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Research Article

Eco-Friendly Management of Fusarium Wilt of Pea (*Pisum sativum* L.) using Biocontrol Agents

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ABSTRACT

Pea (*Pisum sativum* L.), a vital leguminous crop, is severely affected by Fusarium wilt caused by *Fusarium oxysporum* f. sp. *pisi*. In the current study, we tested the effectiveness of biocontrol agents under *in vitro* and pot conditions. The pathogen was isolated from infected plants collected from Faisalabad. The pathogen was identified based on morphological features. Four biocontrol agents were tested using a dual culture technique under *in vitro* conditions: *Bacillus subtilis*, *Janthinobacterium lividum*, *Beauveria bassiana*, and *Verticillium lecanii*. The biocontrol agents were further tested using a seed priming technique on three varieties of peas: Sarsabz, Mateor, and Pea-2009. Results showed that *J. lividum* exhibited maximum inhibitory activity (31.97%) at 12 days of incubation, however, *B. subtilis* consistently showed antagonistic activity (24.80%). In pot experiments, *B. subtilis* significantly reduced disease incidence and severity in all varieties as compared to controls. In addition, *B. subtilis* significantly improved growth parameters in comparison to controls under pot conditions. Among the varieties, Sarsabz showed better results. It can be concluded that *B. subtilis* showed promising potential for the management of Fusarium wilt of pea under controlled conditions, however, further validation under field conditions and molecular-level investigations are required.

Keywords: *Bacillus subtilis*, Biological disease management, Dual culture assay, *Fusarium oxysporum*, *Janthinobacterium lividum*, Pea wilt.

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INTRODUCTION

Pea (*Pisum sativum* L.) is an essential cool-season leguminous crop of the Fabaceae family, which has considerable economic, nutritional, and environmental importance as a nutrient-rich legume seed crop containing high levels of proteins, vitamins, minerals, dietary fiber, and various phytochemicals with antioxidant and anticancer properties (Dahl *et al.*, 2012; Rungruangmaitree and Jiraungkoorskul, 2017). As an inexpensive source of dietary protein, pea has contributed significantly to global food security and sustainable agriculture by virtue of its ability to fix atmospheric nitrogen, thus improving the fertility of the soil and minimizing the application of chemical fertilizers (Graham and Vance, 2003; Zhang *et al.*, 2025).

Pea cultivation on a global scale is practiced mainly in temperate and subtropical climates of the world. According to recent statistics provided by the FAO, along with relevant analysis, the global production of dry peas has averaged around 12-14 million metric tonnes annually over the past few years, with 14.2 million tonnes of dry peas being produced on approximately 7-8 million hectares of land worldwide, with some additional areas under green or fresh pea cultivation (FAOSTAT, 2023; Windsor *et al.*, 2024).

China, India, Canada, and Russia are among the major pea-producing countries worldwide (FAOSTAT, 2023). In Pakistan, pea cultivation has been reported mainly in Punjab and Khyber Pakhtunkhwa provinces, where it has been grown as an important winter crop. However, the yields are not up to international standards due to various biotic and abiotic factors, diseases, lack of proper agricultural practices, and low adoption of improved technology and high-yielding varieties of peas (MNFSR, 2024). Among various biotic factors, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *pisi*, also known as *Fop*, is one of the most destructive diseases of pea worldwide, including Pakistan. *F. oxysporum* f. sp. *pisi*, the causal organism of Fusarium wilt of pea, has the ability to survive as long-lasting chlamydospores in the soil for 10 to 15 years or more, causing substantial loss of yield, ranging from 20 to 100%, through typical symptoms of yellowing of leaves, wilting, vascular discoloration, stunted growth, and finally, death of infected plants (Deng *et al.*, 2022; Sharma, 2011; Usman *et al.*, 2025). In areas like Faisalabad and Punjab, the disease has been exacerbated by the practice of continuous cropping, poor soil management, and favorable environmental conditions of winter season, characterized by cool and moist soils (Merzoug *et al.*, 2014; Usman *et al.*, 2025).

In the past decade (2015-2025), research focus has been given to the application of eco-friendly and sustainable approaches to manage Fusarium wilt in pea and other legumes due to the problems of resistance to chemical fungicides, their toxic nature, and residues. Although chemical fungicides like carbendazim have shown some degree of efficacy in the management of the disease, integrated approaches have come to the limelight (Rauf *et al.*, 2024; Usman *et al.*, 2025). Biological approaches involving antagonistic microorganisms have also come to play a major role in the management of Fusarium wilt in pea. *Trichoderma* spp., including *T. harzianum*, *T. viride*, *T. asperellum*, *T. koningii*, have been evaluated for their efficacy in managing the disease through their mycoparasitic action and their capacity to elicit systemic resistance in plants. In Pakistan, in the Punjab region and neighboring areas, high *in vitro* mycelial inhibition (up to 80-82%), reduced disease severity in pot and field experiments (50-80%), and induced plant growth through the production of cell wall-degrading enzymes, secondary metabolites, and defense enzymes like peroxidase and polyphenol oxidase have been reported (Nawaz *et al.*, 2021; Rauf *et al.*, 2024). *Trichoderma* metabolites, especially aged filtrates, possess dose-dependent antimycotic efficacy against *Fop*. Field trials have revealed substantial wilt suppression and productivity enhancement in pea (Rauf *et al.*, 2024).

Bacillus species, such as *Bacillus subtilis* and close relatives (including *B. amyloliquefaciens*, *B. halotolerans*), have shown great potential. Recent trials from Pakistan and global researchers have reported 50-89% wilt suppression in peas via seed priming or consortia applications, promoting growth attributes (shoot/ root length, biomass up to 80-88%), and triggering ISR through lipopeptide antibiotics (surfactin and fengycin), siderophores, enzymes, and modulation of the plant microbiome (Raza *et al.*, 2024; Riaz *et al.*, 2021; Usman *et al.*, 2025). Vegetable-associated *Bacillus* species have been reported to inhibit root rot disease in peas due to Fusarium solani by using antifungal metabolites and zinc solubilization (Riaz *et al.*, 2021). Recent research findings have confirmed that plant growth-promoting rhizobacteria (PGPR) such as *B. subtilis* can successfully control plant diseases, along with growth and physiological characteristics (Ehsan *et al.*, 2026; Ihsan *et al.*, 2025). Endophytic *B. subtilis* strains have antagonized various soilborne plant pathogens, including *Fusarium* species, and have also triggered defense enzyme activities and PR gene expression in chickpea, which is applicable to other legumes like peas (Usman *et al.*, 2025).

