PLANT GROWTH PROMOTING BACTERIA (PGPB) ENHANCE GROWTH AND YIELD OF STRAWBERRY CULTIVARS

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Abstract. A study was conducted to evaluate the effect of various plant growth promoting bacteria (PGPB) on growth, yield and physicochemical quality attributes of strawberry cultivars. Runners of three commercial strawberry cultivars, viz. 'Chandler', 'Tuft's' and 'Camarosa' were acquired from a certified runner supplier in Swat, KPK, Pakistan and were planted in 10 cm plastic pots. Three plant growth promoting bacterial isolates, viz. *Pseudomonas fluorescens* (T_1), *Bacillus subtilis* (T_2) and *Pseudomonas aeruginosa* (T_3) were applied after two weeks of runners planting with no PGPB application as control (T_0). Results revealed that plants supplied with *Bacillus subtilis* (T_2) had highest number of leaves per plant (38.33), leaf area index (41.69 cm^2), crown diameter (1.36 cm), number of runners (5.42), fruit set (86.11%), average yield (226.97 g), soluble solid contents (SSC) (8.43 ^{0}Brix), ascorbic acid (49.98 mg 100 mL^{-1}), total sugars (5.72%) and Anthocyanin contents (37.55 mg 100 mL^{-1}), while minimum plant growth and physicochemical characteristics were recorded for plants with no PGPB (control). Among cultivars, 'Chandler' responded better to PGPB application compared to two other cultivars. Therefore, use of PGPB, particularly *Bacillus subtilis*, has the potential to affect growth, yield and physicochemical quality characteristics of strawberry cultivars and may be used by strawberry growers for lowering fertilizer cost by alternating with organic PGPB to enhance the growth and yield of strawberry.
Keywords: *bacterial isolates, biochemical attributes, biofertilizer, minor fruit production*

Introduction

Strawberry (*Fragaria ananassa* Duch.) is a valuable minor fruit crop grown worldwide for its delicious fruit having excellent aroma, sweetness and attractive color (Ali et al., 2021). It is a rich source of antioxidants including anthocyanins, phenolic compounds, vitamins and sugars and can be consumed as fresh, juice and as raw material for processing industry to make jam, jelly and syrup (Ayub et al., 2010; Hanif and Budiyati, 2011). Agroclimatic conditions of Pakistan are quite suitable for strawberry cultivation (Aslam and Rasool, 2012) and it is primarily grown in Charsadda, Mansehra, Mardan, Haripur, Swat, Islamabad, Gujrat, Lahore and Karachi. Strawberry cultivars and variable use of chemical fertilizers are the major sources of variability, which affect its yield and quality. There are approximately 500 commercial strawberry cultivars worldwide, however, very few are available in Pakistan, which also need to be evaluated in various agro-climatic conditions for their adaptability in local agro-climatic zones. For successful strawberry cultivation, selection of well adapted and high yielding cultivars is of paramount importance (Galletta and Maas, 1990).

Inadequate application of chemical fertilizers is a serious threat to climate, soils and human health. Therefore, bio-fertilizers are used to protect soils from degradation and

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food contamination. Organic nutrients can increase soil enzyme activity, availability of nitrates, access to total organic carbon and soil fertility quotients (Okwuagwu et al., 2003). Improved plant nutrition by PGPB is mostly due to increased phosphorous absorption through inorganic phosphate solubilization or organic phosphate mineralization. They often release organic acids that contribute to the availability of nutrients and result in increased plant growth by taking water and mineral nutrients (Biswas et al., 2000). PGPB specifically activate growth regulators such as auxin, gibberellins, cytokinin, inorganic phosphorus solubilization and nutrient mineralization along with symbiotic N-fixation (Glick, 1995; Zahir et al., 2004). Application of PGPB improves plant growth, yield, enhances fruit shelf life, texture, and quality (Gupta and Kaushal, 2017).

PGPB have the potential to enhance the yield of important field crops. Generally, PGPB enhance plant growth and yield by synthesizing particular compounds for the plants. They facilitate the uptake of certain nutrients from the soil and protect the plants from diseases (Saravanakumar et al., 2008). Enhanced crop yield was recorded in maize, chickpea, soybean, rice, peanut, sugarcane and wheat as PGPB are able to increase agronomic efficiency by reducing production costs. They are also helpful to reduce environmental pollution, once the use of chemical fertilizers is reduced or eliminated if the inoculants are efficient (De Souza et al., 2015). Vegetable production and quality could be enhanced with supplementation of PGPB by enhancing nutrient uptake and indirect inhibition of pathogen attack during production cycle (Mekonnen et al., 2021). Tomato seedlings provided with 1% liquid PGPB significantly increased biomass, yield and helped in mitigating water deficit irrigation or enhanced water use efficiency (Le et al., 2018). Likewise, the application of PGPB combined with aqueous vermicompost extract markedly increased retaining and uptake of nutrients from the substrate employed for tomato production (Ruiz and Sanjuan, 2022). Recent study revealed that exogenous application of PGPB (*A. brasilense* DSM 2298) combined with variable doses of nitrogen (30 or 60 Kg ha^{-1}) significantly increased yield of lettuce with enhanced total phenolic concentration, ascorbic acid content, chlorophyll, soluble solid contents and total sugars (Consentino et al., 2022).

Considering the research gap, the effect of three PGPB was analyzed on three commercial cultivars grown in Pakistani climatic conditions. Therefore, present study was carried out to evaluate the effect of PGPB on growth, yield and physicochemical quality characteristics of ‘Chandler’, ‘Tuft’s’ and ‘Camarosa’ strawberry cultivars.

Methods

Experiment layout and treatments

The study was conducted at Horticulture Research Area, PMAS-Arid Agriculture University, Rawalpindi, during 2016-17. Prior to the trial, soil samples were taken and different soil physicochemical properties (pH, EC, NPK, bulk density) were estimated (*Table 1*). The runners of three strawberry cultivars, viz. ‘Chandler’, ‘Tuft’s’ and ‘Camarosa’ were sourced from a certified strawberry runner supplier from Swat, KPK, Pakistan, and planted in 10 cm plastic pots. Plants were maintained in a greenhouse set at 25 ± 3°C, no additional fertilizer was applied to the plants except PGPB and were irrigated according to weather conditions and plant requirement based on plant growth stage. Three Plant Growth Promoting Bacterial isolates, viz. *Pseudomonas fluorescens* (T_1), *Bacillus subtilis* (T_2) and *Pseudomonas aeruginosa* (T_3) were applied at 10^6

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$CFU \cdot mL^{-1}$ after two weeks of runners planting. 5 mL of the bacterial suspensions were used to inoculate each treated plant after 15 days of transplanting. Plants receiving no PGPB were considered as control (T_0) and same amount of buffer was provided to uninoculated plants.

