

ETIOLOGY AND ECOLOGY OF FUNGI CAUSING POSTHARVEST DISEASES OF BANANA FRUITS IN EGYPT

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Abstract

Survey of decayed banana fruits of Balady, Maghraby and Williams cultivars of local markets at Gharbeia Governorate, Egypt during 2013 and 2014 summer seasons, several postharvest diseases were observed *i.e.*, crown rot, finger rot, neck rot and flower end rot. Routine isolation of rotten samples of banana fruits yielded mainly three genera of fungi *i.e.*, *Fusarium moniliforme* J. Sheld, *Thielaviopsis paradoxa* (De Seynes) Höhn. and *Colletotricium musae* (Berk. & M.A. Curtis) Arx. Two infection types were recorded on different Cvs. of banana fruits. First infection type caused by single of each fungi, *Fusarium* spp., *C. musae* and *Thialoviopsis paradoxa*. Second infection type caused by *Fusarium* spp. + *C. musae* or *T. paradoxa*. Pathogenicity test indicated that of *C. musae* (No.1) was the most pathogenic fungi caused postharvest diseases on fruits of Balady Cv. followed by *F. moniliforme* (No.4). Pathological synergistic effect of *C. musae* (No.1) + *F. moniliforme* (No.4) on postharvest diseases on banana fruits was recorded. So, ecological relations between pathogenic fungi will be the most consideration of management postharvest diseases program on banana fruits.

Key words : Banana, fruit rot, fungi, postharvest, diseases.

Introduction

Banana (Musa spp. L.) is the fourth food crop after rice, wheat and maize in tropical and subtropical regions (Bakryet al., 2001). Post-harvest diseases destroy 10-30% of the total yield of crops during handling, transportation, storage and marketing (Agrios, 2005). In addition, pathogenic fungi are causing fruit rots and may also produce mycotoxins (Ocran et al., 2011). In Egypt, several reports on banana fruit rots caused by several pathogenic fungi i.e., Gloeosporium musarum (Colletotrichum musae) (Elarosi, 1960), F. oxysporum (Abo-El-Dahab and El-Goorani, 1969), Fusarium semitectum (Abd-Alla et al., 2014). Colletotrichum musae, Fusarium spp. and Thielaviopsis spp. are the major causal agents of crown rot, stem-end rot and blossom-end rot of banana fruits (Sangeetha et al., 2010), in Philippine (Alvindia, 2013), In Ethiopia (Alemu, 2014) and in Senegal (Diedhiou et al., 2014). There are little information of ecological relations between pathogenic fungi on postharvest diseases incidence on banana. In this repect, combination between F. moniliforme + Colletotrichum falcatum was increased stalks red rot disease incidence of sugar cane than each one (Biswas and Samajpti, 1991). The synergestic effect among., *Lasiodiplodia theobromae*, *F. proliferatum* and *C. musae* of crownrot disease incidence of banana fruit were observed than the single pathogen (Anthony *et al.*, 2004 and Niroshini and Karunaratne, 2009). This investigation aimed to survey of postharvest diseases of banana fruits, isolation, identification, pathological potential of causal organisms and their ecological relations between its on postharvest diseases incidence of banana fruits.

Materials and Methods

Survey of postharvest diseases of banana fruits

During 2013-2014 summer seasons, several postharvest diseases *i.e.*, crown rot, neck rot, finer rot and flower end rot were observed in banana fruits of Balady, Maghraby and Williams Cultivars local. In markets at El Gharbia Governorate, Egypt. Percentage of diseased fruits by each postharvest diseases were estimated as following formula:

Disease % = number of diseased banana fruits/total number of banana fruits \times 100

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Disease severity was calculated on linear scale from 0-4 according to percentage of rotten discoloration area of each postharvest diseases (Duamkhanmanee, 2008) with slight modification as follows:

