

BIO BLOCKERS FOR POSTHARVEST ROTS OF BANANA*

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ABSTRACT

Crown rot, anthracnose, and cigar end rot are major postharvest diseases of banana fruits. Fungal antagonists *Trichoderma harzianum* and *Trichoderma koningii* were investigated to control postharvest diseases of bananas. Conidial suspensions containing 10^9 cfu of *Trichoderma harzianum* ml⁻¹ significantly reduced the infestation level of crown rot, anthracnose and cigar end rot. Conidial suspension of *Trichoderma koningii* ml⁻¹ at 10^9 cfu effectively reduced the infestation level of anthracnose disease.

Keywords: Banana, postharvest diseases, crown rot, anthracnose, cigar end rot, bio blockers, *Trichoderma* spp.

INTRODUCTION

Banana is a climacteric fruit that gives off large amounts of ethylene during ripening. It is considered a short shelf-life commodity that undergoes a rapid senescence process immediately after its harvest (Basel *et al.*, 2002; Kleiber *et al.*, 2002). Postharvest diseases reduce the quality postharvest life. The most important of these postharvest diseases are anthracnose (*Colletotrichum musae*), cigar-end rot (*Trachysphaeria fructigena* and *Verticillium theobromae*), crown rot (*Ceratocystis paradoxa*, *C. musae*, *Fusarium pallidoroseum*, *Lasiodiplodia theobromae* and *V. theobromae*), finger rot (*L. theobromae*), Johnson spot (*Magnaporthe grisea*) and squirter disease (*Nigrospora sphaerica*) (Sholberg and Conway, 2004). Rot producing fungi *Lasiodiplodia theobromae*, *Colletotrichum musae*, *Fusarium moniliformae* and *Verticillium theobromae* have been reported from banana during transport and storage in Pakistan (Ilyas *et al.*, 2007). Mohapatra *et al.* (2010) have investigated many postharvest management practices, including delayed ripening, reduced respiration and disease control to increase the shelf life of banana during transport and storage and found promising results with the use of temperature to increase the shelf life. The antagonistic potential of *Trichoderma* sp. has been tested and found to be an effective measure of control of postharvest banana rots (Mortuza and Ilag, 1999).

In this study, we analyzed two biocontrol agents, *Trichoderma harzianum* and *Trichoderma koningii* to determine their antagonistic effects on postharvest banana rots. We used the spray method of conidial suspension on green mature banana at various time intervals. We show that both *Trichoderma harzianum* ml⁻¹ and *Trichoderma koningii* mL⁻¹ reduced the infestation level. Post harvest rot of banana. However, *Trichoderma harzianum* ml⁻¹ was more potent in lowering postharvest rot intensity than *T. koningii*.

MATERIALS AND METHODS

Surveyed marketplaces and storage godowns in Karachi to collect data on postharvest rots that affect banana fruit. Several samples of diseased fruits were collected. Rotted tissues were cut into 2-3 cm long pieces, surface sterilized with 2% Sodium hypochlorite (NaOCl₂) and placed on Petri plates containing Potato Dextrose Agar (PDA). The Petri plates were incubated at 28°C. After 5-7 days of incubation, fungi that populated the diseased tissue were isolated to obtain a pure culture. The fungal species were identified using the method of Barnett and Hunter (1998), Kulwant (1991), Nelson *et al.*, (1983) and Watanabe (2002).

Bio-control agents *Trichoderma harzianum* and *Trichoderma koningii* were tested against postharvest banana rots. Surface sterilized banana hands were inoculated with the isolated rot-producing fungi *Colletotrichum musae*, *Fusarium pallidoroesum*, *Lasiodiplodia theobromae* and *Verticillium theobromae* by plugging 5mm inoculum disc of fungal culture into crown and surface of banana fingers. Conidial suspensions of the biocontrol agents were prepared in sterile water by surface scratching of isolated culture; the number of conidia was adjusted to 10^4 , 10^6 and 10^9 conidia mL⁻¹ with the help of a haemocytometer. The inoculated hands were sprayed with the conidial suspensions. Fruits were assessed for postharvest rots on the 5th and 7th day after fungal spray until the fruits attained maturity, as indicated by their change in colour. The severity (%) of infection and disease severity grade were

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calculated as described below. The banana hands were tied in plastic bags and were incubated at room temperature. There were three replicates for each treatment.