Table 1. *Physicochemical characteristics, viz. pH, EC, NPK, and bulk density of soil samples in study area. Means are averages of three samples*

Treatments	pH	EC (dS m^{-1})	N	P	K	Bulk density
Control (no PGPB) (T_0)	6.3 a[a]	0.96 a	4.36 b	2.36 b	89.23 c	0.94 a
Pseudomonas fluorescens (T_1)	5.9 b	0.88 a	5.80 a	2.33 b	105.33 b	1.01 a
Bacillus subtilis (T_2)	5.9 b	0.48 b	4.16 b	3.23 a	138.26 a	0.64 b
Pseudomonas aeruginosa (T_3)	5.0 b	0.54 b	4.13 b	3.86 a	112.4 b	0.73 b
Mean	5.77	0.71	4.61	2.94	111.30	0.83

[a]Means within a column followed by the same letter are not significant at $P \leq 0.05$

Physical parameters

Plant physical parameters, viz. number of leaves per plant, leaf area index (cm^2), crown diameter (cm), number of runners per plant, plant biomass (g) measured by taking recording both fresh and dry weight, number of flowers per truss, number of trusses per plant, number of flowers per plant, flower diameter (cm), days to flower induction, fruit set (%), number of fruits per plant, fruit weight (g), fruit yield per plant (g) were measured by multiplying average fruit weight and number of fruits per plant. Biomass was measured by taking strawberry plants fresh and dry weights. Plant biomass was measured at the end of harvesting season.

Leaf area index was measured by ADC area AM-100 in which 10 leaves per plant were taken for average estimation and average was recorded from each replication for data analysis. Leaf area index was performed after harvesting the fruits. Formula for calculation of leaf area index is as under;

$$\text{Leaf area index} = \text{Leaf area } (m^2) / \text{Ground area } (m^2)$$

Biochemical analysis of fruit

Soluble solid contents (SSC) of fruit juice was determined by handheld Refractometer (ATAGO, RS-5000 Atago, Japan). Titrable acidity (TA) of the juice was calculated by taking 10 mL of juice in 100 mL conical flask, which was diluted up to 50 mL with distilled water and titrated against 0.1 N NaOH using 2 to 3 drops of phenolphthalein as an indicator till pink color end point was achieved and TA was expressed as percentage (%). Ascorbic acid contents of fruit juice were determined by the method described by Ruck (1969). Strawberry juice @ 10 mL was added to 0.4% oxalic acid solution in 100 mL volumetric flask. Then 5 mL of diluted and filtrated aliquot was titrated against 2, 6–dichlorophenolindophenol dye, to light pink color end point. Juice was extracted by squeezing the fruits using muslin cloth manually. Sugars in juice (extracted through squeezing) were estimated following the method of Hortwitz (1960) in which 10 mL juice was taken in 250 mL volumetric flask and diluted with 100 mL water, 25 mL 25% lead acetate solution and 10 mL 20% potassium oxalate.

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Then the volume was made with distilled water. The filtrate was used for the estimation of different forms of sugars. Sugars were expressed as percentage.

Anthocyanin content

Anthocyanin concentration (mg pf-3-GLE/100 g FW) from fruit juice was measured by the pH differential method (Giusti and Wrolstad, 2001) in spectrophotometer Plasma spectrophotometer Inductively Couple (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT, USA) with distilled water and determining λ max (510 nm and 700 nm), two dilutions (A) and (B) of the extracts were prepared with different buffers. One (A) was prepared by adding 0.4 mol of extract and 3.6 mol of potassium chloride buffer 0.025 M, (pH 1.0 to 4.0) mol cuvettes. The second dilution (B) was prepared in the same way with the addition of sodium acetate buffer (0.4 M, pH 4.5). The dilutions were allowed to equilibrate for 20 min and measurements immediately followed. The reading of distilled water was found to have no difference in relation to buffer. The absorbance of each diluted sample was measured at 510 nm and 700 nm.

Statistical analysis

The experimental layout was completely randomized design with factorial arrangement of treatments replicated three times. Data were analyzed using analysis of variance (ANOVA) and general linear models procedures of SAS (version 9.3, SAS Inst., Inc., Cary, NC, USA) and Fisher's LSD at $P \leq 0.05$ was used to separate means (Steel et al., 1997).

Results and discussion

Leaf NPK contents (%)

Highest NPK was recorded in 'Chandler' (4.93 ± 0.09, 0.57 ± 0.09, 2.37 ± 0.19) followed by 'Tuft' (4.53 ± 0.27) when treated with *Bacillus subtilis* (T_2) (*Fig. 1*). Improved NPK status in 'Chandler' leaves was might be due to more nitrogen fixation, phosphate Solubilization and potassium availability which was facilitated by PGPB. Leaf NPK uptake in strawberry is supported by earlier findings of Consentino et al. (2022), which reported that more nutrient uptake was noted in lettuce which were treated with *A. brasilense* DSM 2298.

Number of leaves per plant and leaf area index (cm^2)

Results revealed that PGPB inoculation enhanced number of leaves per plant. Maximum number of leaves (38.33 ± 0.88) were observed in 'Chandler' followed by 'Camarosa' (36.00 ± 0.58) and 'Tuft' (33.00 ± 1.15), when supplied with *Bacillus subtilis* (T_2) (*Fig. 2*). Increased number of leaves were observed in maize, wheat and pigeon pea by Tilak and Reddy (2006). For leaf area index of strawberry, results depicted significant results in which *Bacillus subtilis* (T_2) produced greatest leaf area index (41.69 ± 0.89) following *Pseudomonas aeruginosa* (T_3) (39.48 ± 0.47) and *Pseudomonas fluorescens* (T_1) (32.85 ± 0.81). Similar findings were observed in earlier reports that PGPB application increased plant growth for lettuce (Consentino et al., 2022), *Agave americana* (La-Torre-Ruiz et al., 2016) and strawberry (Esitken et al., 2010).

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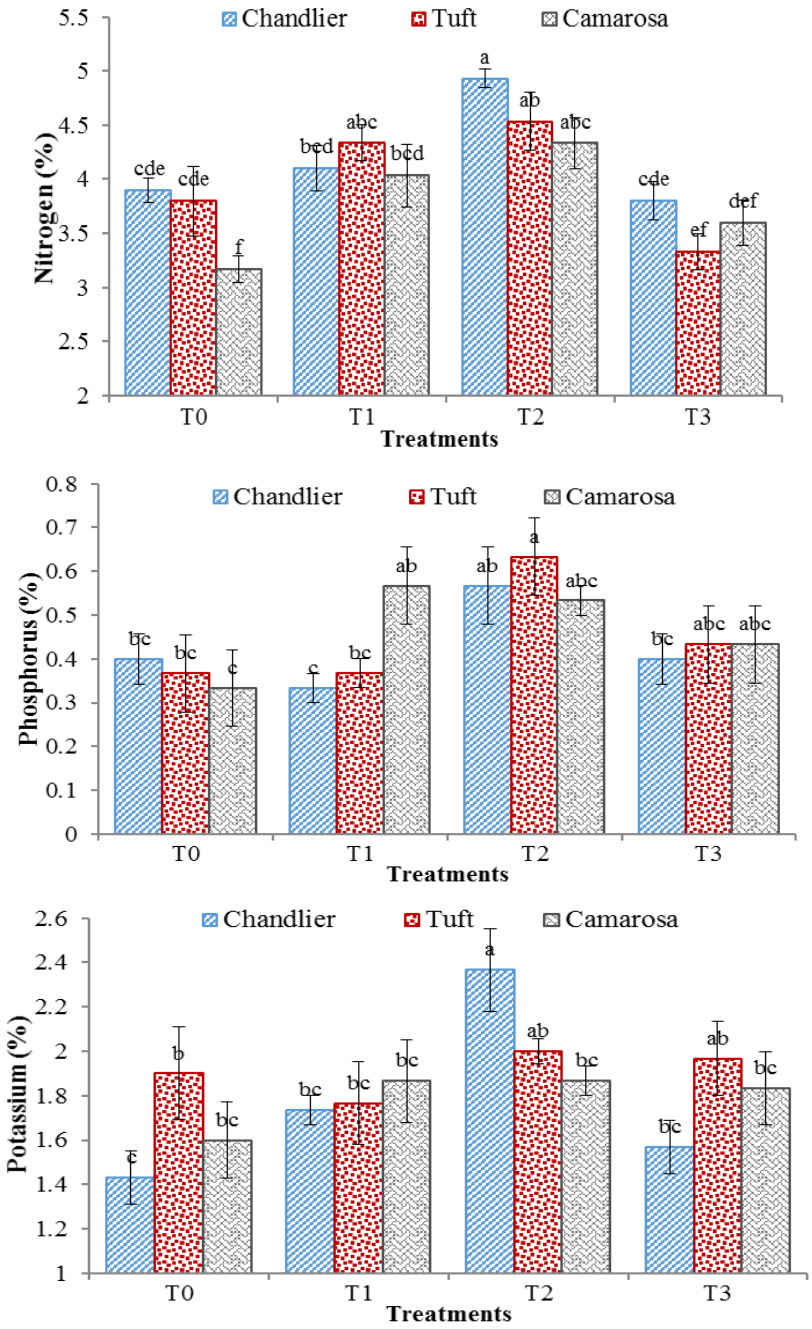

