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Pathological and new biotoxigenic characterization method for *Fusarium* species from banana fruits

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Abstract: There are 19 fungal isolates belong to *Fusarium* genera. The following *Fusarium* species were isolated: *F. semitectum* (5 isolates), *F. proliferatum* (3 isolates), *F. circinatum* (3 isolates), *F. chlamydosporum* (3 isolates), *F. solani* (2 isolates), *F. oxysporum* (2 isolates), *F. thapsinum* (1 isolates). In this respect *F. semitectium*, as the dominant cause of Fusarium rot in stored banana fruits is a typical wound parasite. Maximum growth rate was observed in *F. oxysporum* while the minimum growth rate of *F. chlamydosporum* isolate 2. Temperature × isolate was the most important factor in determining the variation in variation in temperature, *Fusarium* isolate, and their interaction on mycelial dry weight of *Fusarium*. Pathogenicity tests showed typical symptoms of Fusarium rot in most of the inoculated wounded banana fruits. **Nicotiana** seedlings inoculated with *Fusarium* isolates filtration was used to screen mycotoxin producing fungi. Young leaves was affected first and become small and distorted or chlorotic with irregular margins, spotting or necrotic areas.
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Key words: Banana; Mycotoxin; Fusarium, biotoxigenic assay

INTRODUCTION

Musa acuminate (Banana) is one of the most commonly produced and exported fruit in the world, second only to citrus fruit in value terms and the fourth most important export crop after wheat, rice and maize (Santos et al. 2005). Banana fruits affected by great number of fungal diseases (Jones 2000). Furthermore, banana fruits can also be affected by some post-harvest diseases such as 'Fusarium fruit rot' (Hirata et al. 2001; Moretti et al. 2004) and 'Fusarium crown rot', which is a major cause of ripe fruit losses in the producing and consuming countries (Stover, 1972; Ploetz et al. 1994; Ploetz, 2003, 2006; Abd-Elsalam, 2009). More than fifteen *Fusarium* species collected with banana in the world were reported (Ploetz, 2003; Ploetz, 2006; Leslie and Summerell, 2006; Abd-Elsalam, 2009; Li-Sha et al. 2013). Some *Fusarium* species inhabit banana fruit and produce mycotoxins (Ploetz, 2006). To date, there are only a few published articles on secondary metabolites produced by Fusaria in infested bananas fruits (Chakrabarti et al 1986, Vesonder et al. 1995; Jiménez et al. 1997; Hirata et al. 2001; Zakaria et al. 2012).

The most vital mycotoxin-producing *Fusarium* species (*Fusarium verticillioides* (*Gibberella moniliformis, G. fujikuroi* mating population A) has been isolated from bananas by some authors in distant locations such as India (Peshney and Ghaukar 1984), the Windward Islands (Wallbridge, 1981), and Panama, Ecuador, and the Canary Islands (Jiménez et al. 1993). The most important *Fusarium* toxins, in terms of plant health and productivity, are deoxynivalenol (DON), zearalenone (ZEN), fumonisins (FBs) and moniliformin (Vesonder et al. 1995). Morphological identification of *Fusarium* spp., which are typically observed in pure culture, is quite laborious and time-consuming. These method require the skills of a trained plant pathologist, and they are not always suitable for fungal species identification (Bluhm et al. 2002). The need for alternative and/ or complementary techniques for the rapid and accurate identification of toxigenic *Fusarium* species is therefore high, especially in the tropics countries where the species are abundant. Numerous *Fusarium* species found are mycotoxin producers, and, in some cases, an isolate can produce various toxic metabolites. Thus, the development a fast bioassay method for screening toxicogenic isolates is greatly valuable. The aim of the present study was to determine Fusarium species isolated from stored rotten banana fruits using morphological, pathological and new biotoxigenic characterization assays.

MATERIALS AND METHODS

Banana fruit samples

Banana fruits with typical symptoms of fruit rot disease collected from different markets of five locations (south, north, east, west, and center) in Riyadh city were subjected to pathogen isolation. Twenty samples were collected from every location, a minimum of one set of duplicates should be collected. Plant material consisted of commercially available banana fruit originating from Costa Rica, Ecuador, Brazil and Malaysia imported into Saudi Arabia.

Fusarium strains isolation and purification

Pieces from the internal walls of the fruit were surface sterilized via immersion in 2% NaOCl for 1 min, washed in sterile water, dried on sterilized paper, and plated onto potato dextrose agar (PDA). Petri plates were incubated at 25 °C in the dark until colonies developed. From each isolate a single spore culture was prepared and used for the morphological and pathological characterization (Nirenberg and O'Donnell 1998; Leslie and Summerell 2006). Fungal cultures were examined microscopically under low magnification (100-200X) to study morphological features of the aerial mycelia. When sporulation was observed in the cultures, agar blocks containing conidial structures were mounted on a microscopic slide with a drop of sterile water and examined at 400X. Fusarium cultures were maintain on PDA medium at 4°C and then stored as spore suspensions in 15% glycerol at -80°C.

