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### Biological Management of Panama Wilt of Banana Caused by *Fusarium oxysporum* f. sp. *Cubense*

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### Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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### ABSTRACT

Bananas are one of the most important fruit crops grown in tropical and sub-tropical regions of the world. It contributes to the livelihood of many growers and constitutes a larger proportion of fruit orchards in India. Panama wilt of banana is a major fungal disease affecting banana cultivations and farmers facing huge economic losses after the introduction of *Fusarium oxysporum* f.sp.*cubense* Foc TR4 strain B2 in Bihar .In the present investigation, native and commercial isolates of *Trichoderma viride* and *Trichoderma harzianum* are tested against the all isolates of *Fusarium oxysporum* f.sp. *cubense*. Both the biocontrol agents were found highly effective against the Iso. M, Iso A and Iso.K under *in vitro* as well as under natural pot conditions. But in the case of Iso.G (Foc TR 4 strain B2), these bioagents were found moderately effective under *in vitro* and *in vitro* conditions.

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#### **1. INTRODUCTION**

Banana is one of the important tropical fruit crop and economically profitable crop of India having high export potential but diseases are the major limiting factor in the cultivation of bananas and adequate management strategies are necessary to promote the production as well as productivity Banana provides of banana. the most economical source of carbohydrates and provides a much more balanced diet than any other fruit in terms of nutritional point of view with 67 to 137 calories per 100 grams. However, it has low amount of fat and protein, it is a rich source of energy and enclose all essential nutrients including minerals and vitamins A, B<sub>1</sub>, B<sub>2</sub> and vitamin C and thus it is called as "Apple of Paradise" and as well as "Adams fig" [1]. The banana research in India is completely towards the extension in production and productivity. The production restraints also vary in country from region to region. However, numerous issues are identical in nature across the banana producing states of India and also among the various countries of the world. In Bihar state, there are two banana growing region one is old Vaishali belt enriched with tall group of banana cultivars and second new Koshi belt dominated with dwarf Cavendish group of banana cultivars.

"Now a days, global banana production is increasingly constrained by Panama wilt disease and being the most significant" [2,3,4]. "In 2013, TR4 was reported to be in Jordan, the first official report of TR4 outside the Southeast Asia Pacific region and a survey in 2014 revealed another infected area, north of the original outbreak" [5].

Muhammad et al., [6] reported that TR4 is a noteworthy issue for Cavendish banana developing zones in Pakistan and detailed that cv. Basrai to be influenced by TR4 in specific zones in the Sindh territory and create havoc to banana industry in Pakistan and would be devastating economically. In 2018, TR4 was confirmed to be in Myanmar. The analysis of isolates from Laos, Vietnam and Myanmar provided evidence that the particular TR4 strain in these countries was likely introduced from China Zheng et al., [7] Maymon et al., [8] detailed TR 4 in the lower Carmel coastal plain (1,200 ha), western Galilee (500 ha), and Jordan valley (800 ha) of Israel.

"In 2015, a banana grower from Barari village in the Bihar State of India reported the wilting of Cavendish banana cv. Robusta (AAA) and Grand Naine (AAA). The incidence of the disease was ranged from 2 to 26.6 per cent" [9]. "Recently, a highly virulent strain of Foc TR 4 strain B2 identified which was affecting Cavendish group of banana cv. Grand Naine(AAA) and Robusta(AAA) in Koshi belt of Bihar" [10].

"Management of Foc TR 4 is a matter of serious concern and different type of approaches such as chemical, biological and manipulation of cultural practices have been attempted by different research workers. The ability of Foc TR4 is survive in absence of host is a significant factor limiting the successful management through agronomic practices like crop rotation. Therefore, the only alternative approach for management is by use of antagonistic and growth-promoting fungi which have been successfully demonstrated in biological control of soil borne pathogen in different crop plants" [11,12]. This study on the biological management of Panama wilt in bananas caused by Fusarium oxysporum f.sp. cubense(Foc) holds significant scientific relevance due to its implications for sustainable disease management in one of the most economically vital fruit crops globally [13,14,15]. The emergence of the Foc TR4 strain B2 in Bihar has escalated the economic impact of Panama wilt, emphasizing the urgent need for effective control strategies. The utilization of native and commercial isolates of Trichoderma viride and Trichoderma harzianum as biocontrol agents presents a promising avenue for integrated disease management, contributing to the expanding field of biocontrol research. The study's in-depth assessment, both in vitro and in vivo, of the efficacy of these biocontrol agents against different isolates of Foc, including the highly virulent Iso. G, provides valuable insights into the specificity and effectiveness of biological interventions in combatting Panama wilt.

