population, as reported (13). According to a study, the combination of farmyard manure, oil cakes, green manure and press mud resulted in a significant reduction of lesion nematodes in the soil (17). These treatments also improved cane yield, commercial cane sugar percentage and sugar yield compared to the untreated control.

Table 3. Influence of bacterial antagonists for the management of sugarcane lesion nematode

Treatments	Initial Nematode Population	Nematode Population*				Per cent reduction over untreated
		90 DAP	180 DAP	270 DAP	360 DAP	
T_1- *Bacillus firmus* 2.5 kg/ha	407.6	136.40	157.60	170.30	197.40	65.2
T_2- *Bacillus subtilis* 2.5 kg/ha	428.4	115.20	140.70	152.10	168.30	71.3
T_3- *Lysinibacillus fusiformis* 2.5 kg/ha	386.3	131.22	152.21	165.13	189.30	67.3
T_4- Untreated control	398.5	524.10	564.10	608.20	655.60	-
SEM	--	7.16	2.50	4.22	3.96	-
CD (P=0.05)	--	20.12	7.71	14.42	12.33	-

Table 4. Influence of bacterial antagonists for plant growth characters and yield

Treatments	Germination Percentage	Number of tillers/ha	Commercial Cane Sugar (%)	Cane Yield (t/ha)	Sugar Yield (t/ha)
T_1- *Bacillus firmus* 2.5 kg/ha	72.11	154.12	11.6	109.2	14.72
T_2- *Bacillus subtilis* 2.5 kg/ha	85.22	165.32	12.3	118.6	16.66
T_3- *Lysinibacillus fusiformis* 2.5 kg/ha	75.33	157.20	11.9	111.3	15.11
T_4- Untreated control	61.20	143.18	11.0	71.2	8.96
SEM	3.23	0.88	0.05	2.06	0.26
CD (P=0.05)	11.15	2.86	0.22	6.22	0.70

Plant growth characters and yield - Mean of 7 replication

Table 5. Effect of fungal antagonist and integrated management of sugarcane lesion nematode

Treatments	Initial nematode population (Nos/200 g of soil)	Nematode Population (Nos/200 g of soil)				Per cent reduction over untreated control
		90 DAP	180 DAP	270 DAP	360 DAP	
T_1- *Purpureocillium lilacinum* 2.5 kg/ha	544.6	152.2	165.2	182.3	199.3	65.12
T_2- T_1 + Pressmud 15 t/ha	532.2	198.4	203.3	215.4	232.3	62.01
T_3- T_1 + Farm Yard Manure 12.5 t/ha	511.0	185.7	191.0	203.2	222.4	62.56
T_4- T_1 + Intercropping with Marigold	513.0	176.4	172.4	191.3	209.2	63.24
T_5- T_1 + Intercropping with Sunhemp	522.0	232.0	245.3	267.6	276.0	60.09
T_6- T_1 + Neem cake 1 t/ha	507.2	145.3	158.7	176.3	194.2	67.32
T_7- T_1 + Calotrophis leaves 2.5 t/ha	520.3	221.5	236.6	251.2	263.6	60.32
T_8- T_1 + Vermicompost 1 t/ha	525.4	208.2	217.3	229.0	248.3	61.77
T_9- T_1 + Fluensulphone 2% GR 10 kg/ha	561.3	114.0	122.2	138.0	159.2	81.23
T_{10}- T_1 + Carbofuran 33 kg/ha	514.0	120.3	135.3	150.2	173.3	73.17
T_{11}- Carbofuran 33 kg/ha	552.3	126.2	146.5	162.3	186.2	70.12
T_{12}- Untreated control	502.0	528.3	561.4	596.0	632.3	-
SEM	-	6.12	6.92	2.17	4.46	-
CD (P=0.05)	-	17.67	18.44	4.72	12.32	-

