

MYCOFLORA ASSOCIATED WITH INFECTED FRUITS OF DIFFERENT VARIETIES OF *MUSA SAPIENTUM* L. AND THEIR PATHOGENIC POTENTIALITY

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Abstract

A total of 11 fungal species were isolated from the 25 infected fruit samples of three varieties (Sabri, Champa and Sagor) of *Musa sapientum*. The isolated fungi were *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* van Tiegh., *Colletotrichum musae* (Berk. & Curt.) Arx, *Curvularia brachyspora* Boedijn., *Fusarium semitectum* Berk. & Rav. *Fusarium* sp. Link, *Penicillium* sp., *Pestalotiopsis disseminata* (Thum) Steyaert, *Rhizopus* sp. Ehrenb. and *Trichoderma viride* Pers. ex Gray. *Fusarium semitectum* showed the highest (28.61) per cent association frequency in Sabri variety whereas *Fusarium* sp. showed the lowest (3.33). *Curvularia brachyspora* showed the highest (21.11) per cent frequency in Champa variety whereas *A. fumigatus* showed the lowest (2.78). *Fusarium* sp. showed the highest (41.67) per cent frequency in Sagor variety whereas *T. viride* showed the lowest (3.34). Among the isolated fungi *C. musae*, *C. brachyspora*, *F. semitectum*, *Fusarium* sp. and *P. disseminata* were found to be pathogenic to three banana varieties. The presence of *Pestalotiopsis disseminata* (Thum) Steyaert in Sabri variety of banana is the first record in Bangladesh.

Introduction

Banana (*Musa sapientum* L.) is the best-known tropical fruit and belongs to Musaceae⁽¹⁾. It is a nutritious fruit crop in the world and grown in many tropical areas where they are used both as a staple food and dietary supplements⁽²⁾. It is the fifth largest agricultural crop in world trade after cereals, sugar, cocoa and coffee. According to a report, the world production of banana was about 95 million tons and most of the production was consumed locally⁽³⁾. It is one of the cheapest and economically important fruit crops grown in Bangladesh in both homestead and commercial farms. Bangladesh ranks 14th among the top 20 banana producing countries in the world. There are a number of banana cultivars in Bangladesh of which BARI Kola-1, Amritsagar, Sabri, Champa, Kabri and Sagor are commercially important. Like all other commercially important plants, one of the major bottlenecks of banana production is its constant exposure to different biotic and abiotic stresses. Fungal diseases play an important role

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for the severe yield loss of banana. Major fungal diseases of banana are anthracnose (*Colletotrichum musae*), brown blotch (*Pestalotiopsis leprogena*), crown rot (*Colletotrichum musae*, *Curvularia disseminata*, *Fusarium semitectum*), fruit speckle (*Fusarium semitectum*), fruit rot (*Pestalotiopsis disseminata*), panama disease or Fusarium wilt (*Fusarium oxysporum* f. sp. *cubense*), etc. Anthracnose, crown rot and fruit rot are very common diseases in Narsingdi district. In Bangladesh, research on fungal diseases of banana fruits is very limited^(4,5). So, for the sake of economy more information are needed on this regard. Considering the importance of this popular fruit the present investigation was undertaken to record the association of fungi in different banana varieties of Bangladesh.

Materials and Methods

Three varieties of *Musa sapientum* viz., Sabri, Champa and Sagar Kola were selected for the experiment. Twenty five samples were collected and examined from three different Upazila, namely Shibpur, Belabo and Polash of Narsingdi district during May, 2015 to April, 2016. Samples were collected in separate sterile polyethylene bags, labeled properly and then brought to the laboratory for isolation of fungi. The fungi associated with banana were isolated following "Tissue planting method" on PDA medium according to Islam and Shamsi⁽⁶⁾. One hundred inocula, each measuring 2 mm² long were cut with a pair of sterilized scissors from the diseased fruit parts of the banana and kept in a sterile Petri plate. The inocula were washed with sterile water and then surface sterilized by dipping in 10% Clorox solution for three minutes. The inocula were again washed with sterile water for three times. Finally, the inocula were placed inside the folds of a sterile blotting paper to remove the excess surface water. A total of 60 inocula were placed in 20 sterilized Petri plates containing PDA medium. Each Petri plate contained 15 ml of PDA medium with an addition of 1 drop (ca. 0.003 ml) of lactic acid and incubated at 25 ± 2°C for 7 days.

The microscopic structural characters of the isolated fungi were recorded under a digital camera. Morphological structures of the fungi were drawn in detail by the aid of camera lucida. Identities of the isolates were determined following the standard literatures⁽⁷⁻¹²⁾.

