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Innate and learned responses of antagonists against *fusarium musae* causing fusarium fruit rot of banana

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Abstract
Fruit rot disease caused by *Fusarium musae* is one of the post-harvest disease of banana and is adversely affects the fruit quality and the market value. In present study various antagonists were screened to know their bio efficacy in controlling the Fusarium fruit rot of banana.The highest per cent growth inhibition was found in the treatment of *Trichoderma viride* (77.52%) followed by *Pseudomonas fluorescens in vitro.* Whereas, under *in vivo* condition, *T. viride* both in pre inoculation (11.77%) and post-inoculation (11.43%) methods at 8th day after inoculation was found most potent antagonist in reducing the severity and it was remained at par with *P. fluorescens.*

Keywords: banana, antagonists, *Fusarium musae*, *in vitro*, *in vivo*, post-harvest

Introduction

Banana (*Musa paradisiaca* L.) is one of the most important commercial fruit crop grown all over the world in the tropical and subtropical areas. It is the second largest fruit crop, belongs to family *Musaceae* in order *Zingiberales.* It is indigenous to indo-Malayan region.

In India, banana is fourth important food crop in term of gross value exceeding only by paddy, wheat and milk products. It is also a desert fruit for million apart from a staple food owing to it's rich and easy digestibility. The ripe fruits are edible, delicious and very nutritious. The content of carbohydrates 22.84g is very high with a calorific value of 89kcal/100g fruit. It is good source of vitamin A (64 IU/100g of edible portion) and vitamin C (8.7mg/100g of edible portion) and fair source of vitamin B_1, B_2, B_3, B_5, B_6 and B_9. The fruits are rich in magnesium, sodium, potassium and phosphorus. The food value is about three times that of wheat. (Anon., 2019) [1].

Cultivated banana is susceptible to many diseases, mostly fungal pathogen which attacks various part of the plant from root to fruit. Bananas are highly perishable commodities with post-harvest losses estimated to the tune of 25-30 per cent (Kachwaha *et al*., 1991) [4]. Banana fruit suffers from many serious diseases such as fruit rot, crown rot, finger rot, cigar-end rot and pitting disease. The current postharvest problems for bananas are mainly concerned with storage and marketing. Crown rot due to, *Fusarium pallidoroseum* (Cooke) Sacc, *Colletotrichum musae* (Berk. and Curt.), *Verticillium theobromae* (Turc.) Mason and Hughes, *Thielaviopsis paradoxa* (Deseynes) Sacc., *Lasiodiplodia theobromae* (Pat.) Griffth and Maubl, *Fusarium musae* (Syd.) M. B. Ellis and *Fusarium roseum* (Link) Snyder and Hansen pathogens causes losses of bananas in grown for local consumption and export of bananas (Stark *et al*., 2008). Banana fruit majorly infected by *Alternaria alternata* (Fr.) Keissier, *Colletotrichum musae, Fusarium moniliforme* (Cooke) Sacc and *F. oxysporum* (Schlecht. Emend. Snyder and Hansen) pathogens. *Aspergillus flavus* Link., *A. fumigatus* Fresenius., *A. niger* Van. Teigh., *A. terreus* Thom., *Penicillium* spp. Link were dominant pathogens, same as *Curvularia lunata* (Wakker) Boedijn, *Cochliobolus lunatus* (Nelson and Haasis) and *Colletotrichum musae*. *Nigrospora oryzae* (Berk and Ba.) Petch. and *Khuskia oryzae* Hudson were active during the winter while, *Botryodiplodia theobromae* were sporadic whereas, *Deightoniella torulosa* and *Cunninghamella echinulata* (Thaxter) were detected occasionally. *Rhizoctonia solani* (Kuhn) and *Macrophomina phaseolina* (Tassi) Goid. also caused considerable damage to fruits (Sarkar *et al*., 2009) [6].

