OPEN ACCESS Int. J. Phytopathol. 12 (03) 2023. 313-326 DOI: 10.33687/phytopath.012.03.4632

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Available Online at EScience Press

International Journal of Phytopathology

ISSN: 2312-9344 (Online), 2313-1241 (Print)

https://esciencepress.net/journals/phytopath

EFFECTIVENESS OF *BACILLUS PSEUDOMYCOIDES* STRAIN FOR BIOCONTROL OF EARLY BLIGHT ON TOMATO PLANTS

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ARTICLE INFO

Article History

Received: June 03, 2023

Revised: September 19, 2023

Accepted: December 11, 2023

Keywords

Alternaria solani

Bacillus pseudomycoides

Biocontrol

Early Blight

Solanum lycopersicum

Tomato plant

ABSTRACT

Early blight of tomato caused by *Alternaria* spp. is an air-borne and soil-borne pathogen that causes losses and damages that can reach up to 80% in tomato production. In our work, the immediate antagonistic effect of *Bacillus pseudomycoides* was inspected against *Alternaria* early blight of tomato. *Bacillus pseudomycoides* (Bp1) (OQ629426) gave inhibition efficacy against *A. solani* growth, being, 74.22 %. *Invitro*, Bp1 had the capability to produce the endogenous plant auxin (IAA) it was 18.9 (µg/100 mL), the quantity of GA 9.4 (µg/100 mL), 95.8 µ Deferroxamine mesylate. Tomato plants treated with *B. pseudomycoides* registered the least disease severity, being 50 and 40 % in Mancozeb + ALS and Bp1+ ALS treatments with high efficiency to control the severity between 75 and 100 % respectively. Tomato plants treated with *B. pseudomycoides* showed improved growth characteristics as compared with the untreated control. Plants with bacterial treatment conferred 45.6 cm shoot length, 2.9 (g⁄plant) fresh weight and 0.7 (g⁄plant) Dry weight**.** The highest increase in the activity of polyphenol oxidase (PPO) and peroxidase (POD) was observed in the infected leaves of tomato plants treated with *B. pseudomycoides* Bp1 (T4) (4.6, 6.9 m/g f. w.) respectively, followed by treatment of plants with Mancozeb (T3) (3.9, 5.4 m/g f. w.) respectively, compared to other treatments. This study suggested that *B. pseudomycoides* is a promising biocontrol agent against *Alternaria* early blight. This bacterium may represent an important source of potential antimicrobial bio-agent against *Alternaria* early blight disease, also it may play a role in the development of integrated control programs in future studies.

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INTRODUCTION

Tomato plant (*Solanum lycopersicum* L.) is one of the extreme important vegetables crops after potato because of its nutritious value, high levels of antioxidants, vitamins, and minerals (Awan and Shoaib, 2019; Rubén *et al.*, 2020). It belongs to the family Solanaceae and is subjected to infection by many pathogens including fungi, bacteria, viruses, and nematodes. *Alternaria solani* is one of the main fungal pathogens infecting tomato, and it also infects significantly different vegetables crops including pepper, potato, and eggplant (Nashwa and Abo-ElyouSr, 2012). *Alternaria solani* the causes of early blight disease on tomato occur in a variety of climates and can be

extremely destructive if left uncontrolled (Imran *et al.*, 2021). *A. solani* damages older leaves, causing distinctive dark spots and early leaf abscission. The disease spreads from the plant's lower to upper aerial parts. Field or greenhouse losses are severe and *A. solani* can reduce production by up to 80% (Chaerani and Voorrips, 2006). For control of early blight disease of tomato, many approaches have been used. For example, chemical control such as captan, carbendazim, oxychloride, copper, propiconazole, mancozeb, propineb and tebuconazole have been used to keep infection at low rates (Deshmukh *et al.*, 2020). Using Mancozeb with different formulations has significant growth suppression of pathogen of early blight disease in tomato under field conditions (Yang *et al.*, 2019; Sowmya and Chandra, 2021). However, frequent uses of fungicides may lead to developing resistance strains, cause damage to non-target organisms, as well as human health and ecological risk (Prakash *et al.*, 2021; Zhang *et al.*, 2021).

Although it has been reported in many variety climates around the world, there are few reports on its biological control (Lourenço Jr *et al.*, 2009). *Bacillus sp.* is deemed as one of microbiome organism for plant-related bacilli supply capitalization to its plant such as safeguard against plant diseases (Elhadidy, 2019). This is mainly due to the possibility to excrete a broad range of bioactive secondary substances with fixed and harmonious effects (Miao *et al.*, 2023).

