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Plant Growth-Promoting Bacteria in Combination with Optimum N Fertilizer Rates Improves the Growth, Yield and Quality of Tomato

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ABSTRACT

Tomato (Solanum lycopersicum L.) production often relies heavily on chemical nitrogen (N) fertilizers, raising concerns about costs, sustainability, and soil health. Plant growth-promoting bacteria (PGPB) represent a promising alternative that may reduce fertilizer inputs while maintaining fruit yield and quality. This study was undertaken to investigate the influence of two PGPB strains, Bacillus tequilensis (UPMRB9) and Bacillus subtilis (UPMB10), applied under different N levels on tomato performance and soil enzymatic activity. A glasshouse experiment was arranged in a completely randomized design with nine treatments, combining bacterial inoculation and three N application rates (0, 50, and 100% of the recommended dose). Inoculated plants consistently performed better than the uninoculated control, with significant improvements in yield attributes (flower number, fruit number, fruit set, and fruit weight) and fruit quality traits. The combination of UPMRB9 with 50% of the recommended N rate (UPMRB9N50) produced the greatest effects, increasing fruit yield by 27.27%, lycopene content by 12.16%, antioxidant activity by 26.96%, and soil microbial activity by 11.14% compared with the full N dose without inoculation. Higher total soluble solids (5.15 °Brix) were also recorded in UPMRB9N50-treated plants, reflecting better fruit quality. Overall, the findings suggest that microbial inoculation, particularly with UPMRB9, can reduce N fertilizer use by almost 50% while still enhancing tomato yield and fruit quality. Such results provide important preliminary evidence for adopting PGPB as a viable strategy to reduce chemical fertilizer dependency and promote more sustainable tomato production.

Keywords: PGPB, Nitrogen fertilizer, Antioxidants, Enzyme, PCA

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), a member of the Solanaceae family, is one of the most widely cultivated and consumed vegetable crops worldwide (Safari *et al*., 2021). It plays a particularly important role in peri-urban and rural areas of developing countries, where it contributes directly

to household income and food security (Arah *et al*., 2015). Globally, tomatoes were cultivated on 5.03 million hectares, producing 180.77 million tons in 2019 (FAOSTAT, 2019). Beyond their economic value, tomatoes are recognized as a major dietary source of health-promoting antioxidants, including lycopene, phenolic compounds, and vitamin C, which have been linked to reduced risks of cancer and cardiovascular diseases (Santos-Sánchez *et al*., 2013; Cheng *et al*., 2017; Ahmed *et al*., 2018). With rising living standards, consumer demand has shifted from yield alone to fruit quality, particularly attributes related to nutrition and health benefits.

A single tomato can provide approximately 40% of the recommended daily intake of vitamin C, while lycopene, the pigment responsible for its red colour, functions as a potent antioxidant. Lycopene helps protect against cardiovascular disease by reducing low-density lipoprotein oxidation, and phenolic compounds in tomatoes help prevent lipid peroxidation of fatty acids (Martínez-Valverde *et al*., 2002). Despite these benefits, tomato cultivation faces challenges associated with heavy reliance on agrochemicals, which contribute to ecological degradation and pose risks to human health (Villarreal-Romero *et al*., 2010; Bassil *et al*., 2007). Over the last five decades, fertilizer use has increased by nearly 170-fold (FAO, 2010), underscoring the need for alternative, sustainable management practices.

Plant growth-promoting bacteria (PGPB) have gained attention as bio-inoculants that can enhance crop productivity and suppress diseases, thereby reducing the need for synthetic inputs (Worthington, 2001; Vos *et al*., 2014). Commonly used PGPB genera include *Azospirillum*, *Enterobacter*, *Pseudomonas*, and *Bacillus* (Babu *et al*., 2015). Their beneficial effects are attributed to improved nutrient uptake, stimulation of root growth, and production of phytohormones such as cytokinins and gibberellins (Yang *et al*., 2009). By increasing root surface area and water absorption, PGPB improve plant resilience and reduce dependence on chemical fertilizers (Pesakovic *et al*., 2013). Soil enzyme activity, which reflects key biochemical processes, is also an important indicator of soil health and plant stress alleviation (Silva and Fay, 2012). However, limited studies have examined how nitrogen (N) fertilization interacts with PGPB to influence soil enzymatic activity (Khalil *et al*., 2007). This interaction is critical because PGPB effectiveness is closely tied to the level of chemical fertilizer applied, and the combined approach could lower input costs while maintaining productivity (Jogaiah *et al*., 2010). In this context, the present study was designed to evaluate: (1) the effects of PGPB and nitrogen fertilizer on antioxidant activity, phenolic content, ascorbic acid, and lycopene levels in tomato fruits, and (2) the effects of PGPB and nitrogen fertilizer on soil enzyme activity.