The banana fruit infecting fungus *Fusarium musae* was originally known as a distinct population within *Fusarium verticillioides*. However, (Van Hove *et al*., 2011) [9] recently, showed, by multilocus phylogeny and mating experiments that this population represents a unique lineage in the *Fusarium fujikuroi* species complex (FFSC) and consequently they installed the new species *Fusarium musae* being closely related to (i.e. sister species) but distinct from *Fusarium verticillioides*. The first cases of human infection associated with *Fusarium musae* caused through contact with *Fusarium musae* contaminated banana fruits, either being imported or after traveling of the patient to a banana producing country. An alternative hypothesis is that *Fusarium musae* is not only present on banana fruits, but also on other plant hosts or environmental sources. In a more recent survey performed laboratory testing the feasibility of an in house developed MALDITOF MS identification assay and there by using 390 fungal isolates collected between July 2012 and July 2013 from 2 hospitals located in Brussels, one *Fusarium musae* strain was found among the 20 Fusarium isolates identified. This *Fusarium musae* strain was isolated from a blood sample of an immune-suppressed patient, whereas majority of the other fusarioses (Triest *et al*., 2016) [8].

Use of fungicide on harvested fruits to manage the diseases is not desirable from health point of view, also continuous and indiscriminate use has led to the development of fungicide resistant strains of the pathogens. It also reduces the export quality due to high residues. An attempt was made to explore the possibility of using various antagonists for the management of Fusarium fruit rot of banana.

Materials and Methods

In vitro evaluation of antagonists against *Fusarium musae*

Antagonistic effect of different bioagents were tested by dual culture technique for their antagonism against banana fusarium fruit rot pathogen (Dennis and Webster, 1971) [2].

A pure culture of native bio-agents available in the Department of Plant Pathology, N. M. College of Agriculture, NAU, Navsari, were used. It was mass cultured on PDA for one week at 27± 2 °C resulting fungal growth was well developed on Petri-plates. Seven days old culture of the bioagents and the pathogen were employed by following dual culture method. Mycelial disc of 5mm diameter cut from the periphery of both antagonist and test pathogen and were placed at 50mm apart from each other in Petri plates and in case of bacterial bioagents half portion of PDA media plates streaked and 5mm diameter mycelial disc placed at center of Petri plates. In control, only test pathogen was kept in the center of Petri plate. Each treatment was replicated four times. The Petri plates were incubated at 27±2 °C in BOD incubator. The observations on colony diameter (mm) and per cent growth inhibition of *Fusarium musae* recorded after 8 days of incubation. The per cent growth inhibition (PGI) of pathogen in each treatment was calculated by following formula (Vincent, 1947) [10].

$$I = \frac{C - T}{C} \times 100$$

Where,
I = Inhibition per cent
C = Colony diameter (mm) in control plate
T = Colony diameter (mm) in treated plate

Table 1

I	Design	Completely Randomized Design (CRD)
Ii	Treatments	6 (Six) (including control)
Iii	Repetitions	4 (Four)
Iv	Method	Dual culture technique

Testing of antagonists against *Fusarium musae in vitro*

Table 2

Sr. No.	Treatments	Concentration
1	*Trichoderma viride* Pers, ex. Grey NAU isolate	1×10^8cfu/ml
2	*Trichoderma harzianum* Rifai. NAU isolate	1×10^8cfu/ml
3	*Trichoderma longibrachiatum* Rifai. NAU isolate	1×10^8cfu/ml
4	*Pseudomonas fluorescens* Migula NAU isolate	1×10^8cfu/ml
5	*Bacillus subtilis* Ell NAU isolate	1×10^8cfu/ml

In vivo evaluation of antagonists against *Fusarium musae*

Effective antagonists studied *in vitro* were used for further investigation to test their antagonism in controlling Fusarium fruit rot disease of banana following both pre- and post-inoculation methods.

Experimental details

Table 4

1.	Design	Completely Randomized Design (CRD)
2.	Treatments	4 (Four) (including control)
3.	Repetitions	4 (Four)

Testing of antagonists against *Fusarium musae in vivo*

Table 5

Sr. No.	Treatments	Concentration
1.	Trichoderma viride Pers, ex. Grey NAU isolate	1×10^8cfu/ml
2.	Trichoderma harzianum Rifai. NAU isolate	1×10^8cfu/ml
3.	Pseudomonas fluorescens Migula NAU isolate	1×10^8cfu/ml