Consortia and combinations, such as *Bacillus* + *Trichoderma* or *Bacillus* + *Pseudomonas*/AMF, show synergy to obtain 70-80% disease control and yield/anatomical improvement (Raza *et al.*, 2024). Additionally, VOCs of *Bacillus* and other rhizobacteria inhibit *Fusarium* growth, thus showing suppression (Riaz *et al.*, 2021; Usman *et al.*, 2025). In Pakistan, especially Faisalabad/Punjab, indigenous rhizobacteria and fungi of pea rhizosphere soil are being targeted for the management of wilt disease, as seen in global trends focusing on microbial formulations for sustainable agriculture (Usman *et al.*, 2025). However, there are limitations to be considered, such as variability of pathogen races, inconsistent field efficacy, and development of optimized delivery systems (Deng *et al.*, 2022; Usman *et al.*, 2025).

Although numerous studies have been conducted on individual biocontrol agents, few studies have been done on the antagonistic behavior of biocontrol agents over time *in vitro* and their efficacy under pot conditions against pea disease. In addition, the efficacy of bacterial and fungal biocontrol agents against various varieties of pea has not been fully investigated. Hence, the present study was conducted with the aim of evaluating the efficacy of biocontrol agents against *F. oxysporum* f. sp. *pisi* under laboratory and pot conditions. This study, as compared to previous studies, not only focuses on individual biocontrol agents but also presents a comparative evaluation of various bacterial and fungal biocontrol agents against different pea cultivars. In addition, this study presents a comprehensive evaluation of the temporal assessment of antagonistic activity along with plant-level responses. This study hypothesized that *B. subtilis* would offer better consistencies in terms of disease suppression and plant growth promotion under pot conditions compared to other biocontrol agents, in spite of inconsistent *in vitro* antagonistic performance.

MATERIALS AND METHODS

Survey and Sample Collection

Diseased pea plants infected with Fusarium wilt symptoms like yellowing, wilting, and vascular discoloration of the infected plants were collected from different farmer fields in Samundri, Faisalabad (Figure 1). These infected plants were collected in the winter season. After collecting, the infected plants were placed in sterilized polythene bags and transported to the Biocontrol Laboratory, Department of Plant Pathology, University of Agriculture Faisalabad (UAF), and stored at 4°C.

Figure 1: Diseased pea plant samples collected from farmer's fields exhibiting Fusarium wilt disease: (A) infected pea plant, (B) infected pea leaves, (C) infected pea pods.

Isolation, Identification and Purification of the Pathogen

Root and stem segments (5 mm) of infected plants were surface sterilized in ethanol (70%) for 30-60 s. After surface sterilization, the segments were rinsed thrice in sterile distilled water. Finally, the segments were placed on Potato Dextrose Agar (PDA) medium supplemented with 50 mg/L kanamycin. After incubation at 28 ± 2°C for 5-7 days, emerging Fusarium colonies were purified by hyphal tip method. After purification, the pathogen was incubated at 28 ± 2°C for 10-14 days. Identification was based on morphological characteristics. However, the results of the morphological identification may not be precise in the identification of *F. oxysporum*, hence, the lack of molecular confirmation (e.g., ITS rDNA sequencing) is recognized as the limitation of the present study.

In vitro Dual Culture Assay

Antagonism of four biocontrol agents (*Bacillus subtilis, Janthinobacterium lividum, Verticillium lecanii, Beauveria bassiana*) against *F. oxysporum* was investigated by dual culture method in PDA. A 5 mm *F. oxysporum* mycelial disc was placed at the center of the plate, and bacterial agents were streaked at 3-4 cm distance, while fungal agents were placed as discs at the same distance. Control plates were used without biocontrol agents. This experiment was conducted in Completely Randomized Design (CRD) with three replicates. All plates were incubated at 28 ± 2°C. Radial growth was recorded at 4, 8, and 12 days. Percentage Growth Inhibition (PGI) was calculated as:

$$\text{PGI}\ (\%) = \frac{\text{C} - \text{T}}{\text{C}} \times 100$$

Where,

C = Control Growth

T = Treatment Growth

Each treatment had three replicates, and experiment was repeated twice.

Biocontrol Agent Preparation and Seed Priming

Bacterial agents (*B. subtilis* and *J. lividum*) were grown in LB broth at 28°C, 180 rpm for 24-48 hours. Bacterial suspension was adjusted to approximately 10^8 CFU/mL. Seeds of three varieties of pea (Sarsabz, Mateor, Pea-2009) procured from Vegetable Research Institute, Faisalabad, were used in this study. Seeds were soaked in bacterial suspensions (single strains or consortium) for 6-8 hours and then dried in air.

Pot Experiment Setup

Pots of 30 cm diameter and 10 kg capacity were filled with autoclaved sandy loam soil of pH 7.8. Soil samples were artificially infested with *F. oxysporum* sorghum grain inoculum at 10 g/kg of soil (approximately 10^5 CFU/g of soil). Seeds were sown (5-6 seeds per pot) and thinned to 3 uniform seedlings per pot after emergence. The pots were arranged in a Completely Randomized Design (CRD) within the greenhouse to minimize positional effects with three replicates. The pots were also rotated periodically to reduce effects of environmental factors. Greenhouse conditions were maintained (temperature 22 ± 3°C/15 ± 2°C day/night) with natural light and watering every 2 days to field capacity.