Recently, it became critical to improve that plant-derived based chemical whether biotic or biodegradable, with a view to suppress phytopathogens via the incorporated pest management way in order to ameliorate the yield of sustainable cultivation (Anand *et al.*, 2009; Simonetti *et al.*, 2012; Almoneafy *et al.*, 2022). Biological control is regarded as a safe alternative approach to avoiding the use of chemical pesticides in suppressing plant pathogens and support organic crop output (Atia, 2005; Pal and Gardener, 2006; Cawoy *et al.*, 2011; He *et al.*, 2021). Biocontrol via disease suppressive potential strains, such as *Bacillus sp*., is now thought to be an effective management strategy for various plant diseases. This biocontrol agent generates antimicrobial compounds (Daura-Pich *et al.*, 2020; Köhl *et al.*, 2019; Wei *et al.*, 2021). Previous reports suggested that *Bacillus sp*. have strong plant growth promotion and biocontrol effects against many phytopathogens (Wu *et al.*, 2020; Jinal and Amaresan, 2020; Sansinenea, 2019; Shafi *et al.*, 2017; Ali *et al.*, 2014). Among *Bacillus spp., Bacillus pseudomycoides* is one of the possibility bioagents (Rabbee *et al.*, 2019; Hassan *et al.*, 2017). deeming the significance of bio-control agents and elevation of fungicides impedance troubles. In recent work, *Bacillus pseudomycoides* (Bp1) was isolated from tomato roots and *in vitro* screened for opposed effect against *A. solani*. *Bacillus pseudomycoides* (Bp1) was specified by molecular technique. Lipopeptides excreted using *Bacillus pseudomycoides* (Bp1) was studied to determine its antagonistic potential. *A. solani* was selected to determine their impact on early blight disease severity in pot experiment and to contrast bio-agents to a standard fungicide in terms of tomato yield and disease control.

MATERIALS AND METHODS

Pathogen

The fungus *Alternaria solani* strain (MT996276), which causes early blight symptoms was obtained from the Plant Pathology Research Center, ARC, Giza, Egypt. It was revitalized by cultivating it in dark for ten days at 25˚C on Potato Dextrose Agar (PDA) solid medium (potatoes 200 g/L, dextrose 20 g/L, agar15 g/L) and kept at 4˚C until needed. The suspension was made in the manner described by (Nekoval *et al.*, 2022).

Pathogenicity Assessment on Detached Leaves

The virulence of the isolated *Alternaria* spp. was examined in vitro by introducing it to detached leaflets from 30-day-old tomato plants (cv. Super Strain B). Healthy, fully expanded leaflets from greenhouse-grown tomatoes were first surface sterilized using a 2% sodium hypochlorite solution, followed by a rinse with sterilized distilled water (SDW). The spore concentration was standardized to 1 x 10^6 conidia/mL, and a 20 µL droplet was applied to the upper surface of the leaflets. For comparison, a 20 µL SDW droplet served as a control. This procedure was replicated four times, and the entire experiment was conducted twice. Post-inoculation, the leaflets were placed on sterilized Petri dishes with moist, sterilized tissue paper and stored at 25°C, 80% humidity, and a 12-hour light cycle in a growth chamber. Observations were made daily, with symptom documentation occurring after a week. To fulfill Koch's postulate, Alternaria spp. was re-isolated from the affected leaves using PDA media (Sharma *et al.*, 2004). Disease severity was gauged using a 0-5-

point scale (Vakalounakis, 1983), and the Disease Severity Index (DSI) was computed as:

$$DSI\% = \frac{100 \times \text{sum of all ratings}}{N \times \text{maximum disease scale}}$$

Where "n" represents the count of affected leaflets and "N" is the total leaf count.

Preparation of Biocontrol Agent

Bacillus sp. (Bp1) was isolated from phyloshear of healthy tomato plant carried out according to (Gaete *et al.*, 2020). Concise, ten g of soil was collected and mixed in 4 ml of sterilized water using a vortex. Then, transferred 2 ml of the homogenate to a new tube with centrifuged at 5000 rpm for 5 minutes, pure bacterial culture grown on new nutrient agar (NA) (peptone 0.5%, beef extract 0.3%, agar1.5%, pH 6.8 at 25 ˚C) and incubated at 30˚C for 24 h (Urrea *et al.*, 2011). Finally, the Purified *Bacillus sp.* (Bp1) was stored in nutrient agar slants adding 60% glycerol stocks at – 80° C, the concentration was standardized to 6 x 10^8.

