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RESEARCH ARTICLE

Enhancing brinjal resilience to little leaf disease through bio-intensive management

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Abstract

Little leaf disease, caused by *Candidatus Phytoplasma trifolii*, is a major risk to brinjal cultivation, leading to reduced yield and quality. Traditional chemical control methods offer only temporary relief and pose environmental risks. This study aimed to develop a bio-intensive strategy for managing little leaf disease by identifying effective rhizobacterial isolates with plant growth-promoting traits. Among 100 isolates screened, *Bacillus licheniformis* (B 67) demonstrated the highest indole-3-acetic acid (IAA) activity, siderophore activity and phosphorus solubilisation, followed by isolate B 38. Pot culture experiments revealed that treatments involving seedling treatment and drenching with *B. licheniformis* (B 67) or *Bacillus subtilis* (Bbv 57), combined with need-based application of neem seed kernel extract (NSKE 5%), demonstrated significant reductions in disease incidence and improvements in plant health. Field trials validated the efficacy of an Integrated Pest and Disease Management (IPDM) module developed from the findings of pot culture studies. The module comprised seedling treatment with *B. licheniformis* (B 67) and *B. subtilis*, drenching with humic acid, foliar applications of ferrous sulphate and zinc sulphate and targeted chemical sprays. This approach achieved the lowest disease incidence and significantly improved yield compared to untreated controls. The study underscores the potential of bio-intensive management strategies integrating rhizobacteria, micronutrients and eco-friendly sprays to sustainably manage little leaf disease while enhancing crop resilience and productivity. These results provide evidence for environmentally safe and effective alternatives to chemical-based disease management in brinjal cultivation.

Keywords

Bacillus licheniformis; *Bacillus subtilis*; brinjal little leaf; IPDM; rhizobacterial isolates

Introduction

Brinjal (*Solanum melongena* L.) is a globally cultivated vegetable recognized for its nutritional value, including high levels of dietary fiber, vitamins (vitamin C) and phytochemicals (anthocyanin), which contribute significantly to human health. India is the second-largest producer of brinjal after China, contributing approximately 26% of global production. The country cultivates brinjal on an estimated 0.74 million ha, achieving a productivity of 12.8 million tonnes per ha (1). Within India, brinjal is grown on 0.71 million ha, achieving a productivity of 19.1 t/ha and accounting for 8.14% of the total vegetable-growing area, with a

9% contribution to overall vegetable production (2, 3). This short-duration crop is cultivated year-round and serves as a critical source of income for small and marginal farmers.

Despite its economic importance, brinjal cultivation faces significant challenges from biotic stresses, with little leaf disease being a major threat. This disease, first reported in Coimbatore during 1939 (4), has since been documented in several regions across India. Phytoplasmas are wall-less, shapeless bacteria size from 200 to 800 nm in diameter, residing in the sieve tubes of plants and the hemolymph of insect vectors (5, 6). Symptoms of little leaf disease, such as reduced leaf size, chlorosis, malformation, shortened internodes, witches' broom and stunted growth, are well-documented. Severe infections lead to flower malformation (phyllody), reduced fruiting and yield loss, with infected plants often succumbing to the disease (7, 8). Transmission is primarily mediated by the brown leafhopper (*Hishimonus phycitis* Distant) and other leafhoppers, such as *Empoasca devastans* (9, 10).

Management of little leaf disease predominantly relies on chemical insecticides to control vector populations. Although effective in reducing vector numbers, this approach poses environmental risks, including pesticide residues in fruits and the development of insecticide resistance. For example, applications of imidacloprid (18 g/ha) and thiamethoxam (25 g/ha) have been shown to control *Amrasca bigutulla* up to 14 days post-spraying (11, 12). Additionally, antibiotics such as tetracycline (100 ppm) have been used to suppress phytoplasma symptoms, but their effects are temporary and fail to eliminate the pathogen from the host plant (13, 14). Prolonged antibiotic use also increases cultivation costs, leaves residual effects in fruits and is restricted in several countries (15). Other strategies, such as spraying gibberellic acid, have demonstrated partial symptom recovery in infected plants (16).