Disease severity (%) and grade were calculated by using two disease indices. For Crown rot a scale of 0-5 disease grades was used where, 0= apparently healthy fruits, 1= Slight infection, 2= 25% of the crown/fruit infected, 3= 50% of the crown/fruit infected, 4= 100% of the crown/fruit infected, 5= Entire crown/fruit infected and progressing towards the pedicels (Frossard and Laville, 1973). For Anthracnose and Cigar end rot a disease index of 1-5 disease grades was used where, 1= 0% of fruit rotten, 2= 1-25% of fruit rotten, 3=26-50% of fruit rotten, 4= 51-75% of fruit rotten and 5= 76-100% of fruit rotten (Maqbool *et al.*, 2010). Factorial Analysis of Variance analyzed the data, and the means were separated by Duncan's Multiple Range Test (DMRT) ($P \leq 0.05$) by using Statistical Package for the Social Sciences (SPSS) Version 19 (SPSS Inc., USA).

RESULTS

The biocontrol agents *T. herzianum* and *T.koningii* were tested against banana rot diseases. The factorial ANOVA showed the significant effect of biocontrol agents, conidial suspensions, and incubation time. *Trichoderma herzianum* @ 10^9 showed a significant reduction in crown rot disease severity (%) and disease severity grade ($M = .54, .72$; $SD = 0.03, 0.04$; $P \leq 0.05$) on both the 5th and 7th day of storage (Table 1). There was no significant interaction between them. biocontrol agents, conidial suspensions, and incubation time suggesting that reduction in crown rot disease severity (%) and grade can be obtained with the increasing concentrations regardless of the time of application ($F(3, 32) = 5.24$; $P \leq 0.05$) (Table 2).

We found that *Trichoderma herzianum* @ 10^9 treatment led to a significant reduction in anthracnose disease severity (%) and disease severity grade ($M = 9.60, 13.44$; $SD = .6, .11$; $P \leq 0.05$) on both 5th and 7th day of storage than *Trichoderma koningii* (Table 3) spray, which was not as effective as *Trichoderma herzianum*. There was a significant interaction between conidial suspensions and incubation time, suggesting that increasing concentrations of conidial suspensions can considerably and quickly reduce the anthracnose disease severity (%) and grade ($F(3, 32) = 5.5$; $P \leq 0.05$) (Table 4).

We noted the significant effects of biocontrol agents, conidial suspensions, and incubation time as *Trichoderma herzianum* @ 10^9 significantly reduced cigar end rot disease severity (%) and disease severity grade ($M = 12.87, 16.73$; $SD = 0.551, 0.971$; $P \leq 0.05$) on both the 5th and 7th day of storage. However, the effect of *Trichoderma koningii* (Table 5) in reducing the cigar end rot was not very prominent. There was no significant interaction between biocontrol agents, conidial suspensions, and time, showing higher mean values, suggesting that reduction in disease severity (%) and grade can be obtained with increasing concentrations regardless of the time of application ($F(3, 32) = 4.98$; $P \leq 0.05$) (Table 6).

DISCUSSION

Bio-control agents are great alternatives to fungicides in managing postharvest diseases (Spotts and Sanderson, 1994). Considerable interests have been developed in antagonistic microorganisms because of their use in managing postharvest diseases. Biocontrol agents are environment-friendly and do not pose serious health hazards (Coates and Johnson, 1997). It is known that *Trichoderma spp.* produce many volatile and non-volatile compounds that inhibit the growth of pathogenic fungi (Dennis and Webster, 1971a; 1971b). In the present study, we tested two bio-control agents, *Trichoderma harzianum* and *Trichoderma koningii*, against a few major postharvest diseases of bananas. The results obtained confirmed the results proposed by Nath *et al.* (2015), Adebsin *et al.* (2009), Mortuza and Ilag (1999) and Sangeetha *et al.* (2009) in which they evaluated *Trichoderma spp.* as a bio blocker for postharvest rots of banana. It was found that bio blockers can be used as an alternative to traditional postharvest management practices. Using antagonists to control postharvest rots is environment-friendly, less hazardous, and has a greater potential for further investigation.

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Table 1. Mean values and Standard Deviation of the effect of *Trichoderma herzianum* and *Trichoderma koningii* on crown rot disease severity (%).

