Mexican Journal of Phytopathology
ISSN: 2007-8080

Crossref Open access

Scientific Article

Biocontrol of *Fusarium oxysporum* and growth promotion in chili pepper using *Bacillus cereus* and *B. thuringiensis*

Maribel Mendoza-Alatorre[1], Aldo Gutiérrez-Chávez[1], Angélica Anahí Acevedo-Barrera[1], Loreto Robles-Hernández[1], Jared Hernández-Huerta[1]*. [1]Laboratorio de Microbiología Aplicada, Fitopatología y Fisiología Postcosecha, Facultad de Ciencias Agrotecnológicas, Universidad Autónoma de Chihuahua, Campus I s/n, Chih., Chihuahua, CP 31350, México.

***Corresponding Author:**
Jared Hernández-Huerta
jahuerta@uach.mx

Section:
Special Number

Recived:
November 20, 2024
Acepted:
May 22, 2025
Published:
June 06, 2025

Citation:
Mendoza-Alatorre M, Gutiérrez-Chávez A, Acevedo-Barrera AA, Robles-Hernández L and Hernández-Huerta J. 2025. Biocontrol of *Fusarium oxysporum* and growth promotion in chili pepper using *Bacillus cereus* and *B. thuringiensis*. Mexican Journal of Phytopathology 43(4): 71. https://doi.org/10.18781/R.MEX.FIT.2024-15

ABSTRACT

Background/Objective. *Fusarium oxysporum* is the causal agent of chili wilt, and an ecological strategy for its management may involve the use of *Bacillus* spp., as they have demonstrated both biocontrol activity against phytopathogenic fungi and plant growth-promoting (PGP) abilities. The objective was to evaluate the biocontrol and PGP capacity of *B. cereus* and *B. thuringiensis* and their effect on morphological variables in jalapeño pepper and the reduction of wilt severity.

Materials and Methods. Pathogenicity and virulence tests were conducted on jalapeño seedlings using six *F. oxysporum* strains isolated from pepper plants. *In vitro* antagonism assays were performed between native strains *B. cereus* Bc28-2 (Bc28-2), *B. cereus* Bc28-5 (Bc28-5), and *B. thuringiensis* Bt24 (Bt24), using *Bacillus subtilis* QST713 as a positive control (PC), against the *F. oxysporum* strains. PGP assays were also conducted on pepper seedlings using the *Bacillus* strains. Additionally, biocontrol tests against *F. oxysporum* FL2 were conducted under greenhouse conditions. Data were analyzed using ANOVA, mean separation tests ($p < 0.05$), and a principal component analysis (PCA).

Results. All *F. oxysporum* strains were pathogenic, with 100% incidence and 89.9% severity. The PC strain achieved the highest fungal spore degradation (86.7%), followed by Bc28-2 and Bc28-5 (84.2%). Bt24 and Bc28-2 promoted the best development of pepper seedlings, showing increases in vegetative parameters. In the biocontrol of *F. oxysporum* FL2, Bc28-2, and Bc28-5 reduced disease severity by 60.4% and promoted growth comparable to the control, whereas Bt24 showed no efficacy in controlling wilt.

Conclusion. The results indicate the potential of *Bacillus cereus* Bc28-2 and Bc28-5 as biocontrol agents against *F. oxysporum* in jalapeño pepper. However, field validation and exploration of their integration into sustainable agricultural management are necessary.

Keywords: *Bacillus*, Plant growth promotion, Biocontrol, Chili pepper, Antagonism

INTRODUCTION

Chili pepper (*Capsicum annuum*) is the third most cultivated and consumed vegetable worldwide and holds significant economic importance in producer countries such as Mexico (Lozana *et al.*, 2022; Bobadilla-Larios *et al.*, 2017). In 2023, chili production in Mexico reached 3,681,061.47 t, with a market value of 45,997.65 million pesos (SIAP, 2023). However, crop yield is threatened by various biotic factors, with fungal diseases being among the most significant.

Species of the *Fusarium* genus pose one of the main phytosanitary threats to chili cultivation, causing diseases such as wilt and root rot, which can lead to severe yield losses (Sam-on *et al.*, 2024; Gabrekiristos and Demiyo, 2020). Among these, wilt caused by *Fusarium oxysporum* is one of the most destructive, capable of reducing crop yield by 68 to 71% (Sam-on *et al.*, 2024; Adina *et al.*, 2021; Gabrekiristos and Demiyo, 2020). This is because the fungus colonizes the vascular system, blocking the transport of water and nutrients, which leads to the progressive weakening and, in many cases, death of the affected plants (Bashir *et al.*, 2018).

The persistence of *Fusarium* in the soil and its ability to form resistant structures complicate its control, making it a recurring issue in chili-producing areas (Campbell, 1990). Control strategies have relied mainly on chemical fungicides, whose indiscriminate use has caused environmental issues, pathogen resistance, and risks to human health (Shaheen *et al.*, 2021; Sela-Burlage *et al.*, 2001).

As a sustainable alternative, the use of beneficial microorganisms with biocontrol and plant growth-promoting capabilities has been proposed. In this context, the *Bacillus* genus has proven to be a viable option due to its ability to produce antimicrobial compounds, induce plant resistance against pathogens, and enhance plant development (Tiwari *et al.*, 2019; Shafi *et al.*, 2017).

Various *Bacillus* species have shown promising effects in controlling *Fusarium* spp. and promoting plant growth. For example, *Bacillus subtilis* QST713 has been shown to inhibit the growth of *F. oxysporum* through the production of lipopeptide antibiotics such as iturins and fengycins (Kulkova *et al.*, 2023; Zhou *et al.*, 2021). Similarly, *B. amyloliquefaciens* FZB42 induces systemic resistance in tomato and chili plants by producing surfactins that inhibit the growth of *Fusarium* sp. (Chowdhury *et al.*, 2015). In addition, *B. velezensis* S3-1 suppresses *Fusarium* spp. infections through the production of bacillomycins and the activation of plant defense mechanisms (Antil *et al.*, 2022; Kumar *et al.*, 2020).

B. cereus has also been reported as an effective biocontrol agent against *Fusarium* spp., promoting plant growth through phosphorus solubilization and the production of indoleacetic acid (Malik *et al.*, 2022; Yanti *et al.*, 2017). Strains such as *B. cereus* SLBE.1AP have shown antifungal activity and promoted chili seedling growth (Yanti *et al.*, 2021), while *B. cereus* EC9 has induced systemic defense responses in tomato plants (Pazarlar *et al.*, 2022). Likewise, *B. cereus* NK91 was characterized and found to produce chitinases that help control *F. oxysporum in vitro* (Thakur *et al.*, 2022). *B. thuringiensis* has been evaluated not only for its insecticidal action but also as a growth promoter in horticultural crops, inducing increases in biomass and plant height due to the production of phytohormones and siderophores (Hyder *et al.*, 2020; Jo *et al.*, 2020; Praça *et al.*, 2012). Strains such as *B. thuringiensis* CHGP12 have shown effectiveness in controlling

F. oxysporum through lipase production (Fatima *et al.*, 2023), while *B. thuringiensis* IBRC-M11096 induces defense responses in tomato (Zibanezhadian *et al.*, 2020).

However, the use of *B. cereus* and *B. thuringiensis* as biocontrol agents and plant growth promoters (PGPs) in jalapeño chili remains insufficiently studied. This is especially relevant considering that the strains used in this study are native to the soil of the chili-producing region, specifically the south-central area of the state of Chihuahua. This characteristic could provide them with a greater capacity for adaptation and effectiveness against local pathogens such as *F. oxysporum*. Moreover, identifying microorganisms with biocontrol and PGP properties native to the same production area may offer a more efficient and sustainable strategy for managing *Fusarium oxysporum* wilt in jalapeño chili. Based on this premise, it is hypothesized that the application of *B. cereus* and *B. thuringiensis* will reduce the severity of *Fusarium oxysporum* wilt and promote jalapeño chili growth under greenhouse conditions. Therefore, the objective of this study was to evaluate the biocontrol and PGP potential of *B. cereus* and *B. thuringiensis*, as well as their effects on morphological variables of jalapeño chili and the reduction of wilt severity. The findings of this study will provide evidence of the potential of these microorganisms as ecological alternatives to chemical control, contributing to sustainable crop management strategies.

MATERIALS AND METHODS

The strains *Bacillus cereus* Bc28-2 (Bc28-2), *B. cereus* Bc28-5 (Bc28-5), and *B. thuringiensis* Bt24 (Bt24) were used as potential biocontrol agents and plant growth promoters (PGPs). These strains were isolated from soil in the jalapeño chili-producing region of Lázaro Cárdenas, Municipality of Meoqui, Chihuahua, and belong to the Applied Microbiology, Phytopathology, and Postharvest Physiology Laboratory (MAFFP) of the Faculty of Agrotechnological Sciences at the Autonomous University of Chihuahua (UACH) (Hernández-Huerta *et al.*, 2023). The commercial strain *Bacillus subtilis* QST713 (PC), from the product Serenade®, was used as a positive control.