#### 2. MATERIALS AND METHODS

### 2.1 Isolation and Purification the Pathogen

The infected plants parts of banana cultivars like pseudostem, roots and leaves were collected from the field. These infected plant parts were cut in to small pieces in 2-3 mm and surface sterilized with 0.1% mercuric chloride solution for 30 seconds after that washing with distilled water to 2-3 times. These small pieces were placed aseptically on PDA slants with the help of an inoculating needle and incubated at  $28\pm^{\circ}$ C. All the isolates were categorised in to five different groups *viz.*, Iso.M, Iso.A, Iso.K, Iso.G and Iso.R on the basis of host differential of banana cultivars.

### 2.2 Evaluation of Antagonistic Efficacy of Bio-Control Agents Against Foc Isolates

### 2.2.1 Isolation and identification of native and commercial fungal bioagents

Rhizospheric soil from healthy banana plants were collected in poly ethylene bags and brought to the research laboratory of Department of Plant Pathology, RPCAU, Pusa, Samastipur. Serial dilution technique [16] was used to isolate fungal antagonist from the rhizospheric soil of healthy banana plants and shade dried. The mychoflora was isolated on rose Bengal agar medium by utilizing a dilution of  $10^3$  and  $10^4$ . One ml of soil suspension was poured into sterilized Petri plates containing the melted and cooled medium and then rotated gently to get uniform distribution of soil into the medium. At that point, the plates were incubated at  $27\pm2^{\circ}$ C and observed frequently for the development of colonies.

The developed colonies were picked and recognised based on mycological keys described by Gilman [17] and Nelson *et al.* [18] for identification of *Trichoderma viride* and *Trichoderma harzianum.* 

Bioagents were also isolated from two commercial formulation of biocides, *i.e.* Nisarga (Multiplex Agricare Pvt. Ltd. 1%W.P.) and Antagon (Arihant Nature crop Pvt.Ltd.1%W.P.) according to method described in native bioagents isolation.

#### 2.2.2 Evaluation of antagonistic efficacy of *Trichoderma viride* and *Trichoderma harzianum* (native and commercial isolates) against Foc *in vitro*

"For the testing the antagonistic effect of *T*. *harzianum* and *T*. *viride* (Commercial and native isolate) the PDA plate divided into equal halves" [19]. "The first half was independently inoculated with 7 days old culture disc (5mm in diameter) of each fungal bio-control agent, while the later was also independently inoculated with one disc (5 mm in diameter) of 7 days old culture of pathogenic fungus in the opposite side. Control

plate was inculcated with disc of test fungus on PDA medium instead the fungal bio control agents. Three plates were used as replicates for each treatment" [20].

All plates were incubated for  $28 \pm 2^{\circ}$ C until the growth of Foc isolates in the control treatment reached to the edge of Petri dish. The per cent reduction of linear mycelial growth of pathogenic fungi were calculated using formula as given by Vincent [21].

$$I = \left(\frac{C-T}{C}\right) \times 100$$

Where,

I = Per cent growth inhibition

- C = Colony diameter in control Petri plate;
- T = Colony diameter in the treated Petri plate.

Data were subjected to proper statistical analysis of variance according to Snedecor and Cochran [22] Mean of treatments were compared with Ftest and L.S.D. at level of 0.05%.

# 2.2.3 Evaluation of antagonistic efficacy of *Trichoderma viride* and *Trichoderma harzianum* (native and commercial isolates) against Foc *TR* 4 strain B<sub>2</sub> under pot condition

The efficacy of all the bioagents were tested in poly house under pot condition against the isolates Foc TR4 strain B<sub>2</sub>. The mass culture of Foc isolates were prepared as per method described by Haware [23]. "The mass culture of Foc TR4 strain B<sub>2</sub> isolate was blended in steam sterilized soil separately @50 g/kg soil having 10 kg sterile loamy soil / plastic pots (50cm diameter). Each bioagent with 50 g /pot was applied in pathogen-inoculated pots after 15 days of transplanting. Inoculation of pot with sterilized distilled water was served as control. Each treatment was replicated three times with different banana cultivars independently. The disease incidence (%) was calculated by dividing the total number of transplanted plants showing Panama wilt disease symptoms by the total number of plants transplanted and then multiplied by hundred. The data on inhibition per cent over control also treatment recorded in each and data was recorded up to 90 days after transplanting" [20].

### 2.3 Per Cent wilt Index (PWI, External Symptoms)

The intensity of Panama wilt of banana was recorded as per International Musa Testing Program(IMTP) rating in 1-5 scale and per cent wilt index(PWI) was determined in each plot.

