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Evaluation of Rhizobacteria and Beneficial Fungi as Potential Biocontrol Agents against Fusarium Wilt of Peas (*Pisum sativum*)

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ABSTRACT

Pisum sativum **(pea), commonly known as garden pea, is a crop that is being affected by various biotic and abiotic factors, leading to significant economic losses in terms of both quality and quantity of yield worldwide. One particularly devastating biotic factor is pea wilt disease, which can cause a 100% yield loss under favorable conditions. Various management approaches are used to control this pathogen at different stages of crop growth, with chemical control being the most prevalent but not environmentally friendly. In order to promote eco-friendly and sustainable management of pea wilt disease, biocontrol agents were assessed in this study. Field surveys were carried out to investigate the cause and prevalence of pea wilt disease in Sargodha, Pakistan. The pathogenic nature of *Fusarium* spp. was confirmed through pathogenicity tests and Koch's postulates. Both in vitro and in vivo pathogenicity tests were conducted on seedlings and young plants of the pea crop, respectively. Strain F5, identified as *Fusarium oxysporum*, was found to be the most virulent among the strains tested. A dual culture plate assay with five different strains of rhizobacteria demonstrated their biocontrol potential, with strain CA-27, *Bacillus subtilis*, showing the highest inhibition percentage of the pathogen. Additionally, various fungal biocontrol agents were evaluated, with T55 and T54 showing the highest inhibition, while nonpathogenic *Fusarium* species showed the lowest inhibition. A pot bioassay was then conducted using rhizobacterial suspensions to suppress the pathogen's growth and measure disease incidence, severity, and plant health. The results of the in vivo trial showed that CA-62, *Pseudomonas geniculata*, was the most effective in suppressing the growth of *Fusarium* species.**

Keywords: biocontrol, *Fusarium* wilt, *Pisum sativum*, rhizobacteria.

INTRODUCTION

Pea (*Pisum sativum* L.), also known as garden pea, is an herbaceous plant in the Fabaceae family, primarily cultivated for its delicious seeds (Jadon et al., 2020). Peas have their origins in the Mediterranean and near east regions and have been cultivated for feed and food since the early Neolithic period. Carbonized remains provide evidence that peas have been grown alongside cereal crops like barley and wheat since their domestication in 6000-7000 B.C. Peas continue to play a significant role as a nitrogen-fixing rotational crop in modern agriculture. Their high protein concentration makes them a valuable addition to the diets in several developing countries (Ambrose, 2008). Out of 1.5 billion hectares of the world's agricultural land, 66.8 million hectares are dedicated to cultivating legumes, resulting in a production of 61.2 million tons. The most commonly cultivated legumes globally include beans, peas, black-eyed peas, chickpeas, broad beans and lentils. The FAO report for 2021 shows that peas were planted on 2,590,367 hectares. The global cultivation area for peas has been on the rise over the past decade. The pea yield was 79.254 hectograms per hectare. Furthermore, the production of peas reached

