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RESEARCH ARTICLE

Effectiveness of PlantbiotiX formulations as biological solutions for root-knot nematodes in bitter gourd

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Abstract

The root-knot nematode (*Meloidogyne incognita*) is a major pest that adversely affects bitter gourd production in India. Conventional chemical control methods, though effective, raise economic and environmental concerns, making it necessary to adopt integrated nematode management (INM) strategies. This study evaluates the bio-efficacy of PlantbiotiX formulations, including *Bacillus. paralicheniformis* 5 % WP ZBM5, *Bacillus subtilis* 2 % SP ZB87 ½, and Xplorer Glory (vesicular arbuscular mycorrhiza), for controlling root-knot nematodes in bitter gourd. Pot culture and field experiments were performed using various concentrations and application methods of PlantbiotiX formulations . Results revealed significant improvements in plant growth parameters, including vine length (9.13%), fruit weight (6.27%), number of fruits (25.21%), and yield per plant (36.36%) when *B. paralicheniformis* 5 % WP ZBM5 and *B. subtilis* 2 % SP ZB87 ½ were applied in combination. Furthermore, a substantial reduction in nematode populations was observed: soil nematode populations decreased by 72.9%, female nematodes by 65.01%, and egg mass by 60.59%. Scanning Electron Microscope (SEM) analysis of treated roots confirmed biofilm formation by *B. paralichiniformis*, demonstrating its capability to mitigate nematode infestation. This biofilm likely played a role in reducing nematode penetration and survival in the roots. The study indicates that PlantbiotiX formulations serve as a sustainable and environmentally friendly substitute for chemical nematicides, promoting plant growth and efficiently controlling root-knot nematodes, thereby presenting a viable solution for enhancing bitter gourd cultivation while reducing environmental harm.

Keywords

biological control; bitter gourd; management; root-knot nematode

Introduction

Bitter gourd (*Momordica charantia*) is an essential vegetable frequently eaten for its remarkable medicinal qualities. Despite being rich in essential nutrients, this vegetable is often underutilized due to its bitter taste. Bitter gourd contains active phytochemicals, including triterpenes, proteins, and steroids, and is rich in minerals such as copper (Cu), iron (Fe), magnesium (Mg), zinc (Zn), and calcium (Ca). It also contains fatty acids like lauric, myristic, palmitic, stearic, and linoleic acids. Charantin, a compound with

hypoglycemic properties found in this plant, aids in regulating blood glucose levels (1). Bitter gourd fruits are rich in iron and vitamin C, exhibiting significant antioxidant activity (2). It is also well known as a source of anti-diabetic drugs, in the pharmaceutical industry (3), and research is being conducted to enhance bitter gourd applications in areas such as colon cancer prevention and obesity management (4). The fruits and seeds contain over 60 phytochemicals that offer protection against more than 30 diseases, including cancer and diabetes (5).

Various biotic factors, such as fungi, bacteria, insect pests, and nematodes, affect bitter gourd production. Root-knot nematodes (*Meloidogyne* spp.) are particularly destructive, leading to significant yield losses and symptoms like leaf drying and stunted growth. Their wide host range, aggressive nature, and global presence make them a major cause of crop loss (6).Root-knot nematodes exhibit significant pathogenic potential in bitter gourd (7), resulting in yield losses of up to 13.50 percent, equivalent to around Rs. 252.82. Root-knot nematodes exhibit significant pathogenic potential in bitter gourd, resulting in output losses of up to 13.50 percent, equivalent to around Rs. 252.82 (8).

Chemical insecticides are widely used in India to increase yield, but they negatively affected the economy and environment of the nation (9). To reduce the harmful effect of chemical insecticides, INM and biological disease control strategies are gaining popularity, focusing on enhancing rhizosphere biodiversity with organic amendments and biocontrol agents. Biocontrol agents, such as *Purpureocillium lilacinum*, *Pseudomonas fluorescens*, *Trichoderma viride*, and *Pochonia chlamydosporia* have shown promising potential in managing root-knot nematode (10). In recent years, various plant growth-promoting rhizobacteria (PGPR), such as *Bacillus, Pseudomonas*, etc., have demonstrated antagonistic effects on plant pathogens (11). Various rhizobacteria produce biofilms in the rhizosphere, acting as biocontrol agents (12). Moreover, endosphere-forming *Bacillus* strains produce lipopeptides like surfactins, iturins, fengycins, plipastatins, etc., which act as broad-spectrum bio-control agents (13). This study aimed to evaluate the bio-efficacy and mode of action of PlantbiotiX formulations, including *B. paralicheniformis* 5 % WP ZBM5, *B. subtilis* 2 % SP ZB87 ½, and Xplorer Glory, as biocontrol options against root-knot nematode, *M. incognita*, in bitter gourd.

Materials and Methods

Experimental Location

An experiment was conducted using PlantbiotiX formulations, namely *B. paralichiniformis* 5% WP formulation - ZBM5 (strain no. NAIMCC-SB-0043), *B. subtilis* 2 % SP ZB87 ½, and Xplorer Glory (Vesicular Arbuscular Mycorrhiza), to study the mode of action of PlantbiotiX formulations against root-knot nematodes. This experiment was carried out at the Department of Nematology, Glass House, TNAU Coimbatore.