Figure 1. *Effect of various plant growth promoting bacteria (PGPB) on leaf NPK contents of strawberry cvs. Chandler, Tufts and Camarosa. Vertical bars indicate means ± SE. n = 3*

Crown diameter (cm), number of runners and plant biomass (g)

Data regarding crown diameter exhibited greatest crown diameter (1.36 ± 0.10) in 'Chandler' followed by 'Tuft' (1.32 ± 0.05) and 'Camarosa' (1.09 ± 0.04). However, plants subjected with T_2 (*Bacillus subtilis*) produced maximum crown diameter (1.54 ± 0.08) followed by *Pseudomonas aeruginosa* (T_3) (1.31 ± 0.05) and *Pseudomonas fluorescens* (T_1) (1.20 ± 0.05) (*Fig. 3*). Patten and Glick (2002) reported

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that various PGPRs that produce GA, IAA, cytokinin and other plant hormones, play an important role in production of plant material, stimulate plant cell elongation and cell break. In addition, highest number of runners (5.42 ± 0.0.57) were produced in 'Chandler' following 'Tuft' (4.00 ± 0.56) and 'Camarosa' (3.67 ± 0.68). Plants supplied with *Bacillus subtilis* (T_2) yielded greatest number of runners (7.22 ± 0.40) followed by *Pseudomonas aeruginosa* (T_3) (4.56 ± 0.50) and *Pseudomonas fluorescens* (T_1) (3.00 ± 0.41). Lowest number of runners were recorded in control (T_0) (2.67 ± 0.33). Plants supplied with *Bacillus subtilis* (T_2) demonstrated highest plant biomass (120.78 ± 1.74) followed by *Pseudomonas aeruginosa* (T_3) (117.33 ± 0.78) and Control (T_0) (116.00 ± 1.48). 'Chandler' weighed greatest plant biomass (127.00 ± 1.73) followed by 'Tuft' (118.33 ± 1.20) and 'Camarosa' (117.00 ± 1.53) when supplied with *Bacillus subtilis* (T_2) (*Fig. 3*). This is due to P solubilization which was enhanced by *Bacillus* spp. (Egamberdiyeva, 2005).

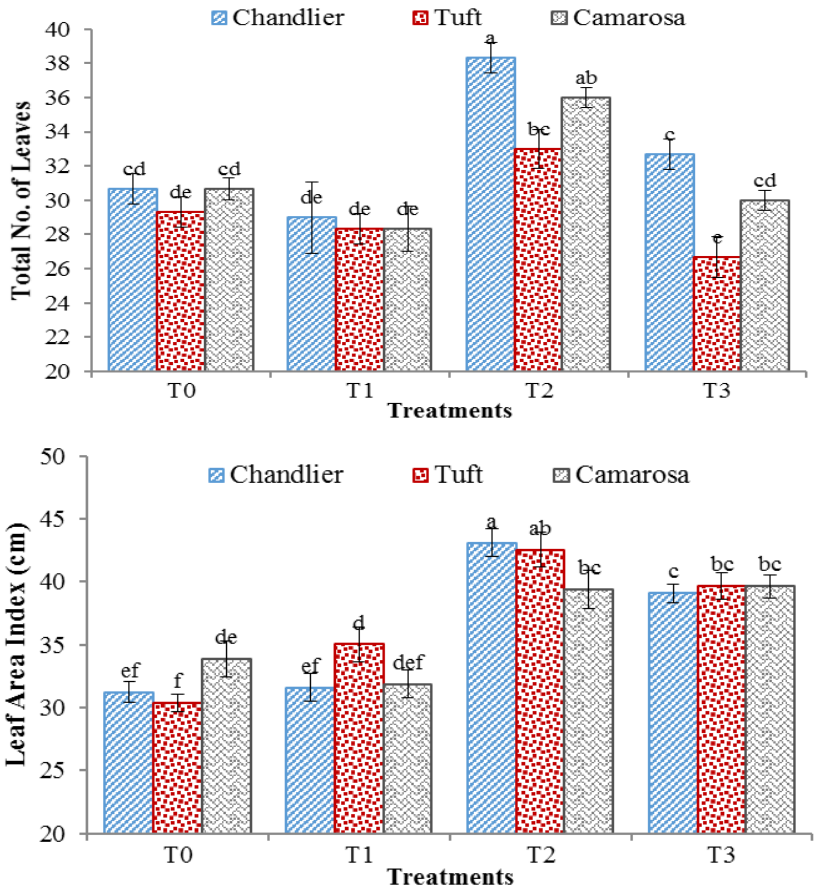

Figure 2. *Total number of leaves and leaf area index of strawberry cvs. Chandler, Tufts and Camarosa treated with various plant growth promoting bacteria (PGPB). Vertical bars indicate means ± SE. n = 30*

Days to flower induction and number of flowers per truss

Strawberry plants grown with *Pseudomonas aeruginosa* (T_3) initiated flowers in least time (99.33 ± 0.83 days) followed by *Bacillus subtilis* (T_2) (99.67 ± 1.24) and *Pseudomonas fluorescens* (T_1) (101.22 ± 0.89), which were statically at par (*Fig. 4*).

