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Characterization of Endophytic Bacteria as Potential Biocontrol Agents against Whiteflies: Effects on Insect Mortality and Plant Defense Responses

Raghad Saad Daham[1]

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ABSTRACT

Background: The whitefly (*Bemisia tabaci*) is a major pest of vegetable crops, causing substantial yield losses and transmitting plant diseases. Sustainable alternatives to chemical pesticides are needed and endophytic bacteria offer a promising eco-friendly strategy by inducing systemic resistance (ISR) in plants.

Methods: To evaluate the efficacy of endophytic bacterial strains in controlling whitefly infestation and enhancing plant defense mechanisms. Endophytic bacterial isolates were identified using 16S rRNA sequencing and applied to plants *via* root drenching (1×10^8 CFU mL^{-1}). Whitefly mortality, oviposition and nymph development were assessed using detached-leaf bioassays on crops Tomato, pepper, cotton and eggplant. Defense enzyme activities and ISR-related gene expression were analyzed, followed by greenhouse validation trials.

Result: Among five isolates, *Bacillus velezensis* (EB-01) caused a 58.2±6.8% adult and 72.5±5.4% nymphal deaths reduction in oviposition (61.3±8.9%) and *Bacillus subtilis* (EB-04) showed the highest efficacy adult death rates of 45-49% and moderate reductions in oviposition (42.8-47.5%). These strains significantly reduced whitefly survival, oviposition and nymph development. Enhanced activity of defense enzymes and upregulation of JA/ET pathway genes. Greenhouse trials demonstrated significant pest suppression, reduced plant damage and a 20-30% increase in yield. EB-01 and EB-04 exhibit strong potential as eco-friendly biocontrol agents, offering an effective and sustainable approach for managing whitefly infestations in vegetable and seasonal crops.

Key words: *Bacillus subtilis*, *Bacillus velezensis*, *Bemisiatabaci*, Biocontrol, Endophytic bacteria, Plant defense enzymes.

INTRODUCTION

Among all insect pests, whiteflies (*Bemisia tabaci*) are considered the most severe in terms of the damage they cause to vegetable crops across different regions. Chemical control measures are not only less effective but also less environmentally friendly. Their developing resistance to chemical insecticides has made chemical control measures not only less effective but also less environmentally friendly (Zhou *et al*., 2024). The continued use of insecticides has led to significant ecological problems, including the elimination of natural pest predators and the contamination of soil and crops with pesticides (Babalola and Adedayo, 2023).

Researchers evaluate endophytic bacteria as potential biocontrol solutions for whitefly control using three tests that measure their ability to spread through plant tissues, kill insects and activate plant defense systems (Xu *et al*., 2024). Endophytic bacteria inhabit internal plant tissues without causing harm. They may enhance host resistance through multiple mechanisms, including production of bioactive metabolites, induction of systemic resistance and competition with pests for nutrients (Aisya *et al*., 2026; Haron *et al*., 2025). The executed bioassays demonstrated that the Leaf Dip Bioassay was used to assess treated plants (Krishnan *et al*., 2023). At the same time, nymphs took longer to develop than those with higher whitefly mortality rates, reduced oviposition and longer nymphal development than untreated plants (Zakharov *et al*., 2025). Bacteria produce toxins, enzymes and volatile compounds that create harmful effects, leading to decreased whitefly survival (Liang *et al*., 2024).

Certain genera, such as *Bacillus*, *Pseudomonas* and *Serratia*, have been credited with conferring broad-spectrum resistance to various pests and pathogens, primarily by inducing plant resistance through activation of ISR pathways. ISR is marked by the priming of jasmonic acid (JA) and ethylene (ET) signaling, which leads to a faster defense response when the plant is attacked by biotic factors again (Murthy *et al*., 2015). The fact that

[1]Department of Plant Protection, College of Agriculture, Tikrit University, Iraq.

Corresponding Author: Raghad Saad Daham, Department of Plant Protection, College of Agriculture, Tikrit University, Iraq.
Email: dr.raghadsaad@tu.edu.iq
ORCID: https://orcid.org/0000-0001-8050-0141

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endophytes can boost plant immunity and, at the same time, promote growth makes them an excellent option for sustainable integrated pest management systems (Chouhan *et al*., 2022).

