



Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology

ISSN: 1019-763X (Print), 2305-0284 (Online)

<https://pjp.pakps.com>

RESEARCH ARTICLE

Biological Control of Fusarium Wilt of Cucumber Using *Trichoderma* spp. and *Paecilomyces* sp. under *in vitro* and High Tunnel Conditions

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Article History:

Submitted: May 03, 2025; Revised: July 05, 2025; Accepted for Publication: November 25, 2025.

ABSTRACT

Cucumber is an important vegetable being affected by Fusarium wilt disease, caused by *Fusarium oxysporum* f. sp. *cucumerinum* (FOC), which is a soil borne disease, that transmits through soil and water and it is one of the most destructive diseases of cucumber plants under high plastic tunnel conditions worldwide including Balochistan, Pakistan. The current study was conducted to evaluate the efficacy of four biocontrol agents such as *Trichoderma harzianum*, *T. hamatum*, *T. viride*, and *Paecilomyces* sp., using a dual culture assay under *in vitro* and naturally infested tunnel conditions in Balochistan in a Completely Randomized Design with four replications. The results under *in vitro* revealed that all the biocontrol agents significantly inhibited the radial growth of FOC as compared to control. The highest inhibition was demonstrated by *T. harzianum* (77%), followed by *Paecilomyces* sp. (75%), *T. hamatum* (72%), and *T. viride* (70%). Based on *in vitro* findings, three most effective agents such as *T. harzianum*, *T. hamatum*, and *Paecilomyces* sp. were further evaluated under high plastic tunnel conditions using soil drench method. The results under tunnel revealed that *T. harzianum* and *T. hamatum* demonstrated the highest effectiveness, reducing the disease incidence (13.2% and 17.0%), plant mortality (10.2% and 21.3%), and root infection (13.97% and 18.00%) compared to the untreated plants, moreover, with respect to the growth parameters, the both bioagents considerably enhanced the shoot and root growth, leading to increase the fresh and dry biomass of the plants. It was concluded from the findings of current study that the *T. harzianum* and *T. hamatum* could be effective biological agents for managing the Fusarium wilt of cucumber in high tunnels.

Keywords: Cucumber, Fusarium wilt, *Trichoderma* spp., *Paecilomyces* sp., Tunnel cultivation.

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is a significant and widely cultivated crop in the *Cucurbitaceae* family. It is being cultivated extensively across various continents, originally native to South Asia. Global cucumber cultivation covers approximately 2.27 million hectares,

with a production of around 83.7 million tonnes and an average yield of 37 tonnes per hectare (Tariq *et al.*, 2020). China leads in global cucumber production while Pakistan ranks 50th with cultivation spanning 3.20 thousand hectares and a total production of 123.67 thousand