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20,529,759 tons (Uskutoğlu and İdikut, 2023). In Pakistan, pea crops are grown in Punjab, Sindh, Khyber Pakhtunkhwa, and Baluchistan, with a total production share of 71.247128 and 11.3 thousand tons (Government of Pakistan, 2020). Peas are susceptible to various fungal pathogens, including common root rot diseases (*A. euteiches*), *Fusarium* wilt, root rot (*R. solani and P. ultimum*), and pea foot rot. Other fungi linked with root rots include foot rot (*Fusarium solani, F. oxysporum* and *F. culmorum*), white mold (*S. sclerotiorum*) and black root rot (*T. basicola*). Pea plants are susceptible to foliar diseases caused by fungi, including *Alternaria* stem and leaf spot (*A. alternata*), Ascochyta blight complex (*P. medicaginis* var. *pinodella, A. pisi* and *M. pinodes*), downy mildew and powdery mildew (Soylu and Dervis, 2011). Peas are susceptible to various diseases such as root rot, powdery mildew, and viral infections (Kharte et al., 2022). *Fusarium* species, including *Fusarium oxysporum* f. sp. *pisi,* cause diseases such as Rhizoctonia seedling blight, bacterial blight, Ascochyta foot rot, downy mildew, powdery mildew, Pythium blight, Aphanomyces root rot, and wilt in pea crops (Soylu and Dervis, 2011). *Fusarium oxysporum* f. sp. *pisi*, the pathogen responsible for wilt in peas, can remain dormant in the soil for over ten years as hard-resting chlamydospores (Gupta and Gupta, 2019). Infected plants show symptoms such as orange or dark red discoloration in the lower stem and root, as well as yellowing, wilting, and downward-curling of leaves during flowering and pod-fill stages (Sampaio et al., 2021). While using disease-free pea cultivars has been effective in treating this disease, there is a persistent risk of resistance due to the emergence of new pathogenic strains (Bani et al., 2018). Plant pathologists have successfully utilized various fungicides to manage this disease. However, the development of fungicide-resistant phytopathogenic strains and the negative impact of pesticides on soil, plant health, and crop products have prompted a search for eco-friendly strategies for disease management. Additionally, the soil-borne nature of the disease has made managing it with fungicides challenging. As a result, there is a focus on developing an integrated management approach (Hamid et al., 2012). Biological disease prevention using antagonistic bacteria is a sustainable and eco-friendly approach to managing pea diseases (Dukare et al., 2020). Numerous studies have been conducted on the interaction between indigenous rhizobacteria and their hosts, but their effect on non-hosts has been rarely explored (Riaz et al., 2021). Plant growth-promoting rhizobacteria (PGPR) can help plants develop faster by providing induced growth factors and promoting systemic resistance to biotic agents (Köhl et al., 2019). The activities of PGPR are highly influenced by different hosts, and they have the potential to be efficient biocontrol agents on non-indigenous hosts (Riaz et al., 2021). Additionally, the chitinolytic ability of microbial antagonists plays a crucial role in pathogen suppression (Dukare and Paul, 2018). Various species of bacteria and fungi, such as *Bacillus*, *Paenibacillus*, *Pseudomonas*, *Azotobacter*, *Streptomyces*, and *Lysobacter*, have been identified as phytostimulators, biofertilizers, and biocontrol agents (Dukare and Paul, 2021). The rhizosphere, where these microorganisms colonize, plays a crucial role in plant health and disease prevention (Nagargade et al., 2018). Furthermore, Gopalakrishnan et al. (2011) reported that rice-associated bacteria considerably reduced sorghum charcoal rot. Therefore, there is a need to recruit rhizobacteria to establish an effective biofungicide from different hosts for sustainable crop production and disease management (Riaz et al., 2021). It is recommended that a comprehensive approach, integrating cultural, prophylactic measures and protective with biological control, be implemented for the successful disease management of the disease (Hamid et al., 2012). Fusarium wilt is a destructive disease that can devastate pea crops. To combat this issue and minimize losses, it is essential to adopt eco-friendly management practices and utilize beneficial rhizobacteria and fungi as biological control agents against pathogenic *F. oxysporum*, while also enhancing plant growth. This study was designed to assess the potential of rhizobacteria and beneficial fungi as biocontrol agents against *Fusarium* wilt of peas. The research also aimed to determine the current disease index and severity in Sargodha, Pakistan, in order to develop effective management strategies for controlling this disease.

MATERIAL AND METHODS

Plant samples were collected from fields with different varieties of pea crops. The fields were located near the Citrus Research Institute, Sargodha, Pakistan. Heavily infested fields with *Fusarium* wilt were identified for sample collection, including the whole plant with roots and soil adhered to the plants at the root zone. The samples were brought to the laboratory of the Department of Plant Pathology, College of Agriculture, University of Sargodha, Pakistan, for further processing. The samples were washed and disinfected prior to isolating the pathogen.

Isolation of pathogenic *Fusarium* strain

Fusarium strains were isolated using two different techniques as described below:

Tissue planting method: Brownish to reddish lesions containing root samples were used for pathogen isolation on PDA media plates. The plates were incubated at 25°C in the incubator. Mycelial growth of pathogenic strains on diseased root pieces was observed after 3-4 days (Aslam et al., 2019).

Soil dilution method: Serial dilutions were prepared by adding one gram of each soil sample to 9 ml of sterile distilled water in a sterilized test tube, shaken well, and serial dilutions up to (1×10^{-4}) were made using the same method. One mL of the (10^{-4}) dilution was poured into each petri dish containing PDA with the help of a sterile pipette. Each sample was replicated on three plates and incubated at 25°C until fungal colonies appeared (Toma and Abdulla, 2012).

Sub culturing: A 2 mm plug was taken from the mycelial growth isolated from infected samples and placed in a Petri dish containing PDA medium. The dishes were then incubated at 25°C for 3-5 days. Pure cultures of isolated strains on PDA plates were developed for observing colony morphology.

Colony morphology and microscopic observation: Fungi were identified based on the cultural and morphological characteristics of *Fusarium* spp. Colony shape, color, substrate color, hyphal morphology, and morphology of sexual spores such as micro and macroconidia were observed. A microscopic study of isolated strains was conducted to observe their spore structure, hyphal growth, and hyphal septation. These characteristics were compared with the literature (Porter et al., 2015).

