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Table: -, Figs : 16

Impact of endemic PGPR isolates on greenhouse tomato (*Solanum lycopersicum* Mill.) cultivation

R. Abdallah [*], S. Messgo-Moumene, D. Saddek [1,] and R. Boukhalfa[2]

Laboratory of Research on Medicinal and Aromatic Plants, University of Blida1,
BP 270 Soumaa road, Ouled Yaich, 09100, Blida, Algeria.
E. mail : raounek_a@yahoo.com, moumene_saida@yahoo.fr

ABSTRACT

The study aimed to test the application of some endemic PGPR isolates on the cultivation of tomato hybrid variety "Tsarine" to improve its growth, production and fruit quality. We used 12 endemic bacterial PGPR isolates of *Pseudomonas* genus, from these bacterial suspensions were prepared and sprayed on seeds, then irrigated the young seedlings separately with each prepared bacterial suspension until the flowering.

The isolate AC6 stimulated plant height and leaf biomass. The isolates AC1, AC2, and AC7, influenced the fruit weight and size. Furthermore, isolate AC8 by increased the sugar content (116.37 ±0.08 mg/ml) unlike other isolates, which reduced it also influenced the fruits secondary metabolite contents. The isolate AC9 induced the highest content of polyphenols (368±130 µg GAE/mg), flavonoids (12.08±0.015µg QE/mg), and flavonols (3.655±1.11µg QE/mg). The isolates AC11 (3.75 mg/100g) and AC9 (1.65 mg/100g) improved the fruits antioxidant power and fruit vitamin C content. Thus, inoculation with isolates "AC11, AC9, AC2, AC7, AC1" could be recommended for better tomato crop performances.

Keywords: Endemic bacterial PGPR, greenhouse cultivation, phytostimulants, secondary metabolites, *Solanum lycopersicum* Mill, Tomato

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is most consumed vegetable crop worldwide (59). Tomato fruit quality depends on colour, size, shape, flavour, storage capabilities, sugar content, vitamin C, minerals, organic acids, amino acids, flavonoids, phenolic compounds, and lycopene (26,46,54). It would be judicious to seek new biological alternatives to harmful agrochemicals for sustainable agriculture (33). The beneficial effects of applied phytostimulants on crops, has provided a potential alternative for better crop quality and yield (24,29). Phytostimulants are divided into two groups: (i). Plant-based and (ii). Microbial stimulants; the last one includes PGPR rhizobacteria with multiple agronomic potentials (35). They stimulate plant growth, improve crop quality and yield, enhance nutrients absorption and increase plant tolerance to abiotic stresses and control phytopathogenic agents, (24,37,45). Hence, this study aimed to evaluate the agronomic potential of some endemic bacterial PGPR isolates of *Pseudomonas* spp. genus on tomato (*Solanum lycopersicum* Mill.). Based on the results, some bacterial isolates were selected as bio-inputs for sustainable and organic tomato agriculture.

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[*]Correspondence author, [1]National Plant Protection Institute, Hacen Badi road El Harrach, Algeria.
[2]Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, 70125 Bari, Italy.

MATERIAL AND METHODS

The experiment was done from end of March 2022 to July 2022 to evaluate the selected study parameters (2) in greenhouse, Department of Biotechnology and Agroecology, SNV Faculty, University of Blida 1 (Algeria), west Algiers (Altitude: 229 m, Latitude: 36.4833, Longitude: 2.83333 36° 28′ 60″ North, 2° 49′ 60″ East). During the experimental period, the minimum temperature was 12° C and a maximum of 26° C, annual rainfall: 641 mm.

We used two biological materials: (i). Plant material and (ii). Microbial material. The plant material was certified seeds of tomato F1 hybrid variety "Tsarine," while the microbial materials were 12-endemic bacterial isolates of *Pseudomonas* spp. (obtained from Prof. Moumene's bacterial collection, Laboratory of Research on Medicinal and Aromatic Plants). These bacteria were part of university research training project (D00L05UN09012210001).

Preparation of bacterial inocula

We prepared a series of preparations of microorganism's suspension. The twelve bacterial isolates were used for seed inoculation and to irrigate young seedlings obtained in pure cultures on King B medium, incubated at 30 °C (12-18 h old). After appropriate dilutions their optical density was measured at 600 nm and adjusted to $1x10^8$ cells/ml concentration (2).

Agronomical potential of PGPR isolates on tomato crop

(i). **Petri-plate bioassay**: Tomato seeds (*Solanum lycopersicum* Mill.) were first sterilized using 2 % sodium hypochlorite solution for 5 min, then 70 % ethanol (v/v) for 10 min, followed by four rinses with distilled water. The sterilized and inoculated seeds were treated separately with each of the 12 bacterial cultures ($1x10^8$ cells/mL concentration) for 30 min. Ten inoculated seeds of tomato were sown in Perti dishes lined with two layers of Whatman filter paper and irrigated with 5 ml distilled water. Each treatment was replicated thrice in complete randomised design. All Petri dishes were placed in dark at 24 °C (32) for 6-days. Thereafter, number of germinated seeds and their radicle length were determined. Germination rates and vigour index (VI) were calculated as under (1):

Germination (%) = (Number of germinated seeds/Total number of seeds) X100

Vigour Index = % Sg X Sl

Where*:* % Sg: Seedling germination rate (%); Sl: Seedling length (cm)

(ii). **Pot culture**: The cultivation was done in pots in greenhouse. Firstly, tomato seeds were inoculated separately with each of the 12 formulated bacterial inocula ($1x10^8$ cells/mL concentration). Then seeds were sown in individual nursery trays filled with peat. The trays were regularly watered with tap water. In addition, for inoculation, the young seedlings were subsequently transferred directly into 10 cm diameter pots, with substrate (mixture of 2/3 soil and 1/3 commercial peat). They were inoculated by watering them with 20 mL of each bacterial suspension from each of the 12 bacterial inocula ($1x10^8$ cells/mL concentration). This inoculation was done once every 15 days and repeated thrice. Four replicates were done for each studied bacterial inoculum and negative control. Seedling were watered daily, as needed. The prepared pots were placed in greenhouse in randomized design. After 2 months, the plantlets were transferred into new pots (15 kg capacity) and regularly watered with tap water.

Growth and yield of tomato plants

After 4-months, growth parameters (plant height, cm number of leaves per plants) were recorded. Additionally, yield parameters (number of fruits per plant, weight of individual fruits i.e yield per plant) were also recorded.

Plant Physiological Parameters

Leaf Pigment Content

Chlorophyll and carotenoid contents were determined at the vegetative stage as per Lichtenthaler (1987). Briefly, 0.5 g freshly harvested leaf sample were grinded with 10 mL 80 % acetone. The mixture was transferred to 25 mL volumetric flask then filtered. The absorbance of supernatants was measured using a UV-VIS spectrophotometer. Chlorophyll and carotenoid contents were calculated as under (31):

$$\text{Chla (mg.g-1 fw)} = 12.25\ A663.2 - 2.79\ A646.8$$
$$\text{Chlb (mg.g-1 fw)} = 21.50\ A646.8 - 5.10\ A663.2$$
$$\text{Chla+b (mg.g-1 fw)} = 7.15\ A663.2 + 18.71\ A646.8$$
$$\text{Cx+c (mg.g-1 fw)} = (1000\ A470 - 1.82\ \text{Chla} - 85.02\ \text{Chlb})/198$$

Physico-chemical parameters of cultivated tomatoes

Physical parameters: For each sample the physical parameters (water, dry matter, mineral matter or ash contents, organic matter and pH). were evaluated asper methods of AOAC (1975) and Rouphael *et al*. (2021) respectively (45).

Chemical parameters

The chemical parameters include sugar content and secondary metabolites.