tonnes, yielding an average of 38.64 tonnes per hectare, mainly in Punjab followed by Balochistan (GoP, 2025). It is commonly consumed as vegetable and due to its high-water content (over 90%), is highly valued for hydrating properties, making it a popular addition to fresh salads and processed into pickles and relishes and it is an ideal choice for weight management and hydration (Sharma *et al.*, 2020). The juice of cucumbers is rich in antacid and carminative properties, helps regulate stomach acidity, offering relief from indigestion and maintaining pH balance in the digestive tract and the essential vitamins. Beyond its nutritional benefits, it is noted for its various medicinal properties (Sotiroudis *et al.*, 2010; Nema *et al.*, 2011; Sharma *et al.*, 2012; Sharma *et al.*, 2020). Cucumbers are often preserved as pickles, typically through fermentation in salt or vinegar, which not only enhances their flavor and texture but also boosts their nutritional profile (Uthpala *et al.*, 2020). The cultivation of cucumber in high plastic tunnels has become increasingly popular in Balochistan due to the increasing demand for cucumbers, limited agricultural land and scarcity of water. The tunnels extend the growing season and enhance yield by providing a controlled environment (Kumar *et al.*, 2020). However, these benefits in tunnels come with the drawback of creating an optimal environment for soil borne diseases, including Fusarium wilt (*Fusarium oxysporum* f. sp. *cucumerinum*), transmitted through soil and water (Shen *et al.*, 2008; Wang *et al.*, 2021). Fusarium wilt causes significant yield losses in cucumber production, with damage ranging from 40 to 70% in affected regions (Sharma and Shukla, 2021). Continuous cropping often leads to the accumulation of soil-borne pathogens and secondary salinization, which further complicates production challenge and increase the diversity of fungal community along with the emergence of pathogenic fungi, declining the plant vigor and making it vulnerable to pest and diseases (Cheng *et al.*, 2020; Meng *et al.*, 2018). The microclimate inside tunnels creates ideal conditions for the pathogen, making it virulent with the increasing temperature and humidity (Leslie and Summerell, 2006; Agrios, 2005; Meyer *et al.*, 2013). The pathogen infects the plants through the roots, leading to systemic wilt symptoms characterized by yellowing and wilting of leaves, stunted growth, and eventually plant death (Katan *et al.*, 2019; Okungbowa and Shittu, 2012). Management of this disease is challenging particularly in an environment which is highly favourable for its

development. Using synthetic fungicides for a long time has made plants resistant against certain fungicides and their continual application has resulted in issues such as environmental contamination and residual toxicity. Harnessing biological control agents is widely viewed as a promising strategy for both enhancing plant development and suppressing plant diseases. (Harish *et al.*, 2009). Implementing biological control strategies offers a sustainable and effective approach to mitigating the Fusarium wilt (Yang *et al.*, 2024). Preferring biological control for protecting from Fusarium wilt disease can be an effective alternative as there are certain species that can significantly control this disease (Raza *et al.*, 2017; Kumar *et al.*, 2020). The mechanisms of biocontrol agents probably involve, suppressing pathogens and activation of plant immune responses (Shafi *et al.*, 2017; Netzker *et al.*, 2020). The beneficial organism not only stop the pathogens but also make the nutrients easily available to the crops, they indirectly enhance the host plant's natural defence systems. As a result, biological control strategies contribute to overall plant health, resilience, and productivity while minimizing the negative environmental impacts and health risks associated with synthetic chemicals (Harman, 2006). Introducing biocontrol agents into the soil ahead of planting is an effective strategy measure against plant diseases (Mazzola *et al.*, 2012). *Trichoderma* spp. are particularly noted for their ability to parasitize and degrade Fusarium species in soil environments, species such as *Trichoderma harzianum* and *Trichoderma viride* are well-documented biocontrol agents for Fusarium wilt. They act through direct antagonism, competition, and by inducing systemic resistance in plants (Harman *et al.*, 2017). Therefore, in this study the different species of *Trichoderma* along with *Paecilomyces* sp. were evaluated against *Fusarium oxysporum* f. sp. *cucumerinum* (FOC), the cause of Fusarium wilt of cucumber, under *in vitro* and high plastic tunnel conditions in Balochistan, Pakistan.

MATERIALS AND METHODS

Isolation and identification of the pathogen: The sample of roots and stems from the infected plants showing characteristic symptoms of *F. oxysporum* were collected in paper envelope from various tunnels across different districts of Balochistan. The collected samples were kept airtight and labeled properly, and brought to the Laboratory of Plant Pathology, Department of Plant Pathology, Sindh Agriculture University Tandojam for pathogen isolation and identification. To expose the