Growth and morphological characteristics of fungi on various media

Nineteen *Fusarium* species isolates were inoculated (agar block containing fungi) in center of five fungal media such as PDA-Potato Dextrose Agar, MEA-Malt Extract Agar, CZA- Czapeks Dox Agar, SDA- Sabourauds Dextrose Agar, RBA- Rose Bengal Agar. The inoculated plates were incubated at room temperature (25ºC) for 7 days of incubation in 24-h intervals, replicated twice. After incubation period, the radial growth (diameter in mm) of each isolate was calculated (Palacios Cabrera et al., 2005).

Effect of pH on Fusarium growth

All the isolates were inoculated into Potato Dextrose Broth (PDB) broth containing different pH ranges (5, 6, 7, 8 and 9) and incubated at room temperature. After incubation for 7 days, the optical density was measured at 600 nm. The fungal fresh and dry weights were also determined.

Effect of temperature on Fusarium growth

All the fungi were inoculated into Potato Dextrose Broth (PDB) broth and the tubes were incubated at different temperature range (20, 30, 40, 50 and 60 °C). After incubation for 7 days, the optical density was recorded at 600 nm. The fungal fresh and dry weights mat were also measured.

Pathogenicity tests

Pathogenicity assay was conducted to confirm the virulence of Fusarium isolates on banana fruits. Healthy banana fruits were surface sterilized by ethanol 70% and washed in the sterile distilled water both for 2 min and then placed on filter paper for drying.

Four replicates for each of 19 selected isolates were used and were resub-cultured onto PDA and incubated at room temperature (25°C) for 7 days. Fungal conidia were collected using 20 mL sterile distilled water and 300 µL conidial solution was inoculated into the inner tissue of the banana using the sterile tips. The infected fruits were incubated for 7 days along with the uninoculated control fruits. The test was repeated twice. The fungus was subcultured on potato dextrose agar. The isolated fungal were re-identified based on their morphological characteristics according to Nirenberg and O'Donnell (1998). The disease severity index (DSI) were measured on a scale of 0 to 4 developed by Amadi et al. (2009) with a slight modification for banana fruits.

Mycotoxin bioassay using Nicotiana seedling

Fusarium isolates were inoculated on potato dextrose broth (PDB) and incubated for 3 weeks and conidiospores were harvested as previously reported by Hilton et al. (1999). Nicotiana seedlings were inoculated by placing 10 µl of conidial suspension (containing 10^8 conidia) on each emerging seedling leaf. Three leaves per seedling were inoculated. Control plants were treated with 10 µl of sterile distilled water. Inoculated plants were incubated in a moist chamber for 72 h and kept in growth chamber at 21°C (day) / 18°C (night) temperatures with day/night regime of 16h/8h.

Results

Fusarium isolated species from post-harvest diseases of banana fruits

Since we identified 19 Fusarium isolates from rotten banana fruit belonging to eight species. The ability of *Fusarium* species isolated from bananas to produce mycotoxins was studied with 19 isolates of the following species: *F. semitectum* (5 isolates), *F. proliferatum* (3 isolates), *F. circinatum* (3 isolates), *F. chlamydosporum* (3 isolates), *F. solani* (2 isolates), *F. oxysporum* (2 isolates), *F. thapsinum* (1 isolates). In this respect *F. semitectium*, as the dominant cause of Fusarium rot in stored banana fruits is a typical wound parasite (Table 1).

Growth and morphological characteristics of fungi on various media

Maximum growth rate was observed in *F. oxysporum* isolate 7 with 84 mm in PDA, 81 mm in diam (MEA), 77 in SDA than other media used. The minimum growth rate of *F. chlamydosporum* isolate 2 (36 and 38 mm in diameter) was observed in MEA and SDA agar plates, respectively (Table 1). Colony morphology of *F. oxysporum* on PDA varies widely. Mycelia may be floccose, sparse or abundant and range in color from white to pale violet (Fig. 1. 1-A). Fusarium cultures usually were grow quickly and produce abundant dense aerial mycelia that initially is off white and becomes beige or brown with age. Brown pigments also may be produced in the agar (Fig. 1. 2-A). Cultures of *F. solani* usually were white to cream with sparse mycelium. Sporodochia often were produced in abundance and may be cream, blue or green (Fig. 1. 3-A). White mycelium, but may produce violet pigment in the agar. Grows relatively rapidly (Fig. 1. 4-A).

Effect of temperature on the fresh and dry weight of different *Fusarium* species isolates

Colony growth of 19 isolates of eight *Fusarium* species was studied at five different

temperature viz. 20°, 30°, 40°, 50°, and 60°C. In this study, the maximum growth was observed in temperature range of 30ºC after 7 days of incubation (Table 2). In this temperature study, two *F. chlamydosporum* isolates showed maximum fresh weights of the fungi growth with 1.40, 1.70 mg/g respectively. Of all the tested species, *F. semitectum* isolate number 3was the slowest-growing species at both temperatures with 0.55 mg/g fresh weight. *F. chlamydosporum* isolate 2 showed maximum dry weights of the fungi growth with 1.240 mg/g respectively. Of all the tested species, *F. semitectum* isolate number 3 was the slowest-growing species at both temperatures with 0.400 mg/g dry weight (Table 3).