(I). **Sugar content**. It was determined in tomato fruit juice as per Dubois *et al*. (1956). For each sample, 2 mL fruit juice was mixed with 1 mL 5 % phenol solution and 5 mL 10 % diluted sulfuric acid. The tubes were incubated at 30 °C for 10 min. The absorbance of solutions was measured at 485 nm using a UV-Vis spectrophotometer. Sugar concentration was calculated from a glucose calibration curve ($y = 0.0248x + 0.0003$) (16).

(II). **Secondary metabolites**. Their content includes total polyphenols, flavonoids, flavanols and vitamin C. Total polyphenol content was determined according to the Folin-Ciocalteu method (51), adapted for 96-well microplate assay, as per Muller *et al*. (2010). For each sample, 20 µL extract was mixed with 100 µL diluted Folin-Ciocalteu reagent (1:10) and 75 µL 7.5 % Na_2CO_3 solution. The mixture was incubated in dark for 2 h. Absorbance was measured at 765 nm using a microplate reader (Perkin Elmer, Enspire) (52,35). Total polyphenols concentration was calculated from the gallic acid calibration curve ($y = 0.0075x + 0.0289$). Results were expressed in µg of gallic acid equivalent per mg of fresh weight.

(i). **Total flavonoids.** The content was evaluated using a colorimetric method (55) for 96-well microplate analysis. For each sample, 50 µL extract was mixed with 130 µL methanol (MeOH), 10 µL CH_3COOH and 10 µL Al $(NO_3)^2$, $9H_2O$. After 40 min incubation at room temperature, absorbance was measured at 415 nm using a UV-VIS spectrophotometer. Flavonoid concentration was calculated for each sample using the quercetin calibration curve ($y = 0.2953x + 0.252$). Results were expressed in µg of quercetin equivalents per mg of fresh weight.

(ii). **Flavonols:** The content was evaluated using the aluminum trichloride (AlCl3) colorimetric method, with absorbance measured using a microplate reader (Perkin Elmer, Enspire). To determine total flavonol content, 50 µL extract was mixed with 50 µL $AlCl_3$ and 150 µL $C_2H_3NaO_2$. After incubation in darkness for two and a half hours, absorbance was measured at 440 nm using a UV-Vis spectrophotometer (30). Sample concentration was calculated using the quercetin calibration curve ($y = 0.2953x + 0.252$) and results were in µg of quercetin equivalents/ mg FW.

(iii). **Vitamin C**: Its assay requires following preparations:

(a). Ascorbic acid standard was prepared using 500 mg USP reference standard. The mixture was diluted with 30 ml diluent and sonicated for 15 min.

(b). Standard preparations of 0.01 %, 0.002 %,0.004 %,0.006 % and 0.008 % concentrations were also prepared.

(c). Diluent preparation was done by weighing 0.56 g disodium ethylenediaminetetraacetate dihydrate and 2.04 g monopotassium phosphate. The volume was made 1000 ml with distilled water.

(d). In each sample (0.5 g), 30 ml diluent was added and shaked for 20 min, passed through 0.45 µm porosity membrane filter into an Erlenmeyer flask, discarding the first 5 ml.

(e). One ml of each filtered, treated and control fruit sample was pipetted into 50 ml volumetric flask and diluted to 80 ml volume with mobile phase. Samples were placed in vials and injected into HPLC for injection (6).

(iv). **Antioxidant activity:** It was evaluated by mixing 40 µL fruit sample with 60 µL methanolic solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) prepared at 0.04 mg/mL. After 30 min incubation in dark at room temperature, absorbance was measured at 517 nm with UV-VIS spectrophotometer. This method allows estimation of the extracts' capacity to scavenge the DPPH free radical, an indicator of their antioxidant potential (50). Results were expressed as inhibition (%) of DPPH radical and calculated according to the formula (53):

$$I (\%) = (A0-A1) \times 100/A0$$

Where: I (%): inhibition percentage, A0: blank absorbance, A1: sample absorbance

Statistical Analysis

Analysis of variance (ANOVA) was performed on all data. The significance of differences between means was evaluated by Tukey's test using MINITAB version 19 software, with a significance threshold of $P \leq 0.05$. Results are presented as mean ± standard deviation (43).

RESULTS AND DISCUSSION

Agronomical potential of PGPR isolates

(i). Germination rate

The effects of inoculation of PGPRs on tomato seed germination rate is presented in Figure 1. The results showed significant variations with applied treatments. The germination ranged from 40 % to 100 %. Tukey's test classified the treatments applied to the plants into only two distinct homogeneous groups based on tomato seed germination rate, with bacterial isolate AC1 showing the lowest percentage of 40 %.

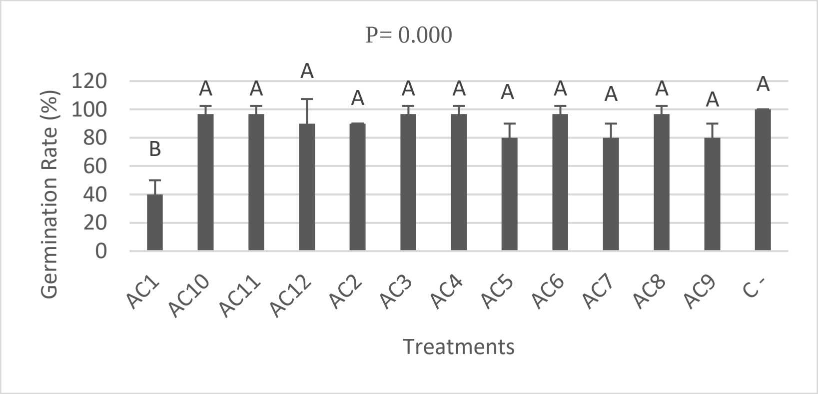


Figure 1. Effects of PGPR treatments. AC1, AC2, AC3, AC4, AC5, AC6, AC7, AC8, AC9, AC10, AC11, AC12: PGPR strains on Tomato seed germination rates, C: Negative control. Data are means of 3-replicates. In each treatment, column with different letters is significantly different ($p \leq 0.05$, Tukey's test).

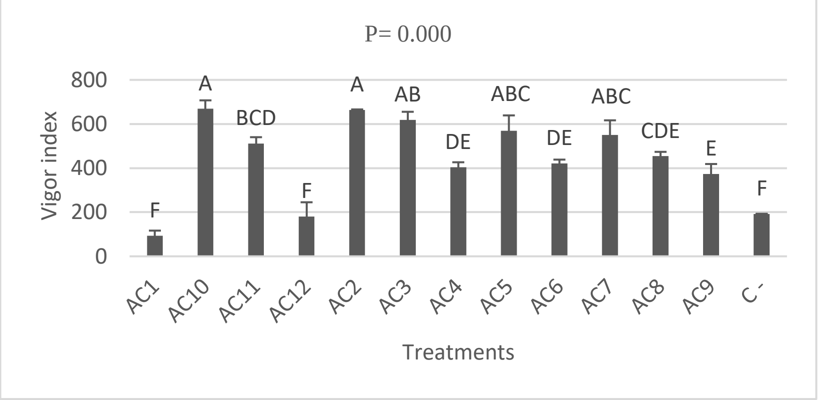


Figure 2. Effects of PGPR treatments. AC1, AC2, AC3, AC4, AC5, AC6, AC7, AC8, AC9, AC10, AC11, AC12: PGPR strains on Tomato seed vigour index, C: Negative control. Data are means of 3-replicates. In each treatment, column with different letters is significantly different ($p \leq 0.05$, Tukey's test)

(ii). Vigour Index

The vigour index of tomato seeds showed significant variations (92.9 to 668.9) with applied treatments (Figure 2). The two isolates AC10 and AC2 demonstrated the highest vigour index values of 668.9 and 663.3 respectively, while isolates AC12 and AC1 and the negative control, showed the lowest values.