In vitro Assay

To evaluate the antagonistic properties of *B. pseudomycoides* (Bp1) strain as biocontrol agent *in vitro* on the tested *A. solani* strain, the experiment was conducted using potato dextrose agar (PDA) medium in 9 cm diameter Petri plates. Disks (0.6 cm) were placed two cm from the edge of the plate. On the opposite side of the Petri plate a streak of the bacterium was placed. Disks were obtained from *A. solani* six days old culture and inoculated at 25°C in darkness. *B. pseudomycoides* (Bp1) was grown in nutrient agar (N.A.) plates for 24 hours. Negative control with no bacteria. The diameter of colony (cm) of fungus on PDA plates for treatments and control were measured. Treatments were replicated five times, with conducted was twice. The average of both diameters was evaluated after 3, 5, and 7 days The antifungal effect of biocontrol agent was calculated using the following formula inhibition % (Mari *et al.*, 1996).

$$\text{Inhibition } (\%) = \frac{C - T}{C - 6} \times 100$$

Where: C is the fungal colony of control, 6 is the diameter of fungal disk, T is the fungal colony with treatments.

Biochemical Characteristics of *B. pseudomycoides* (Bp1)

The plant growth promotion agents were the main biological control mechanisms such as Indole Acetic Acid (IAA) (Zhang *et al.*, 2021). IAA was quantified according to the method described by Bric (Bric *et al.*, 1991). Briefly**,** *B. pseudomycoides* (Bp1) was transferred in liquid medium for one day and transferred 10 µl into liquid LB containing 100 µg mL−1 of L-tryptophan for seven days at 28°C on 200 rpm/min. with Using (49 ml of 35% $HClO_4$, 1 ml of 0.5M $FeCl_3$) as a Salkowski reagent was transferred the supernatant to an ELISA plate at room temperature for 35 min. Three replicates were used. The growth promotion can stimulate the production of phytohormones such as the (IAA). The amount of gibberellic acid (GA) in the ethyl acetate phase was measured by the UV spectrophotometer at 254 nm according to (Mitter *et al.*, 2002). The concentration of siderophores of the bacterial isolate filtrate was determined by modified CAS assay method (Alexander and Zuberer, 1991) and Hydrogen cyanid (HCN) production of the bacterial isolate was assessed according to (Wei *et al.*, 2021).

Molecular Identification of *Bacillus* sp.

DNA from *B. pseudomycoides* (Bp1) bacterial colonies was extracted as described by (Courtois *et al.*, 2001). A fragment of primers was used to amplify 16S rRNA by polymerase chain reaction (PCR) proceeded with forward primer forward: - AGA GTT TGA TCC TGG CTC AG and reverse primer: - GGT TAC CTT GTT ACG ACT T. extension was carried out in a Thermal Cycler T100 machine using Maxima Hot Start Master Mix (Thermo) (2X). The PCR reaction was as follows: 95°C for 10 minutes; 35 rounds of 95°C for 30 seconds, 55°C for one minute, and 72°C for 1.5 minutes; and a final supplementary at 72°C for 10 minutes. The ABI 3730 DNA sequencer was used to sequence the clarified PCR fragment (Applied Biosystems, Thermo Fisher Scientific, Korea). The 16S rRNA gene sequence was specified using GenBank database (Miranda *et al.*, 2008; Morgan *et al.*, 2009; Altschul *et al.*, 1997)**.** The phylogenetic tree was completed using the neighbor-joining technique and the entire 16S rRNA sequence (Feng *et al.*, 2011; Phanse *et al.*, 2013).

***In vivo* Assay**

Plastic pots of 25 cm were filled up with 5 kg of sterilized silty-clay soil mixed with 0.2 g of diammonium phosphate fertilizer (DAF). After 4-5 weeks seedlings of Super Strain B tomato plants were transplanted, until the plants had 4-5 real leaves. Using five pots as replicates for each treatment and the untreated as a control. Complete randomized block design was used. Tomato plants were treated with *B. pseudomycoides* (Bp1) once every 14 days before infection and determine the efficiency of tomato early blight disease

severity. A suspension Spores of *A. solani* were prepared according to EL-Tanany (El-Tanany *et al.*, 2018)at a rate (3×10^5 spore/mL). Fresh culture of bacteria was prepared at rate (6×10^8 CFU/ml). 5 mL of liquid culture consisting of 5 g peptone, 3 g beef extract, and 10 g glucose of (Bp1) was added to the pots by supplementing to the soil in a 2-cm hole beside the seedlings after transplanting. Treatments were (pathogen *A. solani* ALS) (T1), Control untreated plants (T2), Mancozeb + ALS (T3) and Bp1+ ALS (T4). Three replications of each hybrid were evaluated and inoculated with 30 ml of spore suspension was applied using a hand atomizer. Plants sprayed with the same amount of distilled water were used as control. Disease severity (%) recorded were done every two weeks after inoculation. This was based on the severity of six categories rated as follows; (% of shoot wilted, using a scale of 0 to 5 (0 = no symptoms, 1 = symptoms less than 10% of the leaf area, 2 = symptoms more than 10% >20%, 3= symptoms 20% >30%, 4 = symptoms 30% >40% and 5 = symptoms < 40% of the leaf area) (Moročko, 2006). Disease severity was determined due to formula described by Townsend (1943).