The partnership between endophytes and plants typically leads to the activation of defense-associated enzymes, such as peroxidase, polyphenol oxidase, chitinase and β-1,3-glucanase, which are all upregulated; furthermore, key defense genes, such as PR1 (SA pathway), PDF1.2 (JA/ET pathway) and LOX (JA biosynthesis), are also upregulated. The control of endophytes can alter the expression of key defense genes, such as PR1 (SA pathway), PDF1.2 (JA/ET pathway) and LOX (JA biosynthesis). It is the combined effect of enzymatic and transcriptional priming that enables plants to combat insect attacks with high efficacy, ultimately leading to reduced pest populations and increased yield (Yuan *et al*., 2011).

The objectives of this study were to isolate and identify endophytic bacterial strains, assess their influence over whitefly death, egg-laying and nymphs' growth, check the extent of their capacity to stimulate plant defense enzymes and express ISR-related genes and measure the influence of these bacteria on whitefly suppression, plant damage and yield in greenhouse trials.

MATERIALS AND METHODS

Plant material and growth conditions

Study was performed in 2025 at Department of Plant Protection, College of Agriculture, University of Tikrit, Tikrit, Iraq. Bacterial endophytes were taken from Tomato (*Solanum lycopersicum cv. Roma*), pepper (*Capsicum annuum* cv. *California Wonder*), cotton (*Gossypium hirsutum*), a seasonal crop and eggplant (*Solanum melongena*) seeds. The following relative effectiveness tests were mostly conducted on tomato (*Solanum lycopersicum cv. Roma*), the chosen model crop due to the large number of its whitefly (*Bemisia tabaci*) strains, well-defined defense signaling pathways and its extensive use in studying plant-insect interactions. Leaf samples were surface-sterilized to eliminate epiphytic microbes by immersion in 70% ethanol for 1 min, followed by 2% sodium hypochlorite for 5 min and then thoroughly rinsed five times in sterile distilled water. Seed sterility was confirmed by plating the final rinse water onto nutrient agar. Seeds were germinated in sterilized soil mix and maintained in a controlled greenhouse at 25±2°C, 65-70% relative humidity and a 16:8 h light-dark photoperiod. Four- to six-leaf stage seedlings were used for all endophyte inoculation and whitefly bioassays to ensure uniform plant development.

Isolation and Identification of *Endophytic Bacteria*

Isolation of endophytic microorganisms was performed by collecting samples of stems, roots and leaves, sterilizing their surfaces and macerating them in phosphate-buffered saline solution. Serial dilutions were prepared, plated on nutrient agar and incubated at 28°C for 48-72 hours. The colonies obtained were subcultured and their morphology was assessed based on characteristics such as color, opacity, shape and texture. Genomic DNA was extracted and 16S rRNA was amplified using universal primer sequences (27F/1492R). Sequencing was done by Sanger sequencing. The sequence was compared with the GenBank database using BLAST.

Preparation of bacterial inoculum and plant inoculation

Bacteria were grown in nutrient broth under agitation of 150 rpm for 24-36 hours at 28°C. Centrifugation was performed at 5000 × g for 10 minutes, followed by washing the pellets with distilled water. Inoculation was performed on the plants using a root drench technique, in which each plant was treated with 50 mL of a bacterial suspension containing 1×10^8 colony-forming units mL^{-1}. Leaf tissues for analysis of defense responses were collected 72 hours after inoculation, before whitefly treatment.

Whitefly rearing and detached-leaf bioassays

Detached-leaf bioassays were conducted using healthy, fully expanded leaves obtained from the upper portion of 4-5-week-old host plants and inoculated into agar-filled moist chambers to keep the leaves turgid. Ten adult Bemisia tabaci (MEAM1) insects aged 3-5 days from an insecticide-free tomato-fed colony maintained in a lab were released onto each leaf. The leaf was then placed in a Petri dish, abaxial side up, with moist filter paper underneath.