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Among cultivars, 'Chandler' flowered earlier after 95.33 ± 0.88 days of planting followed by 'Tuft' (101.00 ± 1.15) and 'Camarosa' (102.67 ± 1.20) with *Bacillus subtilis* (T_2). However, longest time (107.33 ± 0.67 days) were taken by 'Camarosa' and 105.67 ± 1.20 days by 'Tuft' for flower induction. Similar results were also reported by Kurokura et al. (2017). Maximum number of flowers (4.00 ± 0.62) per truss were recorded in 'Chandler' following 'Tuft' (3.42 ± 0.43) and 'Camarosa' (3.08 ± 0.42). Plants supplied with *Bacillus subtilis* (T_2) had highest number of flowers (5.44 ± 0.53) per truss followed by *Pseudomonas aeruginosa* (T_3) (3.56 ± 0.41) and control (T_0) (2.67 ± 0.37). Least number of flowers per truss were recorded in plants supplied with *Pseudomonas fluorescens* (T_1) (2.33 ± 0.33). Among cultivars, 'Chandler' had highest number of flowers (7.00 ± 0.58) per truss following 'Tuft' (5.00 ± 0.58) and 'Camarosa' (4.33 ± 0.88) when grown with *Bacillus subtilis* (T_2). 'Camarosa' had least number of flowers (2.00 ± 0.58) per truss as compared to 'Tuft' (2.33 ± 0.88) and 'Chandler' (2.67 ± 0.33) when supplied with *Pseudomonas fluorescens* (T_1).

Total number of flowers, flower diameter (cm) and number of trusses per plant

Highest number of flowers (33.08 ± 2.25) were recorded in 'Chandler' followed by 'Tuft' (28.67 ± 2.05) and 'Camarosa' (28.00 ± 2.39). Plants grown with *Bacillus subtilis* (T_2) had highest number of flowers (37.56 ± 1.31) followed by *Pseudomonas aeruginosa* (T_3) (36.22 ± 0.68) and *Pseudomonas fluorescens* (T_1) (24.33 ± 1.52) (*Fig. 5*). Rahman and Islam (2019) stated that PGPB applied to strawberry improved total number of flowers. Maximum flower diameter (1.88 ± 0.14) was recorded in 'Chandler' followed by 'Camarosa' (1.73 ± 0.07) and 'Tuft' (1.65 ± 0.10). Plants supplied with *Bacillus subtilis* (T_2) produced maximum flower diameter (2.12 ± 0.14) followed by *Pseudomonas aeruginosa* (T_3) (1.78 ± 0.08) and *Pseudomonas fluorescens* (T_1) (1.66 ± 0.09). Minimum flower diameter was recorded in plants without PGPB application (Control) (1.45 ± 0.06) (*Fig. 5*). Among cultivars, 'Chandler' produced maximum flower diameter (2.61 ± 0.08) followed by 'Tuft' (1.96 ± 0.11) and 'Camarosa' (1.80 ± 0.21) when supplied with *Bacillus subtilis* (T_2). Highest number of trusses (6.75 ± 0.35) were recorded in 'Chandler' followed by 'Tuft' (5.08 ± 0.34) and 'Camarosa' (4.50 ± 0.36). 'Chandler' showed maximum number of trusses (8.00 ± 0.58) followed by 'Tuft' (6.00 ± 0.58) and 'Camarosa' (3.33 ± 0.33) when supplied with *Pseudomonas fluorescens* (T_1) (*Fig. 5*). 'Camarosa' had minimum number of trusses (3.33 ± 0.33) compared to 'Tuft' (6.00 ± 0.58) and 'Chandler' (8.00 ± 0.58) when grown with *Bacillus subtilis* (T_2). Among PGPB, *Pseudomonas fluorescens* proved a best performing treatment in respect to emergence of flowers and fruit trusses. Bhattacharyya and Jha (2012) reported that PGPB application can increase flower trusses and root growth.

Fruit parameters

Fruit set (%) and average number of fruits per plant

Application of *Bacillus subtilis* (T_2) exhibited highest fruit set percentage (86.11 ± 0.87) followed by *Pseudomonas aeruginosa* (T_3) (85.87 ± 0.25) and *Pseudomonas fluorescens* (T_1) (85.78 ± 0.72), which were statistically at par (*Fig. 6*). Minimum fruit set percentage was recorded in plants which were grown without any PGPB application (control) (71.37 ± 2.49). Among cultivars, 'Chandler' had highest fruit set percentage (88.50 ± 1.19) followed by 'Tuft' (85.20 ± 1.10) and 'Camarosa' (84.63 ± 1.43), when supplied with *Bacillus subtilis* (T_2). Mena-Violente and Olade-

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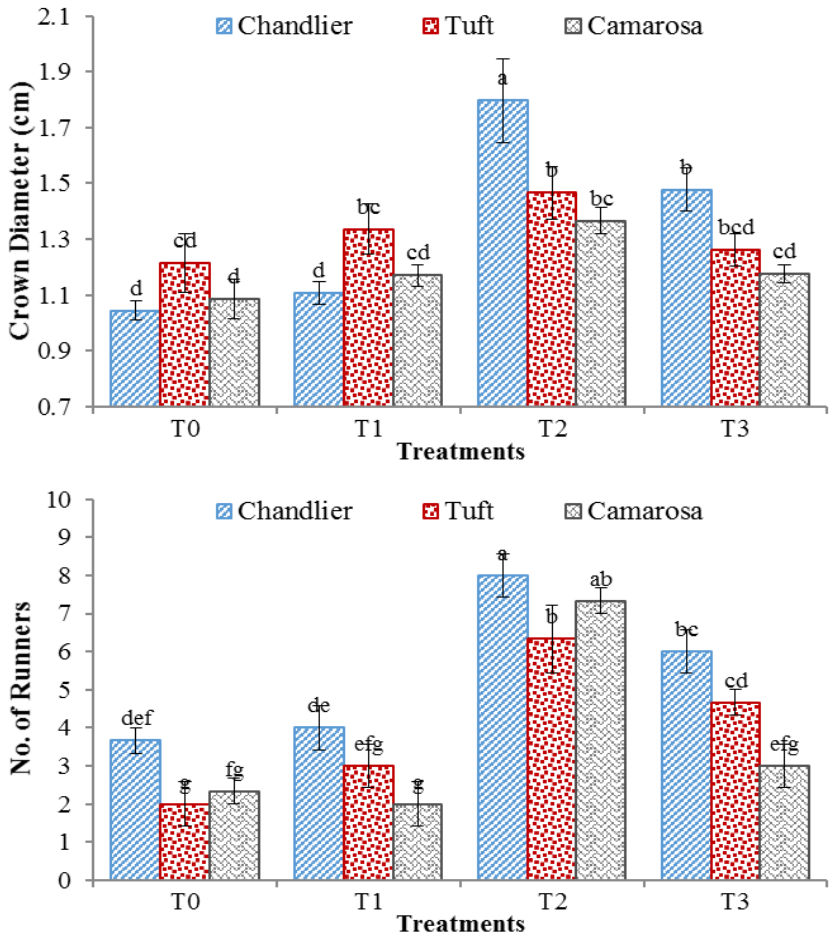
Portugal (2007) obtained similar results and found that the addition of *Bacillus subtilis* improved tomato yield. Maximum average number of fruits (27.25 ± 2.7) were also recorded in 'Chandler' followed by 'Tuft' (23.83 ± 2.15) and 'Camarosa' (23.25 ± 2.23). Bacterial isolate *Bacillus subtilis* (T_2) produced maximum average number of fruits (32.89 ± 1.51) followed by *Pseudomonas aeruginosa* (T_3) (31.22 ± 0.68) and *Pseudomonas fluorescens* (T_1) (19.67 ± 1.39) (*Fig. 6*).