Cultural practices, including rouging of infected crop, adjusting planting dates, using healthy propagating material, crop rotation with non-preferred species and weed removal, are effective in limiting disease spread. However, the obligate parasitic nature of phytoplasmas complicates their management compared to fungal or bacterial pathogens. Thus, a holistic, integrated approach combining cultural, physical, biological, chemical and resistance-based strategies is essential for sustainable disease management (17). In this study, we explore a bio-intensive management approach by integrating growth-promoting rhizobacteria and growth-inducing chemicals to develop a sustainable and effective strategy for managing little leaf disease in brinjal.

Materials and Methods

Isolation of rhizobacterial isolates

Rhizobacterial cultures were isolated from the rhizosphere soil of brinjal plants collected from Pudukkottai, Dindigul, Theni and Thoothukudi districts during the summer months. Soil samples were taken from a depth of 10-15 cm and bacterial cultures were isolated using the standard serial dilution method. The dilutions were plated on nutrient agar (NA) medium and isolate colonies were obtained within 2 days under room temperature conditions. The isolates were subsequently subcultured on NA medium to maintain pure cultures.

Assessment of growth-promoting traits

Seed bacterization using rhizobacterial isolates : Pure rhizobacterial isolates subcultured on NA medium were kept at 30 °C for 24 hr. A loopful of the bacterial culture was shifted to NA broth and kept at 28 °C for a day in a shaker at 50 rpm. The resulting broth culture, adjusted to an OD600 of 0.6 (3×10^8 cfu/mL) by dilution (Model no: Double Beam UV-VIS Spectrophotometer 2205, Company: Systronics), was used for seed bacterization. Brinjal seeds were soaked overnight in the bacterial culture and successively placed on moist blotters arranged in a roll towel setup. A control group was maintained using seeds treated with sterile water. After 10 days of incubation in a growth chamber, the Seedling Vigour Index (SVI) was measured using the equation (18):

Vigour index (SVI) =

(Mean root length + Mean shoot length) x Percent Seed germination

Biochemical characterization of rhizobacterial isolates

IAA production : The bacterial isolates were cultured in tryptic soy broth (TSB) enriched with 100 µg/mL tryptophan and incubated at 28 ± 2 °C. After the maturation period, 1 mL of the culture filtrate was collected and mixed with 2 mL of Salkowski reagent, which was prepared by dissolving 1 mL of 0.5 M $FeCl_3$ in 50 mL of 35% perchloric acid. The mixture was allowed to react at room temperature for 30 min. The resulting colour intensity was measured spectrophotometrically at 530 nm and IAA concentration was assessed by referring to a standard calibration curve (19).

Siderophore production : Siderophore synthesis by the bacterial isolates was evaluated through a qualitative plate assay (20). A 10 µL aliquot of a 48 hr old bacterial culture was applied to succinate agar medium supplemented with chrome azural S (CAS), ferric ion (Fe^{3+}) and hexadecyl trimethyl ammonium bromide. A shift in the medium's color from blue to fluorescent yellow indicated the presence of siderophores. The diameter of the color change was measured (mm^2) to estimate the production capacity.

Phosphorus solubilization : The ability of rhizobacterial culture to solubilize phosphorus was tested by inoculating them onto Pikovskaya's agar medium containing insoluble tricalcium phosphate as the substrate. The inoculated plates were incubated at 28 ± 2 °C for 48 hr. Solubilization was observed as a clear halo zone around the bacterial colonies. The Phosphate Solubilization Index (PSI) was calculated using the following formula (21, 22):

$$PSI = \frac{(\text{Colony diameter} + \text{halo zone})\ (mm)}{\text{Colony diameter}\ (mm)}$$

Genomic characterization of rhizobacterial isolates : The 16S rDNA region of the bacterial culture was amplified using specific primers (16SrDNA-F and 16SrDNA-R). The PCR amplicon (1500 bp) was cleared and sequenced using the ABI

3730xl Genetic Analyzer. Sequences were aligned and analyzed using Clustal W to generate a phylogenetic tree in MEGA 10. The BLAST tool (NCBI) was used to identify the closest relatives of the isolates based on sequence similarity.

Management of brinjal little leaf disease

In vivo pot culture studies : Three consecutive pot culture experiments were conducted from 2022 to 2023 to evaluate the management strategies for brinjal little leaf disease. A completely randomized design (CRD) with 9 treatments and 3 replications was carried out. The treatments included seed and seedling treatments with effective rhizobacterial isolates, foliar sprays and drenching with microbial and chemical formulations. The local brinjal variety susceptible to little leaf disease was used and *Bacillus subtilis* (Bbv57) from TNAU was included as a standard check.