MATERIALS AND METHODS

Set up of the experiment and plant establishment

The pot experiment was carried out in the glasshouse at Field 15 (03°00′12.6″ N; 101°47′22.4″ E), Faculty of Agriculture, Universiti Putra Malaysia (UPM). The soil used, classified as sandy clay loam, was collected from the *Ladang Kongsi* at UPM. Its properties were: pH 4.91, total nitrogen 0.199%, total carbon 2.31%, available phosphorus 39.71 mg kg^{-1}, and exchangeable K, Ca, and Mg of 0.23, 0.31, and 0.10 meq 100 g^{-1}, respectively. Plastic pots (27 × 27 cm) were filled with 10 kg of soil and maintained at field capacity throughout the study. Tomato seeds were first germinated in plastic trays, and seedlings were transplanted into pots at three weeks old, with one seedling established per pot.

Experimental Design

A completely randomized design (CRD) was used with each treatment replicated three times. The treatments are presented in **Table 1**.

Table 1: Description of the treatments applied in the study

Treatments	Treatments description
B0N0	Control
B0N50	Uninoculated control applied with 50% of nitrogen fertilizer as per the recommended rate of 180 kg ha^{-1} in three split applications
B0N100	Uninoculated control applied with 100% nitrogen fertilizer as per the recommended rate of 180 kg ha^{-1} in three split applications
UPMB10N0	*Bacillus subtilis* (10^8 CFU mL^{-1}) inoculated without nitrogen fertilizer
UPMB10N50	*Bacillus subtilis* (10^8 CFU mL^{-1}) inoculated with 50% of nitrogen fertilizer as per the recommended rate of 180 kg ha^{-1} in three split applications
UPMB10N100	*Bacillus subtilis* (10^8 CFU mL^{-1}) inoculated with 100% nitrogen fertilizer as per the recommended rate of 180 kg ha^{-1} in three split applications
UPMRB9N0	*Bacillus tequilensis* (10^8 CFU mL^{-1}) inoculated without nitrogen fertilizer
UPMRB9N50	*Bacillus tequilensis* (10^8 CFU mL^{-1}) inoculated with 50% of nitrogen fertilizer as per the recommended rate of 180 kg ha^{-1} in three split applications
UPMRB9N100	*Bacillus tequilensis* (10^8 CFU mL^{-1}) inoculated with 100% nitrogen fertilizer as per the recommended rate of 180 kg ha^{-1} in three split applications

Fertilizer management

Fertilizer application rates were determined based on soil pH and nutrient status (FAO, 2004), with recommended levels of 180, 180, and 225 kg ha^{-1} for N, P, and K, respectively. On a per-pot basis, this corresponded to 0.783 g (50% N), 1.565 g (100% N), 4.13 g (100% P_2O_5), and 3.74 g (100% K_2O) supplied using urea, triple superphosphate (TSP), and muriate of potash (MOP). Forty-eight hours before transplanting, TSP and MOP were incorporated into the soil. Nitrogen was applied in three split doses: the first at seven days after transplanting (DAT), followed by two additional applications at 14-day intervals. Manual weeding was carried out as needed, and plastic nets were placed around the pots to minimize pest infestation.

Preparation of inocula and application to the plant

The bacterial strains UPMB10 and UPMRB9 were each cultured in 250 mL conical flasks containing 100 mL of tryptic soy broth (TSB). Cultures were incubated on an orbital shaker (Model OSI-503 LD; Firstek Scientific, Japan) at 28 °C and 150 rpm for 24 hours. The cell density was standardized to an optical density of 1.0 at 600 nm (OD_{600}) using a UV-visible spectrophotometer. Each plant was inoculated with 28.57 mL of live inoculant suspension (10^8 CFU mL^{-1}) per pot, applied at 14-day intervals until harvest. Control plants received sterile distilled water in place of bacterial inoculants.

Total N and C content of soils

Total soil carbon (C), nitrogen (N), and sulfur (S) were determined using LECO's TruMac CNS Macro Analyzer (LECO, 2018) together with the LECO FP-2000 (LECO Corp., Michigan, USA). For each analysis, approximately 0.1 g of soil was combusted at 1350 °C in an oxygen-rich environment using a C-free combustion boat. The resulting gases were quantified for total C, N, and S, and the combustion boat was introduced into the TruMac CNS analyzer via an auto-sampler stand.