xylem tissue of cucumber plants the samples were cut longitudinally into thin (5 mm) sections near the base of the stem including xylem vessels, then they were exposed to 1% solution of sodium hypochlorite for 1 min followed by washing two times in sterilized water. Three to five tissue sections were put on autoclaved Petri plates of potato dextrose agar (PDA) media. These plates were put in an incubator at 27 °C for 7 days. Once the mycelial growth was observed, the samples were transferred to fresh PDA plates to obtain pure culture. PDA plates having the robust mycelial growth of the fungus were taken and the morphological identification was carried out by first carefully examining the culture plates with the naked eye, followed by a detailed observation under the microscope. The characteristics of the colony were examined using a stereoscope, while the morphological and microscopic features including shape and size of microconidia, macroconidia, and chlamydo spores were studied under a compound microscope following the identification method described by Leslie and Summerell (2006). The confirmed and identified pure sub-cultures were kept in refrigerator at 4 °C for further study. The pathogenicity was confirmed by Koch's postulate.

Preparation of the fungus conidial suspension: Conidial suspension of the test fungus was prepared from 10 days old cultures taken from the PDA plates. Sterile water in the amount of 10 ml was poured into the plates and a glass rod was used to remove the mycelia of the fungus in order to receive the spores. The suspension of spores was filtered with muslin cloth and transferred into tubes and the spore concentrations were fixed to 1×10^6 spores ml^{-1} with the help of hemocytometer (Vargas-Inciarte *et al.*, 2019).

Preparation of spore suspension of biocontrol agents: Biocontrol agents such as *Trichogermia harzianum*, *T. hamatum*, *T. viride* and *Paecilomyces* sp. were procured from the laboratories of Plant Pathology departments of Balochistan Agriculture College, Quetta and Sindh Agriculture University, Tandojam. They were first revived and multiplied on PDA then spore suspension of the biocontrol agents was prepared from 8 days old cultures taken from the PDA plates. 10ml sterile water was applied into the plates and the mycelia was scraped with a sterile glass rod to obtain the spores. The suspension of spores was filtered with the help of muslin cloth, poured into tubes and the concentration was adjusted using a hemocytometer to 1×10^6 spores ml^{-1} (Xie *et al.* 2023).

In vitro evaluation of different biocontrol agents

against FOC: Biocontrol agents like, *Trichogermia harzianum*, *T. hamatum*, *T. viride* and *Paecilomyces* sp. were tested against radial mycelial growth of *F. oxysporum* f. sp. *cucumerinum* using the dual culture technique. A 5 mm disc of each biocontrol agent, along with an equally sized disc of the pathogen, was excised from a fresh culture by using a cork borer and then positioned 20 mm apart on plates containing PDA medium. For control, pathogen was plated separately without the biocontrol agent. Afterwards, plates were placed in an incubator for 7 days at 27 °C and later for the assessment of the antagonistic potentiality of biocontrol agents, the radial growth of the fungus was measured. The fungal development was monitored on both treated and untreated (control) plates. The extent of growth suppression was determined by calculating the percentage inhibition using the formula below (Yelmame *et al.*, 2010).

$$\text{Inhibition \%} = \frac{C-T}{C} \times 100$$

Whereas, C = Radial growth of pathogen in control and T= Radial growth of pathogen in treatment.

Evaluation of selected biocontrol agents against Fusarium wilt of cucumber in high plastic tunnel: Based on the results of *in vitro* experiment on the effectiveness of tested biocontrol agents, among the four biocontrol agents three most effective bio-control agents *viz.*, *Trichoderma harzianum*, *Paecilomyces* sp. and *T. hamatum* that showed the highest inhibition% against FOC were selected for further study. For this purpose, the seeds of susceptible cucumber cultivar "Mawasem" were chosen to evaluate the efficacy of selected biocontrol agents (BCAs) against Fusarium wilt of cucumber in high plastic tunnel at the field site of Vegetable Seed Production-ARI-Quetta, Balochistan in March to April 2024. The high tunnel selected in this study has been under continuous cucumber monocropping for over nine years, leading to natural soil infestation with FOC. In short, prior to sowing the seeds, they were surface-sterilized in 2% sodium hypochlorite for 3 min, rinsed three times in sterile distilled water and let them dry, and then sown on the flat beds in the tunnel. Fertilization and irrigation were done in the soil according to the cucumber requirement through the drip irrigation system. Completely Randomized Design (CRD) was applied as the design for the experiment and 4 replications for each treatment were maintained. The experimental area comprised of 16 plots, keeping 25 ft^2 area per plot, and