The *Fusarium oxysporum* strains FM1, FM2, FL1, FL5, FL6, and FL2, belonging to the MAFFP laboratory, were used in this study. These strains were isolated from jalapeño chili plants exhibiting characteristic wilt symptoms in the chili-producing agricultural area of Irrigation District 005 in Delicias, Chihuahua. For the assays, jalapeño chili seeds of cultivar M (Southern Star Seeds S. de R.L. de C.V., Mexico) were used. This cultivar is a common commercial variety in the chili-producing region where the microorganisms were obtained.

Preparation of the bacterial inoculum. *Bacillus* strains were cultured in nutrient broth (NB; BD Difco Laboratories, Sparks, Maryland, MD, USA) for 72 h at 28 °C. The bacterial suspensions were then centrifuged at 7,000 rpm for 10 min at 4 °C, and the supernatant was discarded. The resulting pellet was resuspended in 20 mL of sterile 0.85% saline solution and adjusted to a concentration of 1×10^8 CFU mL^{-1}, corresponding to an optical density of 0.4 at 600 nm (Chandrasekaran *et al.*, 2017).

Preparation of *Fusarium* spore suspensions. Spore suspensions were obtained by culturing the fungi on potato dextrose agar (PDA; BD Difco Laboratories, Sparks, Maryland, MD, USA) for 7 days at 28 °C. Spores were collected by scraping and filtering

with sterile fiberglass. The spore concentration was then adjusted to 1×10^6 spores mL^{-1} using a Neubauer chamber (Weber Scientific International Ltd., Teddington, UK).

Pathogenicity and virulence tests of *Fusarium* strains. This test was conducted during the germination stage of jalapeño chili seeds under *in vitro* conditions. Seeds were surface-disinfested with 4% (v/v) NaClO under agitation for 5 min, followed by three rinses with sterile distilled water, each lasting 5 min. The sterile seeds were then immersed in fungal spore suspensions and agitated at 120 rpm for 20 min. After inoculation, the seeds were dried and placed in Petri dishes containing moist sterile paper, with five seeds per dish. The dishes were sealed and placed in a growth chamber under a photoperiod of 16 h light at 28 °C and 8 h darkness at 18 °C.

Disease incidence was evaluated as the percentage of infected seeds per dish, and the severity index (SI) was determined 10 days after inoculation using the scale by Robles-Hernández *et al.* (2015): 0 = asymptomatic germinated seed; 1 = seed with mycelial growth on the seed coat; 2 = isolated necrotic spots and/or necrotic lesions of 1–5 mm; 3 = necrotic lesions of 6–10 mm; and 4 = germinated seeds with total necrosis, necrotic lesions over 11 mm, or seedlings with necrotic hypocotyls. The SI was calculated using the formula proposed by Galanihe *et al.* (2004).

$$IS = \sum [\frac{PQ}{MN}] \text{ x } 100$$

where IS = severity index, P = severity category, Q = number of plants in the same category, M = total number of plants observed, and N = maximum value on the rating scale.

To assess disease progression caused by each fungal strain over time, the area under the disease progress curve (AUDPC) was calculated using the formula proposed by Saner and Finney (1997) and the method of Campbell and Madden (1990).

The assay was conducted under a completely randomized design (CRD) with four replicates per treatment (Petri dishes with inoculated seeds). Treatments consisted of fungal spore suspensions, and sterile distilled water was used as the control.

***In vitro* dual culture antagonism assay between *Bacillus* and *Fusarium*.** The antagonism assay was conducted in sterile 96-well microplates (Biologix Steril 07-6096, Biologix Research) under *in vitro* conditions. Each well was filled with 150 µL of bacterial suspensions (1×10^8 CFU mL^{-1}). Then, 150 µL of fungal spore suspensions (1×10^6 spores mL^{-1}) prepared in potato dextrose broth (BD Difco Laboratories, Sparks, Maryland, MD, USA) were added. The inoculated microplates were sealed and incubated at 28 °C for 48 h in darkness. To assess fungal spore degradation, 10 µL were taken from each well, and the spores present were counted directly using a Neubauer chamber.

The assay was conducted using a 4×6 completely randomized factorial design, evaluating two factors: bacterial strains (Bc28-2, Bc28-5, Bt24, and PC) and fungal isolates (FM1, FM2, FL1, FL5, FL6, and FL2), resulting in 24 unique treatments corresponding to each bacteria–fungus combination. Fungal spore suspensions alone were used as controls. All treatments were performed in triplicate.

Growth promotion in chili seedlings by *Bacillus* spp. To evaluate the effect of bacteria on the development of jalapeño chili seedlings, sterile seeds were sown in 20-cavity polystyrene trays filled with sterile horticultural perlite (1 h at 120 °C, 15 lb/in²). The trays were irrigated with a nutrient solution (Nutrient Solution for Vegetables®; pH 5.5 and electrical conductivity of 1.5 mS). The seedlings were grown under greenhouse conditions at 27 °C ± 2 °C and 75% relative humidity (RH).

Seedlings were inoculated at the stem base with 5 mL of bacterial suspensions of Bc28-5, Bc28-2, Bt24, and the PC (1×10^8 CFU mL^{-1}) at 10 days after sowing. Inoculation was repeated every six days for a total of five applications. Thirty days after the first inoculation, morphological variables were evaluated: seedling height, root length, stem diameter, number of leaves, leaf area (Canopeo App; https://canopeoapp.com), and fresh and dry biomass of leaves, stem, and root. Photosynthetic pigment content (chlorophyll a, chlorophyll b, and carotenoids) was also assessed following the method of Lichtenthaler and Wellburn (1983).

The assay was established under a completely randomized design (CRD) with four replicates per treatment. Treatments consisted of bacterial solutions applied to the seedling trays, and uninoculated seedlings were used as the control.

Biocontrol of *Fusarium* by *Bacillus* spp. under greenhouse conditions. This assay used 40-day-old jalapeño chili seedlings that had been previously grown under the described conditions and inoculated with strains Bc28-2, Bc28-5, Bt24, and PC. The seedlings were transplanted into 10 cm diameter pots filled with sterile peat moss previously inoculated with spores of *Fusarium oxysporum* FL2—a strain characterized by a high severity index, the largest area under the disease progress curve, and resistance to spore degradation.

Before transplanting, the sterile peat moss (1 h at 120 °C, 15 lb/in²) was inoculated with a fungal spore suspension (1×10^6 spores mL^{-1}) and incubated at 28 °C for five days. The inoculated seedlings were then placed under greenhouse conditions at 27 °C ± 2 °C and 75% relative humidity. Plants were irrigated every third day with nutrient solution.

Thirty days after transplanting, the disease severity index (SI) was evaluated using the following scale: 0 = healthy root system; 1 = 1–25% root damage; 2 = 25–50% root damage; 3 = 50–75% root damage; and 4 = 75–100% root damage. The SI was calculated using the formula proposed by Galanihe *et al.* (2004).

Morphological variables were also assessed, including seedling height, root length, stem diameter, number of leaves, leaf area (Canopeo App), root volume, and fresh and dry biomass of leaves, stem, and root. Photosynthetic pigment content was determined following the methodology of Lichtenthaler and Wellburn (1983). The experiment was conducted under a completely randomized design (CRD) with four replicates per treatment, as described in Table 1.

Table 1. Treatments used for the biocontrol of *Fusarium oxysporum* FL2 applied to jalapeño chili plants grown under greenhouse conditions.

Treatments	Treatment code
Fusarium oxysporum FL2	FL2
Bacillus subtilis QST713 + *F. oxysporum* FL2	CP/FL2
B. cereus Bc28-2 + *F. oxysporum* FL2	Bc28-2/FL2
B. cereus Bc28-5 + *F. oxysporum* FL2	Bc28-5/FL2
B. thuringiensis Bt24 + *F. oxysporum* FL2	Bt24/FL2
Plants without microbial treatment	Control

Statistical analysis. The data from the assays were subjected to Shapiro-Wilk and Levene's tests to assess normality and homogeneity of variance prior to statistical analysis.

Data from the *in vitro* pathogenicity and virulence tests were analyzed using the non-parametric Kruskal-Wallis test followed by the Conover-Iman test ($p < 0.05$). Results from the *in vitro* antagonism assay evaluating fungal spore degradation were analyzed using a factorial analysis to assess the main effects (bacteria and fungi) and their interactions on spore degradation. Multiple comparisons were then performed using the Scott-Knott test ($p < 0.05$).

Data from the assay on seedling growth promotion by *Bacillus* spp. and from the greenhouse assay evaluating growth promotion in plants interacting with *F. oxysporum* were analyzed by analysis of variance (ANOVA), followed by Tukey's post hoc test for mean separation ($p < 0.05$).