Yield parameters

The number of flowers per plant was recorded at final harvest by averaging counts from eight clusters on two tagged plants. Fruit number was determined by averaging two fruits per plant at harvest, while fruit diameter was measured using a digital Vernier caliper (Model CD-6''CS, Mitutoyo Corporation, Neuss, Germany) from three randomly selected fruits, with the mean

value recorded. The percentage of fruit set was determined at harvest utilizing the equation mentioned below,

$$\% \text{ fruit set} = \frac{Number\ of\ fruit\ per\ plant}{Number\ of\ flowers\ per\ plant} \times 100$$

Fruit weight per plant was calculated as the average weight of all harvested fruits per treatment. Total soluble solids (TSS) were measured using a portable refractometer (Model PAL-1, B926422, ATAGO Co. Ltd., Japan). Fresh tomato juice was extracted with a garlic pestle, and a drop was placed on the refractometer lens. Readings were expressed as °Brix (%), following the method of Nirupama et al. (2010).

Quality parameters

Antioxidant properties and activity

Ascorbic acid

Fully mature tomato fruits were used to determine ascorbic acid content following the direct colorimetric method with 2,6-dichlorophenol-indophenol (DCPIP) dye (Ding and Mashah, 2016). Two grams of the sample were homogenized with 20 mL of 2% metaphosphoric acid (HPO_3) to a final volume of 40 mL. The extract was filtered through cotton wool and adjusted to volume with an additional 2% HPO_3. For analysis, 0.5 mL of extract was mixed with 3 mL of 2% HPO_3 and 2 mL of DCPIP dye. Absorbance was read at 518 nm using a UV spectrophotometer (Model 1510, Thermo Fisher Scientific, Vantaa, Finland). Ascorbic acid concentration was expressed as mg g^{-1} fresh weight, calculated against a standard curve.

$$AA\ (\frac{mg}{g\ FW}) = \frac{[(conc.of\ sample\ sol.as\ ppm\ derived\ through\ the\ standard\ curve) \times Final\ vol.calc.in\ L\ (0.04\ l)]}{sample\ weight\ (g)}$$

Lycopene

Ripe tomato fruits were analyzed for lycopene content following the method of Nagata and Yamashita (1992), with slight modifications. Approximately 1 g of sample was homogenized using a mortar and pestle in 10–20 mL of an acetone–hexane mixture (4:6, v/v). The pigments were extracted, and the supernatant was automatically separated for analysis. The optical density was promptly measured at wavelengths of 663 nm, 645 nm, 505 nm, and 453 nm using a UV spectrophotometer, and the quantity of lycopene was calculated according to the equation provided below:

$$Lycopene\ \left(\frac{mg}{100g}\right) = -0.0458A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453}$$

The absorbances at 663 nm, 645 nm, 505 nm, and 453 nm are designated A_{663}, A_{645}, A_{505}, and A_{453}, respectively. The result acquired as mg per 100 mL was then transformed to data mg per 100 mL × sample volume = data mg per 100 g

Total phenolic content (TPC)

Total phenolic content (TPC) was determined following Musa et al. (2011) with minor modifications. A 150 µL aliquot of the supernatant was mixed with 750 µL of 10% Folin–Ciocalteu reagent (FCR) and incubated for 5 minutes. Subsequently, 600 µL of sodium carbonate (Na_2CO_3, 7.5% w/v) was added, and the mixture was incubated in the dark for 30 minutes. Absorbance was then measured at 765 nm using a UV spectrophotometer. Results were expressed as milligrams of gallic acid equivalents per 100 g of fresh weight (mg GAE 100 g^{-1} FW).

DPPH radical scavenging assay

Antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay described by Musa et al. (2011). A stock solution of 1 mM DPPH was prepared by dissolving 40 mg DPPH in 100 mL of 80% methanol and stored under refrigeration until use. For analysis, 1 mL of supernatant was mixed with 1 mL of the DPPH solution and incubated in the dark for 30 minutes. The absorbance was measured immediately after incubation using a UV spectrophotometer set to a wavelength of 517 nm, and the following equation was applied to determine the percentage of antioxidant activity:

$$\text{DPPH scavenging activity (\%)} = \frac{Absorbance\ (blank) - Absorbance\ (sample)}{Absorbance\ (blank)} \times 100$$

Soil microbiological parameters

Enzyme assays for acid phosphatase (FTA), dehydrogenase (DHA), and β-glucosidase (β-GLU) were performed 14 weeks after transplanting. Soil samples were collected from the root zone, transported in plastic bags, homogenized, and sieved through a 2 mm mesh to remove visible debris. Samples were stored at 4 °C until analysis. Acid phosphatase activity was determined at pH 6.0 and 11.0 using p-nitrophenol phosphate as substrate, following Tabatabai and Bremner (1996), and expressed as μg p-nitrophenol released g^{-1} soil h^{-1}, measured at 440 nm. Dehydrogenase activity was quantified as triphenyl formazan (TPF) production, following Klein et al. (1971), while β-glucosidase activity was assayed according to Eivazi and Tabatabai (1988). Total enzyme activity was measured by fluorescein diacetate (FDA) hydrolysis (Adam and Duncan, 2001). For this, 1 g of soil was mixed with 7.5 mL of 60 mM sodium phosphate buffer (pH 7.6) and 0.1 mL FDA, then shaken at 150 rpm for 40 minutes. The reaction was stopped with chloroform–methanol (2:1), and the mixture was centrifuged at 8000 rpm for 10 minutes. Hydrolyzed FDA was measured at 490 nm using a UV spectrophotometer against a fluorescein standard curve.