Relative contribution of temperature, isolate, and their interaction to variation in mycelial dry weight of *Fusarium*

Relative contribution of each source of variation to variation in temperature, *Fusarium* isolates, and their interaction on mycelial dry weight of *Fusarium* is shown in figure 2. Temperature × isolate was the most important factor in determining the variation in variation in temperature, *Fusarium* isolate, and their interaction on mycelial dry weight of *Fusarium*. It accounted for 53.00% of the explained (model), temperature was the second in importance as a source of variation in temperature, *Fusarium* isolates, and their interaction on mycelial dry weight of *Fusarium*. It accounted for 34.35%.

Pathogenicity test

In the virulent test, a total of 76 banana fruits were inoculated with 19 isolates of the *Fusarium* species (Table 4) with the control fruits. All the inoculated and non-inoculated fruits were observed from day after inoculation (day) 1 to 7 every 24h. on day 1, there was no growth either of necrosis or mycelia on the epicarp of the majority of banana fruits (0% DSI) except in 11 isolates with 7 - 8% DSI. On day 2 and 3, there were growth and development of necrosis on most of the fruits (10 isolates with 26 - 36% DSI) with few exception associated with white mycelia of fungal (3 isolates with 30 - 37% DSI). Moderate infection dominated majority of the inoculated banana fruits (19 isolates with 42 - 62% DSI) and others in few instances with spore mass appeared (5 isolates with 60 - 65% DSI) on day 5. Severe infection covered almost half of the fruits (18 isolates with 69 - 80% DSI) and few others very devastating in which their necrotic tissues were soft and decay with fungal mass appeared on day 6. The non-inoculated controls showed no symptoms of fruit rot from day 1 to 7 (Table 4).

Mycotoxin bioassay using *Nicotiana* seedling

The filtration of liquid culture media for *F. proliferatum* isolate 3 and *F. circinatum* was used to screen Zearalenone and fumonisin producing fungi respectively. Young leaves was affected first and become small and distorted or chlorotic with irregular margins, spotting or necrotic areas. The initial symptoms were the yellowing of the entire leaf including veins usually starting with the younger leaves. Leaf tips may yellow and curl downward. Leaf size was reduced and overall growth will be stunted. Leaves yellowing or distortion of leaf shape (**Fig. 2**).

Table 1. Growth and morphological characteristics of *Fusarium* species isolated from banana fruits on five cultural media

Isolates number	Isolate identification	Cultural media					Mean diam
		PDA	MEA	CZA	SDA	RBA	
1	*F. chlamydosporum*	43	46	49	37	34	48.8
2	*F. chlamydosporum*	48	36	55	38	45	**44.4**
3	*F. circinatum*	53	46	58	62	60	55.8
4	*F. circinatum*	55	49	60	60	74	59.6
5	*F. circinatum*	77	25	38	49	52	48.2
6	*F. oxysporum*	86	79	56	68	49	67.6
7	*F. oxysporum*	84	81	45	77	69	**71.2**
8	*F. semitectum*	65	44	67	75	82	66.6
9	*F. semitectum*	57	55	74	62	56	60.8
10	*F. semitectum*	63	48	69	66	78	64.8
11	*F. semitectum*	48	56	64	62	68	59.6
12	*F. semitectum*	66	56	60	59	58	59.8
13	*F. solani*	55	78	46	36	59	49.4
14	*F. solani*	63	57	43	65	61	57.8
15	*F. thapsinum*	53	48	38	56	49	48.8
16	*F .proliferatum*	78	57	77	63	48	64.6
17	*F. proliferatum*	68	45	48	35	49	49.0
18	*F. proliferatum*	55	39	65	68	70	59.4
19	*Fusarium* spp*	78	77	65	52	68	68.0

PDA-Potato Dextrose Agar, **MEA**-Malt Extract Agar, **CZA**- Czapeks Dox Agar, **SDA**- Sabourauds Dextrose Agar, **RBA**- Rose Bengal Agar. The values are represented in mm in diameter.