The treatments were as follows: T1 - Seed treatment and seedling dip with streptocycline (100 ppm) followed by foliar sprays of streptomycin sulphate + tetracycline hydrochloride (150 ppm) as needed. T2 - Foliar sprays of gibberellic acid (50 ppm) at 30, 60 and 90 days after transplanting (DAT) with need-based application of NSKE (5%). T3 - Soil drenching with humic acid (3 mL/L) at 30, 60 and 90 DAT with need-based NSKE sprays. T4 - Foliar application of ferrous sulphate (0.5%) and zinc sulphate (0.5%) at 30, 60 and 90 DAT, supplemented with NSKE sprays. T5 and T6 - Treatments with 2 PGPR strains (1 and 2) for seedling treatment and soil drenching at 30, 60 and 90 DAT along with NSKE sprays. T7 - Seedling treatment and drenching with *Bacillus subtilis* combined with NSKE sprays. T8 - Dimethoate (30 EC) systemic insecticide spray (1 mL/L) at 30, 60 and 90 DAT. T9 - Untreated control.

Field trial for integrated disease management : The best-performing treatments from the pot culture experiments were evaluated in field trials conducted in 3 locations (Kalanjipatti, Kannivadi and Muthanampatti, Dindigul district) using a randomized block design (RBD) with 3 treatments and 7 replications. The treatments included: T1 - Integrated Pest and Disease Management (IPDM) approach combining seedling treatments with isolate B67 and *Bacillus subtilis* (Bbv57), soil drenching, foliar sprays of ferrous sulphate and zinc sulphate and NSKE applications along with dimethoate (1 mL/L). T2 - Farmer's practice. T3 - Untreated control.

Results

Isolation of rhizobacteria and seed bacterization

A total of 100 rhizobacterial isolates were successfully isolated from rhizosphere soil collected from diverse brinjal-growing regions of Tamil Nadu, including Allavayal, Elamanam, Periyanayakipuram, Agarapatti, Vadakadu, Thiruvarangulam (Pudukkottai district); Srirengapuram, Erumalainayakkanpatti and E. Pudupatti (Theni district); Kannivadi, Thumbichipalayam, Muthanampatti, Kalanjipatti, Oddanchathiram (Dindigul district) and Vallanadu (Thoothukudi district). These isolates were periodically sub-cultured and preserved on NA slants at 4 °C for subsequent investigations.

Seed bacterization trials conducted on the CO 2 variety of brinjal revealed that rhizobacterial isolates B 38 (Pudukkottai district) and B 67 (Dindigul district) significantly enhanced germination rates and vigor indices associated to the untreated control (Table 1). These results indicate the effect of these cultures to promote early seedling growth in brinjal.

Biochemical characterization of rhizobacterial cultures for growth-promoting traits

Biochemical assays demonstrated that isolate B 67 exhibited the highest IAA accumulation at 48.50 µg/L×10^8 cfu, followed by isolate B 38 with 43.00 µg/L×10^8 cfu. Regarding siderophore production, isolate B 67 showed superior activity (545 mm^2) compared to isolate B 38 (544 mm^2). Additionally, phosphorus solubilization assays revealed that isolate B 67 achieved the highest solubilization index (2.13) among the tested isolates (Table 2). These findings underscore the promising plant growth-promoting properties (PGPR activities) of isolates B 38 and B 67. Based on these effects, these 2 isolates were selected for further *in vitro* and *in vivo* studies.

In vivo studies (Pot culture)

Pot culture experiments conducted over 2 consecutive years (2022-2023) demonstrated the efficacy of integrated treatments in managing little leaf disease caused by phytoplasma. Treatment T6, which included seedling treatment with isolate B 67, drenching with isolate B 67 at 30, 60 and 90 days after transplanting and need-based application of NSKE 5%, resulted in the most effective disease management. Similarly, T7, involving seedling treatment with *Bacillus subtilis* (Bbv57), drenching with *B. subtilis* (Bbv57) at 30, 60 and 90 days after transplanting and need-based spraying with NSKE 5%, also performed well in reducing disease incidence (Table 3, Fig. 1).

