EGYPTIAN JOURNAL OF BOTANY (EJBO)

Microbial bio-inducers and seaweed extract enhance tomato immunity and physiological responses against *Fusarium oxysporum*

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Fusarium oxysporum significantly reduces tomato crop yields. While chemical fungicides can suppress the pathogen, they often harm the environment and lead to increased fungal resistance. Biocontrol, using antagonistic bacteria, offers an eco-friendly alternative by enhancing plant resistance and suppressing *Fusarium* through competition and antimicrobial activity. This study evaluated the effectiveness of plant growth-promoting rhizobacteria (PGPR) and *Ascophyllum nodosum* (Alga Mix®) in boosting tomato plant immunity. Twenty bacterial isolates were collected from the tomato rhizosphere in Eltal Elkbeer, Ismailia, Egypt, and assessed for their ability to produce hydrocyanic acid production (HCN), siderophores, and Indole-3-acetic acid (IAA). Two isolates exhibited the highest activity and antifungal properties against *F. oxysporum*. Molecular identification confirmed them as *Bacillus subtilis* and *Achromobacter insuavis*. Under greenhouse conditions, infected tomato plants treated with these agents showed reduced disease severity and improved physiological parameters. Alga Mix® was particularly effective, enhancing chlorophyll content, carotenoids, and phenolic compounds. Treatments also boosted enzymatic defenses and mitigated oxidative stress. These findings support the potential of PGPR and Alga Mix® as sustainable strategies for managing Fusarium wilt in tomatoes.

Keywords: *Achromobacter, Ascophyllum, Bacillus,* Biocontrol*, Fusarium,* Therapeutic nutrition

ARTICLE HISTORY

Submitted: March 27, 2025

Accepted: July 3, 2025

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DOI: 10.21608/ejbo.2025.371153.3243

EDITED BY: Sawsan Abdel Ghany

INTRODUCTION

One of the most significant challenges to sustainability and world food security is the loss of crops due to pathogen attacks, particularly from fungi (Attia et al., 2024). Infections caused by fungi severely affect vegetables, often resulting in complete or partial crop failure, with tomatoes especially vulnerable (Panno et al., 2021). In Egypt, tomatoes are a major agricultural commodity, with the country ranking among the top 10 producers globally, contributing 6.4 million tons of tomatoes annually (Ali et al., 2021). However, fungal diseases such as *F oxysporum* pose a significant threat, significantly reducing the quality and quantity of tomato yields (Abdelaziz et al., 2024b). With the added stress of climate change and the prevalence of such diseases, there is an urgent need to enhance crop productivity while reducing reliance on chemical pesticides (Shah et al., 2021). Biological control, which involves using beneficial microorganisms to manage plant diseases, offers a promising alternative (Heydari & Pessarakli, 2010). The indiscriminate and excessive use of chemical pesticides negatively impacts soil health, plant vitality, and human well-being (Khalil et al., 2015). On the other hand, natural inducers can enhance plant defenses against pathogens and boost productivity while preserving soil health, fertility, and therapeutic nutrient levels (Alrashidi et al., 2022; Khattab et al., 2022). eTherapeutic

nutrition refers to providing plants with specific nutrients and fertilizers that activate physiological processes, improving their resilience to stress, reducing disease risks, and mitigating side effects associated with diseases (Attia et al., 2016; Kumar & Verma, 2018). Plant growth-promoting rhizobacteria form beneficial relationships with plants in the rhizosphere (Sharma et al., 2024; Yu et al., 2024). Enhancing crop productivity through various direct and indirect mechanisms. Macroalgae extracts produce substances that inhibit pathogen progression, improve stress tolerance, and reduce oxidative damage in cells (Abdelaziz et al., 2024a). Algae also release compounds such as phenols and their oxidized derivatives, which are toxic to plant pathogens (Chan et al., 2022). Recent research highlights the potential of natural biological agents, including PGPR and *Ascophyllum nodosum*, in mitigating fungal diseases by activating plant defense mechanisms (Waller, 1999). This study aims to isolate and identify PGPR from soil and evaluate their effects, along with *Ascophyllum nodosum*, on the physiological processes of tomato plants infected with *F oxysporum*, focusing on photosynthetic pigments, metabolic indicators, and phenolic compounds.

MATERIALS AND METHODS

Isolation and molecular identification of PGPR

Soil samples were collected from the tomato-cultured soil in Eltal Elkbeer, Ismailia, Egypt (30°32'46.0"N 31°50'02.4"E). A 10-gram soil sample was mixed with 90 milliliters of sterile distilled water, followed by serial dilution (10^{-2} to 10^{-6}). Diluted samples (0.1mL) were plated on Nutrient Agar Medium and incubated at 35°C for 48 hours. PGPR isolates were tested for hydrocyanic acid production (HCN) using glycine-supplemented nutrient agar, with picric acid-treated filter paper detecting color changes (Trivedi et al., 2008). Indole-3-acetic acid (IAA) production was assessed calorimetrically (Leveau & Lindow, 2005). While siderophore presence was confirmed using the $FeCl_3$ test (Attia et al., 2025b). The 16S rRNA gene of the selected bacterial isolates was amplified using PCR with universal forward and reverse primers (Abbas et al., 2024).