Disease Assessment

Disease severity was assessed using a modified 0–9 Fusarium wilt disease rating scale (Shahbaz *et al.*, 2025). The growth parameters were assessed at harvest including Shoot length (cm), Shoot Weight (g), Root Length (cm), Root Weight (g), No. of Primary Branches, No. of Secondary Branches and No. of Pods.

Table 1. Disease rating scale used for assessment of Fusarium wilt in pea caused by *F. oxysporum*.

Rating scale	Disease response	Symptom on plants
0	Immune	No plant wilting
1	Highly Resistant	1% plants wilted
3	Resistant	2-20% plants wilted
5	Moderately Resistant	20.1-30% plants wilted
7	Susceptible	30.1-50% plants wilted
9	Highly Susceptible	50.1% or more plants wilted

Statistical Analysis

The data were analyzed using analysis of variance (ANOVA) with R software. Before subjecting the data to ANOVA, normality and homogeneity of variance were tested using Shapiro-Wilk and Levene's tests, respectively. Where appropriate, percentage data were transformed using arcsine-square root transformation. Multiple comparisons of means were carried out using Tukey's Honestly Significant Difference (HSD) at $p \leq 0.05$. Furthermore, two-way ANOVA was used for *in vitro* data to evaluate the impact of treatment and time. Principal Component Analysis (PCA) was used to evaluate the relationship between variables.

RESULTS

Morphological Identification

Morphological identification confirmed the pathogen as *F. oxysporum* based on colony and microscopic examination for septate hyphae and typical macroconidia (Figure 2). Identification of the pathogen was based on morphological characteristics using standard taxonomic keys, but molecular identification using methods such as ITS sequencing was not carried out and is considered a limitation of the study.

Antagonistic test

Two-way ANOVA revealed that both treatment and incubation time had significant effects ($p \leq 0.05$) on mycelial growth inhibition, indicating that antagonistic activity varied significantly over time. The *in vitro* dual culture assay indicated that the four biocontrol agents had differential antagonistic activities towards *F. oxysporum* during the 12-day period shown in Table 2 and Figure 3. Inhibition activities changed over time. At 4 days, *B. subtilis* showed the highest inhibition (30.17%), followed by *B. bassiana* (25.87%), *V. lecanii* (24.73%), and *J. lividum* (22.03%).

At 8 days, *J. lividum* showed the strongest inhibition (27.57%), while *B. subtilis* recorded the lowest inhibition (18.80%). The inhibitions of the two entomopathogenic fungi continued to decline (*B. bassiana* 15.73%, *V. lecanii* 15.07%). At 12 days, *J. lividum* showed the highest inhibition (31.97%), followed by *B. subtilis* (24.80%). The inhibitions of the two entomopathogenic fungi continued to be moderate (*B. bassiana* 21.27%, *V. lecanii* 19.87%).

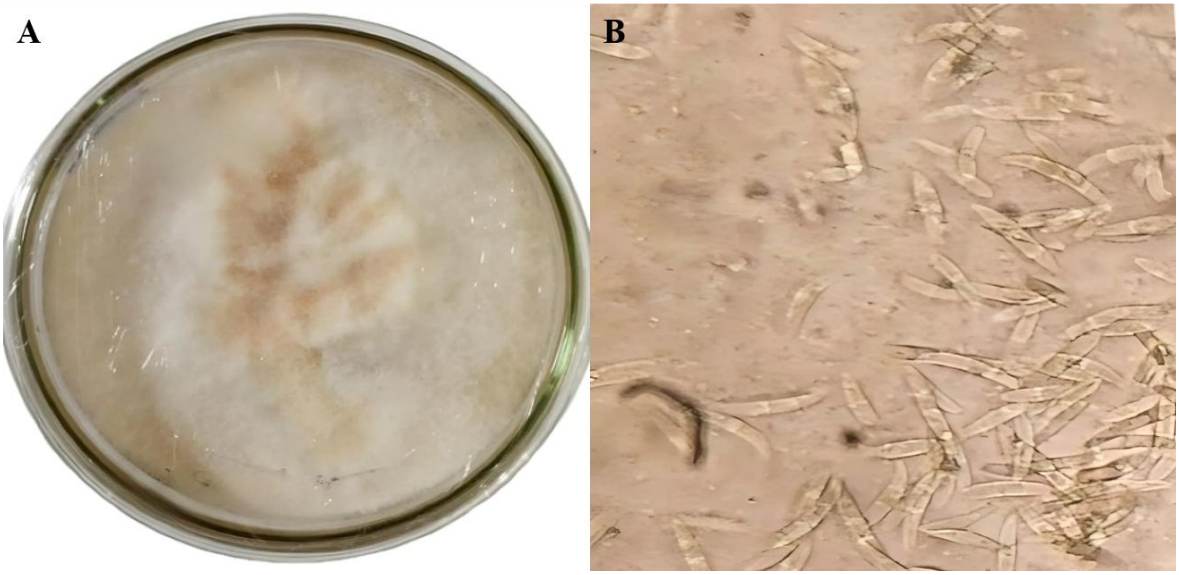

Figure 2. Morphological characteristics of *F. oxysporum*: (A) pure culture of *F. oxysporum* on PDA medium, (B) microscopic view of *F. oxysporum* macroconidia.

Table 2. Percentage inhibition of radial growth of *F. oxysporum* by various biocontrol agents after 4, 8, and 12 days of inoculation.

Treatments	4 Days	8 Days	12 Days
B. subtilis	30.17a	18.80b	24.80b
J. lividum	22.03c	27.57a	31.97a
B. bassiana	25.87b	15.73c	21.27c
V. lecanii	24.73b	15.07c	19.87c
Control	0d	0d	0d

Values followed by different letters are significantly different at $p \leq 0.05$ according to Tukey's HSD test.