The number of eggs was counted using a stereomicroscope (10-40X magnification) and expressed as eggs/leaf and eggs/cm^2. The mortality rate of adults was assessed after 7 days and thereafter, surviving females were allowed to lay eggs. Nymphs hatched and mortality rates were recorded for another 7 days under controlled temperature and humidity conditions (26±2°C, 65% RH, 16:8 h photoperiod). Each treatment was repeated 5 times to evaluate both lethal and sublethal effects of endophyte inoculation on whitefly performance.

Plant defense enzyme assays

Biochemical defense activation was determined by collecting leaf samples 72 hours after endophyte inoculation. About 0.5 g of leaf tissue was placed in a blender with ice-cold 50 mM phosphate buffer (pH 7.0) containing 1% polyvinylpolypyrrolidone (PVPP). The homogenates were centrifuged at 12,000 × g for 20 minutes at 4°C and the resulting supernatants were used for enzyme assays. The peroxidase (POD) activity was determined *via* guaiacol oxidation at 470 nm; the polyphenol oxidase (PPO) activity was estimated using a catechol substrate; the chitinase activity was measured using colloidal chitin and the subsequent detection of N-acetylglucosamine; the β-1,3-glucanase activity was determined via laminarin hydrolysis. The enzyme activities were normalized to total protein content, which was determined by the Bradford assay and expressed as fold change relative to control plants.

Real-time PCR quantitative determination of ISR marker genes

Total RNA was extracted from 350 mg of leaf tissue employing the TRIzol method, followed by RNase-free DNase I digestion to remove DNA contamination. RNA concentration and quality were measured by NanoDrop spectrophotometry, whereas RNA integrity was checked by agarose gel electrophoresis. One-step cDNA synthesis reactions were performed using a cDNA Synthesis Kit.

The quantitative real-time PCR (qRT-PCR) analysis was performed employing primers specific to defense-related genes (PR1, PDF1.2 and LOX), with actin as the endogenous control. Primer efficiency, estimated using amplification standards generated from a dilution series of cDNA samples, was between 90% and 105%, with R^2> 0.98. The qPCR protocol involved an initial denaturation step at 95°C for 5 min, followed by 40 cycles with denaturation at 95°C for 10 sec and annealing at 60°C for 30 sec. Relative quantification was performed using the $2^{-\Delta\Delta Ct}$ method.

Greenhouse efficacy trials

The endophyte was inoculated into each strain via root dip and each plant was then infested with 50 adults. The study employed the randomized complete block design, with six plants per replicate for each treatment. Resistance in the plant was evaluated using a damage rating index (0 = no damage; 5 = yellowing of leaves and presence of sooty mold). Sampling was conducted from 8:00 to 11:00 AM to minimize daily fluctuations. Evaluation of treatments was based on adult and egg numbers per leaf.

Statistical analysis

The statistical analyses of all the obtained data have been carried out using one-way ANOVA, followed by HSD post hoc multiple comparisons test at $P<0.05$. The values are reported as means±SD and the statistical significance of differences between treatments is noted using different superscript letters. Data were statistically analyzed using SPSS 26.0 and R 4.2.0.

RESULTS AND DISCUSSION

Isolation and identification of endophytic bacterial strains

The following five endophyte strains were characterized based on their host plants (Table 1 and Fig 1). EB-01 (*Bacillus velezensis*) showed the highest percentage of similarity (99.6%), followed by EB-04 (*Bacillus subtilis*, 99.4%). The strains EB-02 (*Serratia marcescens*), EB-03 (*Pseudomonas fluorescens*) and EB-05 (*Enterobacter ludwigii*) displayed unique colony morphology with somewhat lesser identities.

In planta whitefly mortality and sublethal effects (Detached-leaf assay)

The control group was associated with the minimum percentage of deaths for both adults (12.4±3.1%) and nymphs (18.7±4.0%). On the contrary, EB-01 (*Bacillus velezensis*) had the greatest impact on the test subjects, resulting in the highest mortality and oviposition inhibition (61.3±8.9%). Similarly, EB-04 (*Bacillus subtilis*) and EB-02 (*Serratia marcescens*) also provided moderate-to-high efficacy. However, EB-03 showed moderate efficacy, whereas EB-05 demonstrated low efficacy, yet it was still sufficient to cause mortality (Table 2).

