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Comparative Analysis of Various Strains of Plant Growth Promoting Rhizobacteria on the Physiology of Garlic (*Allium sativum*)

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ABSTRACT

Garlic is a most important medicinal herb belonging to the family *Liliaceae*. Both its leaves and bulb are edible. The current study was based on evaluating the growth promoting potential of plant growth promoting rhizobacteria (PGPR) on garlic (*Allium sativum* L.) growth and biochemical contents. Garlic cloves were inoculated with 3 kinds of PGPRs, *Pseudomonas putida* (KX574857), *Pseudomonas stutzeri* (Kx574858) and *Bacillus cereus* (ATCC14579) at 10^8 cells/mL prior to sowing. Under natural conditions, plants were grown in the net house. The PGPR significantly enhanced % germination, leaf and root growth and their biomass also increased the diameter of bulb and fresh and dry weight. The flavonoids, phenolics, chlorophyll, protein and sugar content were also significantly increased due to PGPR inoculation. The *Pseudomonas stutzeri* was found most effective for producing longer leaves with moderate sugar, high flavonoids (129%) and phenolics (263%) in bulb over control (Tap). The *Pseudomonas putida* exhibited a maximum increase in bulb diameter and bulb biomass with maximum phenolics and flavonoid contents.

1. INTRODUCTION

Allium sativum L. a spice and flavoring agent for food also has some medicinal benefits. Garlic is cultivated all over the world, and it produces hermaphrodite flowers and a compound bulb with fine leaves. The leaves, flowers and cloves of garlic containing organosulfur compounds are used in ancient times for the treatment of various diseases [1]. Asparagus plant leaf area index, yield, leaf and root weight were enhanced with PGPR and cow manure application [2]. Garlic enriches in numerous phytonutrients known

as a vital element of diet and contains thio-sulfinates and volatile sulfur compounds of medicinal value in the treatment of several disorders, including heart disease, cancer, obesity, hypercholesterolemia, disturbances of the gastrointestinal tract, cataracts, diabetes type 2 and hypertension. Garlic contains thio-sulfinates, which present the advantages to be stable to cook and pungent-free [3]. Purple garlic cultivars contain low ferulic acid and phenolic content compared to Chinese garlic cultivars and white garlic cultivars [4]. There are numerous kinds of garlic verities. Purple garlic contains low ferulic acid and phenolic content compared to Chinese and white garlic [5].

The PGPR synthesizes plant hormones, fixes atmospheric nitrogen, improves plant resistance to stress, inhibits harmful microorganism and promotes the uptake of nutrients [6]. PGPR promotes maize photosynthesis and development with their ability to synthesize indole-3-acetic acid (IAA) [7]. PGPR enhances phosphorus and nitrogen supply and controls pathogens [8]. The PGPR inoculated plants displayed yield and growth increase significantly between 29.29% and 9.6%. The PGPR application gives promising results for environmentally friendly agricultural approaches [9, 10].

The *Pseudomonas species* is hypothesized to be ideal because of having wide range of plant growth potential (Nitrifying, IAA and siderophores synthesis) [11]. This work subsidizes the management of garlic yield with bioinoculants. We systematically compared the accretion features of rhizobacteria on garlic growth and biochemical parameters.

2. MATERIALS AND METHOD

2.1. Inoculation of PGPR

The clove of garlic (Desi variety) was washed in autoclaved distilled water. Bacterial inocula of *Pseudomonas putida*, *Pseudomonas stutzeri* and *Bacillus cereus* were prepared in Luria Bertani (LB) media by inoculating with 48 h old bacterial colonies and placed in a shaking incubator. Garlic cloves were soaked in bacterial inocula for 2 h. For uninoculated plants, cloves were soaked in LB for the same time.

Treatments Detail Provided to Plants Are Shown in Table 1.

2.2. Percent Germination

Percent germination was considered as: Germinated cloves count/total cloves ×100.

2.3. Biochemical Analysis

2.3.1. Flavonoid in Leaf

Bulb and leaf flavonoids concentration was determined following the method of Zhishen and Jianming [12]. The leaf and bulb homogenates were prepared in methanol (80%) and centrifuged at 2000 rpm for 20 min. $AlCl_3$ reagent (1.5 ml) and 0.6 ml crystalline sodium acetate, liquified in 100 mL of $AlCl_3$ reagent. 1.6 ml of $AlCl_3$ reagent and 0.6 ml of water were added to the supernatant (3.5 ml). The optical density was measured at 430 nm against blank. Flavonoids were represented as mg quercetin per g leaf (mg QE/g).

Table 1. Treatments detail provided to plants.