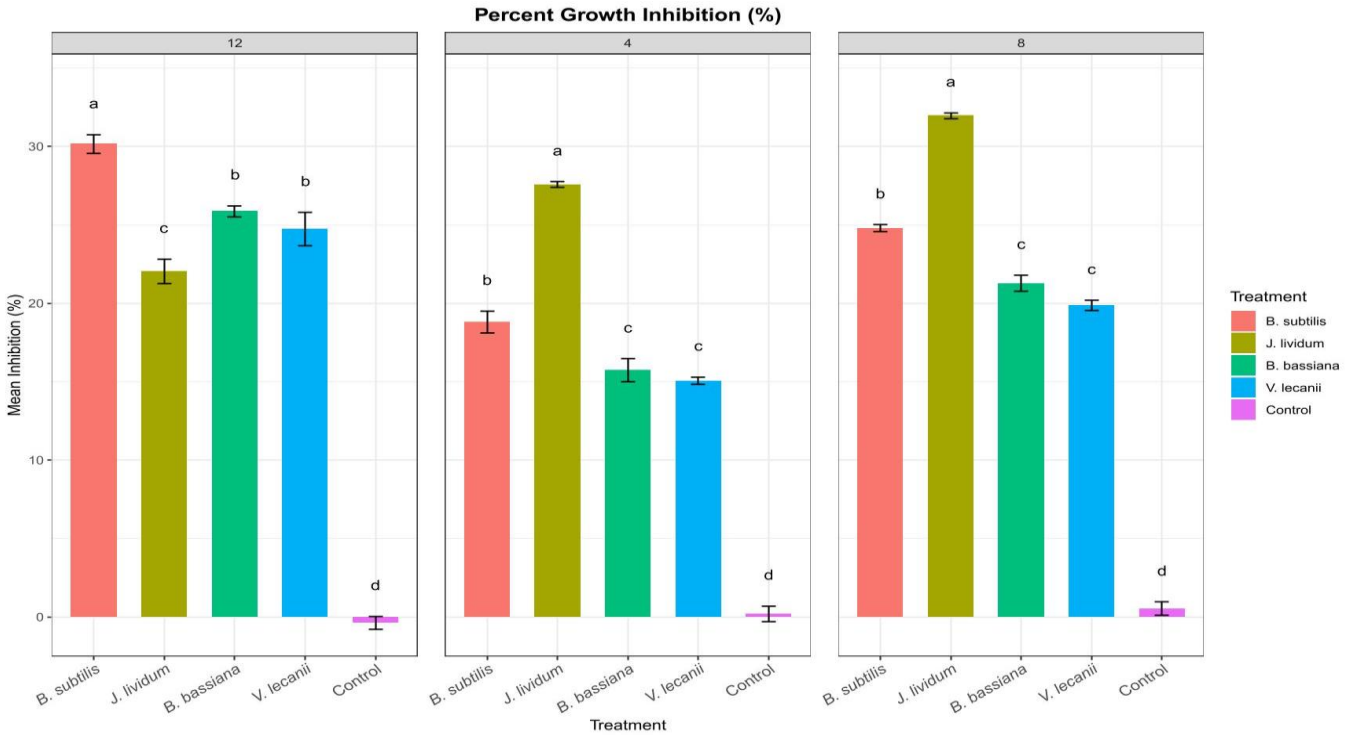

Figure 3. Percentage inhibition of radial growth of *F. oxysporum* by biocontrol agents at 4, 8, and 12 days after inoculation.

Table 3. Effect of biocontrol agents on disease incidence and disease severity of Fusarium wilt in pea plants.

Varieties	Treatments	Incidence	Severity
Mateor	*B. subtilis*	32c	17.2c
	J. lividum	52.3b	35.6b
	B. subtilis & *J. lividum*	41.5c	26.8b
	Control	87.2a	74.1a
Pea-2009	*B. subtilis*	34.8d	18.6d
	J. lividum	55.4b	37.9b
	B. subtilis & *J. lividum*	44.7c	28.3c
	Control	90.1a	76.5a
Sarsabz	*B. subtilis*	22.1c	9.6c
	J. lividum	41.8b	27.7b
	B. subtilis & *J. lividum*	33.6b	20.9b
	Control	79.8a	67.7a

Values followed by different letters are significantly different at $p \leq 0.05$ according to Tukey's HSD test.