Isolation of rhizobacteria: For the isolation of rhizobacteria, roots of healthy plants along with rhizospheric soil were collected. This soil samples were further processed using the soil dilution method mentioned above, and bacterial streaks were cultured on Nutrient agar (NA) medium. The plates were incubated at 28 ± 2°C until the appearance of bacterial colonies (Majeed et al., 2015).

Management of the pathogen

Dual culture assay: Dual culture assays were conducted to evaluate the antagonistic mechanism of bacteria against *Fusarium* wilt of peas. A single round agar plug of the pathogenic isolate was placed in the center of a petri plate, and a loop full of bacteria was inoculated around the agar plug in a circle, keeping about a 1-inch gap. Control plates were inoculated with only the pathogenic isolate. Plates were wrapped and kept incubated for 5 and 7 days at 25°C. The mycelial growth was recorded, and the percent inhibition of the pathogen was calculated using the formula described by Dukare et al. (2020).

$$\text{Percent inhibition (I)} = \frac{\text{C(Control)-T(Treatment)}}{\text{T(Treatment)}} \times 100$$

Greenhouse trial

In a greenhouse pot study, the biological agents (*Bacillus licheniformis*, *B. subtilis*, *Pseudomonas fluorescens*, *P. putida*, and *P. geniculata*) were investigated. Twenty-one pots were used for biological control, and five pots served as controls. One pot was placed as a negative control in which neither inoculum nor biocontrol was added. Fifteen pots were allocated for five strains (three replicates for each). The rhizobacterial spore suspension was calculated based on the number of spores per ml of water. Healthy seeds were dipped first in 10 mL of the spore suspension of rhizobacteria before sowing. After one week, the same suspension of the concerned *Fusarium* was injected into the soil and roots, and plants were watered daily. The rhizobacterial suspension was again poured into the soil. After the appearance of symptoms, root and shoot length, their weight, and the incidence of plant death were recorded. The trial included an untreated control group for comparison. The experiment lasted for the management of the disease, and the results of the pot trials were collected for future research (Dukare et al., 2020).

***In-vitro* evaluation of non-pathogenic *Fusarium, Paecilomyces spp.* and different *Trichoderma spp.* against pathogenic Fusarium**

A dual culture technique was used to evaluate the efficacy of a biological control agent (*Fusarium oxysporum* and different *Trichoderma* spp.) against *Fusarium* wilt of peas (Table 1). Data on pathogen inhibition were obtained after seven days and 14 days of evaluation (Popiel et al., 2008).

Table 1. Strains used as biocontrol agents with their characterization

Species	Colony color	Growth pattern
T. atroviridae	Green	Thin in center, thicker at edges
T. asperellum	Dull Green	Cottony
T55 (*Trichoderma* spp.)	Merged (white + green)	Circular
T. harzianum	Dark Green	Concentric rings
T. viridae	Dark Green	Ring like (circular)
Paecilomyces lilacinus	Purplish pink	Scattered spots
Non-pathogenic *Fusarium*	Brownish white	Creamy white having powdery granules in the center & boundary

RESULTS AND DISCUSSION

Fusarium wilt of peas is a significant and destructive disease that causes deleterious losses in pea-growing regions of the world, including Pakistan. Pea fields with a heavy infestation of wilt were visited, observed, and identified based on the symptoms of the disease (Figure 1).

Figure 1. Pea plant was heavily infected with *Fusarium* species under fields condition identified based on the symptoms

Identification of different isolates of Fusarium oxysporum: During the field survey, diseased root samples from different sites were collected. After isolating the pathogen from the samples in the laboratory, ten isolates were selected from all the isolates. They were characterized based on colony morphology, growth pattern, and color. Data were recorded after seven days of inoculation (Table 2 and Figure 2).

Table 2. Fusarium isolates with their names and colony color

	Species	Colony color
F1	*F. pallidosporum*	Pale yellow
F2	*F. solani*	White
F3	*F. Equisiti*	Pinkish white
F	*F. avenaceum*	Creamy
F5	*F. oxysporum*	White
F6	Not identified	White
F7	Not identified	Pale yellow
F8	Not identified	Dull white
F9	Not identified	Pale yellow
F10	Not identified	Dull white

Figure 2. Isolation of *Fusarium* species from diseased samples on PDA plate under laboratory condition

In-vivo pathogenicity test: The most virulent isolate was evaluated in vivo to confirm the *Fusarium* species. After 15 days of inoculation of the pathogen on the roots of healthy plants, data were recorded. Wilting, yellowing, and stunted growth were clearly observed. The aggressive nature of all the isolates responsible for plant wilting, stunting, and discoloration, especially isolate F5 (*Fusarium oxysporum*), was noticed because the pathogens directly attached to the roots, produced the symptoms, and ultimately, the inoculated plants died, as shown in (Figure 3A and 3B).