Experiment details

Hybrid Palee was selected to assess the efficacy of the *B. paralicheniformis* 5 % WP ZBM5 formulation, applied *via* both soil and seed treatment. Seed treatments were conducted at concentrations of 2.5, 5.0, and 7.5g/Kg seeds. Soil applications were performed at concentrations of 1.0, 2.0, and 4.0 g /L of water.

Pot culture studies

Pots were disinfected and filled with sterilized soil. Plants were inoculated with root-knot nematodes (2J2 /g soil) 15 days after sowing. Soil application of the formulation was done at three concentration levels (1.0, 2.0, and 4.0 g /L of water), while seed treatment was conducted at concentrations of 2.5, 5.0, and 7.5 g / Kg of seed. Observations were recorded 45 days after plant emergence, focusing on vine length (cm), root length (cm), and nematode populations. Roots were washed, stained with 0.1% acid fuschin, and then left in lactophenol for at least a day to discolor. Galls and egg masses per plant were counted using a stereoscopic binocular microscope. Soil from each treatment was thoroughly mixed, and 200 cc was taken to estimate nematode populations using Cobb's sieving and decanting technique, followed by Baermann's funnel method.

Scanning Electron Microscopy

The SEM utilized for analysis was the FEI Quanta 250, developed by the Field Electron and Ion Company, Czech Republic. This model is equipped with an Everhart- Thornley Detector (ETD), designed to collect secondary electrons for creating high-resolution images. The SEM operates with a tungsten electron source, which provides a stable electron required for imaging. Root samples were initially sputter-coated and then spread on double-sided conductive carbon tape, fixed onto a stub, and placed in the SEM sample chamber. After attaining a high vacuum pressure of 3.99e-4 Pa, the filament was turned on, and various parameters such as electron beam intensity, spot size, voltage, and emission current were adjusted. A magnification of 7220× provided detailed imaging of the microstructure of the samples. Images were then captured and depicted.

Field studies

The study was conducted using a completely randomized block design with seven treatments and five replications. *B. paralicheniformis* 5 % WP ZBM5 was applied both separately and in combination with *B. subtilis* 2 % SP ZB87 ½ and Xplorer Glory and was compared with a chemical nematicide (Fluopyrum 34.48% SC) and a bio-nematicide (*P. chlyamdosporia*). Initial soil testing was done to evaluate the nematode populations. Fields were plowed, and pits were dug at 2×1 m spacing. Two to three hybrid Palee seeds were planted per pit and thinned to one plant after 15 days. The first treatment was applied 15 days after sowing (DAS), followed by additional treatments on the 30th and 45th day. Recommended practices were maintained throughout. Nematode populations and growth parameters were observed.

Statistical analysis

The glasshouse experiment was conducted using a com-

pletely randomized block design with three replications, while the field experiments were carried out in a randomized block design with five replications to study the bioefficacy of PlantbiotiX products. Statistical significance was assessed using the F-value at the 5% level, along with the critical difference (CD). AGRESS software was used to perform the statistical analysis.

Results

Mode of action of PlantbiotiX products against M. incognita in bitter gourd: Pot culture studies

A pot culture experiment was conducted to study the mode of action of PlantibiotiX formulations against *M. incognita* in bitter gourd. Among the different methods, seed treatment performed better than soil application. The longest vine length was recorded in T3 (*B. paralicheniformis* 5 % WP ZBM5, seed treatment at 7.5g/kg seed) with a 12.10% increase over the control, followed by T6 (*B. paralicheniformis* 5 % WP ZBM5, soil application at 4g/kg) with a 10.36% increase over the control. The maximum root length was recorded in T3, showing a 12.99% increase over the control, followed by T6 with a 10.56 % increase (Table 1 & Figs. 1 - 3). The maximum reduction in soil nematode population (72.25%), female nematode population (59.77%), and egg mass (54.76%) was observed in T3, showing a significant reduction over the control. This was

Table 1. Effect of PlantbiotiX formulations on growth and nematode population of bitter gourd (Palee): Pot culture studies.

Treatments	Vine length (cm)	Root length (cm)	Soil nematode population	Root gall index	Number of female nematodes /5g of roots (30 days)	Number of egg masses/ 5g of roots (30 days)
T_1 - *B. paralicheniformis* 5 % WP ZBM5- seed treatment-2.5g/kg seed	178.15	8.79	156.10	3	9.8	8.1
T_2 - *B. paralicheniformis* 5 % WP ZBM5- seed treatment-5g/kg seed	180.46	9.01	145.97	2	8.1	7.6
T_3 - *B. paralicheniformis* 5 % WP ZBM5- seed treatment-7.5g/kg seed	190.64	9.74	95.97	1	7.0	5.7
T_4 - *B. paralicheniformis* 5 % WP ZBM5- soil application-1g/ L	176.43	8.71	170.15	3	9.8	9.2
T_5 - *B. paralicheniformis* 5 % WP ZBM5- soil application-2 g/ L	182.25	8.99	166.44	3	9.3	8.7
T_6 - *B. paralicheniformis* 5 % WP ZBM5- soil application-4g/ L	186.93	9.53	124.43	2	7.9	6.5
T_7 - Sterile soil without nematodes	173.21	8.7	0.00	0	0	0
T_8 - Sterile soil with nematodes	167.56	8.6	345.90	4	17.4	12.6
SE(d)	4.24	0.15	4.25		0.18	0.18
CD (5%)	8.99	0.31	9.01		0.39	0.39