The severity index data from the greenhouse interaction between bacterial strains and *F. oxysporum* FL2 were analyzed using the Kruskal-Wallis test and the Conover-Iman test ($p < 0.05$).

A principal component analysis (PCA) was conducted to assess the influence of bacterial treatments on biocontrol and plant growth promotion in interaction with *F. oxysporum* FL2. Prior to PCA, Bartlett's test of sphericity ($p < 0.01$) was performed and the Kaiser-Meyer-Olkin (KMO) index was calculated, with KMO values above 0.60 considered acceptable (Tabachnick and Fidell, 2007).

All statistical analyses were performed using InfoStat software (InfoStat 2021v, Grupo InfoStat, Argentina) and JAMOVI version 2.5.2.0 (The Jamovi Project, 2024).

RESULTS

Pathogenicity and virulence tests of *Fusarium* strains. The *in vitro* pathogenicity and virulence tests conducted with *Fusarium* isolates during jalapeño chili seed germination showed that all fungal strains caused disease symptoms 10 days after inoculation, with no significant differences among strains. Observed symptoms included necrotic spots on the radicle and hypocotyl, complete necrosis of the radicle, mycelial growth on the seed coat (either alone or accompanied by exudate production), and ungerminated seeds with internal necrosis. All treatments showed 100% disease incidence and an average severity of 89.9%.

Disease progression, measured as the area under the disease progress curve (AUDPC), had an average value of 26.7, indicating rapid and severe disease development (Table 2). In contrast, the control group showed healthy seed germination and seedling development. After the assay, fungi were re-isolated from infected plants, and their morphological characteristics matched those of the original inoculum, thus fulfilling Koch's postulates.

Table 2. Incidence, severity, and progression of wilt in chili seeds 10 days after inoculation with *Fusarium oxysporum.*

Isolated	Incidence (%)	Severity (%)	ABCDE[x]
F. oxysporum FM1	100	95.8[a]	30.2[a]
F. oxysporum FM2	100	89.6[a]	27.3[a]
F. oxysporum FL1	100	89.6[a]	27.4[a]
F. oxysporum FL5	100	81.3[a]	21.0[a]
F. oxysporum FL6	100	87.5[a]	23.7[a]
F. oxysporum FL2	100	95.8[a]	30.5[a]
Control	0	0[b]	0

[x]AUDPC = area under the disease progress curve. Values in each column followed by the same letter are not significantly different according to the Kruskal-Wallis and Conover-Iman tests ($p \leq 0.05$).

***In vitro* antagonism of *Bacillus* vs. *Fusarium*.** The analysis of *Fusarium* spore degradation by *Bacillus* spp. showed that the commercial strain PC exhibited the highest spore degradation percentages compared to the native *Bacillus* strains evaluated (Figure 1). In interaction with PC, no significant statistical differences were observed among the fungal isolates, with an average degradation rate of 86.7%.

In contrast, when interacting with strain Bc28-5, the fungus FM2 showed greater resistance to degradation, with an average of 41.4%, while the remaining fungi were degraded at an average rate of 84.2%. In the case of strain Bc28-2, FL6 showed the highest resistance to spore degradation at 18%, followed by FL2 and FL1 with 42.9%. The other fungal isolates were degraded at an average of 84.9%.

Bt24 was the least effective strain in degrading spores. It only succeeded in degrading two fungi (FL1 and FM1) at a rate of 92.85%, followed by FL6 and FL2 at 73.15%. The most resistant fungi were FL5 and FM2, with spore degradation percentages of 32.8% and 52.2%, respectively.

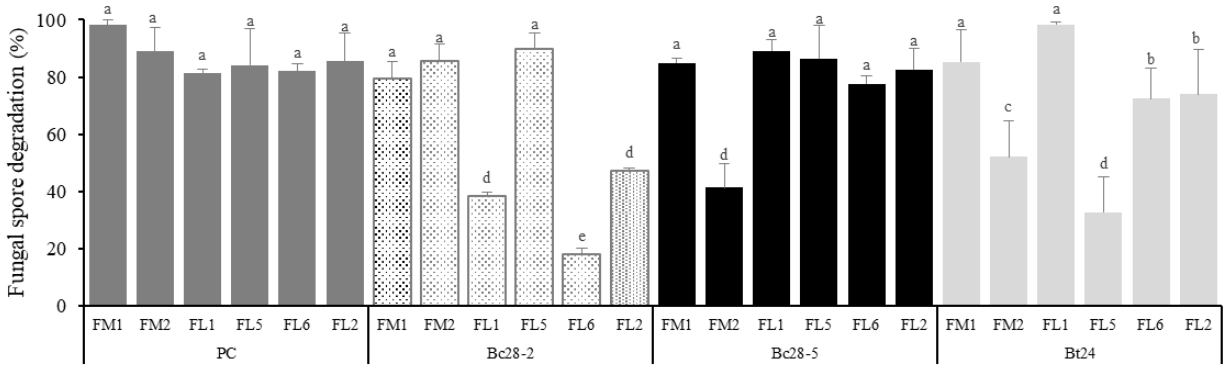

Figure 1. Spore degradation of *Fusarium oxysporum* by *Bacillus* spp. in nutrient broth after 48 hours of *in vitro* interaction. PC = *B. subtilis* QST713; Bc28-2 and Bc28-5 = *B. cereus*; Bt24 = *B. thuringiensis*; *F. oxysporum* = FM1, FM2, FL1, FL5, FL6, FL2. Bars with different letters indicate significant differences according to the Scott-Knott test ($p < 0.05$).

Growth promotion in chili seedlings. Bacterial inoculation significantly improved chili seedling growth under controlled conditions at 30 days (Table 3).

Table 3. Effect of *Bacillus* spp. on the growth of jalapeño chili seedlings under controlled conditions 30 days after inoculation.

Parameters	Control	*Bacillus subtilis* PC	*B. cereus* Bc28-2	*B. cereus* Bc28-5	*B. thuringiensis* Bt24	*LSD*
SH (cm)	8.2±0.5^{bc}	6.6±0.7^{d}	8.9±1.1^{ab}	7.7±0.5^{cd}	9.8±0.2^{a}	1.1
RL (cm)	8.9±0.8^{a}	8.9±1.5^{a}	9.9±0.8^{a}	9.5±1.4^{a}	9.4±1.4^{a}	2.0
SD (mm)	2.2±0.1^{c}	2.0±0.3^{c}	2.8±0.3^{ab}	2.3±0.5^{bc}	2.8±0.3^{a}	0.5
NL	8.0±0.6^{b}	10.0±2.8^{ab}	12.0±1.3^{a}	9.3±1.5^{ab}	12.0±0.9^{a}	2.7
LA (cm^2 plant^{-1})	51.1±7.1^{c}	48.9±2.1^{c}	115.1±6.7^{a}	76.3±8.5^{b}	103.6±10.7^{a}	20.5
LFW (g)	0.6±0.1^{c}	0.6±0.3^{c}	1.6±0.1^{a}	1.1±0.1^{b}	1.5±0.2^{a}	0.3
SFW (g)	0.3±0.03^{c}	0.3±0.1^{c}	0.7±0.2^{a}	0.5±0.1^{b}	0.7±0.1^{a}	0.2
RFW (g)	0.2±0.1^{c}	0.2±0.09^{c}	0.6±0.1^{a}	0.4±0.1^{b}	0.7±0.1^{a}	0.2
LDW (g)	0.1±0.01^{c}	0.1±0.03^{c}	0.3±0.1^{a}	0.2±0.03^{b}	0.3±0.1^{a}	0.1
SDW (g)	0.03±0.01^{b}	0.02±0.01^{b}	0.1±0.01^{a}	0.03±0.01^{b}	0.1±0.01^{a}	0.01
RDW (g)	0.02±0.01^{cd}	0.02±0.003^{d}	0.1±0.01^{b}	0.03±0.004^{c}	0.1±0.01^{a}	0.01
Chl a (mg/g FW^{-1})	1.5±0.1^{b}	1.7±0.1^{ab}	2.1±0.4^{a}	2.2±0.3^{a}	1.9±0.2^{ab}	0.6
Chl b (mg/g FW^{-1})	0.9±0.3^{a}	0.9±0.1^{a}	1.2±0.4^{a}	1.2±0.2^{a}	1.3±0.1^{a}	0.5
Carotenoids (mg/g FW^{-1})	0.2±0.1^{a}	0.2±0.03^{a}	0.2±0.2^{a}	0.2±0.1^{a}	0.1±0.1^{a}	0.2

SH= seedling height; RL = root length; SD = stem diameter; NL = number of leaves; LA = leaf area; LFW = leaf fresh weight; SFW = stem fresh weight; RFW = root fresh weight; LDW = leaf dry weight; SDW = stem dry weight; RDW = root dry weight; Chl a = chlorophyll a; Chl b = chlorophyll b; LSD = least significant difference. PC = *B. subtilis* QST713; Bc28-2 and Bc28-5 = *B. cereus*; Bt24 = *B. thuringiensis*. Data represent the means ± standard deviation of four replicates. Values in rows followed by different letters indicate significant differences ($p \leq 0.05$) according to ANOVA and Tukey's test.