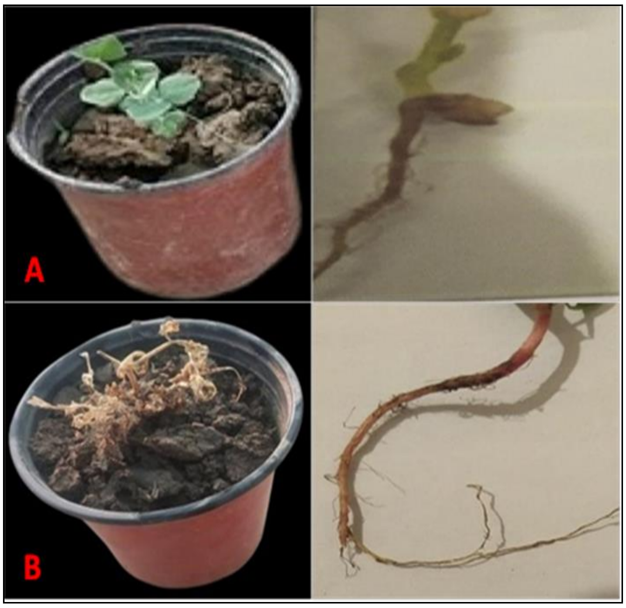

Figure 3. Healthy plant and root of Pea (A), plant and root were infected with *Fusarium* species (B) under greenhouse condition

Microscopy: For clear identification of the fungus, cultures of all the isolates were observed under a light microscope after seven days of the appearance of a mycelial colony on PDA media. Microconidia and macroconidia were observed, which were curved, oval, and flaccid in shape. Some were septate, and some of them were coenocytic. Macroconidia had 3-5 septations, while microconidia had 1-2 septations in them (Figure 4).

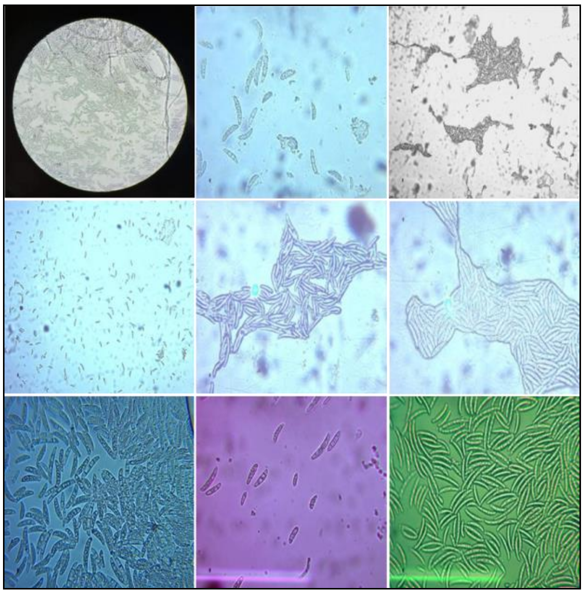

Figure 4. Microscopic view of mycelium, macro and microconidia spores of *Fusarium* spp., isolated from Pea plants

Dual culture antagonism assay with potential biocontrol agents: Different *Trichoderma* spp., *Paecilomyces lilacinus,* and nonpathogenic *Fusarium oxysporum* were evaluated in a dual-culture assay in vitro as biocontrol agents to suppress the disease caused by the highly pathogenic F5 isolate, according to the results of pathogenicity. The fungal biocontrol agents were morphologically identified based on their growth pattern. Moreover, microscopic observations were conducted to confirm the biocontrol strains at the genus level. In vitro studies showed that each of the fungal biocontrol agents significantly reduced the mycelial colony of the pathogenic F5 isolate (Figure 5). The inhibition percentage was recorded on the 3rd, 5th, and 7th day, showing significant variation. The pathogen colony size was remarkably reduced. Among the seven biocontrol agents, *Trichoderma* spp. (T55), *T. asperellum* (T54), and *T. atroviridae* showed inhibition percentages of 55.45%, 52.89%, and 49.52%, respectively, while the nonpathogenic *Fusarium oxysporum* showed the least inhibition percentage, i.e., 28.99%, when compared to the control (Figure 6).

