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Efficacy of *Pseudomonas fluorescens* and *Trichoderma asperellum* against Fusarium Wilt of Cucumber in Semi-Arid Zone

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Abstract: Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cucumerinum* (FOC), poses a significant threat to cucumber (*Cucumis sativus* L.) cultivation, particularly under continuous cropping systems. In the present study, talc-based bioformulations of *Pseudomonas fluorescens* (Pf6) (OP002308) and *Trichoderma asperellum* (Th d) (OP012701) were developed and evaluated under polyhouse conditions for the management of Fusarium wilt (FOC, OP002306) in cucumber. The combined application of seed treatment and seedling dip with Pf6 significantly reduced wilt incidence (2.5%) compared to the control (25%) and outperformed chemical fungicide (Carbendazim 50% WP). Pf6 also enhanced crop earliness and recorded the highest yield (129.98 q/ha). Rhizosphere competency studies revealed that Pf6 maintained higher colony-forming units (CFU/g) over time compared to Th d, indicating superior root colonization. The biocontrol efficacy is attributed to mechanisms such as competition, antibiosis, and induction of systemic resistance. These findings establish *P. fluorescens* Pf6 as a promising, eco-friendly alternative for managing Fusarium wilt and enhancing productivity in cucumber cultivation under protected conditions.

Keywords: *Fusarium oxysporum* f.sp. *cucumerinum* (FOC), *Pseudomonas fluorescens, Trichoderma asperellum,* Bioformulation, Rhizosphere colonization

Cucumber (*Cucumis sativus* L.), a member of the family Cucurbitaceae, is a commercially important vegetable crop cultivated worldwide, especially in tropical and subtropical regions (Hin et al., 2023). India, the primary center of origin, has cultivated cucumber for over 3000 years and exhibits considerable genetic diversity (Kaur and Dhall 2017). Despite its nutritional and economic significance, cucumber cultivation is challenged by several diseases, among which Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cucumerinum* (FOC) is particularly damaging (Al-Tuwaijri 2015). FOC is a soil-borne pathogen that invades plant roots, colonizes the vascular tissue, and produces chlamydospores capable of surviving in soil for over a decade (Chung et al., 2008). It disrupts water transport through xylem blockage and secretes phytotoxins such as fusaric acid, leading to wilting, necrosis, and eventual plant death (Lievens et al., 2007). In continuous cucumber cropping systems, root exudates like cinnamic and *p*-hydroxybenzoic acids accumulate in the rhizosphere, increasing pathogen virulence and contributing to soil sickness (Liu et al., 2010).

Historically, control of Fusarium wilt relied on chemical fungicides and soil fumigation, including methyl bromide, which is now banned due to its environmental hazards (Zhang et al, 2008). Breeding for host resistance has had limited success due to the rapid emergence of new pathogen races (Ling et al., 2010). In this context, biological control offers a promising and sustainable alternative for managing soil-borne pathogens (Huang et al., 2012). Biocontrol agents such as *Trichoderma* spp. and *Pseudomonas* spp. have demonstrated efficacy in suppressing FOC through mechanisms including competition, mycoparasitism, production of cell wall-degrading enzymes, antibiotics, and induced systemic resistance (Cao et al., 2011). *Trichoderma* spp. are highly adaptive soil fungi that colonize the rhizosphere and antagonize pathogens through secretion of chitinases, β-glucanase, and secondary metabolites (Waghunde et al, 2016). However, their efficacy is sometimes limited by soil fungistasis and nutrient availability, necessitating improved formulation strategies (Pan et al., 2006). Similarly, *Pseudomonas* spp., particularly fluorescent strains, exhibit strong rhizosphere competence and suppress FOC through the production of compounds like 2,4-diacetylphloroglucinol (DAPG), siderophores, and hydrogen cyanide (Singh et al., 2015). The present investigation was undertaken to develop bioformulations of *Pseudomonas fluorescens* Pf 6 and *Trichoderma asperellum* Th d and to standardize their application techniques under polyhouse conditions for effective management of fusarium wilt in cucumber.

MATERIAL AND METHODS

Virulent pathogen *Fusarium oxysporum* f.sp. *cucumerinum* (FOC) (accession no. OP002306) and two most potent native biocontrol agents i.e. *Pseudomonas fluorescens* (Pf 6) (accession no. OP002308) and *Trichoderma asperellum* (Th d) (accession no. OP012701)

were procured from Biocontrol Lab, Department of Plant Pathology, Punjab Agricultural University, Ludhiana and were used in the evaluation.