Genomic sequencing of the bacterial isolates using 16S rDNA analysis

Molecular characterization of isolate B 67 through 16S rDNA sequencing and subsequent BLAST analysis revealed a high grade of comparison with *B. licheniformis* based on nucleotide similarity and phylogenetic alignment (Table 4, Fig. 2). These findings confirm that isolate B 67 belongs to the species *B. licheniformis*.

Field trial for the integrated management of little leaf of brinjal

Field trials were conducted in 3 distinct locations to validate the findings from pot culture studies. The integrated pest and disease management (IPDM) approach (T1), which included seedling treatment with *B. licheniformis* isolate B 67 and *B. subtilis* (Bbv57), drenching with 3 mL humic acid, foliar spraying of 0.5% ferrous sulfate and 0.5% zinc sulfate, drenching with *B. licheniformis* isolate B 67 and *B. subtilis* (Bbv57) at 30, 60 and 90 days after transplanting and need-based spraying with NSKE 5% and dimethoate 30 EC at 1 mL/L, showed the lowest incidence of little leaf disease (4.48%). This treatment also achieved a significantly higher yield (22.05 t/ha) compared to the control.

Table 1. Growth promotion effect of rhizobacterial isolates on brinjal

Isolate	Germination Percentage (%)	Vigour Index	Isolate	Germination Percentage (%)	Vigour Index
B1	62.00	440.82	B 51	58.00	446.02
B 2	80.00	422.40	B 52	88.00	567.60
B 3	84.00	706.44	B 53	70.00	364.71
B 4	56.00	351.68	B 54	96.00	402.24
B 5	60.00	467.40	B 55	82.00	675.68
B 6	65.00	358.80	B 56	80.00	655.20
B 7	83.50	543.59	B 57	84.00	477.12
B 8	46.00	225.60	B 58	76.00	417.24
B 9	68.00	508.64	B 59	70.00	306.60
B 10	78.00	210.00	B 60	88.00	843.04
B 11	56.00	341.04	B 61	54.00	237.06
B 12	82.00	533.82	B 62	66.00	276.54
B 13	46.00	124.20	B 63	80.00	457.60
B 14	96.00	926.30	B 64	70.00	337.40
B 15	94.00	703.12	B 65	58.00	208.22
B 16	80.00	271.20	B 66	30.00	108.60
B 17	54.00	316.44	B 67	**100.00**	**1012.00**
B 18	56.00	310.24	B 68	60.00	256.20
B 19	88.00	628.32	B 69	96.00	516.48
B 20	62.00	404.24	B 70	48.00	218.56
B 21	88.00	454.08	B 71	62.00	332.32
B 22	86.00	884.08	B 72	92.00	757.16
B 23	84.00	546.84	B 73	86.00	559.00
B 24	54.00	244.62	B 74	70.00	316.08
B 25	84.00	337.68	B 75	86.00	485.04
B 26	88.00	484.88	B 76	62.00	345.98
B 27	74.00	277.50	B 77	78.00	452.40
B 28	46.00	233.68	B 78	80.00	508.00
B 29	62.00	345.96	B 79	84.00	577.08
B 30	92.00	706.56	B 80	76.00	473.48
B 31	92.00	648.60	B 81	70.00	304.50
B 32	40.00	105.64	B 82	88.00	655.60
B 33	92.00	419.52	B 83	52.00	226.72
B 34	30.00	103.50	B 84	66.00	370.26
B 35	90.00	847.80	B 85	88.00	756.60
B 36	94.00	903.34	B 86	62.00	352.78
B 37	88.00	706.64	B 87	84.00	524.16
B 38	100.00	984.00	B 88	72.00	336.96
B 39	92.00	560.28	B 89	44.00	204.60
B 40	91.60	525.60	B 90	60.00	263.40
B 41	90.00	445.50	B 91	90.00	644.40
B 42	94.00	773.62	B 92	78.00	439.14
B 43	64.00	305.92	B 93	62.00	359.60
B 44	74.00	506.90	B 94	80.00	520.00
B 45	88.00	737.76	B 95	86.00	566.74
B 46	100.00	859.00	B 96	74.00	434.38
B 47	86.00	842.80	B 97	72.00	435.60
B 48	84.00	460.32	B 98	50.00	280.00
B 49	78.00	255.84	B 99	58.00	281.30
B 50	70.00	326.90	B 100	78.00	510.12
			Bbv7	94.00	940.00
			Control	84.00	766.08
			CD (0.05)	**17.75**	**8.40**
			SE.d	**8.94**	**4.23**