Figure 5. Paecilomyces lilacinus (A), *Trichoderma harzianum* (B), T55 (C), T54 (D), nonpathogenic *Fusarium oxysporum* (E), *Trichoderma atroviridae* (F) and Control (G)

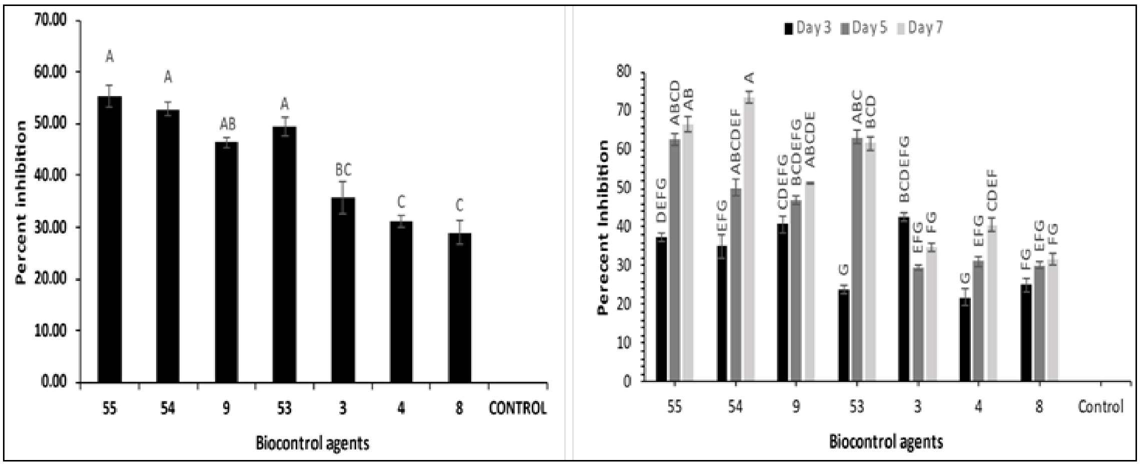

Figure 6. Inhibition zone (%) on the 3rd, 5th, and 7th day of potential fungal biocontrol agents (BCA's) against pathogenic F5 isolate

***In-vitro* evaluation of rhizobacteria antagonistic to pathogenic *Fusarium oxysporum*:** Different strains of rhizobacteria were used to check efficacy and vigor against the pathogenic F5 isolate through a dual culture assay conducted (Table 3). The dual culture assay revealed the inhibition potential of the pathogen through different strains of rhizobacteria on the 3rd, 5th, and 7th day (Figure 7). All strains of rhizobacteria were significantly effective in maximum inhibition of the pathogen, but *Bacillus subtilis* compared to others inhibited the maximum mycelia, i.e., 83.63%, while *P. geniculata*, *P. fluorescence*, *Bacillus licheniformis*, and *P. putida* had 70.51%, 74.82%, 71.48%, and 75.42%, respectively, on the 7th day (Figure 8).

Table 3. Bacterial strains as biocontrol agents

	Isolates	Strains	Accession No
1	CA09	*Bacillus licheniformis*	MT380163
2	CA26	*Pseudomonas flouresence*	MT197384
3	CA27	*Bacillus subtilis*	MT197386
4	CA41	*Pseudomonas putida*	MT197386
5	CA62	*Pseudomonas geniculata*	MT197388