***Sed**: Standard error of deviation; **CD**: Critical difference. Source: AGRESS Software*

Fig. 1. Effect of PlantbiotiX formulations on plant growth of bitter gourd (Palee): pot culture studies. **A**: T3 plant sample; **B**:T8 plant sample

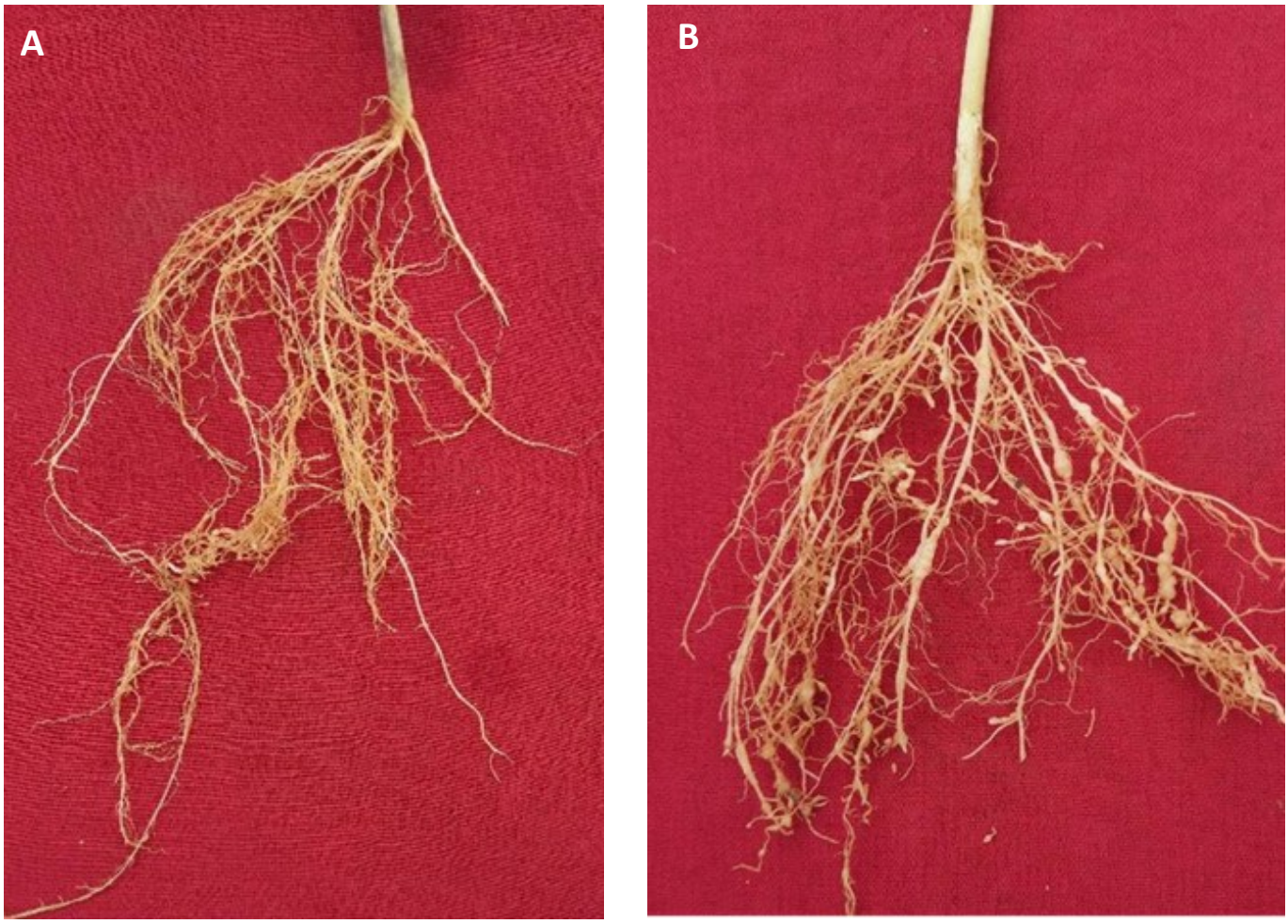

Fig. 2. Effect of PlantbiotiX formulations on roots of bitter gourd (Palee): Pot culture studies. **A**: T3 plant sample; **B**: T8 plant sample.