Pathogenicity test of the fungal isolates has been done following "detached fruit inoculation technique"⁽¹³⁾. Healthy banana were collected from the selected market and sterilized with 10% Chlorox. The sterilized bananas were placed into the germ-free desiccators for drying. Three holes were made on the sterilized surface of fruits with a sharp cork borer (5 mm diameter) at a depth of 4 mm. For preparing inocula, 5 mm agar disc cut from the margin of actively growing culture of the test fungi. The prepared fungal mycelial agar disc was inoculated by sterilized inoculation loop in each 5 mm hole on the healthy banana surface. Three replications were maintained in each test pathogens. In control set, fresh PDA agar disc was used instead of fungal mycelial agar

disc on the sterilized banana surface. Then both the control and the treated sets of banana were kept in separate sterilized plastic boxes. Characteristic symptoms of fruit rot appeared on inoculated fruits after 5 - 7 days of incubation at $25 \pm 2^\circ\text{C}$. The fungi were isolated from artificially inoculated fruits and compared with naturally infected fruits.

Results and Discussion

A total of 11 fungi were isolated from the fruits of three infected varieties of *Musa sapientum*. The isolated fungi were *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Colletotrichum musae*, *Curvularia brachyspora*, *Fusarium semitectum*, *Fusarium* sp., *Penicillium* sp., *Pestalotiopsis disseminata*, *Rhizopus* sp. and *Trichoderma viride* (Tables 1 - 3).

Table 1. Per cent association frequency of fungi associated with infected Sabri kola collected from Shibpur Upazila, Narsingdi.

Name of fungi	% frequency of fungi at different intervals				Mean
	05.08.15	16.08.15	11.10.15	21.10.15	
<i>Aspergillus flavus</i>	13.33	6.67	27.78	13.33	15.28
<i>A. fumigatus</i>	13.33	-	11.11	6.67	7.78
<i>A. niger</i>	20.00	-	11.11	13.33	11.11
<i>Curvularia brachyspora</i>	13.33	40.00	-	-	13.33
<i>Fusarium semitectum</i>	26.67	13.33	27.78	46.67	28.61
<i>Fusarium</i> sp.	-	-	-	13.33	3.33
<i>Penicillium</i> sp.	6.67	20.00	-	6.67	8.33
<i>Pestalotiopsis disseminata</i>	6.67	20.00	22.22	-	12.22

'-' = No fungal growth.

Table 1 shows that eight fungal species were isolated from the infected Sabri variety. The per cent frequency of *F. semitectum* was highest (28.61) which was followed by *A. flavus* (15.28), *C. brachyspora* (13.33), *P. disseminata* (12.22), *A. niger* (11.11), *Penicillium* sp. (8.33), *A. fumigatus* (7.78) and *Fusarium* sp. (3.33).

Nine fungal species were recorded from the infected Champa variety. The per cent frequency of *C. brachyspora* was highest (21.11) which was followed by *Rhizopus* sp. (17.22), *A. flavus* (12.78), *Penicillium* sp. (11.67), *Fusarium* sp. (10), *A. niger* (8.89), *F. semitectum* and *T. viride* (7.78) and *A. fumigatus* (2.78) (Table 2).

Seven fungal species were isolated from the infected Sagor variety. The highest per cent frequency was observed in *Fusarium* sp. (41.67) which was followed by *C. musae* (25), *Penicillium* sp. (11.67), *A. flavus* and *Rhizopus* sp. (6.67), *A. fumigatus* (5) and *Trichoderma viride* (3.34) (Table 3).

Table 2. Per cent frequency of fungi associated with infected Champa Kola collected from Belabo upazila, Narsingdi.

Name of fungi	% frequency of fungi at different intervals				Mean
	17.08.15	08.11.15	20.11.15	27.11.15	
<i>Aspergillus flavus</i>	-	40.00	-	11.11	12.78
<i>A. fumigatus</i>	-	-	-	11.11	2.78
<i>A. niger</i>	13.33	-	-	22.22	8.89
<i>Curvularia brachyspora</i>	53.33	20.00	-	11.11	21.11
<i>Fusarium semitectum</i>	20.00	-	-	11.11	7.78
<i>Fusarium sp.</i>	-	-	40.00	-	10.00
<i>Penicillium sp.</i>	6.67	20.00	20.00	-	11.67
<i>Rhizopus sp.</i>	-	20.00	26.67	22.22	17.22
<i>Trichoderma viride</i>	6.67	-	13.33	11.11	7.78

'-' = No fungal growth.