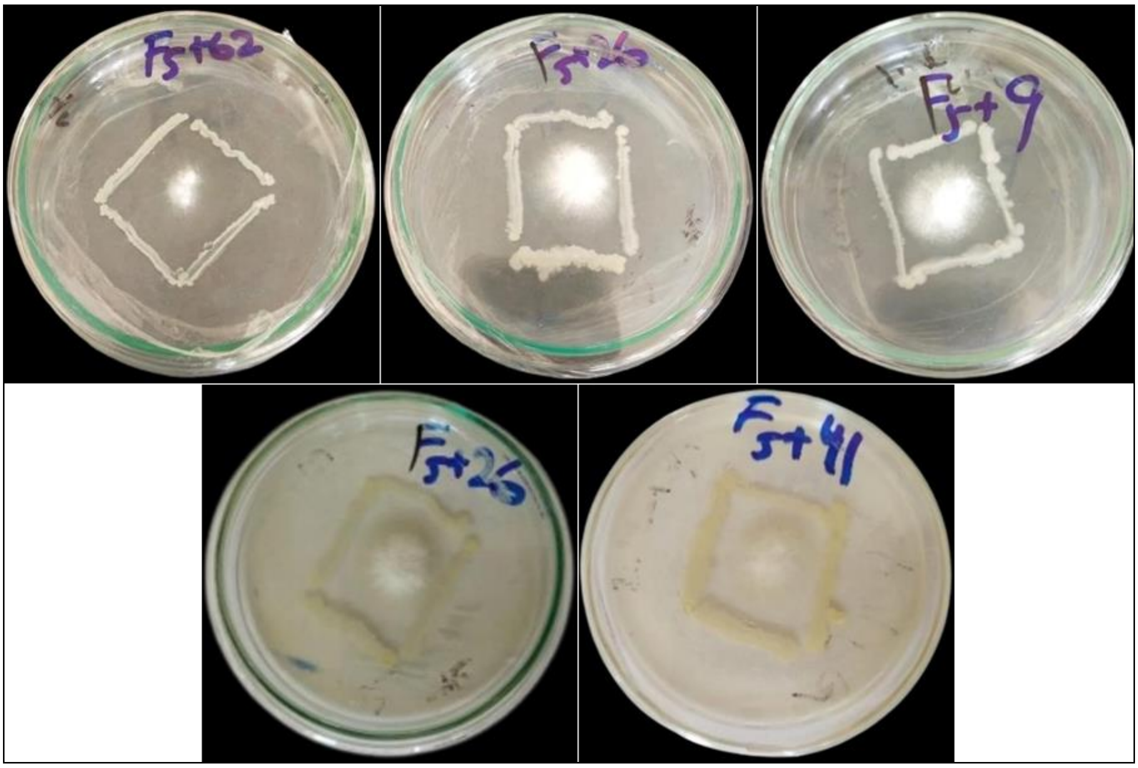

Figure 7. Dual culture assay with rhizobacteria against *Fusarium oxysporum* on PDA plate under laboratory condition

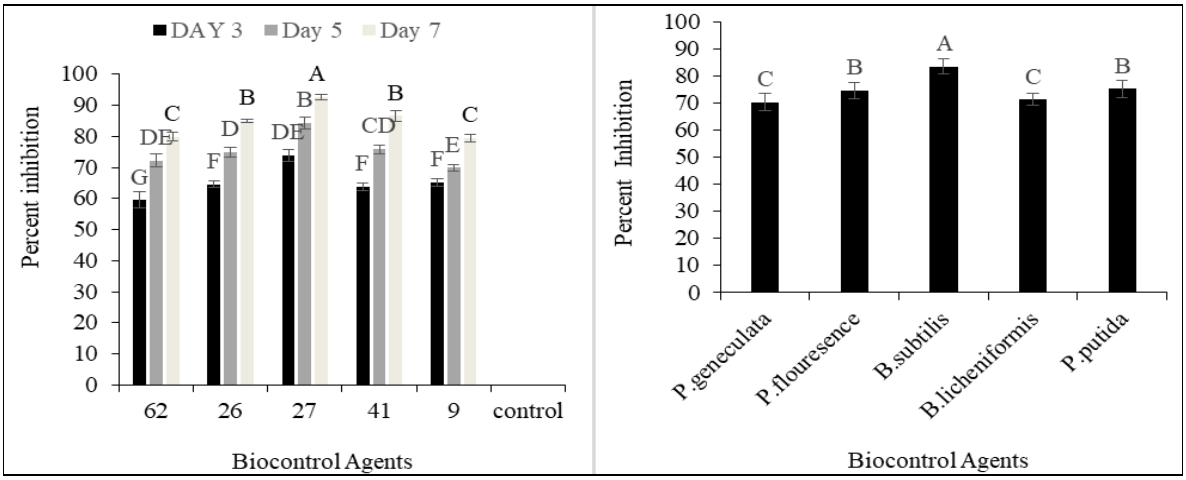

Figure 8. Inhibition percentage bacterial biocontrol agents against *F. oxysporum* on 3rd, 5th and 7th day (A) and rhizobacteria as biocontrol agent (B)

In-vivo evaluation of rhizobacteria: All strains of rhizobacteria in the pot experiment demonstrated the importance and effectiveness of natural biocontrol in disease suppression, root growth, shoot growth, and pathogen inhibition. This experiment significantly enhanced plant growth. Disease incidence and severity were also measured in this assay. The disease incidence was lowest for strain 62, while it was highest in the positive control where only the pathogen was applied. No symptoms were observed where neither the pathogen nor the biocontrol was applied. (Table 4 and Figure 9). The root length and weight of pea plants were measured to check efficacy the of rhizobacteria. The highest healthy root length was recorded in the pot where seeds were coated with strain, measuring 195 mm, while the least was seen in the case of the positive control, measuring 95 mm where only the pathogen was applied. Among all strains, strain 26 of bacteria showed the best results, while strain 41 showed the minimum inhibition of the diseased measured in the pot assay (incidence). Similarly, the root weight estimated for strain 62 was the maximum among all, but there was variation in the corresponding root length and weight with respect to strains (Figure 10A). The shoot length and weight of pea plants were measured to check the efficacy of rhizobacteria. The maximum fresh root (healthy) length was recorded in the pot where seeds were coated with a suspension of strain 26. Among other strains tested, strain 27 CA showed the best results, while strain 9 exhibited minimal inhibition of disease in the pot assay. Similarly, the shoot weight estimated for strain 62 was the highest among all strains, but there was variation in the corresponding shoot length and weight with respect to the strains (Figure 10B). There was a clear difference in the length of shoots of plants, but only slight variation in the weight of shoots of plants inoculated with rhizobacterial strains.