Pre- inoculation of antagonists on banana fruit

The healthy, semi-matured uniform size of banana fruits were surface sterilized by dipping in 0.1 per cent NaOCl solution for one min. followed by three washings with distilled sterile water and inoculated separately with the pathogen by the stylar-end pricking method. The fruits were first inoculated with spore suspension (1 X 10^8cfu/ml) of seven days old culture of different antagonists separately and after 12hrs, the fruits were inoculated at the same site with spore suspension (10^6cfu/ml) of seven days old culture of test pathogens. Control was maintained separately with pathogen. The interval between antagonists treatment and inoculation was kept twelve hours. The severity of fruit rots was recorded on 4^{th} and 8^{th} day after inoculation with the help of assessment key.

Post-inoculation of antagonists on banana fruit

The procedure mentioned in above was followed except that the fruits were first inoculated with pathogen and then with antagonists.

Assessment key used for severity of fusarium fruit rot disease of banana

Table 6

Scale	Per cent infection
0	0%
1	1-10%
2	11-20%
3	21-40%
4	41-50%
5	> 60%

$$\text{Severity (\%)} = \frac{\text{Area of infected fruits}}{\text{Total area of fruit tissue}} \times 100$$

Results and Discussion

Effect of bio-agents on growth inhibition of *Fusarium musae* under *in vitro*

Five antagonists *viz.*, *Trichoderma viride*, *T. harzianum*, *T. longibrachiatum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated against *F. musae* by dual culture method. The observations on mycelium growth and per cent growth inhibition (PGI) recorded after eight days of incubation and the results obtained are presented in table 1. All the antagonists significantly inhibiting the mycelial growth of *F. musae.* Out of five bio-agents, highest per cent inhibition of mycelial growth of *F. musae* was found in *T. viride* with 77.52 per cent. The next best bio-agent in order of merit was *P. fluorescens* with 73.64 per cent followed by *T. harzianum* and *T. longibrachiatum i.e.,* 70.93 and 55.81 per cent inhibition mycelial growth of *F. musae*, respectively. Whereas, *B. subtilis* was found to be comparatively less effective and recorded minimum per cent inhibition of mycelial growth of *F. musae i.e.,* 47.29 per cent.

Thus, it shows that *T. viride* was found to be the more superior because it was observed lowest mycelial growth and highest per cent growth inhibition of *F. musae* as compared to rest of bio-agents. From this experiment, it is very clearly shown that the main mechanisms of action present in the antagonists are antibiosis, production of lytic enzymes, competence by nutrients and space, or the induction of resistance mechanisms. Bio-agents of *T. viride*, *T. harzianum* and *P. fluorescens* having different mode of action, *i.e.,* competition (pseudobactin, pyoverdins), lysis (chitinase, β-1-3 glucanase), antibiosis (gliotoxin, viridin, trichodermin and phenazine-l- carboxylic acid) and mycoparasitism (proteases, endochitinases, exochitinases) and which directly affects the growth of the pathogen.

These findings are conformities by Singh (2011) [7] screened five antagonists *viz*., *Trichoderma viride*, *T. harzianum*, *T. virens*, *Pseudomonas fluorescens* and *Bacillus subtilis* against *Fusarium moniliforme* infecting banana fruits by dual culture method. All the antagonists significantly helped in inhibiting the mycelial growth of *F. moniliforme* over control. Significantly highest mycelial growth inhibition was recorded in *T. harzianum* (53.06%) followed by *T. virens* (50.09%) and *T. viride* (49.72%) after 7 days of inoculation, while *P. fluorescens* gave (22.07%) inhibition of mycelial growth.

Effect of antagonists on growth inhibition of *Fusarium musae in vivo*

The effective *Trichoderma viride*, *T. harzianum* and *Pseudomonas fluorescens* studied *in vitro* were further tested at 1 × 10^8 cfu/ml. All the antagonists were found significantly superior in reducing the fusarium fruit rot severity at 4^{th} and 8^{th} day after inoculation in pre-and post-inoculation treatments (Table 2).