Biocontrol agents	Suspensions	Time	Mean	Std. Deviation
<i>Trichoderma herzianum</i>	10*4	5th Day	0.7900	0.03606
		7th Day	1.2067	.09452
		Total	.9983	.23702
	10*6	5th Day	.5567	.02082
		7th Day	.7767	.03786
		Total	.6667	.12356
	10*9	5th Day	.5433	.03055
		7th Day	.7267	.04163
		Total	.6350	.10559
	Control	5th Day	1.5100	.07937
		7th Day	2.4300	.13000
		Total	1.9700	.51303
	Total	5th Day	.8500	.41295
		7th Day	1.2850	.72110
		Total	1.0675	.61613
<i>Trichoderma koningii</i>	10*4	5th Day	.8667	.04509
		7th Day	1.2467	.05508
		Total	1.0567	.21295
	10*6	5th Day	.6533	.05033
		7th Day	.7733	.07506
		Total	.7133	.08710
	10*9	5th Day	.6333	.03215
		7th Day	.8333	.07024
		Total	.7333	.11994
	Control	5th Day	1.7067	.10693
		7th Day	2.9600	.12767
		Total	2.3333	.69451
	Total	5th Day	.9650	.46070
		7th Day	1.4533	.93120
		Total	1.2092	.76055
Total	10*4	5th Day	.8283	.05565
		7th Day	1.2267	.07257
		Total	1.0275	.21697
	10*6	5th Day	.6050	.06317
		7th Day	.7750	.05320
		Total	.6900	.10479
	10*9	5th Day	.5883	.05672
		7th Day	.7800	.07797
		Total	.6842	.11935
	Control	5th Day	1.6083	.13674
		7th Day	2.6950	.31233
		Total	2.1517	.61228
	Total	5th Day	.9075	.43187
		7th Day	1.3692	.81902
		Total	1.1383	.68844

Table 2. Factorial ANOVA for the effect of *Trichoderma herzianum* and *Trichoderma koningii* on crown rot disease severity (%).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	22.106 ^a	15	1.474	277.736	0.000	.992
Intercept	62.199	1	62.199	11721.750	0.000	.997
Biocontrol agents	.241	1	.241	45.387	0.000	.586
Suspensions	17.357	3	5.786	1090.336	0.000	.990
Time	2.558	1	2.558	482.004	0.000	.938
Biocontrol agents x Suspensions	.201	3	.067	12.623	0.000	.542
Biocontrol agents x Time	.009	1	.009	1.608	0.214	.048
Suspensions x Time	1.658	3	.553	104.142	0.000	.907
Biocontrol agents x Suspensions x Time	.084	3	.028	5.246	0.005	.330
Error	.170	32	.005			
Total	84.474	48				
Corrected Total	22.276	47				

a. R Squared = 0.992 (Adjusted R Squared = 0.989)

Table 3. Mean values and Standard Deviation of the effect of *Trichoderma herzianum* and *Trichoderma koningii* on anthracnose disease severity (%).

Biocontrol agents	suspensions	Time	Mean	Std. Deviation
<i>Trichoderma herzianum</i>	10*4	5th Day	11.30	.608
		7th day	15.40	.600
		Total	13.35	2.310
	10*6	5th Day	12.90	.265
		7th day	19.80	1.114
		Total	16.35	3.848
	10*9	5th Day	9.47	.611
		7th day	14.17	1.909
		Total	11.82	2.869
	control	5th Day	25.67	2.517
		7th day	52.00	6.245
		Total	38.83	15.039
	Total	5th Day	14.83	6.752
		7th day	25.34	16.470
		Total	20.09	13.429
<i>Trichoderma koningii</i>	10*4	5th Day	14.60	.600
		7th day	20.10	.854
		Total	17.35	3.084
	10*6	5th Day	13.33	.611
		7th day	17.33	1.815
		Total	15.33	2.503
	10*9	5th Day	11.03	.551
		7th day	16.57	1.358
		Total	13.80	3.169
	control	5th Day	36.33	3.512
		7th day	51.33	2.082
		Total	43.83	8.612
	Total	5th Day	18.83	10.756
		7th day	26.33	15.199
		Total	22.58	13.436
Total	10*4	5th Day	12.95	1.887
		7th day	17.75	2.658
		Total	15.35	3.333
	10*6	5th Day	13.12	.483
		7th day	18.57	1.908
		Total	15.84	3.140