Seedlings inoculated with Bt24 exhibited the greatest height, reaching an average of 9.8 cm, which represents a 19.5% increase compared to the control (8.2 cm). In contrast, the PC treatment resulted in the shortest height (6.6 cm), reflecting a 19.5% reduction compared to the control. Regarding root length, treatments showed no significant differences, with an average of 9.3 cm. Stem diameter was significantly greater in the Bc28-2 and Bt24 treatments (2.8 mm), representing a 27.3% increase compared to the control (2.2 mm).

The number of leaves was significantly higher in seedlings treated with Bt24 (12) and Bc28-2 (12), representing a 50% increase compared to the control (8 leaves). Similarly, leaf area was greater in seedlings inoculated with Bc28-2 and Bt24 (109.4 cm² plant^{-1}), representing a 114.0% increase compared to the control (51.1 cm² plant^{-1}).

Regarding fresh and dry biomass, leaf fresh weight was significantly higher in the Bt24 and Bc28-2 treatments (1.6 g), which corresponds to a 166.7% increase compared to the control (0.6 g). Stem fresh weight also increased with Bt24 and Bc28-2 (0.7 g), representing a 133.3% increase compared to the control (0.3 g). Root fresh weight was significantly higher as well in the Bt24 and Bc28-2 treatments (0.7 g), a 250% increase over the control (0.2 g).

Leaf dry weight showed a significant increase with Bt24 and Bc28-2 (0.3 g), representing a 200% increase over the control (0.1 g). Stem dry weight also increased significantly with Bt24 and Bc28-2 (0.1 g), reflecting a 233.3% increase compared to the control (0.03 g). For root dry weight, the Bt24 treatment (0.1 g) showed the highest increase, with a 400% gain compared to the control (0.02 g).

In terms of photosynthetic pigment content, chlorophyll a was significantly higher in the Bc28-5 and Bc28-2 treatments (2.2 mg/g FW^{-1}), a 46.7% increase over the control (1.5 mg/g FW^{-1}). In contrast, chlorophyll b content showed no significant differences among treatments, with an average of 1.1 mg/g FW^{-1}. Carotenoid content also showed no significant differences, with an average of 0.2 mg/g FW^{-1}.

Growth of chili plants in interaction with *Fusarium*. Bacterial inoculation significantly improved chili plant growth in interaction with the fungus (Table 4).

Table 4. Effect of *Bacillus* spp. on the growth of chili plants under greenhouse conditions 30 days after inoculation with *Fusarium oxysporum* FL2, the causal agent of chili wilt.

Parameters	Control	FL2	PC/FL2	Bc28-2/FL2	Bc28-5/FL2	Bt24/FL2	*LSD*
SH (cm)	22.1 ± 1.7^{b}	14.4 ± 1.9^{cd}	26.4 ± 1.0^{a}	23.1 ± 1.3^{ab}	17.8 ± 1.3^{c}	13.0 ± 2.3^{d}	3.7
RL (cm)	19.4 ± 1.7^{a}	11.5 ± 0.8^{b}	18.4 ± 1.4^{a}	17.9 ± 1.5^{a}	18.1 ± 1.4^{a}	11.6 ± 0.6^{b}	2.9
SD (mm)	4.5 ± 0.5^{bc}	5.1 ± 0.7^{abc}	5.9 ± 0.6^{ab}	4.7 ± 0.5^{abc}	6.1 ± 1.0^{a}	3.7 ± 0.7^{c}	1.6
NL	35.5 ± 3.3^{a}	20.3 ± 2.8^{b}	35.0 ± 5.7^{a}	25.0 ± 2.2^{b}	37.3 ± 3.3^{a}	19.0 ± 2.6^{b}	7.9
LA (cm^2 $plant^{-1}$)	516.3 ± 88.7^{ab}	196.1 ± 21.0^{c}	564.0 ± 77.0^{a}	405.4 ± 58.7^{b}	583.8 ± 79.5^{a}	252.2 ± 19.2^{c}	143.2
LFW (g)	10.5 ± 0.9^{a}	3.0 ± 1.0^{d}	9.7 ± 0.9^{ab}	6.5 ± 0.8^{c}	7.5 ± 1.7^{bc}	3.6 ± 0.4^{d}	2.3
SFW (g)	6.9 ± 1.2^{abc}	3.2 ± 1.0^{cd}	10.0 ± 2.8^{a}	5.6 ± 1.2^{bcd}	9.4 ± 1.5^{ab}	3.0 ± 0.9^{d}	3.8
RFW (g)	4.0 ± 0.5^{ab}	1.6 ± 0.4^{c}	4.8 ± 0.7^{a}	3.1 ± 0.7^{b}	4.3 ± 0.5^{ab}	1.1 ± 0.4^{c}	1.2
LDW (g)	1.9 ± 0.2^{a}	0.6 ± 0.3^{c}	1.6 ± 0.1^{ab}	0.8 ± 0.1^{c}	1.4 ± 0.2^{b}	0.5 ± 0.1^{c}	0.4
SDW (g)	0.8 ± 0.3^{abc}	0.3 ± 0.2^{c}	1.0 ± 0.3^{ab}	0.5 ± 0.1^{abc}	1.0 ± 0.3^{a}	0.5 ± 0.2^{bc}	0.5
RDW (g)	0.5 ± 0.1^{ab}	0.2 ± 0.03^{c}	0.5 ± 0.1^{ab}	0.4 ± 0.1^{b}	0.5 ± 0.04^{a}	0.1 ± 0.04^{c}	0.1
RV (cm^3)	5.4 ± 0.6^{a}	1.5 ± 0.6^{c}	4.8 ± 0.7^{ab}	3.9 ± 0.5^{b}	4.1 ± 0.6^{ab}	1.1 ± 0.6^{c}	1.4
Chl a (mg/g FW^{-1})	2.0 ± 0.5^{a}	1.8 ± 0.4^{a}	2.0 ± 0.4^{a}	1.8 ± 0.4^{a}	2.0 ± 0.4^{a}	1.8 ± 0.3^{a}	0.9
Chl b (mg/g FW^{-1})	0.9 ± 0.3^{a}	1.0 ± 0.3^{a}	1.2 ± 0.3^{a}	1.0 ± 0.1^{a}	1.2 ± 0.3^{a}	1.0 ± 0.4^{a}	0.7
Carotenoids (mg/g FW^{-1})	0.3 ± 0.1^{a}	0.1 ± 0.1^{a}	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}	0.1 ± 0.03^{a}	0.3 ± 0.1^{a}	0.2

SH = seedling height; RL = root length; SD = stem diameter; NL = number of leaves; LA = leaf area; LFW = leaf fresh weight; SFW = stem fresh weight; RFW = root fresh weight; LDW = leaf dry weight; SDW = stem dry weight; RDW = root dry weight; RV = root volume; Chl a = chlorophyll a; Chl b = chlorophyll b; FL2 = *F. oxysporum*; PC = *B. subtilis* QST713; Bc28-2 and Bc28-5 = *B. cereus*; Bt24 = *B. thuringiensis*; LSD = least significant difference. Data represent the means ± standard deviation of four replicates. Values in rows followed by different letters indicate significant differences according to ANOVA and Tukey's test ($P < 0.05$).

Plants inoculated with PC/FL2 showed the greatest height, averaging 26.4 cm, which represents a 20% increase compared to the control (22.1 cm). In contrast, the treatments with FL2 alone and the Bt24/FL2 interaction recorded the lowest heights (14.4 cm and 13.0 cm, respectively), reflecting a reduction of 34.8% and 41.2%, respectively, compared to the control.

Regarding root length, most bacteria–pathogen interaction treatments maintained a length similar to the control (18.6 cm), except for Bt24/FL2 and FL2 alone, which showed a 40.5% reduction (11.6 cm) compared to the control. Stem diameter was significantly greater in the Bc28-5/FL2 treatment (6.1 mm), representing a 35.6% increase compared to the control (4.5 mm). On the other hand, the Bt24/FL2 interaction resulted in the smallest stem diameter (3.7 mm), a 17.8% decrease relative to the control.

The number of leaves did not differ significantly in the PC/FL2 and Bc28-5/FL2 treatments compared to the control, with an average of 35.9 leaves. However, the Bc28-2/FL2 and Bt24/FL2 interactions, as well as the FL2 pathogen alone, showed a significant reduction, averaging 21.4 leaves, which represents a 39.6% decrease compared to the control (35.5 leaves).

Leaf area was significantly greater in the Bc28-5/FL2 and PC/FL2 treatments (573.9 cm^3). In contrast, the Bt24/FL2 and FL2 treatments showed the lowest leaf area (224.2 cm^3), reflecting a 43.4% reduction compared to the control (516.3 cm^3).