Statistical analysis

Data was analyzed using SAS version 9.4 (Statistical Analysis Software) following the procedures of Gomez and Gomez (1984). Treatment means were compared using Duncan's Multiple Range Test (DMRT) at a 5% significance level. Principal component analysis (PCA) was performed and visualized with JMP version 14 (SAS Institute Inc., Cary, NC, USA, 2021).

RESULTS

Quality parameters

Antioxidant properties and activity

The results showed that the ascorbic acid levels, lycopene, total phenolic content, and DPPH Scavenging activity were significantly affected ($P \leq 0.05$) by the treatments. The highest concentration of ascorbic acid was in UPMRB9N0 (0.5800 mg g^{-1} FW), followed by UPMB10N0 (0.55333 mg g^{-1} FW) and B0N0 (0.53667 mg g^{-1} FW). The lowest levels were in B0N100 (0.34667 mg g^{-1} FW), UPMB10N100 (0.38000 mg g^{-1} FW), and UPMRB9N100 (0.40333 mg g^{-1} FW) (**Table 2**). Similarly, all treatments significantly influenced ($P \leq 0.05$) the lycopene content of the fruit, with the highest concentration in UPMRB9N50 (4.85 mg per 100 g FW), followed by UPMB10N50 (4.68667 mg per 100 g FW). UPMRB9N50 had 57.73% and 12.16% higher lycopene than B0N0 and B0N100, respectively. The two inoculated controls (UPMRB9N0, UPMB10N0) showed no significant difference between them. In terms of the total phenolic

content, UPMRB9N50 had the highest with 4.85 mg per 100 g FW, followed by UPMB10N50 with 4.68667 mg per 100 g FW. Similarly, UPMRB9N50 had 12.83% and 26.96% higher DPPH activity than the control and B0N100 treatments, respectively.

Table 2: Lycopene, ascorbic acid, total phenolic content (TPC), and DPPH scavenging activity of tomato as affected by the treatments

Treatments	Lycopene (mg per 100 g FW)	Ascorbic acid (mg g^{-1} FW)	Total phenolic content (mg g^{-1} FW)	DPPH scavenging activity (%)
B0N0	2.050±0.006 h	0.537±0.003c	0.353±0.003 f	57.55±0.017f
B0N50	3.240±0.021 f	0.440±0.006f	0.450±0.006 e	60.65±0.029c
B0N100	4.263±0.035 c	0.347±0.009i	0.5100±0.006 c	48.217±0.038i
UPMB10N0	2.100±0.012 gh	0.553±0.003b	0.360±0.006 f	58.397±0.015e
UPMB10N50	4.687±0.020 b	0.470±0.006e	0.567±0.003 b	65.913±0.029b
UPMB10N100	3.850±0.017 e	0.380±0.006h	0.467±0.003 d	50.293±0.030h
UPMRB9N0	2.130±0.012 g	0.580±0.006a	0.367±0.003 f	58.71±0.023d
UPMRB9N50	4.850±0.012 a	0.500±0.006d	0.620±0.006 a	66.017±0.071a
UPMRB9N100	3.950±0.012 d	0.403±0.003g	0.477±0.003 d	50.713±0.020g

Note: A column sharing the same lower-case letter(s) indicates no significant difference at P ≤0.05 based on Duncan's multiple range test means separation techniques

Soil microbiological parameters

UPMRB9N50 had significantly higher (P≤ 0.05) dehydrogenase (DHA), acid phosphatase, β-glucosidase (β-GLU), and total enzyme activity than the other treatments (**Table 3**). The UPMRB9N50 had an average DHA level of 254.333 µg TPF g^{-1} soil 24 h^{-1}, which was 39.97% and 11.14% higher than the control and B0N100 treatments, respectively, followed by UPMB10N50 at 246.333 µg TPF g^{-1} soil 24 h^{-1}. In terms of phosphatase enzyme activity (FTA), the UPMRB9N50 had 15.46% and 6.43% higher enzymatic activity, and 17.65% and 8.05% higher beta-glucosidase activity, than the control and B0N100 treatments, respectively. Likewise, the UPMRB9N50 had the highest FDA concentration (0.613333 µg L^{-1}), which was significantly higher (P≤ 0.05) than that of other treatments and showed a 72.83% increase compared to the control (0.166667 µg L^{-1}) (**Figure 1**).