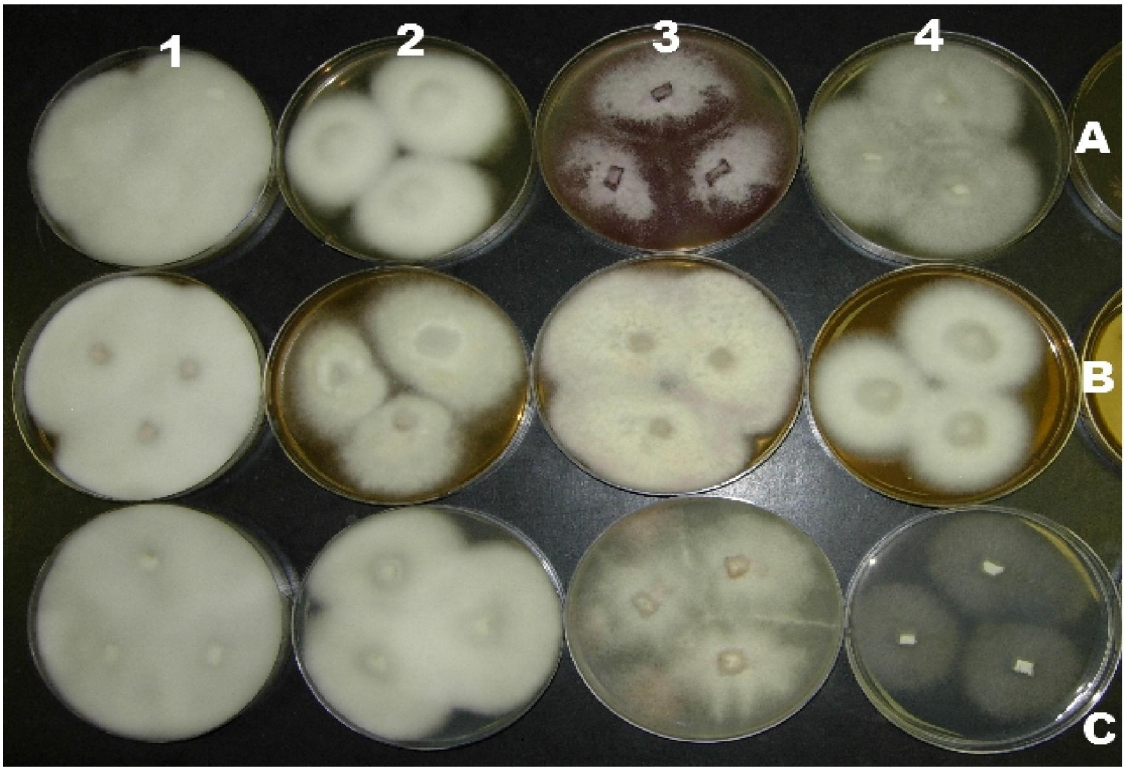

Figure 1. Four *Fusarium* species including 1) *F. oxysporum*, 2) *F. semitectium* 3) *F. solani* 4) *F. circinatum* were cultured on **A**=PDA-Potato Dextrose Agar, **B**= MEA-Malt Extract Agar, **C**= CZA- Czapeks Dox Agar,

Table 2. Effect of temperature on fresh weight of *Fusarium* species isolated from banana fruits

Isolates number	Isolate identification	Temperature					Mean
		20°C	30°C	40°C	50°C	60 °C	
1	*F. chlamydosporum*	0.83	1.40	1.12	0.92	0.58	**0.916**
2	*F. chlamydosporum*	0.90	1.70	1.55	1.00	1.10	**1.250**
3	*F. circinatum*	0.65	0.93	0.96	0.77	0.59	0.780
4	*F. circinatum*	0.89	0.92	0.90	0.89	0.67	0.900
5	*F. circinatum*	0.92	0.99	0.85	0.80	0.62	0.800
6	*F. oxysporum*	0.55	0.89	1.20	1.00	0.97	0.922
7	*F. oxysporum*	0.78	0.93	1.11	0.99	0.83	0.928
8	*F. semitectum*	0.56	0.68	0.78	0.45	0.37	0.568
9	*F. semitectum*	0.67	0.73	0.81	0.49	0.42	0.624
10	*F. semitectum*	0.49	0.55	0.67	0.44	0.40	**0.510**
11	*F. semitectum*	0.77	0.85	0.63	0.52	0.49	0.652
12	*F. semitectum*	0.85	0.94	0.77	0.45	0.40	0.682
13	*F. solani*	0.90	1.20	0.89	0.55	0.52	0.812
14	*F. solani*	0.79	0.84	0.88	0.79	0.66	0.792
15	*F. thapsinum*	0.39	0.68	0.97	0.75	0.57	0.672
16	*F .proliferatum*	0.77	0.91	1.33	0.88	0.50	0.878
17	*F. proliferatum*	0.89	0.95	0.98	0.76	0.63	0.842
18	*F. proliferatum*	0.89	0.92	0.90	0.89	0.67	**0.900**
19	*Fusarium* spp*	0.75	1.30	1.10	0.89	0.72	0.952

The values are represented in mg/g.