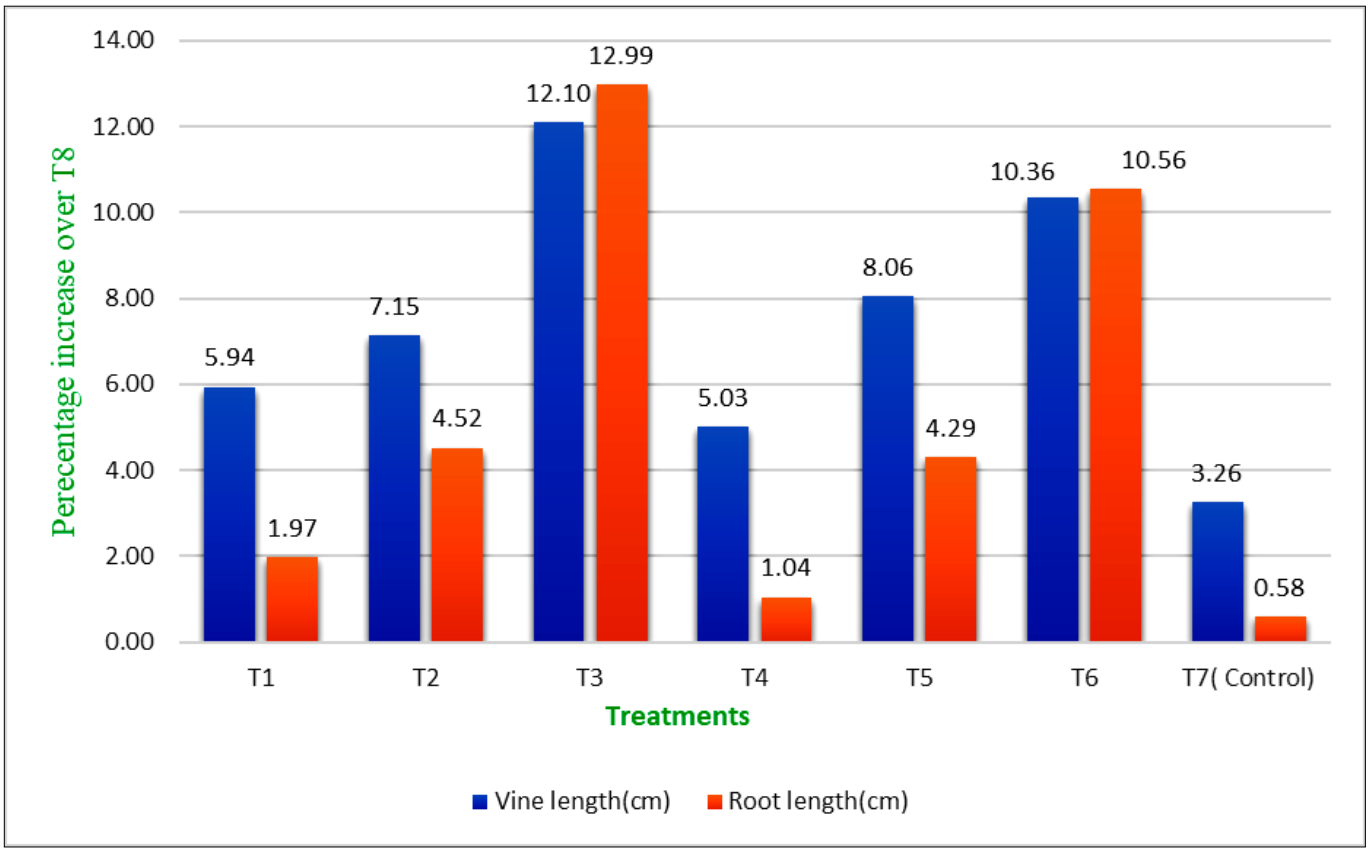

Fig. 3. Effect of PlantbiotiX formulations on vine length and root length of bitter gourd (Palee): Pot culture studies.

followed by T6, with decreases of 64.03%, 54.60%, and 48.41%, respectively, compared to the untreated control (Table 1 & Fig. 4).