Mean of 3 replication; DAP- Days After Planting

Table 6. Effect of fungal antagonist for plant growth characters and yield

Treatments	Germination percentage	Tiller Count ('000/ha)	Commercial cane sugar%	Cane yield (t/ha)	Sugar yield (t/ha)
T_1- *Purpureocillium lilacinum* 2.5 kg/ha	73.18	148.61	12.40	96.3	14.10
T_2- T_1 + Pressmud 15 t/ha	72.16	131.00	12.12	87.6	12.12
T_3- T_1 + Farm Yard Manure 12.5 t/ha	76.06	135.18	12.26	91.3	12.45
T_4- T_1 + Intercropping with Marigold	71.22	137.12	12.35	92.4	13.12
T_5- T_1 + Intercropping with Sunhemp	69.26	130.56	11.20	78.3	10.21
T_6- T_1 + Neem cake 1 t/ha	74.18	150.33	12.60	98.3	14.12
T_7- T_1 + Calotrophis leaves 2.5 t/ha	68.20	129.44	11.52	80.5	10.73
T_8- T_1 + Vermicompost 1 t/ha	70.33	130.27	11.18	83.6	11.65
T_9- T_1 + Fluensulphone 2% GR 10 kg/ha	80.12	164.14	12.76	122.3	16.52
T_{10}- T_1 + Carbofuran 33 kg/ha	76.32	160.24	12.72	111.2	15.71
T_{11}- Carbofuran 33 kg/ha	77.17	156.27	12.61	102.2	15.31
T_{12}- Untreated control	68.66	111.12	11.00	70.2	8.76
SEM	4.14	3.17	0.14	3.14	0.87
CD (P=0.05)	12.32	7.89	0.64	9.14	1.66

*Nematode population - Mean of 3 replication; DAP- Days After Planting

Organic amendments combat plant- parasitic nematodes through several mechanisms. The breakdown of microorganisms or organic amendments releases organic acids, including butyric, propionic, acetic and formic acids into the soil. Additionally, the decomposition of organic matter in soil releases gases such as ammonia and hydrogen sulphide, which are toxic to plant-parasitic nematodes and contributes to the reduction of nematode population (18, 19). The incorporation of organic matter also stimulates the rapid growth of microbes antagonistic to nematodes. Furthermore, organic fertilizers enhance soil quality and promote plant growth by supplying essential nutrients to crops. According to a study, the incorporating of organic amendments adds 10 to 12 tonnes of biomass/ha, improving the soil's physicochemical properties (20). The application of *Pseudomonas fluorescens* was minimized the lesion nematode population with increased cane and sugar yield (21).

The impact of *P. fluorescens* combined with neem cake aligns with the findings of (20), who reported that applying *P. fluorescens* (Pf1) at 2.5 kg/ha significantly reduced *Pratylenchus zeae* populations while increasing millable cane yield and sugar yield. They also noted that *Calotropis*, FYM, press mud, Neemin and Neemark were effective organic amendments against *P. zeae*. Reports from Bihar (21, 22) demonstrated that integrating press mud at 200 q/ha with carbofuran 3G at 1 kg a.i./ha during planting significantly reduced nematode populations, including *P. zeae*. This approach also improved growth, yield, juice quality and CCS% in sugarcane. The present findings on the application of *Pseudomonas fluorescens* combined with neem cake to control sugarcane nematodes, while enhancing yield and cane quality, are consistent with the reports of (10, 23).

Conclusion

Lesion nematodes cause significant damage to sugarcane root, leading to a reduction in economical yield in terms of both quality and quantity. The application of *P. lilacinum* reduced nematode populations by up to 81% in sugarcane fields, both with/without organic amendments and nematicides, and resulted in the highest cane yield compared to untreated controls. The application of *P. lilacinum* provides continuous protection to sugarcane cultivation against biotic stress caused by nematodes. Hence, these findings can be effectively incorporated into integrated management strategies for controlling sugarcane nematodes.

Acknowledgements

Authors wish to thank Professor and Head of the Institutes for providing support, guidance and advice during the research and manuscript writing process.

Authors' contributions

JJ carried out the experiment, took observations and analysed the data. TS formulated the research concept, finalised the manuscript. RV carried out interpretation and statistical analysis of the findings. SG designed, coordinated and summarized the experiment and revised the manuscript. GM participated in the sequence alignment and drafted the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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