Fruit weight (g) and average yield (g)

Maximum fruit weight (9.70 ± 0.51) was recorded in 'Camarosa' followed by 'Tuft' (9.25 ± 0.66), while 'Chandler' had lowest fruit weight (8.77 ± 0.90). Among PGPB, *Bacillus subtilis* (T_2) had greatest fruit weight (11.18 ± 0.82) followed by *Pseudomonas aeruginosa* (T_3) (9.64 ± 0.45) and *Pseudomonas fluorescens* (T_1) (9.31 ± 0.77) (*Fig. 7*). Raspberry using *Bacillus* M3 and *Bacillus* OSU-142 produced maximum fruit weight, yield and fruit mineral contents. Mena-Violente and Olade-Portugal (2007) found that the introduction of *Bacillus subtilis* improved the weight of tomatoes. Maximum average yield (263.19 ± 49.74) recorded in 'Chandler' followed by 'Camarosa' (226.97 ± 25.07) and 'Tuft' (226.74 ± 31.18). Among PGPBs, *Bacillus subtilis* (T_2) produced greatest average yield (376.86 ± 41.16) followed by *Pseudomonas aeruginosa* (T_3) (299.76 ± 15.10) and *Pseudomonas fluorescens* (T_1) (174.10 ± 6.25) (*Fig. 7*). Lowest average yield was recorded in plants with no PGPB (control) (104.15 ± 6.25).



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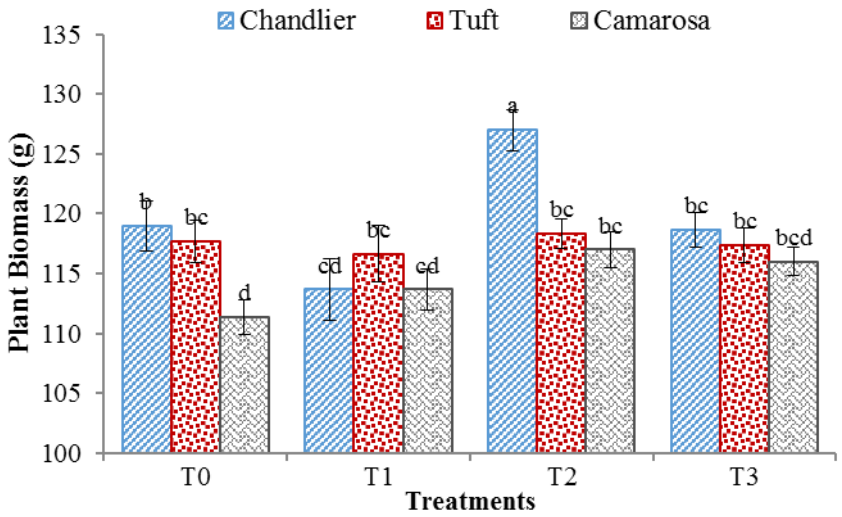

Figure 3. *Crown diameter, number of runners and plant biomass data of strawberry cvs. Chandler, Tufts and Camarosa treated with various plant growth promoting bacteria (PGPB). Vertical bars indicate means ± SE. n = 30*

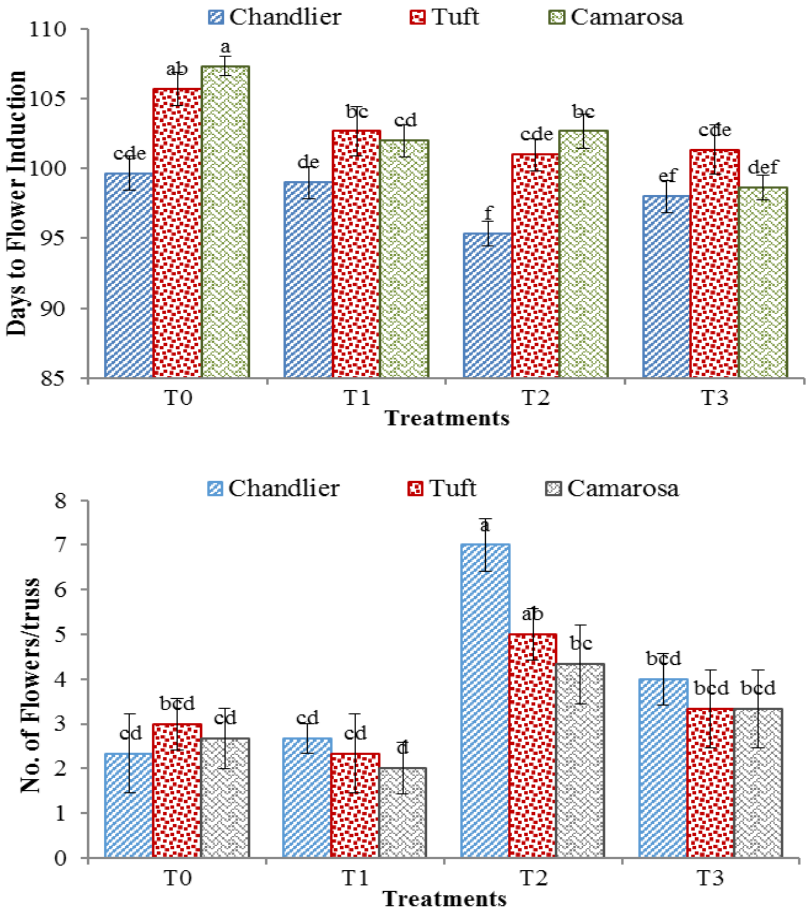

Figure 4. *Days to flower induction and number of flower per truss data of strawberry cvs. Chandler, Tufts and Camarosa treated with various plant growth promoting bacteria (PGPB). Vertical bars indicate means ± SE. n = 30*