Scanning electron microscopic analysis

The surface morphology was closely examined using SEM

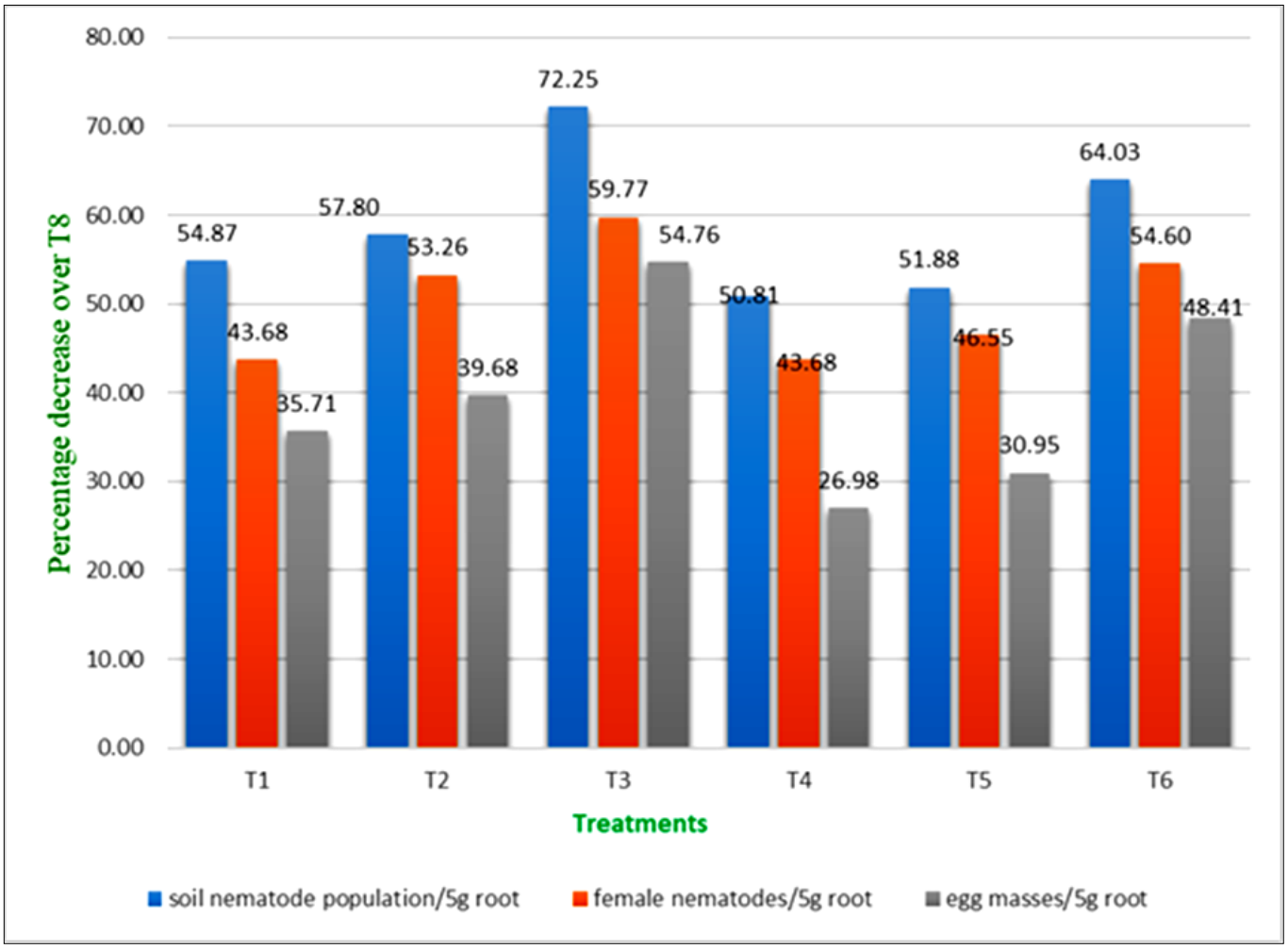

Fig. 4. Effect of PlantbiotiX formulations on soil nematode population, female nematodes, and egg masses: Pot culture studies.

images. Root samples from T6 (*B. paralicheniformis* 5 % WP ZBM5, soil application at 4g/kg) and T8 (sterile with nematodes) were observed at various magnification levels. The root sample from T6 (*B. paralicheniformis* 5 % WP ZBM5) showed root colonization, with *B. paralichinformis* forming a biofilm on the root surface. This biofilm prevented the action of *M. incognita*, preventing further infection. In contrast, the root sample from T8 (sterile with nematodes) showed high infection, with root nematodes forming gall symptoms (Fig. 5). The biofilm formation and colonization patterns of *Bacillus amyloliquefaciens* FZB42 on the roots of *Arabidopsis thaliana* was previously examined using a SEM (14). The potential of biofilm formation by biological control agents and their role in effective pest management strategies is further supported by another investigation (15).

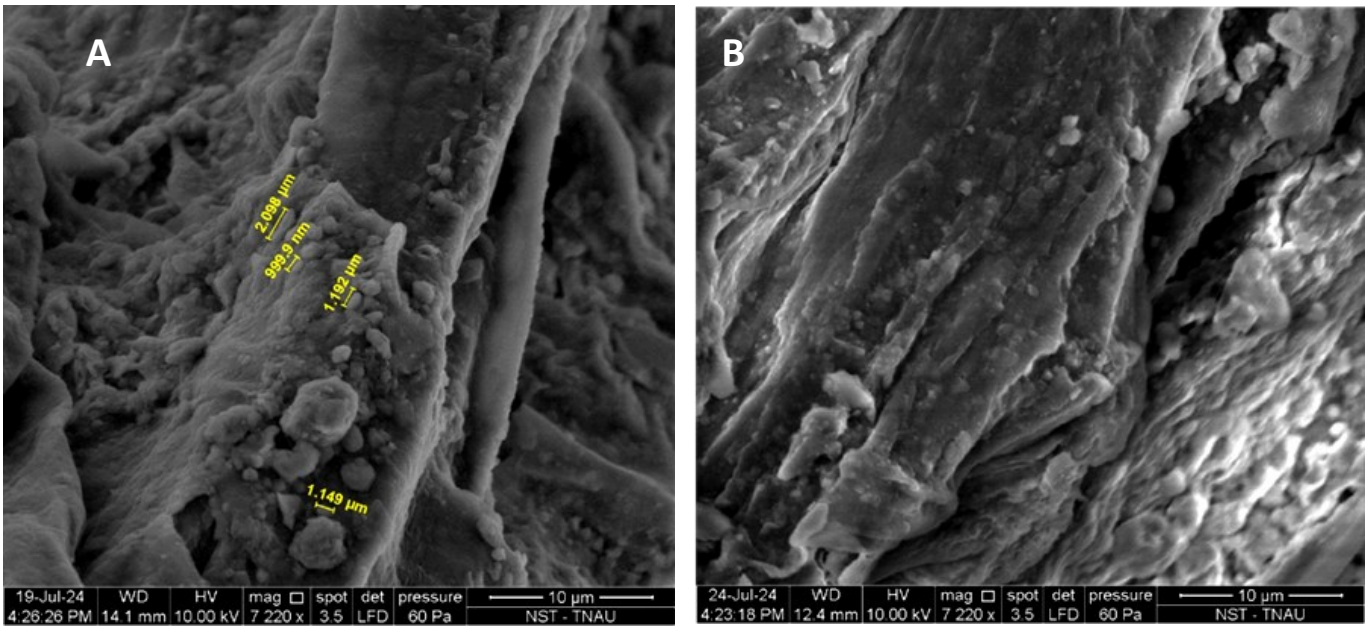

Fig. 5 Effects of PlantbiotiX formulations on root samples of bitter gourd (Palee). **A**: T3 root sample showing biofilm formation of *B. paralichiniformis*, imaged at a magnification of 7220×; **B**:T8 control without biofilm formation, imaged at a magnification of 7220