Regarding fresh biomass, only the PC/FL2 treatment showed a leaf fresh weight similar to the control (9.7 g). The Bt24/FL2 interaction and FL2 treatment alone showed the lowest leaf fresh weight (3.3 g), corresponding to a 68.6% decrease compared to the control (10.5 g). For stem and root fresh weight, only the PC/FL2 and Bc28-5/FL2 treatments maintained values comparable to the control (9.7 g and 4.55 g, respectively), while Bt24/FL2 and FL2 recorded the lowest values: 3.1 g for stem and 1.35 g for root, representing reductions of 44.9% and 66.3%, respectively.

As for dry biomass, leaf dry weight did not show significant increases in any treatment, except for PC/FL2, which matched the control (1.6 g). The lowest leaf dry weight was observed in Bc28-5/FL2, Bt24/FL2, and FL2 (0.6 g), a 66.7% decrease relative to the control (1.9 g). For stem dry weight, most treatments showed no significant differences (0.8 g), except for Bt24/FL2 and FL2, which resulted in a 50% reduction. In the case of root dry weight, only PC/FL2 and Bc28-5/FL2 maintained values similar to the control (0.5 g), while Bt24/FL2 and FL2 showed the lowest values (0.15 g), representing a 70% reduction.

Root volume did not show significant increases, remaining comparable to the control in the PC/FL2 and Bc28-5/FL2 treatments (4.5 cm^3). The lowest root volume was observed in the Bt24/FL2 and FL2 treatments (1.3 cm^3), representing a 75.9% reduction compared to the control (5.4 cm^3). Regarding photosynthetic pigment content, no significant differences were observed among treatments for chlorophyll a (2.28 mg/g FW^{-1}), chlorophyll b (1.26 mg/g FW^{-1}), or carotenoids (0.24 mg/g FW^{-1}).

The severity index results showed significant differences among treatments (Figure 2a). Plants treated with FL2 alone exhibited a severity index (SI) of 87.5%, significantly higher than all other treatments. In contrast, the bacteria–pathogen interaction treatments PC/FL2, Bc28-2/FL2, and Bc28-5/FL2 reduced the SI by 60.4% compared to the pathogen (FL2) alone. Conversely, the Bt24/FL2 treatment did not show a significant reduction in SI, with a value of 81.3%, which was statistically equivalent to the pathogen treatment.

Plant development in interaction with the pathogen also varied depending on the treatment applied (Figure 2b). Plants that did not receive microbial treatment developed healthily and vigorously. In contrast, plants treated with the pathogen alone exhibited severe disease symptoms, particularly in the root system, resulting in stunted growth. Plants treated with the bacteria–pathogen combinations PC/FL2, Bc28-2/FL2, and Bc28-5/FL2 showed development similar to the control treatment, characterized by healthy aerial growth and reduced disease symptoms in the root system. In contrast, plants treated with Bt24/FL2 displayed severe symptoms comparable to those observed in plants treated with the pathogen alone.

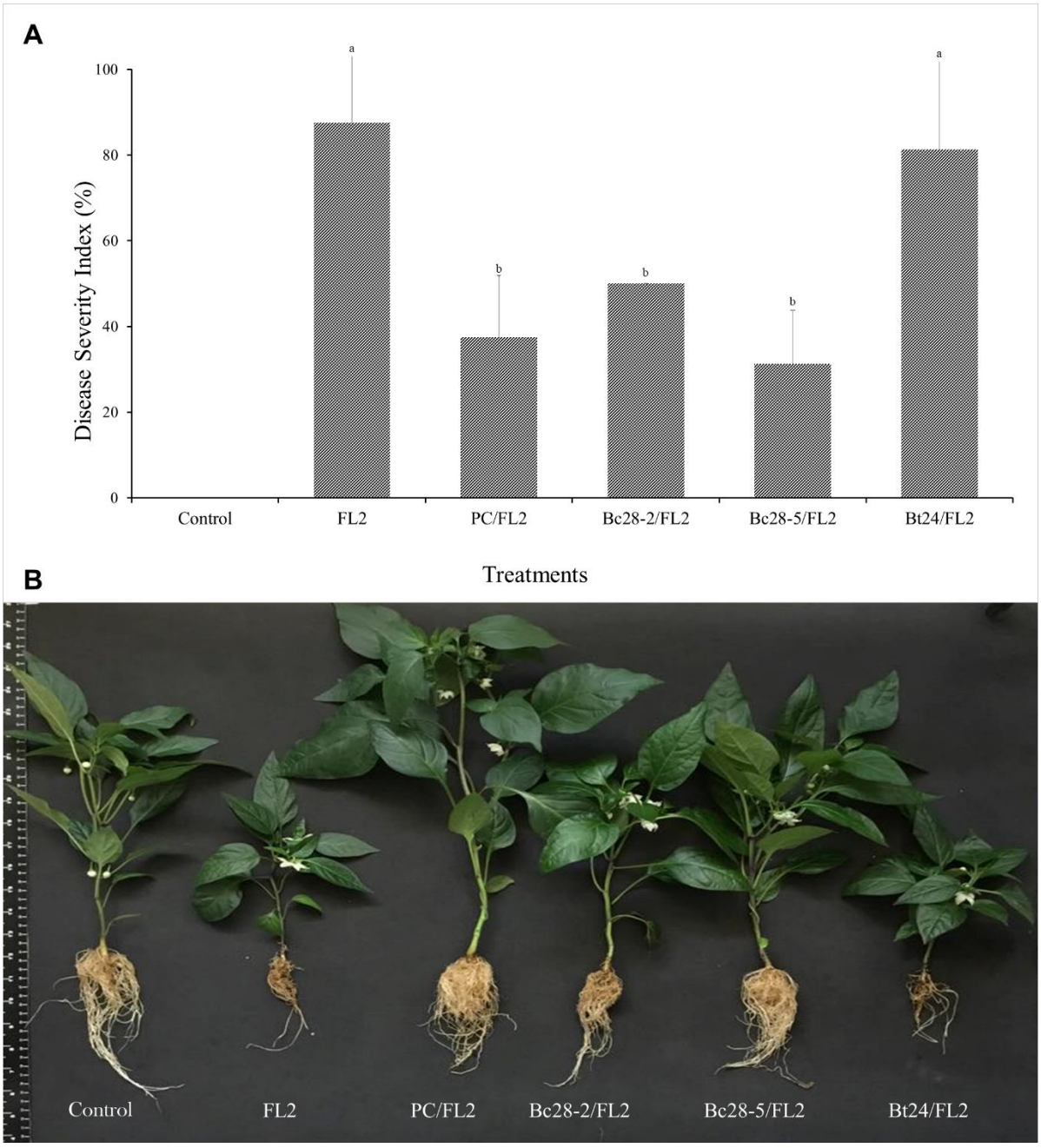

Figure 2. Effect of *Bacillus* spp. on the control of chili wilt caused by *Fusarium oxysporum* FL2 under greenhouse conditions, 30 days after inoculation. A) Severity index of chili wilt in interaction with *Bacillus* spp. F = *Fusarium* sp. FL2; PC = commercial product (*B. subtilis* QST713®); Bc28-2 and Bc28-5 = *B. cereus*; Bt24 = *B. thuringiensis*. Error bars represent standard deviation. Columns with different letters indicate significant differences according to the Kruskal-Wallis and Conover-Iman tests ($p < 0.05$). B) Symptoms of chili wilt caused by *F. oxysporum* FL2 and its interaction with *Bacillus* spp. under greenhouse conditions.

Principal component analysis allowed visualization of the variability in the developmental response of jalapeño chili plants to the different *Fusarium* biocontrol treatments (Figure 3). The first principal component (Dim 1) explained 75.4% of the variability, while the second component (Dim 2) accounted for 11.3%, together capturing 86.7% of the total variability.

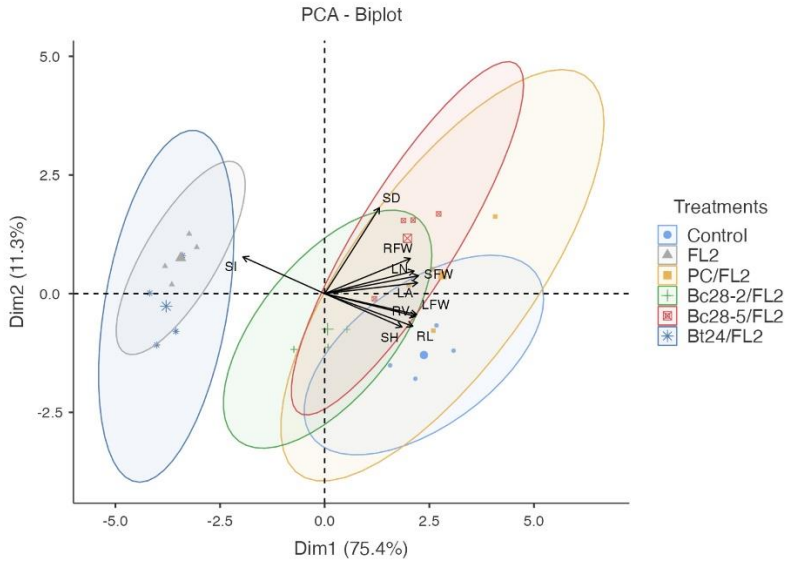

Figure 3. Principal Component Analysis (PCA) of chili plant growth inoculated with *Fusarium* sp. FL2 (F), commercial product (PC; *B. subtilis* QST713), *B. cereus* (Bc28-2, Bc28-5), or *B. thuringiensis* (Bt24) ($\chi^2 = 291$, df = 45, $p < .001$). SI = severity index; SH = seedling height; RL = root length; SD = stem diameter; NL = number of leaves; LA = leaf area; LFW = leaf fresh weight; SFW = stem fresh weight; RFW = root fresh weight; RV = root volume.