$DS\ (\%) = (\Sigma\ (nv) / NV) \times 100$

Where, n - degree of symptom according to the scale; v - number of samples per each category; V - total number of plants; N - the highest score of the categories.

Then efficacy of biological control agents and different concentrations of chemical inducers was calculated using Song's formula (Song *et al.*, 2004) as follows:

$Efficacy\ (\%) = (X - Y) / X \times 100$

Where, X = disease severity in untreated control. Y = disease severity in each treatment. Also, some growth parameters i.e., Plant high, fresh, and dry weight g/plant of shoot of tomato plants were recorded.

Enzyme Activity Assays

To determine (PPO and POD) enzymes activities and total phenol content, fresh tissue of Super Strain B tomato plants CV was used for treated and control treatment. All steps were executed at 0−4° C.

Polyphenol Oxidase Activity (PPO)

PPO efficacy was measured following by Maxwell and Bateman (Maxwell and Bateman, 1967). Briefly, sodium phosphate buffer (pH 7) was added to crude enzyme. PPO was estimated using a spectrophotometer absorbance at 495 nm.

Estimate Peroxidase Activity (POD)

Peroxidase activity was estimated by the method described by Attia (Attia *et al.*, 2020). The peroxidase efficacy was recorded using spectrophotometrically and showed as the change in absorbance at 425nm.

Estimation of Total Phenols Content

Phenols content was carried out following the method of Alhaithloul (Alhaithloul *et al.*, 2019). Briefly, 5g of fresh weight tissue was used for each treatment mixed with 80 mL methanol one day. Then the solution was filtered and mixed with to 1.4 mL of distilled water, Folin-Ciocalteu phenol reagent was used. Five minutes later, the $NaCo_3$ solution was mixed at 0.3 mL w/v. and incubated for 60 min at 45 ◦C and homogenized for 1 min at 800 rpm then, the total phenols content was estimated as the difference in color and absorption the color and absorbance change were at 765 nm.

Statistical Analysis

Data were analyzed by using Completely Randomized Design (CRD). And it was expressed as mean ± standard error (SE). Mean comparisons of several parameters were conducted using the procedures of SPSS statistical analysis software version 16. Mean separation was estimated using one-way ANOVA and Duncan's multiple range test and Differences were deemed statistically remarkably ($P<0.05$) (Duncan, 1955).

RESULTS

Pathogenicity Tests on Detached Leaves

The *A. solani* isolate manifested early blight symptoms on the detached tomato leaves, regardless of their original host plant. In contrast, control leaflets treated with SDW remained symptom-free (Figure 1). A diverse range of pathogenic responses was noted upon symptom evaluation. By the 4th day post-inoculation, yellow flecks appeared, which subsequently enlarged into sunken lesions. These lesions later evolved, showcasing a gray center encircled by a yellow halo. The virulence of *A. solani* on tomato leaves was evident, with a DSI of 83.2%.

***In vitro* assay**

Bacillus pseudomycoides (Bp1) strain was evaluated *in vitro,* for its antagonistic effect averse to *Alternaria solani* which are utmost virulent pathogenic isolate by dual culture inoculation technique. The antifungal effectiveness of *Bacillus pseudomycoides* (Bp1) strain as biocontrol agent was determined as the percentage inhibition % (Figure 2). *Bacillus pseudomycoides* (Bp1) gave the inhibition efficacy against *A. solani* growth, being, 74.22 %.

Biochemical Characteristics of *Bacillus pseudomycoides* (Bp1) for Growth Promotion

The *Bacillus pseudomycoides* (Bp1) qualitatively displayed varied plant growth-promoting efficiency like indole acetic acid (IAA) amount, gibberellic acid (GA), Siderophore and hydrogen cyanide (HCN). It was moreover examined to determine the plant growth-promoting values (Table 1). After seven days of incubation at 28 °C, *Bacillus pseudomycoid*es had the capability to produce the endogenous plant auxin (IAA) it was 18.9 (µg/100 mL), the quantity of (GA) 9.4 (µg/100 mL), 95.8 Deferroxamine mesylate (µ MDFOM) was the concentricity of siderophores and production of (HCN) was calculated (Table 1). IAA is popular amongst the plant growth promoting bacteria (PGPB) for the reason that it assists in root extension and uptake of minerals.