Table 3. Effect of temperature on dry weight of *Fusarium* species isolated from banana fruits

Isolates number	Isolate identification	Temperature					Mean
		20°C	30°C	40°C	50°C	60 °C	
1	*F. chlamydosporum*	0.63	1.00	0.92	0.62	0.38	0.710
2	*F. chlamydosporum*	0.63	1.24	1.15	0.70	0.80	**0.904**
3	*F. circinatum*	0.45	0.63	0.78	0.63	0.39	0.576
4	*F. circinatum*	0.62	0.73	0.69	0.53	0.42	0.598
5	*F. circinatum*	0.72	0.79	0.54	0.70	0.41	0.632
6	*F. oxysporum*	0.32	0.68	0.89	0.77	0.80	0.692
7	*F. oxysporum*	0.62	0.63	0.77	0.74	0.61	0.694
8	*F. semitectum*	0.36	0.46	0.63	0.25	0.17	0.374
9	*F. semitectum*	0.42	0.51	0.62	0.30	0.22	0.414
10	*F. semitectum*	0.30	0.40	0.49	0.31	0.25	**0.350**
11	*F. semitectum*	0.55	0.69	0.49	0.34	0.37	0.488
12	*F. semitectum*	0.70	0.77	0.63	0.34	0.28	0.544
13	*F. solani*	0.79	0.90	0.72	0.48	0.37	0.652
14	*F. solani*	0.62	0.71	0.62	0.59	0.47	0.602
15	*F. thapsinum*	0.28	0.43	0.78	0.59	0.46	0.508
16	*F .proliferatum*	0.60	0.79	1.10	0.68	0.31	0.696
17	*F. proliferatum*	0.64	0.65	0.68	0.62	0.47	0.612
18	*F. proliferatum*	0.72	0.67	0.71	0.66	0.44	0.640
19	*Fusarium* spp*	0.61	0.97	0.88	0.64	0.51	0.722

The values are represented in mg/g.

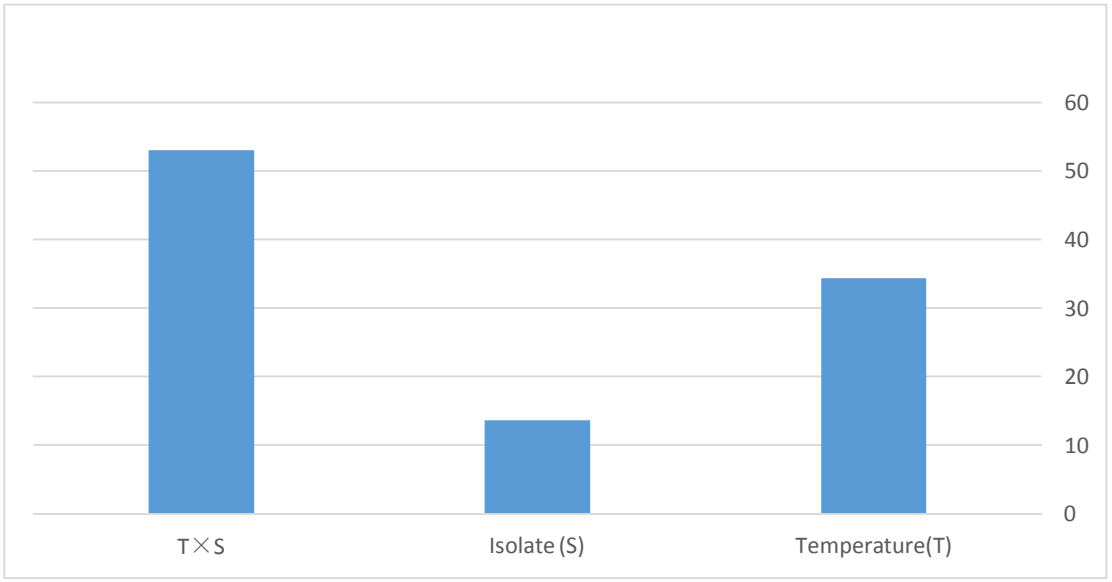

Figure 2. Relative contribution of temperature, *Fusarium* isolate, and their interaction on mycelial dry weight of *Fusarium* grown on potato dextrose broth medium for 7 days at five different temperature.

Table 4. Banana fruit rot disease severity index (DSI) for day 1 – 7.

Isolate number	*Fusarium* species	Disease severity index (DSI%) for each day						
		1	2	3	4	5	6	7
1	*F. chlamydosporum*	0.00	24.00	24.00	46.00	48.00	65.00	75.00
2	*F. chlamydosporum*	0.00	27.00	31.00	51.00	55.00	67.00	77.00
3	*F. circinatum*	0.00	25.00	25.00	50.00	52.00	72.00	80.00
4	*F. circinatum*	0.00	24.00	24.00	50.00	50.00	69.00	77.00
5	*F. circinatum*	0.00	28.00	33.00	42.00	48.00	59.00	67.00
6	*F. oxysporum*	0.00	29.00	29.00	44.00	49.00	62.00	70.00
7	*F. oxysporum*	0.00	23.50	23.50	50.00	56.00	77.00	79.00
8	*F. semitectum*	0.00	20.00	20.00	50.00	57.00	73.00	77.00
9	*F. semitectum*	0.00	18.00	18.00	40.00	44.00	71.00	78.00
10	*F. semitectum*	0.00	26.00	26.00	50.00	50.00	69.00	75.00
11	*F. semitectum*	7.20	33.50	37.00	62.00	65.00	80.00	82.00
12	*F. semitectum*	0.00	24.00	24.00	50.00	60.00	71.00	81.00
13	*F. solani*	0.00	25.00	25.00	50.00	54.00	70.00	76.00
14	*F. solani*	5.59	36.00	39.00	59.00	62.00	69.00	73.00
15	*F. thapsinum*	0.00	24.00	24.00	55.00	55.00	75.00	75.00
16	*F .proliferatum*	0.00	28.00	28.00	47.00	50.00	76.00	80.00
17	*F. proliferatum*	0.00	23.00	23.00	50.00	50.00	71.00	74.00
18	*F. proliferatum*	0.00	29.00	34.00	50.00	54.00	75.00	79.00
19	*Fusarium* spp*	0.00	24.00	27.00	49.00	60.00	80.00	82.00

