

Biofertilizer and Biocontrol Potential of Bacillus Isolates on Tomato Plant Growth

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ABSTRACT

Tomato *Solanum lycopersicum* is an important crop throughout the world and losses result from pathogenic microorganisms. Excessive chemical fertilizers and pesticides are detrimental to environment and human health and need to be replaced by alternatives and biological products which can be sustained. In the greenhouse, nine *B.* isolates were tested for their biofertilizer and biocontrol potential to growth of tomato plants. Bacterial suspensions were injected into the stems and sprayed onto the leaves of the plants, morphological parameters were measured at 30 and 60 days after inoculation. Multivariate Analysis of Variance (MANOVA) was used to statistically compare the root, shoot and fruit parameters in all the treatments and the number of branches and fruits. Highly significant differences were observed between the results obtained for the eight out of nine isolates and the untreated controls in all growth parameters. The isolate *B. species* (C4PWK-8) was consistently the strongest growth stimulator for all parameters measured. *B. thuringiensis* (SSG-7) had the lowest activity with comparable results to the control on some parameters. The findings suggest that *Bacillus* based biofertilizers are an effective and environmentally friendly alternative to chemical fertilizers in tomato production.

Keywords: *Bacillus*, Biofertilizer, Biocontrol, Manova, *Solanum Lycopersicum*, Plant Growth Promotion, Rhizobacteria

Introduction

Increased food production and food quality are needed to meet the nutritional demands of the growing world population, expected to reach more than 10 billion

people by 2050. Tomato (*Solanum lycopersicum*) is the second most common vegetable grown worldwide and an important part of food security. Tomatoes have many nutritional and medicinal properties, including high levels of vitamins A, C, E, potassium, iron, flavonoids, hydroxycinnamic acids, lycopene and other carotenoids (Ochoa-Velasco et al., 2016; Obeng et al., 2018).

In 2016, the worldwide tomato production was estimated at 179,508,401 tonnes and China, India, Pakistan, Turkey and the United States are the top five producers (Sharma et al., 2019). However, tomatoes are highly vulnerable to biotic stress factors, including bacterial diseases like *Pseudomonas syringae*, *Erwinia carotovora*, *Staphylococcus* sp., *Klebsiella* and *Salmonella* (Obeng et al., 2018; Vessey, 2003). These diseases can result in significant losses, impacting food security and farmer livelihoods.

Traditional solutions have depended a great deal on use of synthetic chemical fertilizers and pesticides. They are effective in the short-term, but their repeated use causes longterm phytotoxic residues, soil and water contamination, and other ecological harm (Sebilo et al., 2013; Mengistie et al., 2017). This has sparked the need for adequate eco-friendly solutions.

Biological control agents such as plant growth-promoting rhizobacteria (PGPR) have proven to be an attractive option. Of them, *Bacillus* sp. are of special interest not only for their biofertilizing properties but also for their bioinsecticidal ones. They do this in a variety of ways, such as synthesizing lipopeptide antibiotics (iturins, fengycins, surfactins), secreting hydrolytic enzymes, producing phytohormones (auxins, gibberellins), and nitrogen fixation, as well as solubilizing phosphates (Ben et al., 2016; Radhakrishnan et al., 2017). *Bacillus* species including *B. amyloliquefaciens*, *B. subtilis*, *B. cereus*, *B. safensis*, *B. pumilus* and *B. thuringiensis* are well documented as biocontrol agents and growth promoters (Jamal et al., 2018; Alejandro et al., 2011).

The present study was prompted by Hussein (2021) findings that the use of biofertilizers has improved yields of cereal crops by 10 – 20%. Based on this evidence the study will seek to further expand to tomato culture the nine *Bacillus* isolates obtained from the rhizospheric soil and natural spring water samples from Swat and Dir districts, K-P-Khawaja, Pakistan, that will promote the growth of tomato in a controlled greenhouse environment.

1.1 Study Objectives

The primary objectives of this investigation were:

To apply *Bacillus* isolates on tomato plants and analyze biofertilizer properties by monitoring morphological changes after inoculation.

To assess the impact of selected *Bacillus* isolates on plant growth parameters relative to untreated control plants.

To statistically compare the efficacy of nine isolates across multiple morphological variables using MANOVA

Review of Literature

Jamal et al. (2018) In pot experiments, *Bacillus amyloliquefaciens* Y1 had a significant positive effect on soil properties, pepper seedling growth, and rhizosphere bacterial flora, as evidenced by increases in soil NPK content, chlorophyll content, flower number, and root/shoot biomass. The results indicated that *B. amyloliquefaciens* is a promising alternative to chemical fertilizers. It was shown by