**1 - 55: Pudukkottai isolates; 55 - 72: Dindigul isolates; 73 - 91: Theni isolates; 91 - 100: Thoothukudi isolates, 101 - Bacillus subtilis (Bbv7), ** Mean of 2 replications*

Table 2. Plant growth-promoting traits of rhizobacterial isolates

Sl. No.	PGPR isolates	IAA* (µg/1x10^{8}cfu)	Siderophore production* (mm^2)	Phosphorus solubilization Index
1.	14	41.60^b	512^b	1.84^a
2.	36	32.50^c	106^d	0.0^c
3.	38	43.00^b	544^a	1.79^a
4.	46	11.50^d	142^c	1.38^b
5.	67	48.50^a	545^a	2.13^a
CD (0.05)		**3.22**	**4.46**	**0.37**
SE.d		**1.51**	**2.09**	**0.17**

** Mean of 4 replications*

Table 3. *In vivo* studies on the management of little leaf disease in brinjal (2022-2023)

Sl. No.	Treatment particulars	Percentage Incidence (PI)			
		Pot culture I	Pot culture II	Pot culture III	Pooled mean
1.	T1- Seed treatment with streptocycline 100 ppm for 30 min + seedling treatment for 20-30 min with (Streptomycin sulphate + tetracycline hydrochloride at 150 ppm) + need based foliar spray of Streptomycin sulphate + tetracycline hydrochloride at 150 ppm	41.67^{cd} (6.42)	50.00^{cd} (6.94)	55.56^b (7.40)	49.07^{bcd} (44.47)
2.	T2 – Foliar spray of 50 ppm gibberellic acid at 30, 60 and 90 days after transplanting + need based spraying with NSKE 5%	41.67^{cd} (6.42)	41.67^{bcd} (6.40)	55.56^b (7.40)	46.30^{bc} (42.47)
3.	T3 - Spray drenching with 3 mL of humic acid at 30, 60 and 90 days after transplanting + need based spraying with NSKE 5%	33.33^{bc} (5.70)	33.33^{abc} (5.73)	44.78^b (6.63)	37.15^b (37.50)
4.	T4 - Foliar spray of 0.5% ferrous sulphate + .0.5% Zinc sulphate at 30, 60 and 90 days after transplanting + need based spraying with NSKE 5%	25.00^{abc} (5.00)	33.33^{abc} (5.73)	44.78^b (6.63)	34.37^b (35.75)
5.	T5 - Seedling treatment with isolate B 38 + drenching with isolate B 38 at 30, 60 and 90 days after transplanting + need based spraying with NSKE 5%	41.67^{cd} (6.42)	58.33^{cd} (7.10)	77.78^b (8.81)	59.26^{bcd} (50.62)
6.	T6 - Seedling treatment with isolate B 67 + drenching with isolate B 67 at 30, 60 and 90 days after transplanting + need based spraying with NSKE 5%	08.33^a (2.15)	16.67^a (3.60)	11.11^a (2.41)	12.04^a (22.08)
7.	T7 - Seedling treatment with *Bacillus subtilis* (Bbv57) + drenching with *Bacillus subtilis* (Bbv57) at 30, 60 and 90 days after transplanting + need-based spraying with NSKE 5%	16.67^{ab} (3.60)	25.00^{ab} (5.05)	11.11^a (2.41)	17.59^a (24.52)
8.	T8 – Systemic insecticide spray with dimethoate 30 EC at 1 mL/L	41.67^{cd} (6.42)	50.00^d (7.63)	55.56^b (7.40)	49.07^{cd} (50.62)
9.	T9 - control	58.33^d (7.63)	58.33^d (7.63)	77.78^b (8.81)	64.82^d (53.82)
	CD (0.05)	**23.34**	**24.75**	**33.23**	**10.66**
	SE.d	**11.11**	**11.78**	**15.82**	**5.07**