Field evaluation of the talc bioformulations: Talc-based bioformulations of *P. fluorescens* (Pf 6) and *T. asperellum* (Th d) were prepared individually as per Singh et al, (2024). Under polyhouse conditions, the variety used was Defender Improved F1 (Parthenocarpic cucumber). The field experiment was conducted by Randomized Block Design with six treatments and four replications (10 plants per replication) during 2020-21 at the Razapur Bet (30.955823, 75.708365), Ludhiana. The bioformulations of *P. fluorescens* (Pf 6) (6.1×10^9/g) and *T. asperellum* (Th d) (9.2×10^{11}/g) were applied as seed treatment (15g bioformulations per kg of seed) + seedling dip (15g bioformulations per litre of water for 4 hours). Th - *T. harzianum* and Tv - *T. viride* were also included in the study, which were used earlier as standard biocontrol agents in the Biocontrol Lab. A chemical fungicide, Carbendazim 50% W.P. (1.5 g per kg seed) was kept as a standard check. An untreated treatment was also maintained as a control. The per cent disease incidence, plant growth promotion parameters and cucumber yield were recorded.

Rhizosphere competency evaluation: To assess rhizosphere colonization, root-adjacent soil and small root sections (approximately 10 mm in length) were collected. These samples were suspended in 10 ml of 10 mM phosphate buffer (pH 7.2) and agitated at 150 rpm on a rotary shaker at 30°C for one hour. Following serial dilution, aliquots were spread onto nutrient agar (NA) plates, which were then incubated at 28 ± 2°C for 48 hours (Das et al, 2010). Colony counts were used to estimate the population density of *Pseudomonas fluorescens* (Pf 6) and *Trichoderma asperellum* (Th d) per gram of soil, following the methodology described by Killani et al. (2011).

$$\text{CFU per gram of soil} = \frac{\text{No. of colonies} \times \text{Dilution made} \times \text{Fresh wt. of the soil}}{\text{Oven-dry weight of the soil}}$$

Statistical Analysis: The treatment means of various parameters were separated by Duncan's Multiple Range Test (DMRT) and determined by the magnitude of the F value ($p \leq 0.05$) using statistical software SPSS version 26.0 (Kara and Arici 2019).

RESULTS AND DISCUSSION

The data in minimum wilt incidence was in the *P. fluorescens* Pf 6 (2.5%) and Tv (2.5%) as compared to the control (25%) (Table 1). Wilt incidence in Th d and chemical (Carbendazim 50% WP) was 7.5 per cent and 5 per cent, respectively. The plants treated with Pf 6 start giving yield 14 days earlier than the control plant, followed by Tv and Th d, which were at par with each other (start giving yield 8 days earlier than the control plants). The yield was highest in the plants treated with Pf 6 (129.98 q/ ha), followed by Tv (126.72q/ha). All the treatments increased the yield significantly as compared to the control (84.08 q/ha).

After the seedling dip treatment, the initial CFU/g count was 9.4×10^7 and 7.1×10^9 of *T. asperellum* (Th d) and *P. fluorescens* (Pf 6), respectively (Table 2). The population density decreased after 15 and 30 days of treatment in case of the antagonists. After 45 days, the population of *T. asperellum* decreased (7.5×10^6 CFU/g), but that of *P. fluorescens* increased (8.6×10^8 CFU/g of soil). After 75 and 90 days of treatment, the population density of *T. asperellum* increases (6.5×10^6 and 7.6×10^6 CFU/g, respectively). But the CFU count of *P. fluorescens* decreases after 75 and 90 days (5.7×10^6 and 7.2×10^5 CFU/g, respectively).

The effective management of Fusarium wilt observed in this study underlines the potential of *Trichoderma* and *Pseudomonas* spp. as practical biocontrol solutions in cucumber cultivation. Earlier studies laid the foundation for understanding the mechanisms behind this success. Liu et al. (1995) first demonstrated induced resistance in cucumber due to *Pseudomonas putida*, providing evidence of systemic defense activation as a biocontrol mechanism. Later, Yedidia et al. (2000) reported increased activity of defense enzymes such as chitinase, glucanase, cellulase, and peroxidase following *Trichoderma harzianum* colonization, further strengthening the role of induced resistance in pathogen

Table 1. Effect of seed + seedling dip treatments on disease incidence and plant growth promotion under polyhouse conditions

