

MYCOFLORA ASSOCIATED WITH POST-HARVEST DISEASE OF PAPAYA (*CARICA PAPAYA* L.) AND THEIR PATHOGENIC POTENTIALITY

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Keywords: Mycoflora, Diseased fruits, *Carica papaya*, Pathogenic potentiality.

Abstract

Nineteen species of fungi namely, *Alternaria alternata* (Fr.) Keissler, *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* Van. Tieghem, *Colletotrichum dematium* (Pers.) ex. Fr., *C. gloeosporioides* (Penz.) Sacc., *Corynespora citricola* M.B. Ellis, *Curvularia lunata* Wakker, *Fusarium flocciferum* Corda, *F. nivale* (Fr.) Ces., *Fusarium* Link, *Lasiodiplodia theobromae* (Pat.) Griff & Moub., *Monilia* Pers., *Mucor* Fresen, *Penicillium* Link, *Pestalotiopsis guepinii* (Desm.) Stay., *Rhizoctonia solani* J.G. Kuhn, *Rhizopus stolonifer* Bull. and *Syncephalastrum* Schroet were found to be associated with the diseased fruits of *Carica papaya* L. Among the isolated fungi *C. gloeosporioides*, *F. nivale* and *Fusarium* sp. were found to be pathogenic for both red and yellow cultivar Shahi papaya. Association of *C. lunata*, *F. nivale*, *P. guepinii* and *Syncephalastrum* sp. with papaya is a new record. The present report is the first record of *Corynespora citricola* and *Fusarium flocciferum* from Bangladesh.

Introduction

Papaya (*Carica papaya* L.) is one of the most important fruits cultivated throughout the tropical and subtropical regions of the world (Ahmed *et al.* 2008). It is consumed at unripe and ripe stages (Mendoza 2007). The edible portion of the ripe papaya fruit contains Na, K, Ca, Mg, P, Fe, Cu, Zn and Mn. It is a source of carotenoids, vitamin C, thiamine, riboflavin, niacin, vitamin B-6 and vitamin K (Bari *et al.* 2006). Bangladesh is one of the major papaya producing countries along with Brazil, China, India, Indonesia, Mexico, Thailand etc. Bangladesh produces 133370 million tones of ripe papaya fruits annually from 3126 acres of land (Anon. 2015). In Bangladesh, Shahi papaya released by BARI is only a single recommended variety of the crop (Ara *et al.* 2013). Post-harvest losses due to fungal infections are significantly high in papaya fruits. Various fungi cause rots in fruits of papaya (Sawant and Gawai 2011). The post-harvest pathogens like *Colletotrichum gloeosporioides* (Penz.) Sacc., *Botryodiplodia theobromae* Pat., *Alternaria*, *Phomopsis*, *Fusarium*, *Aspergillus*, *Stemphylium* and *Pestalotiopsis* attack the fruits and cause considerable damage to fruit production and quality (Chowdhury *et al.* 2014). Six post-harvest diseases of papaya *viz.*, Anthracnose, Aspergillus rot, Fusarium rot, Penicillium rot, Rhizopus rot and stem end rot were recorded in Bangladesh (Hamim *et al.* 2014). The post-harvest fruit rots bring about a big loss in fruit business which provokes price hike (Uddin 1995). Investigation on post-harvest diseases and associated mycoflora of Shahi papaya is limited in Bangladesh (Chowdhury *et al.* 2014, Hamim *et al.* 2014). For the above reasons present research was undertaken to find out the association of fungi with Shahi papaya fruits and their pathogenic potentiality.

Materials and Methods

Fruits of red and yellow Shahi papaya variety were collected from five different markets of Dhaka namely, Segunbagicha bazaar, Shantinagar bazaar, Karwan bazaar, Sham bazaar (Shoarighat) and Polashi bazaar during April, 2016 to November, 2016.

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Each market was visited four times about one-month interval to collect samples. During every visit, one for each of the red and yellow Shahi papaya showing anthracnose and rot was collected randomly. Fungi associated with diseased papaya were isolated immediately after collection following Tissue planting method (CAB 1968).

The microscopic structural characters of the isolated fungi were recorded with a digital camera. Morphological structures of the fungi were drawn in detail with the help of camera lucida. Identities of the isolates were determined following standard literatures (Thom and Raper 1945, Booth 1971, Ellis 1971, Barnett and Hunter 1972, Sutton 1980).

For pathogenicity test healthy papaya were collected from the selected five markets. Conidia of different fungi cultured on PDA plates were taken and conidial suspensions (10^5 /ml) were made separately from hemocytometer count.