Currently, interest in utilizing beneficial microorganisms in agriculture is rapidly expanding and could provide promising alternatives to chemical fertilizers by promoting plant growth, resistance, and tolerance to various stresses (18). Among these microorganisms, rhizobacteria, known as PGPR, such as *Pseudomonas* spp. and *Bacillus* sp., have demonstrated their ability to accelerate germination and improve tomato plant yield (18,56). PGPR bacteria or their products have been commercialized in many countries worldwide, as Amase®, manufactured by Lantmännen BioAgri in Sweden and formulated with *Rhizobium*, *Azotobacter*, *Pseudomonas*, *Bacillus* and *Chaetomium* isolates for growth stimulation, rapid development of a substantial and vigorous root system, and increased stress tolerance in cucumber, lettuce, tomato, pepper, eggplant, cabbage and broccoli crops and PGA® (manufactured by Organica technologies in the USA) formulated with *Bacillus* sp. for fruits and vegetables to enhance biomass accretion and stress tolerance (24). Zhaoyu et *al* (60) showed that treatment with *Bacillus velezensis* FX-6 promoted tomato seed germination, with germination rates exceeding 90 %, which was higher than control. It growth (plant height, stem thickness, taproot length and fresh weight), which were 1.57, 1.31, 1.26 and 1.69 times greater than control, respectively (60).

Growth and Yield Parameters of Tomato Plants

(i). Tomato Plant Height

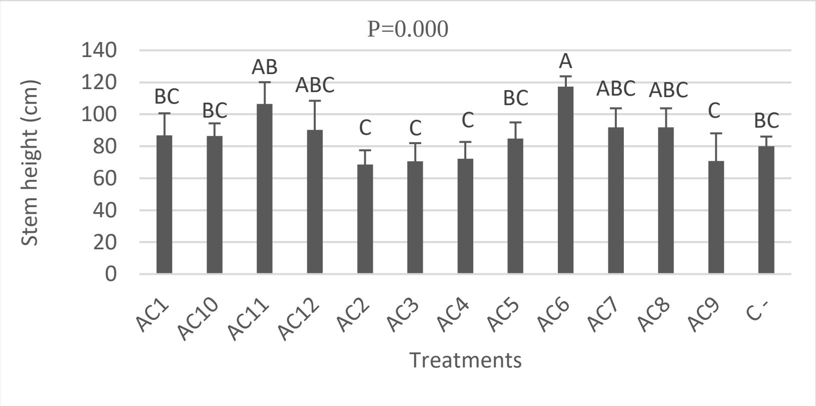


Figure 3. Effects of PGPR treatments. AC1, AC2, AC3, AC4, AC5, AC6, AC7, AC8, AC9, AC10, AC11, AC12: PGPR strains on Tomato plant height, C: Negative control. Data are means of 3-replicates. In each treatment, column with different letters is significantly different ($p \leq 0.05$, Tukey's test).

The results of tomato plant growth showed significant variations depending on the treatments applied, with heights ranging from 68.63 to 117.25 cm (Figure 3). Treatment with isolate AC6 proved the most effective in promoting tomato plant height, followed by isolate AC11, while isolates AC2, AC3, AC4, and AC9 showed limited effect among the tested treatments.

(ii). Number of leaves

Figure 4 illustrated the influence of PGPR inoculation on the number of leaves per plant, which showed significant results, with counts between 92.75 and 157.25 leaves per plant. Treatment with isolate AC6 in promoted leaf production, followed by AC7, while other treatments had intermediate or weak effects than the negative control.

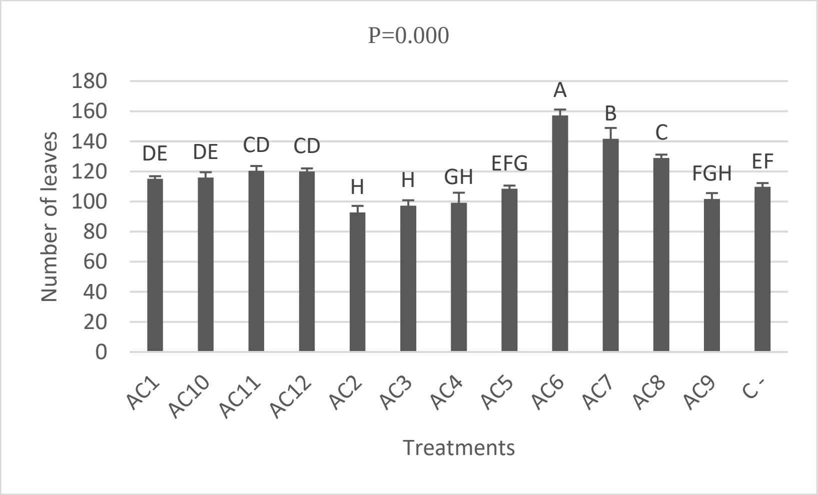


Figure 4. Effects of PGPR treatments. AC1, AC2, AC3, AC4, AC5, AC6, AC7, AC8, AC9, AC10, AC11, AC12: PGPR strains on Number of tomato leaves, C: Negative control. Data are means of 3-replicates. In each treatment, column with different letters is significantly different ($p \leq 0.05$, Tukey's test).

(iii). Fruits per plant

The applied treatments did not have a significant impact on the number of fruits produced, with recorded values varying between 1.5 and 4.75 fruits per plant, grouped into a single group according to Tukey's test. According to the results in Figure 5, fruit weight showed significant variability depending on the treatments applied, with a maximum weight of 51.328 g obtained with isolate AC2, followed by treatments AC1 and AC7, while AC9 and AC10 had the most limited effects on this parameter.

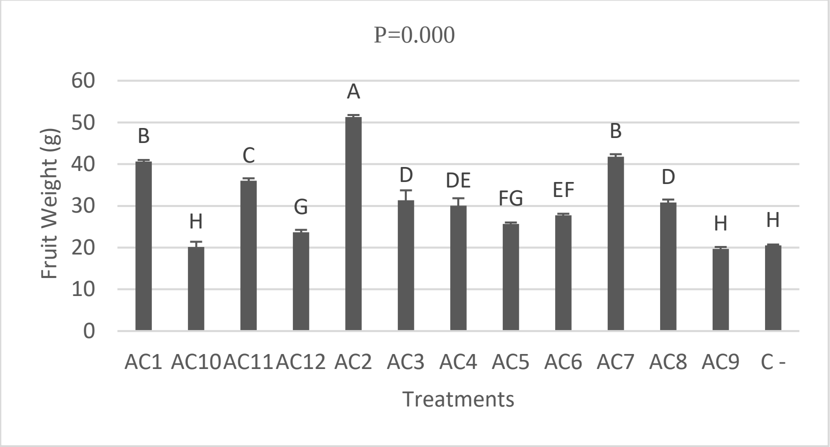


Figure 5. Effects of PGPR treatments. AC1, AC2, AC3, AC4, AC5, AC6, AC7, AC8, AC9, AC10, AC11, AC12: PGPR strains on Tomato plant fruit weight, C: Negative control. Data are means of 3-replicates. In each treatment, column with different letters is significantly different ($p \leq 0.05$, Tukey's test).

(iv). Morphological characteristics of fruits

The applied treatments did not impact the fruit length (11 and 15.33 mm), grouped into a single group according to Tukey's test. However, fruit diameter significantly varied (between 11.667 and 16.667 mm), depending on treatments applied, where treatment with bacterial isolate AC2 resulted in the highest diameter (Figure 6).