Source of pathogen (*F. oxysporum*)

A highly virulent isolate of *F. oxysporum* RCMB (008 001) was obtained from RCMB, Al-Azhar University, and then it was recognized through a pathogenicity test.

Antifungal activity of the PGPR

The PGPR isolates *Bacillus subtilis* (Bs) and *Achromobacter insuavis* (Ai) were cultured in Nutrient broth (Sigma Aldrich) and incubated on a shaker at 35°C for 48 hours. After incubation, the cultures' filtrate was centrifuged at 20,000 rpm for 10min, and the supernatant was filtered through a 0.22µm microbiological filter.

Dual culture assay

The antifungal assay was performed on PDA medium. *F oxysporum* was inoculated on one side of the Petri plate. In contrast, bacterial PGBR was inoculated on the opposite side of the Petri plate at the same time, simultaneously. Three replicates were prepared for each strain, and plates inoculated only with *F oxysporum* were used as controls. Plates inoculated only with F oxysporum were used as controls. The plates were incubated at 28°C for 7 days. The mycogonistic activity was expressed by the formula: (R1-R2)/R1 × 100, where R1 and R2 were the *F oxysporum* growth in the control and the presence of the PGPR, respectively.

Experimental design

Three-week-old *Solanum lycopersicum* L. var. 023 were planted in 40 × 40cm pots containing a sand-clay mixture (1:3). The experiment was conducted in a greenhouse at Al-SALAM International, Egypt. Conditions were maintained at 22°C (day) and 18°C (night) with 70–85% humidity. Seedlings were irrigated regularly and left untreated for seven days. A randomized design with six replicates as follows: T1: Control healthy, T2: Control infected, T3: healthy seedlings treated with the Bs; T4: healthy seedlings treated with Ai; T5: healthy seedlings treated with Alga Mix®; T6: infected seedlings treated with Bs; T7: infected seedlings treated with Ai; T8: infected seedlings treated with Alga Mix®.

Disease symptoms and disease index

Disease indicators were recorded, and the severity of the disease, along with the protection percentage, was calculated according to Abbas et al. (2024). Using the following equation:

Protection% % = (A-B) / A × 100%.

where, A= PDI (Percent disease index) in diseased control plants, B= PDI in diseased-treated plants.

Biochemical defense indicators

Chlorophyll and carotenoid pigments were measured using a modified method by Attia et al.

(2021). Tomato Fresh leaves, weighing one gram, were finely chopped and ground in a mortar with 80% aqueous acetone (v/v) to extract the pigments. The resulting homogenate was then filtered using a Buchner funnel and Whatman No. 1 filter paper. The final volume was adjusted to 100mL with 80% acetone. The optical density of the plant extract was determined using a spectrophotometer at wavelengths of 470, 649, and 665nm.

The total soluble carbohydrate content in dried leaves was assessed using the anthrone method outlined by Irigoyen (Irigoyen et al., 1992). One gram of dried plant was mixed with phenol water (5mL of 2%) and trichloroacetic acid (10mL of 30%), left overnight, filtered, and adjusted to 50 ml. Ten milliliters of filtrate were shaken with activated charcoal, filtered, and combined with anthrone reagent (4mL). The mixture was heated, cooled, and the absorbance was measured at 620 nm. The measurement of total protein content in dried leaves was performed using the protocol by Lowry et al. (1951). One gram of dried plant powder was mixed with phenol (5 ml of 2%) and distilled water (10mL), left overnight, filtered, and adjusted to 50mL. The extract was combined with (50mL of 2% sodium carbonate in 0.1 N sodium hydroxide and 0.5g copper sulfate dissolved in 1% sodium potassium tartrate), then treated with 0.5mL of Folin reagent. Absorbance was measured at 750nm. The proline content in dry shoots was assessed using the method described in Bates (1973). The total phenol content in dry shoots was analyzed following the method outlined by Dai et al. (1993).

Determination of antioxidant indicators

The extraction and analysis of antioxidant enzymes (POD, PPO, SOD, and CAT) were performed as follows: Two grams of tomato leaves were homogenized in 10mL of 0.1M phosphate buffer (pH 6.8) and centrifuged at 2000 rpm for 20min. POD activity was measured by mixing 0.2mL enzyme extract with 2mL of 20mM pyrogallol, 5.8mL of 50mM phosphate buffer, and H_2O_2, recording absorbance at 470nm. PPO activity was assessed by incubating the enzyme extract with phosphate buffer and pyrogallol, stopping the reaction with 1mL of 5% H_2SO_4, and measuring absorbance at 430nm. For CAT, A reaction mixture with a total volume of 10mL was prepared by adding 40µL of crude enzyme extract to 9.96mL of H_2O_2-phosphate buffer (pH 7.0), which was composed of 0.16mL of 30% H_2O_2 dissolved in 100mL of 50mM phosphate buffer. The CAT activity was assessed by monitoring the change in H_2O_2 absorbance over 60sec at 250nm. For SOD, A 10mL reaction mixture was prepared, consisting of 3.6mL of distilled water, 0.1mL of enzyme extract, 5.5mL of 50mM phosphate buffer (pH 7.8), and 0.8mL of 3mM pyrogallol dissolved in 10mM HCl. The rate of pyrogallol reduction was monitored at 325nm. The method (Hu et al., 2004) was used to determine the amount of MDA in fresh pepper leaves. Fresh pepper leaves were tested for hydrogen peroxide H_2O_2 content (Mukherjee & Choudhuri, 1983). ANOVA analyzed all results, LSD test compared means, and Co-State assessed significance.