Figure 1. Display of early blight symptoms induced by *A. solani* on detached tomato leaves. Comparisons include non-inoculated (a:0 scale) and *A. solani*-inoculated leaves (b:1scale, c:2scale, d:3scale, e:4scale, f:5scale). The test utilized 30-day-old tomato plant leaves (cv. Super strain B). Photographs were taken 7 days post-inoculation, and disease severity was evaluated using a 0-5-point system.

Figure 2. Effectiveness of *Bacillus pseudomycoides* (Bp1) versus *Alternaria solani* ALS *in vitro.*

Table 1. Growth-promoting characteristics of bacterial isolate *Bacillus pseudomycoides* (Bp1).

Isolate	IAA* production (µg/100 mL)	Gerbilline production (µg/100 mL)	Siderophore µ MDFOM	Hydrogen cyanide (HCN)
Bacillus pseudomycoides (Bp1)	18.9	9.4	95.8	+++

Identification of Endophytic Bacterium by Molecular Method

The tested Bp1 isolate was identified as a *B. pseudomycoides* with a rod cell with spore production and positive reaction to gram stain. The partial (1510 bp), 16S rRNA genes were used to identify the strain. The efficiency of 16S rRNA sequences was definitively matched with *B. pseudomycoides* with an ambiguous 98% similarity. Bootstrap values (1000 replicates) are shown on the branches. The scale bar indicates the genetic distance using the database of 16S rRNA in EzBioCloud and it was recorded on the NCBI Gen Bank with accession number (OQ629426). The phylogenetic tree was built by comparing the isolate Bp1 with 16S rRNA gene sequences to sequences from the NCBI GenBank (Figure 3).

***In vivo* Assay**

Our results show that the plants which treated with *B. pseudomycoides* (OQ629426) registered the least disease severity, being 50 and 40 % in treatments T3&T4 with high efficiency to control the disease between 75 and 100 % respectively (Table 2 and Figure 4).

Effect of *Bacillus pseudomycoides* (OQ629426) Strain on Vegetative Parameters of Tomato *in vivo*

Bacillus pseudomycoides offered remarkably higher values in utmost of the variables recorded as compared to control plants (Table 3). Symbiotically plants with *B. pseudomycoides* conferred 45.6 cm shoot length, 2.9 (g/plant) fresh weight and 0.7 (g/plant) Dry weight.

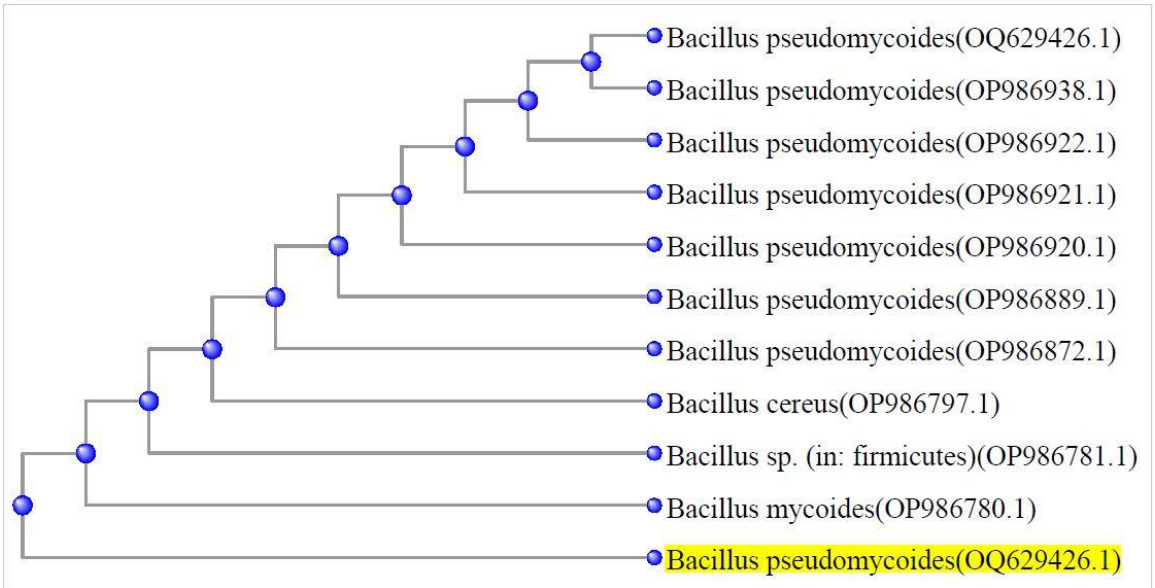

Figure 3. Phylogenetic tree based on the partial 16S rDNA sequences of the Bp1 isolate.