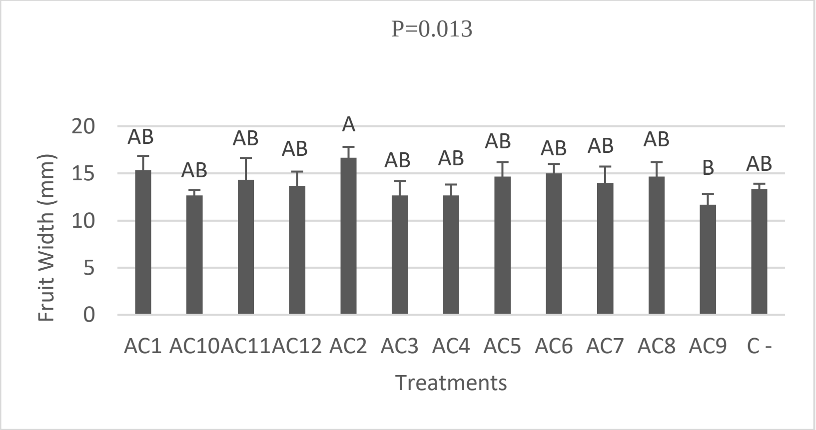


Figure 6. Effects of PGPR treatments. AC1, AC2, AC3, AC4, AC5, AC6, AC7, AC8, AC9, AC10, AC11, AC12: PGPR strains on Morphological characteristics of tomato fruits, C: Negative control. Data are means of 3-replicates. In each treatment, column with different letters is significantly different ($p \leq 0.05$, Tukey's test).

The beneficial effects of rhizobacteria on plant growth are achieved through direct and indirect mechanisms. Direct mechanisms are nitrogen fixation, phosphate and potassium solubilization, siderophore production and phytohormone production (Auxins, cytokinins, gibberellins, or ethylene) (33,44). PGPR of the genera *Arthrobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Microbacterium, Pseudomonas, Rhizobium, Rhodococcus* and *Serratia* may be used to increase phosphate solubilization and thus enhance growth and yields. They also reduce the effects of inhibited growth and plant development caused by numerous pathogenic microorganisms. Improved yields and fruit size would result from the facilitation of plant nutrition by PGPR via direct and indirect methods (12,34).

The seed inoculation with these bacterial isolates of genus *Pseudomonas* spp. and frequent irrigation of young seedlings with bacterial inoculum, significantly ($p < 0.05$) improved plant height, leaf number and fruit characteristics [(individual fruit weight (g) and fruit width (cm)] than non-inoculated controls. Current results highlighted the great capacity of *Pseudomonas* strains to promote plant growth, which has been reported in cereals, peppers and tomatoes (5,23,58). Research has also confirmed the promoting effect of *Bacillus* sp. in improving growth, biomass, and yield of lettuce by increased production of plant hormones (24).

Plant height has relationship with crop yield (25,54). Our results showed that the application of PGPR of the genus *Pseudomonas* spp. significantly increased plant height, due to the presence of substances promoting and improving plant growth processes, such as cell division and cell elongation (54). Our results are similar to Gashash *et al*. (18); inoculation of *Pseudomonas alcaligenes*, *B. subtilis* and *B. amyloliquefaciens* significantly improved the tomato plant height and leaf number; plant height increased to 117 cm after inoculation with the mixture of *B. subtilis* + *B. amyloliquefaciens* than non-inoculated plants (77 cm). Our results showed more leaves (61 leaves) after treatment with the mixture of *B. subtilis* + *B. amyloliquefaciens*. However, their results significantly improved the tomato fruit quantity (76 %) in treated plants, while fruit weight showed 33 % increase, and fruit diameter improved by 50 % compared to non-inoculated plants (18). This is due to the production of plant hormones such as gibberellins (GAs), which influences seed germination, flowering, stem elongation and fruit formation (3).

Similarly, Singh *et al*. (51) confirmed that *Pseudomonas* sp. and *Trichoderma harzianum* increased stem length, root length, and fresh and dry weight of plants. Other studies, by Cervantes-Vázquez *et al.* (10), showed that the PGPR inoculation (seed inoculation, spraying, or both) significantly influences several growth parameters such as plant height, stem diameter, root displaced volume, fresh and dry weight of leaves, stems and roots, and leaf surface area.

According to Widnyana (2019), the inoculation of tomato seeds in a suspension of *Pseudomonas alcaligenes* showed significant effects on leaf number (167.6 cm) and tomato plant height (114.1 cm) (57,56). In another study, inoculation with *Azotobacter* *chroococcum* in phosphorus-poor soil increased the dry weight of inoculated plants compared to non-inoculated plants. The increase in biomass of inoculated seedlings could be due to hormone production, nitrogen fixation, and phosphorus solubilization by PGPRs (20). *Bacillus pumilus* and *Pseudomonas pseudoalcaligenes* inoculation stimulated rice growth and production through phosphate solubilization and production of auxins, gibberellins, siderophores, and ACC utilization (24). *Azospirillum brasilense* produces various plant hormones (indole-3-acetic acid, abscisic acid, and gibberellins), they improved plant’s ability to absorb water and nutrients, showing a more significant effect when directly inoculated at the root level (13,36,47).

Our results align with those of Katsenios *et al*. (27), where treatment with *Bacillus subtilis* gave the highest fruit weight (93.77 g/fruit), followed by *Bacillus amyloliquefaciens* and *Bacillus licheniformis*. Additionally, results of Oancea *et al*. (37), confirmed an increase in tomato fruit weight and improvement in its dimensions when applying *Azospirillum lipoferum* DO12 and *Brevibacillus parabrevis* B50. Other similar results reported that using a combination of AMF and *Pseudomonas* (7,8) could increase tomato fruit weight and size. This increase is associated with signalling pathways that modify cell expansion (auxins) and sucrose synthase enzyme, which play a central role in tomato fruit development (10). The application of *Bacillus subtilis*, *B. amyloliquefaciens*, *B. licheniformis* and *Priestia megaterium* increased the average fruit weight per plant by 26.78 to 30.70 % compared to the control (27).

(v). **Plant Physiological Parameters**

Leaf Pigments Contents

The effects of PGPR inoculation on photosynthetic pigments of tomato plants is shown in Figure 7. Leaf pigment content revealed extremely significant variability in total chlorophyll content and carotenoid contents. This analysis highlighted marked differences in these pigment levels, emphasizing the impact of treatments on leaf pigment composition. The recorded contents ranged between 13.096 and 31.37 mg/ml of fresh weight for chlorophyll content. Among the treatments, isolate AC3 showed the highest chlorophyll content, followed by treatments with isolates AC1, AC2, and AC4, while isolates AC10 and AC11 showed the lowest chlorophyll content (Figure 7a). The carotenoid contents were 4.285 and 7.813 mg/ml of FW. Unlike chlorophyll, isolate AC10 increased the carotenoid, other treatments were lower than control (Figure 7b).