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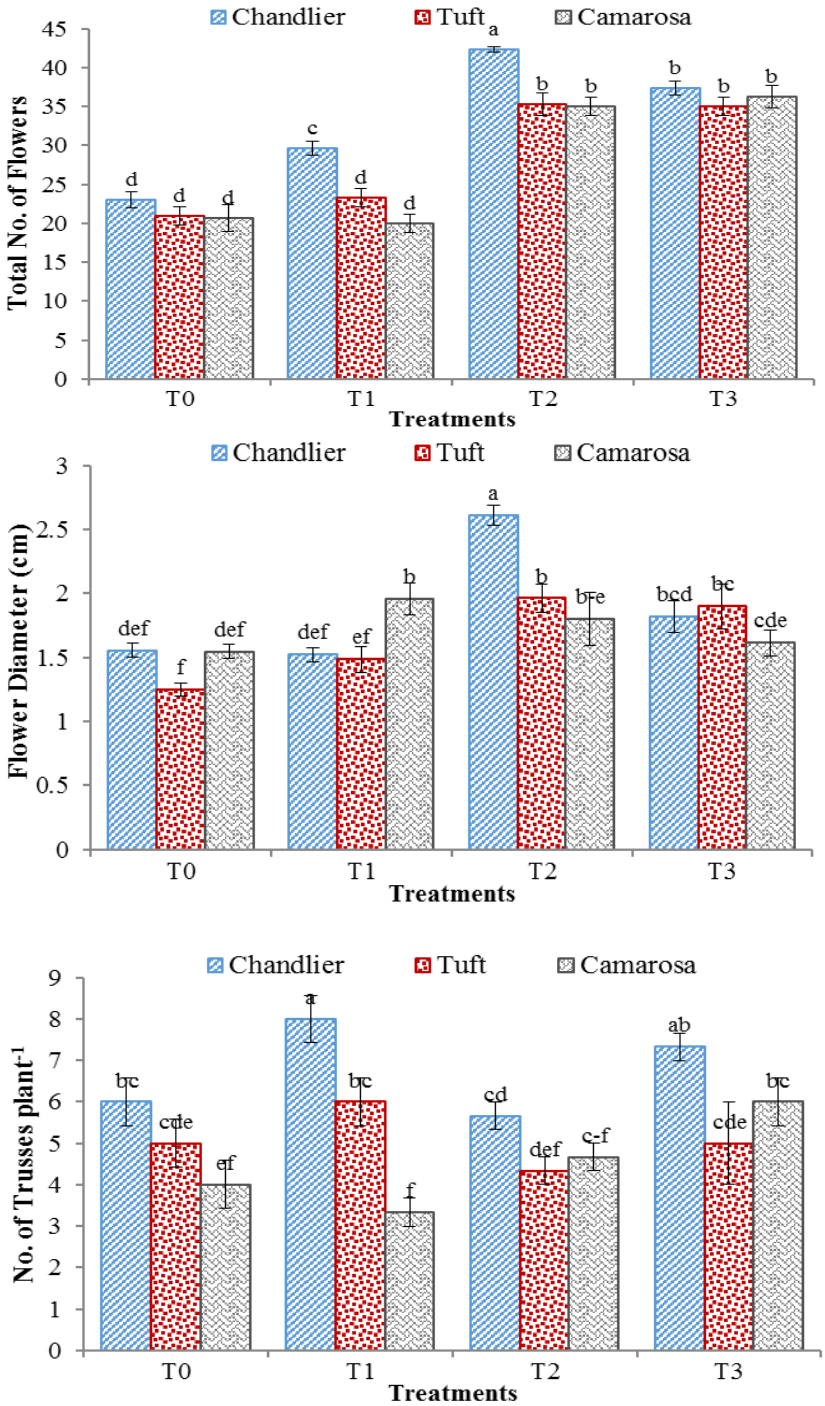

Figure 5. *Total number of flowers, flower diameter (cm) and number of trusses plant^{-1} data of strawberry cvs. Chandler, Tufts and Camarosa treated with plant growth promoting bacteria (PGPB). Vertical bars indicate ± SE of means. n = 3 replicates*

Biochemical analysis of fruit

Soluble solid contents (^{o}Brix)

Maximum total soluble solid contents (8.43 ± 0.45) were recorded in fruit of 'Chandler' followed by 'Tuft' (8.38 ± 0.45) and 'Camarosa' (7.64 ± 0.28). Among

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PGPB, *Bacillus subtilis* (T_2) had highest soluble solid contents (9.35 ± 0.34) followed by *Pseudomonas fluorescens* (T_1) (8.35 ± 0.49) and *Pseudomonas aeruginosa* (T_3) (8.20 ± 0.30) (*Fig. 8*). Consentino et al. (2022) reported that PGPB application markedly increased total soluble solids (TSS) and total sugars in lettuce which is in-line with our finding in strawberry fruits.

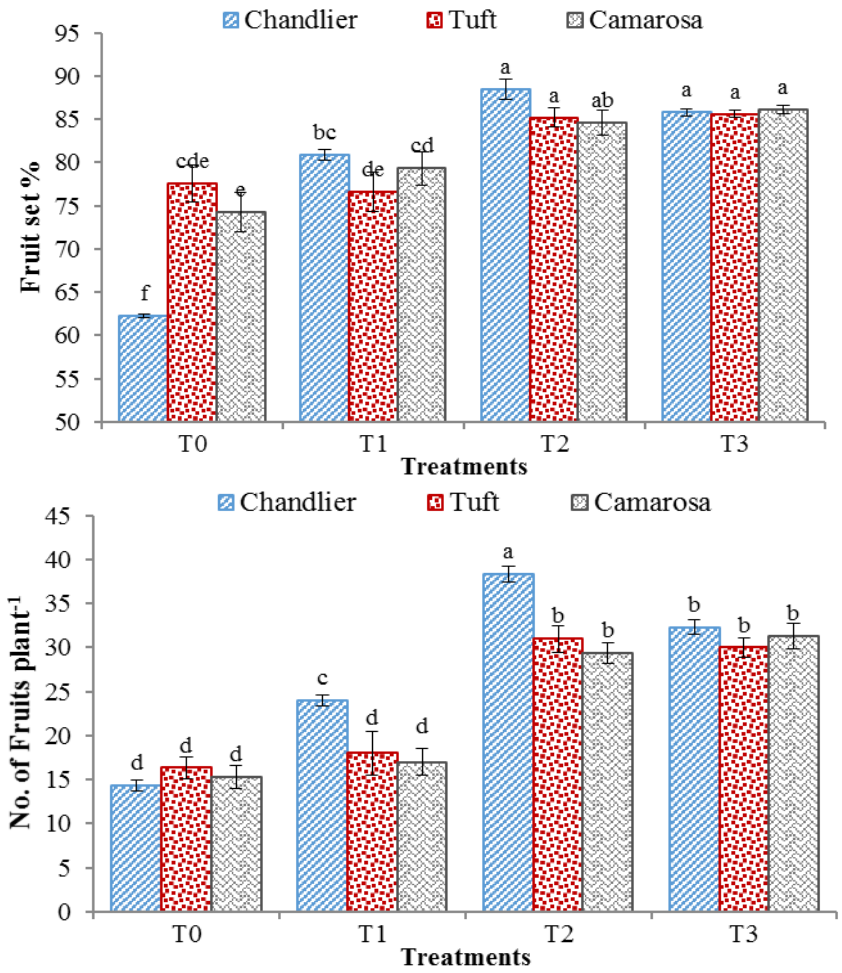

Figure 6. *Fruit set (%) and number of fruits plant^{-1} data of strawberry cvs. Chandler, Tufts and Camarosa treated with plant growth promoting bacteria (PGPB). Vertical bars indicate ± SE of means. n = 3 replicates*

Titrable acidity (%)

Plants supplied with *Pseudomonas fluorescens* (T_1) produced fruits with lowest titrable acidity as compared to other PGPB supplied plants. Among cultivars, 'Chandler' fruit had lowest titrable acidity (0.72 ± 0.06) as compared to 'Camarosa' (0.72 ± 0.07) and 'Tuft' (0.76 ± 0.0.04) (*Fig. 8*).

TA/TSS ratio

Minimum TA/TSS ratio (9.48 ± 0.24) was recorded in 'Camarosa' fruit followed by 'Chandler' (10.21 ± 0.56) and 'Tuft' (10.56 ± 0.45). Fruits of strawberry cultivars which were grown without any PGPB had lowest TA/TSS ratio (8.47 ± 0.27) followed by

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Pseudomonas aeruginosa (T_3) (10.06 ± 0.48) and *Bacillus subtilis* (T_2) (10.68 ± 0.48). Among cultivars, 'Chandler' fruit had highest TA/TSS ratio (12.22 ± 0.71) followed by 'Tuft' (9.93 ± 0.49) and 'Camarosa' (9.89 ± 0.50) when supplied with *Bacillus subtilis* (T_2) (*Fig. 8*).