Table 3. Per cent frequency of fungi associated with infected Sagar Kola collected from Polish upazila, Narsingdi.

Name of fungi	% frequency of fungi at different intervals				Mean
	15.09.15	08.10.15	30.11.15	06.12.15	
<i>Aspergillus flavus</i>	13.33	6.67	-	6.67	6.67
<i>A. fumigatus</i>	13.33	-	-	6.67	5.00
<i>Colletotrichum musae</i>	46.67	53.33	-	-	25.00
<i>Fusarium sp.</i>	-	40.00	53.33	73.33	41.67
<i>Penicillium sp.</i>	20.00	-	13.33	13.33	11.67
<i>Rhizopus sp.</i>	-	-	26.67	-	6.67
<i>Trichoderma viride</i>	6.67	-	6.67	-	3.34

'-' = No fungal growth.

Taxonomic descriptions of pathogenic fungi associated with three infected varieties of *Musa sapientum*.

1. *Colletotrichum musae* (Berk. & Curt.) Arx, Verh. Akad. Wet. Amst. **51**: 107 (1957).

(Fig. 1A)

Colonies often with abundant white aerial mycelium becoming grey with age, with abundant ochreous to cinnamon conidial masses, usually coalescing. Sclerotia absent. Setae absent. Conidia straight, cylindrical, obtuse at apices, 12 - 17 × 4.5 - 5.5 μm. Appressoria common, medium to dark brown, irregular in shape, often with large or deep lobes, 9 - 13 × 9 - 11.5 μm, often becoming complex.

Specimen examined: Isolated from diseased fruits of *Musa sapientum*, Belabo and Polash upazila, Narsingdi district, MA Ameen 01, Nov - Dec. 2015.

2. ***Curvularia brachyspora*** Boedijn. (Fig. 1B)

Colonies effuse grayish black. Conidiophores septate, mostly unbranched, brown, solitary, straight or slightly undulating, up to 97.2 μm long, 4.8 - 5.6 μm thick. Conidia olivaceous brown, mostly three septate, mostly straight or slightly curved, third cell from the base is broader and darker than others, smooth, 20 - 26 into 10 - 14 μm .

Specimen examined: Isolated from diseased fruits of *Musa sapientum*, Belabo, Shibpur and Polash upazila, Narsingdi district, MA Ameen 02, July - Dec. 2015.

3. ***Fusarium semitectum*** Berk. & Rav. In Berkeley, Gravillea 3: 98, 1875. (Fig. 1C)

Cultures at first white peach tinge and peach coloured from below. Aerial mycelium floccose, peach gradually changing to avellaneous and finally becoming buff brown (14 - 21 days). Sporodochia absent, brown stromatic pustules occasionally developed. Red, blue or violet pigmentation not observed.

Macroconidia formed in aerial mycelium from loosely branched conidiophores. Each branch terminates in a conidiogenous cell 19 - 24 \times 2 - 4 μm which appears to form a conidium from a single apical pore and then to form successively a second, third or even fourth pore thus forming a polyblastic sympodial cell. The conidia vary from 3 to 5 septate and are curved with a wedge-shaped but not pedicellate basal cell and pointed apex. They measure: 3 septate 17 - 28 \times 2.5 - 4 μm and 5 septate 22 - 40 \times 3.7 - 4 μm .

In older cultures especially after mass transfers a greater variation of conidial shape and size occurs, many 1 septate pyriform to obovate conidia, 10-12 \times 2.5-3.5 μm , being formed.

Chlamydo spores, often sparse, are globose, intercalary, 5 - 10 μ diameter formed singly or in chains.

Specimen examined: Isolated from diseased fruits of *Musa sapientum*, Belabo, Shibpur and Polash upazila, Narsingdi district, MA Ameen 03, July - Dec. 2015.

4. ***Fusarium sp.*** Link (Fig. 1D)

Colony initially pink colored and brownish white from below. Growth of colony was fluffy and reached 2 - 3 cm from below the Petri plate within 5 days. Microconidia and macroconidia were found. Aerial mycelium floccose, macroconidia formed in aerial mycelium from loosely branched conidiophores. Each branch terminates in a conidiogenous cell which appears to form a conidium from a single apical pore. The conidia vary from 2 to 3 septate and are not curved with nearly straight-shaped.