	10*9	5th Day	10.25	1.003
		7th day	15.37	1.981
		Total	12.81	3.063
	control	5th Day	31.00	6.450
		7th day	51.67	4.179
		Total	41.33	11.972
	Total	5th Day	16.83	9.016
		7th day	25.84	15.507
		Total	21.33	13.348

Table 4. Factorial ANOVA for the effect of *Trichoderma herzianum* and *Trichoderma koningii* on anthracnose disease severity (%).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	8224.247 ^a	15	548.283	117.029	0.000	.982
Intercept	21845.333	1	21845.333	4662.825	0.000	.993
Biocontrol agents	74.501	1	74.501	15.902	0.000	.332
Conidial suspensions	6463.612	3	2154.537	459.880	0.000	.977
Time	973.801	1	973.801	207.855	0.000	.867
Biocontrol agents x Conidial suspensions	63.401	3	21.134	4.511	0.010	.297
Biocontrol agents x Time	27.000	1	27.000	5.763	0.022	.153
Conidial suspensions x Time	544.301	3	181.434	38.726	0.000	.784
Biocontrol agents x Conidial suspensions x Time	77.632	3	25.877	5.523	0.004	.341
Error	149.920	32	4.685			
Total	30219.500	48				
Corrected Total	8374.167	47				

a. R Squared = 0.982 (Adjusted R Squared = 0.974)

Table 5. Mean values and Standard Deviation of the effect of *Trichoderma herzianum* and *Trichoderma koningii* on cigar end rot disease severity (%).

Biocontrol agents	suspensions	Time	Mean	Std. Deviation
<i>Trichoderma herzianum</i>	10*4	5th Day	20.43	.839
		7th day	24.37	1.305
		Total	22.40	2.367
	10*6	5th Day	16.43	1.069
		7th day	19.13	1.501
		Total	17.78	1.883
	10*9	5th Day	12.87	.551
		7th day	16.73	.971
		Total	14.80	2.232
	control	5th Day	31.00	2.000
		7th day	46.00	7.000
		Total	38.50	9.418
	Total	5th Day	20.18	7.175
		7th day	26.56	12.472
		Total	23.37	10.470
<i>Trichoderma koningii</i>	10*4	5th Day	24.40	1.311
		7th day	28.67	3.215
		Total	26.53	3.207
	10*6	5th Day	19.13	1.332
		7th day	24.00	2.000
		Total	21.57	3.068
	10*9	5th Day	16.03	1.626
		7th day	20.67	1.332
		Total	18.35	2.865

	control	5th Day	37.33	2.082
		7th day	58.33	4.041
		Total	47.83	11.856
	Total	5th Day	24.22	8.611
		7th day	32.92	15.799
		Total	28.57	13.212
Total	10*4	5th Day	22.42	2.385
		7th day	26.52	3.219
		Total	24.47	3.447
	10*6	5th Day	17.78	1.831
		7th day	21.57	3.099
		Total	19.67	3.130
	10*9	5th Day	14.45	2.046
		7th day	18.70	2.393
		Total	16.58	3.071
	control	5th Day	34.17	3.920
		7th day	52.17	8.472
		Total	43.17	11.312
	Total	5th Day	22.20	8.021
		7th day	29.74	14.294
		Total	25.97	12.082

Table 6. Factorial ANOVA for the effect of *Trichoderma herzianum* and *Trichoderma koningii* on cigar end rot disease severity (%) and grade

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6654.479 ^a	15	443.632	68.940	0.000
Intercept	32375.241	1	32375.241	5031.117	0.000
Biocontrol agents	324.480	1	324.480	50.424	0.000
Conidial suspensions	5110.541	3	1703.514	264.726	0.000
Time	681.013	1	681.013	105.830	0.000
Biocontrol agents x Conidial suspensions	68.855	3	22.952	3.567	0.025
Biocontrol agents x Time	16.101	1	16.101	2.502	0.124
Conidial suspensions x Time	438.545	3	146.182	22.717	0.000
Biocontrol agents x Conidial suspensions x Time	14.944	3	4.981	0.774	0.517
Error	205.920	32	6.435		
Total	39235.640	48			
Corrected Total	6860.399	47			

a. R Squared = 0.970 (Adjusted R Squared = 0.956)

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