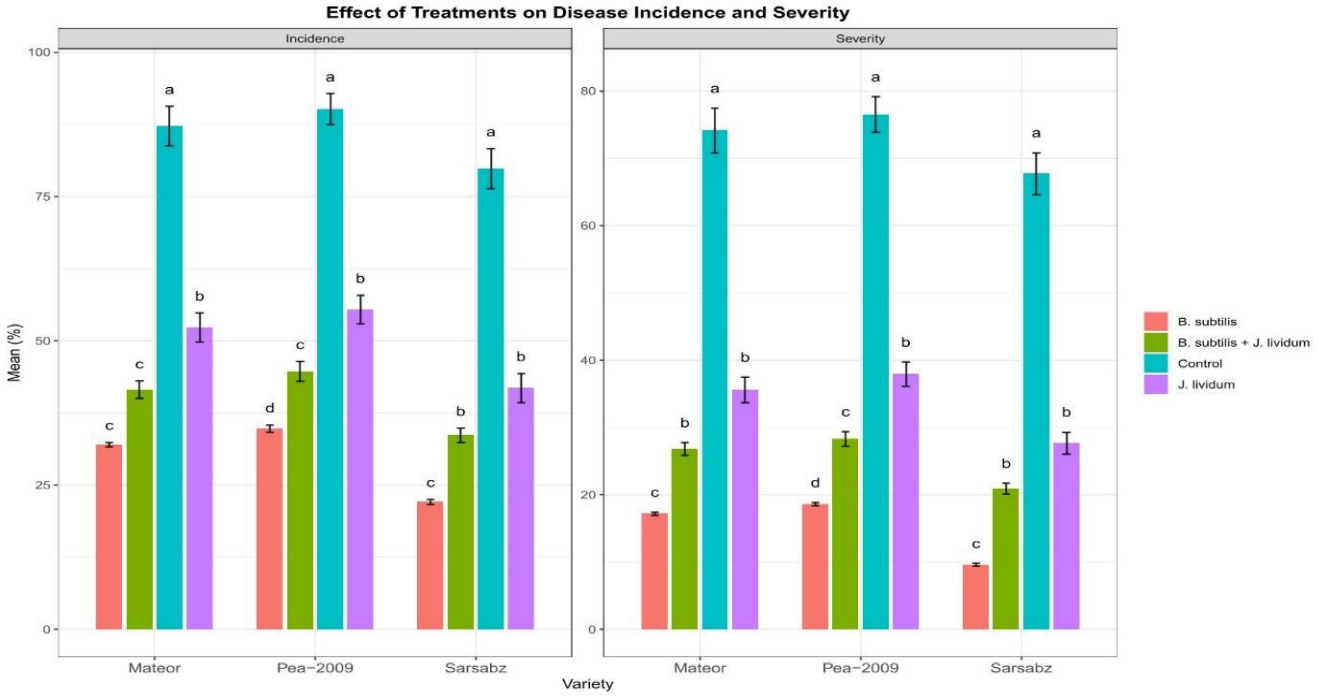

Figure 4. Effect of biocontrol treatment on disease incidence and disease severity of Fusarium wilt disease in pea varieties under pot conditions.

J. lividum showed the highest inhibition at 12 days; however, it was not significantly different from other treatments based on Tukey's HSD test. *B. subtilis* had strong and relatively stable inhibitory activities during the entire period. In contrast, the inhibitory activities of the entomopathogenic fungi *B. bassiana* and *V. lecanii* started high but declined over time. No inhibition of radial growth was observed in the control treatment.

Pot Trial

In the pot experiment, the plants inoculated with *F. oxysporum* alone showed typical symptoms of Fusarium wilt disease. These symptoms appeared as pale yellowing of leaves, which later turned straw-colored. Leaflets and stems showed drooping, while plants were stunted, and no pod formation was observed. Dark brown to black vascular discoloration was observed upon longitudinal sectioning of stems and roots. In the late stages, total (100%) wilting was observed, while in the intermediate stages, 30% and 50% wilting were observed, as depicted in Figure 5. However, the application of biocontrol agents delayed disease appearance and reduced severity in all varieties (Table 3 and Figure 4).

Figure 5. Fusarium wilt disease in pea plants under pot conditions: (A) 30% infection, (B) 50% infection, (C and D) complete wilting of pea plants due to pathogen infection.

Table 4. Effect of biocontrol treatments on growth parameters of three pea varieties.

Varieties	Treatments	Shoot Length (cm)	Root Length (cm)	Shoot Weight (g)	Root Weight (g)	No. of Primary Branches	No. of Secondary Branches	No. of Pods/Plant
Mateor	*B. subtilis*	28.67a	15.67a	23.33ab	5.03ab	3.67ab	10.67ab	6.67ab
	J. lividum	25.67bcd	12.33d	18.67cd	3.63def	3abcd	9abc	6abc
	B. subtilis & *J. lividum*	27.33ab	13.33cd	20.67bc	4.8abc	3.33abc	9.67abc	6abc
	Control	23.33de	12d	17def	2.93fg	2.33cd	8bc	5.33bc
Pea-2009	*B. subtilis*	27.33ab	15abc	22.33ab	4.7abc	3.33abc	10.33abc	6.33abc
	J. lividum	24.33cde	13.67bcd	18cdef	3.57def	2.67bcd	8bc	6abc
	B. subtilis & *J. lividum*	25.33bcd	14.67abc	17.33def	4.27bcd	2.67bcd	9.33abc	6abc
	Control	22.67e	12d	15.33f	2.57g	2d	7.67c	4.67c
Sarsabz	*B. subtilis*	28.67a	15.33ab	24.33a	5.25a	3.67ab	11.33a	7.67a
	J. lividum	26bc	13.67bcd	18.33cde	4.07cde	3.33abc	9.67abc	6.33abc
	B. subtilis & *J. lividum*	27.33ab	15abc	18.67cd	4.77abc	4a	9.67abc	6.67ab
	Control	23.67cde	12.33d	15.67ef	3.3efg	2.33cd	7.67c	5.33bc

Values followed by different letters are significantly different at $p \leq 0.05$ according to Tukey's HSD test.

The application of biocontrol agents significantly reduced disease incidence and severity compared with the pathogen-inoculated control for all varieties (Table 3). In the Mateor variety, *B. subtilis* showed disease incidence and severity at 32% and 17.2%, respectively, which were significantly lower than the control (87.2% incidence and 74.1% severity). *J. lividum* showed higher values for disease incidence and severity at 52.3% and 35.6%, respectively, while the consortium treatment (*B. subtilis* + *J. lividum*) showed intermediate values for disease incidence and severity at 41.5% and 26.8%, respectively.

In Pea-2009, *B. subtilis* recorded the lowest disease incidence (34.8%) and severity (18.6%), followed by combination of both treatment disease incidence (44.7%) and severity (28.3%). *J. lividum* showed relatively higher disease incidence (55.4%) and severity (37.9%). The maximum disease incidence (90.1%) and severity (76.5%) were shown by the control treatment. In Sarsabz variety too, *B. subtilis* showed the lowest disease incidence (22.1%) and severity (9.6%), followed by consortium treatment disease incidence (33.6%) and severity (20.9%). *J. lividum* showed relatively higher disease incidence (41.8%) and severity (27.7%), whereas the maximum disease incidence (79.8%) and severity (67.7%) were shown by the control treatment.

The above results, however, indicate that all the biocontrol agents significantly reduced disease incidence and severity in all varieties in comparison to the control treatment. Among all biocontrol agents, *B. subtilis* showed lower disease values in all varieties; however, in a few cases, it showed statistically at par results in comparison to consortium treatment based on Tukey's HSD test ($p \leq 0.05$). Seed priming with the biocontrol agents resulted in significant promotion of vegetative and reproductive growth over the pathogen-inoculated control. Among the three varieties, B. subtilis showed the strongest promotion, followed by the *B. subtilis* + *J. lividum* consortium, and finally, *J. lividum* alone (Figure 6 and Table 4).