Category	Reaction
1	Healthy
2	Slight chlorosis and wilting with no petiole buckling
3	Moderate chlorosis and wilting with some petiole buckling and or splitting of leaf base
4	Severe chlorosis, severe wilting, petiole buckling and dwarfing of newly emerged leaf
5	Dead

Per cent wilt index =Total sum of numerical rating / Total number of plants observed × maximum category in the score chart × 100

The data was analysed by CRD design.

### 3. RESULTS AND DISCUSSION

### 3.1 Antagonistic Effect of *Trichoderma viride* and *Trichoderma harzianum* on Different Isolates of Foc *in vitro*

#### 3.3.1 Effect of native isolate of *Trichoderma viride* and *Trichoderma harzianum* on different isolates of Foc *in vitro*

The antagonistic activity of native isolates of *Trichoderma viride* and *Trichoderma harzianum* against the various isolates of Foc (Iso. M, Iso. A, Iso. K, Iso. R and Iso. G) were determined by the dual culture technique *in vitro*. Five mm disc of seven days old cultures of bioagent (*T. viride* and *T. harzianum*) and isolates of *Foc* were placed equidistant in sterilized Petri plate containing PDA medium. Suitable control was also maintained without antagonist. The growth of the pathogen was measured at 24 hrs interval up to 240 hrs of inoculation. Percent inhibition of mycelial growth of pathogen was calculated. Data are presented in (Table 1).

In case of native isolate of *Trichoderma viride* maximum inhibition was observed in Iso. K (64.6%) followed by Iso. A (62.2%), Iso. M (59.8%) and Iso. R (51.0%). The minimum

inhibition was recorded in Iso. G (50.2%) over control after 240 hrs. While in case of native isolate of T. harzianum, the maximum inhibition was observed in Iso. K (57.6%) followed by Iso. A (56.4%), Iso. M. (55.2%) and Iso. R (44.0%). The minimum inhibition was recorded in Iso. G (43.6%). The result clearly showed that the native isolates of T. viride and T. harzianum were highly effective against the Iso. M, Iso. A and Iso. K. But these native isolates of bioagents were found moderately effective against the Iso. R and Iso. G (TR4 strain B<sub>2</sub>) of Foc. Data pertaining to inhibition per cent over control revealed that the radial growth of Iso. M, Iso. A and Iso. K were significantly inhibited against the native isolate of T. viride and T. harzianum. However, the differences between Iso. R and Iso. G was found nonsignificant or remained significantly at par. The maximum inhibition was recorded in Iso. K closely followed by Iso. A or the Iso. A and Iso. K varied significantly.

#### 3.3.2 Antagonistic effect of *Trichoderma viride* (commercial) and *Trichoderma harzianum* (commercial) on different isolates of Foc *in vitro*

The antagonistic effect of *Trichoderma viride* (commercial) and *Trichoderma harzianum* (commercial) on Foc isolates (Iso. M, Iso. A, Iso. K, Iso. G and Iso. R) were studied by dual culture method and results presented in the (Table 2).

In case of Trichoderma viride (commercial) isolate, maximum inhibition was recorded in Iso. K (55.1%) followed by Iso. A (54.2%), Iso. M (53.2%), Iso. R (42.8%) while minimum inhibition was noticed in Iso. G (42.3%) over control. But in case of commercial isolate of Trichoderma harzianum, the inhibition per cent was found maximum in Iso. K (46.1%) followed by Iso. A (45.5%), Iso. M (43.9%) and Iso. R (33.7%). The minimum inhibition was recorded in Iso. G (TR4 strain B<sub>2</sub>) *i.e.* 33.2% over control. Both the commercial isolates of T. harzianum and T. viride were found less effective against the lso. M (race 1), Iso. A (race 1) and Iso. K (race 2). Data pertaining to inhibition per cent over control against the commercial isolate of T. viride and T. harzianum showed that considerable variation in all the isolates of Foc. The differences between Iso. R and Iso G was found non significant or Iso. G at par with Iso. R and minimum inhibition were recorded in Iso. G.