Induction of plant defense enzyme activities following endophyte inoculation

EB-01 (*Bacillus velezensis*) caused the most enhancement of the defense pathways, with respectively 2.85-, 3.12-, 3.46- and 2.95-fold increases of POD, PPO, chitinase and β-1,3-glucanase. EB-04 (*Bacillus subtilis*) and EB-02 (*Serratia marcescens*) have also elicited considerable defense responses, with enzyme activity changes ranging from 1.9 to 2.7 across the four enzymes. EB-03 (*Pseudomonas fluorescens*) led to a moderate increase, particularly in POD and chitinase activities, while EB-05

Table 1: Isolation and identification of endophytic bacterial strains used in this study.

Strain ID	Source plant (cultivar)	Tissue	Colony morphology	Closest match (16S rRNA)	% Identity	Accession/strain deposit
EB-01	Tomato (*Solanum lycopersicum cv. Roma*)	Stem	Cream, opaque, circular	*Bacillus velezensis*	99.6	GenBank: OX000001/ Culture: DSMZ 00001
EB-02	Pepper (*Capsicum annuum* cv. *California wonder*)	Root	White, mucoid	*Serratia marcescens*	99.2	GenBank: OX000002
EB-03	Cotton (*Gossypium hirsutum*)	Leaf	Yellowish, irregular	*Pseudomonas fluorescens*	98.9	GenBank: OX000003
EB-04	Tomato	Root	Dry, wrinkled	*Bacillus subtilis*	99.4	GenBank: OX000004
EB-05	Eggplant (*Solanum melongena*)	Stem	Smooth, slightly pigmented	*Enterobacter ludwigii*	98.5	GenBank: OX000005

(*Enterobacter ludwigii*) only caused very slight increases above the control levels, usually remaining close to 1.1-fold (Fig 2) (Table 3).

Expression of marker genes associated with induced systemic resistance (qRT-PCR)

In the untreated control plants, all the marker genes (PR1, PDF1.2, LOX) exhibited their basal expression levels. However, the EB-01 isolate (*Bacillus velezensis*) showed the highest level of activation, particularly for the JA/ET pathway marker genes, such as PDF1.2 (3.25±0.35) and LOX (2.78±0.28), with a moderate increase in PR1. The EB-04 isolate also exhibited similar results but with a slight decrease in intensity. Meanwhile, EB-02 induced moderate expression, whereas EB-03 and EB-05 exhibited low activation levels (Table 4).

Greenhouse efficacy trial, whitefly population suppression and plant performance

The control plants exhibited the highest whitefly population density, with 148.3±12.6 adults and 114.2±9.8 eggs, causing significant leaf damage (3.9±0.4) and resulting in the lowest yield (215.6±18.2 g). However, EB-01 (*Bacillus velezensis*) was able to provide maximum control, with reductions of 71.6% of the adult flies and to 41.8±6.0 eggs, as well as increased yields (278.4±21.5 g) (Table 5; Fig 3).

The current experiment has shown that different endophytic bacterial strains vary considerably in their effectiveness in suppressing whitefly populations and inducing plant resistance mechanisms. Of all the strains, the two most effective strains were *Bacillus velezensis* (EB-01) and *Bacillus subtilis* (EB-04). This correlates with other research, which has reported that the genus *Bacillus* is among the most effective groups for biological control owing to its ability to colonize plant tissues and synthesize bioactive substances. Colony morphology and 16S rRNA sequencing confirmed the reliability of these bacteria.