Treatments	Disease Incidence (%)	Yield (q/ha)	Shoot length (m)	Root length (m)	Total length (m)
Th d	07.50 (2.74)^{bc}	122.14 ± 2.59^{ab}	6.54 ± 0.44^{ab}	0.56 ± 0.02^{c}	7.10
Th	10.00 (3.16)^{cd}	114.39 ± 6.69^{c}	6.20 ± 0.85^{bc}	0.53 ± 0.06^{d}	6.73
Tv	02.50 (1.58)^{a}	126.72 ± 1.48^{a}	6.65 ± 0.59^{ab}	0.62 ± 0.07^{b}	7.28
Pf 6	02.50 (1.58)^{a}	129.98 ± 2.18^{a}	7.15 ± 0.14^{a}	0.75 ± 0.02^{a}	7.90
Carbendazim 50% WP	05.00 (2.24)^{ab}	121.37 ± 3.46^{b}	5.65 ± 0.31^{c}	0.63 ± 0.05^{b}	6.28
Control	25.00 (5.00)^{e}	84.08 ± 5.05^{d}	5.23 ± 0.57^{d}	0.55 ± 0.04^{cd}	5.79

Th: *T. harzianum*, Tv: *T. viride* (standard in lab), alphabets represent DMRT

Table 2. Rhizosphere competence of antagonists applied as seed + seedling dip

Seedling Dip Treatment (Days)	Antagonist (CFU/g of soil)*	
	Razapur Bet	
	Th d	Pf 6
0	9.4×10^7 (18.37)	7.1×10^9 (22.69)
15	9.2×10^7 (18.34)	6.8×10^9 (22.64)
30	8.7×10^6 (15.95)	5.4×10^8 (20.00)
45	7.5×10^6 (15.83)	8.6×10^8 (20.57)
60	4.1×10^5 (12.92)	6.3×10^6 (15.66)
75	6.5×10^6 (15.68)	5.7×10^6 (15.55)
90	7.6×10^6 (15.82)	7.2×10^5 (13.46)
105	8.9×10^5 (13.71)	5.9×10^5 (13.29)
120	4.7×10^5 (13.08)	2.7×10^5 (12.51)
CD (p=0.05)	Th d = 0.12, Pf 6 = 0.26, Th d * Pf 6 = 0.37	

Values in parenthesis are Log_{10} transformed values

suppression. Randhawa et al. (2007) observed improved seed germination, reduced seedling mortality, and effective management of wilt disease with *T. viride* and *T. harzianum*, establishing the field relevance of these antagonists. Gul et al. (2013) also emphasized the growth-promoting and disease-suppressing roles of bioagents under field conditions. Around the same period, Tanwar et al. (2013) recorded better nutrient uptake and biomass accumulation with *T. viride* and *P. fluorescens*, which aligns with the enhanced growth responses observed in our study. The significance of rhizosphere colonization in sustaining biocontrol effects was highlighted by Gary et al. (2004), who found consistent root association and resistance enhancement by *T. viride* and *T. harzianum*. Similarly, Zhang et al. (2013) linked increased IAA production by mutant *Trichoderma* strains with higher plant biomass and improved microbial populations in the rhizosphere, suggesting that growth promotion and disease suppression are closely connected. Arya et al. (2018) reported siderophore synthesis and inhibition of *Fusarium* by *P. fluorescens*, confirming the dual role of nutrient competition and antagonism. Lian et al. (2023) demonstrated improved fresh and dry weights and chlorophyll content in *Trichoderma*-treated plants, supporting the plant growth promotion observed in our study. Furthermore, Miftakhov et al. (2023) highlighted competition for root exudates and inhibitory compound production as critical modes of action in biocontrol, corroborating the mechanisms by which the applied bioformulations reduced wilt incidence in cucumber. Taken together, these findings indicate that the integration of *Trichoderma* and *Pseudomonas* not only reduces disease severity but also improves plant vigor through multiple mechanisms such as induced resistance, growth promotion, and rhizosphere colonization.

CONCLUSION

The present study validates and extends earlier reports by demonstrating that the application of *Pseudomonas fluorescens* Pf 6 provides consistent suppression of fusarium wilt and promotes overall plant growth under field conditions. The strain exhibited strong rhizosphere persistence and advanced crop performance, underlining its potential as a sustainable and eco-friendly alternative to chemical fungicides in cucumber cultivation.

AUTHOR'S CONTRIBUTIONS

N. Singh and D.S. Buttar were responsible for the conception and design of the study. G.S. Brar and A.K. Choudhary were responsible for the acquisition of data, performed the analysis, and wrote the original draft of the manuscript. All authors have reviewed and approved the final manuscript.

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