RESULTS

Screening of bacteria for plant growth promotion activity

HCN-producing PGPR are considered promising bio fertilizers that enhance plant growth by improving root and increasing nutrient availability. Siderophores are powerful bio fertilizers that enhance iron availability and stimulate plant defense, thus resulting in suppressing plant pathogens. IAA is a vital plant hormone that manages root stem development, and growth, which results in plant stress resistance. Twenty bacterial isolates (P1 to P20) were screened for HCN, siderophore, and IAA production. The most potent bacterial isolates were P1 and P19.

Antifungal activity of PGPR

P1 and P19 recorded antifungal activity against *F oxysporum* as shown in Figure 1. P1 more potency than P19; where P1 caused 33.33%, P19 resulted to 22.22%. Bs showed an identity of 99.79% to 100% and coverage of 99% to 100% with various strains of the same species, including one with GenBank accession number PQ199504, as shown in Figure 1. Additionally, The P191MS strain of Ai exhibited an identity of 99.72% to 100% and coverage of 99% to 100% with several strains of the same species, including the type of strain with GenBank accession number PQ203277 as shown in Figure 1.

Disease symptoms and disease index

As shown in Table 1, the disease index reached 90% due to infection by *F oxysporum*. The results indicated that *F oxysporum* impacts plant roots, causing yellowing, root rot, and eventually leading to plant death. Also highlights that treatments with Bs, Ai, and Alga Mix® significantly mitigated the severity of root rot caused by *F oxysporum* by 37.5%, 27.5%, and 30%, respectively. Moreover, these treatments improved plant resistance to the disease by 58.3%, 69.4%, and 66.6%, respectively (Table 1).

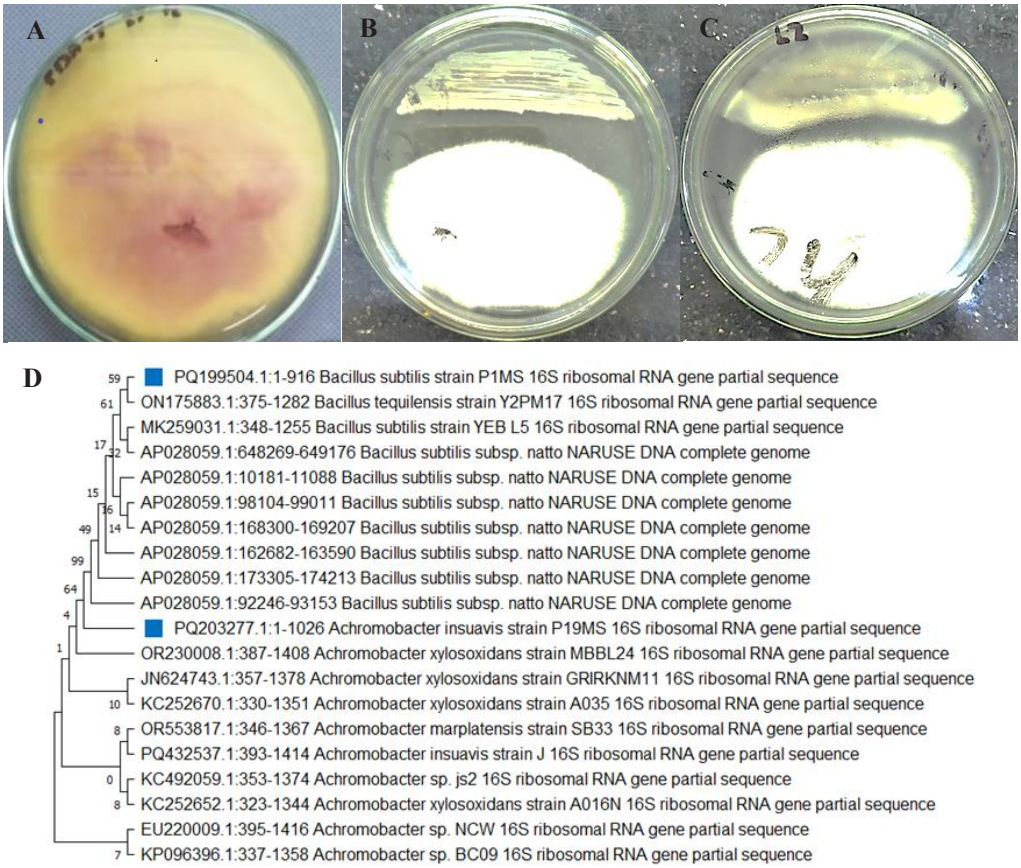

Figure 1. Dual culture of PGPR of *F. oxysporum*, where A) (control) *F. oxysporum*, B) *F. oxysporum* and P1, and C) *F. oxysporum* and P19, D) A phylogenetic tree of Bs with GenBank accession number PQ199504 and Ai with GenBank accession no. PQ0203277

