Original Research Article

Biological management of Panama wilt of Banana caused by *Fusarium oxysporum* f.sp. *cubense*

Abstract

Banana is 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* TR4strain B2 in Bihar .In present investigation , native and commercial isolates of *Trichoderma viride* and *Trichoderma harzianum* are tested against the all isolates of *Fusarium oxxysporum* f.sp. *cubense*.Both the bio control agentswere found highly effective against the Iso.M,Iso A and Iso.K under *in vitro* as well as under natural pot conditions.But in case of Iso.G(Foc TR 4 strain B2), these bio agents were found moderately effective under *in vivo* and *in vitro* conditions.

Key Word:-*Trichoderma*, strain ,TR4

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 banana and adequate management strategies are necessary to promote production as well as productivity of banana.Banana provides most economical source of carbohydrates and provides much balanced diet than any other fruit in term 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 vitamin A, B_1, B_2 and vitamin C and thus it is called as “Apple of Paradise” and as well as “Adams fig” (Bose and Mitra, 2001). 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.

Global banana production is increasingly constrained by Panama wilt disease and being the most significant. Due to the perennial nature of bananas and the polycyclic nature of the disease, effective, long-term management of this disease remains a challenge and requires the development of new alternative management strategies (Ghag *et al.,* 2015). Due to the ease with which the disease spreads, the global banana industry is under serious threat from this soil-borne fungal disease (García-Bastidas *et al.,* 2014; Ordonez *et al.,* 2015).

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(Ploetz *et al*., 2015).

Muhammad *et al.,* (2017) 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.,* 2018).Maymon*et al.,* (2018) 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 (Thangavelu *et al.,* 2019).Recently, a highly virulent strain of *Fusarium oxysporum* f.sp. *cubense* TR 4 strain B2 identified which was affecting Cavendish group of banana cv. Grand Naine(AAA) and Robusta(AAA) in Koshi belt of Bihar (Shukla and Singh,2019).

Management of *Fusarium oxysporum*f.sp. *cubense*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 F*usarium oxysporum* f.sp. *cubense* TR4 is survive in absence of host is a significant factor limiting the successful management through agronomic

practices like crop rotation. Therefore,the only alternatives 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 (Tan *et al.*,2015,Khan*et al.,* 2018). In this context, we evaluate the bio agents like *Trichoderma* isolates against the *Fusariumoxysporum* f.sp. *cubense* causing Panama wilt of banana.

MATERIAL AND METHODS

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 inoculating needle and incubated at 28±ºC. 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.

Evaluation of antagonistic efficacy of bio-control agents against *Fusarium oxysporum* f.sp. *cubense* isolates

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 (Johnson and Curl, 1972) was used to isolate fungal antagonist from 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±2°C and observed frequently for the development of colonies.

The developed colonies were picked and recognised based on mycological keys described by Gilman (1957) and Nelson *et al*. (1983) 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.

Evaluation of antagonistic efficacy of *Trichoderma viride* and *Trichoderma harzianum* (native and commercial isolates) against *Fusarium oxysporum* f.sp. *cubense 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 (Karunanithi and Usman, 1999). 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.

All plates were incubated for 28 ±2°C until the growth of *Fusarium oxysporum* f.sp. *cubense* 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 (1927).

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

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 (1980). Mean of treatments were compared with F-test and L.S.D. at level of 0.05%.

Evaluation of antagonistic efficacy of *Trichoderma viride* and *Trichoderma harzianum* (native and commercial isolates) against *Fusarium oxysporum* f.sp. *cubense TR* 4 strain B_2 under pot condition

The efficacy of all the bioagents were tested in poly house under pot condition against theisolates*Fusarium oxysporum* f.sp. *cubense* TR4 strain B_2. The mass culture of *Fusarium oxysporum* f.sp. *cubense*isolates were prepared as per method described by Haware (1980). The mass culture of *Fusarium oxysporum* f.sp. *cubense* TR4 strain B_2 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 were 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 cultivar independently. The disease incidence (%) was calculated by dividing the total number of transplanted plants showing Panama wilt disease symptoms by the total plant transplanted and then multiplied by hundred. The data on inhibition per cent over control also recorded in each treatment and data was recorded up to 90 days after transplanting.

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

$$\text{Per cent wilt index} = \frac{\text{Total sum of numerical rating}}{\text{Total number of plants observed} \times \text{maximum category in the score chart}} \times 100$$

The data was analysed by CRD design.