Figure 4. Disease severity % on infected tomato plants with *A.solani* ALS4 under greenhouse condition. T1*: pathogen ALS, T2*: Control untreated plants, T3*: Mancozeb + ALS, T4*: Bp1+ ALS

Table 2. Disease severity % and Efficacy % on infected tomato plants with *A. solani* ALS4 *in vivo*.

Treatments	Disease severity%	Efficacy %
Pathogen (T1)*	80 a	0.0 c
Control (T2)*	10 d	0.0 c
Mancozeb + ALS (T3)*	50 b	75 b
Bp1+ ALS (T4)*	40 c	100 a

T1*: pathogen ALS, T2*: Control untreated plants, T3*: Mancozeb + ALS, T4*: Bp1+ ALS. Values in a column followed by the same letter are not significantly different according to the LSD test (P=0.05).

Table 3. Effect of *B. pseudomycoides* on shoot length, fresh weight and dry weight of tomato plants (cv. Super Strain B).

Treatments	shoot length (cm)	Fresh weight (g/plant)	Dry weight(g/plant)
pathogen (T1)*	20.5 c	1.1 c	0.3 c
Control (T2)*	40.5 d	1.4 c	0.4 bc
Mancozeb + ALS (T3)*	45.6 b	2.9 b	0.6 b
Bp1+ ALS (T4)*	51.3 a	3.6 a	0.9 a

T1*: pathogen ALS, T2*: Control untreated plants, T3*: Mancozeb + ALS, T4*: Bp1+ ALS ; *Means with the same letter are not significantly different

Phenolic Determination and Enzymes Activities

The average polyphenol oxidase (PPO) and peroxidase (POD) enzymes and phenolic content in the tomato roots infected with *A. solani* are presented in (Table 4). Tomato plants treated with *B. pseudomycoides* (OQ629426) showed remarkable boost in their PPO and POD activity and total phenolic content compared with control plants. As for activity of oxidative enzymes, the highest increase in the activity of PPO and POD was observed in the leaves of tomato plants treated with Bp1+ ALS (T4) (4.6, 6.9 m/g f. w.) respectively, followed by treatment of plants with Mancozeb + ALS (T3) (3.9, 5.4 m/g f. w.) respectively, compared to other treatments. The treatment of *B. pseudomycoides* on the tomato plants stimulated their phenolic values and raised POD and PPO activities.

Table 4. Effect of antagonistic bacteria on polyphenol oxidase (PPO), peroxidase (POD) and total soluble phenol content in tomato leaves artificially inoculated with *Alternaria solani* under greenhouse conditions.

Treatments	Total phenol (mg/100g f. w.)	PPO* (m/g f. w.*)	POD* (m/g f. w.*)
pathogen (T1)*	85.9 c	1.2 c	2.3 c
Control (T2)*	63.1 d	0.9 c	1.4 d
Mancozeb + ALS (T3)*	136.4 b	3.9 b	5.4 b
Bp1+ ALS (T4)*	145.6 a	4.6 a	6.9 a

T1*: pathogen ALS, T2*: Control untreated plants, T3*: Mancozeb + ALS, T4*: Bp1+ ALS ; PPO*: polyphenol oxidase, POD*: peroxidase, f. w.*: fresh weight; *Means with the same letter are not significantly different

DISCUSSION

The use of beneficial microorganisms in organic agriculture can help to protect crops from phytopathogens. Several researchers have proved that the microbiome associated with plants can improve their growth and development in addition to suppressing the growth of phytopathogenic agents. The ability of new antagonistic strains holds enormous potential to improve the efficiency of these biological control agents in repression of plant diseases (Beneduzi *et al.*, 2012; Choudhary and Johri, 2009).

Bacillus pseudomycoides recorded high antagonistic activity against *A. solani* 75%. acquired results are in harmony with (Tozlu *et al.*, 2018) who recorded that several *Bacillus sp.* strains were the most effective agent against *A. solani* and inhibited the fungal growth by 73.87%. *Bacillus* species are reported as the paramount antagonistic agent for using in repression of plant diseases and they appear to have special features over other bacteria used against pathogens due to their wide - spectrum antibiotic activity and endospore figuration (Pane and Zaccardelli, 2015). The results showed that *B. pseudomycoides* significantly inhibited pathogen mycelial growth. The impact of microbiome *Bacillus* sp strains on mycelial growth (mm) of *Alternaria* sp. pathogen is greatest with different antagonistic activity compared to untreated plants (Yazici *et al.*, 2011).