the row-to-row (R×R) and plant-to-plant (P×P) distances were maintained as 75 and 45 cm, respectively. After one week of sowing, as the seeds sprouted, the soils at the plant root zone were drenched with the BCAs in the amount of 100 ml (1×10⁶ spores ml⁻¹) except for control in the plastic tunnel. During the experimental period,

plants were regularly observed for any visible symptom attributable to *FOC*. After one month from the application of bioagents, the plants were uprooted, the disease incidence and infection percentage were calculated, and the data of plant growth – shoot and root length, and weight of root and shoot were recorded.

$$\text{Disease Incidence \%} = \frac{\text{Number of infected cucumber plants}}{\text{Total number of cucumber plant}} \times 100$$

$$\text{Plant Mortality \%} = \frac{\text{Number of dead plants}}{\text{Total number of plants observed}} \times 100$$

$$\text{Root infection \%} = \frac{\text{Number of root pieces colonized by the fungus}}{\text{Total number of root pieces observed}} \times 100$$

STATISTICAL ANALYSIS

All experiments were carried out in Completely Randomized Design (CRD). Analysis of variance (ANOVA) was employed to differentiate the outcomes of various treatments. Statistical comparisons of the mean values were carried out using the LSD method (P < 0.05) in Statistix 8.1 package. The data for different experiments are presented in the graphical form, and the standard errors have been marked on the graph bars.

RESULTS

***In vitro* evaluation of biocontrol agents against *FOC*:**

The experimental finding showed that radial colony growth of *FOC* was inhibited by all the biological control agents over the control (Table 1). There were no significant variations in inhibition% among all the BCAs observed, the range of inhibition% on the colony growth of the fungus was 70 to 77% by all the BCAs. The separate analysis revealed that the most effective biocontrol agent was *T. harzianum* followed by *Paecilomyces* sp. and *T. hamatum* that represented 77.50%, 74.72% and 72.22% inhibition respectively over the control plate (0.00%) (Figure 1).

Table 1. Effect of biocontrol agents on the growth inhibition % of *F. oxysporum* f.sp. *cucumerinum* (*FOC*)

Biocontrol agents	Radial colony growth (mm)		Growth inhibition % over control
	<i>FOC</i> BCAs		
<i>Trichoderma harzianum</i>	20.25	69.75	77.50 a
<i>Trichoderma hamatum</i>	25.00	65.00	72.22 a
<i>Trichoderma viride</i>	27.00	63.00	70.00 a
<i>Paecilomyces</i> sp.	22.75	67.25	74.72 a
Control	90	---	0.00 b

*Mean values followed by same letters within the column are not significantly different, as determined by the LSD test (P < 0.05).

Evaluation of selected biocontrol agents against *Fusarium* wilt of cucumber in high plastic tunnel: The findings of *in vitro* provided a productive insight for selecting the most effective biocontrol agents such as *Trichoderma harzianum*, *T. hamatum* and *Paecilomyces* sp. which were further evaluated for their potentiality against the most devastating disease *Fusarium* wilt of cucumber in high plastic tunnels. The findings under the tunnels mentioned in Table. 2, demonstrates that the disease incidence% was significantly lower (13.17-31.62%) in plants treated with the biocontrol agents over the control plants (80.20%). Plants treated with *T. harzianum* represented the lowest disease incidence (13.17%) followed by *T. hamatum* (17.02%), whereas

relatively the maximum disease incidence (31.62%) was observed in plants treated with *Paecilomyces* sp. Moreover, the range of plant mortality% was significantly lower (10.22-38.30%) in plants treated with the biocontrol agents over the control plants (68.57%). *T. harzianum* represented the lowest plant mortality (10.22%) followed by *T. hamatum* (21.27%), whereas *Paecilomyces* sp. showed the highest plant mortality (38.30%). With regards to the examination of roots, the range of infection% was considerably lower (13.97-26.12%) in plans treated with the biocontrol agents over the control plants (71.95%). *T. harzianum* represented the lowest root infection followed by *T. hamatum* and *Paecilomyces* sp. representing 13.97, 18.00 and 26.12%,

respectively. These reductions in disease incidence, plant mortality, and root infection were statistically significant