Treatments	Details
C	Cloves soaked in a liquid broth of LB.
T1	Cloves soaked in *Pseudomonas putida* (KX574857) inocula.
T2	Cloves soaked in *Pseudomonas stutzeri* (Kx 574858) inocula.
T3	Cloves soaked in PGPR *Bacillus cereus* (ATCC14579) inocula.

2.3.2. Phenolic Content

Bulb and leaf phenolic content were determined following the method of Singleton and Jones [13] method considering Gallic acid as a standard. The aqueous extract (1 mL) of fresh leaves was homogenized with 9 mL water (deionized) and Folin-Ciocalteu reagent (1 mL), and 10 mL sodium-carbonate (7%) was further added in the mixture. The mixture was incubated at 28˚C for 90 min, optical density was noted at 765 nm. The total phenolics content was stated as mg gallic acid per g of the leaf.

2.3.3. Chlorophyll and Carotenoid Contents

Content of leaf chlorophyll *a*, *b* and carotenoid were measured by the method of Arnon [14]. Leaves (100 mg) were homogenized in 80% of acetone, incubated (5 min) at 90˚C in a water bath. The extracts were centrifuged at 3000 rpm for 15 min. The optical density of supernatant was noted for carotenoid and chlorophyll *a*, *b* contents at 480, 663 and 645 nm against acetone (80%) blank respectively.

Chlorophyll *a* (mg/g FW) $(12.7 \times OD^{663}) - (2.69 \times OD^{645}) \times$ sample volume/FW of leaves.

Chlorophyll *b* (mg/g fresh weight) $(22.9 \times OD^{645}) - (4.68 \times OD^{663}) \times$ sample volume/FW of leaves.

Total chlorophyll (mg/g fresh weight) $(12.7\ OD^{663}) - (22.9\ OD^{645})$ sample volume/FW of leaves.

Carotenoids (mg/g fresh weight) $= 4 \times OD^{480} \times$ sample volume/FW of leaves.

2.3.4. Determination of Sugars Content in Leaf and Bulb

Bulb and leaf sugars were determined by following the method of Dubois *et al.* [15] using glucose as standard. Samples of bulb and leaves were mixed in 10 mL deionized water, grinded and centrifuged at 3000 rpm for 10 min. In 0.1 mL of supernatant 1 mL of 0.5% (v/v) phenol was further supplemented. The reaction mixture was incubated at 24˚C for 2 h at room temperature and then 89% sulfuric acid (5 mL) was supplemented. The optical density was measured at 420 nm against blank.

2.3.5. Determination of Proteins Content in Leaf and Bulb

Bulb and leaf protein contents were determined following the method of Lowry *et al.* [16] considering bovine serum albumin (BSA) as a standard. 0.1 g sample of leaves and bulbs were homogenized in phosphate buffer 1 mL with a pH value of 6.4. The mixture was centrifuged for 10 min at 3000 rpm. A mixture of 50 ml of Na_2CO_3, NaOH and NaK-tartarate and 1 ml of $CuSO_{4.5}H_2O$ was homogenized with the supernatant solution (0.1 ml) and vortexed for 10 min. Incubation of Folin-phenol reagent (0.1 mL) was done for 30 min. The absorbance of samples was measured at 650 nm. Following formula used for soluble protein.

$$\text{Protein content } (\text{mg} \cdot \text{g}^{-1}) = K \text{ value} \times \text{Absorbance} \times \text{Dilution Factor}/\text{sample weight}$$

where's K value is 19.6.

2.4. Statistical Analysis

The statistical analyses of the research data were made with Statistix software, version 8.1. There are 3 replicates per treatment and 6 plants per pot. The pots were settled in a complete randomized design (CRD). The bar on the graph represents standard error while the letters (a, b and c) signify the statistical means ($p > 0.05$) of Tukey's HSD tests. Alike alphabets on the bar do not vary significantly.

3. RESULTS

3.1. Analysis of Percent Germination

The results presented in **Table 2** showed that all the inoculated plants resulted in a significant improvement in % germination. The maximum rise (83%) in germination rate was recorded in *Bacillus cereus* treatment and the least increase (42%) was recorded in *Pseudomonas stutzeri* treatment over control (C).

Table 2. Effect of PGPR on % germination, leaf number, bulb diameter (cm), length (cm), bulb weight (g) root weight (g) and leaf weight (g) of garlic under various treatments.