The tested microbiome strain demonstrated immediate activity against the pathogen *A. solani*, suppressing its growth *in vitro*. It ensured that the antagonists produce a variety of venomous substances that have an antimicrobial effect on the pathogen, resulting in the antibiosis phenomenon. The bioactive compounds are allegedly derived from lipopeptides that are toxic to pathogens (Ongena *et al.*, 2005; Andrić *et al.*, 2020). Biocontrol agents from *Bacillus, Pseudomonas*, and *Streptomyces* have been identified by defining inhibition *in vitro* (Saraf *et al.*, 2014). *In vitro* tests revealed that

bacteria such as *B. subtilis* and *B. cereus-GC*, were highly effective on *A. solani* (Larkin, 2020; Donmez *et al.*, 2015). *Bacillus pseudomycoides* had the ability to produce indole acetic acid (IAA) and results were quite similar to find of Ahmed (Ahmed *et al.*, 2022) who reported that *Bacillus amyloliquefaciens* WS-10 promote the tobacco plant's growth and dry matter contents. Growth promotion in tobacco plants related to the *in vitro* production of IAA (ug/mL). Similarly, *Bacillus* sp. PG-8 produced 2.78g/ml IAA with adding 0.1% (w/v) tryptophan after 120 hours at 30°C (Gohil *et al.*, 2022). *Bacillus pseudomycoid* B1302 was able to produce IAA as Plant growth-promoting (PGP) (Yi *et al.*, 2022). The study found that even within the same species, strains of *Bacillus* sp. vary in their ability to give and excrete active metabolites (Sun *et al.*, 2021). Zhou indicated *Bacillus cereus* YN917 had siderophores, indole and some enzymes production (Zhou *et al.*, 2021). Sharma *et al.* (2019) indicated that *Bacillus pseudomycoides* produced enzymes, siderophore. He showed that, IAA production and fixed nitrogen, solubilize phosphorus activities increase nutrient content in tissues, guide to improved yield and stress impedance Seeds of wheat were germinated faster than control seeds when treated with filtrate of *Bacillus* B1302. The reason for this could be production of IAA and activate specific enzymes that promote early germination. The intension plant length and weight in the potted plants experiment suggested that the B1302 strain might promote growth. Furthermore, when compared to commercial fungicides, *B. mojavensis* B1302 strain has high control efficiency. The disease index could be reduced by using *B. mojavensis* B1302 filtrate. Furthermore, they investigated the efficacy of bacterial filtrate and trade fungicide. The efficacy of *Bacillus* filtrate was the best 65.25% of control treatment. As a result, *B. mojavensis* had superior control impact against *Rhizoctonia sp*, which is symmetrical with the antifungal action observed *in vitro*.

Gibberellic acid is a phytohormone that helps in seed germination and plant growth (Vishal and Kumar, 2018). *Bacillus velezensis* (Bv1) had the ability to produce 9.4 (µg/100 mL), results are similar to the findings of Gohil (Gohil *et al.*, 2022)who reported that the *Bacillus* sp. PG-8 was able to produce the highest gibberellic acid 0.70 mg/ml at 30◦C after 72 h in nutrient broth. They also proved that *B. velezensis* (Bv1) had the ability to produce siderophores, results are similar to the findings of Andrić *et al.* (2023) who hypothesize that *B. velezensis* relies on such chelator sensing to accurately identify competitors, illustrating a new facet of siderophore-mediated interactions beyond the concept of competition for iron and siderophore piracy.

Overall, the results showed that *B. pseudomycoides* was effective at reducing early blight severity and raising shoot and root weight of tomato plants. These findings are consistent with those of Khalila and Adbelghany (Khalil and Adbelghany, 2021), who discovered that the bacteria *B. megaterium* and *B. subtilis* control early blight on tomato and recorded disease severity of 26.14 and 29.86%, respectively, and efficacy of 51.86 and 54.00%. These findings are consistent with (El-Tanany *et al.*, 2018), who indicated that several strains of bio-agents on *A. solani* growth and concluded that *B. subtilis* were reducing *A. solani* growth by bio agents. Data elucidated that using the beneficial microbiome *B. pseudomycoides* on tomato prior to infection with *Alternaria* sp. in pot experiment reduced the disease severity of early blight compared to control plants. Data are in harmony with EL-Tanany and Moustafa, they recorded that adding several strains of bacteria had significantly suppressed disease severity of early blight on tomatoes *in vivo*. Antibiotic microbiome had a remarkable role in the control of plant diseases (El-Tanany *et al.*, 2018; Moustafa *et al.*, 2018).