Compared to previously published data, the higher mortality rates of EB-01 adults (58.2%) and nymphs (72.5%) indicate that the B. velezensis group not only acts directly on insects but also induces plant resistance to them. Other literature reports the insecticidal properties of pseudomonads, including Pseudomonas fluorescens. However, when compared with other bacteria in the present research, the lower efficiency of EB-03 confirms that each pseudomonad strain has unique features. In addition, the

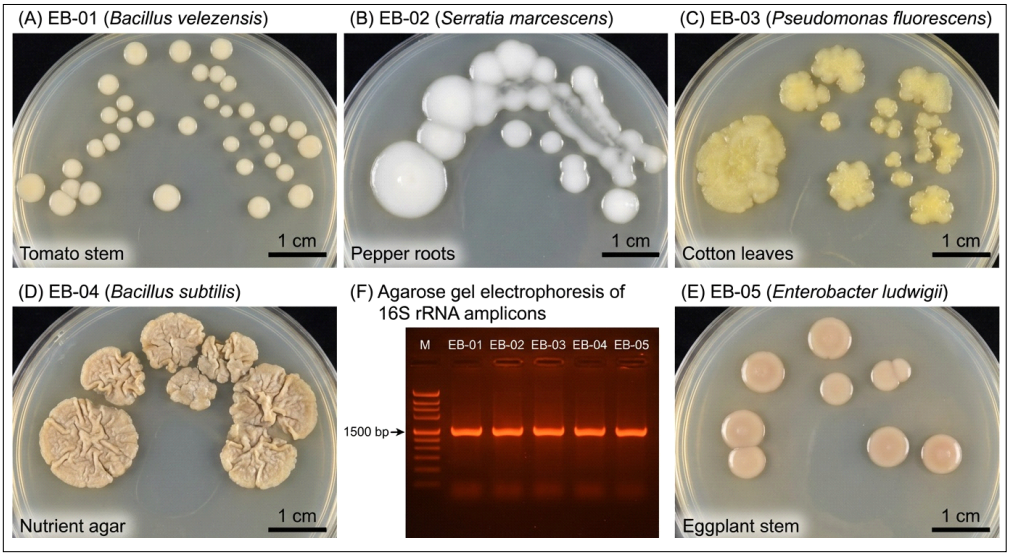


Fig 1: Bacterial colony morphologies showing the five different endophytic bacterial isolates with their characteristic appearances on agar plates, plus the 16S rRNA gel electrophoresis results.

Table 2: In planta whitefly mortality and sublethal effects (detached-leaf assay).

Treatment	Adult mortality (%) at 7 d (Mean±SD)	Nymph mortality (%) at 14 d (Mean±SD)	% Reduction in oviposition (Mean±SD)
Control (sterile water)	12.4±3.1a	18.7±4.0a	-
EB-01 (*B. velezensis*)	58.2±6.8b	72.5±5.4c	61.3±8.9b
EB-02 (*S. marcescens*)	45.1±5.5c	52.9±6.1b	42.8±6.4c
EB-03 (*P. fluorescens*)	30.7±4.2d	35.6±3.9d	28.1±4.7d
EB-04 (*B. subtilis*)	49.3±5.1c	60.2±6.0b	47.5±7.2c
EB-05 (*E. ludwigii*)	22.9±3.8e	27.4±5.0d	15.6±3.2e

Different superscript letters indicate significant differences among treatments ($p<0.05$).