0 = healthy fruit free rotten and discoloration

1 = 1-25% rotten and discoloration area

2 = 26-50% rotten and discoloration area

3 = 51-75% rotten and discoloration area

4 = 76-100% rotten and discoloration area

Isolation and identification of fungi associated with banana diseased fruits

Banana fruits with different rotting symptoms, softness and brown discoloration were collected from local markets at Gharbeia governorate, Egypt. Isolation of rotten tissues was carried cut by using the methods described by Gamagae et al. (2003). Pieces of fruit were surface sterilized with sodium hypochlorite 2% for 2 minutes then rinsed several times in sterile distilled water then dried between two layers of sterilized filter papers. Pieces of fruit were plated out on potato dextrose agar (PDA) supplemented with streptomycin sulfate for eliminationbacterial contamination. Plates were incubated at 25°C for 3-5 days and checked regularly from fungal growth development. Isolates of fungi obtained were purified and identified according to their cultural andmorphological characterization (Nelson et al., 1993 and Barnett and Hunter, 1998). Colonization and frequency of isolated fungi was recorded using the following formula:

Colonization % = number of infected pieces by fungi/ total No pieces tested \times 100

Frequency % = total fungal colonies / total fungal colonies of each cultivar \times 100

Pathogenicity test

Pathogenicity potential of pure cultures of isolated fungi *i.e.*, *C. musae*, *F. moniliformae*, *T. paradoxa*, *F. roseum* and *F. solani*were tested. Ten apparent ripe healthy fruits (Balady Cv.) were used as replicates for each fungal isolates tested. Fruits were surface sterilized with sodium hypochlorite (2%), for 2 minutes, then rinsed in sterilized distilled water and left to dry at room temperature. Each fruit wounded by small scratch, then inoculated by spore suspension of each fungal isolates (1×10⁶ spores/ml) from 7 days old cultures. Banana fruits were incubated at 28°C. Percentage of diseased fruits by each postharvest diseases and disease severity were estimated after 4 days as mentioned before according to Duamkhanmanee (2008).

Interaction between pathogenic fungi on postharvest diseases incidence of banana fruits

Spore suspension $(1 \times 10^6/\text{ml})$ of C. *musae* and *F. moniliforme* were prepared from 7 days old on PDA cultures. Banana fruits (Balady Cv.) were surface sterilized as mentioned before. Ten fruits were used of each treatment as replicates and ten fruits was served free infestation as a control. Banana fruits were infested by each single and combined isolates then incubated mentioned before as follows:

- Control (free treatment),

- C. musae. single isolate comprised.

- F. moniliforme. single isolate comprised.

- *C. musae* then *F. moniliforme* (after ore hour) at the same time .

- *F. moniliforme* then *C. musae* (after ore hour) at the same time .

- C. musae and F. moliniforme.

In combination postharvest diseases incidence of banana fruits were estimated as mentioned before.

Statistical analysis

Data were analysis of variance (ANOVA). Comparisons among means were made using Duncan's multiple range test at P = 0.05 according to Snedecor and Cocharn (1980).

Results

Survey of banana postharvest diseases

Survey of some Egyptian markets in El Gharbeia, Governorate, Egypt during summer seasons 2013 and 2014 on postharvest diseases of banana fruit *i.e.*, crown rot, neck rot, finger rot and flower end rot are the common on banana fruits *i.e.*, Balady, Maghraby and Williams cultivars as shown in fig. 1. Results presented in table 1 indicated that the high percentage of infected fruits and disease severity of crown rot, finger rot, neck rot and flower end rot were significantly on banana fruits of Balady followed by Williams cultivars. Meanwhile, Maghraby fruit cultivar recorded the lowest incidence of postharvest diseases on banana fruits.

Frequency of fungi associated with postharvest diseases on banana fruits

Results in table 2 indicated that the most fungi associated with postharvest diseases symptoms on banana fruits (Balady Cv.) *i.e.*, crown rot, neck rot, finger rot and flower end rot were *Fusarium* spp., *Colletotrichum musae*, *Penicillium* spp. and *Rhizopus stolonifer*. *Fusarium* spp. highly frequency of fungi associated with all postharvest diseases on banana fruits followed by *C*.

	eetion.									
	Postharvest diseases incidence of some banana fruits									
Cultivars	Crown rot		Neck rot		Finger rot		Flower end rot			
	%	D.S	%	D.S	%	D.S	%	D.S		
Balady	35.0a	2.0a	41.0a	2.0a	51.0a	3.0a	48.0a	2.0a		
Williams	23.0b	1.0b	25.0b	1.0b	41.0b	2.0b	45.0b	2.0a		
Maghraby	21.0c	1.0b	18.0c	1.0b	36.0c	2.0b	31.0c	2.0a		

Table 1 : Postharvest diseases incidence of banana fruits under natural fungus associated with first and second infection

Values in each column followed by the same letter are not significantly different at P≤0.05 according to Duncan's multiple rangtest.