Liu et al. (2018) that the growth promotion and fungal and bacterial pathogen suppression capability of mixtures of PGPR were more effective than that of individual PGPR, suggesting a possible synergistic effect of applying two or more PGPR. Rufus et al (2018) tested 12 strains of *Bacillus* spp. isolated from wheat rhizosphere for their ability to stimulate the growth of corn, wheat and soybean. Their results warned that physiological in-vitro assays were inadequate to make a strain selection and highlighted the importance of greenhouse and field pot tests. Ge et al. (2016) employed a novel *Bacillus methylotrophicus* strain, NKG-1 to reduce the severity of *Botrytis cinerea* gray mold by 60%, and promote tomato seedling growth in greenhouse trials. Ochoa-Velasco et al. (2016) found that tomato quality was enhanced with the use of N-fixing bacteria along with biofertilizers, thus providing a foundation for integrated use of biofertilizers. Weselowski et al. (2016) described *P. polymyxa* CR1 as a nitrogen fixing, phosphate solubilizing, IAA producing and antimicrobial bacterium that shows high potential for sustainable agriculture against a wide range of plant pathogens such as *B. cereus*. Nagendranatha et al. (2016) isolated *B. safensis* K7 from microbial electrolysis cells and its biofertilizer potential was confirmed by the presence of nitrogen fixation, IAA production, antifungal, and siderophore forming abilities. Khalid et al. (2004) have shown that the ability of rhizobacterial production of indole acetic acid (IAA) greatly influenced the growth of wheat in laboratory, pot and field conditions and suggested the production of IAA as a reliable screening parameter for selecting PGPRs. Dawwam et al. (2013) also isolated plant growth promoting bacteria (PGPB) from potato roots and used IAA production and phosphate solubilization potential of *B. cereus* strains.

Materials and Methods

Plant Material and Growth Conditions

The plant selected for the test was tomato (*Solanum lycopersicum*) as it is a nutritious crop, commercially important and susceptible to bacterial diseases. Seeds were collected from Plant Pathology Department, University of Agriculture Peshawar and were sown in trays filled with non-sterile soil and grown under screen house conditions. The daytime temperature range was kept at 21–27°C (70–80°F); the night time temperature range was 16–18°C (60–65°F). These warm season crops were grown in full sun as required.

Bacterial Isolates

Seven *Bacillus* isolates were obtained from natural springs and two rhizospheric (root region) soil samples were collected from Swat and Dir districts of KPK province, Pakistan and tested. All isolates were made into a 24-hour old suspension culture.

Inoculation Methods

Two inoculation methods were used for tomato seedlings of 4-5 cm height (1) stem injection: superficially wounding plant stems and injecting 0.5ml bacterial suspension through a 1ml syringe to achieve a sub-surface tissue contact and (2) foliar spraying: spraying plants evenly with the inocula. After inoculation, the plants were then separately placed under plastic bags to ensure moist environments that are conducive

to bacterial growth and colonization. Plants without inoculation were used as controls. Growth conditions for all plants were the same in the greenhouse with the same soil, temperature and light.

Data Collection

The morphological features were recorded at 30 and 60 days after inoculation. These dependent variables were measured: Note: Root parameters: Root dry weight, Root fresh weight, Root length - Meristem weight, shoot length, shoot fresh weight, and shoot dry weight. Leaf parameters are the length of the leaf, width of the leaf and number of leaves. Parameters affecting reproduction (reproduction parameters): Number of branches, Number of fruits Triplicates (n = 3) were used to conduct all treatments.

Statistical Analysis

All morphological variables were obtained by descriptive statistics (arithmetic means). Visual comparison was done in the form of bar charts. Multiple dependent variables were examined for significance among bacterial strains using Multivariate Analysis of Variance (MANOVA). The choice of Pillai's Trace statistic as the main test statistic for MANOVA was because it is robust to the assumption of homogeneity of the covariance matrix.

Results

from MANOVA were significant and one-way ANOVAs were run separately for each dependent variable with Levene's test yielding homogeneity of error variances. Post hoc Least Significant Difference (LSD) tests were used to detect specific pairwise differences. The statistical analysis was carried out with SPSS 22 version. Growing Degree Days (GDD) Shoot height was significantly higher in all inoculated plants than uninoculated plants during 56 days of observation. The isolate B. specie (C4PWK-8) produced the maximum shoot height (86 cm at Day 56) which was almost double that of the control (44 cm). The plant inoculated with B. thuringiensis (SSG-7) had the lowest shoot height, close to the control. 4. Results

Shoot Height Over Time

All inoculated plants demonstrated greater shoot height compared to uninoculated controls throughout the 56-day observation period. The isolate B. specie (C4PWK-8) produced the highest shoot height (86 cm at Day 56), nearly double the control (44 cm). B. thuringiensis (SSG-7) performed lowest among the inoculated plants, with a final shoot height nearly equivalent to the control.

Table 1. Shoot height (cm) of tomato plants treated with nine Bacillus isolates measured at weekly intervals over 56 days.

Code	Species	Day 1 (cm)	Day 7 (cm)	Day 14 (cm)	Day 21 (cm)	Day 28 (cm)	Day 35 (cm)	Day 42 (cm)	Day 49 (cm)	Day 56 (cm)
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Code	Species	Day 1 (cm)	Day 7 (cm)	Day 14 (cm)	Day 21 (cm)	Day 28 (cm)	Day 35 (cm)	Day 42 (cm)	Day 49 (cm)	Day 56 (cm)
Control	<i>Not treated</i>	4.5	5.5	6.5	14	27	40	41	42	44
C4PWK-8	<i>B. specie</i>	5.5	16.5	26	52	65	73	79	84	86
PWG-7	<i>B. safensis</i>	5.5	10	15	29	41	53	55	57	60
SSG-7	<i>B. thuringiensis</i>	4.5	7.5	10	23	34	36	38	40	44
C1SSK-8	<i>B. pumillus</i>	6	14.5	23	35	43	50	53	56	61
C2SWK-8	<i>B. thuringiensis</i>	4.5	12	17	34	42	47	50	52	57
C2PMW-8	<i>B. pumillus</i>	4.5	6	9	17	34	56	58	60	64
C2PWD-7	<i>B. safensis</i>	6	14	23.5	38	47	55	57	59	63
PWK-8	<i>B. cereus</i>	4.5	10	14.5	32	47	55	57	60	64
C1PWK-7	<i>B. pumillus</i>	6	11	19	35	46	60	62	64	67