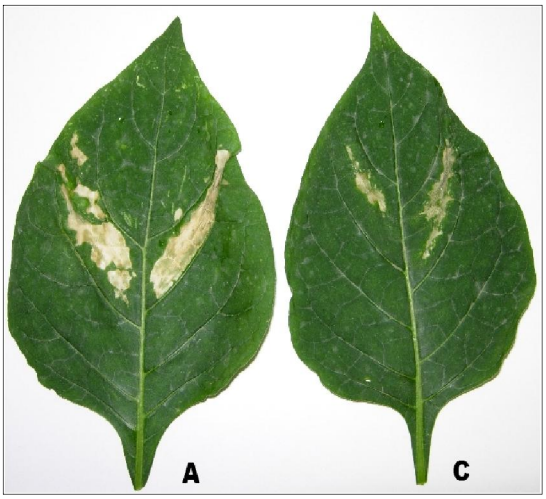

Figure 3. Mycotoxin-induced lesion formation in **Nicotiana** seedlings, the leaves infected with *F. circinatum* isolate 3 have abnormal colors (A). Leafs inoculated with water (C).

Table 5. Biotoxigenic assay using Nicotiana seedlings for nineteen isolates of Fusarium which collocted from banana fruits imported into Saudi Arabia market/2013.

Isolates code	*Fusarium* species	biotoxigenic assay		
		Fumonisin concentration (ppb)	Zearalenone concentration (ppb)	Deoxynivalenol concentration (ppb)
1	*F. chlamydosporum*	■	■	■
2	*F. chlamydosporum*	■	■	■
3	*F. circinatum*	■	■	■
4	*F. circinatum*	■	■	■
5	*F. circinatum*	■	■	■
6	*F. oxysporum*	□	□	□
7	*F. oxysporum*	□	□	□
8	*F. semitectum*	■	■	■
9	*F. semitectum*	■	■	■
10	*F. semitectum*	■	■	■
11	*F. semitectum*	■	■	■
12	*F. semitectum*	■	■	■
13	*F. solani*	■	■	■
14	*F. solani*	■	■	■
15	*F. thapsinum*	■	■	■
16	*F .proliferatum*	■	■	■
17	*F. proliferatum*	■	■	■
18	*F. proliferatum*	■	■	■
19	*Fusarium* spp*	■	■	■

Toxigenic isolate (■) non toxigenic isolate (□)

Discussion

Some *Fusarium* species can cause banana fruit rot while stored. Fusarium diseases are a major cause of yield loss in many perennial crops such as banana (Mirete et al 2003). The most serious disease affecting this fruit is the so-called "rotten banana" The aim of this study was to identify *Fusarium* species from rotten bananas, based on combined morphological characteristics and pathological data. The following Fusarium species were isolated: *F. semitectum* (5 isolates), *F. proliferatum* (3 isolates), *F. circinatum* (3 isolates), *F. chlamydosporum* (3 isolates), *F. solani* (2 isolates), *F. oxysporum* (2 isolates), *F. thapsinum* (1 isolates). In this respect *Fusarium semitectium*, as the dominant cause of Fusarium rot in stored banana fruits is a typical wound parasite.

Our results showed that, *F. semitectum* cultures usually were grow rapidly and produce abundant dense aerial mycelia that initially is off white and becomes beige or brown with age. Brown pigments also may be produced in the agar. While, white mycelium, but may produce violet pigment in the agar and growing relatively rapidly by *F. citrinum*. *F. thapsinum* was formed abundant white mycelium on PDA media which may darken (violet pigments) with age.

Temperature × isolate was the most important factor in determining the variation in variation in temperature, *Fusarium* isolate, and their interaction on mycelial dry weight of *Fusarium*. It accounted for 53.00% of the explained (model), temperature was the second in importance as a source of variation in temperature, *Fusarium* isolates, and their interaction on mycelial dry weight of *Fusarium*. It accounted for 34.35%.

The pathogenicity of 19 *Fusarium* species isolates was also confirmed on commercial banana fruits. Many dark brownish spots were observed on the epidermis. Whitish mycelia were also frequently observed on the larger spots. Some larger brown spots, especially on the wounded parts, had whitish mycelial colonies and were often surrounded by halos. Severe infection covered almost half of the fruits (18 isolates with 69 - 80% DSI) and few others very devastating in which their necrotic tissues were soft and decay with fungal mass appeared on day 6. The non-inoculated controls showed no symptoms of fruit rot from day 1 to 7. To date, pathogenicity tests have not been performed with *F. concentricum* and *F. musarum*, and although *F. camptoceras* has been recovered from decayed bananas, it is a presumed saprophyte (Leslie and Summerell 2006).