RESULT AND DISCUSSION

Antagonistic effect of *Trichoderma viride* and *Trichoderma harzianum* on different isolates of *Fusarium oxysporum* f.sp. *cubensein vitro*

Effect of native isolate of *Trichoderma viride* and *Trichoderma harzianum* on different isolates of *Fusarium oxysporum* f.sp. *cubense in vitro*

The antagonistic activity of native isolate of *Trichoderma viride* and *Trichoderma harzianum* against the various isolates of *Fusarium oxysporum* f.sp. *cubense* (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 culture of bioagent (*T. viride* and *T. harzianum*) and isolates of *Fusarium oxysporum* f.sp. *cubense* were placed equidistant in sterilized Petri plate containing PDA medium. Suitable control was also maintained without antagonist. The growth of pathogen was measured at 24 hrs interval up to 240 hrs of inoculation. Per cent 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_2) of *Fusarium oxysporum* f.sp. *cubense*. 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 non significant 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.

Antagonistic effect of *Trichoderma viride* (commercial) and *Trichoderma harzianum* (commercial) on different isolates of *Fusariumoxysporum* f.sp. *cubensein vitro*

The antagonistic effect of *Trichoderma viride* (commercial) and *Trichoderma harzianum* (commercial) on *Fusarium oxysporum* f.sp. *cubense* 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_2) *i.e.* 33.2% over control. Both the commercial isolates of *T. harzianum* and *T. viride* were found less effective against the Iso. 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 *Fusarium oxysporum* f.sp. *cubense*. 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.

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.

Effectiveness of native and commercial isolates of *T. viride* and *T. harzianum* against the *Fusarium oxysporum* f.sp. *cubense* TR 4 strain B_2 (Iso. G) under pot condition

Mass culture of *Fusarium oxysporum* f.sp. *cubense* 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 harzianum* and *Trichoderma viride* 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 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 *Fusarium oxysporum* f.sp. *cubense* TR4 strain $B_{2,}$ 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 *Fusarium oxysporum* f. sp. *cubense* TR4 strain B_2 followed by native isolate of *T. harzianum*. Commercial isolate of *T. viride* and *T. harzianum* were found less effective against the *Fusariumoxysporum* f.sp. *cubense* TR4 strain B_2. Data pertaining to inhibition over control under pot condition revealed that the T_2 and T_3 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_3 at par with T_1.

Nurbailis *et al., (*2016) observed that highest surface colonization capability of *T. viride* (T1sk) found in Barangan and Kepok cultivars and endophyte colonization found in Kepok cultivar. The surface colonization capacity of *T. viride* (T1sk) on the roots of Barangan and kepok reached 93, 33% and the ability of being endophyte 43, 33% and 38, 33% could reduce *Fusarium* wilt disease on banana seedling and increase seedling growth.Khan *et al.,* (2017) detailed that the *T. harzianum* created up to 75.5% hindrance growth of the pathogen followed by incubation for 72 hrs at 28+2°C *in vitro*. In pot culture *T. harzianum* considerably reduced disease severity. It proves that it is a potential biological control agent against banana wilt pathogen.

CONCLUSION

During the evaluation of biocontrol agents like*Trichoderma viride* and *T. harzianum* (Native and commercial isolates) against the different isolates of *Fusarium oxysporum* f.sp. *cubense* 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 *Fusarium oxysporum* f.sp. *cubense* TR 4 strain B_2, 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 *Fusarium oxysporum* f.sp. *cubense* TR 4 strain B_2.

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Table 1. Effect of native isolate of *Trichoderma viride* and *Trichoderma harzianum* on different isolates of *Fusarium oxysporum* f.sp. *cubense in vitro*

Isolates	Radial growth(mm)*					
	Trichoderma 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

*Mean of three replications

Table 2. Antagonistic effect of *Trichoderma viride* (commercial) and *Trichoderma harzianum* (commercial) on different isolates of *Fusariumoxysporum* f.sp. *cubensein vitro*

Isolates	Radial growth(mm)*					
	Trichoderma viride (Commercial)			*Trichoderma harzianum* (Commercial)		
	120 hrs	240 hrs	Inhibition over control (%) at 240 hrs	120 hrs	240 hrs	Inhibition over control (%) 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

Table 3. Effectiveness of native and commercial isolates of *T. viride* and *T. harzianum* against the *Fusarium oxysporum* f.sp. *cubense* TR 4 strain B_2 (Iso. G) under pot condition

Bioagent		Malbhog* (Silk Group)			Alpan* (Mysore Group)			Kothia* (Bluggoe group)			Grand Naine* (Dwarf Cavendish Group)			Robusta* (Dwarf Cavendish Group)		
		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_1	*T. viride* (Native)	56	46	52.6	59	33	62.1	58	34	57.5	51	46	52.6	54	42	56.3
T_2	*T.harzianum* (native)	49	53	45.4	51	53	39.1	44	47	41.3	34	53	45.4	36	50	47.9
T_3	*T. viride* (Commercial)	46	57	41.2	49	35	59.8	48	39	51.3	37	57	41.2	38	52	45.8
T_4	*T.harzianum* (Commercial)	40	60	38.1	41	58	33.3	39	57	28.8	41	60	38.1	43	58	39.6
T_5	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

*Mean of three replications