Table 3: Treatment effects on enzymatic activities in soil

Treatments	Dehydrogenase activity in soil (µg TPF g^{-1} soil $24h^{-1}$)	Acid Phosphatase (µg PNP g^{-1} h^{-1})	Beta-glucosidase (µg PNP g^{-1} h^{-1})
B0N0	152.667 ± 0.667 i	324.3333 ± 0.667 h	266 ± 0.577 i
B0N50	204 ± 1 f	346.6667 ± 0.333 e	285 ± 0.577 f
B0N100	226 ± 0.577 c	359 ± 0.577 c	297 ± 0.577 c
UPMB10N0	165.333 ± 0.882 h	332.6667 ± 0.667 g	273.333 ± 0.882 h
UPMB10N50	246.333 ± 0.667 b	374 ± 1 b	314.333 ± 1.202 b
UPMB10N100	211.333 ± 0.667 e	351 ± 0.577 d	288.667 ± 0.882 e
UPMRB9N0	170.667 ± 0.667 g	337 ± 0.577 f	277 ± 1 g
UPMRB9N50	254.333 ± 0.667 a	383.6667 ± 0.882 a	323 ± 1.155 a
UPMRB9N100	214.333 ± 0.667 d	353 ± 0.577 d	292.667 ± 0.667 d

Note: A column sharing the same lower-case letter(s) indicates no significant difference at P ≤0.05 based on Duncan's multiple range test means separation techniques

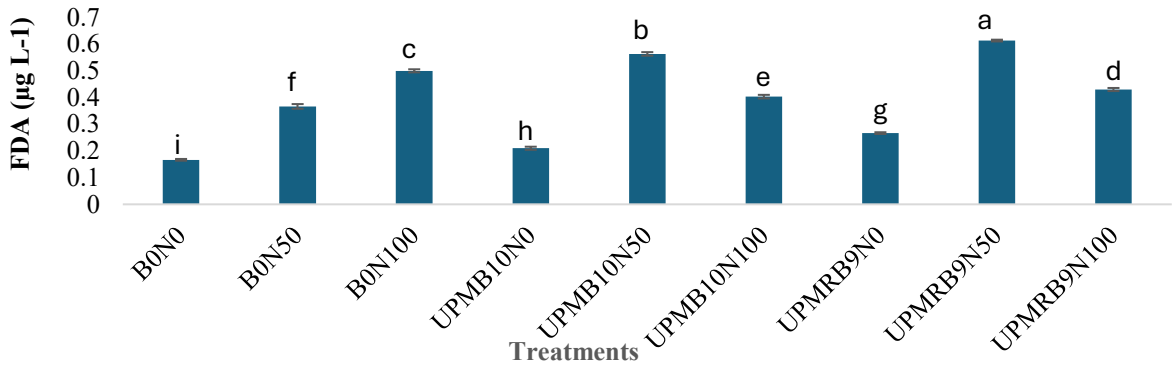

Figure 1: Total enzyme activity in soil determined by FDA analysis test

Note: The standard error of the means is shown by the bars. A bar sharing the same lower-case letter(s) indicates no significant difference at P ≤0.05 based on Duncan's multiple range test means separation techniques

Yield parameters

The use of PGPB and different nitrogen levels significantly affected (P≤ 0.05) tomato flower production. The highest flower production was in UPMRB9N50-treated plants, followed by UPMB10N50. The lowest yield was in B0N0-treated plants. Full N fertilizer application increased flower production by 54.88% compared to the control, with UPMRB9N50 enhancing it by 64.08% (**Table 4**). PGPB and nitrogen levels significantly influenced (P≤ 0.05) fruit yield per tomato plant. Results showed that UPMRB9N50 produced the most fruits (18.33), followed by B0N100 (13.33), and the least was observed in the control (4.33). UPMRB9N50 and B0N100 increased fruit production by 76.36% and 67.50%, respectively, compared with the control. There was no significant difference in fruit setting percentage between UPMRB9N50 and UPMB10N50. Fruit set percentages ranged from 35.04% in B0N0 (control) to 53.83% in UPMB10N50. The result shows that UPMRB9N50 had the highest fruit weight at 564.67 g, followed by UPMB10N50 at 503.07 g, while the lowest was observed in the B0N0 (control) at 133.47 g. UPMRB9N50 had 72.73% and 27.27% higher than B0N0 and B0N100, respectively. In terms of total yield (t ha^{-1}), UPMRB9N50 recorded 12.0467 t ha^{-1}, which differed significantly (P≤ 0.05) from other treatments.