In Sarsabz, the best-performing variety, *B. subtilis* priming resulted in 28.67 cm shoot length, 21.1% greater than the control 23.67 cm; 15.33 cm root length, 24.3% greater than the control 12.33 cm; 24.33 g shoot fresh weight, 55.2% greater than the control 15.67 g; 5.25 g root fresh weight, 59.1% greater than the control 3.3 g; 3.67 primary branches, 57.5% greater than the control 2.33; 11.33 secondary branches, 47.8% greater than the control 7.67; and 7.67 pods per plant, 43.9% greater than the control 5.33. Mateor and Pea-2009 varieties showed similar trends with lower absolute values and percentage increases. For instance, in Mateor, the shoot length was 28.67 cm (22.9% increase over 23.33 cm control), root weight 5.03 g (71.7% increase over 2.93 g control), and pods 6.67 (25% increase over 5.33 control); in Pea-2009, the shoot length was 27.33 cm (20.6% increase over 22.67 cm control), root weight 4.7 g (82.9% increase over 2.57 g control), and pods 6.33 (35.6% increase over 4.67 control).

Generally, the consortium treatment had intermediate effects; the growth increase was 10 to 30% lower than that of *B. subtilis*. The above effects may be associated with possible interactions between the two bacterial strains. *J. lividum* alone showed growth increase over control but remained lower than that of *B. subtilis* and the consortium in almost all growth parameters.

The first two principal components, PC1 and PC2, accounted for most of the variability among treatments, where PC1 is linked to growth parameters and PC2 is linked to disease-related traits. Principal component analysis of growth parameters (Figure 7) and correlation matrix (Figure 8) showed that *B. subtilis* treatment had strong positive correlation with all positive growth parameters and negative correlation with disease severity indicators of the control.

Out of all the varieties screened, Sarsabz showed the highest positive response to *B. subtilis* treatment. This study thus suggests that *B. subtilis* can be employed as an effective biocontrol agent for management of Fusarium wilt in pea, particularly in areas where the disease is common, such as Faisalabad, in an eco-friendly manner. The results showed that *B. subtilis* showed consistent antagonistic activity under *in vitro* conditions.

This was consistent with the reduced disease incidence and plant growth promotion under pot conditions. The above effects may be associated with possible mechanisms such as antibiosis, induced systemic resistance, and phytohormone production, as reported in previous studies; however, these mechanisms were not directly investigated in the above study.

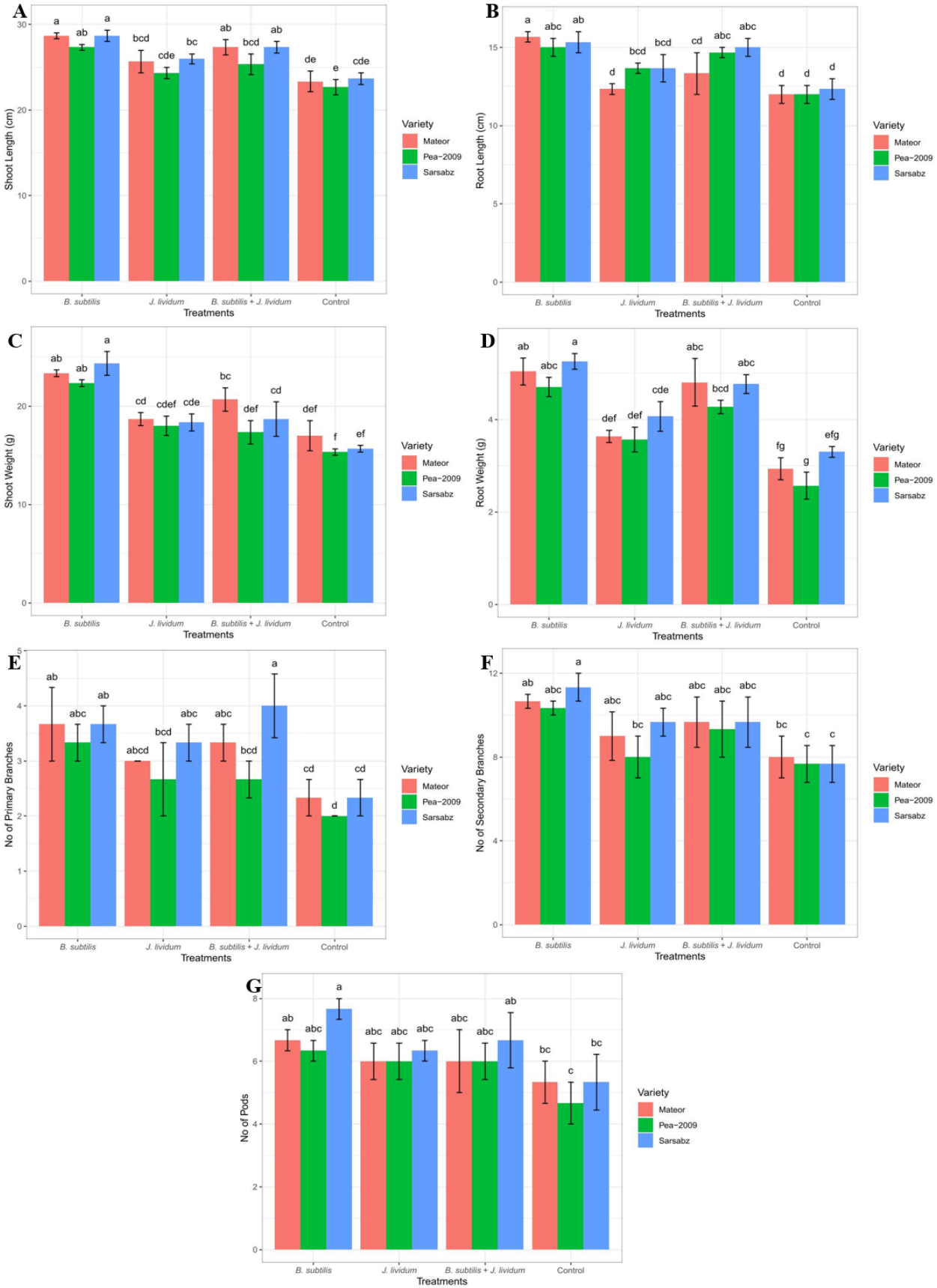

Figure 6. Effect of biocontrol treatment on growth parameters of pea plants: (A) shoot length, (B) root length, (C) shoot weight, (D) root weight, (E) number of primary branches, (F) number of secondary branches, and (G) number of pods per plant.

