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Effectiveness of Fungal and Bacterial Biocontrol agents in Suppressing *Fusarium oxysporum f. sp. cubense* in *In vitro* Condition

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ABSTRACT

Fusarium wilt of banana also known as Panama wilt, is a deadly fungal disease caused by the soil-borne fungus *Fusarium oxysporum* f.sp. cubense (Foc) and is one of the most widespread and destructive disease of banana plantain. Present study was taken up to effectiveness of fungal and bacterial biocontrol agents in suppressing *Fusarium oxysporum f. sp. cubense* in *In vitro* condition to include in integrate disease management strategy as it is eco-friendly technique. The causal organism of Panama wilt was tested along with various fungal and bacterial bioagents in dual culture method for testing the efficacy. Results revealed that *Trichoderma viride* reduced 84.74 % radial growth of the causal pathogen followed by *Trichoderma harzianum* (83.05%) and *Trichoderma reesei* (81.48%) while *Bacillus subtillis* inhibited lowest radial growth (62.23 %).

Key words: *Banana, Panama wilt, FOC, Bioagents, IDM, Eco-friendly*

Introduction

Banana and plantain (*Musa* spp.) are among the most economically important fruit crops cultivated in tropical and subtropical regions, serving as both staple food and a vital source of livelihood for millions of people worldwide. However, their production is severely constrained by various diseases, among which Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *cubense* (Foc), is considered the most destructive. Commonly known as Panama wilt, this soil-borne fungal disease colonizes the roots, rhizomes, and pseudostem tissues of banana plants, leading to progressive wilting symptoms followed by plant death (Wardlaw, 1961; Stover, 1962), The disease is highly persistent due to the long-term survival of chlamydospores in the soil and is difficult to eradicate once established. Believed to have originated in Southeast Asia, Panama wilt has rapidly spread to major banana-growing regions of the world through infected rhizomes (Stover, 1962), posing a serious threat to global banana cultivation, particularly in fields planted with susceptible cultivars such as 'Gros Michel' and 'Cavendish'. Management of the disease primarily relies on the use of disease- free planting material and resistant cultivars; however, resistant varieties often lack market acceptability among farmers and consumers limiting

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their widespread adoption. In this context, biological control using antagonistic microorganisms has gained increasing attention as an eco-friendly and sustainable approach for disease management. Bioagents such as *Trichoderma spp.*, *Pseudomonas fluorescens*, and *Bacillus subtilis* have demonstrated promising bioefficacy against Foc through mechanisms like mycoparasitism, antibiosis, and induced systemic resistance. These agents not only suppress pathogen development but also enhance plant vigor and soil health. *In vitro* screening of fungicides and bioagents provides a rapid and cost-effective method to assess their potential in inhibiting the mycelial growth of the pathogen under controlled conditions, thereby serving as a preliminary step for field-level evaluation. Considering the severity of Fusarium wilt and the limitations of conventional control methods, the present investigation was undertaken to evaluate the *In vitro* efficacy of selected fungicides and biological control agents against *Fusarium oxysporum* f.sp. *cubense*, with the objective of identifying effective options for integrated management of the disease in banana cultivation.

Materials and Methods

The present investigation was carried out to evaluate the *In vitro* bioefficacy of selected fungal and bacterial antagonists against *Fusarium oxysporum* f. sp. *cubense* (Foc), the causal agent of Fusarium wilt (Panama disease) in banana. A total of five bioagents were tested, which included two bacterial antagonists-*Pseudomonas fluorescens* and *Bacillus subtilis*-obtained from the Department of Plant Pathology and Agricultural Microbiology, MPKV, Rahuri, and three fungal antagonists-*Trichoderma viride* and *Trichoderma harzianum* from the College of Agriculture, Dhule, and *Trichoderma reesei* supplied by the Central Institute for Subtropical Horticulture (CISH), Lucknow.