Table 4: Treatment effects on the number of flowers, number of fruits, percentage fruit set, fruit weight per plant, and yield of tomato

Treatments	Number of flowers per plant	Number of fruits per plant	Percentage of fruit set (%)	Fruit weight per plant (g)	Yield (t ha^{-1})
B0N0	12.3333 ± 0.333 h	4.3333 ± 0.333 e	35.04 ± 1.710 de	133.47 ± 10.267 e	2.85 ± 0.220 e
B0N50	20.6667 ± 0.667 e	8.6667 ± 0.333 d	41.97 ± 1.538 c	266.93 ± 10.267 d	5.6933 ± 0.217 d
B0N100	27.3333 ± 0.333 c	13.3333 ± 0.333 c	48.76 ± 0.617 b	410.67 ± 10.267 c	8.76 ± 0.220 c
UPMB10N0	13.6667 ± 0.333 g	4.6667 ± 0.333 e	34.063 ± 1.647 e	143.73 ± 10.267 e	3.07 ± 0.220 e
UPMB10N50	30.3333 ± 0.333 b	16.3333 ± 0.333 b	53.833 ± 0.503 a	503.07 ± 10.267 b	10.73 ± 0.220 b
UPMB10N100	24.3333 ± 0.333 d	9.3333 ± 0.333 d	38.333 ± 0.833 d	287.47 ± 10.267 d	6.13 ± 0.220 d
UPMRB9N0	15.3333 ± 0.333 f	5.3333 ± 0.333 e	34.72 ± 1.390 de	164 ± 10.267 e	3.5033 ± 0.213 e
UPMRB9N50	34.3333 ± 0.333 a	18.3333 ± 0.333 a	53.39 ± 0.450 a	564.67 ± 10.267 a	12.0467 ± 0.217 a
UPMRB9N100	25.3333 ± 0.333 d	9.6667 ± 0.333 d	38.153 ± 1.165 d	297.73 ± 10.267 d	6.35 ± 0.220 d

Note: A column sharing the same lower-case letter(s) indicates no significant difference at P ≤0.05 based on Duncan's multiple range test means separation techniques

The significantly higher fruit diameter of 66.667 mm was recorded in UPMRB9N50 with 36.50% higher than B0N0 and 11.50% higher than B0N100 (**Figure 2**).

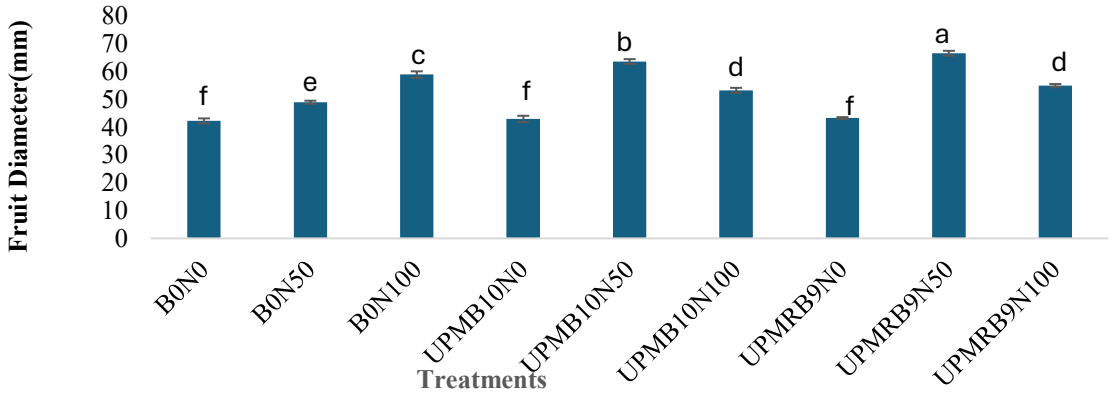

Figure 2: Impact of treatments on the fruit diameter.
Note: The standard error of the means is shown by the bars. A bar sharing the same lower-case letter(s) indicates no significant difference at P ≤0.05 based on Duncan's multiple range test means separation techniques

The treatments had significantly (P≤ 0.05) influenced the total soluble solids (TSS) (**Figure 3**). The result shows that UPMRB9N50 had a significantly (P≤ 0.05) higher TSS value of 5.14, which differs from the other treatments, while the control (B0N0) had the lowest with 3.99. There was no significant difference (P>0.05) between UPMRB9N50 and UPMB10N50. The control had 22.55 % lower TSS than UPMRB9N50.