Treat.	% Germination	Leaf count	Bulb diameter	Length		Bulb		Root		shoot	
				Root	Shoot	FW	DW	FW	DW	FW	DW
C	6.96^{c} (±0.35)	4.68^{b} (±0.15)	0.25^{c} (±0.01)	9.76^{c} (±0.51)	36.76^{c} (±0.31)	4.43^{b} (±0.13)	0.25^{c} (±0.01)	7.97^{c} (±0.46)	0.48^{cd} (±0.54)	32.08^{c} (±0.26)	2.31^{b} (±0.16)
T1	12.74^{a} (±0.35)	5.78^{b} (±0.05)	1.04^{a} (±0.09)	27.00^{b} (±0.41)	65.00^{b} (±0.11)	18.21^{a} (±0.20)	1.04^{a} (±0.09)	18.28^{b} (±0.42)	1.54^{b} (±2.46)	63.16^{a} (±0.56)	5.47^{a} (±0.22)
T2	12.49^{a} (±0.86)	5.52^{b} (±0.01)	0.51^{b} (±0.05)	18.00^{ab} (±0.24)	76.41^{a} (±0.86)	13.16^{ab} (±2.34)	0.51^{b} (±0.05)	23.03^{a} (±0.54)	2.14^{a} (±2.11)	55.56^{a} (±1.76)	4.48^{ab} (±0.02)
T3	9.90^{b} (±0.20)	7.88^{a} (±0.01)	0.67^{ab} (±0.10)	19.63^{a} (±0.29)	59.60^{b} (±0.88)	6.52^{b} (±0.11)	0.67^{ab} (±0.10)	15.80^{b} (±0.11)	0.76^{c} (±1.48)	51.06^{b} (±1.10)	5.83^{b} (±0.33)

3.2. Analysis of Leaf Number and Bulb Diameter

The results presented in Table 2 showed that all the treatments increased leaf number and bulb diameter, the maximum increase (50%) in leaf number was recorded in *Bacillus cereus* while *Pseudomonas putida*, *Pseudomonas stutzeri* exerted no significant effect on leaf number over control (C). the maximum increase (311%) in bulb diameter was recorded in *Pseudomonas stutzeri* and the least increase (120%) resulted in *Pseudomonas putida* as compared to control (C).

3.3. Measurement of Root and Leaf Length

Root and shoot length were significantly improved in all the treatments. The maximum rise (155%) in root length resulted in *Pseudomonas stutzeri* and the least increase (42%) was shown in *Bacillus cereus* over control (C). The maximum increase (86%) resulted in *Pseudomonas putida* and the least increase (83%) was shown in *B. cereus* as compared to control.

3.4. Measurement of Bulb Fresh and Dry Weights

The significant stimulatory effect of PGPR was observed in treated plants. The maximum increase (297%) in bulb fresh weight (FW) was noted in *Pseudomonas putida* and the least increase (47%) resulted in *Bacillus cereus* treatments over control (C). The maximum increase (34.6-fold) in bulb dry weight was noted in *Pseudomonas putida* and the least increase (1.9 fold) resulted in *Bacillus cereus* over control Table 2.

3.5. Measurement of Root Fresh and Dry Weights

The results presented in Table 2 showed that all the treatments resulted in an increase in the fresh and dry weight of the root. The maximum increase (1.9 fold) in root fresh weight was recorded in *Pseudomonas stutzeri* and the least increase was observed in *Bacillus cereus* as compared to control (C). Root dry weight showed maximum increase (153%) was in *Pseudomonas stutzeri* and the least increase (58.3%) resulted in *Bacillus cereus* as compared to control.

3.6. Measurement of Shoot Fresh and Dry Weights

All the inoculated plants resulted in a noteworthy increase in the fresh and dry weight of the shoot

over control (C). Shoot fresh weight showed maximum increase (1.9 fold) was recorded in *Pseudomonas putida* and the least increase was observed in *Bacillus cereus* and *Pseudomonas putida* as compared to control (C). Soot dry weight showed maximum increase (1.5 fold) in T3 and least increase 92% noted in T2 Table 2.

The data represent the means of 3 replicates and ± represents their standard error values. Measurements were made in the vegetative phase after 3 weeks of germination. Treatment's detail: C = Cloves soaked in a liquid broth of LB, T1 = Cloves soaked in *Pseudomonas putida* (KX574857) inocula, T2 = Cloves soaked in *Pseudomonas stutzeri* (Kx 574858) inocula, T3 = Cloves soaked in PGPR *Bacillus cereus* (ATCC14579) inocula. Alphabetical letters represent the statistical means of the Tukey HSD test ($p < 0.05$) run on Statistix 8.1 software. Letters with similar alphabets do not differ significantly.