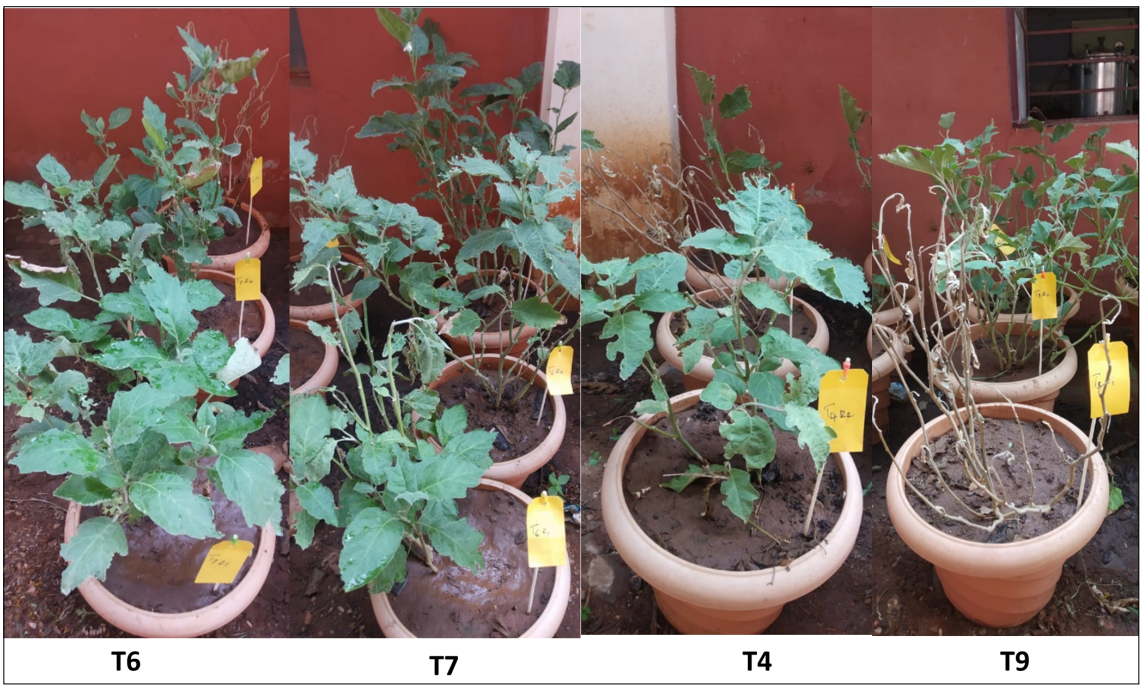

Fig. 1. Pot culture studies on the management of little leaf disease in brinjal. T6 - Seedling treatment with isolate B 67 + drenching with isolate B 67 at 30, 60 and 90 days after transplanting + need based spraying with NSKE 5%. T7 - Seedling treatment with *Bacillus subtilis* (Bbv57) + drenching with *Bacillus subtilis* (Bbv57) at 30, 60 and 90 days after transplanting + need-based spraying with NSKE 5%. T4 - Foliar spray of 0.5% ferrous sulphate + .0.5% Zinc sulphate at 30, 60 and 90 days after transplanting + need based spraying with NSKE 5%. T9- Control.

Table 4. NCBI sequence alignments showing significant matches with isolate B 67

Description	Max score	Total score	Query cover	E value	Per. indent	Accession
Bacillus licheniformis strain Sua-BAC006	2503	2503	100%	0.0	99.42%	EU870503.1
Bacillus paralicheniformis strain A4-3	2503	2503	100%	0.0	99.42%	MN121188.1
Bacillus licheniformis strain FC14167	2503	2503	100%	0.0	99.42%	MT704412.1
Bacillus sp. XJ1-05	2503	2503	100%	0.0	99.42%	EF591780.1
Bacillus licheniformis strain APSAC 04	2503	2503	100%	0.0	99.42%	KY886137.1
Bacillus paralicheniformis strain J27TS1	2499	2499	100%	0.0	99.28%	LC58821.1
Bacillus licheniformis strain K10	2497	2497	100%	0.0	99.35%	DQ351930.2
Bacillus licheniformis strain Sua-BAC008	2497	2497	100%	0.0	99.35%	EU870504.1
Bacillus sp. bB25(2011)	2497	2497	100%	0.0	99.35%	JF772466.1
Bacillus sp. KP22	2497	2497	100%	0.0	99.35%	KJ777154.1