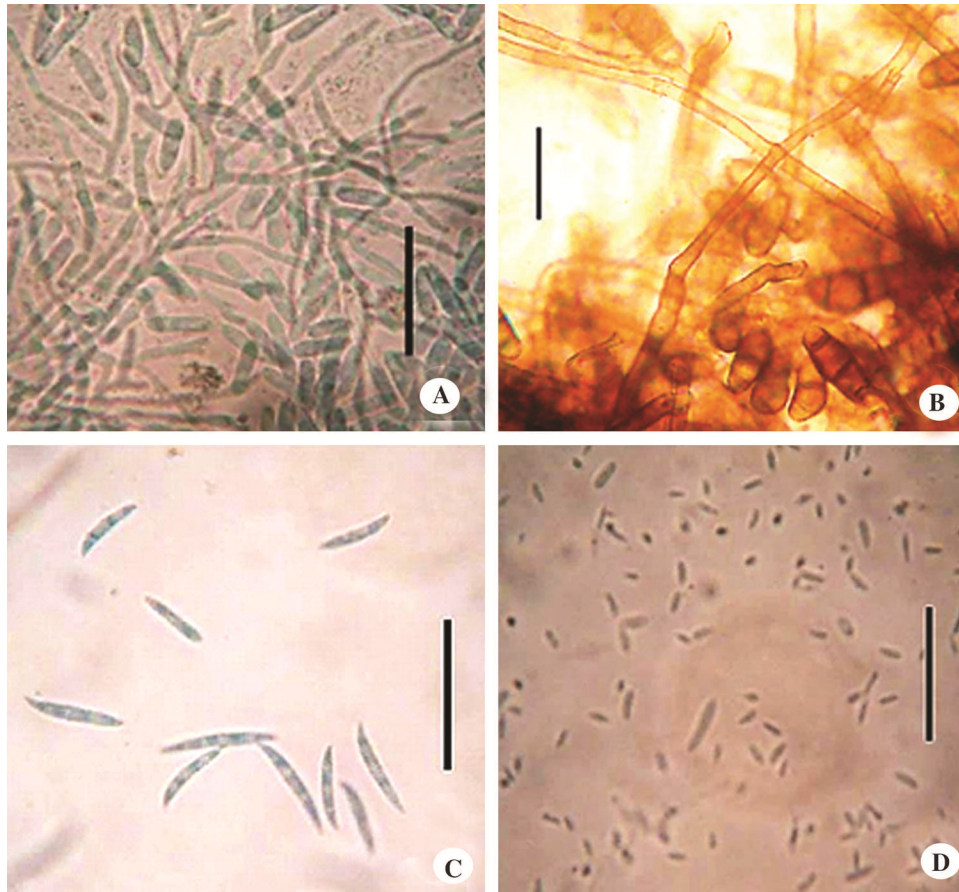


Fig. 1. Conidiophores and conidia of *Colletotrichum musae* (A) and *Curvularia brachyspora* (B), conidia of *Fusarium semitectum* (C) and *Fusarium* sp. (D) (Bar = 50 μm).

Macroconidiophores were simple, lateral, subulate phialides formed on the aerial hyphae; they may form short lateral branches. Macroconidia were formed under optimum growing conditions and these can readily be observed *in situ* under the low power of the microscope.

Microconidia formation was very common. The conidiophores may consist of a single basal cell bearing 2 - 3 apical phialides or it may form 2 - 3 metulae which in turn bear simple to obclavate phialides. Microconidia were thin-walled, with an elongated, often sharply curved apical cell and pedicellate basal cell. They are 1 septate. Chlamydospores were absent both in mycelium and conidia.

Specimen examined: Isolated from diseased fruits of *Musa sapientum*, Belabo, Shibpur and Polash upazila, Narsingdi district, MA Ameen 04, June - Dec. 2015.

5. *Pestalotiopsis disseminata* (Thum) Steyaert

(Fig. 2)

Colonies white, cottony, reverse white. Hyphae septate, branched, hyaline. Acervuli becoming black and granular. Conidia end cells hyaline, intermediate three cells pale brown, apical setulae up to 20 μm long, basal appendages 5 - 10 μm long. Conidia 24 - 28 \times 7 - 8 μm .

Specimen examined: Isolated from healthy and diseased fruits of *Musa sapientum*, Belabo, Shibpur and Polash upazila, Narsingdi district, MA Ameen 05, July - Dec. 2015.