Table 2 : Frequency of fungal associated with postharvest diseases on banana fruits (Cv. Balady).

Banana diseases	Frequency of fungi %						
	Crown rot	Neck rot	Finger rot	Flower end rot			
C. musae	26.7b	8.3b	8.3c	19.5b			
Fusarium spp.	43.3a	41.7a	66.7a	52.7a			
Penicillium spp	10.0d	41.7a	16.7b	19.5b			
Rhizopus spp.	20.0c	8.3b	8.3c	8.3c			

Values in each column followed by the same letter are not significantly different Weak fungi causing postharvest diseases of at $P \le 0.05$ according to Duncan's multiple range.

musae. Saprophytic fungi *i.e.*, Penicillium spp. and Rhizopus stolonifer were also, associated with all postharvest diseases of banana fruit. Penicillium spp. recorded high frequency like Fusarium with neck rot followed by finger rot the flower end rot .Meanwhile, the lowest frequency of Penicillium spp. are associated with crown rot. On the other hand, Rhizopus stolonifer was the lowest frequency of all postharvest diseases on banana fruits.

Fungal infection types of diseased banana fruit cultivars

Data in table 3 indicated that two infection types were observed on different banana cultivars *i.e.*, Balady, Maghraby and Williams are the common banana fruits in Egyptian markets. First infection type war recorded by single fungus i.e., Fusarium spp., Colletotrichum musae and T. paradoxa. Fusarium spp. caused highly infection on Balady followed by Maghraby then Williams cultivars. Colletotrichum musae recorded high infection on Williams cultivars, Meanwhile, T. paradoxa recorded moderate percentage of infection on Maghraby with no infection recorded on Balady and Williams cultivars . The second infection types was observed by Fusarium spp. + Colletotrichum musae of all fruits of banana cultivars with high percentage on Balady followed by William then Maghraby cultivars, respectively. In addation, Fusarium spp.+T. paradoxa were recorded on Maghraby and Williams only. In general, Fusarium spp. was the most

infection types of all postharvest diseases on tested banana fruit cultivars .

Pathogenicity test of isolated fungi on banana fruit Cv. Balady

Data in table 4 showed that all tested fungal isolates of T. paradoxa, F. moniliforme, F. roseum, F. solani and C. musae caused postharvest disease on fruit of banana Balady cultivar. The highest percentage of fungal infection caused by F. moniliforme isolate (No.4), crown rot (90.0%) and disease severity (4) followed by C. musae isolate (No.1) recorded (50%) of crown rot, disease severity (2) and neck rot (50%), disease severity (2), finger rot 50%, disease severity (2) and flower end rot) 50%, disease severity (2). Also, data in table 4 indicated that two isolates of Colletotrichum musae were causing flower end rot with no observation by another tested fungal isolates. banana fruits of Cv. Balady was T. paradoxa

causing neck rot and finger rot only, with no crown and flower end rot diseases. Two isolates of Fusarium roseum (No.6) and Fusarium solani (No.8) caused crown rot incidence (30 and 50%) respectively, with the same disease severity grade (2.0), no observation of flower end rot, finger end rot and neck rot diseases. Meanwhile, another isolate of F. solani (No. 7) recorded finer rot (2.0%) and disease severity (1.0) with no observation of crown rot, neck rot and flower end rot disease. In general, as shown in table 4 and fig. 2, Colletotrichum musae isolate (No.1) and F. moniliforme isolate (No.4) were the most fungal isolates that caused crown, neck, finger and flower end rots of banana fruits Cv. Balady.