($P < 0.05$), as determined by ANOVA followed by LSD tests.



Figure 1. Effect of biocontrol agents (BCAs) on the growth inhibition % of *F. oxysporum* f.sp. *cucumerinum* (FOC) compared to control. The top row shows control plates with FOC alone. The bottom row shows dual culture plates with BCAs (left) and FOC (right)

Table 2. Effect of selected biocontrol agents against Fusarium wilt of cucumber in high plastic tunnel

Biocontrol agents	Disease incidence%	Plant mortality%	Root infection%
<i>Trichoderma harzianum</i>	13.17 d	10.22 d	13.97 d
<i>Paecilomyces</i> sp.	31.62 b	38.30 b	26.12 b
<i>Trichoderma hamatum</i>	17.02 c	21.27 c	18.00 c
Control	80.20 a	68.57 a	71.95 a

*Mean values followed by different letters within the same column indicate a statistically significant difference, as determined by the LSD test ($P < 0.05$)

Shoot and root length (cm): Fusarium wilt disease was significantly ($P < 0.05$) suppressed and the shoot (38.25 to 54.20 cm) and the root (10.55 to 15.00 cm) lengths were enhanced by all the biocontrol agents as compared to untreated plants (shoot 21.70 cm and root 5.10 cm). Moreover, the maximum shoot (54.20 cm) and root (15.00 cm) lengths were observed in plants treated with *Trichoderma harzianum*, whereas *Paecilomyces* sp. represented 38.25 and 10.55 cm shoot and root lengths respectively (Table 3).

Fresh shoot and root weight (g): The fresh shoot weights were significantly ($P < 0.05$) enhanced by all the biocontrol agents. The range of fresh shoot and root weights were recorded 111.25 to 170.75 g and 7.95 to

11.00 g respectively as compared to untreated plants (shoot 51.62 g and root 4.00). Moreover, *T. harzianum* followed by *T. hamatum* demonstrated the highest shoot and roots weights representing 170.75 and 11.00 g respectively, whereas the minimum shoot (111.25 g) root (7.95 g) weights were recorded by *Paecilomyces* sp. (Table 3).

Dry shoot and root weight (g): The dry shoot (72.07 g) and root (2.75 g) weights were significantly ($P < 0.05$) enhanced by *T. harzianum* followed by *T. hamatum* as compared to untreated plants (shoot 25.70 and root 0.65 g), whereas the minimum dry shoot (47.32 g) and root (1.52 g) weights were represented by *Paecilomyces* sp. (Table 3).

Table 3. Effect of selected biocontrol agents on the growth parameters of cucumber plants in high plastic tunnels

Biocontrol agents	Shoot	Root length	Fresh shoot	Fresh root	Dry shoot	Dry root
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	length (cm)	(cm)	weight (g)	weight (g)	weight (g)	weight (g)
<i>Trichoderma harzianum</i>	54.2 a	15.00 a	170.75 a	11.00 a	72.07 a	2.75 a
<i>Paecilomyces</i> sp.	38.25 c	10.55 c	111.25 c	7.95 b	47.32 c	1.52 c
<i>Trichoderma hamatum</i>	42.82 b	11.40 b	128.55 b	8.30 b	54.30 b	2.12 b
Control	21.7 d	5.10 d	51.62 d	4.00 c	25.70 d	0.65 d