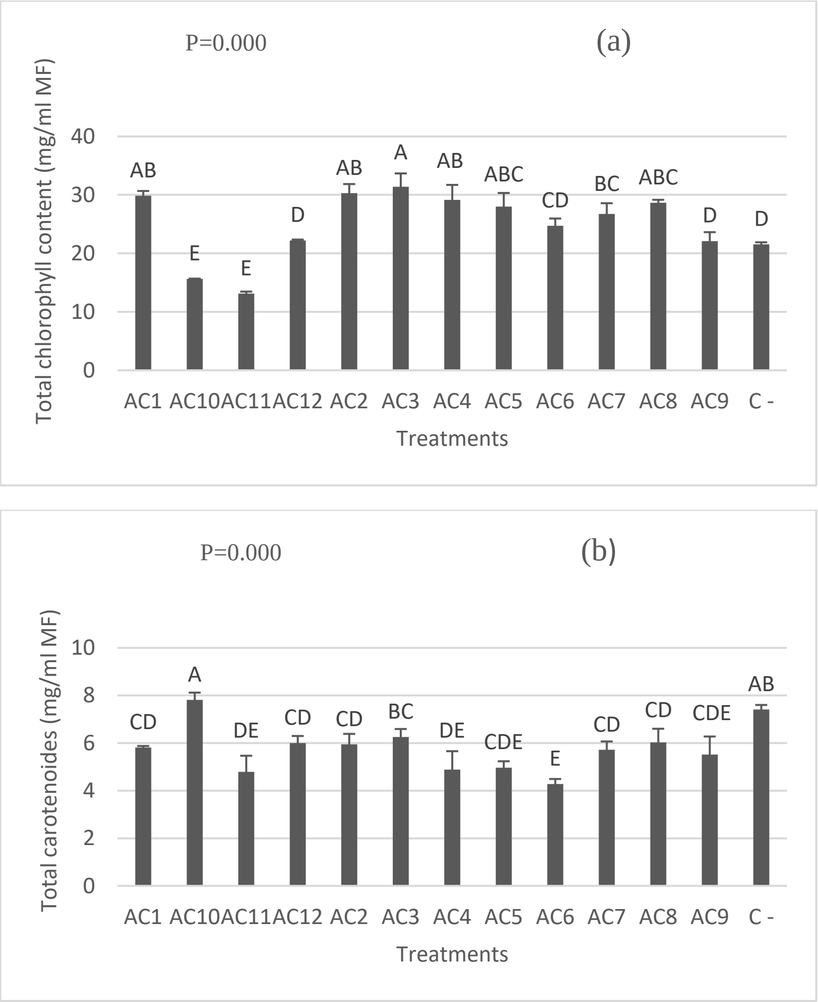


Figure 7. Effects of PGPR treatments. AC1, AC2, AC3, AC4, AC5, AC6, AC7, AC8, AC9, AC10, AC11, AC12: PGPR strains on Tomato Leaf pigment contents, C: Negative control. Data are means of 3-replicates. In each treatment, column with different letters is significantly different ($p \leq 0.05$, Tukey's test).

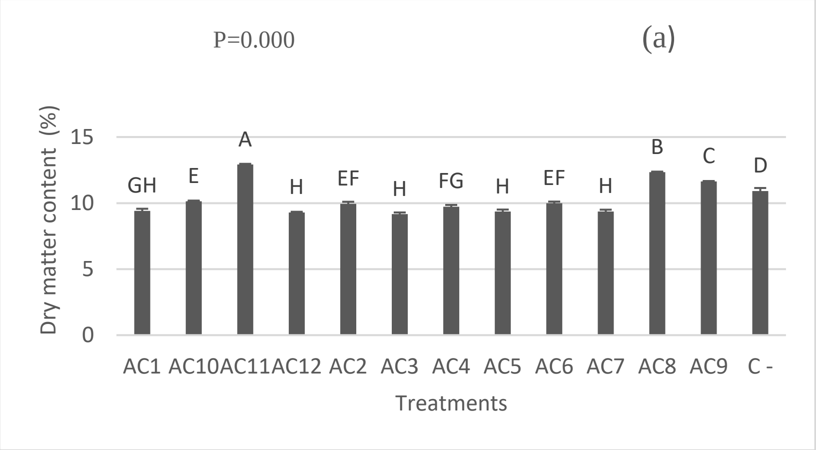
Leaf gas exchanges, particularly photosynthesis, constitute a potential indicator that determines the rate of assimilate production and the plant's capacity to produce good yield. According results, plants treated with PGPR of genus *Pseudomonas* spp. significantly stimulated the photosynthetic rate by improving total chlorophyll content and carotenoid content. These high levels are explained by changes in the photosynthetic mechanism and improvement in plant physiology, notably a reduction in stomatal closure, an increase in leaf surface area, and better maintenance of cellular hydration (54). Our results are similar to Gashash *et al*. (18), who showed that the combined application of *B. subtilis* and *B. amyloliquefaciens* significantly increased the total chlorophyll content (a + b) and carotenoids in tomato leaves (1600 and 1200 µg/g FW, respectively), indicating an increase in photosynthetic activity of (18). The application of *Streptomyces thermocarboxydus* increased total chlorophyll content in tomato plants than than uninoculated controls (41).

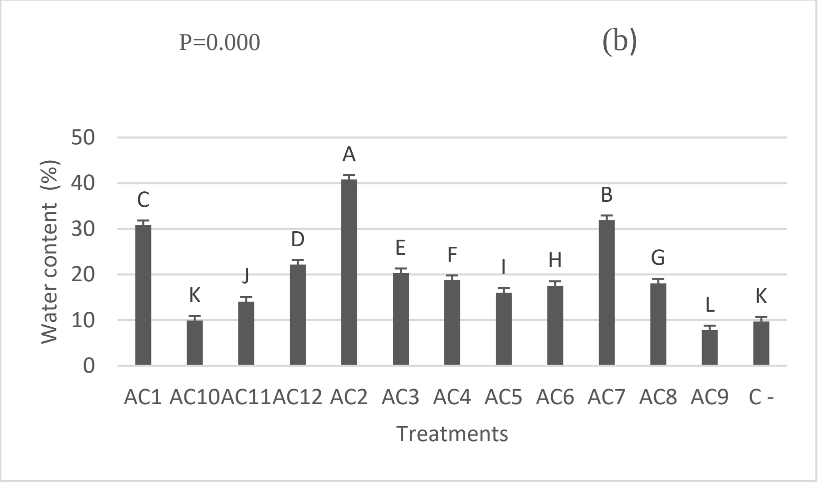
Previous studies of Mekureyaw *et al*. (33), have demonstrated a correlation between PGPR inoculation and increased chlorophyll levels in plants, which appears as higher leaf greenness. It was also demonstrated by Pérez-Rodriguez *et al*. (42), that in combined PGPR treatment, the total chlorophyll content was 11.12 mg/ml FW, better than the uninoculated control of 9.51 mg/ml. Other works reported an increase in chlorophyll and carotenoid levels after PGPR treatment in various species, notably in potato and *Arabidopsis thaliana* (13).

Quality parameters of tomato fruits (biochemical parameters)

(i). Organoleptic parameters

PGPR treatments showed very significant variability in water content, dry matter content and pH of cultivated plants (Figure 8). However, the applied treatments did not impact the organic matter and mineral matter content. The results showed that the majority of treatments induced significant variations in water content except for treatment with isolate AC9, which decreased water content (to 7.803 %) compared to negative control. Moreover, treatment with isolate AC11 increased the highest dry matter content (12.91 %), followed by treatments with isolates AC8, AC9 compared to negative control. Regarding pH values, treatment with isolate AC11 induced the highest pH of 4.453, followed by treatments with isolates AC10 and AC2, while other treatments showed lower pH values closer to the controls. The contents of mineral matter and organic matter were similar, 0.061 and 0.224 % for mineral matter and between 99.776 and 99.938 % for organic matter.





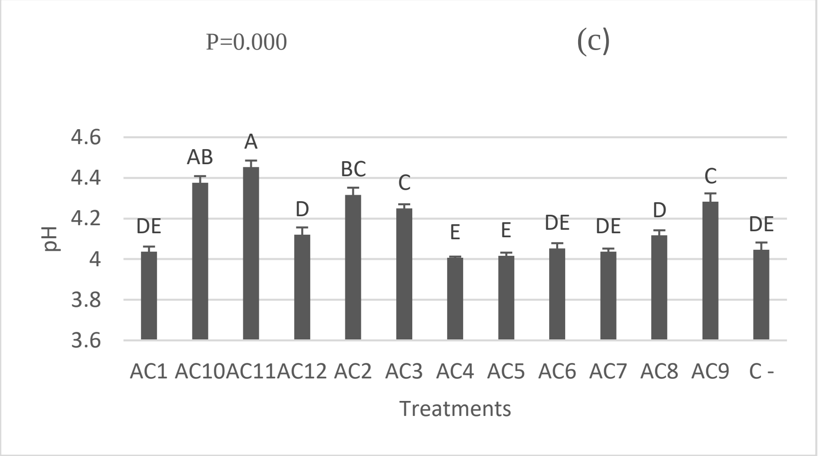


Figure 8. Effects of PGPR treatments. AC1, AC2, AC3, AC4, AC5, AC6, AC7, AC8, AC9, AC10, AC11, AC12: PGPR strains on Tomato fruits Quality criteria, C: Negative control. Data are means of 3-replicates. In each treatment, column with different letters is significantly different ($p \leq 0.05$, Tukey's test).