Interaction between pathogenic fungi on postharvest incidence on banana (Cv. Balady)

Data in table 5 indicated that Colletotrichum musae isolate (No.1) and Fusarium moniliforme isolate (No.4) were tested individually and in combinations. Also, data in table 5 indicated that, all artificial infestation of banana fruits by each fungal isolate or in combination significantly caused postharvest diseases of banana fruits *i.e.*. crown, neck, finger and flower end rots than the control. Combination between two fungi had synergistic effect for incidence different postharvest diseases on banana fruits than the individual fungal infestation. Artificial infestation of banana fruit with Colletotrichum musae then Fusarium moniliforme recorded the high at postharvest incidence

Model infection	Fungal isolates	Frequency of fungal infection of banana fruits %					
	Tungar isolates	Balady	Maghraby	Williams			
	C. musea	11.40c	00.00e	48.20a			
First	Fusarium spp.	50.80a	29.60b	12.00d			
	T. paradoxa	00.00d	25.20c	00.00e			
Second	C. musae + Fusarium spp.	37.80b	11.30d	15.80c			
	Fusarium spp.+T. paradoxa	00.00d	33.90a	24.00b			

Table 3 : Fungal infection types of banana fruits cultivars.

Values in each column followed by the same letter are not significantly different at $P \le 0.05$ according to Duncan's multiple range test.

Table 4 : Pathogenicity test of fungal isolates on banana fruits of (Cv. Balady)

Fungi	Strain No.	Postharvest diseases incidence on banana fruits (Cv. Balady)							
		Crown rot		Neck rot		Finger rot		Flower end rot	
		%	D.S	%	D.S	%	D.S	%	D.S
Control	0	00.0 e	0.0 d	0.0 f	0.0 e	0.0f	0.0 c	0.0c	0.0b
C. musae	1	50.0b	2.0b	50.0c	2.0c	50.0a	2.0a	50.0a	2.0a
C. musae	2	30.0c	2.0 b	30.0 d	2.0c	30.0 c	2.0a	30.0b	2.0a
F. moniliforme	3	50.0b	2.0 b	70.0b	3.0 b	50.0a	2.0a	0.0c	0.0b
F. moniliforme	4	90.0a	4.0a	100.0 a	4.0a	20.0 d	1.0b	0.0c	0.0b
F. moniliforme	5	20.0 d	1.0c	20.0 e	1.0d	40.0b	2.0a	0.0c	0.0b
F. roseum	6	30.0 c	2.0b	0.0 f	0.0 e	0.0 f	0.0 c	0.0c	0.0b
F. solani	7	00.0 e	0.0 d	0.0 f	0.0 e	20.0d	1.0b	0.0c	0.0b
F. solani	8	50.0b	2.0b	0.0 f	0.0 e	0.0 f	0.0 c	0.0c	0.0b
T. paradoxa	9	00.0 e	0.0 d	20.0 e	1.0d	10.0 e	1.0b	0.0c	0.0b

Values in each column followed by the same letter are not significantly different at $P \le 0.05$ according to Duncan's multiple range test.

Table 5 : Interaction between fungi on postharvest diseases incidence on banana Cv. Balady 4 day after infestation.

	Postharvest diseases incidence on banana fruits (Cv. Balady)								
Fungi	Crown rot		Neck rot		Finger rot		Flower end rot		
	%	D.S	%	D.S	%	D.S	%	D.S	
Control	0.0d	0.0c	0.0b	0.0b	0.0 d	0.0d	0.0d	0.0d	
C. musae (C)	60.0c	3.0b	100.0a	4.0a	100.0a	40.0a	60.0a	3.0a	
F. moniliforme (F)	90.0b	4.0a	100.0a	4.0a	20.0c	1.0c	0.0d	0.0d	
C+F	100.0a	4.0a	100.0a	4.0a	60.0b	3.0b	40.0b	2.0b	
F+C	100.0a	4.0a	100.0a	4.0a	20.0c	1.0c	0.0d	0.0d	
C=F	100.0a	4.0a	100.0a	4.0a	60.0b	3.0b	20.0c	1.0c	

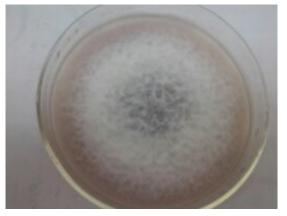
Values in each column followed by the same letter are not significantly different at $P \ge 0.05$ according to Duncan's multiple range test.