The pathogen and the Bt24/FL2 treatment exhibited similar behavior patterns, showing a negative relationship with most vegetative parameters and a positive relationship with the severity index (SI), indicating that Bt24 did not contribute to disease control. In contrast, a strong positive interrelationship was observed between the bacterial treatments PC/FL2, Bc28-2/FL2, and Bc28-5/FL2, as well as the control, across vegetative parameters, along with a negative relationship with the SI. This indicates effective disease control and plant growth promotion by the bacterial treatments.

DISCUSSION

The results of this study show that inoculation with native strains of *Bacillus cereus* (Bc28-5 and Bc28-2) and *B. thuringiensis* (Bt24) in jalapeño chili plants under controlled conditions has a significant impact on vegetative growth and on reducing the severity of wilt caused by *Fusarium oxysporum*. Additionally, some *Bacillus* strains demonstrated the ability to effectively degrade *Fusarium* spores in *in vitro* assays. However, the effectiveness varied depending on the bacterial and fungal strain involved.

The pathogenicity and virulence tests confirmed that all *Fusarium* strains were pathogenic, causing 100% disease incidence and an average severity of 89.9%. This confirms the virulence of the evaluated strains on jalapeño chili seedlings under *in vitro* conditions. Observed symptoms included necrosis in the radicle and hypocotyl, which is consistent with previous studies on the ability of *Fusarium oxysporum* to affect the root system of crops such as jalapeño chili (Robles-Hernández *et al.*, 2015).

The antagonistic capacity of *Bacillus* strains in degrading *Fusarium* spores varied significantly. The commercial strain PC showed the highest spore degradation percentage (86.7%) compared to the native strains. Antagonistic activity was also evident with Bc28-

5, whereas Bt24 demonstrated lower effectiveness, particularly against the more resistant *Fusarium* strains (FL5 and FM2).

The degradation mechanism may be associated with the production of antifungal lipopeptides, as previous studies have shown that *B. cereus* and *B. thuringiensis* can control *F. oxysporum* f. sp. *lycopersici* through the production of antimicrobial peptides (Sarangi *et al.*, 2017). Ajesh *et al.* (2013) reported that soil-derived *B. cereus* produces the lipopeptide kurstakin, which has antifungal activity. In addition, *Bacillus* spp. can produce lytic enzymes such as chitinases, glucanases, and proteases that hydrolyze fungal cell walls (Chenniappan *et al.*, 2019; Ku *et al.*, 2018). In the case of *B. thuringiensis*, recent studies have reported its ability to control phytopathogenic fungi through the production of bacteriocins, lactonases, chitinases, antibiotics, and hydrogen cyanide (Gupta *et al.*, 2024; Azizoglu *et al.*, 2023; Yoshida *et al.*, 2019).

In terms of growth promotion, Bt24 showed the greatest increase in seedling height and biomass. Strains Bc28-2 and Bc28-5 also significantly promoted growth by increasing leaf area and both fresh and dry plant biomass. This effect may be linked to plant growth-promoting mechanisms previously reported for *B. cereus*. Kumar *et al.* (2020) noted that *B. cereus* can produce indole-3-acetic acid (IAA) and gibberellins as mechanisms for promoting plant growth. Similarly, Ku *et al.* (2018) reported that this bacterium solubilizes both organic and inorganic phosphorus, which supports plant development. Yanti and Nasution (2017) indicated that this group of bacteria rapidly colonizes the plant rhizosphere due to exopolysaccharide production, and *B. cereus* has been shown to form biofilms that facilitate root colonization (Gao *et al.*, 2019; Wijman *et al.*, 2007).

On the other hand, the growth-promoting effect of *B. thuringiensis* may be associated with its ability to produce IAA and 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which act as plant growth regulators (Cherif-Silini *et al.*, 2016). It has also been reported to produce siderophores that enhance nutrient uptake by the plant (Delfim, 2021), and to solubilize soil phosphorus through the production of organic acids, pH modification, and the secretion of acid and alkaline phosphatases (Cherif-Silini *et al.*, 2016; Delfim, 2020).

In addition, the biocontrol of *Fusarium* in jalapeño chili plants under greenhouse conditions showed that treatments with *B. cereus* (Bc28-2 and Bc28-5) significantly reduced disease severity caused by *Fusarium* FL2, compared to the treatment with the pathogen alone. These results may be attributed to the ability of *B. cereus* to reduce fungal disease severity through the production of antimicrobial compounds, as mentioned earlier, and by inducing systemic resistance (ISR) in plants (Boulahouat, 2023).

Several studies have shown that *B. cereus* induces ISR against fungi such as *Botrytis cinerea* in tomato through the production of oxalic acid (Yu *et al.*, 2022; Nie *et al.*, 2017). Likewise, *B. cereus* MH778713 has been reported to help control *F. oxysporum* in tomato via ISR (Nie *et al.*, 2017), and *B. cereus* EC9 has been identified as an inducer of resistance against *F. oxysporum* in kalanchoe plants (Madriz-Ordeñana *et al.*, 2022). Furthermore, *B. cereus* C1L has been reported to induce ISR through the production of volatile compounds such as dimethyl disulfide (Piechulla *et al.*, 2017).

The growth observed during the interaction between *Fusarium* spp. FL2 and *B. cereus* may be explained by findings from Leibman-Markus *et al.* (2023), who suggest that growth promotion and induced resistance can be codependent processes. In some cases,

inoculation with bacteria such as *Bacillus* can enhance developmental processes and improve plant performance in biocontrol interactions (Gupta *et al.*, 2022).

However, the Bt24/FL2 treatment showed an unexpected pattern, with results similar to the pathogen-only treatment in terms of both disease severity and growth parameters. This may be due to the limited antagonistic capacity of Bt24 against certain *Fusarium* strains, highlighting the importance of evaluating the specificity of bacteria–pathogen interactions.

The results suggest that the use of specific *Bacillus* strains can contribute to the integrated management of *Fusarium* in agricultural systems by enhancing plant growth while reducing disease severity. This opens the possibility of developing targeted formulations, particularly with strains such as Bc28-2 and Bc28-5, for application under both greenhouse and field conditions.

CONCLUSIONS

This study demonstrated that all *Fusarium oxysporum* strains isolated from soil are pathogenic to jalapeño chili seedlings, causing 100% disease incidence and an average severity of 89.9%. Disease progression showed a rapid increase under controlled conditions.

The *in vitro* biocontrol assay revealed that *B. subtilis* (PC) achieved the highest spore degradation of *F. oxysporum* (86.7%), followed by *B. cereus* (84.2%), while *B. thuringiensis* was less effective. This suggests that antagonistic effects depend on the specific antagonist and pathogen strains evaluated.

Greenhouse biocontrol with *Bacillus* spp. showed variability in effectiveness depending on the strain used. *B. cereus* Bc28-2 and Bc28-5 reduced disease severity by 60.4% and promoted chili plant growth. In contrast, Bt24 did not reduce disease severity, showing results similar to the pathogen-only treatment. However, both Bt24 and Bc28-2 promoted vegetative growth in chili seedlings.

These findings highlight the potential of *Bacillus cereus* as a bioinoculant for jalapeño chili. Nonetheless, further validation under field conditions is necessary, as well as exploration of its integration into sustainable agricultural management strategies.

Limitations

Although the assays in this study were conducted under controlled conditions, the results must be validated under field conditions. Moreover, the variation in the response among different strains suggests that biocontrol efficiency may be influenced by pathogen-specific factors. Therefore, it is recommended to investigate the molecular mechanisms involved in spore degradation by *Bacillus* spp., as well as their effectiveness under variable environmental conditions. In addition, long-term evaluation in the field is essential to determine the practical viability of these treatments in agricultural systems.

Conflict of interest

The authors declare no conflict of interest.

Financing

This research received no external funding.