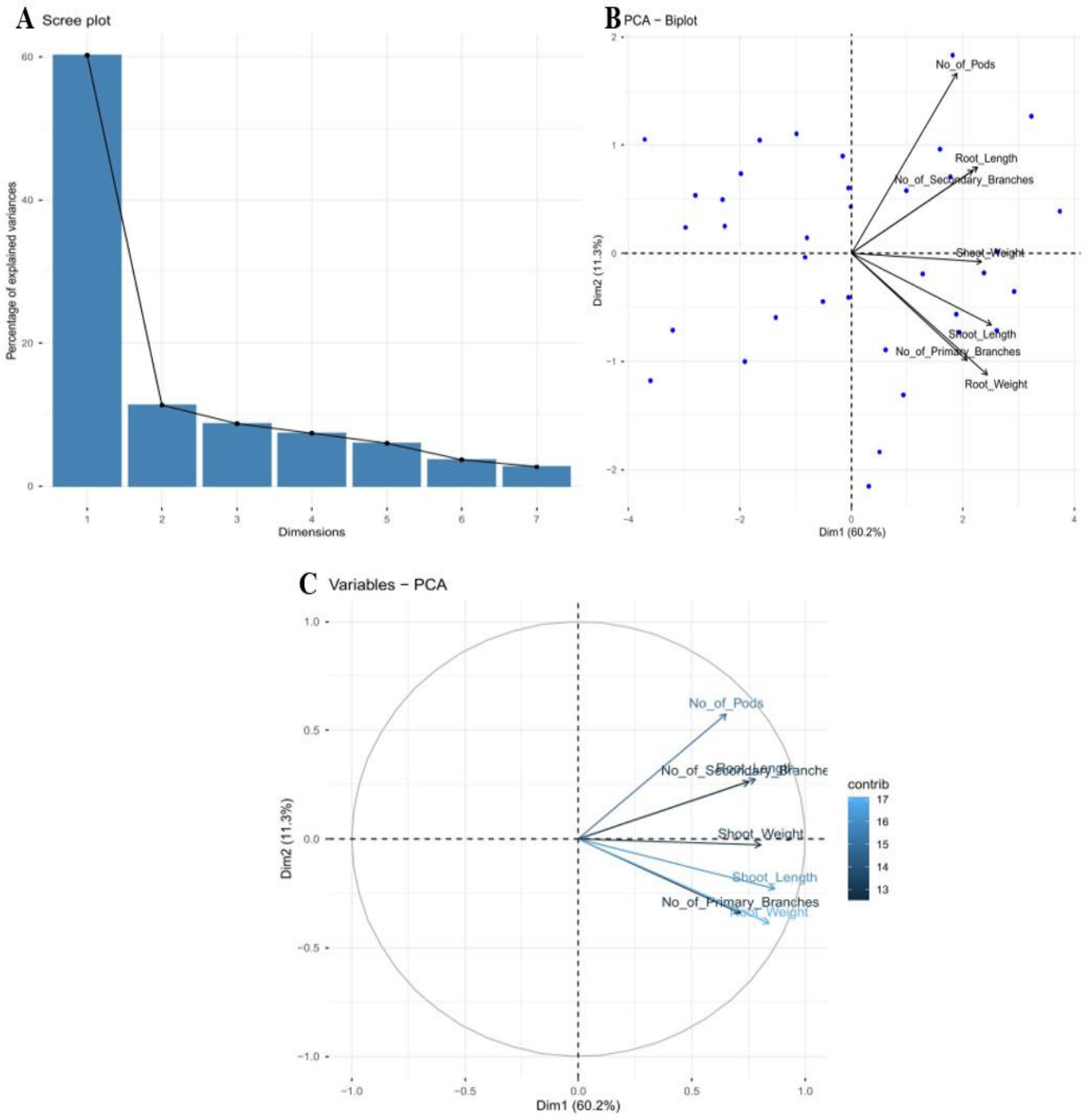

Figure 7. Principal Component Analysis (PCA) of growth parameters of pea plants under different treatment conditions: (A) scree plot, (B) biplot, (C) contribution of variables.

DISCUSSION

In the *in vitro* dual culture assay, the antagonistic potential of the biocontrol agents against Fop showed distinct time-course responses. *J. lividum* showed strong initial antagonism, whereas *B. subtilis* showed sustained antagonism over time of the dual culture assay. In contrast, the antagonistic potential of the two fungal biocontrol agents, *B. bassiana* and *V. lecanii*, showed moderate antagonism, which declined after a short period, returning to the control level. These findings are consistent with the well-established modes of actions of the biocontrol agents. The rapid antagonism of *J. lividum* may be associated with the production of secondary metabolites such as violacein, as reported in previous studies, violacein, which has potent antifungal activity against a wide range of fungi, including *Fusarium* spp., owing to its ability to damage the membrane of the cell of the pathogen (Becker *et al.*, 2009; Durán *et al.*, 2022).