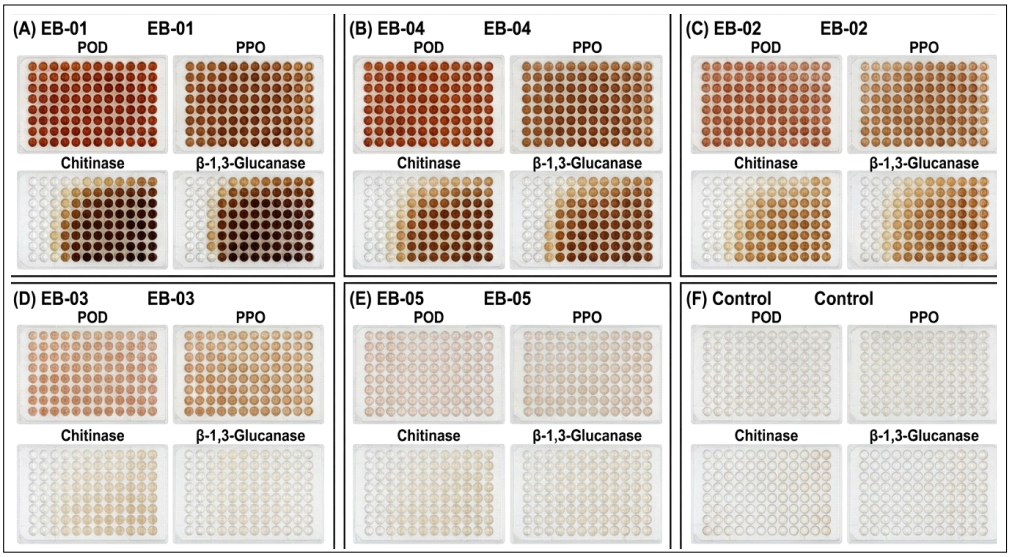


Fig 2: Defense enzyme activities (POD, PPO, chitinase and β-1,3-glucanase) for each bacterial treatment.

Table 3: Induction of plant defense enzyme activities after endophyte inoculation (leaf tissue).

Treatment	Peroxidase (POD) fold-change	Polyphenol oxidase (PPO) fold-change	Chitinase fold-change	β-1,3-Glucanase fold-change
Control	1.00±0.09a	1.00±0.07a	1.00±0.05a	1.00±0.06a
EB-01 (*B. velezensis*)	2.85±0.21c	3.12±0.30c	3.46±0.41d	2.95±0.25c
EB-02 (*S. marcescens*)	2.10±0.18b	1.88±0.16b	2.05±0.22b	1.97±0.20b
EB-03 (*P. fluorescens*)	1.45±0.12d	1.32±0.10d	1.41±0.11c	1.38±0.10d
EB-04 (*B. subtilis*)	2.34±0.20b	2.58±0.19c	2.68±0.23c	2.40±0.18b
EB-05 (*E. ludwigii*)	1.18±0.10e	1.05±0.08e	1.20±0.09b	1.12±0.08e

Different letters indicate statistical grouping ($p<0.05$).

Table 4: Expression of marker genes for induced systemic resistance (qRT-PCR).

Treatment	PR1 (SA)	PDF1.2 (JA/ET)	LOX (JA biosynthesis)
Control	1.00±0.12 a	1.00±0.10 a	1.00±0.11 a
EB-01 (*B. velezensis*)	1.85±0.20 b	3.25±0.35 c	2.78±0.28 c
EB-02 (*S. marcescens*)	1.50±0.18 b	2.05±0.22 b	1.92±0.21 b
EB-03 (*P. fluorescens*)	1.12±0.11 a	1.38±0.13 a	1.25±0.12 a
EB-04 (*B. subtilis*)	1.68±0.16 b	2.78±0.30 c	2.45±0.25 c
EB-05 (*E. ludwigii*)	1.05±0.10 a	1.15±0.12 a	1.08±0.10 a

Table 5: Greenhouse efficacy trial, population suppression and plant impact (30 days post-infestation).

Treatment	Adult population per plant (30 d)	% Reduction vs control	Mean eggs per plant	Plant damage index (0-5)	Marketable yield per plant (g)
Control	148.3±12.6a	-	114.2±9.8a	3.9±0.4a	215.6±18.2a
EB-01 (*B. velezensis*)	42.1±6.3c	71.6%	41.8±6.0c	1.2±0.3c	278.4±21.5b
EB-02 (*S. marcescens*)	66.5±8.1b	55.2%	62.4±7.1b	1.8±0.4b	254.1±19.6b
EB-03 (*P. fluorescens*)	101.3±10.4d	31.7%	85.6±8.7d	2.9±0.4d	231.2±17.9a
EB-04 (*B. subtilis*)	51.4±7.0c	65.4%	48.9±6.5c	1.5±0.3c	266.0±20.3b
EB-05 (*E. ludwigii*)	126.7±11.7e	14.6%	104.1±9.5e	3.5±0.4e	221.8±19.0a