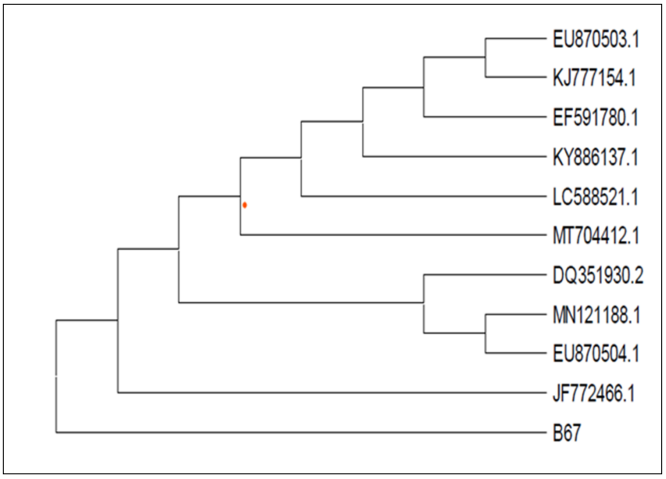

Fig. 2. Phylogenetic tree depicting nucleotide sequence similarities of isolate B 67.

Economic analysis further revealed that integrated pest and disease management (IPDM) approach recorded the highest benefit-cost (BC) ratio of 3.15, outperforming both the farmers' practice (2.65) and the untreated control (2.28). Consistent results were observed across all 3 trial locations, as evidenced by pooled analysis data (Table 5).

Discussion

Brinjal cultivation is severely impacted by various biotic and abiotic factors, with little leaf disease caused by phytoplasma being an important global challenge. The disease manifests through distinctive symptoms, including reduced leaf size, witches' broom, flower malformation, phyllody, oversize calyxes and buds, leading to substantial reductions in both yield and quality (7). Despite various control measures, including chemical fertilizers and pesticides, sustainable and eco-friendly solutions remain limited. The phytoplasma's biotrophic nature complicates management as it relies on living hosts, making integrated approaches essential for addressing this issue comprehensively.

Challenges in managing little leaf disease

Chemical approaches, such as the application of tetracyclines, have shown temporary remission of symptoms. For instance, tetracycline at 500 ppm successfully restored normal leaf size and internode length within 30-37 days after treatment; however, this remission lasted only 20-30 days, necessitating frequent applications (23, 24). Similarly, controlling vectors through systemic insecticides, such as carbaryl and dimethoate, reduced disease severity but did not eliminate reinfections, which persisted due to vector activity (17, 25). The reliance on chemical-based solutions raises environmental concerns, including residue accumulation in harvested produce, which can negatively impact human health by causing chronic exposure to harmful chemicals and pollution, which disrupts ecosystems. This underscores the need for bio-intensive strategies.

Role of Plant Growth-Promoting Rhizobacteria (PGPR)

PGPR, including *B. subtilis* (Bbv 57) sourced from the TNAU commercial product and *B. licheniformis* isolated from brinjal rhizosphere in the present study, have emerged as promising bio-intensive alternatives for managing phytoplasma-associated diseases. In this study, under *in vivo* conditions, *B. subtilis* (Bbv 57) and *B. licheniformis* isolate B 67 effectively reduced disease incidence in brinjal. PGPR enhance plant development and yield through direct mechanisms, such as producing antibiotics, enzymes, hydrogen cyanide, volatile organic compounds and ammonia to suppress pathogens (26-28). They also indirectly induce systemic and acquired resistance, inhibit quorum sensing and disrupt biofilm formation (29). The ability of PGPR to withstand environmental stressors, coupled with their broad-spectrum activity and compatibility with other rhizobacteria, underscores their utility in sustainable disease management (30).

Several studies highlight the antagonistic potential of PGPR. For example, *B. subtilis* strains produce lipopeptides, such as fengycin, surfactin and iturin, which exhibit antifungal properties (31). Specifically, iturin A produced by *B. subtilis*

Table 5. Field trials for integrated management of little leaf disease in brinjal across different locations