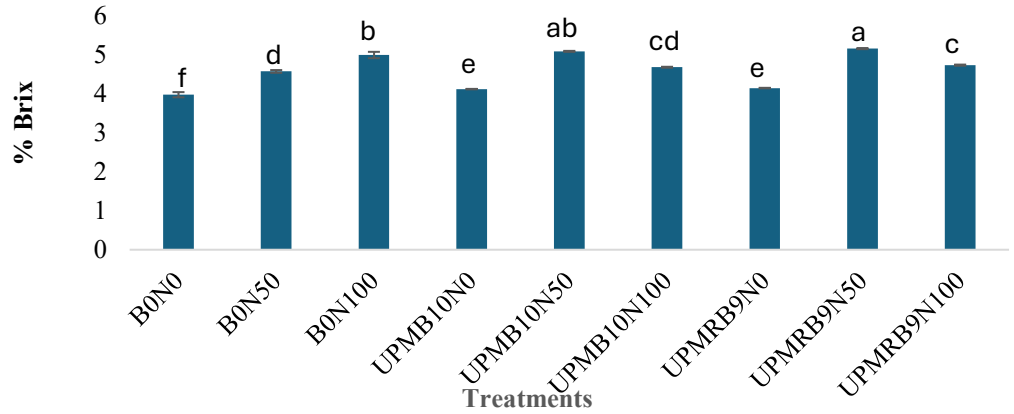

Figure 3: Impact of treatments on total soluble solids
Note: The standard error of the means is shown by the bars. A bar sharing the same lower-case letter(s) indicates no significant difference at P ≤0.05 based on Duncan's multiple range test means separation techniques

Principal component analysis

Principal component analysis (PCA) revealed relationships among yield, fruit diameter, total soluble solids, antioxidant properties, and enzymatic activities, all influenced by plant growth-promoting bacteria (PGPB) at various nitrogen levels in tomato cultivation (**Figure 4**). Each point on the loading plot represents a variable's contribution to the score, including yield, fruit diameter, total soluble solids, antioxidant traits like lycopene and ascorbic acid, total phenolic content, DPPH scavenging activity, and enzymatic activities such as dehydrogenase and acid phosphatase. Each dot on the score plot represents a specific treatment. The first principal component (PC1) explains 58.1% of the variation, while the second (PC2) accounts for 25.4%, for a total of 83.5% of the variance. Results show that UPMRBB9N50 and UPMB10N50

improved yield, fruit diameter, total soluble solids, antioxidant properties, and enzymatic activities (**Figure 4**). Most parameters were strongly correlated, except ascorbic acid (AA), which showed only weak correlations with the others. The PCA results grouped treatments into three categories: group 1 (B0N100, UPMB10N50, UPMB10N100, UPMRB9N50) showed increases in most parameters, including DPPH and lycopene; group 2 (UPMRB9N100) was linked to TSS and DHA; and group 3 (B0N0, B0N50, UPMB10N0, UPMRB9N0) was associated only with AA.

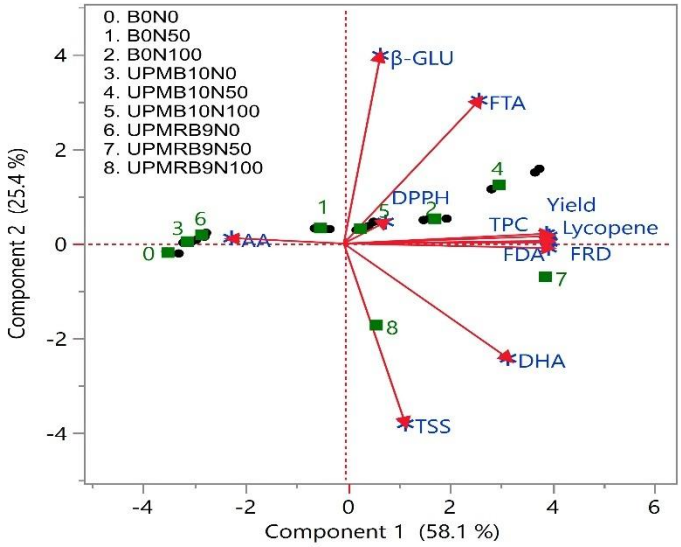

Figure 4: Principal component analysis biplot of the effects of PGPB strains application under different N levels on tomato plants.
Note: Yield (t ha^{-1}): Yield; Fruit diameter(mm): FRD; Total soluble solids (% Brix): TSS; Ascorbic acid: AA; Lycopene; Total phenolic content: TPC; DPPH scavenging activity(%): DPPH; Dehydrogenase:DHA; Acid phosphatase: FTA; Beta-glucosidase: β-GLU; FDA value: FDA

DISCUSSION

This study demonstrated that applying plant growth-promoting bacteria (PGPB) under different nitrogen levels improved tomato yield, fruit quality, antioxidant properties, and soil enzymatic activity. Among the treatments, UPMRB9 combined with 50% of the recommended nitrogen rate (UPMRB9N50) consistently produced the highest values across most parameters, while the uninoculated control recorded the lowest. These results suggest that the bacterial inoculant performed optimally when supported with moderate nitrogen, enhancing plant growth, fruit set, and overall quality. Inoculated plants outperformed uninoculated ones across all measured traits, underscoring the role of PGPB in reducing fertilizer dependence while maintaining productivity, in agreement with Aggani *et al*. (2013), who emphasized the value of integrating biological and chemical fertilizers.