3.7. Determination of Leaf Carotenoids and Chlorophyll Contents

The results presented in Figure 1 revealed that all the treatments lead to in an increase in carotenoids and chlorophyll (*a*, *b*, total chlorophyll) content, the maximum increase (60%) in chlorophyll a was found in *Pseudomonas putida* inoculated plants while minimum increase (50%) was recorded in *Bacillus cereus* over control (C). The maximum increase (1.8%) in chlorophyll b was recorded in *P. putida* treatment and the least increase (1.5%) resulted in *B. cereus* over control. The maximum increase (50%) in total chlorophyll was recorded in *Pseudomonas putida* treatment as compared to the control. The maximum increase in carotenoid content was due to *Bacillus cereus* and the least increase (20%) was in *Pseudomonas putida* treatment over control.

3.8. Analysis of Sugar in Bulb and Leaf

All the treatments significantly increased the sugar content of bulb and leaves Figure 2. The maximum increase (40.3-fold) in bulb sugar was recorded in T1 and the minimum increase (2-fold) resulted in

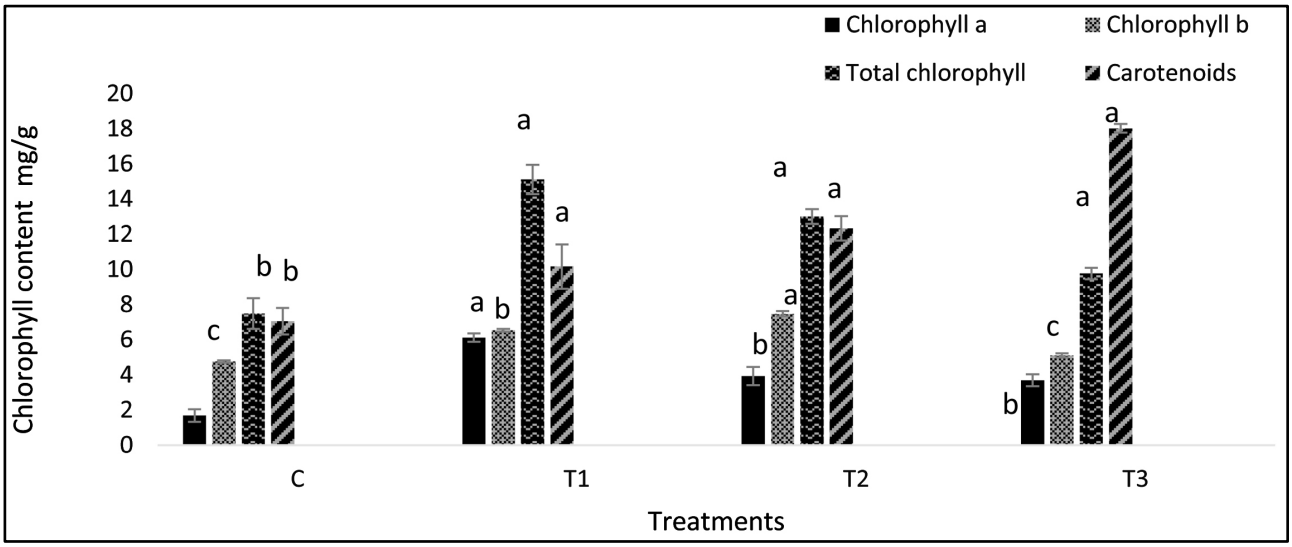

Figure 1. Effects of various strains of PGPR on carotenoids and chlorophyll content of leaves. The data represent the means of 3 replicates and ± represents standard error values. Measurements were made at the vegetative phase after 3 weeks of seed germination. Treatment's detail: C = Cloves soaked in a liquid broth of LB, T1 = Cloves soaked in *Pseudomonas putida* (KX574857) inocula, T2 = Cloves soaked in *Pseudomonas stutzeri* (Kx 574858) inocula, T3 = Cloves soaked in PGPR *Bacillus cereus* (ATCC14579) inocula. Alphabetical letters represent the statistical means of the test (Tukey HSD) $p < 0.05$ run on Statistix 8.1. Letters with alike alphabets do not vary significantly.