Table 1. The impact of treatments on disease index and protection % of seedling infected with *F. oxysporum* wilt

Treatments	Disease symptoms classes					DI (%)	Protection (%)
	0	1	2	3	4		
Control infected	0	0	1	2	7	90	0
B. subtilis	3	3	1	2	1	37.5	58.3
A. insuavis	5	2	1	1	1	27.5	69.4
Alga Mix ®	4	3	1	1	1	30	66.6

Biochemical defense indicators

Photosynthetic pigments

Figure 2 shows that *F. oxysporum* infection significantly decreased chlorophyll a and b while increasing carotenoid content. In uninfected plants, Bs, Ai, and Alga Mix® boosted chlorophyll and carotenoids, with Alga Mix® being most effective. In infected plants, all treatments mitigated chlorophyll loss, with Alga Mix® increasing chlorophyll a and b by 107% and 128%, respectively, and enhancing carotenoids by 65%.

Total soluble protein

Results presented in Figure 3 indicated that *Fusarium oxysporum* infection significantly reduced total soluble protein content. In uninfected plants, treatments with Bs, Ai, and Alga Mix® increased protein levels, with Alga Mix® being the most effective in enhancing protein content, followed by Ai and Bs. For infected plants, all treatments mitigated the reductions in protein content caused by *F. oxysporum* infection. Alga Mix® was the most effective, increasing protein levels by 110%, followed by Bs with an increase of 54%, and Ai with a of 41%.

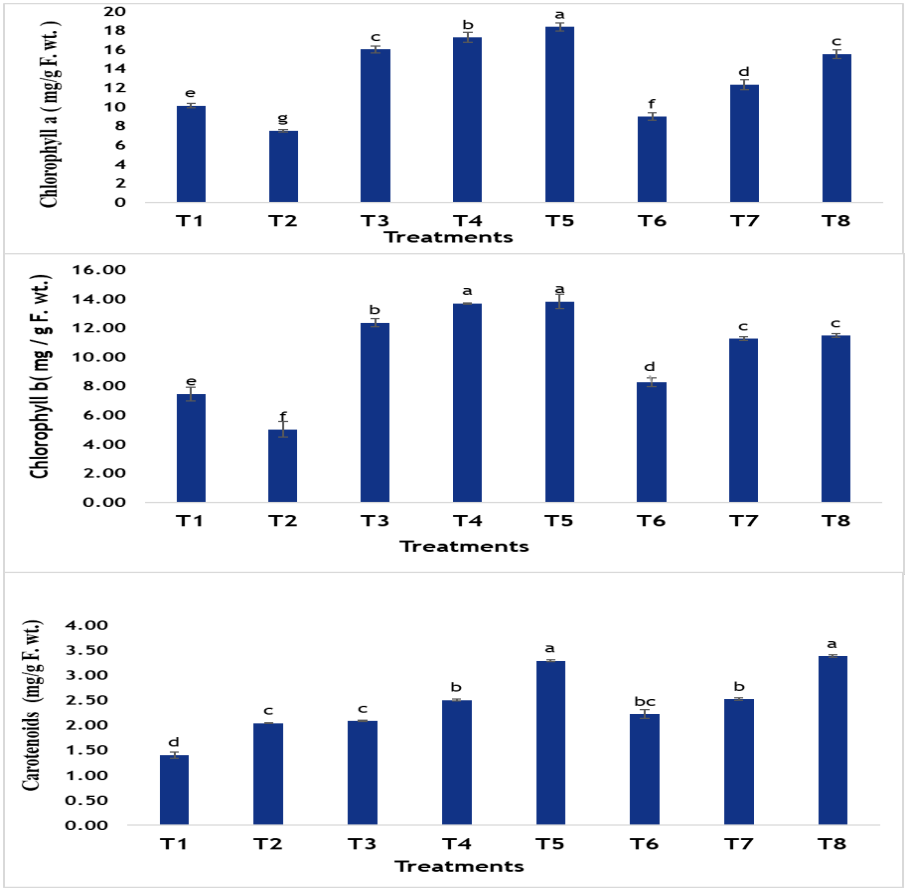

Figure 2. Effect of tested treatments on chlorophyll a , chlorophyll b , and carotenoid content in infected and uninfected pepper plants [T1: Control healthy, T2: Control infected, T3: Healthy seedlings treated with the Bs; T4: Healthy seedlings treated with Ai; T5: Healthy seedlings treated with Alga Mix®; T6: Infected seedlings treated with Bs; T7: Infected seedlings treated with Ai; T8: Infected seedlings treated with Alga Mix®. Different letters indicate significant statistical differences]