Author contributions

Conceptualization, J.H.H., A.G.C., and L.R.H.; methodology, A.A.A.B., M.M.A., and J.H.H.; software, J.H.H., L.R.H., and A.A.A.B.; validation, M.M.A., J.H.H., and A.G.C.; formal analysis, M.M.A. and L.R.H.; investigation, M.M.A., A.G.C., and J.H.H.; resources, J.H.H. and A.A.A.B.; data curation, J.H.H., L.R.H., and A.A.A.B.; writing—original draft preparation, M.M.A., J.H.H., and A.G.C.; writing—review and editing, J.H.H., L.R.H., and A.A.A.B.; visualization, M.M.A.; supervision, J.H.H. and A.G.C.; project administration, J.H.H.; funding acquisition, J.H.H. and L.R.H. All authors have read and agreed to the published version of the manuscript.

REFERENCES

Adina GD, Yalew M and Berhan. 2021. Survey on major diseases of vegetable and tuber crops in South Gondar zone, Ethiopia. In: Birhanu et al. (Eds.), *Results of Plant Protection Research.* Proceedings of the Completed Plant Protection Research Activities, pp. 106–111 https://doi.org/10.31254/njes.2021.8201

Ajesh K, Sudarslal S, Arunan C and Sreejith K. 2013. Kannurin, a novel lipopeptide from *Bacillus cereus* strain AK1: isolation, structural evaluation and antifungal activities. Journal of Applied Microbiology 115(6): 1287–1296. https://doi.org/10.1111/jam.12324

Antil S, Kumar R, Pathak DV, Kumar A, Panwar A and Kumari A. 2022. Plant growth-promoting rhizobacteria-*Bacillus cereus* KMT-5 and *B. megaterium* KMT-8 effectively suppressed *Meloidogyne javanica* infection. Applied Soil Ecology 174: 104419. https://doi.org/10.1016/j.apsoil.2022.104419

Azizoglu U, Salehi JG, Sansinenea E, Sanchis-Borja V. 2023. Biotechnological advances in *Bacillus thuringiensis* and its toxins: Recent updates. Reviews in Environmental Science and Biotechnology 22(2): 319–348. https://doi.org/10.1007/s11157-023-09652-5

Bashir MR, Atiq M, Sajid M, Mohsan M, Abbas W, Alam MW, Bashair M. 2018. Antifungal exploitation of fungicides against *Fusarium oxysporum* f. sp. *capsici* causing *Fusarium* wilt of chilli pepper in Pakistan. Environmental Science and Pollution Research 25: 6797–6801. https://doi.org/10.1007/s11356-017-1032-9

Bobadilla-Larios V, Esparza-Ibarra E, Delgadillo-Ruiz L, Gallegos-Flores P, Ayala-Lujan JL. 2017. Variedades de chile (*Capsicum annuum* L.) identificadas mediante marcadores RAPD. Tropical and Subtropical Agroecosystems 20(3): 465–473. Available online: https://www.redalyc.org/pdf/939/93953814014.pdf (accessed on 1 April 2025)

Boulahouat S, Cherif-Silini H, Silini A, Bouket AC, Luptakova L, Alenezi FN, Belbahri L. 2023. Biocontrol efficiency of rhizospheric *Bacillus* against the plant pathogen *Fusarium oxysporum*: a promising approach for sustainable agriculture. Microbiology Research 14(3): 892–908. https://doi.org/10.3390/microbiolres14030062

Campbell CL, Madden LV. 1990. Introduction to Plant Disease Epidemiology. 1st ed.; John Wiley and Sons: New York, USA, pp. 1–532.

Chandrasekaran M, Belachew ST, Yoon E, Chun SC. 2017. Expression of β-1.3-glucanase (GLU) and phenylalanine ammonia-lyase (PAL) genes and their enzymes in tomato plants induced after treatment with *Bacillus subtilis* CBR05 against *Xanthomonas campestris* pv. *vesicatoria*. Journal of General Plant Pathology 83: 7–13. https://doi.org/10.1007/s10327-016-0692-5

Chenniappan C, Narayanasamy M, Daniel GM, Ramaraj GB, Ponnusamy P, Sekar J, Ramalingam PV. 2019. Biocontrol efficiency of native plant growth promoting rhizobacteria against rhizome rot disease of turmeric. Biological Control 129: 55–64. https://doi.org/10.1016/j.biocontrol.2018.07.002

Cherif-Silini H, Silini A, Yahiaoui B, Ouzari I, Boudabous A. 2016. Phylogenetic and plant-growth-promoting characteristics of Bacillus isolated from the wheat rhizosphere. Annals of Microbiology 66: 1087–1097. https://doi.org/10.1007/s13213-016-1194-6

Chowdhury SP, Hartmann A, Gao X, Borriss R. 2015. Biocontrol mechanism by root-associated *Bacillus amyloliquefaciens* FZB42–a review. Frontiers in microbiology 6: 780. https://doi.org/10.3389/fmicb.2015.00780

Delfim J, Gerding M, Zagal E. 2020. *Phosphorus fractions in Andisol and Ultisol inoculated with Bacillus thuringiensis and phosphorus uptake by wheat*. Journal of Plant Nutrition 43(18): 2728–2739. https://doi.org/10.1080/01904167.2020.1793176

Delfim J. Dijoo ZK. 2021. *Bacillus thuringiensis as a biofertilizer and plant growth promoter.* In *Microbiota and Biofertilizers, Vol 2: Ecofriendly Tools for Reclamation of Degraded Soil Environs*; Springer International Publishing: Cham, Switzerland, pp. 251–265. https://doi.org/10.1007/978-3-030-61010-4_12

Fatima R, Mahmood T, Moosa A, Aslam MN, Shakeel MT, Maqsood A, Al-Shehri M. 2023. *Bacillus thuringiensis* CHGP12 uses a multifaceted approach for the suppression of *Fusarium oxysporum* f. sp. *ciceris* and to enhance the biomass of chickpea plants. Pest Management Science 79(1): 336–348. https://doi.org/10.1002/ps.7203

Gabrekiristos E, Demiyo T. 2020 *Hot pepper fusarium wilt (Fusarium oxysporum f. sp. capsici): Epidemics, characteristic features and management options.* Journal of Agricultural Science 12(10): 347–360. https://doi.org/10.5539/jas.v12n10p347

Galanihe LD, Priyantha MGDL, Yapa DR, Bandara HMS, Ranasinghe JADAR. 2004. Insect pest and disease incidences of exotic hybrids chilli pepper varieties grown in the low country dry zone of Sri Lanka. Annals of Sri Lanka, *6*, 99–106. Available online: https://www.sphinxsai.com/2022/ch_vol15_no2/1/(67-75)V15N2CT.pdf (accessed on 6 April 2025)

Gao T, Ding M, Yang CH, Fan H, Chai Y, Li Y. 2019. The phosphotransferase system gene ptsH plays an important role in MnSOD production, biofilm formation, swarming motility, and root colonization in *Bacillus cereus* 905. Research in Microbiology 170(2): 86–96. https://doi.org/10.1016/j.resmic.2018.10.002

Gupta R, Elkabetz D, Leibman-Markus M, Jami E, Bar M. 2022. Cytokinin-microbiome interactions regulate developmental functions. Environmental Microbiome 17(1): 2. https://doi.org/10.1186/s40793-022-00397-2

Gupta R, Keppanan R, Leibman-Markus M, Matveev S, Rav-David D, Shulhani R, Elad Y, Ment D, Bar M. 2024. *Bacillus thuringiensis* promotes systemic immunity in tomato, controlling pests and pathogens and promoting yield. Food Security https://doi.org/10.1007/s12571-024-01441-4

Hernández-Huerta J, Tamez-Guerra P, Gomez-Flores R, Delgado-Gardea MCE, Robles-Hernández L, Gonzalez-Franco AC, Infante-Ramirez R. 2023. Pepper growth promotion and biocontrol against *Xanthomonas euvesicatoria* by *Bacillus cereus* and *Bacillus thuringiensis* formulations. PeerJ 11: e14633. https://doi.org/10.7717/peerj.14633

Hyder S, Gondal AS, Rizvi ZF, Ahmad R, Alam MM, Hannan A, Ahmed W, Fatima N, Inam-Ul-Haq M. 2020 *Characterization of native plant growth promoting rhizobacteria and their anti-oomycete potential against Phytophthora capsici affecting chilli pepper (Capsicum annum* L.). Scientific Reports 10(1). https://doi.org/10.1038/s41598-020-69410-3

Jo H, Tagele SB, Pham HQ, Kim M, Choi S, Kim M, Park Y, Ibal J., Park G, Shin J. 2020. Response of soil bacterial community and pepper plant growth to application of *Bacillus thuringiensis* KNU-07. Agronomy 10(4): 551. https://doi.org/10.3390/agronomy10040551

Ku Y, Xu G, Tian X, Xie H, Yang X, Cao C. 2018. Root colonization and growth promotion of soybean, wheat and Chinese cabbage by *Bacillus cereus* YL6. PLoS One, *13*(11), e0200181. https://doi.org/10.1371/journal.pone.0200181

Kulkova I, Dobrzyński J, Kowalczyk P, Bełżecki G, Kramkowski K. 2023. Plant growth promotion using *Bacillus cereus*. International Journal of Molecular Sciences 24(11): 9759. https://doi.org/10.3390/ijms24119759

Kumar P, Pahal V, Gupta A, Vadhan R, Chandra H, Dubey RC. 2020. Effect of silver nanoparticles and *Bacillus cereus* LPR2 on the growth of *Zea mays*. Scientific Reports 10(1): 20409. https://doi.org/10.1038/s41598-020-77460-w

Leibman-Markus M, Schneider A, Gupta R, Marash I, Rav-David D, Carmeli-Weissberg M, Elad Y, Bar M. 2023. Immunity priming uncouples the growth–defense trade-off in tomato. Development 150(21). https://doi.org/10.1242/dev.201158

Lichtenthaler H, Wellburn AR. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Portland Press Limited, London. https://doi.org/10.1042/bst0110591.