Figure 9. Evaluation of rhizobacteria against *Fusarium oxysporum* on healthy Peas plants under greenhouse condition

Table 4. Disease severity measured in pot under green house condition

Factors	**62**			**9**			**41**			**27**			**26**			**(+) Ve**		**(-) Ve**			
	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3
Number of damaged roots	6	3	2	6	7	5	2	3	4	2	3	2	5	4	4	6	7	7	0	0	0
Number of wilted leaves	3	4	2	5	4	5	3	2	3	4	2	4	4	6	4	9	10	7	0	1	0
Aver. Lesion size on roots	2.8	3.125	2.8	3.1	3.4	3.62	3.08	3.1	2.6	2.7	3.55	2.6	4.1	2.9	3.1	Completely wilted	Completely wilted		0.5	1.75	0
Dead plant	1:3				2:3		1:3			1:3			2:3			3:3		0:3			

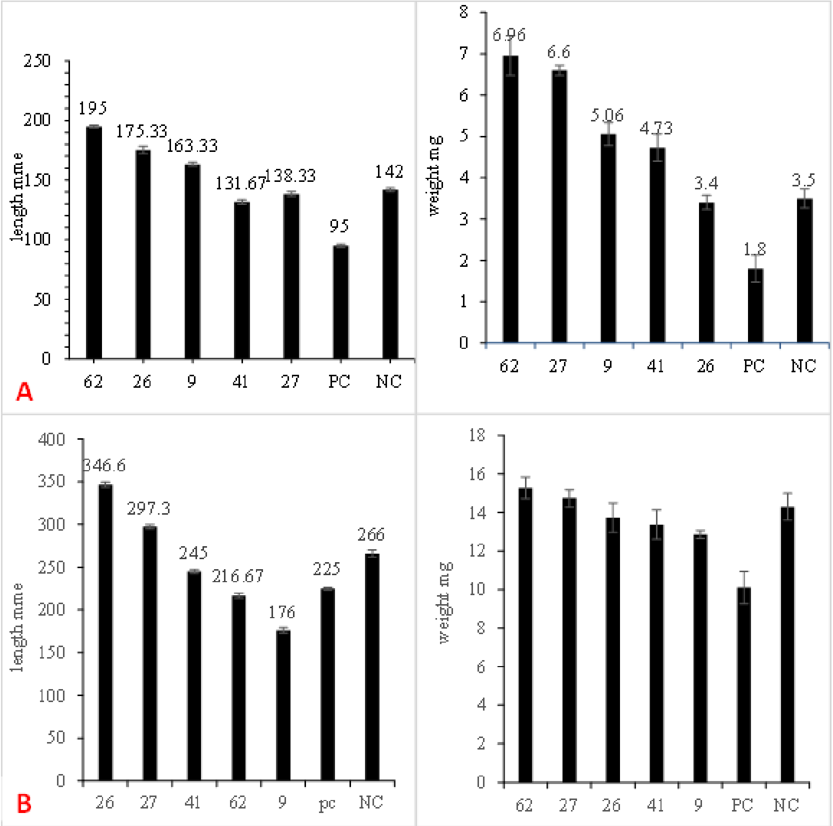

Figure 10. Root length, weight (A), Shoot length and weight (B) of pea plants were measured to check the efficacy of rhizobacteria