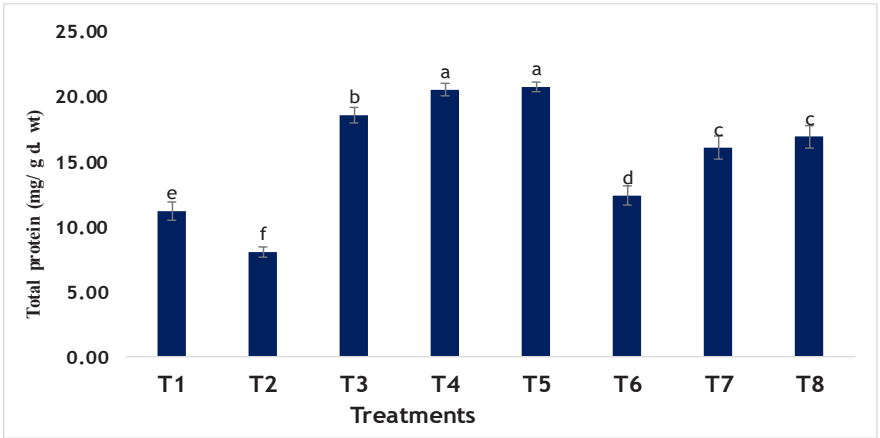

Figure 3. Effect of tested treatments on total protein in infected and uninfected pepper plants [T1: Control healthy, T2: Control infected, T3: Healthy seedlings treated with the Bs; T4: Healthy seedlings treated with Ai; T5: Healthy seedlings treated with Alga Mix®; T6: Infected seedlings treated with Bs; T7: Infected seedlings treated with Ai; T8: Infected seedlings treated with Alga Mix®. Different letters indicate significant statistical differences]

Total soluble carbohydrates

Figure 4 shows that *F. oxysporum* infection significantly reduced total soluble carbohydrates. In uninfected plants, Bs, Ai, and Alga Mix® increased carbohydrate content, with Alga Mix® being most effective. In infected plants, all treatments mitigated carbohydrate loss, with Alga Mix® increasing levels by 112%, followed by Ai (99%) and Bs (54%).

Free proline

Results depicted in Figure 5 showed that *F. oxysporum* infection significantly reduced free proline content. In uninfected plants, treatments with Bs, Ai, and Alga Mix® enhanced free proline levels. Among these, Alga Mix® was the most effective in increasing free proline content, followed by Bs and Ai. In infected plants, all treatments mitigated the reductions in free proline content caused by *F. oxysporum* infection. Alga Mix® was the most effective, increasing free proline levels by 187%, followed by Ai with an increase of 140%, and Bs with a rise of 31%.

Phenol content

As shown in Figure 6, *F. oxysporum* infection increased phenol content by 24% compared to the healthy control. Applying Bs, Ai, and Alga Mix® resulted in a significant increase in phenol content. In infected plants, Alga Mix® treatment caused a substantial rise in phenol content by 159%, followed by Ai with a 134.3% increase, and Bs with a 32% increase, compared to the untreated infected control.

Effect of treatments on Antioxidant enzymes

The results in Table 2 showed that *Fusarium*-infected plants exhibit significantly higher antioxidant enzymatic activity to healthy controls. The application of biocontrol agents and Alga Mix® further enhanced the activity of these enzymes, particularly in infected plants. Among the treatments, Alga Mix® exhibited the highest peroxidase (1.93 unit/ g. f. wt. /hour), and polyphenol oxidase (2.03 unit/ g. f. wt. /hour) activity, while Bs treatment led to the highest superoxide dismutase activity (1.12 unit/ g. f. wt. /hour), and Catalase (1.27 unit/ g. f. wt. /hour).

Effect of treatments on MDA and H_2O_2

The results in Table 3 indicated a significant increase in oxidative stress markers in Fusarium-infected plants, with MDA (11.21mg/100 g) and H_2O_2 (1.46mg/g) levels being the highest compared to all treatments. Applying biocontrol agents and Alga Mix® significantly reduced oxidative damage, with Alga Mix®-treated plants showing the lowest MDA (6.12 mg/100 g) and H_2O_2 (0.52mg/g) levels in healthy plants. In infected plants, Bs treatment mitigated *Fusarium*-induced oxidative stress through MDA and H_2O_2 levels of 9.42mg/100 g and 1.27mg/g, respectively, indicating a protective role against oxidative stress.