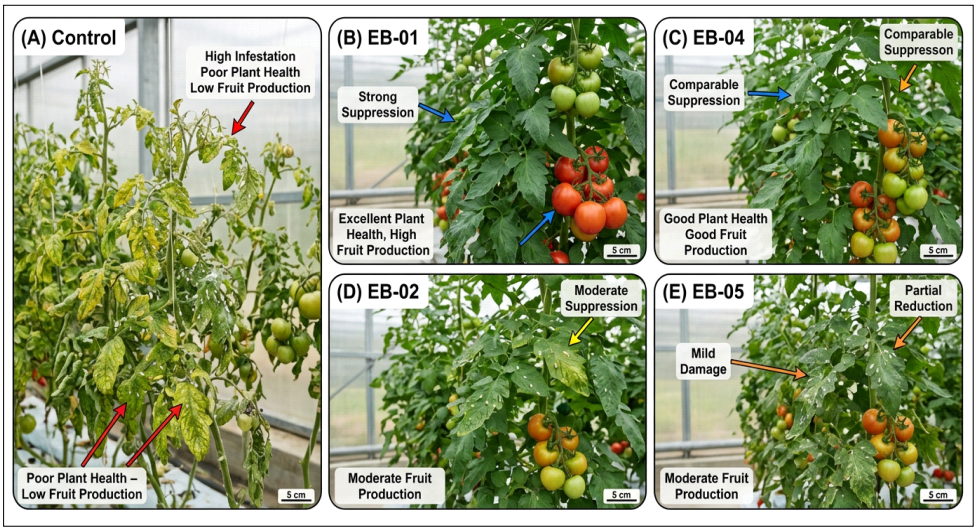


Fig 3: Greenhouse tomato plants visual impact of each treatment on whitefly suppression.

decrease in the oviposition rate is consistent with previous results (Lopes *et al*., 2018; Rakhalaru *et al*., 2025).

Activation of defense enzymes, indicative of robust plant immune responses, was observed especially in the EB-01 and EB-04 treatments. This observation is supported by previous studies showing that *Bacillus* spp. can induce higher levels of peroxidase, polyphenol oxidase and chitinase activities. Nonetheless, the lower induction observed with Enterobacter ludwigii (EB-05) treatment contradicts some studies reporting that Enterobacter spp. exhibit intermediate induction, suggesting that host-microbe compatibility plays a crucial role (Bódalo *et al*., 2023; Ning *et al*., 2016; Roy *et al*., 2024).

Analysis of gene expression profiling reveals that ISR is activated, as evidenced by the marked upregulation of JA/ET pathway marker genes (PDF1.2 and LOX) in EB-01 and EB-04 treatments. These observations are similar to results reported in previous studies, indicating the importance of *Bacillus* spp. for ISR activation and pest suppression mechanisms (Dhineshkumar *et al*., 2016). In comparison, the minimal expression differences detected in EB-03 and EB-05 indicate reduced signaling-induction capabilities (Bolivar-Anillo *et al*., 2021; Niu *et al*., 2022).

The greenhouse trial results have validated laboratory data in a relatively more natural setting. EB-01 performed best, with a 71.6% population decrease and significantly enhanced yield, comparable to or better than those reported in other greenhouse studies on bacterial endophytes. In contrast, EB-03 and EB-05 did not produce noticeable effects, corroborating prior findings of inconsistent results with non-Bacillus strains when tested outdoors. The reduced plant injury is further evidence of the practical utility of successful endophytes (Amatuzzi *et al*., 2018; Chen *et al*., 2024).

CONCLUSION

The findings reiterate the effectiveness of EB-01 and EB-04 as pest control agents, both of which are environmentally friendly and sustainable, making them a very good choice for integrated pest management compared with synthetic insecticides. The next step in research should be on-site validation of results, formulation development and characterization of the mechanisms of secondary metabolites to facilitate their acceptance in commercial vegetable production.

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Clinical trial number

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Data availability statement

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

Consent to participate

All the co-authors are willing to participate in this manuscript.

Consent for publication

All authors are willing for the publication of this manuscript.

Conflict of interest

The authors declare no conflict of interest.

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