Pea is an important winter season annual autogamous (2n=14) pulse crop that belongs to the legume family. Pea improves soil fertility through nitrogen fixation by *Rhizobium leguminosarum* present in root nodules, reducing the amount of fertilizer required (Williamson-Benavides et al., 2020). *Fusarium* spp. are soil-inhibiting wilt pathogens that affect vascular tissues and are among the most significant plant pathogenic and damaging fungi (Nelson et al., 1994). The *F. avenaceum* isolate had the lowest growth rate in vitro and showed the least virulence in the glasshouse experiment, while the *F. culmorum* isolate had the second lowest growth rate in vitro and exhibited intense aggressiveness in causing disease in field trials. Bai and Shaner (1996) also reported a direct relationship between in vitro growth and in vivo pathogenicity of *F. graminearum* isolates. Similarly, in this study, in vitro pathogenicity showed a direct relation with in vivo pathogenicity assay, as demonstrated by the isolate F5, *F. oxysporum*, which exhibited greater virulence compared to other species. The rhizosphere is a significant breeding ground for bacteria capable of degrading chitin (Hong et al., 2023), with agricultural fields showing high concentrations of chitinolytic bacteria (Sriwati et al., 2023). According to research, the highest incidence of wilt was observed in the pathogen-only inoculation treatment (67.34%). In this study, the highest wilt incidence was measured at 66.6% when *Pseudomonas putida* was used against *Fusarium oxysporum*. Two bacteria, *Pseudomonas* spp. NS-1 and *Bacillus* spp. NS-22, significantly reduced *Fusarium* propagules in the rhizosphere and controlled the incidence of wilt disease (Dukare and Paul, 2018). Similar results were obtained in these experiments, as *Bacillus subtilis* and *Pseudomonas geniculata* significantly ($p<0.005$) inhibited pathogen growth. *Bacillus* and *Pseudomonas* prevent crops from being infected by harmful fungi by producing antibiotics, lytic enzymes, siderophores, biosurfactants, inducing host defense, and efficiently colonizing the rhizosphere under greenhouse and field conditions. Additionally, the root zone colonization of *R. irregularis* increased with the application of S. viridosporus (Radhakrishnan et al., 2017). In this research, both rhizobacteria significantly ($p<0.005$) suppressed the disease, but *Bacillus* spp. demonstrated better in vivo efficacy compared to *Pseudomonas*. Our results showed up to 75% inhibition of *Fusarium oxysporum* by *P. putida*. Various soilborne phytopathogens like *Rhizoctonia solani* and *F. solani* are known to be suppressed by their antifungal metabolites (Al-Fadhal et al., 2019). The impact of various parameters, including growth rate, enzyme production, and secondary metabolite synthesis, was found to be relevant in the interaction between toxic *Fusarium* species and *Trichoderma* competitors (Ghazalibiglar et al., 2016). In this study, *Trichoderma* spp. were found to be more competitive and have a faster growth rate than *Fusarium* spp. in dual culture plate assays. The antagonism of fungal interactions was examined by physically separating pathogenic and nonpathogenic *F. oxysporum* strains while growing in a medium with the same quantities of glucose. Nonpathogenic *F. oxysporum* WCS816 appeared to be more antagonistic towards pathogenic *F. oxysporum* WCS816 according to literature, but our research showed that the non-pathogenic *Fusarium oxysporum* strain was less competent than the pathogenic one, exhibiting reduced inhibition and less potential. Glucose competition may play a role in the antagonistic relationship between pathogenic *F. oxysporum* WCS816 and nonpathogenic *F. oxysporum* Fo47blO, although there was no distinct role of glucose for both strains. Both strains were provided with the same amount of glucose, yet a significant difference was observed in their effectiveness. Alabouvette et al. (2009) proposed that different strains of *F. oxysporum* compete for carbon molecules and suggested that carbohydrates are the first nutrient restricting *F. oxysporum* development in sterilized soil. The inhibitory effect of *Trichoderma* spp. on *F. oxysporum* f. sp. *pisi* was likely due to mycoparasitism, hyperparasitism, competition for food and space, and the production and release of antagonistic chemicals into the environment. *Trichoderma* species produce unsaturated monobasic acid, chitinase, cellulose, peptides (Alamethicine), antibiotic compounds (*Trichodermin*), and extracellular enzymes (chitinase, cellulose), which are believed to contribute positively to the biota. *Trichoderma* strains played a significant role in this research when evaluated against *Fusarium oxysporum* f. sp. *pisi*. However, further research is needed to determine if rhizosphere microbiota can effectively prevent *Fusarium* wilt in legumes, particularly in field circumstances, despite their involvement in the early induction of plant defense mechanisms showing promising results in the suppression of soilborne diseases. Effective management of *Fusarium* wilt in legumes requires the combination of various disease management techniques. Some microbiome, fungal, and bacterial species are remarkable strategies against *F. oxysporum*. Crop rotation, another crucial agricultural practice for disease prevention, also requires further study.

CONCLUSIONS

Fusarium wilt of peas is a major and devastating disease caused by soilborne pathogens. This research was conducted to assess the effectiveness of biological control agents (BCAs) against this disease. *Fusarium oxysporum* isolates were found to be the most aggressive and virulent in causing the disease. Various fungal antagonists, including T55, *T. atroviride*, T54, *T. harzianum, T. viride, Paecilomyces lilacinus,* and non-pathogenic *Fusarium oxysporum*, were tested. Among these, *Trichoderma* spp., *T55*, and *T54* showed the highest inhibition of the pathogen. *T. harzianum* exhibited the least suppression, while the non-pathogenic *Fusarium oxysporum* showed the lowest inhibition among all the fungal BCAs. In addition, five different strains of rhizobacteria (*Pseudomonas putida, Pseudomonas fluorescens, Pseudomonas geniculata, Bacillus subtilis* and *Bacillus licheniformis*) were evaluated using a dual culture technique, with *Bacillus subtilis* showing the highest percentage inhibition. The research concluded that field conditions may yield different results due to various climatic factors that can influence disease development and plant health. Factors such as competition among *Bacillus* strains, enzymatic activity, metabolite production, antibiosis, and other inhibition mechanisms may have influenced the outcomes.

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