Figure 1: Shoot Height Over Time (56 Days)

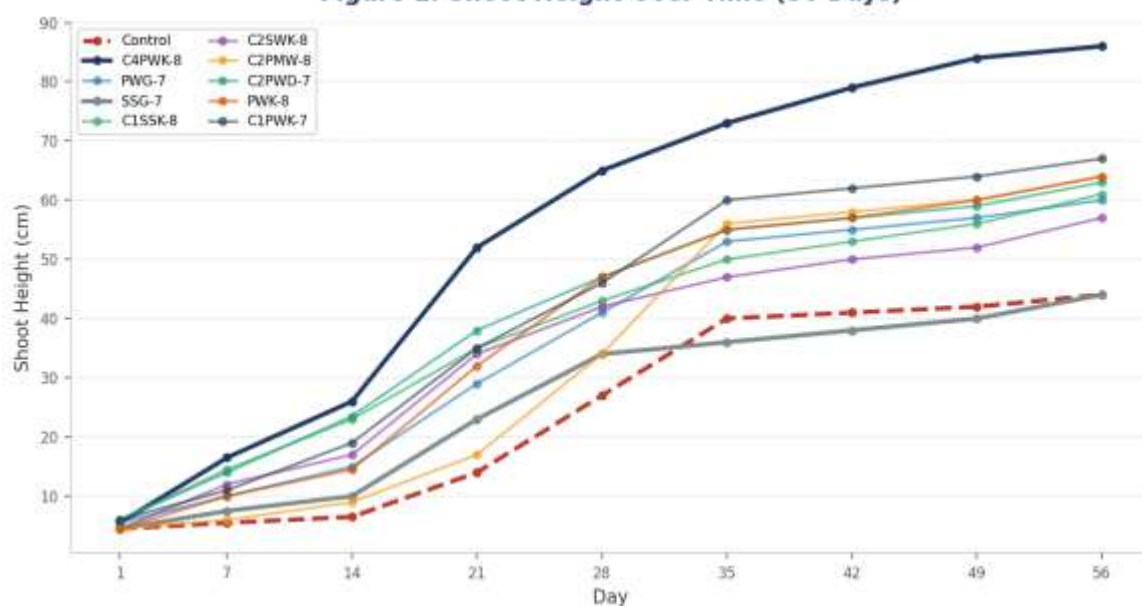


Figure 1. Shoot height (cm) of tomato plants inoculated with nine *Bacillus* isolates over 56 days. C4PWK-8 (dark navy) showed the highest growth trajectory; SSG-7 (grey) closely tracked the control (red dashed line).

Root Parameters

MANOVA of the root parameters gave a Pillai's Trace of 2.852 ($F = 35.899$, $p < 0.001$, partial $\eta^2 = 0.942$), which showed that the differences between the bacterial strain treatment conditions explained about 94% of the variation in the combined root measures. All root dependent variables were found to be homogenous of variance using Levene's Test. Subsequent univariate ANOVAs showed that root dry weight ($F = 76.133$, $p < 0.001$), root fresh weight ($F = 33.709$, $p < 0.001$), and root length ($F = 104.778$, $p < 0.001$) were all significantly affected by bacterial treatment.

Table 2. Mean root parameters of tomato plants treated with nine *Bacillus* isolates.

Code	Species	Root Dry Wt. (g)	Root Fresh Wt. (g)	Root Length (cm)
Control	<i>Not treated</i>	0.3	1.3	13.9
C4PWK-8	<i>B. specie</i>	1.5	7.6	16.5
PWG-7	<i>B. safensis</i>	0.5	2.0	16.5
SSG-7	<i>B. thuringiensis</i>	0.3	1.2	15.2
C1SSK-8	<i>B. pumillus</i>	0.7	3.3	24.1
C2SWK-8	<i>B. thuringiensis</i>	0.7	3.1	20.3
C2PMW-8	<i>B. pumillus</i>	1.5	10.2	17.8
C2PWD-7	<i>B. safensis</i>	1.4	6.2	25.4
PWK-8	<i>B. cereus</i>	1.5	6.5	27.9
C1PWK-7	<i>B. pumillus</i>	1.0	4.5	25.4

Table 3. Tests of Between-Subjects Effects for Root Parameters (ANOVA).