*Mean values followed by different letters within the same column indicate a statistically significant difference, as determined by the LSD test ($P < 0.05$)

DISCUSSION

The high plastic tunnels are widely adopted in Balochistan, as they provide a controlled environment, save the water, increase the cucumber production and extend the growing season. However, the unique environmental conditions inside the tunnels favour the soil borne pathogens such as *Fusarium oxysporum* f. sp. *cucumerinum* (FOC), causing wilt in cucumber plants and the continuous monocropping can intensify the virulence of the pathogen (Shen *et al.*, 2008; Meng *et al.*, 2018; Cheng *et al.*, 2020; Wang *et al.*, 2021). *Fusarium* wilt has the potential to infect the cucumber plants at any stage and bring huge losses to the production (Din *et al.*, 2020). *Fusarium* is a fungus that inhabits in soil and disseminates through soil and water, and enters the plants via the roots and stems and quickly spreads throughout vascular system, and then it persists in the soil and becomes difficult to be eliminated (Wang *et al.*, 2012).

In the present study, the *in vitro* antagonistic activity demonstrated that growth of FOC was significantly inhibited by all the biocontrol agents, bringing up to 77.50% reduction. Among them *Trichoderma harzianum* represented the highest antagonism, followed by *Paecilomyces* sp. and *T. hamatum*. Our results agree with the previous studies by Haque (2023) and Abro *et al.* (2019) who studied several endophytic fungal species and found that *Trichoderma* isolates were significantly effective under *in vitro* conditions against *F. oxysporum*. Moreover, our results agree with the previous studies by Waris *et al.* (2025) who studied several species of *Trichoderma* spp. and found that *T. harzianum* isolate significantly effective under *in vitro* conditions against the fungal pathogen. The suppression mechanisms of *Trichoderma* spp. are well documented against the *F. oxysporum* that include the mycoparasitism, degradation of pathogen structure, enzyme secretion and inducing systemic resistance (Vinale *et al.*, 2014; Harman *et al.*, 2017). The genus

Trichoderma includes the highest number of BCAs among all the other fungal genera (Sharma *et al.*, 2022; Woo *et al.*, 2023). Under high plastic tunnel conditions, *T. harzianum* and *T. hamatum* significantly reduced the disease incidence, plant mortality, and root infection and as well as enhanced the growth and the weight of the shoot and root. Although *Paecilomyces* sp. was less effective compared to *Trichoderma*, as its efficacy was less competitive in the rhizosphere, however it showed moderate suppression of the *Fusarium* wilt and improved plant growth compared to untreated plants. Our results are in confirmation with Abed *et al.* (2019), demonstrating that *T. harzianum* reduced the *Fusarium* wilt severity and promoted cucumber growth. A similar study by Redda *et al.* (2018) and Patil *et al.* (2021) confirmed that the cucumber plants treated with *Trichoderma* isolates exhibited significantly increased height and the biomass in seedlings and shoots were also increased under the greenhouse conditions. *Paecilomyces* sp. showed moderate effectiveness in the current study. A similar study reported that *P. variotii* demonstrated a strong ability to produce siderophores and indole acetic acid (IAA) which improved the seed germination, seedling vigor, and root and shoot length (Moreno-Gavira *et al.*, 2020). *T. harzianum* and *T. hamatum* are good alternatives to chemical fungicides and have strong potential to suppress the *Fusarium* wilt in Balochistan.

CONCLUSION

Based on the findings, the current research concludes that the *Trichoderma* spp. could be effective biocontrol agents against the *Fusarium* wilt of cucumber. Particularly *T. harzianum* and *T. hamatum* that demonstrated significant effectiveness to suppress the *F. oxysporum* and reduced the disease incidence, plant mortality, and root infection and also enhanced the plant growth under high plastic tunnel conditions. The applications of these biocontrol agents can serve environmentally safe alternatives to chemical

fungicides in managing the Fusarium wilt disease in Balochistan.

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