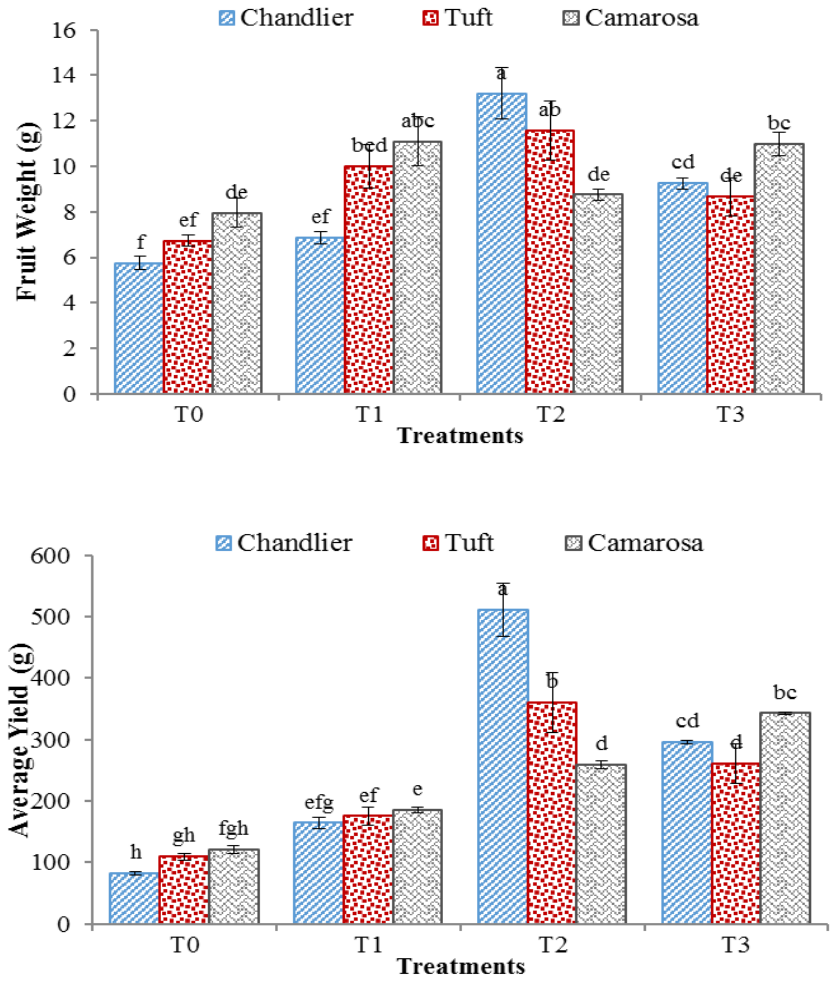

Figure 7. *Fruit weight (g) and average yield (g) data of strawberry cvs. Chandler, Tufts and Camarosa treated with plant growth promoting bacteria (PGPB). Vertical bars indicate ± SE of means. n = 3 replicates*

Ascorbic acid (mg 100 mL^{-1})

Plants grown with application of *Bacillus subtilis* (T_2) had highest ascorbic acid (57.41 ± 1.21) followed by *Pseudomonas fluorescens* (T_1) (53.32 ± 1.17) and *Pseudomonas aeruginosa* (T_3) (51.75 ± 0.62) (*Fig. 9*). Among cultivars, 'Chandler' fruit had lowest ascorbic acid (49.98 ± 1.30) compared to 'Tuft' (52.83 ± 0.64) and 'Camarosa' (56.82 ± 1.64). Ascorbic acid is known as one of the bioactive compounds also stated as non-enzymatic antioxidant which has imperative position in fruits (Hasan et al., 2021). Our results of higher ascorbic acid contents in strawberry fruits harvested from PGPB treated plants are also supported by finding of Consentino et al. (2022) in lettuce grown under application of PGPB with varying doses of nitrogen exhibited higher ascorbic acid content as compared to control.

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Total sugars (%)

Highest total sugars (5.72 ± 0.13) were recorded in 'Chandler' fruit followed by 'Camarosa' (5.57 ± 0.11) and 'Tuft' (5.54 ± 0.12) (*Fig. 9*). Among bacterial isolates, *Bacillus subtilis* (T_2) resulted in highest total sugars (5.93 ± 0.15) followed by *Pseudomonas aeruginosa* (T_3) (5.78 ± 0.11) and no PGPB (Control) (5.44 ± 0.11).