Dependent Variable	Sum of Squares	Mean Square	F	Sig.	Partial Eta Squared
Root Dry Weight	6.852	0.761	76.133	.000	0.972
Root Fresh Weight	239.067	26.563	33.709	.000	0.938
Root Length (cm)	678.960	75.440	104.778	.000	0.979

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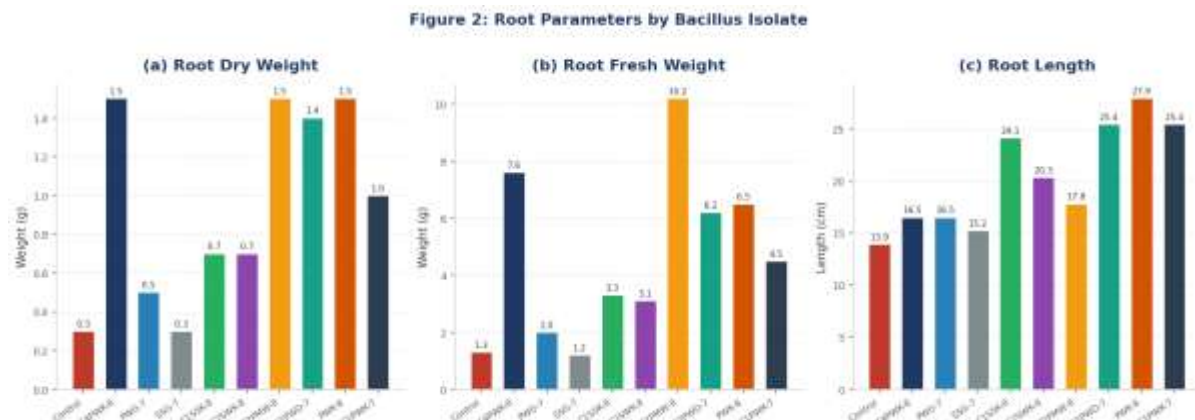
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Figure 2. Root dry weight (g), fresh weight (g), and root length (cm) of tomato plants inoculated with nine *Bacillus* isolates. C2PMW-8 produced the highest fresh weight; PWK-8 achieved the greatest root elongation.

Post hoc LSD analysis revealed that only *B. thuringiensis* (SSG-7) showed no significant difference from the control in root dry weight. All others showed a significantly greater number of isolates than the control. *B. pumillus* (C2PMW-8) produced the highest root fresh weight (10.2 g), which was significantly better than all other isolates. *B. cereus* (PWK-8) had the longest root length (27.9 cm), followed by *B. safensis* (C2PWD-7) and *B. pumillus* (C1PWK-7) with root length of 25.4 cm each.

Shoot Parameters

MANOVA of shoot parameters revealed a Pillai's Trace value of 2.960 ($p < 0.001$, $\eta^2 = 0.987$), which means that bacterial treatment accounted for 98.7% of the variations in the shoot growth. Separate ANOVAs confirmed significant treatment effects for shoot dry weight ($F = 117.090$, $p < 0.001$), shoot fresh weight ($F = 210.312$, $p < 0.001$), and shoot length ($F = 426.000$, $p < 0.001$).

Table 4. Mean shoot parameters of tomato plants treated with nine *Bacillus* isolates.

Code	Species	Shoot Length (cm)	Shoot Fresh Wt. (g)	Shoot Dry Wt. (g)
Control	Not treated	44	18.2	3.5
C4PWK-8	<i>B. specie</i>	86	162.3	23.5
PWG-7	<i>B. safensis</i>	60	46.3	8.1
SSG-7	<i>B. thuringiensis</i>	40	28.4	3.4
C1SSK-8	<i>B. pumillus</i>	61	43.9	6.7
C2SWK-8	<i>B. thuringiensis</i>	57	94.5	7.5
C2PMW-8	<i>B. pumillus</i>	64	62.7	9.4
C2PWD-7	<i>B. safensis</i>	63	106.0	9.4

Code	Species	Shoot Length (cm)	Shoot Fresh Wt. (g)	Shoot Dry Wt. (g)
PWK-8	<i>B. cereus</i>	64	85.2	12.9
C1PWK-7	<i>B. pumillus</i>	67	53.5	8.1

Table 5. Tests of Between-Subjects Effects for Shoot Parameters (ANOVA).

Dependent Variable	Sum Squares	of F	Sig.	Partial Squared Eta
Shoot Dry Weight	886.608	117.090	.000	0.981
Shoot Fresh Weight	49904.603	210.312	.000	0.990
Shoot Length	3834.000	426.000	.000	0.995

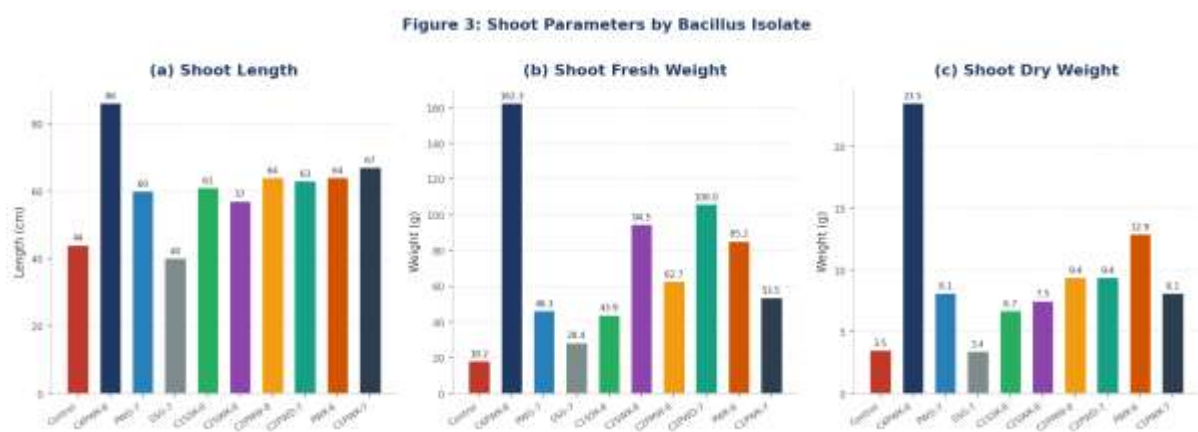


Figure 3. Shoot length (cm), fresh weight (g), and dry weight (g) of tomato plants treated with nine *Bacillus* isolates. C4PWK-8 substantially outperformed all other isolates on every shoot metric.