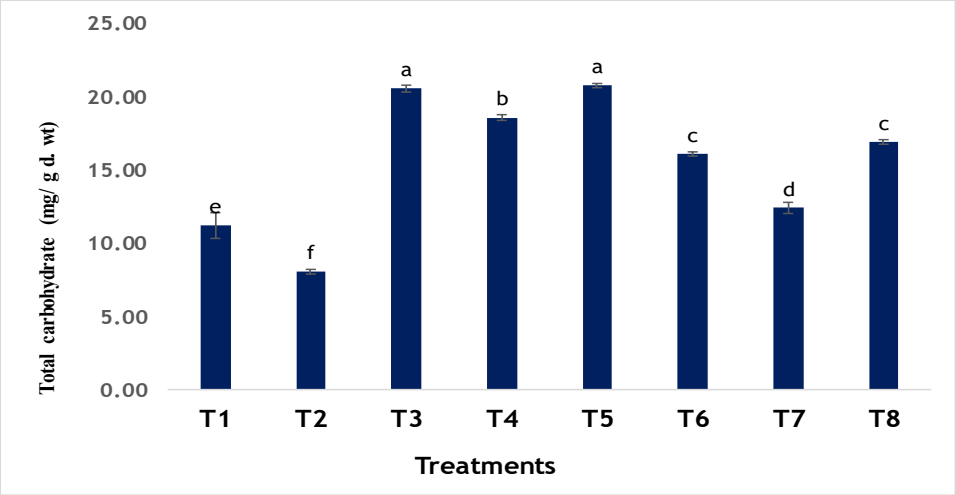

Figure 4. Effect of tested treatments on total carbohydrates in infected and uninfected pepper plants [T1: Control healthy, T2: Control infected, T3: Healthy seedlings treated with the Bs; T4: Healthy seedlings treated with Ai; T5: Healthy seedlings treated with Alga Mix®; T6: Infected seedlings treated with Bs; T7: Infected seedlings treated with Ai; T8: Infected seedlings treated with Alga Mix®. Different letters indicate significant statistical differences]

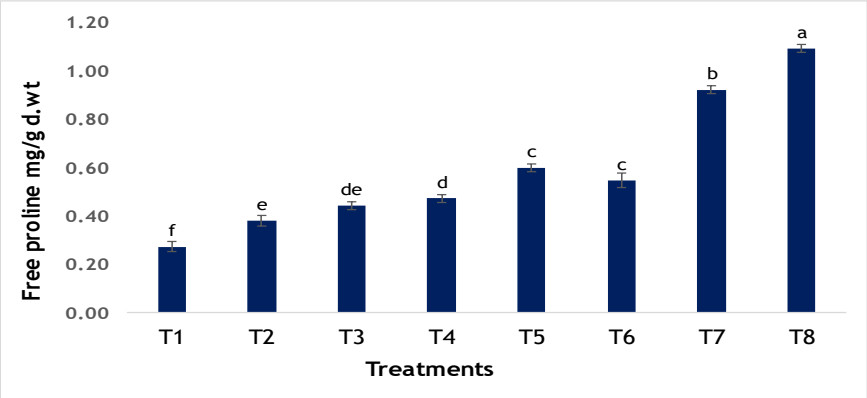

Figure 5. Effect of tested treatments on free proline in infected and uninfected pepper plants [T1: Control healthy, T2: Control infected, T3: Healthy seedlings treated with the Bs; T4: Healthy seedlings treated with Ai; T5: Healthy seedlings treated with Alga Mix®; T6: Infected seedlings treated with Bs; T7: Infected seedlings treated with Ai; T8: Infected seedlings treated with Alga Mix®. Different letters indicate significant statistical differences]

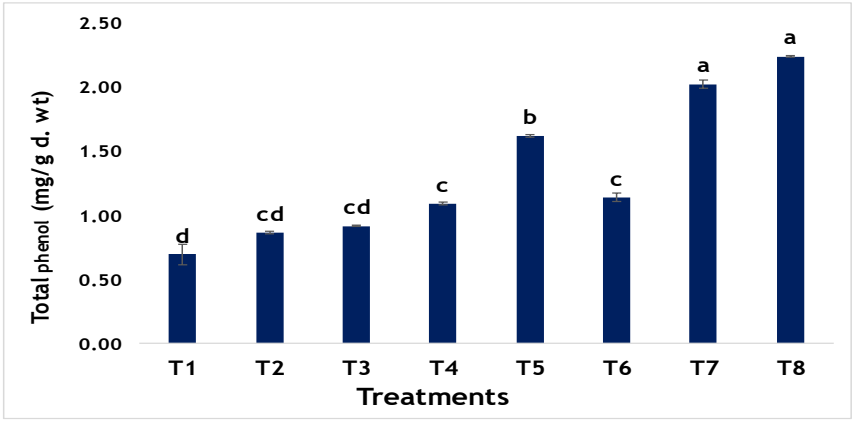

Figure 6. Effect of tested treatments on phenols in infected and uninfected pepper plants [T1: Control healthy, T2: Control infected, T3: Healthy seedlings treated with the Bs; T4: Healthy seedlings treated with Ai; T5: Healthy seedlings treated with Alga Mix®; T6: Infected seedlings treated with Bs; T7: Infected seedlings treated with Ai; T8: Infected seedlings treated with Alga Mix®. Different letters indicate significant statistical differences]