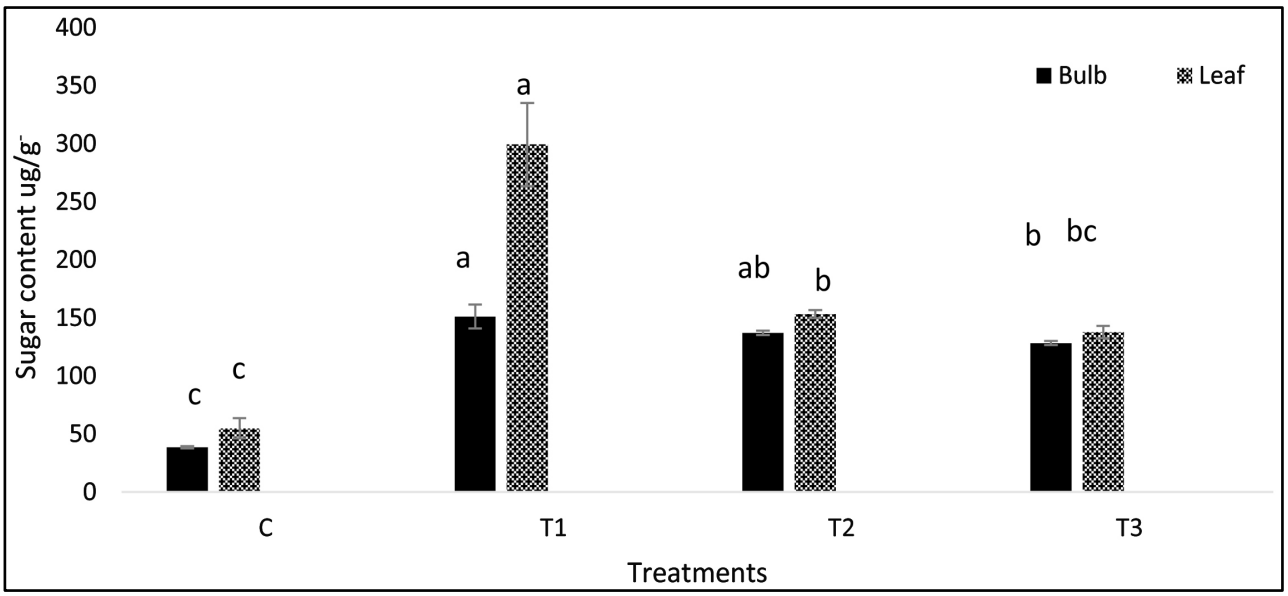

Figure 2. Effect of various strains of PGPR on the sugar content of bulb and leaves of garlic. The data represent the means of replicates (3) and ± signifies standard error values. Measurements were made after 3 weeks of seed germination (vegetative phase). Treatment's detail is given in Figure 1. Alphabetical letters represent the statistical means of the Tukey HSD test ($p < 0.05$) run on Statistix 8.1. Letters with alike alphabets do not vary significantly.

T3 = T2 over control (C). The maximum increase (54 fold) in leaves was recorded in T1 and the least increase (1.2-fold) was in T3 = T2 over control (Figure 2).

3.9. Analysis of Flavonoids Bulb and Leaves

The results presented in Figure 3 showed that all the inoculated plants showed a significant increase in flavonoid content of bulb and leaves over control (C). The maximum increase (1.2 fold) in flavonoid content of the bulb was recorded in T2 and the least increase (1 fold) was in T3 over control. The maximum increase (60%) in flavonoid content of leaves was recorded in T1 = T3 while the least increase (21%) was in the T2 treatment as compared to the control.

3.10. Analysis of Phenolics Content in Bulb and Leaves

The results presented in Figure 4 showed that all the treatments resulted in an increase in phenolics contents, the maximum increase (1.75 fold) in phenolics content of bulb was noted in T1 and the least increase (70%) was in T2 over control (C). The maximum increase (263%) in phenolics content of leaves was in T2 and the least increase (48%) was in T3 over control.

3.11. Estimation of the Protein Content in Bulb and Leaves

The results presented in Figure 5 showed all the treatments resulted in an increase in the protein content of bulb and leaves. The maximum increase (38 fold) in protein content of the bulb was recorded in T2 and the least increase (21%) was in T1 as compared to control. The maximum increase in protein content of leaves was recorded in T1 and the least increase was in T3 over control.

3.12. Analysis of Leaves Condition under Field

The results presented in Figure 5 showed that T1 (*Pseudomonas putida* inoculated garlic) showed