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Isolates		Radial growth(mm)*													
	Trichoderma	a viride (Native	)	Trichoderma harzianum (Native)											
	120 hrs	240 hrs	Inhibition over control (%) at 240 hrs	120 hrs	240 hrs	Inhibition over control (%) at 240 hrs									
Iso. M (race 1)	15.6	35.6	59.8	21.4	39.6	55.2									
Control	35.3	88.4		35.4	88.3										
Iso. K (race 2)	13.5	31.4	64.6	19.5	37.6	57.6									
Control	33.4	88.5		33.5	88.5										
Iso. A (race 1)	14.2	33.4	62.2	20.5	38.5	56.4									
Control	34.3	88.5		34.5	88.4										
Iso. G (race 4)	24.6	44.3	50.2	28.4	50.4	43.6									
Control	37.4	89.1		37.5	89.4										
Iso. R (race 4)	23.4	43.3	51.0	27.6	49.7	44.0									
Control	36.3	88.4		36.6	88.7										
CD at 5%	1.68	1.29	2.21	1.73	1.27	2.09									
S.Em. (±)	0.57	0.43	0.70	0.58	0.43	0.66									
C.V. (%)	3.67	1.19	2.10	3.41	1.12	2.21									

### Table 1. Effect of native isolate of Trichoderma viride and Trichoderma harzianum on different isolates of Foc in vitro

\*Mean of three replications

### Table 2. Antagonistic effect of Trichoderma viride (commercial) and Trichoderma harzianum (commercial) on different isolates of Foc in vitro

Isolates	Radial growth(mm)*													
	Trichoder	ma viride (Co	mmercial)	Trichoderma harzianum (Commercial)										
	120 hrs	240 hrs	Inhibition	120 hrs	240 hrs	Inhibition								
			over		over									
			control (%)			control (%)								
			at 240 hrs			at 240 hrs								
Iso. M (race 1)	22.5	41.4	53.2	25.8	49.6	43.9								
Control	35.5	88.4		35.6	88.5									
Iso. K (race 2)	20.5	39.7	55.1	23.6	47.7	46.1								
Control	33.5	88.4		33.6	88.4									
Iso. A (race 1)	21.3	40.5	54.2	24.6	48.6	45.1								
Control	34.5	88.4		34.6	88.5									
Iso. G (race 4)	30.5	51.5	42.3	32.7	59.4	33.2								
Control	37.5	89.3		37.5	88.9									
Iso. R (race 4)	29.5	50.5	42.8	31.7	58.8	33.7								
Control	36.6	88.4		36.7	88.6									
CD at 5%	1.88	1.28	2.13	1.94	1.20	1.98								
S.Em. (±)	0.63	0.43	0.67	0.65	0.40	0.62								
C.V. (%)	3.62	1.12	2.33	3.57	0.99	2.65								

\*Mean of three replications

But in case of Iso. R (race 4) and Iso. G (TR4 strain  $B_2$ ) showed ineffectiveness against the commercial isolates because these isolates were showed only little amount of mycelial growth reduction in dual culture techniques.

## 3.3.3 Effectiveness of native and commercial isolates of *T. viride* and *T. harzianum* against the Foc TR 4 strain $B_2$ (Iso. G) under pot condition

Mass culture of Foc TR4 strain  $B_2$  (Iso. G) was multiplied on sand maize medium and added to

steam sterilized soil (15 psi for 30 minutes) in pots @ 5% (w/w). Soil mixture without inoculums served as control. Each pot was planted with one-month old banana sucker of different cultivars viz., Malbhog (AAB), Alpan (AAB), Kothia (ABB), Robusta (AAA) and Grand Naine (AAA). After 15 days of transplanting different native and commercial isolates of Trichoderma and Trichoderma viride harzianum were multiplied on sorghum sand medium mixed in each inoculum containing pots @ 50 gm/pot. Observation was done based on the first appearance of symptom of disease, per cent

		Malbh (Silk G				Alpan* (Mysor	e Group	)			Kothia* (Bluggoe group)					Grand Naine* Dwarf Cavendish Group)				Robus (Dwarf		sh Group	)
	Bioagent	Frist appearance of disease (DAT)		Incidence (%)	Inhibition over control (%)	Frist appearance of disease (DAT)	Incidence (%)		Inhibition over control (%)		Frist appearance of disease (DAT)	Incidence (%)		Inhibition over control (%)		Frist appearance of disease (DAT)	Incidence (%)		Inhibition over control (%)	Frist appearance of disease (DAT)	Incidence (%)		Inhibition over control (%)
T <sub>1</sub>	T. viride	56	46		52.6	59	33	62.1		58		34	57.5		51	46		52.6		54	42	56.3	
T <sub>2</sub>	(Native) <i>T.harzianu</i>	49	53		45.4	51	53	39.1		44		47	41.3		34	53		45.4		36	50	47.9	
T <sub>3</sub>	<i>m</i> (native) <i>T. viride</i> (Commerci al)	46	57		41.2	49	35	59.8		48		39	51.3		37	57		41.2		38	52	45.8	
T <sub>4</sub>	<i>T.harzianu m</i> (Commerci al)	40	60		38.1	41	58	33.3		39		57	28.8		41	60		38.1		43	58	39.6	
T₅	Control	26	97			27	87			29		80			22	97				24	96		
CD at 5	%		2.27		4.38		1.99	5.82				2.27	5.26			2.27		5.16			2.36	5.29	
S.Em. (:	±)		0.71		1.32		0.62	1.76				0.71	1.59			0.71		1.62			0.74	1.60	
C.V. (%	)		1.96		5.16		1.95	6.26				2.39	6.16			1.96		6.63			2.14	5.84	