Meanwhile, Zhang et al., 2021 reported that in pot experiment, the fermentation broth of *Bacillus subtilis* ZD01 significantly reduced *A. solani* pathogenicity. In comparison to the treatments, leaf chlorosis had shown with *A. solani* the disease index of potato decreased notably (p 0.05) to 34.17%, contrast to the control treatment (83.02%). There were no remarkable differences between the 5 10^3 and 5 10^5 CFU/mL treatments. When the lesion areas spread to 0.90 cm^2, in bacterial treatments 5 × 10^3, 5 × 10^5 and 5 × 10^7 CFU/mL, the lesion areas were restricted to 0.33 cm^2, 0.31 cm2 and 0.42 cm^2, respectively.

Bacillus pseudomycoides had significantly improved vegetative parameters in contrast to control plants (Table 3). Tomatos grown in symbiosis with *Bacillus pseudomycoid* produced 45.6 cm shoot length, 2.9 (gplant) fresh weight, and 0.7 (g/plant) dry weight. Data consistent with (Akram *et al.*, 2015), who recorded that root inoculation of *Bacillus fortis* IAGS162 and *B. subtilis* IAGS174 significantly promoted growth attributes and showed notably raises in length of shoot and tomato

yield in greenhouse experiments. Antibiotic microorganisms are crucial in the control of diseases. They usually have a broad range of suppressive pathogens. The conservative impact of microbiome comprises a variety of tools, including the production of venomous substances that forthrightly counteract pathogen growth (Thesaurus *et al.*, 2019). *B. pseudomycoides* give an adequate and more efficacious biocontrol and vegetative-promoting organism for tomato cultivate than others agents, being an safe and prospective agent in contemporary cultivation (Rashad *et al.*, 2022).

The application of *B. pseudomycoides* to tomato plants increased their phenolic content and POD and PPO activities. These findings are consistent with those of Rashad (Rashad *et al.*, 2022), who found that the application of *B. subtilis* SR22 increased the expression of the interactive factor JERF3 (11fold) as well as the protection -related genes POD (9 fold) and PR1 (4.5-fold) in tomato cultivates. Antioxidant activity has been reported in several growth-promoting bacteria (Mirzaei *et al.*, 2020; Ren *et al.*, 2019; Heidari and Golpayegani, 2012; Ordookhani and Zare, 2011).

POD are accountable for production of phenolic substances that participate in the strengthening of cell wall and, as a result, allow disease resistance. It also included in the injuring stress action (Gainza *et al.*, 2015; Saltveit, 2015; Padró *et al.*, 2021). That, PAL, POD, and PPO are the protection concerning enzymes correlated to phenolic and lignin values in plants tissues confrontation biotic and abiotic stress (Mandal *et al.*, 2010; Padró *et al.*, 2021).

Furthermore, bacteria increased the total phenolic amount (76.8%) and antioxidant enzyme response (56%) and Poly phenol oxidase (29.2%) in roots, appear a protection-inducing impact on tomato cultivate (Rashad *et al.*, 2022). According to Awan(Awan *et al.*, 2022), the interacting leverage of *Bacillus subtilis* BS-01 with nutrients allow a notably different degree of pliability in infected plants opposed to *Alternaria* blight by efficiently raising the values of hole phenolics as well as the actions of antioxidant enzymes (POD and PPO). *Bacillus sp.* Gave 30% of the radicals even at little concentrations and can neutralize 100% of the radicals at higher concentrations. To assess the impacts of *B. mojavensis* on wheat, a potted plant experiment was carried out. This study previously demonstrated this strain's ability to give exoenzymes and disclose plant growth promoting capabilities. Strain B1302 was found to be capable of producing many enzymes (Yi *et al.*, 2022).

CONCLUSION

Plant growth-promoting endophytic bacteria can offer incredible benefits to plants and can support environmentally friendly approaches for sustainable agriculture. They can be used as tools that could be an alternative way to reduce the use of chemicals. It is obvious that, under the current climate changes and increasing world population, the sustainability of agriculture should be based on innovative environment-friendly approaches and should consider crop biodiversity and highly nutritious crops such as tomatoes and other non-conventional crops. Our study focused on the effect of *B. pseudomycoides* on tomato growth and resistance to early blight disease. It proved that *B. pseudomycoides* can be used as a promising bioagent to limit this disease and can be used as a biotechnology technique in tomato seedling production. *Bacillus pseudomycoides* proved effective in reducing the severity of early blight and improving growth of the tested tomato cultivar. The application of *B. pseudomycoides* to tomato plants increased their phenolic content and POD and PPO activities. *B. pseudomycoides* is a promising *Alternaria* early blight combating agent and growth promoter of plants. Future research is needed to evaluate the bioformulation and large areas of application of this bioagent.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally to the research and compiling the data as well as editing the manuscript.

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