B. specie (C4PWK-8) was the most effective isolate for all shoot parameters, with a shoot length of 86 cm (vs. 44 cm for the control), shoot fresh weight of 162.3 g (vs. 18.2 g), and shoot dry weight of 23.5 g (vs. 3.5 g). Post hoc analysis identified *B. thuringiensis* (SSG-7) as the only isolate failing to show a significant improvement in dry shoot weight compared to the control.

Leaf Parameters

MANOVA for leaf parameters gave a Pillai's Trace of 1.497 ($p = 0.005$, partial $\eta^2 = 0.499$). Univariate ANOVAs confirmed a significant effect on number of leaves ($F = 153.242$, $p < 0.001$). Leaf width and leaf length showed marginal significance as individual parameters ($p = 0.217$ and $p = 0.111$, respectively), though the combined multivariate effect was statistically significant.

Table 6. Mean leaf parameters of tomato plants treated with nine *Bacillus* isolates.

Code	Species	Leaf Length (cm)	Leaf Width (cm)	No. of Leaves
Control	Not treated	4.80	2.50	70
C4PWK-8	<i>B. specie</i>	7.33	3.33	220
PWG-7	<i>B. safensis</i>	5.83	3.00	110
SSG-7	<i>B. thuringiensis</i>	4.67	2.33	65
C1SSK-8	<i>B. pumillus</i>	5.10	2.33	115
C2SWK-8	<i>B. thuringiensis</i>	6.33	3.17	125
C2PMW-8	<i>B. pumillus</i>	5.80	2.67	140
C2PWD-7	<i>B. safensis</i>	7.00	2.50	240
PWK-8	<i>B. cereus</i>	6.83	3.33	270
C1PWK-7	<i>B. pumillus</i>	5.30	2.33	190

Figure 4: Leaf Parameters by *Bacillus* Isolate

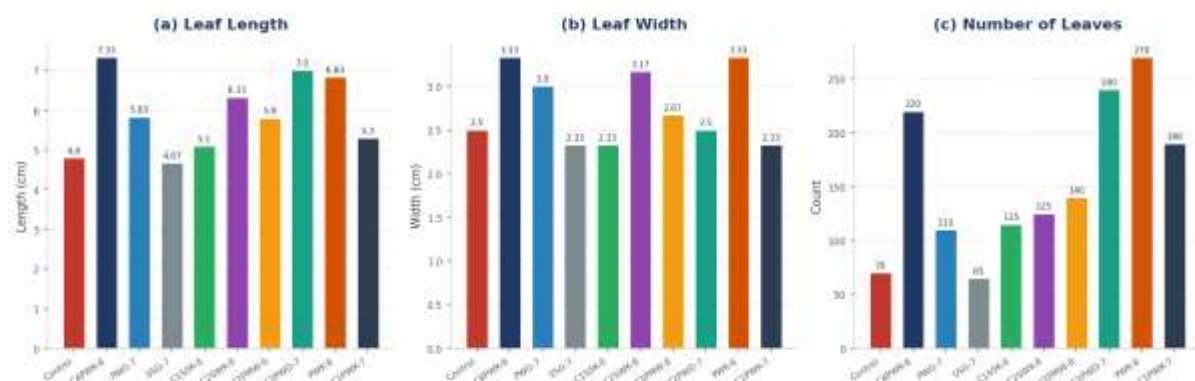


Figure 4. Leaf length (cm), leaf width (cm), and number of leaves in tomato plants inoculated with nine *Bacillus* isolates. PWK-8 and C2PWD-7 produced the most prolific leaf development.

The maximum number of leaves (270) was recorded in *B. cereus* (PWK-8)-treated plants, followed by *B. safensis* (C2PWD-7) with 240 and *B. specie* (C4PWK-8) with 220 leaves. *B. thuringiensis* (SSG-7) produced 65 leaves, below the control (70 leaves). Maximum leaf length (7.33 cm) and leaf width (3.33 cm) were observed in C4PWK-8 and PWK-8 treated plants.

Number of Branches and Fruits

The MANOVA for reproductive parameters produced a Pillai's Trace of 1.647 ($p < 0.001$, partial $\eta^2 = 0.824$). Separate ANOVAs confirmed significant treatment effects for number of fruits ($F = 42.074$, $p < 0.001$) and number of branches ($F = 6.874$, $p < 0.001$).

Table 7. Mean number of branches and fruits in tomato plants treated with nine *Bacillus* isolates.