The pathogenicity of *F. thapsinum* isolate was studied by inoculation of banana fruits; both isolates were pathogenic to wounded fruits. Inoculations of banana fruits with *F. thapsinum* produced a soft rotten circular area with brown colour. *F. verticillioides* and *F. oxysporum* are serious pathogens affecting only wounded fruits (Alvindia *et al.* 2000). This is the first reported occurrence of *F. thapsinum* from commercial banana imported into Saudi Arabia. The degree of pathogenicity is dependent on growth stage (Tarekegn *et al.* 2004) and genetic background of the host (Tesso *et al.* 2004). *F. thapsinum* can show significantly reduced germination (Prom *et al.* 2003). *Fusarium* spp. have been isolated from bananas by several authors in different countries such as India (Peshney *et al.* 1984), the Windward Islands (Wallbridge 1980), Panama, Ecuador and the Canary Islands (Jiménez *et al.* 1993; Jiménez *et al.* 1997), and Mexico (Hirata *et al.* 2001). Seven strains of *Fusarium* isolated from rotten banana fruits imported into Japan from Mexico were identified as *F. verticillioides* based on morphological and molecular characterization (Hirata *et al.* 2001). *F. proliferatum* was isolated from banana samples collected from 12 localities in Sri Lanka (Anthony *et al.* 2004). Phytopathogenic fungi produce a wide range of phytotoxic compounds, such as AAL-toxin and fumonisin B1 (Stone et al. 2000). Disease symptoms often result from the effects of these fungal toxins. Moniliformine, which is toxic towards tobacco plants (Cole et al 1973). Thus, detection tools for multi-mycotoxins analysis that allow for the evaluation of the toxigenic potential of *Fusarium* spp. growing in and on bananas is greatly valuable.

In conclusion *F. semitectum*, followed by *F. proliferatum*, *F. circinatum*, and *F. chlamydosporum* are potential major pathogens of banana fruits. *F. semitectium*, as the predominant cause of Fusarium rot in stored banana fruits is a distinctive wound parasite. Based on biotoxigenic assay, Fusarium isolates were more prevalent than non-toxigenic isolates, only two *F. oxysporum* isolates were found to be non-toxigenic.

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REFERENCES

[1]. ABD-ELSALAM KA (2009). First report of *Fusarium thapsinum* on imported banana fruits into Saudi Arabia. Pest Technol. 3:25–27.

[2]. ALVINDIA DG, KOBAYASHI T, YAGUCHI Y. AND NATSUAKI KT (2000). Symptoms and the associated fungi of post-harvest diseases on non-chemical bananas imported from the Philippines. Jpn. J. Trop. Agric., 44:87–93.

[3]. AMADI JE, ADEBOLA, MO AND EZE CS (2009). Isolation and identification of a bacterial blotch organism from watermelon

(*Citrulluslanatus* (Thunb.) Matsum and Nakai). Afr. J. Agric. Res. 4(11): 1291-1294

[4]. ANTHONY S, ABEYWICKRAMA K, DAYANANDA R, WIJERATNAM SW, ARAMBEWELA L 2004. Fungal pathogens associated with banana fruit in Sri Lanka, and their treatment with essential oils. Mycopathologia 157: 91-97.

[5]. BLUHM BH, FLAHERTY JE, COUSIN MA AND WOLOSHUK CP (2002). Multiplex polymerase chain reaction assay for the differential detection of trichothecene and fumonisin-producing species of *Fusarium* in cornmeal. J. Food Prot. 65: 1955–1961.

[6]. CHAKRABARTI DK, SHIBNATH-GHOSAL AND GHOSAL S. (1986). Occurrence offree and conjugated 12,13-epoxytrichothecenes and zearalenone in banana fruits infected with Fusarium moniliforme. Appl. Environ. Microbiol. 51: 217-219.

[7]. COLE RJ, KIRKSEY JW, CUTLER HG, DOUPNIK BL AND PECKHAM JC, (1973). Toxin from *Fusarium moniliforme*: effects on plants and animals. Science 179 (4080), 1324-1326.

[8]. HILTON AJ, JENKINSON P, HOLLINS TW AND PARRY DW (1999). Relationship between cultivar height and severity of Fusarium ear blight in wheat. Plant Pathol. 48:202-208.

[9]. HIRATA T, KIMISHIMA E, AOKI T, NIRENBERG HI, O'DONNELL K (2001). Morphological and molecular characterization of *Fusarium verticillioides* from rotten banana imported into Japan. Mycoscience 42, 155-166.

[10]. JIMÉNEZ M., HUERTA T., AND MATEO R. (1997). Mycotoxin production by *Fusarium* species isolated from Bananas. *Applied and Environmental Microbiology* 63, 364-369.