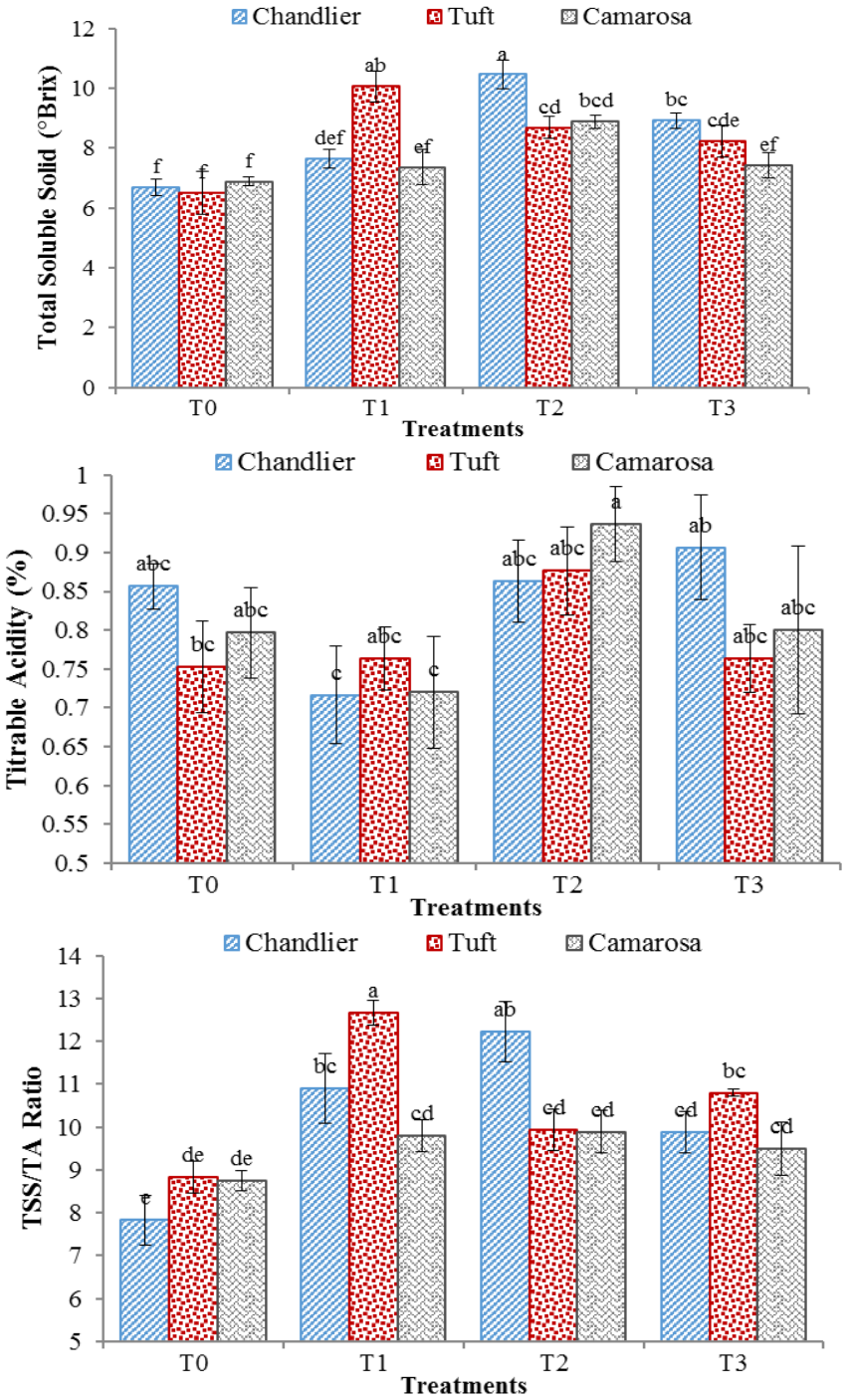

Figure 8. *Biochemical Analysis (TSS, TA, TSS/TA ratio) of fruits strawberry cvs. Chandler, Tufts and Camarosa treated with plant growth promoting bacteria (PGPB). Vertical bars indicate ± SE of means. n = 3 replicates*

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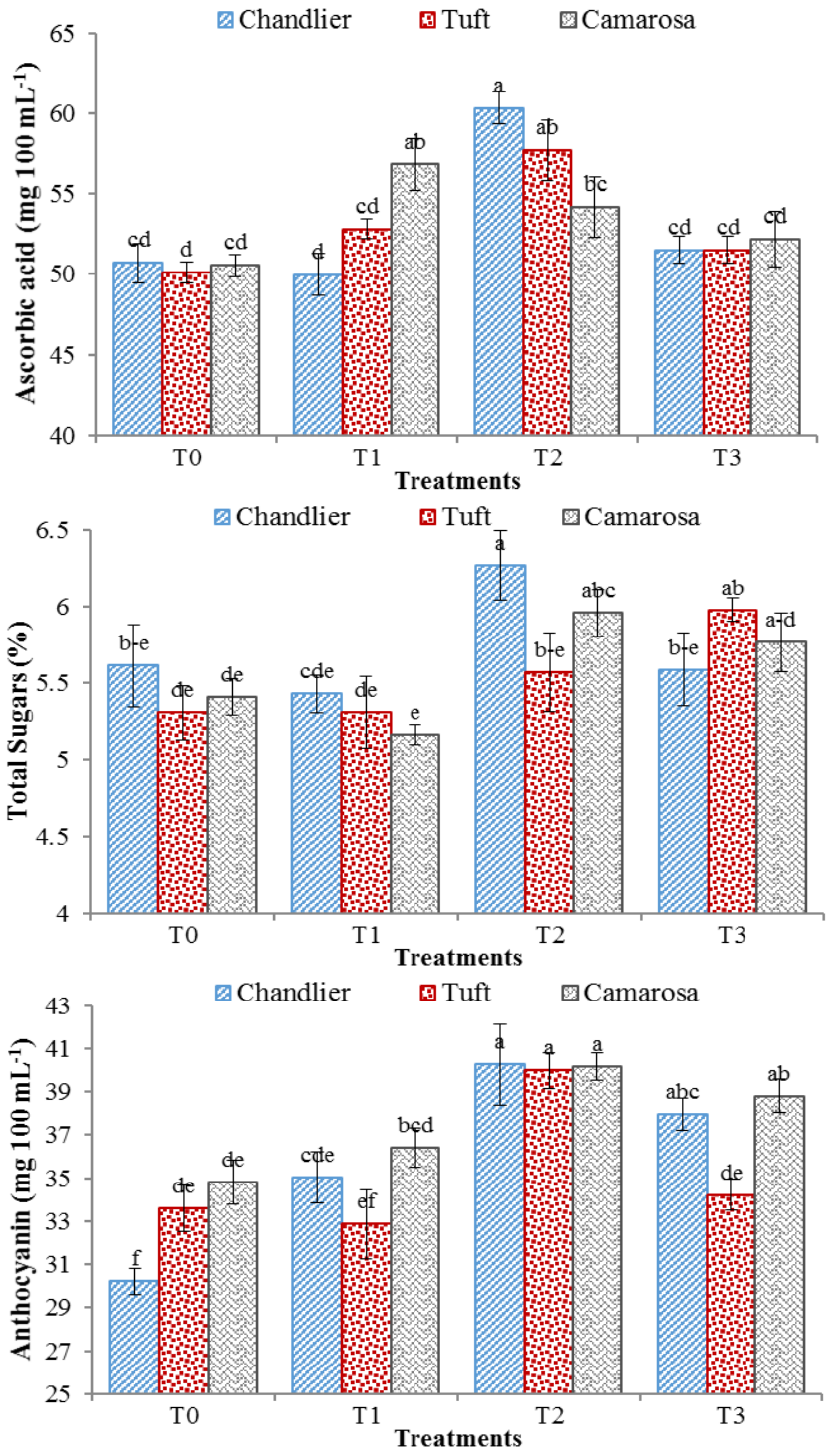

Figure 9. *Biochemical Analysis (ascorbic acid, total sugars, anthocyanin) of fruits strawberry cvs. Chandler, Tufts and Camarosa treated with plant growth promoting bacteria (PGPB). Vertical bars indicate ± SE of means. n = 3 replicates*

Anthocyanin contents (mg 100 mL^{-1})

Highest anthocyanin contents (37.55 ± 0.72) were recorded in 'Camarosa' fruit followed by 'Chandler' (35.88 ± 1.24) and 'Tuft' (35.16 ± 0.98). While, among

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bacterial isolates, plants supplied with *Bacillus subtilis* (T_2) had highest anthocyanin contents (40.14 ± 0.62) followed by (*Pseudomonas aeruginosa* (T_3) (37.00 ± 0.80) and *Pseudomonas fluorescens* (T_1) (34.77 ± 0.82) (*Fig. 9*). Minimum anthocyanin contents were recorded in plants with no PGPB application (control) (32.87 ± 0.83). Red colored berries contains more anthocyanin contents which might be affected in different production and postharvest conditions (Ali et al., 2016). PGPB treated strawberry cultivars enhanced anthocyanin contents in addition to the increased production, and has been found similar as earlier revealed by Lingua et al. (2013) who stated that arbuscular mycorrhizal (AM) fungi in combination with *Pseudomonas* strains displayed enhanced anthocyanin contents in strawberry fruits.

Conclusion

In summary, PGPB significantly improved growth and resulted in better performance of strawberry cultivars. Among tested PGPBs, *Bacillus subtilis* proved best for enhancing performance of strawberry, while among cultivars, 'Chandler' is best one having higher yield of quality fruit. Therefore, PGPBs may be used for commercial production of 'Chandler' strawberry for lowering its fertilizer requirements and improving yield and quality.

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