Table 2. Effect of biological agents and algal extract on antioxidant enzymes

Treatments	POD (unit/ g. fresh wt./hour)	PPO (unit/ g. fresh wt./ hour)	SOD (unit/ g. fresh wt./hour)	CAT (unit/ g. fresh wt./ hour)
T1	0.63± 0.03 ^{h}	0.73± 0.01 ^{h}	0.28± 0.02 ^{h}	0.26± 0.01 ^{h}
T2	1.23± 0.03 ^{d}	1.57± 0.08 ^{d}	0.62± 0.01 ^{d}	0.50± 0.005 ^{d}
T3	0.94± 0.02 ^{f}	1.12± 0.04 ^{f}	0.54± 0.05 ^{e}	0.42± 0.01 ^{e}
T4	0.88± 0.04 ^{g}	1.03± 0.069 ^{g}	0.46± 0.03 ^{f}	0.39± 0.002 ^{f}
T5	1.02± 0.15 ^{e}	1.35± 0.16 ^{e}	0.37± 0.01 ^{g}	0.30± 0.002 ^{g}
T6	1.85± 0.026 ^{b}	1.82± 0.06 ^{b}	1.12± 0.02 ^{a}	1.27± 0.01 ^{a}
T7	1.67± 0.05 ^{c}	1.76± 0.09 ^{c}	0.93± 0.02 ^{b}	1.16± 0.003 ^{b}
T8	1.93± 0.17 ^{a}	2.03± 0.11 ^{a}	0.74± 0.02 ^{c}	0.89± 0.04 ^{c}
LSD at 5%	0.0499	0.0413	0.0470	0.0196

- T1: Control healthy, T2: Control infected, T3: Healthy seedlings treated with the Bs; T4: Healthy seedlings treated with Ai; T5: Healthy seedlings treated with Alga Mix®; T6: Infected seedlings treated with Bs; T7: Infected seedlings treated with Ai; T8: Infected seedlings treated with Alga Mix®.
- Different letters indicate significant statistical differences

Table 3: Effect of biological agents and algal extract on MDA and H_2O_2

Treatments	MDA mg/g. f. wt.	H_2O_2 mg/g. f. wt.
T1	4.24± 0.55 [g]	0.33± 0.02 [g]
T2	11.21± 0.09 [a]	1.46± 0.04 [a]
T3	7.48± 0.36 [d]	0.84± 0.22 [d]
T4	6.93± 0.13 [e]	0.67± 0.06 [e]
T5	6.12± 0.55 [f]	0.52± 0.48 [f]
T6	9.42± 0.48 [b]	1.27± 0.15 [b]
T7	8.54± 0.22 [c]	1.11± 0.36 [c]
T8	7.95± 0.13 [d]	0.88± 0.48 [d]
LSD at 5%	0.5425	0.0697

- T1: Control healthy, T2: Control infected, T3: Healthy seedlings treated with the Bs; T4: Healthy seedlings treated with Ai; T5: Healthy seedlings treated with Alga Mix®; T6: Infected seedlings treated with Bs; T7: Infected seedlings treated with Ai; T8: Infected seedlings treated with Alga Mix®.

- Different letters indicate significant statistical differences

DISCUSSION

Tomato plants are vulnerable to *F. oxysporum*, causing severe root rot and yield loss. This study assessed Bs, Ai strain, and Alga Mix® for disease control. In vitro tests showed Bs and Ai inhibited *F. oxysporum* growth by 33.33% and 22.22%, respectively. Our results can be explained by Mardanova et al. (2016) recording the anti-fusarial activity of Bs by producing hydrolytic enzymes, siderophore, ammonia, and HCN. The present results demonstrated the high virulence of *F. oxysporum*, with a disease index (DI) reaching 90% in untreated control plants. Applying Bs, Ai, and Alga Mix® significantly reduced disease severity and increased protection percentages. Bs reduced DI by 37.5% and provided 58.3% protection. The observed reductions in disease severity underscore the potential of Bs as a biocontrol agent for managing *F. oxysporum*. Bs produces a range of antimicrobial compounds, including lipopeptides that directly inhibit the growth of *F. oxysporum* by disrupting fungal membranes (Dimkić et al., 2022). Bs and Ai effectively compete with pathogens for essential nutrients and space in the rhizosphere, limiting the resources available for pathogen survival (Attia et al., 2023). These include improving nutrient uptake, enhancing stress-related metabolite production, and priming systemic defenses to counteract pathogen invasion (Zarraonaindia et al., 2023). Similar levels of disease reduction by *Bacillus subtilis* have been reported in tomato by Wu et al. (2021), who recorded a 60–70% reduction in Fusarium wilt incidence through the production of iturin and fengycin. Likewise, the partial efficacy of *Achromobacter* strains in disease suppression was noted by Al Daghari (2023), although our study is among the first to document its specific role against *F. oxysporum* in tomato. The present study demonstrated that *F. oxysporum* significantly reduced chlorophyll a and b levels while increasing carotenoid content. Alga Mix® demonstrated the highest efficacy, increasing chlorophyll a and b levels by 107% and 128%, respectively. Ai and Bs also improved chlorophyll levels to a lesser extent, with Ai increasing chlorophyll b by 124% and Bs contributing a 64.3% rise in the same pigment. Alga Mix® led with a 65% increase in carotenoids, followed by Bs at 58%. These findings align with studies indicating that PGPR and bio-inducers combat pathogens and strengthen photosynthetic efficiency by promoting chlorophyll biosynthesis and reducing oxidative stress (Sun et al., 2024). Restoration of chlorophyll content following treatment is a marker of improved photosynthetic efficiency and membrane stability, both of which are compromised under Fusarium infection. The observed pigment recovery supports the findings of Hoeger et al. (2021), who reported similar effects of seaweed-based biostimulants on stressed plants. Soluble proteins are critical components for plant stress responses, serving as building blocks for enzymes and structural proteins involved in repair and growth processes (Chanthini et al., 2024). This improvement likely results from the activation of stress-responsive pathways, including those involved in protein biosynthesis and turnover (Abbas et al., 2024). *Fusarium*-infected plants showed higher