Sl. No.	Treatment particulars	Percentage Incidence* (PI)				Yield* per t/ha				BC ratio
		I	II	III	Pooled mean	I	II	III	Pooled mean	
1.	T1	4.29^{a}	4.57^{a}	4.57^{a}	4.48	22.14^{a}	21.86^{a}	22.14^{a}	22.05	3.15
2.	T2	9.05^{b}	10.29^{b}	9.71^{b}	9.68	20.50^{a}	19.29^{b}	19.86^{b}	19.88	2.65
3.	T3	13.81^{c}	16.00^{c}	15.43^{c}	15.08	15.00^{b}	14.71^{c}	14.79^{c}	14.83	2.28
CD (0.05)		**3.18**	**3.49**	**3.68**		**1.89**	**1.66**	**2.20**		
SE.d		**1.46**	**1.60**	**1.69**		**0.86**	**0.76**	**1.01**		

**Locations - Kalanjipatti (I), Kannivadi (II) and Muthanampatti (III) of Dindigul district, Tamil Nadu, India*

strains PCL1608 and PCL1612 has been shown to inhibit *Fusarium oxysporum* and *Rosellinia necatrix* (32). Furthermore, *B. licheniformis* strain ML3, isolated from weed rhizospheres, demonstrates plant growth-promoting traits, including IAA production, phosphate solubilisation and siderophore activity (33-35).

Efficacy of humic acid and micronutrient applications

Humic acid emerged as a crucial component in this study, improving soil physicochemical and biological properties as reported in a previous study (36). It promotes plant growth and enhances stress resilience by increasing nutrient availability and uptake. For example, combining NPK fertilizers with humic acid foliar applications enhanced chlorophyll content and growth in lettuce (37). Similarly, the foliar application of ferrous sulfate and zinc sulfate in conjunction with humic acid demonstrated a significant reduction in little leaf disease incidence in brinjal, with reductions of 37.15% (T3) and 34.37% (T4). These results align with previous studies on sesame and cucumber, where phytoplasma infections were linked to reduced water content, chlorophyll levels and mineral concentrations (38, 39).

Development of an integrated management module

Building on these findings, an Integrated Pest and Disease Management (IPDM) module was developed to effectively mitigate little leaf disease. The module integrated seedling treatment with *B. subtilis* (Bbv 57) and *B. licheniformis* isolate B 67, drenching with humic acid, foliar applications of ferrous sulfate and zinc sulfate and need-based spraying of neem seed kernel extract (NSKE 5%) and dimethoate 30 EC. Under field conditions, systemic insecticide sprays with dimethoate achieved a 49.07% reduction in disease severity, complementing the bio-intensive components.

Advantages of Bio-Intensive Approaches

The bio-intensive IPDM module offers several advantages over conventional methods. It minimizes environmental impacts by reducing chemical usage while enhancing plant resilience through the synergistic effects of PGPR, humic acid and micronutrients. This approach aligns with sustainable agricultural practices, ensuring improved crop health and productivity. The module's success underscores the importance of combining biological and chemical strategies to manage vector-mediated diseases effectively.

This study highlights the potential of bio-intensive approaches, particularly the integration of PGPR, humic acid and micronutrients, for sustainable management of little leaf disease in brinjal. Future research could focus on further optimizing the module for large-scale field applications and exploring its efficacy against other phytoplasma-associated diseases.

Conclusion

Little leaf disease in brinjal, poses a significant challenge due to its biotrophic nature and dependence on living hosts. Traditional chemical-based management practices have proven insufficient, often leading to reinfections, environmental pollution and residue accumulation in harvested produce, raising concerns about food safety and sustainability. This study highlights the potential of bio-intensive strategies as sustainable alternatives for managing this disease. The integration of PGPR such as *B. licheniformis* and *B. subtilis*, along with humic acid, micronutrient applications and need-based chemical sprays, significantly reduced disease incidence and enhanced plant health through synergistic mechanisms. The IPDM module developed in this research comprising seedling treatments, foliar applications of humic acid and micronutrients and judicious use of systemic insecticides showed to be exceedingly effective in reducing the disease intensity. This holistic approach not only minimizes the environmental impact but also supports sustainable agricultural practices by improving crop resilience, productivity and soil health. These findings demonstrate that the adoption of bio-intensive management strategies can bridge the gap between effective disease control and environmental safety, offering a scalable solution for managing little leaf disease in brinjal. Future research should focus on optimizing these approaches for large-scale implementation and exploring their potential in managing other phytoplasma-associated diseases across various crops.

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Authors' contributions

AV, MK conceptualized the review and defined the structure of the manuscript. VR, SP, CR, JR and SS helped to improvise language and content. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest.

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