Code	Species	No. of Branches	No. of Fruits
Control	Not treated	10	0
C4PWK-8	<i>B. specie</i>	25	13
PWG-7	<i>B. safensis</i>	14	3
SSG-7	<i>B. thuringiensis</i>	9	2
C1SSK-8	<i>B. pumillus</i>	17	3
C2SWK-8	<i>B. thuringiensis</i>	18.67	3
C2PMW-8	<i>B. pumillus</i>	16	5
C2PWD-7	<i>B. safensis</i>	16	5
PWK-8	<i>B. cereus</i>	17	6
C1PWK-7	<i>B. pumillus</i>	17	2

Figure 5: Number of Branches and Fruits by *Bacillus* Isolate

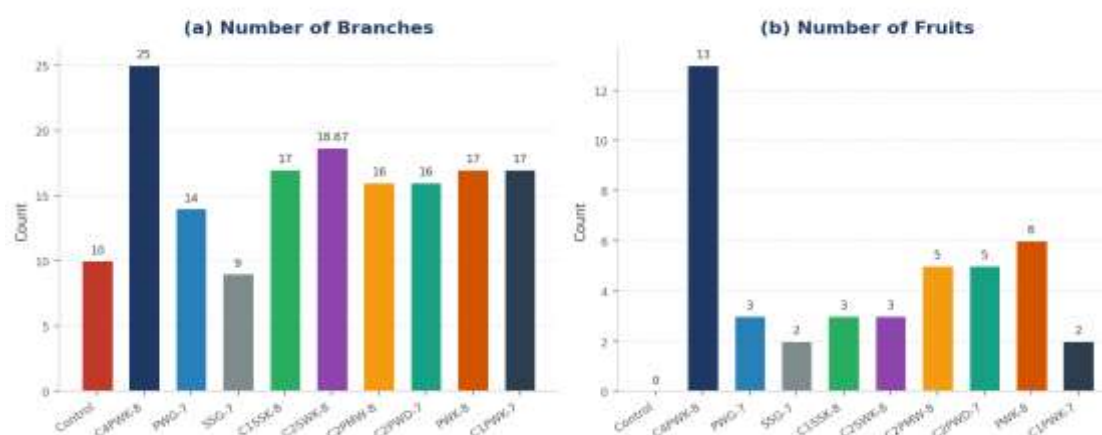


Figure 5. Number of branches and number of fruits in tomato plants treated with nine *Bacillus* isolates. C4PWK-8 produced the most branches (25) and fruits (13); the untreated control produced no fruits.

B. specie (C4PWK-8) produced the most branches (25) and fruits (13). The control produced no fruits during the observation period. Post hoc LSD tests showed that only *B. safensis* (PWG-7) and *B. thuringiensis* (SSG-7) did not significantly differ from the control in number of branches, while all isolates produced significantly more fruits than the control.

MANOVA Effect Sizes Across All Parameters

Figure 6 summarises the Partial Eta Squared values (η^2) from MANOVA analyses across all morphological parameter groups. Shoot parameters exhibited the highest effect sizes (η^2 up to 0.995 for shoot length), followed by root and leaf parameters.

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Number of branches showed the lowest effect size ($\eta^2 = 0.756$), though still substantial.

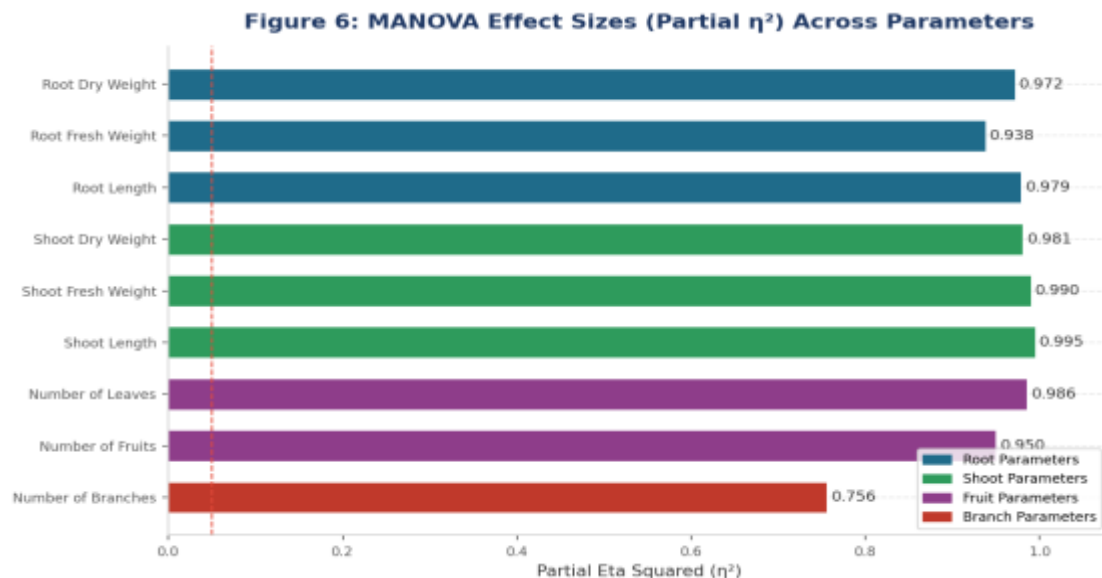


Figure 6. Partial Eta Squared values (partial η^2) from MANOVA/ANOVA analyses across all dependent variables. All values exceed 0.75, indicating large effect sizes for bacterial strain treatment.

Comparative Performance Across Isolates

Figure 7 presents a normalised radar chart comparing four isolates (C4PWK-8, PWK-8, SSG-7, and the control) across five key growth metrics. C4PWK-8 consistently occupies the outermost area of the radar, confirming its broad-spectrum growth-promoting superiority. The control and SSG-7 are constrained to a narrow inner arc, while PWK-8 shows particular strength in root length and leaf count dimensions.