(ii). Sugar content

The applied bacterial isolates also influenced the total sugar content of cultivated tomato plants, showing highly significant difference. The recorded total sugar contents varied between 42.25 and 116.373 mg/ml, with isolate AC8 showed the greatest influence on total sugar contents and higher than negative control (Figure 9).

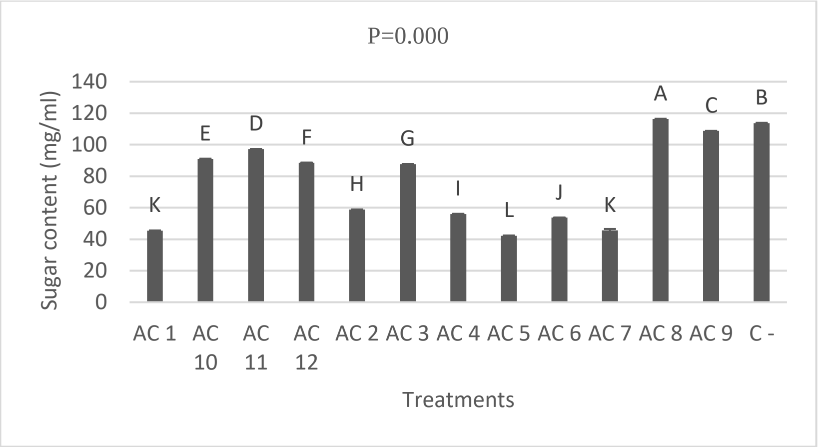
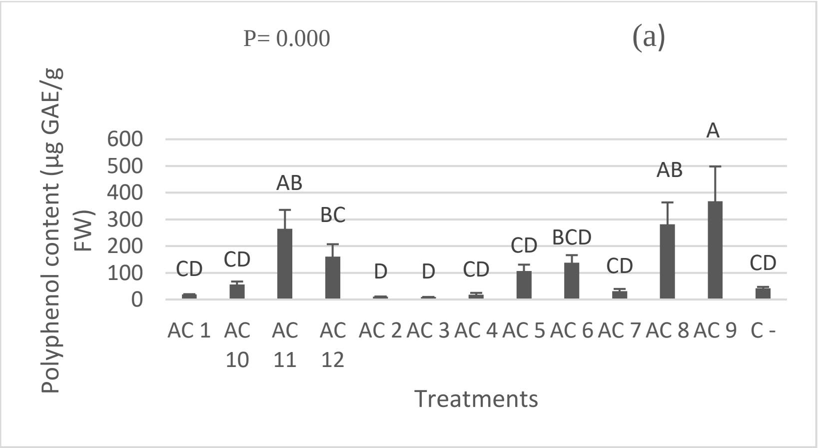


Figure 9. Effects of PGPR treatments. AC1, AC2, AC3, AC4, AC5, AC6, AC7, AC8, AC9, AC10, AC11, AC12: PGPR strains on Tomato fruits Sugar content, C: Negative control. Data are means of 3-replicates. In each treatment, column with different letters is significantly different ($p \leq 0.05$, Tukey's test).

(iii). Secondary metabolites

The treatments applied to cultivated tomato plants also significantly influenced the content of secondary metabolites, particularly polyphenols, flavonoids, and flavonols (Figure 10). The treatments showed significant variations in total polyphenol content, ranged from 9.055 to 368.1 µg GAE/g FW (Figure 10 a). Total flavonoid contents ranged from 3.29 to 12.08 µg QE/g FW (Figure 10 b), and total flavonol contents ranged from 0.094 to 3.655 µg QE/g FW (Figure 10 c). Treatment with isolate AC9 was the best to get highest content of these secondary metabolites.



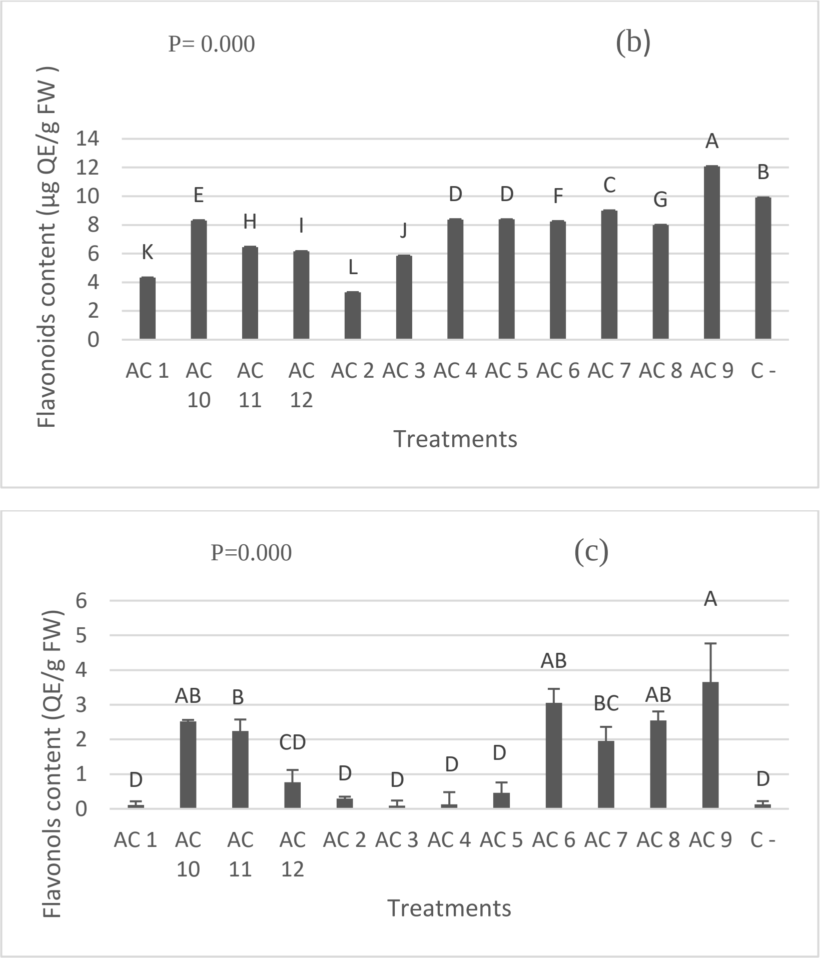


Figure 10. Effects of PGPR treatments. AC1, AC2, AC3, AC4, AC5, AC6, AC7, AC8, AC9, AC10, AC11, AC12: PGPR strains on Tomato Secondary metabolite contents, C: Negative control. Data are means of 3-replicates. In each treatment, column with different letters is significantly different ($p \leq 0.05$, Tukey's test).

(iv). **Antioxidant activity**

The treatments applied to cultivated tomato plants also influenced antioxidant activity and highly significant differences were recorded. All treatments significantly influenced the antioxidant activity of tomato fruit, with values ranging between 5.889 % and 58.85 % (Figure 11). The highest antioxidant activity was noted in treatment with isolate AC11, while isolate AC4 and the negative control showed the lowest percentages.

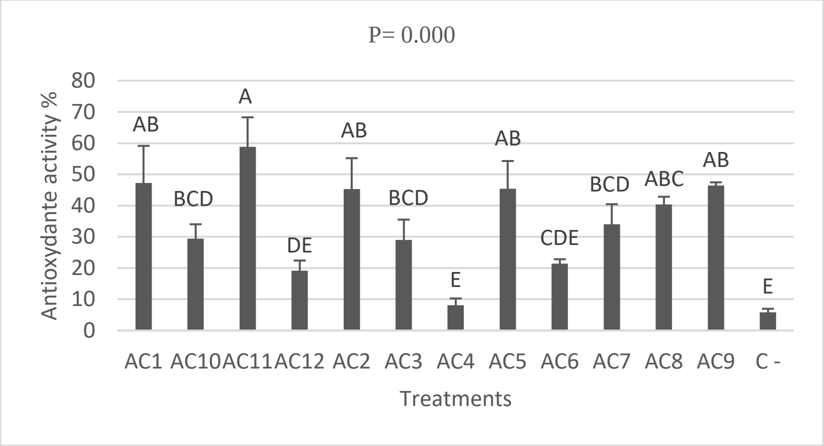


Figure 11. Effects of PGPR treatments. AC1, AC2, AC3, AC4, AC5, AC6, AC7, AC8, AC9, AC10, AC11, AC12: PGPR strains on Tomato fruits Antioxidant activity, C: Negative control. Data are means of 3-replicates. In each treatment, column with different letters is significantly different ($p \leq 0.05$, Tukey's test).