Bioefficacy of PlantbiotiX products against M. incognita in bitter gourd at field condition

Plant growth parameters

The longest vine length among the PlantbiotiX formulations was recorded in T3 *B. paralicheniformis* 5 % WP ZBM5, *B. subtilis* 2 % SP ZB87 ½ (2 times each), showing a 7.84% increase over the control, followed by T4 (*B. paralicheniformis* 5 % WP ZBM5, Xplorer Glory, applied 2 times each), with a 4.98% increase. The highest fruit weight was observed in T3 (*B. paralicheniformis* 5 % WP ZBM5, *B. subtilis* 2 % SP ZB87 ½ , applied 2 times each), with a 5.62% increase over control, followed by T4 (*B. paralicheniformis* 5 % WP ZBM5, Xplorer Glory, applied 2 times each), with a 3.10% increase. The maximum number of fruits was recorded in T3(*B. paralicheniformis* 5 % WP ZBM5, *B. subtilis* 2 % SP ZB87 ½, applied 2 times each), with a 25.21% increase over the control, followed by T4 (*B. paralicheniformis* 5 % WP ZBM5, Xplorer Glory, applied 2 times each), showing a 17.58% increase. The highest yield per plant was recorded in T3 (*B. paralicheniformis* 5 % WP ZBM5, *B. subtilis* 2 % SP ZB87 ½, applied 2 times each), with a 36.36% increase over control, followed by T4 (*B. paralicheniformis* 5 % WP ZBM5, Xplorer Glory, applied 2 times each), with a 24.32% increase. All the observations on growth parameters were comparable to the chemical control (Table 2).

The maximum reduction among the PlantbiotiX formulations was observed with *B. paralicheniformis* 5 % WP ZBM5 and *B. subtilis* 2 % SP ZB87 ½ (2 times each), resulting in a decrease in the soil nematode population (70.7%), female nematode population (61.16%), and egg mass (60.36%) compared to the untreated control, with results comparable to the chemical control (Fluopyrum 34.48% SC). This was followed by *B. paralicheniformis* 5 % WP ZBM5 and Xplorer Glory (2 times each), which reduced the soil nematode population by 57.3%, *B. paralicheniformis* 5 % WP ZBM5 (3 times), which showed a 55.07% reduction in the female nematode population, and *P. chlyamdosporia,* which reduced egg mass by 35.99% compared to the untreated control (Table 3).

Nematode population at harvest

The maximum reduction among the PlantbiotiX formulations was observed with the combination of *B. paralicheniformis* 5 % WP ZBM5 and *B. subtilis* 2 % SP ZB87 ½ (2 times each), which resulted in a decrease in the soil nematode population (72.9%), female nematode population (65.01%), and egg mass (60.59%) compared to the untreated control. This combinations outperformed the chemical control (Fluopyrum 34.48% SC). It was followed by *B. paralicheniformis* 5 % WP ZBM5 and Xplorer Glory (2 times each), which reduced the soil nematode population by 57.3%, and *B. paralicheniformis* 5% WP ZBM5 (applied three times), which showed a 57.72% reduction in the female nematode population. *P. chlamydosporia* also showed a reduction in egg mass (32.87%) compared to the untreated control (Table 3).

Table 2. Effect of PlantbiotiX formulation on plant growth and yield of bitter gourd (Palee) under field conditions.

Treatments	Vine length (cm)	Percent increase over control	Fruit weight (g)	Percent increase over control	No. of fruits per plant	Percent increase over control	Yield per plant (kg)	Percent increase over control
T_1 *B. paralicheniformis* 5 % WP ZBM5 100g/acre (2 times)	581.48	2.32	153.31	1.86	25.95	14.17	2.78	19.42
T_2 *B. paralicheniformis* 5 % WP ZBM5 100g/acre (3 times)	588.39	3.46	154.79	2.79	25.17	11.54	2.64	15.15
T_3 *B. paralicheniformis* 5 % WP ZBM5 100g/acre, *B. subtilis* 2 % SP ZB87 ½ 2.5g/L (2 times each)	616.34	7.84	159.42	5.62	29.78	25.21	3.52	36.36
T_4 *B. Paralicheniformis* 5 % WP ZBM5 100g/acre. Xplorer Glory 2 g/L (2 times each)	597.76	4.98	155.29	3.10	27.02	17.58	2.96	24.32
T_5 Fluopyrum 34.48% SC 1ml /L	625.07	9.13	160.54	6.27	28.72	22.46	3.28	31.71
T_6 *P. Chlyamdosporia* 5kg/ha	579.73	2.02	154.45	2.58	25.05	11.10	2.92	23.29
T_7 Non treated	568.01	0.00	150.46	0.00	22.27	0.00	2.24	0.00
SE.d	6.48		1.79		0.24		0.11	
CD (5%)	13.16		3.64		0.49		0.23	