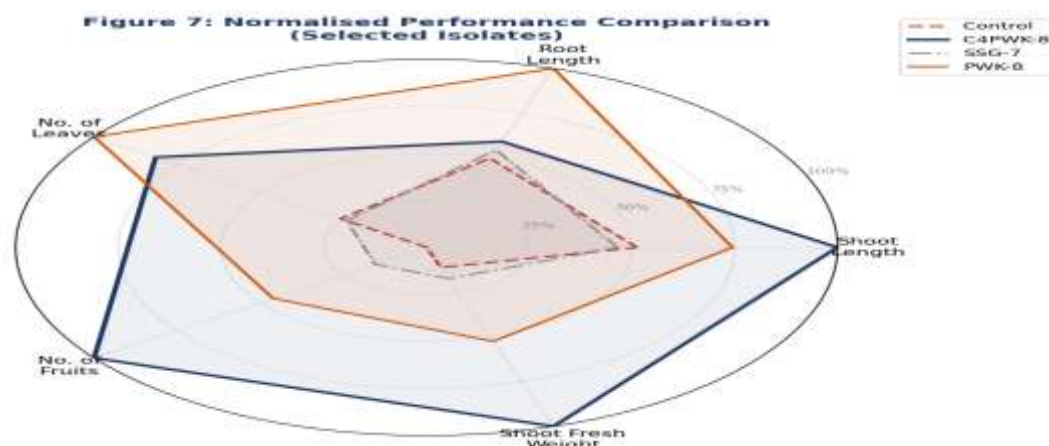


Figure 7. Normalised radar chart comparing four selected isolates across five key growth metrics (normalised to 0–1 scale). C4PWK-8 dominates across all axes; SSG-7 performs closest to the untreated control.

Discussion

The findings of the present study are able to give convincing evidence that *Bacillus* spp. isolated from the natural springs and rhizospheric soils of Swat and Dir districts can significantly improve the growth of tomato plants on all the monitored morphological parameters. The results were in line with the large volume of literature reporting on the growth promotion effect of PGPR (Radhakrishnan et al., 2017; Jamal et al., 2018; Liu et al., 2018).

B. specie (C4PWK-8) exhibited a high efficiency on all parameters (root, shoot, leaf and reproductive parameters) indicating that this isolate owns a wide range of plant growth promoting capabilities, such as efficient auxin production, phosphate solubilization and biofilm formation which is important for root colonization. Broad-spectrum activity has been reported for *B. amyloliquefaciens* (Jamal et al., 2018), and for *Bacillus* strains that produce volatile organic compounds that modulate auxin homeostasis (Alejandro et al., 2011).

It is interesting to note that *B. thuringiensis* (SSG-7) has performed poorly in each of the parameters. *B. thuringiensis* is a well-established bioinsecticide, accounting for about 70% of all biopesticide products used in the world, but its growth promotion activity seems to be less pronounced than that of other species of *Bacillus* (Thakore, 2006). The use value of the strain might be mainly the suppression of pathogens, but not necessarily in nutrient delivery or phytohormone production.

The MANOVA results for the root and shoot parameters indicated high and consistent treatment effects by the bacterial treatments (Partial Eta Squared = 0.942 for root parameters and 0.987 for shoot parameters). The effect sizes observed here are one of the largest reported in greenhouse PGPR studies and validate the efficacy of the isolates as well as the analytical framework of MANOVA for multi-trait agronomic studies.

Fruits were also absent in the control treatment at the time of the second harvest, which suggests that the bacterial isolates have growth acceleration properties. Isolates like C4PWK-8 and PWK-8 could be useful for agricultural application in commercial tomato production, as they enable faster fruit development, directly resulting in economic benefits.

Conclusion

The results of this study have showed that 9 *Bacillus* isolates isolated from Khyber Pakhtunkhwa, Pakistan have significant plant growth promoting (PGP) attributes when applied to tomato under greenhouse condition. All morphological parameters, such as root dry weight, root fresh weight, root length, shoot dry weight, shoot fresh weight, shoot length, leaf dimensions, leaf count, branch count and fruit yield were statistically significantly improved using MANOVA. The most growth promoting was consistently isolate *B. specie* (C4PWK-8) and the least growth promoting was *B. thuringiensis* (SSG-7). The outcomes are encouraging for the development of *Bacillus* based biofertilizers as eco-friendly and sustainable options to synthetic agrochemicals which could be used to a wide extent throughout the crop types and regions.

Recommendations

B. specie (C4PWK-8) and *B. cereus* (PWK-8) are recommended for field-scale trials on tomato.

Testing should be repeated on other vegetable and cereal crops (such as wheat, corn, potato, etc.) to test the generalizability of growth promotion effects of the same isolates.

Further research needs to include molecular typing of the isolates to determine which gene clusters contribute to plant growth promoting attributes.

To make the findings more generalizable to the province of KP, the study should be expanded to other districts of the province.

Combined inoculation of various isolates should be investigated to determine the synergistic effect as recommended by Liu et al. (2018).