[11]. JIMÉNEZ M, A. LOGRIECO, AND BOTTALICO A.(1993). Occurrence and pathogenicity of *Fusarium* species in banana fruits. J. Phytopathol. 137:214–220.

[12]. JONES DR (2000) 'Diseases of Banana, Abaca and Enset.' (CAB International: Wallingford, Oxon, UK).

[13]. LESLIE JF, AND SUMMERELL, BA (2006). The Fusarium Lab Manual. Blackwell, Ames, IA.

[14]. LI-SHA Z, ZHAO ZHI-HUI, LÜ SHUN, XI ZHUO-JUN, LI MIN-HUI, XI PING-GEN, JIANG ZI-DE (2013). The Fusarium species isolated from banana and their phylogenetic relationships. Mycosystema 32: 617-632

MIRETE S, VÁZQUEZ C, MULÈ G, JURADO M, AND GONZÁLEZ-JAÉN MT (2004). Differentiation of *Fusarium verticillioides* from banana fruits by IGS and EF-1 alpha sequence analyses. Eur J Plant Pathol 110:515–523.

[15]. MORETTI A, MULE G, SUSCA A, GONZA´LEZ-JAE´N M.T. AND LOGRIECO A (2004) Toxin profile, fertility and AFLP analysis of *Fusarium verticillioides* from banana fruits. Eur. J. Plant Pathol. 110:601–609.

[16]. O'DONNELL K, CIGELNIK E AND NIRENBERG HI (1998) Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. Mycologia 90: 465–493.

[17]. PALACIOS-CEBRERA H, TANIEAKI MH, HASHIMOTO TM, AND MENEZERA HC (2005). Growth of *Aspergillus ochraceus, A. carbonarius* and *A. niger* on culture media at different water activities, temperature. Braz. J. Microbiol. 36(1): 67-71.

[18]. PESHNEY NL, AND GHAUKAR KB (1984). Black heart of banana caused by *Fusarium moniliforme*. Indian Phytopathol. 37:682–683.

[19]. PLOETZ RC (2003). Diseases of tropical fruit crops. CABI Publishing,Wallingford, UK. 1-519.

[20]. PLOETZ RC (2006). *Fusarium*-induced diseases of tropical, perennial crops. Phytopathology 96:648-652.

[21]. PROM LK., WANISKA RD, KOLLO AI, AND ROONEY WL (2003). Response of eight sorghum cultivars inoculated with *Fusarium thapsinum*, *Curvularia lunata*, and a mixture of the two fungi. Crop Prot. 22:623-628.

[22]. SANTOS, CMR, MARTINS NF, HöRBERG HM, DE ALMEIDA ERP, COELHO MCF, TOGAWA RC, DA SILVA FR, CAETANO AR, MILLER RNG, SOUZA MT (2005) Analysis of expressed sequence tags from *Musa acuminata* sp. *burmannicoides*, var. *Calcutta* 4 (AA) leaves submitted to temperature stresses. Theoretical Appl. Genet. 110, 1517-1522.

[23]. SANTOS, CMR, MARTINS NF, HöRBERG HM, DE ALMEIDA ERP, COELHO MCF, TOGAWA RC, DA SILVA FR, CAETANO AR, MILLER RNG, AND SOUZA MT (2005). Analysis of expressed sequence tags from *Musa acuminata* sp. *burmannicoides*, var. *Calcutta* 4 (AA) leaves submitted to temperature stresses. Theoretical Appl. Genet. 110: 1517-1522.

[24]. STONE JM, HEARD JE, ASAI T, AND AUSUBEL, FM (2000). Simulation of fungal-mediated cell death by fumonisin B1 and selection of fumonisin B1-resistant (fbr) Arabidopsis mutants. Plant Cell 12:1811-1822.

[25]. TAREKEGN G, MCLAREN NW AND SWART WJ (2004). Relationship between grain

development stage and sorghum cultivar susceptibility to grain mould. Afr. Plant Prot. 10: 53-62.

[26]. TESSO T, CLAFLIN LE, AND TUINSTRA MR (2004). Estimation of combining ability for resistance to Fusarium Stalk Rot in grain sorghum. Crop Sci. 44:1195–1199.

[27]. VESONDER RF, LOGRIECO A, BOTTALICO A, ALTOMARE C, AND PETERSON SW (1995). Fusarium species associated with banana fruit rot and their potential toxigenicity. Mycotoxin Res. 11:93-98.

[28]. WALLBRIDGE A (1981). Fungi associated with crown-rot disease of boxed bananas from the Windward Islands during a two-year survey. Trans. Br. Mycol. Soc. 77:567–577.

[29]. ZAKARIA L, CHIK MW, HENG KW AND SALLEH B (2012). *Fusarium* species Associated with Fruit Rot of Banana (Musa spp.), Papaya (*Carica papaya*) and Guava (*Psidium guajava*). Malaysian J. Microbiol. 8:127-130.

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