on banana fruits followed by infestation by two isolate eat the same time. Meanwhile, infestation banana fruits by *F. moniliforme* then *C. musae* recorded diseases incidence by crown rot, and neck rot and finger rot with no observation of flower end rot. In general, *F. moniliforme* encouraged *C. musae* for causing crown rot with higher of percentage (100%) and disease severity (4) and reducing incidence of finger rot and suppress incidence of flower end rot of banana fruits. Meanwhile, *Colletotrichum musae* encouraged *F. moniliforme* for causing finger rot and flower end rot in case of *Colletotrichum musae* was infested banana fruits one hour before *F. moniliforme* or at the same time.





Fig. 1 : Postharvest diseases on banana Balady Cv. under natural infection showing crown, finger and neck rots (left) compared with the healthy fruits (right).



F. moniliforme

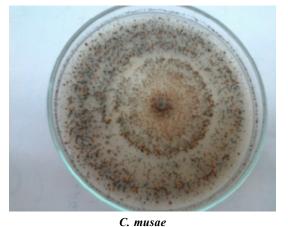


Fig. 2 : Cultures of highly aggressive fungi causing postharvest diseases of banana 10 days old on (PDA).

Discussion

Postharvest diseases of banana fruits *i.e.*, crown rot, neck rot, finger rot (Anthracnose) and flower end rot were the major diseases on famous banana cultivars in Egypt. Postharvest diseases caused high losses during storage, transportation and marketing before consumption worldwide, 10-30% of the total yield of crops and in some perishable crops especially in developing countries, they destroy more than 30% of the crop yield (Agrios, 2005). Postharvest diseases of banana fruits caused by several fungi C. musae, F. moniliforme, F. solani, F. roseum and T. paradoxa, F. moniliforme and C. musae were the most isolated fungi of banana cultivars i.e., Balady, Maghraby and Williams. F. moniliforme isolate (No.4) and C. musae isolate (No. 1) were the most pathogenic fungi that caused crown rot, neck rot, finger rot and flower end rot diseases on banana fruits(Cv. Balady). Meanwhile, F. solani, T. paradoxa and F. roseum were the lowest isolates that caused postharvest disease on banana fruits. These results

are in agreement with results obtained by in Egypt (Elarosi, 1960; Abo-El-Dahab and El-Goorani, 1969 and Abd-Alla et al., 2014). In Philippine (Alvindia, 2013). In Ethiopia (Alemu, 2014) and in Senegal (Diedhiou et al., 2014). Two types of fungal infection models were observed on fruits of Balady, Maghraby and Williams banana cultivars. First model of infection types were caused by each individual fungi Fusarium spp., C. musae and T. paradoxa. Highest occurrence of first infection type was recorded by Fusarium spp. on Balady followed by Maghraby then Williams cultivars. Meanwhile, C. musae recorded single infection with high significances on Williams cultivar, and low infection on (Balady Cv.) as well no single infection was recorded on Maghraby cultivar. On the other hand, T. paradoxa recorded single infection type with moderately occurrence on Maghraby Cv. with no observation Balady and Williams. Second infection type by two fungi were occurred mainly by Fusarium spp. + C. musae or T. paradoxa followed by

Fusarium spp. + C. musae or Fusarium spp. + T. paradoxa. The highest frequency of second infection type was Fusarium spp. + C. musae on fruits of Balady cultivar followed by Williams then Maghraby cultivars. Meanwhile, Fusarium spp. + T. paradoxa are recorded second model of infection type on Maghraby and Williams cultivars only, with no observation on Balady cultivar. These infection types of banana may be attributed to ecological relations among pathogenic fungi, natural host and environmental conditions under natural conditions. These results are in agreement with results reported that the synergistic on red rot disease incidence of sugar cane in combination between F. moliniforme + Colletotrichum falcatum (Biswas and Samajpti, 1991) and synergetic effect among Lasiodiplodia theobromae, Fusarium proliferatum and Colletotrichum musae on crown rot disease incidence of banana fruit (Anthon et al., 2004 and Niroshini and Karunaratne, 2009). So, ecological relations between pathogenic fungi will be the most consideration in management of postharvest diseases program on banana fruits.

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