References

- Ajmal, M., Ali, H. I., Saeed, R., Akhtar, A., Tahir, M., Mehboob, M. Z., & Ayub, A. (2018). Biofertilizer as an alternative for chemical fertilizers. *Journal of Agriculture and Allied Sciences*, 7(1), 1–7.
- Aseri, G. K., Jain, N., Panwar, J., Rao, A. V., & Meghwal, P. R. (2008). Biofertilizer improve plant growth, fruit yield, nutrition, and metabolism and rhizosphere enzyme activities of pomegranate in Indian Thar Desert. *Scientia Horticulturae*, 117(2), 130–135.
- Bisbis, M. B., Gruda, N., & Blanke, M. (2018). Potential impacts of climate change on vegetable production and product quality — A review. *Journal of Cleaner Production*, 170, 1602–1620.
- Dawwam, G. E., Elbeltagy, A., Emara, H. M., Abbas, I. H., & Hassan, M. M. (2013). Beneficial effect of plant growth promoting bacteria isolated from the roots of potato plant. *Annals of Agricultural Sciences*, 58(2), 195–201.
- Doolotkeldieva, T., Bobusheva, S., & Konurbaeva, M. (2015). Effects of *Streptomyces* biofertilizer to soil fertility and rhizosphere functional biodiversity of agricultural plants. *Advances in Microbiology*, 5(7), 555.
- Ge, B., Liu, B., Nwet, T. T., Zhao, W., Shi, L., & Zhang, K. (2016). *Bacillus methylotrophicus* strain NKG-1, isolated from Changbai Mountain, China, has potential applications as a biofertilizer or biocontrol agent. *PLOS ONE*, 11(11), e0166079.
- Jamal, Q., Lee, Y. S., Jeon, H. D., & Kim, K. Y. (2018). Effect of plant growth-promoting bacteria *Bacillus amyloliquefaciens* Y1 on soil properties, pepper seedling growth, rhizosphere bacterial flora and soil enzymes. *Plant Protection Science*, 54(3), 129.
- Kesaulya, H., Zakaria, B., & Syaiful, S. A. (2015). Isolation and physiological characterization of PGPR from potato plant rhizosphere in medium land of Buru Island. *Procedia Food Science*, 3, 190–199.
- Khalid, A., Arshad, M., & Zahir, Z. A. (2004). Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *Journal of Applied Microbiology*, 96(3), 473–480.
- Liu, K., McInroy, J. A., Hu, C. H., & Kloepper, J. W. (2018). Mixtures of plant-growth-promoting rhizobacteria enhance biological control of multiple plant

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- diseases and plant-growth promotion in the presence of pathogens. *Plant Disease*, 102(1), 67–72.
- Mengistie, B. T., Mol, A. P., & Oosterveer, P. (2017). Pesticide use practices among smallholder vegetable farmers in Ethiopian Central Rift Valley. *Environment, Development and Sustainability*, 19(1), 301–324.
- Nagendranatha, C. N., Arunasri, K., Kumar, Y. D., Krishna, K. V., & Mohan, S. V. (2016). Qualitative in vitro evaluation of plant growth promoting activity of electrogenic bacteria from biohydrogen producing microbial electrolysis cell towards biofertilizer application. *Journal of Energy and Environmental Sustainability*, 1, 47–51.
- Obeng, F. A., Gyasi, P. B., Olu-Taiwo, M., & Ayeh-Kumi, F. P. (2018). Microbial assessment of tomatoes sold at some central markets in Ghana. *BioMed Research International*, 2018.
- Ochoa-Velasco, C. E., et al. (2016). Effect of nitrogen fertilization and *Bacillus licheniformis* biofertilizer addition on antioxidant compounds and activity of greenhouse cultivated tomato fruits. *Scientia Horticulturae*, 201, 338–345.
- Pérez-García, A., Romero, D., & De Vicente, A. (2011). Plant protection and growth stimulation by microorganisms: biotechnological applications of *Bacilli* in agriculture. *Current Opinion in Biotechnology*, 22(2), 187–193.
- Radhakrishnan, R., Hashem, A., & Abd_Allah, E. F. (2017). *Bacillus*: A biological tool for crop improvement through bio-molecular changes in adverse environments. *Frontiers in Physiology*, 8, 667.
- Schütz, L., et al. (2018). Improving crop yield and nutrient use efficiency via biofertilization — A global meta-analysis. *Frontiers in Plant Science*, 8, 2204.
- Sebilo, M., Mayer, B., Nicolardot, B., Pinay, G., & Mariotti, A. (2013). Long-term fate of nitrate fertilizer in agricultural soils. *Proceedings of the National Academy of Sciences*, 110(45), 18185–18189.
- Sharma, S., & Bhattarai, K. (2019). Progress in developing bacterial spot resistance in tomato. *Agronomy*, 9(1), 26.
- Thakore, Y. (2006). The biopesticide market for global agricultural use. *Industrial Biotechnology*, 2(3), 194–208.
- Vessey, J. K. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil*, 255(2), 571–586.
- Weselowski, B., et al. (2016). Isolation, identification and characterization of *Paenibacillus polymyxa* CR1 with potentials for biopesticide, biofertilization, biomass degradation and biofuel production. *BMC Microbiology*, 16(1), 1–10.
- Wu, S. C., Cao, Z. H., Li, Z. G., Cheung, K. C., & Wong, M. H. (2005). Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. *Geoderma*, 125(1–2), 155–166.
- Zhao, K., et al. (2018). Actinobacteria associated with *Glycyrrhiza inflata* Bat. are diverse and have plant growth promoting and antimicrobial activity. *Scientific Reports*, 8(1), 1–13.