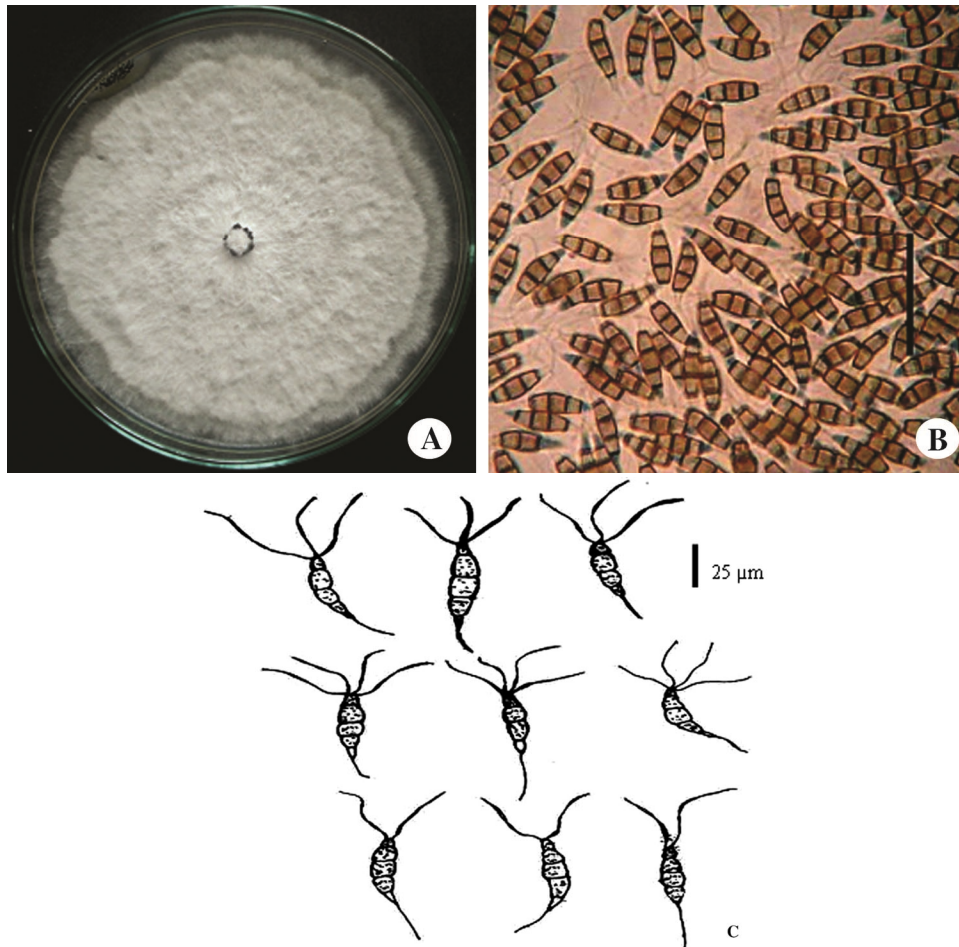


Fig. 2. Colony (A), conidia (B) and camera lucida drawing of conidia (C) of *Pestalotiopsis disseminata* (Bar = 50 μm).

The fungi isolated from three infected banana varieties were tested for their pathogenic potentiality following detached fruit technique. Among the isolated fungi *C. musae*, *C. brachyspora*, *F. semitectum*, *Fusarium* sp. and *P. disseminata* were found to be

pathogenic to all the tested banana varieties. Control set remains fresh without showing any fungal growth (Fig. 3).



Fig. 3. Pathogenicity test of banana fruits of different varieties. Control (A), inoculated fruits with *Colletotrichum musae* (B), *Curvularia brachyspora* (C), *Fusarium semitectum* (D), *Fusarium* sp. (E) and *Pestalotiopsis disseminata* (F).

Alvindia *et al.*⁽¹⁴⁾ and Hirata *et al.*⁽¹⁵⁾ reported *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl., *Colletotrichum musae* (Berk. & Curt.) Arx, *Thielaviopsis paradoxa* (De Seynes) Höhn. *Fusarium* spp., as well as other fungi, associated with post-harvest diseases of banana. Nelson *et al.*⁽¹⁶⁾ reported *Acremonium*, *Colletotrichum musae*, *Curvularia*, *Fusarium* and *Verticillium* from the crown rot diseases of banana. Odebode and Sanusi⁽¹⁷⁾ stated that *Botryodiplodia theobromae* was a major organism causing spoilage of banana in south-west part of Nigeria. They described that *B. theobromae*, *Rhizopus oryzae*, *Aspergillus niger*, *A. flavus* and *Fusarium equarii* were found to be associated with the ripening of banana and also caused rot during storage.

The findings of the present investigation slightly differed from others. Present results reveal that out of 11 fungal species five were pathogenic to the three varieties of *Musa sapientum*. The presence of *Pestalotiopsis disseminata* is the first record in Sabri Kola from Bangladesh.

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