Pre-inoculation of antagonists on banana fruit

In case of pre-inoculation, *T. viride* was found superior and significantly reducing the fusarium fruit rot severity with 7.77 per cent on 4^{th} day and 11.77 per cent on 8^{th} day and it was at par with *P. fluorescens* recorded 8.80 per cent on 4^{th} day and 13.06 per cent on 8^{th} day after inoculation. However, *T. harzianum* found least effective in reducing the fusarium fruit rot severity with 10.08 per cent on 4^{th} day and 16.97 per cent on 8^{th} day.

Post- inoculation of antagonists on banana fruit

The trend similar to that observed in pre-inoculation was noted in post-inoculation treatment. *T. viride* was found superior and significantly reducing the fusarium fruit rot severity with 8.05 per cent on 4^{th} day and 11.43 per cent on 8^{th} day and it was at par with *P. fluorescens* recorded 9.19 per cent on 4^{th} day and 13.80 per cent on 8^{th} day after inoculation. However, *T. harzianum* found least effective in reducing the fusarium fruit rot severity with 10.19 per cent on 4^{th} day and 15.25 per cent on 8^{th} day. The results of present investigations corroborate with the results obtained by Filho *et al.* (2019) [3] evaluation different isolates of *Trichoderma* spp. *viz.*, T2, T5, T9 and T15 against *Colletotrichum musae* and observed that T9 isolate presented higher antagonism (60.06%), followed by T2 isolates (39.11%), T5 isolates (28.8%) and T15 isolates (26.02%). Mortuza and Ilag (1999) [5] observed that *Trichoderma harzianum* exhibited the highest inhibition in dual culture. *Trichoderma viride* reduced rotting by 29.07 to 65.06 per cent in artificially inoculated banana fruits before 4 hours with *L. theobromae* provided better protection than simultaneous application of treatment 4 hours after inoculation.

Table 7: Effect of antagonists on per cent growth inhibition of *Fusarium musae in vitro*

Sr. No.	Antagonists	Colony diameter (mm) 8 DAI	Per cent growth Inhibition
1	*Trichoderma viride*	3.87** (14.50)*	77.52
2	*Trichoderma harzianum*	4.38 (18.75)	70.93
3	*Trichoderma longibrachiatum*	5.38 (28.50)	55.81
4	*Pseudomonas fluorescens*	4.18 (17.00)	73.64
5	*Bacillus subtilis*	5.87 (34.00)	47.29
6	Absolute control	8.06 (64.50)	
	SEm ±	0.09	
	CD at 5 %	0.28	
	CV %	3.59	

*Figure in parenthesis is original value & **outside is square root transform value

DAI: Day after incubation

Table 8: Effect of antagonists on severity of fusarium fruit rot *in vivo*

Sr. No.	Antagonists	Fusarium fruit rot severity (%)			
		Pre-Inoculation		Post-Inoculation	
		4^{th} DAI	8^{th} DAI	4^{th} DAI	8^{th} DAI
1	*Trichoderma viride*	16.17** (07.77)*	20.05** (11.77)*	16.47** (08.05)*	19.75** (11.43)*
2	*Trichoderma harzianum*	18.50 (10.08)	24.32 (16.97)	18.57 (10.19)	22.95 (15.25)
3	*Pseudomonas fluorescens*	17.24 (08.80)	21.17 (13.06)	17.63 (09.19)	21.79 (13.80)

4	*Control*	22.08 (14.13)	30.78 (26.23)	22.14 (14.22)	30.43 (25.72)
	SEm ±	0.38	0.66	0.56	0.83
	CD at 5 %	1.17	2.02	1.73	2.54
	CV %	4.11	5.45	6.00	6.95

*Figure in parenthesis is original value & **outside is arcsine transform value
DAI: Day after incubation

Conclusions

Five antagonists *viz.*, *Trichoderma viride*, *T. harzianum*, *T. longibrachiatum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated *in vitro* against *F. musae* by dual culture technique. The highest per cent growth inhibition was found in *T. viride* with 77.52 per cent. The next best bio-agent in order of merit was *P. fluorescens* with 73.64 per cent under *in vitro*. *In vivo* condition, *T. viride* (11.77 & 11.43 %) was found most potent antagonist in reducing the severity and it was at par with *P. fluorescens* (13.06 & 13.80%) in pre- and post-inoculation treatments after 8 days of inoculation, respectively.

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