***Sed**: Standard error of deviation; **CD**: Critical difference. Source: AGRESS Software*

Nematode population at 30 DAT

The maximum reduction among the PlantbiotiX formulations was observed with the combination of *B. paralicheniformis* 5 % WP ZBM5 and *B. subtilis* 2 % SP ZB87 ½ (2 times each), resulting in a decrease in the soil nematode population (68.7%), female nematode population (48.99%), and egg mass (60.39%) compared to the untreated control, with results comparable to the chemical control (Fluopyrum 34.48% SC). This was followed by *B. paralicheniformis* 5 % WP ZBM5 and Xplorer Glory (2 times each), which showed reductions of 54.1%, 39.52%, and 31.55%, respectively, compared to the untreated control (Table 3).

Nematode population at 60 DAT

Discussion

The beneficial effects of *B. paralicheniformis* and *B. subtilis* are well-documented in multiple studies on various plants. For instance, *B. paralicheniformis* TRQ65, isolated from wheat fields in Mexico, contains genes linked to stress response and growth-promoting substances (16). The *B. paralicheniformis* RP01 strain promoted growth on the root surface of *Brassica chinensis*, significantly increasing root length and brassinosteroid (BR) content (17). Similar-

Table 3. Effect of PlantbiotiX formulation on root-knot nematode populations at field condition.

Treatments	Soil nematode population			Root gall index			Number of females/5g of root			Number of egg masses/ 5g of root		
	DAYS			DAYS			DAYS			DAYS		
	30	60	90	30	60	90	30	60	90	30	60	90
T_1 *B. paralicheniformis* 5 % WP ZBM5 100g/acre (2 times)	170.1 (49.9)	164.4 (52.0)	155.6 (55.7)	3	3	3	9.64 (48.1)	9.09 (53.7)	8.93 (56)	9.19 (32.7)	8.96 (30.6)	8.47 (27.6)
T_2 *B. paralicheniformis* 5 % WP ZBM5 100g/acre (3 times)	166.4 (51.0)	155.9 (54.4)	147.2 (58.1)	3	3	3	9.34 (49.7)	8.84 (55.0)	8.58 (57.7)	8.85 (35.2)	8.43 (34.6)	8.23 (29.7)
T_3 *B. Paralicheniformis* 5 % WP ZBM5 100g/acre, *B. subtilis* 2 % SP ZB87,½ 2.5g/L (2 times each)	106.4 (68.7)	100.3 (70.7)	95.1 (72.9)	2	2	2	9.48 (48.9)	7.64 (61.1)	7.10 (65.0)	5.42 (60.3)	5.12 (60.3)	4.61 (60.5)
T_4 *B. Paralicheniformis* 5 % WP ZBM5 100g/acre, Xplorer Glory 2 g/L (2 times each)	156.1 (54.1)	146.1 (57.3)	132.8 (62.2)	3	3	3	11.24 (39.5)	9.23 (53.1)	8.71 (57.0)	9.36 (31.5)	8.29 (35.7)	7.89 (32.6)
T_5 Fluopyrum 34.48% SC 1ml /L	86.0 (74.7)	82.5 (75.9)	120.2 (65.8)	1	1	1	6.43 (65.4)	5.62 (71.4)	8.29 (59.1)	4.71 (65.5)	4.44 (65.5)	8.85 (24.4)
T_6 *P. Chlyamdosporia* 5kg/ha	193.0 (43.2)	185.6 (45.8)	149.5 (57.4)	3	3	3	11.64 (37.3)	10.11 (48.6)	9.63 (52.5)	8.63 (36.8)	8.26 (35.9)	7.86 (32.8)
T_7 Non treated	339.9 (0.0)	342.2 (0.0)	351.1 (0.0)	4	4	4	18.58 (0.00)	19.68 (0.00)	20.29 (0.00)	13.68 (0.00)	12.90 (0.00)	11.71 (0.00)
SE.d	1.70	1.78	2.36				0.13	0.17	0.15	0.12	0.11	0.12
CD (5%)	3.46	3.62	4.79				0.27	0.35	0.30	0.25	0.22	0.25

***Sed**: Standard error of deviation; **CD**: Critical difference. Source: AGRESS Software*

ly, (18) *B. paralicheniformis* SX21 was observed to significantly enhance root structures, photosynthetic parameters, growth rate, and biomass in cucumber . Supporting these findings, inoculation with *B. paralicheniformis* LBEndo1 and *Aeromonas caviae* KBEcto4 led to increased fruit production in tomato plants (19). In line with these observations, our study also found that *B. paralicheniformis* improved plant growth and yield in bitter gourd. Moreover, *B. paralicheniformis* MDJK30 was shown to promote plant growth by enhancing of carbohydrate and amino acid metabolism (20).