The biological activity of these bioagents against Foc was assessed using the dual culture technique, as described by Dennis and Webster (1971) [3]. In this method, seven-day-old cultures of both the bioagents and the pathogen were inoculated aseptically on potato dextrose agar (PDA) medium in sterile Petri plates. For fungal bioagents, a 5 mm mycelial disc was placed at one end of the plate, while a similar disc of the pathogen was placed directly opposite maintaining a distance of 1 cm from the edge. In the case of bacterial bioagents, instead of placing a mycelial disc, the bacterial cultures were streaked using a flame-sterilized inoculation needle on one side of the plate, with the fungal pathogen placed on the opposite side.

All treatments were replicated four times and incubated at 27 ± 1 °C for seven days. Observations on the radial growth of both the pathogen and the bioagents were recorded, and the zone of inhibition, if any, was measured. Plates inoculated only with the pathogen served as untreated control. The percentage inhibition of pathogen growth by each bioagent was calculated using the formula proposed by Arora and Upadhyay (1978):

$$I = \frac{CT}{C} \times 100$$

I = Per cent inhibition of mycelial growth,
C = Radial growth of pathogen in control plate (mm), and
T = Radial growth of pathogen in treatment plate (mm).

Results and Discussion

The *In vitro* efficacy of various bioagents in inhibiting the radial growth of *Fusarium oxysporum* f.sp. *cubense* (Foc), the causal organism of Panama wilt in banana, was assessed through dual culture technique. Among the five bioagents tested, *Trichoderma viride* exhibited the highest antifungal activity, significantly reducing the radial growth of the pathogen to 13.73 mm, which corresponds to a percent inhibition of 84.74%. This was followed closely by *Trichoderma harzianum* and *Trichoderma reesei*, which recorded mean radial growths of 15.25 mm and 16.66 mm, with percent inhibition of 83.05% and 81.48%, respectively.

On the other hand, the bacterial bioagents *Pseudomonas fluorescens* and *Bacillus subtilis* demonstrated comparatively lower antagonistic activity. *Pseudomonas fluorescens* recorded a radial growth of 31.85 mm with 64.61% inhibition, while *Bacillus subtilis* showed 33.99 mm radial growth with 62.23% inhibition. In contrast, the untreated control exhibited complete colonization of the Petri plate with 90.00 mm radial growth, indicating the absence of any inhibitory effect.

These results clearly indicate that fungal antagonists belonging to the genus *Trichoderma* were highly effective in suppressing the radial growth of Foc

under *In vitro* conditions. Among them, *Trichoderma viride* emerged as the most potent bioagent, which may be attributed to its rapid colonization ability, production of lytic enzymes such as chitinases and glucanases, and strong mycoparasitic behavior. The significant inhibition observed with *T. harzianum* and *T. reesei* further reinforces the potential of *Trichoderma* spp. as efficient biocontrol agents against soil-borne pathogens like Foc.

The bacterial antagonists also exhibited noteworthy suppression of Foc, albeit to a lesser extent than *Trichoderma* spp. Their antagonistic effect may be attributed to the production of antibiotics, siderophores, hydrogen cyanide, and other antifungal metabolites that inhibit fungal growth. Despite their relatively moderate efficacy, both *Pseudomonas fluorescens* and *Bacillus subtilis* demonstrated statistically significant inhibition of pathogen growth, and may be considered as valuable components in integrated disease management (IDM) strategies, particularly when used in combination with fungal bioagents or organic amendments.

Overall, the study highlights the superior performance of *Trichoderma viride* in suppressing the mycelial growth of *Fusarium oxysporum* f.sp. *cubense*, suggesting its strong potential as a core biocontrol agent in IDM practices for managing Fusarium wilt in banana. These findings are in agreement with earlier reports by Nayak *et al*. (2020), who also reported *T. viride* as the most effective antagonist against Foc. Similar results were also documented by Chand *et al*. (2021), who observed complete inhibition of Foc mycelial growth when co- cultured with *Trichoderma* spp. under dual culture conditions. These consistent results across studies strongly support the inclusion of *Trichoderma viride* in sustainable disease management programs for banana cultivation.