Tomatoes are a valuable dietary source of ascorbic acid. In this study, ascorbic acid levels ranged from 0.580 to 0.346 mg g^{-1} FW, consistent with Ochoa-Velasco *et al*. (2016), who reported 73.85–116.79 mg kg^{-1} FW, and the Batateiro variety reported by Pinela *et al*. (2012) (108.6 ± 0.9 mg kg^{-1} FW). Increasing nitrogen reduced ascorbic acid content in uninoculated plants, with levels declining from 0.536 ± 0.003 to 0.346 ± 0.008 mg g^{-1} FW (B0N0 to B0N100). Similar reductions at high N fertilization (>270 kg ha^{-1}) have been reported by Ibrahim *et al*. (2013). Conversely, PGPB inoculation increased ascorbic acid levels, supporting findings by Bona *et al*. (2015), who observed higher vitamin C content in strawberries inoculated with PGPB under reduced fertilizer regimes.

Lycopene, a key antioxidant in tomatoes, was also enhanced by inoculation. Our findings are consistent with Verma et al. (2015), who reported a 35.52% increase in lycopene when 50% inorganic N was combined with microbial inoculants. Similarly, Ahmed *et al*. (2017) found that PGPB improved quality traits, including lycopene and phenolic content, corroborated by our PCA results.

Antioxidant activity measured through DPPH declined with higher nitrogen levels, consistent with Ibrahim *et al*. (2013), who showed that >90 kg N ha^{-1} reduced antioxidant activity in *Labisia pumila*. In contrast, organic fertilization enhanced antioxidant capacity, while PGPB inoculation increased DPPH activity, as also reported by Katsenios *et al*. (2021), who observed up to a 31.25% increase. Our findings confirm earlier reports that Bacillus strains enhance antioxidant activity in tomato (Ochoa-Velasco *et al*., 2016).

Enzyme assays provided further evidence of PGPB benefits. UPMRB9N50-treated soils showed higher dehydrogenase activity (TPF production), reflecting larger microbial populations and oxidative activity. Acid phosphatase activity (FTA) also varied significantly with PGPB treatments, confirming observations by Ruiz and Salas (2019). β-glucosidase activity responded similarly to PGPB and nitrogen interactions. These findings align with those of Sekaran *et al*. (2019), who attributed increased enzyme activity to microbial processes such as nitrogen fixation, nutrient mineralization, and the metabolism of root exudates. FDA hydrolysis confirmed greater microbial activity in UPMRB9N50 soils, followed by UPMB10N50, consistent with Sarin and Riddech (2018), who reported enhanced fertility when rhizobacteria were combined with chemical fertilizer.

Yield-related parameters also improved markedly with inoculation. UPMRB9N50 produced the highest number of flowers, fruits, fruit set percentage, and fruit weight per plant, yielding 76.34% more than the control (2.85 t ha^{-1}). These results parallel Bona *et al*. (2018), who reported increased flower and fruit production under reduced fertilization with microbial inoculation, and Katsenios *et al*. (2021), who found yield increases of 45–52% with Bacillus strains. Fruit size was similarly enhanced by inoculation, in line with Bona *et al*. (2018). Overall, the results demonstrate that combining PGPB with 50% nitrogen fertilization not only sustains tomato yield but also improves fruit quality, antioxidant levels, and soil health. This highlights PGPB's potential to reduce fertilizer inputs while promoting more sustainable tomato production.

CONCLUSION

The isolation and application of plant growth-promoting bacteria (PGPB) provide an important step toward reducing reliance on chemical fertilizers in tomato production. In this study, the two bacterial isolates tested enhanced both yield and quality attributes, with performance strongly influenced by nitrogen (N) fertilization rates. Notably, combining PGPB with 50% of the recommended N increased fruit yield by 27.27% compared to full N fertilization, underscoring the potential of microbial inoculants to sustain productivity while lowering input costs. In contrast, excessive N appeared to suppress microbial activity in the rhizosphere, reducing the effectiveness of beneficial bacteria. The findings highlight the practical, sustainable approach of integrating PGPB with reduced chemical inputs as a novel solution for tomato cultivation. Broader testing across different crops, soil types, and agro-climatic zones is needed to confirm the consistency of these results. Future research should also examine microbial adaptability under variable field conditions, formulation strategies for longer shelf-life, and interactions with other nutrient sources. Such work will be essential in advancing the commercialization of microbial inoculants and in supporting greener, more resilient farming systems.

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