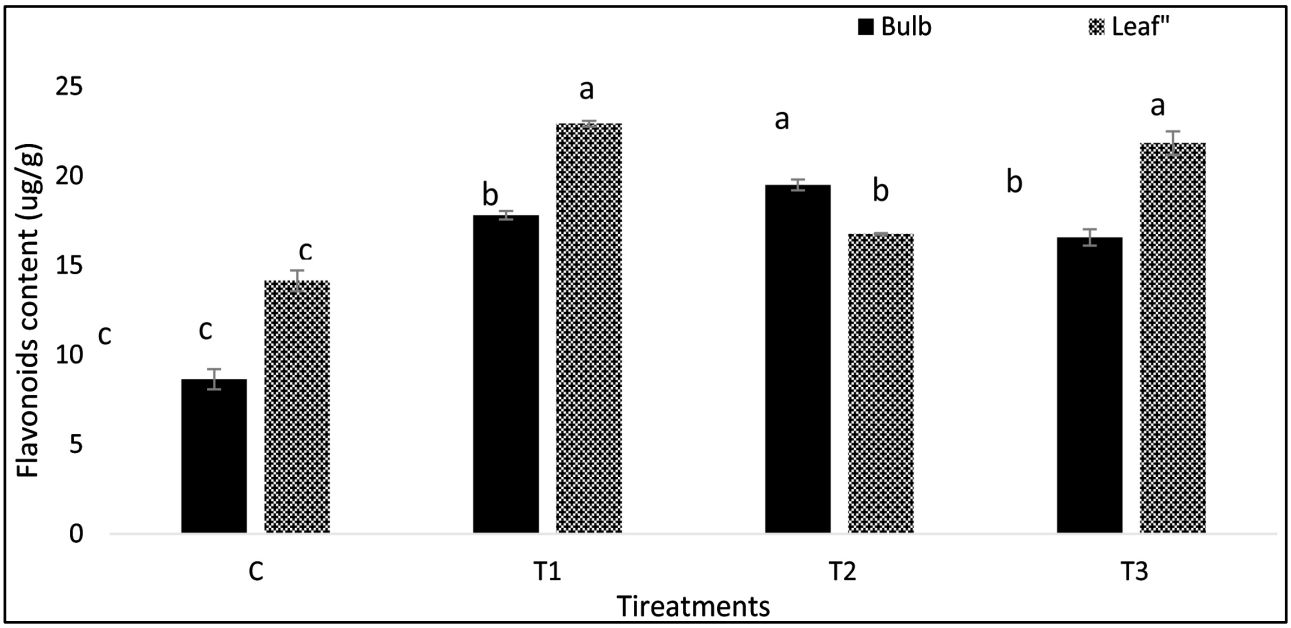

Figure 3. Effect of different strains of PGPR on flavonoid content of bulb and leaves. The data represent the means of 3 replicates and ± represents their standard error values. Measurements were made after 3 weeks of seed germination at vegetative phase. Treatment details as in Figure 1. Alphabetical letters represent statistical means of the Tukey HSD test ($p < 0.05$) run on Statistix 8.1 (software). Letters with similar alphabets do not vary significantly.