Table 3. Effectiveness of native and commercial isolates of *T. viride* and *T. harzianum* against the Foc TR 4 strain B<sub>2</sub> (Iso. G) under pot condition

\*Mean of three replications

incidence of the disease and per cent inhibition over control. Results are presented in (Table 3).

Results revealed that the native and commercial isolates of *T. viride* and *T. harzianum* were significantly lower in comparison to the control.

Among the different isolates of T. viride and T. harzianum, the native isolate of T. viride and T. harzianum were found highly and moderately effective against the Foc TR4 strain B<sub>2</sub>. respectively. Because Panama wilt disease symptom was not observed up to 59 days in cv. Alpan followed by cv. Kothia (58 DAT), cv. Malbhog (56 DAT), cv. Robusta (54 DAT) and cv. Grand Naine (51 DAT) against the native isolate of T. viride. The native isolate of T. viride was found significantly effective in disease suppression against the Foc TR4 strain B2 followed by native isolate of *T. harzianum*. Commercial isolate of T. viride and T. harzianum were found less effective against the Foc TR4 strain B<sub>2</sub>. Data pertaining to inhibition over control under pot condition revealed that the  $T_2$ and T<sub>3</sub> was found non significant or significantly at par in cultivar Malbhog (AAB), Grand Naine (AAA) and Robusta (AAA). However, the differences between  $T_1$  and  $T_3$  in cultivar Alpan (AAB) was found non significant or T<sub>3</sub> at par with T<sub>1</sub>. In comparing this study with existing research on soil quality and Fusarium wilt in bananas from Latin America, an intricate interplay between biological management Araya-Alman et al. [24] Rey et al., [25] and environmental factors becomes apparent [26]. Soil quality studies often elucidate the role of soil microbiota and physicochemical properties in influencing plant health [27,28]. The integration of biocontrol agents, such as Trichoderma viride and Trichoderma harzianum, the in present investigation aligns with the broader understanding that soil health directly impacts plant-pathogen interactions. Additionally, the moderate efficacy observed against the highly virulent Iso. G underscores the complexity of Fusarium wilt dynamics, suggesting that soil quality may influence the success of biocontrol strategies. Comparative analyses with Latin American studies could shed light on regional variations in soil microbiota, offering nuanced insights into the adaptability and efficacy of biological management practices in diverse agroecosystems [29,30].

Considering the influence of agro-environmental factors on *Fusarium* wilt in bananas from Latin America, the present study contributes to the

discourse bv addressing the practical applicability of biocontrol agents under different environmental conditions [31,32]. The observed efficacy of Trichoderma viride and Trichoderma harzianum against multiple isolates of Fusarium oxysporum f.sp. cubense in both controlled (in vitro) and natural pot (in vivo) conditions indicates a robust potential for biological management across diverse agro-environments. However, the moderate effectiveness against the Iso. G strain highlights the need for a nuanced understanding of the agro-environmental factors influencing the interaction between biocontrol agents and specific Fusarium strains. Collaborative research efforts between regions facing Fusarium wilt challenges could yield valuable insights into the adaptability of biocontrol strategies to varying environmental contexts, enhancing the global applicability of such interventions [33,34].

### 4. CONCLUSION

During the evaluation of biocontrol agents like Trichoderma viride and T. harzianum (Native and commercial isolates) against the different isolates of Foc under in vitro as well as in artificial pot condition. Among the both biocontrol agents, T. viride (native) was found highly effective against the Iso. M and Iso. A and Iso. K under in vitro as well as under pot condition. Under pot condition, the native isolate of T. viride and T. harzianum were found highly and moderately effective against the Foc TR 4 strain B<sub>2</sub>, respectively. Because Panama wilt symptom not develop up to 59 days in cv. Alpan followed by cv. Kothia (58 DAT), cv. Malbhog (56 DAT), cv. Robusta (54 DAT) and cv. Grand Naine (51 DAT) against the T. viride (native). Commercial isolate of both bio control agents was found less effective against the Foc TR 4 strain B<sub>2</sub>.

### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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