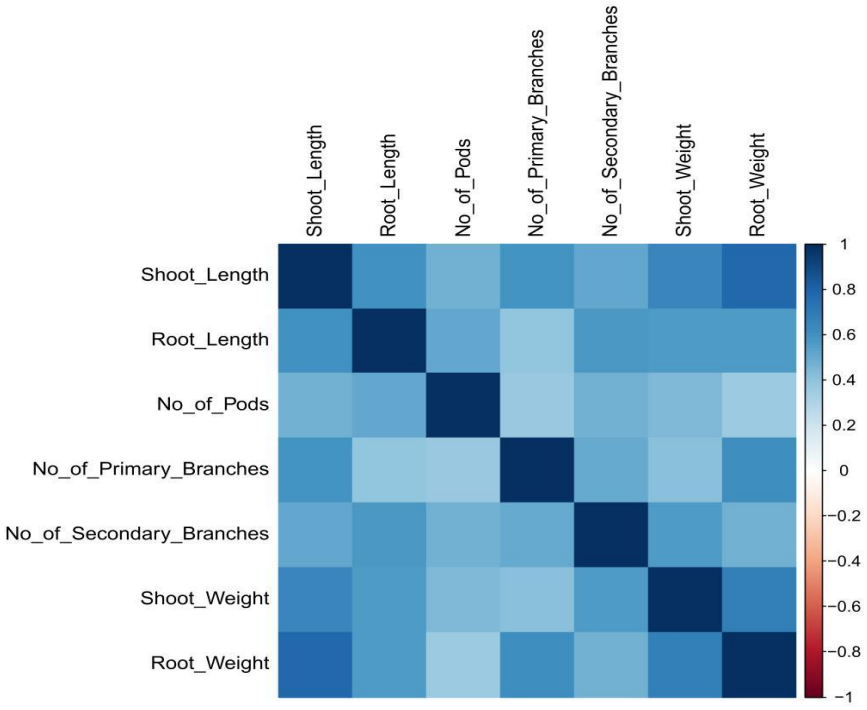

Figure 8. Correlation heatmap showing relationships among plant growth parameters and disease traits under different treatments.

In contrast, the sustained antagonism of *B. subtilis* may be associated with the production of stable cyclic lipopeptides, including surfactin, iturin, and fengycin, as well as the production of siderophores, which may contribute to pathogen suppression through nutrient competition and, thereby inhibiting the growth of the pathogen, as well as the production of enzymes, which inhibit the growth of the pathogen through the degradation of the cell walls of the pathogen (Ongena and Jacques, 2008; Raza *et al.*, 2024; Xiao *et al.*, 2021). These mechanisms have been extensively documented in recent studies on *Bacillus*-mediated biocontrol of soil-borne *Fusarium* pathogens of legumes and other crops (Riaz *et al.*, 2021; Usman *et al.*, 2025).

Under pot conditions, *B. subtilis* priming showed the most effective control of Fusarium wilt and the most enhanced plant growth and yield components compared to the application of *J. lividum* alone or the bacterial consortium. This difference in performance under *in vivo* conditions, notwithstanding the potent early *in vitro* efficacy of *J. lividum*, reflects the significance of competence for the plant-rhizosphere and the persistence of metabolites and induced systemic resistance. *B. subtilis* has been widely reported as an effective biocontrol agent due to its ability to effectively colonize plant roots, to synthesize plant growth hormones such as indole-3-acetic acid to stimulate root and shoot elongation, to solubilize phosphate for enhanced plant nutrition, and to induce plant defense responses such as peroxidase, polyphenol oxidase, and pathogenesis-related proteins to mitigate disease pressure while enhancing plant growth and yield (Fan *et al.*, 2025; Raza *et al.*, 2024; Riaz *et al.*, 2021).

The apparent dominance of *B. subtilis* over consortium treatment could also imply possible mild antagonistic or competitive interactions between two strains of bacteria in the rhizosphere, which might reduce synergistic potential. Similar results have also been recorded in other experiments assessing multi-strain consortium efficacy in controlling Fusarium wilt in pea and other legume crops, where in some cases, single-strain applications were found to be more effective compared to consortium treatments owing to possible niche overlap and colonization efficiency (Elbouazaoui *et al.*, 2022; Raza *et al.*, 2024).

Among these three varieties, Sarsabz always demonstrated the best response in growth to *B. subtilis* priming. This could be attributed to genetic vigor, partial resistance, or a more compatible microflora in the rhizosphere of Sarsabz compared to Mateor and Pea-2009. Specific responses of genotypes to biocontrol agents have already been demonstrated and should be taken into consideration for variety selection (Elbouazaoui *et al.*, 2022; Landa *et al.*, 2006).

These findings agree with recent studies that showed that *B. subtilis* and other strains can suppress Fusarium wilt, increase vegetative biomass, and enhance yield parameters through a combination of antibiosis, PGP, and induced systemic resistance (Raza *et al.*, 2024; Riaz *et al.*, 2021; Usman *et al.*, 2025). The results of this study agree with previous research findings, which showed that *B. subtilis* was successful in controlling disease severity along with growth characteristics under controlled and field conditions (Ihsan *et al.*, 2025; M. Usman *et al.*, 2025).

In conclusion, *B. subtilis* has been found to be the most effective bacterial biocontrol agent for the management of fusarium wilt of pea under controlled pot conditions at the Faisalabad region. The use of *B. subtilis* provides a promising alternative to the use of chemical fungicides (Rauf *et al.*, 2024). However, the results are only indicative and need to be confirmed by conducting field trials. One of the limitations of the present investigation is the lack of molecular confirmation of the pathogen based only on the morphological characteristics of the pathogen. It is suggested that future investigations should include the use of molecular identification techniques for accurate pathogen characterization, as well as the examination of microbial populations in the field.

CONCLUSION

The results of the present study proved that *Bacillus subtilis* effectively reduced the disease incidence and severity and improved the growth parameters of pea plants. Although *Janthinobacterium lividum* possessed significant antagonistic potential *in vitro*, the results of the pot study were not as promising. Among all the tested varieties, Sarsabz was found to be the most responsive. However, the results are based on controlled conditions and morphological characteristics of the pathogen. Further studies need to be carried out for molecular confirmation of the pathogen, mode of action of the biocontrol agent, and field studies.

AUTHOR CONTRIBUTIONS

Abdul Rahman conceived and designed the study. Muhammad Usman conducted statistical analysis, contributed to data interpretation and writing original draft. Numaad Ihsan and Muhammad Talha Ashraf performed the experiments and collected data. Ayesha Khalid assisted in manuscript writing and literature review. Muhammad Ehetisham-Ul-Haq provided technical guidance and resources. Huma Abbas assisted in data compilation and formatting. Amjad Abbas supervised the research work and finalized the manuscript. All authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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