peroxidase (1.23 unit/g), polyphenol oxidase (1.57 unit/g), superoxide dismutase (0.62 unit/g), and catalase (0.50 unit/g) compared to healthy plants. These findings align with previous studies suggesting that *Fusarium* attack induces oxidative stress, enhancing enzymatic responses (Farrag et al., 2017). Bs, Ai, and Alga Mix® significantly boosted enzyme activity in healthy and infected plants. Alga Mix® exhibited the highest peroxidase (1.93 unit/g) and polyphenol oxidase (2.03 unit/g) activity, likely due to its bioactive compounds enhancing plant immunity (Abdelaziz et al., 2024a). The strong efficacy of Alga Mix® is likely due to its complex composition of alginates, laminarins, betaines, and micronutrients, which are known to activate the salicylic acid and jasmonic acid defense pathways (Shukla et al., 2019; Attia et al., 2025a). These molecules not only induce systemic resistance but also enhance antioxidant enzyme activities, which was evident in our study. Bs treatment exhibited the highest SOD (1.12 unit/g) and CAT (1.27 unit/g) activity, reducing oxidative stress in infected plants. *Fusarium*-infection increased lipid peroxidation and H_2O_2 accumulation, with MDA levels rising from 4.24mg/100g in healthy plants to 11.21mg/100g in infected plants, indicating severe oxidative damage (Sharma et al., 2012). The elevation of peroxidase and polyphenol oxidase activities indicates the activation of the phenylpropanoid pathway, which strengthens the plant's cell walls against pathogen penetration (Manguro et al., 2006). Meanwhile, increased SOD and CAT levels reflect an active ROS detoxification system, minimizing cellular damage during infection (Abdelaziz et al., 2024a). The integration of PGPR and bio-based stimulants aligns with sustainable disease management strategies that reduce reliance on chemical fungicides and minimize environmental impact (Mendes et al., 2013). This supports the global transition toward eco-friendly agriculture (Attia et al., 2020).

CONCLUSION

This study provides clear evidence that integrating plant growth-promoting rhizobacteria (PGPR) and bio- products like Alga Mix® can effectively support tomato plants in coping with *Fusarium* wilt. Rather than only suppressing the pathogen, these biological agents enhance the plant's immune systems and physiological responses. The findings underscore the potential of sustainable, environmentally safe solutions that align with modern agricultural practices aiming to reduce chemical inputs. In particular, the synergistic use of microbial biocontrol agents and algal extracts emerges as a strategic approach for strengthening plant immunity, reducing oxidative stress, and fostering healthier, more productive crops under disease pressure.

Acknowledgments: The authors thank the Botany and Microbiology Department, Faculty of Science, Al-Azhar University, for their support—special thanks to Eng. Mahmud M. Elsayed for his assistance. Appreciation is also extended to Al-SALAM International. The authors are also grateful to Hydro Fert company, Via dei Fornai, Barletta BT, Italia.

Competing interests: All authors proclaim that there is no conflict of interest.

Authors' contributions: Conceptualization: Mohamed S. Attia, Salah M. Elsayed, Amer M. Abdelaziz and Mustafa A. Nouh, Methodology: Mohamed S. Attia, Salah M. Elsayed, Amer M. Abdelaziz and Mustafa A. Nouh, Mohamed M.Ali, Maryam M .Elsayed; Formal analysis and investigation: Mohamed S. Attia, Salah M. Elsayed, Amer M. Abdelaziz and Mustafa A. Nouh; Writing - original draft preparation: Mohamed S. Attia, Salah M. Elsayed, Amer M. Abdelaziz and Mustafa A. Nouh; Writing - review and editing: Mohamed S. Attia, Salah M. Elsayed, Amer M. Abdelaziz, Mustafa A. Nouh; Resources: Mohamed S. Attia, Salah M. Elsayed, Amer M. Abdelaziz, Mustafa A. Nouh; Supervision: Mohamed S. Attia.

Availability of data and material: All data were included within the manuscript.

Ethics approval and consent to participate: not applicable.

Funding: not applicable.

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