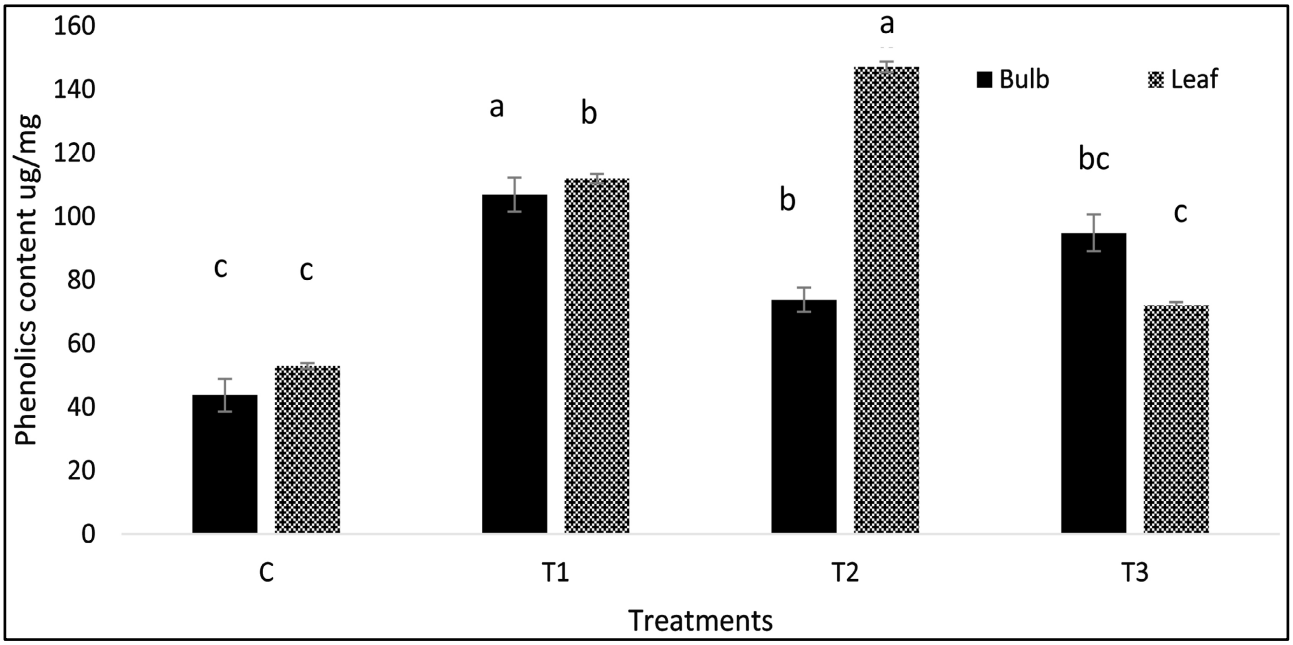

Figure 4. Effect of various strains of PGPR on phenolics content of bulb and leaves of garlic. The data represent the means of 3 replicates standard error values (represented as ±). Measurements were made after 3 weeks of seed germination (at vegetative phase). Treatment details as in Figure 1. Alphabetical letters represent statistical means of the Tukey HSD test ($p < 0.05$) run on Statistix 8.1 (software). Letters with similar alphabets do not vary significantly.

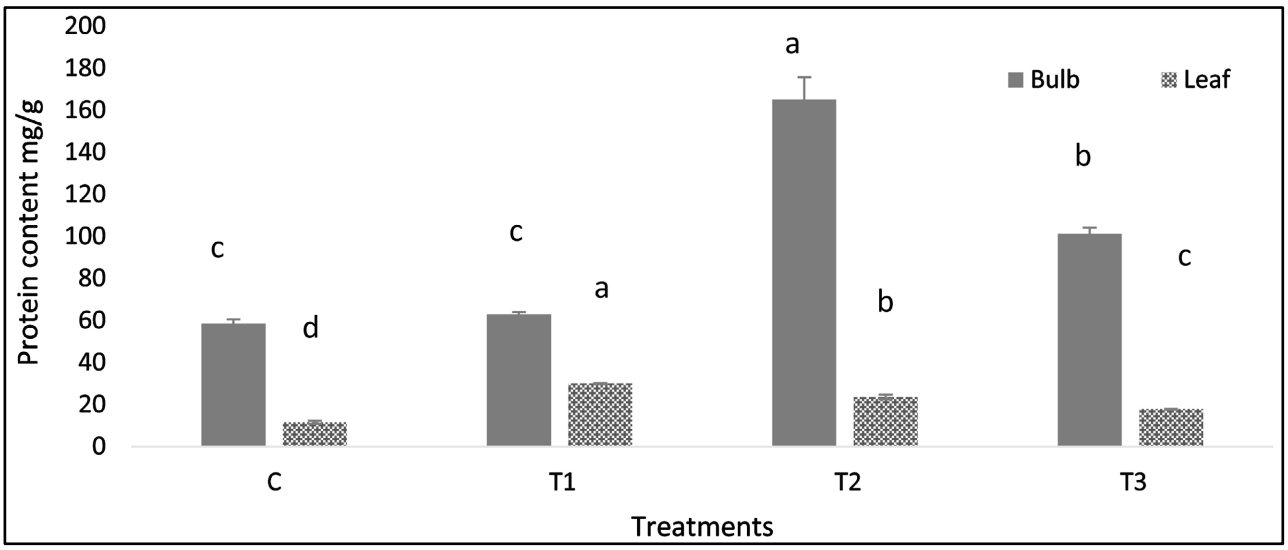

Figure 5. Effect of various strains of PGPR on the protein content of bulb and leaves of garlic. The data represent the means of 3 replicates and ± represents standard error values. Measurements were made 3 weeks of seed germination (at vegetative phase). Treatment details as in Figure 1. Alphabetical letters represent statistical means of the Tukey HSD test ($p < 0.05$) run on Statistix 8.1 software. Letters with similar alphabets do not vary significantly.

Figure 6. Effect of various PGPR strains on leaves of garlic. Measurements were made after 3 weeks (vegetative phase) of seed germination. Treatment details as in Figure 1.

significant increase in shoot length and diameter. Higher leaf count with darker green color was observed in T1 over C (Figure 6).