Lozada DN, Bosland PW, Barchenger DW, Haghshenas-Jaryani M, Sanogo S, Walker S. 2022. Chile pepper (*Capsicum*) breeding and improvement in the "multi-omics" era. Frontiers in Plant Science 13: 879182. https://doi.org/10.3389/fpls.2022.879182

Madriz-Ordeñana K, Pazarlar S, Jørgensen HJL, Nielsen TK, Zhang Y, Nielsen KL, Hansen LH, Thordal-Christensen H. 2022. The *Bacillus cereus* strain EC9 primes the plant immune system for superior biocontrol of *Fusarium oxysporum*. Plants 11(5): 687. https://doi.org/10.3390/plants11050687

Malik MS, Haider S, Rehman A, Rehman SU, Jamil M, Naz I, Anees M. 2022. Biological control of fungal pathogens of tomato (*Lycopersicon esculentum*) by chitinolytic bacterial strains. Journal of Basic Microbiology 62(1): 48–62. https://doi.org/10.1002/jobm.202100512

Nie P, Li X, Wang S, Guo J, Zhao H, Niu D. 2017. Induced systemic resistance against *Botrytis cinerea* by *Bacillus cereus* AR156 through a JA/ET- and NPR1-dependent signaling pathway and activates PAMP-triggered immunity in *Arabidopsis*. Frontiers in Plant Science 8: 238. https://doi.org/10.3389/fpls.2017.00238

Pazarlar S, Madriz-Ordeñana K, Thordal-Christensen H. 2022. *Bacillus cereus* EC9 protects tomato against Fusarium wilt through JA/ET-activated immunity. Frontiers in Plant Science 13: 1090947. https://doi.org/10.3389/fpls.2022.1090947

Piechulla B, Lemfack MC, Kai M. 2017. Effects of discrete bioactive microbial volatiles on plants and fungi. Plant, Cell & Environmen 40(10): 2042–2067. https://doi.org/10.1111/pce.13011

Praca LB, Gomes ACMM, Cabral GB, Martins ES, Sujii ER, Pontes RGMS. 2012. Endophytic colonization by Brazilian strains of *Bacillus thuringiensis* on cabbage seedlings grown *in vitro*. Bt Research 3(3): 11–19. http://doi.org/10.5376/bt.2012.03.0003

Robles-Hernández L, Hernández-Huerta J, González-Franco AC, Hernández-Rodríguez OA, Núñez-Barrios A, Pérez-Leal R. 2015. *Streptomyces* PRIO41 as plant growth promoter of jalapeño pepper plants and as biocontrol agent of *Fusarium*. Phyton 84(2): 253. Available online: https://www.proquest.com/docview/2398004132?pq-origsite=gscholar&fromopenview=true&sourcetype=Scholarly%20Journals (accessed on 5 April 2025)

Sam-on MFS, Mustafa S, Yusof MT, Hashim AM, Aizuddin KNAK. 2024. Exploring the global trends of microbial agents for pest and pathogen control in chili cultivation. Saudi Journal of Biological Sciences 104046. https://doi.org/10.1016/j.sjbs.2024.104046

Sarangi T, Ramakrishnan S, Nakkeeran S. 2017. Antimicrobial peptide genes present in indigenous isolates of *Bacillus* spp. exhibiting antimicrobial properties. International Journal of Current Microbiology and Applied Sciences 6(8): 1361–1369. https://doi.org/10.20546/ijcmas.2017.608.166

Sela-Buurlage MB, Budai-Hadrian O, Pan Q, Carme-Goren L, Vunsch R, Zamir D, Fluhr R. 2001. Genome-wide dissection of *Fusarium* resistance in tomato reveals multiple complex loci. Molecular Genetics and Genomic 265*:* 1104–1111. https://doi.org/10.1007/s004380100509

Shafi J, Tian H, Ji M. 2017. *Bacillus* species as versatile weapons for plant pathogens: A review. Biotechnology & Biotechnological Equipment 31(3): 446–459. https://doi.org/10.1080/13102818.2017.1286950

Shaheen N, Khan UM, Azhar MT, Tan DKY, Atif RM, Israr M, Yang SH, Chung G, Rana IA. 2021. Genetics and genomics of *Fusarium* wilt of chilies: A review. Agronomy 11: 2162. https://doi.org/10.3390/agronomy11112162

Saner G, Finney RE. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67(8): 1051–1056. Available online: https://acortar.link/M8Yvo6 (accessed on 2 April 2025)

SIAP. Sistema de Información Agroalimentaria y Pesquera. Panorama Agroalimentario. Available online: https://nube.siap.gob.mx/panorama_siap/pag/2020/Atlas-Agroalimentario-2020 (accessed on 1 April 2025).

Tabachnick BG, Fidell LS Using Multivariate Statistics, 5th ed.; Pearson Education, Inc.: Boston, MA, USA, 2007; Available online: https://acortar.link/Ynt78M (accessed on 12 January 2025).

Thakur N, Nath AK, Chauhan A, Gupta R. 2022 Purification, characterization, and antifungal activity of *Bacillus cereus* strain NK91 chitinase from rhizospheric soil samples of Himachal Pradesh, India. Biotechnology and Applied Biochemistry, 69(5): 1830–1842. https://doi.org/10.1002/bab.2250

Tiwari S, Prasad V. 2019. and Lata, C. *Bacillus*: Plant growth promoting bacteria for sustainable agriculture and environment. In *New and Future Developments in Microbial Biotechnology and Bioengineering*; Elsevier: Amsterdam, The Netherlands, pp. 43–55. https://doi.org/10.1016/B978-0-444-64191-5.00003-1ç

Wijman JG, Leeuw PP, Moezelaar R, Zwietering MH.and Abee. 2027 T. Air-liquid interface biofilms of *Bacillus cereus*: formation, sporulation, and dispersion. Applied and Environmental Microbiology 73(5): 1481–1488. https://doi.org/10.1128/AEM.01781-06

Yanti Y, Habazar T, Reflinaldon R, Nasution, C.R. and Felia, S. 2017. Indigenous *Bacillus spp.* ability to growth promoting activities and control bacterial wilt disease (*Ralstonia solanacearum*). Biodiversitas 18(4): 1562–1567. https://doi.org/10.13057/biodiv/d180434

Yanti Y, Hamid H, Syarif Z, Afeland SN. 2021. Selected formulations of *Bacillus cereus* strain SLBE3.1AP with different storage durations for control *Fusarium oxysporum* f. sp. *capsici* Chili Plants. International Journal of Environment, Agriculture and Biotechnology 6: 6. https://dx.doi.org/10.22161/ijeab

Yoshida S, Koitabashi M, Yaginuma D, Anzai M. 2019. and Fukuda, M. Potential of bioinsecticidal *Bacillus thuringiensis* inoculum to suppress gray mold in tomato based on induced systemic resistance. Journal of Phytopathology 167(11–12): 679–685. https://doi.org/10.1111/jph.12864

Yu Y, Si F, Wang N, Wang T, Jin Y, Zheng Y, Yang W, Luo Y, Niu D, Guo J. 2022. and Jiang, C. Bacillus-Secreted oxalic acid induces tomato resistance against gray mold disease caused by *Botrytis cinerea* by activating the JA/ET Pathway. Molecular Plant-Microbe Interactions 35(8): 659–671. https://doi.org/10.1094/mpmi-11-21-0289-r

Zhou H, Ren Z, Zu X, Yu X, Zhu H, Li X, Zhong J. and Liu, E. 2021. Efficacy of plant growth-promoting bacteria *Bacillus cereus* YN917 for biocontrol of rice blast. Frontiers in Microbiology 12. https://doi.org/10.3389/fmicb.2021.684888

Zibanezhadian M, Pakdaman Sardrood B, Taheri H, Farkhari M. 2020. Anti-oxidative Response of *Bacillus thuringiensis*-Primed Tomato Plants to *Fusarium oxysporum* f. sp. *lycopersici*. Journal of Plant Molecular Breeding 8(2): 29–37. https://doi.org/10.22058/jpmb.2022.543818.1245