(v). Vitamin C

The treatments applied to tomato plants cultivated with isolates AC11 and AC9 significantly increased the vitamin C content of fruits. Other treatments gave lower values than negative control (Figure 12) and chromatograms (Figures 13, 14, 15 and 16).

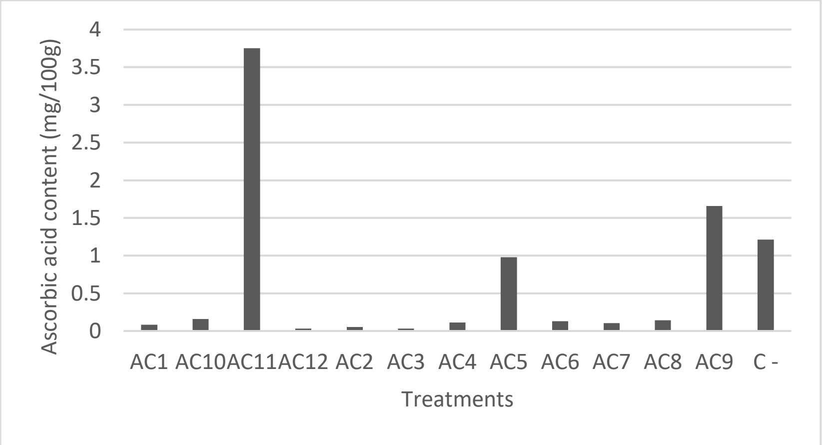


Figure 12**.** Effects of PGPR treatments. AC1, AC2, AC3, AC4, AC5, AC6, AC7, AC8, AC9, AC10, AC11, AC12: PGPR strains on Tomato Vitamin C content, C: Negative control. Data are means of 3-replicates. In each treatment, column with different letters is significantly different ($p \leq 0.05$, Tukey's test).

The vitamin C concentration of fruit treated with isolate AC11 was 3.753 mg/100g (Figure 15 c), followed by isolate AC9 with a concentration of 1.658 mg/100g (Figure 15 a) compared to the negative control 1.212 mg/100g (Figure 16).

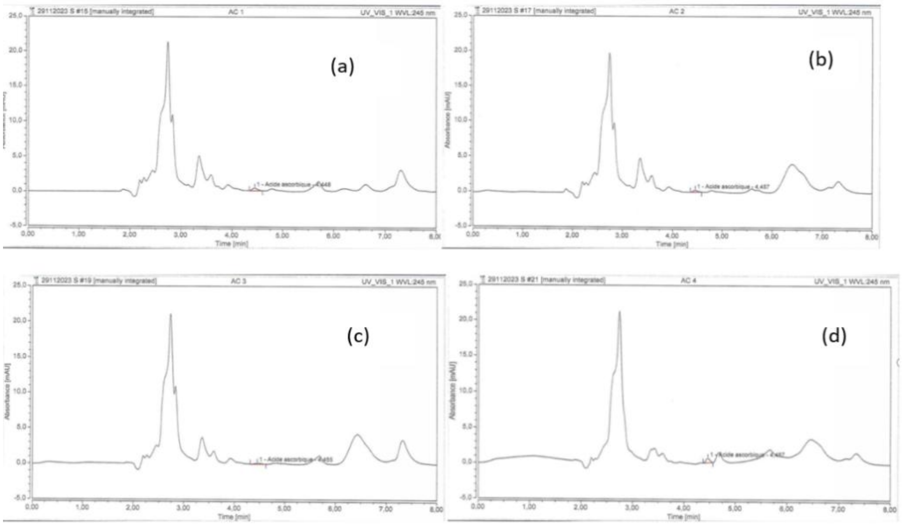


Figure 13. Chromatographic profile of ascorbic acid (Vitamin C) in tomato treated with isolates AC1, AC2, AC3, AC4.

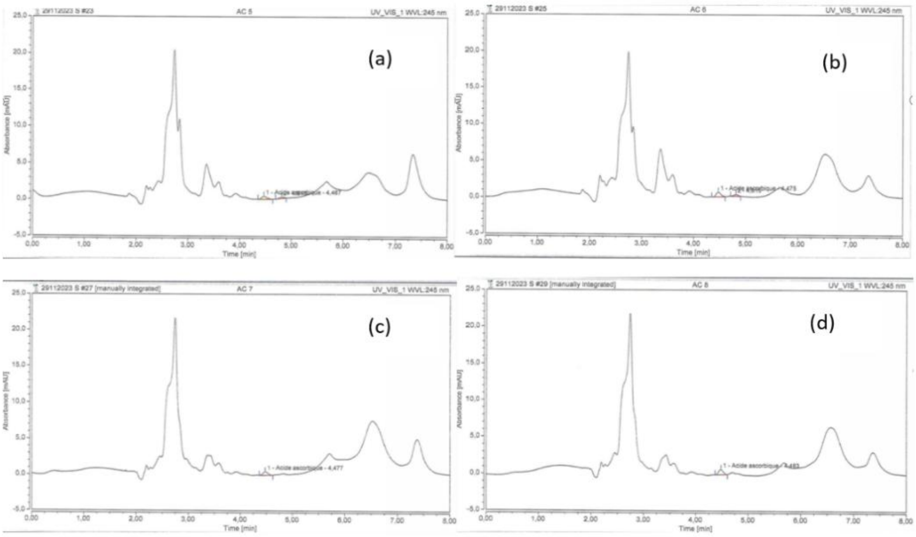


Figure 14. Chromatographic profile of ascorbic acid (Vitamin C) in tomato treated with isolates AC5, AC6, AC7, AC8.

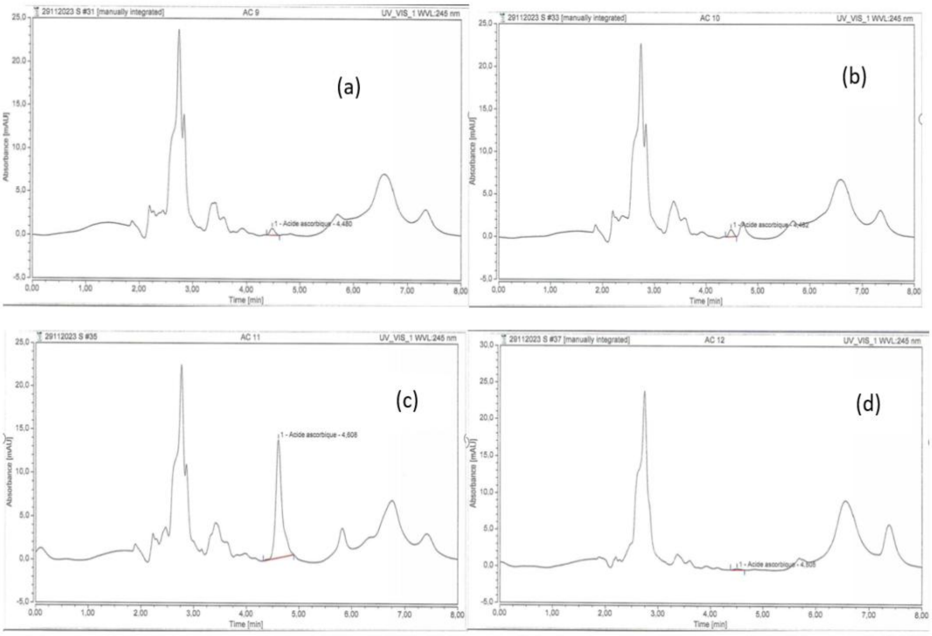


Figure 15. Chromatographic profile of ascorbic acid (Vitamin C) in tomato treated with isolates AC9, AC10, AC11, AC12.