4. DISCUSSION

The research was conducted in greenhouse with 4 replicates comprising garlic irrigated with tap water treated as control and T1, T2 and T3 comprising plants (garlic) inoculated with *Pseudomonas putida*, *Pseudomonas stutzeri and Bacillus cerus*. The PGPR acts an important role in germination and establishment either directly or indirectly enhancing plant growth and development [17]. It was observed during the present investigation that both *Pseudomonas* sp. were more effective than that *Bacillus cereus*. Bulb

diameter and weight of bulb and length (root and shoot) were also greater in *Pseudomonas* species treatment. The observed higher increase in bulb size and dry weight in *Pseudomonas putida* inoculation may be attributed to the higher increase in shoot weight following this treatment. *Bacillus subtilis* and *Trichoderma harzianum* application in potatoes reduce common scabs and enhance yield and beneficial bacteria [18].

The *Pseudomonas putida* treated plant showed a maximum increase in root length while *Pseudomonas stutzeri* treated plants resulted in a significant increase in shoot length (**Table 1**). Longer roots enable plants to derive water deeper down the soil and hence plant can adapt better to moisture deficit condition. Plant biomass and root length were significantly enhanced in tomato plants with the application of PGPR [19]. *Bacillus subtilis* CBR05 (PGPR strain) improved fruit quality [20]. *Bacillus megaterium* inoculation in *Arabidopsis thaliana* induced root growth development increased root count and hair length. The observed increase in bud diameter and its biomass may be attributed to the improvement in growth parameters like root and shoot growth.

Rhizobacteria e.g., *Bacillus*, *Pseudomonas*, *Pantone*, *and Arthobacter* produce phytohormones and induce tolerance to abiotic stress effectively [21]. The PGPR enhances plant dry weight, chlorophyll and sugar content which ultimately enhance the growth of plants [22]. Plants treated with *Pseudomonas putida* showed a higher increase in dry weight and chlorophyll. PGPR produces phytohormones and fixes atmospheric nitrogen and subsequently enhances plant growth [23]. It was studied that PGPR assesses chlorophyll production in *Sesbania sesban* L. [24]. The PGPR inoculation in *Althaea officinalis* L. enhances amino acid and sugar content on the other hand alone and in combination with different fertilizers enhances sugar production [22-25]. The maximum increase in leaf sugar recorded in T1 (*Pseudomonas putida*) inoculation may be attributed to higher chlorophyll content in both chlorophyll *a* and *b*. The PGPR enhances dry weight, chlorophyll and sugar content that ultimately enhance plant development and growth over control (Tap) (**Figure 1**). The PGPR inoculation in *Althaea officinalis* L. enhances amino acid and sugar content alone and in combination with different fertilizers [25]. Carotenoids are photo-protective pigments their production was enhanced in *Sesbania sesban* with the PGPR application [26].

The *Bacillus cereus* inoculation resulted in a higher increase in leaf carotenoids and phenolics. The PGPR strains *Bacillus subtilis* and *Bacillus licheniformis* promote flavonoid production and enhance fruit quality [27]. *Pseudomonas putida* showed maximum flavonoid production. T1 (*Pseudomonas putida*) exhibited maximum flavonoids in leaves and also had higher flavonoids in the bulb (**Figure 2**). Flavonoids possess medicinal benefits e.g., anticancer, antioxidant, anti-inflammatory, and antiviral properties [27].

Among all PGPR strains, phenolics content in *Pseudomonas stutzeri* treated leaf was maximum while *Pseudomonas putida*-treated bulbs were rich in phenolics (**Figure 3**). Phenolics are the antioxidants in plants that enhance fruit quality, seed germination, pollination, plant development and reproduction [28, 29]. Phenolics prevent oxidative damage and provide protection against cancer and cardiac disease and defend plants against phytopathogens and develop resistance against various stresses [19]. The *Pseudomonas putida* is efficient in bulb protein production while *Pseudomonas stutzeri* is highly effective in leaf protein production. PGPR enhances stress-related proteins [30].

In C4 plant PGPR enhance drought tolerance almost equal to a well-watered condition and assist to recover drought stress in a much better way [31]. The PGPR enhances bulb and leaf protein content approximately 38-fold. Bulb show more positive effects of PGPR over leaf to enhance protein (**Figure 4**). The PGPR increase garlic leaf diameter and length over control (TAP) (**Figure 5**). In wheat phenolics, carotenoids and protein content increased with PGPR treatment that assists plant to alleviating heavy metal toxicity [21].

5. CONCLUSIONS

It is contingent from the present findings that T2 (*Pseudomonas stutzeri*) may be good for the production of longer leaves with moderate sugar and high flavonoids and phenolics contents for bulb production it is demonstrated that inoculation treatment with T1 (*Pseudomonas putida*) is better as it showed

maximum increase in bulb diameter, bulb biomass with maximum phenolics and flavonoids contents.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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