The inoculation of the B. paralichiniformis RP01 strain in cotton plants led to elevated concentrations of growth hormones, specifically brassinosteroids (BRs) and auxin (IAA), with increases of 43.9% and 5.1%, respectively (17). Genomic analysis of this strain identified the trpABCDEF pathway for L-TRP, spermine synthase (speE), and agmatinase (speB), which can catalyze the conversion of amino acids into plant growth-promoting compounds (17). The microbial diversity of the rhizosphere is an important ecological bioindicator for the healthy plant growth (18). After 44 days of inoculation with *B. paralicheniformis* 2R5, the diversity of beneficial microorganisms such as *Nitrospira*, *Ramlibacter*, *Sphingomonas*, *Massilia*, and *Terrimonas* increased in the rhizosphere of canola (21). Genomic analysis of *B. paralichiniformis* BP9 revealed the presence of genes for secondary metabolites such as bacillibactin, lantibiotics, and bacitracin, contributing to its antibacterial potential (22). *B. subtilis* enhances plant growth by improving nutrient uptake through mechanisms such as nitrogen fixation, phosphorus solubilization, and siderophore production (23). Volatile substances synthesized by *B. subtilis* SST2, such as albuterol and 1,3-propanediol, enhance plant development by elevating gibberellin and auxin levels, as well as photosynthetic rates (24). Additionally, *B. subtilis* AH18 and *B. licheniformis* K11 have been shown to promote growth in tomatoes and peppers by producing auxins (IAA, IBA, IPA), antifungal β-glucanase, siderophores, and solubilizing phosphates (25). Complementing this, a study emphasized the effectiveness of using multiple microorganisms together, highlighting the genomic and plant growth-promoting traits of *B. paralicheniformis* ES- 1 (26).

The nematicidal properties of *B. paralicheniformis* and *B. subtilis* have been extensively studied, showcasing their potential in managing plant-parasitic nematodes. *B. paralichiniformis* has demonstrated potential as a biocontrol agent against phytopathogenic nematodes, a degrader of polystyrene, and a plant growth promoter (27). A study found that the secretome of *B. paralicheniformis* TB197 exhibited over 95% nematicidal activity against *M. incognita* and effectively suppressed *M. enterolobii* infections in tomatoes (28). Similarly, it was reported from another study that *B. paralicheniformis* FMCH001 and *B. subtilis* FMCH002 hampered the establishment of nematodes by reducing egg-hatching and juvenile survival (29). This finding supports the notion that *B. paralicheniformis* is effective in inhibiting the life cycle of nematodes, thus providing a viable biocontrol strategy. Furthermore, a comparative study between *B. paralicheniformis* TB197 and *B. subtilis* ATCC 21332 showed that TB197 exhibited greater nematicidal action (30). The nematicidal activity of *B. paralichiniformis* TB197 is primarily attributed to its high polarity, which disrupts the cell membranes of *Caenorhabditis elegans* larvae, affecting their viability. Mutagenisis experiments in *B. subtilis* OKB105 identified the purL gene as crucial for nematicidal activity (31). In another study, tomato plants treated with bacterial suspensions of *B. subtilis* (MTCC-441) and *Pseudomonas putida* (MTCC-102) before J2 inoculation exhibited enhanced growth character-

istics and high nematicidal activity (32).

Gas chromatography-mass spectrometry (GC-MS) analysis identified several nematicidal volatile compounds, like ketones, alkyls, sulfides, and heterocyclic compounds, in *B. subtilis* Bs-1 strain (33). Additionally, the genomic study of *B. subtilis* GEB5 identified 21 homologs of nematode-virulent proteases, suggesting a potential mechanism for its nematicidal activity (34). This further underscores the superior efficacy of *B. paralicheniformis* and *B. subtilis* in managing nematode populations.

Nonetheless, one drawback of the pot cultivation study is the controlled environmental condition, which may not accurately represent the variability found in wild field situations . Additionally, the study duration was limited to a single growing season, which may not capture the long-term effects of the formulations on nematode activity and plant growth performance.

Conclusion

The study compared PlantbiotiX formulations with a chemical nematicide (Fluopyrum 34.48% SC) and a biological control agent (*P. chlamydosporia*). Fluopyrum 34.48% SC effectively reduced the nematode population; however, its effectiveness declined after 60 days. In contrast, the combination of *B. paralicheniformis* 5 % WP ZBM5 and *B. subtilis* 2 % SP ZB87 ½ (T3) demonstrated sustained efficacy throughout the crop cycle.

Future research could explore the combined use of *B. paralichiniformis* with other biocontrol agents such as *B. subtilis, P. chlymadosporia, P. lilacinum*, *P. fluorescens*, and *T. viride* to further reduce nematode populations and enhance plant growth in bitter gourd. Additionally, the sustainability of *B. paralichiniformis* formulations can be examined through long-term field trials across different regions and environmental conditions. Future studies could also investigate the molecular mechanisms behind the nematicidal activity of *B. paralichiniformis* to identify key bioactive compounds.

Further research on formulations that enhance shelf life and stability across various soil types, as well as studies on their synergistic effect with other organic amendments, would be valuable for practical agricultural applications. Moreover, assessing the impact of PlantbiotiX formulations on other economically important crops could expand its potential in INM strategies.

In conclusion, cumulative evidence from these studies suggests that PlantbiotiX formulations hold significant potential for reducing root-knot nematode damage while improving plant growth and yield of bitter gourd.

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Authors' contributions

For the Authors' contributions, we suggest the following format (please use initials to refer to each author's contribution): AB carried out the molecular genetic studies, participated in the sequence alignment, and drafted the manuscript. JY carried out the immunoassays. MT participated in the sequence alignment. ES participated in the design of the study and performed the statistical analysis. FG conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

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

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