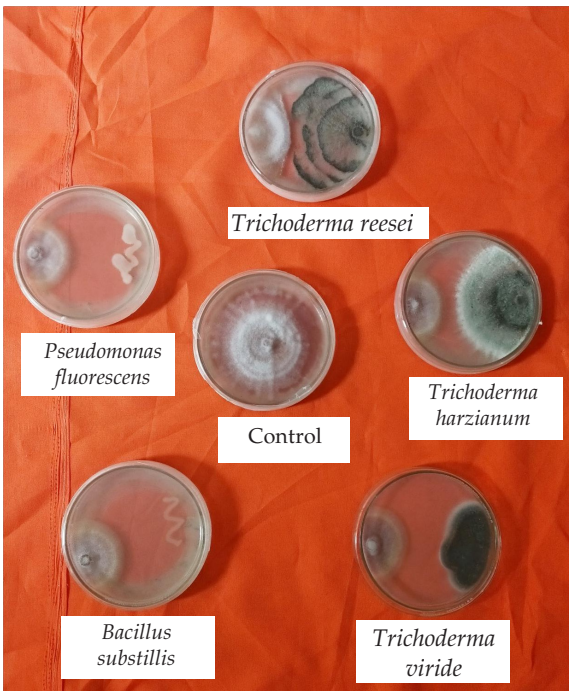

Fig. 1. Bioefficacy of bioagents against radial growth of *Fusarium oxysporum f.sp cubense (Foc)*

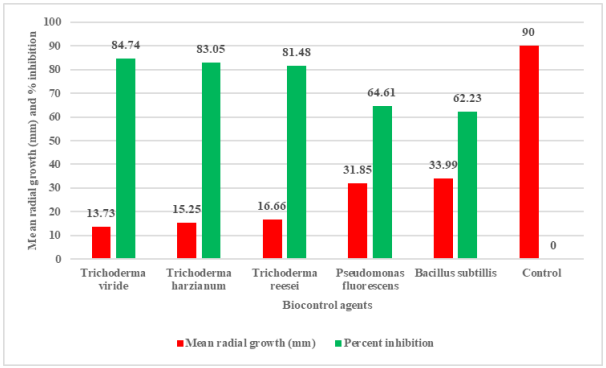

Fig. 2. Efficacy of different bioagent against radial growth of *Fusarium oxysporum* f.sp *cubense In vitro.*

Conclusion

The in vitro evaluation of bioagents against *Fusarium*

Table 1. Efficacy of different bioagents against radial growth of *Fusarium oxysporum* f sp. *cubense*

Treatments	Biocontrol agents	Mean radialgrowth (mm)	Percentinhibition
T1	*Trichoderma viride*	13.73	84.74
T2	*Trichoderma harzianum*	15.25	83.05
T3	*Trichoderma reesei*	16.66	81.48
T4	*Pseudomonas fluorescens*	31.85	64.61
T5	*Bacillus subtillis*	33.99	62.23
	Control	90.00	-
	S Em ±	0.79	-
	CD at 1 %	3.22	-

oxysporum f.sp. *cubense* revealed that fungal antagonists, particularly *Trichoderma viride*, were highly effective in suppressing the mycelial growth of the pathogen. *T. viride* recorded the highest inhibition (84.74%), followed by *T. harzianum* and *T. reesei*, confirming the superior antagonistic potential of *Trichoderma* spp. over bacterial bioagents. Although *Pseudomonas fluorescens* and *Bacillus subtilis* showed moderate inhibition, their efficacy indicates potential when used in integrated disease management strategies. These findings highlight the promising role of *Trichoderma viride* as a key biocontrol agent for the effective management of Panama wilt in banana.

Conflict of Interest – None

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