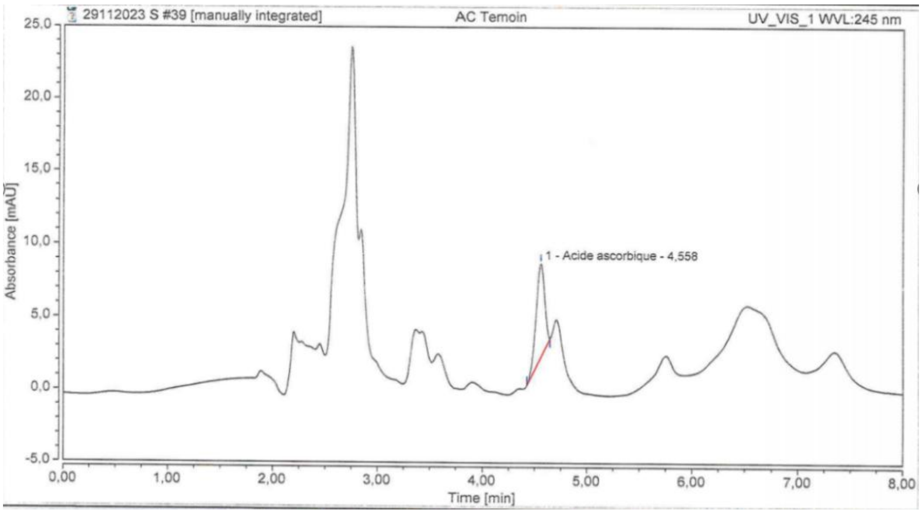


Figure 16. Chromatographic profile of ascorbic acid (Vitamin C) in untreated tomato (negative control).

The application of PGPR is an interesting strategy to naturally manipulate the level of bioactive molecules in plants (40). In our study, inoculation with PGPR of genus *Pseudomonas* spp. significantly improved tomato fruit quality parameters (water content, mineral matter, dry matter, organic matter and pH). Moreover, these treatments increased sugar content, when treated with isolate AC8. The treatments also influenced the content of secondary metabolites (polyphenol, flavonoid, and flavonol content), antioxidant activity, and vitamin C content. Studies on cucumber showed that PGPR applications positively influenced the pH and firmness of cucumber fruits (48). Furthermore, inoculation with *B. cepacia* significantly increased the pH value to 4.07 than control 3.75 in tomato fruits (58). Previous studies of Ordookhani *et al.* (38), showed that increased minerals in inoculated plants can improve tomato fruit quality. PGPR applications had positive effects on the mineral content of tomatoes; N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, and B differed in root, leaf, and fruit samples than uninoculated controls (48). Combined treatments can also improve mineral matter content; inoculation with *Pseudomonas* + *Azospirillum* mixture increases K, Ca, Mg content, and treatment with *Pseudomonas* + *Azotobacter* + *Azospirillum* was best for N, P, and K absorption in tomato stems (49). Plant inoculation with *B. subtilis* + *B. amyloliquefaciens* improves N, P, and K composition in tomato fruits (18). Khan (28) reported that *Pseudomonas* and *Acinetobacter* increased iron, zinc, magnesium, calcium, potassium, and phosphorus contents in the plants. Other studies highlighted the importance of using a 30/70 mix of chemical fertilizers/bio-compost to improve the mineral matter (nitrogen, phosphorus, and organic matter) of tomato fruits compared to treatment with pure chemical fertilizers (25).

Sugars are the predominant soluble solids in tomatoes and contribute significantly to their flavour. Among the main free sugars are sucrose, glucose, and fructose (21). A very significant increase in glucose and sucrose was observed in tomatoes and peppers following biostimulant application. In UCO variety, inoculation with Rt6M10 significantly increased sugars (14). This increase is due to photosynthetic efficiency and increased CO_2 absorption, leading to greater sugar accumulation in fruits (54). This was also confirmed by González Rodríguez *et al*. (22), where they observed an increase (22,66 %) in reducing sugars following inoculation with *Aeromonas* sp. strain, compared to uninoculated control. This increase can be attributed to the fact that PGPR leads to improved photosynthetic efficiency and thus higher chlorophyll content due to high levels of CO_2 capture, leading to greater sugar accumulation in fruits. The application of combined PGPR + AMF treatment increased higher sugar content than control; the improvement in sugar content after AMF inoculation is influenced by increased photosynthesis production, nutrients solubilization (P and K) and phytohormone concentration: abscisic acid; the latter plays important role in fruit development and maturation and it also increases fruit sugar synthesis (fructose and sucrose) while reducing fruit acidity (4).

The observed antioxidant activity is explained by the presence of bioactive compounds in the fruits. It has been noted that immature fruits contain a large quantity of antioxidant compounds, as fruit ripening leads to the degradation of several of these compounds (11). Ordookhani and Zare (39), demonstrated that fruit antioxidant activity increased (50.73 %) with combined treatments of *Pseudomonas* + *Azotobacter* + AMF compared to control. In their study, Katsenios *et al*. (27) explained that increase in antioxidant activity was correlated to carotenoid, lycopene, and polyphenol concentrations in tomatoes inoculated with PGPR such as *B. licheniformis*, *B. subtilis*, and *B. amyloliquefaciens* (27).

PGPR treatments showed a positive and significant effect on flavonoid quantities and total phenolic compound content. These bioactive molecules are very important for human health due to their antioxidant, anticancer, antidiabetic, and cardiovascular protective effects (17), conferring improved nutritional characteristics to treated tomatoes. Oancea (37) proved that the application of biostimulants based on *Azospirillum lipoferum* DO12 and *Brevibacillus parabrevis* B50 increased polyphenol content by 17.2 %. Vitamin C (or ascorbic acid) is involved in many plants physiological processes such as genetic regulation and molecule transport, and is considered effective antioxidant. According to these researchers, its production can be induced by different environmental factors (11). Organically grown tomatoes generally have higher concentrations of ascorbic acid and lycopene than conventionally produced tomatoes, which could be related to each microorganism's ability to synthesize phytohormones (22).

Studies on PGPR treatment showed that inoculation with a mixture of two *Bacillus strains* (*B. subtilis* + *B. amyloliquefaciens*) increased ascorbic acid content by 75 %. Moreover, inoculation with *B. amyloliquefaciens* alone increased ascorbic acid content by 50 % than uninoculated plants (18). Furthermore, *Azospirillum lipoferum* DO12 and *Brevibacillus parabrevis* B50 played an important role in increasing vitamin C by 15.4 % compared to control (18). Additionally, inoculation with *B. cepacia* increased vitamin C values to 29.81 mg/100g compared to uninoculated control (58). Moreover, inoculation with the *Phyllobacterium endophyticum* strain to increase the yield and quality of strawberry plants, with a significant increase in vitamin C content (11).

CONCLUSIONS

The study confirmed the agronomic potential of endemic isolates of *Pseudomonas* spp. These stimulated germination and growth parameters size, leaf biomass, photosynthetic pigment content and fruit weight of tomato plants. Furthermore, certain PGPR isolates improved quality very useful to consumer well-being and health. There was possibility of choosing between tomatoes with high sugar content or low sugar content for diabetics. Certain bacterial isolates improved the content of phenolic compounds, free radical scavenging power, and vitamin C content compared to control. In this regard, endemic PGPR isolates AC11, AC9, AC2, AC1 may be recommended for sustainable and eco-friendly tomato cultivation.

AUTHORS’ CONTRIBUTIONS

This work was carried out in collaboration with all authors. All authors finally approved and drafted the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest. All authors agree to publish it.

DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

ETHICAL STATEMENT

This is to inform you that in this study, we have not been involved in any animal and human studies.

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