Papayas were washed thoroughly in running tap water and dipped in one liter of 10% Chlorox solution for three min for surface sterilization. Then papayas were washed three times in sterilized distilled water to remove 10% Chlorox solution from the surface. Excess water from the surface of the papaya was removed by pressing them between two sheets of previously sterilized filter paper. Suitable sized plastic boxes were wiped with 70% alcohol and sterilized wet cotton was placed in a corner of box to maintain moisture content. Before spraying, the fruits were scratched with sterilized needle. Then fruits were inoculated by spraying the conidial suspension (10^5 conidia/ml) of 19 isolated fungi from diseased Shahi papaya with an atomizer (Tarl rops, Germany) and placed into separate plastic boxes. In control set, sterilized distilled water was used instead of conidial suspension on the sterilized papaya surface. The inoculated fruits with plastic boxes were incubated at 25 - 28°C for 7 - 10 days. Observation was made after 7 - 10 days of inoculation and symptoms developed on the fruits surface were recorded.

Re-isolation of the pathogen was made from the artificially infected papaya following the usual procedure. The morphological characters of the re-isolated fungi were compared with the original isolation by which they were inoculated.

Results and Discussion

Anthracnose and fruit rot symptoms were recorded on diseased Shahi papaya fruits. The fungi associated with diseased papaya and their per cent frequencies of occurrence are presented in Tables 1 - 2. A total of 19 fungi *viz.*, *Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Colletotrichum dematium*, *C. gloeosporioides*, *Corynespora citricola*, *Curvularia lunata*, *Fusarium flocciferum*, *F. nivale*, *Fusarium* sp., *Lasiodiplodia theobromae*, *Monilia* sp., *Mucor* sp., *Penicillium* sp., *Pestalotiopsis guepinii*, *Rhizoctonia solani*, *Rhizopus stolonifer* and *Syncephalastrum* sp. were isolated from the diseased papaya. *C. dematium* and *F. flocciferum* were isolated from yellow Shahi papaya whereas *C. lunata*, *Monilia* sp. and *Rhizoctonia solani* were isolated from red Shahi papaya.

Sixteen fungi were isolated from diseased yellow Shahi papaya collected from five different markets of Dhaka city (Table 1). The per cent frequency of *C. gloeosporioides* was highest (40.91) whereas *A. niger* was lowest (4.47) in Segunbagicha bazaar. In Shantinagar bazaar the per cent frequency of *C. gloeosporioides* was highest (24.46) whereas *Rhizopus stolonifer* was lowest (2.60). *Fusarium* sp. showed highest (17.44) per cent frequency whereas *Syncephalastrum* sp. showed the lowest (3.35) in Karwan bazaar's samples. *Fusarium* sp. showed highest (24.21) per cent frequency and *R. stolonifer* showed lowest (0.55) in the samples collected from Sham bazaar. In the sample collected from Polashi bazaar, the per cent frequency of *C. gloeosporioides* was highest (30.92) and *Penicillium* sp. was lowest (3.34).

Table 1. Per cent frequency of fungi associated with diseased yellow Shahi papaya collected from different markets of Dhaka city.

Name of fungi	Segunbagicha bazaar	Shantinagar bazaar	Karwan bazaar	Sham bazaar	Polashi bazaar
<i>Alternaria alternata</i>	-	4.69	5.28	6.67	5.00
<i>Aspergillus flavus</i>	5.71	-	-	1.48	3.67
<i>A. fumigatus</i>	21.73	7.00	11.50	7.87	5.95
<i>A. niger</i>	4.47	4.51	6.86	9.39	10.89
<i>Colletotrichum dematium</i>	-	-	16.40	-	-
<i>C. gloeosporioides</i>	40.91	24.46	-	16.57	30.92
<i>Corynespora citricola</i>	-	9.20	10.45	-	13.41
<i>Fusarium flocciferum</i>	-	10.45	-	20.30	-
<i>F. nivale</i>	22.23	12.36	15.12	-	12.98
<i>Fusarium</i> sp.	-	-	17.44	24.21	10.13
<i>Lasiodiplodia theobromae</i>	-	20.74	-	11.10	-
<i>Mucor</i> sp.	-	-	-	-	3.67
<i>Penicillium</i> sp.	9.86	-	-	-	3.34
<i>Pestalotiopsis guepinii</i>	-	-	10.45	1.91	-
<i>Rhizopus stolonifera</i>	-	2.60	3.67	0.55	-
<i>Syncephalastrum</i> sp.	-	3.97	3.35	-	-

- = No fungal growth.

Seventeen fungi were isolated from diseased red Shahi papaya (Table 2). The per cent frequency of *C. gloeosporioides* was highest (37.49) and *A. niger* was lowest (5.01) in Segunbagicha bazaar. In Shantinagar bazaar the per cent frequency of *C. gloeosporioides* was highest (24.70) whereas *Monilia* sp. was lowest (1.05). *Colletotrichum gloeosporioides* showed highest (27.08) per cent frequency whereas *A. alternata* showed the lowest (3.46) from Karwan bazaar's samples. *Colletotrichum gloeosporioides* showed highest (27.88) per cent frequency and *Monilia* sp. showed lowest (3.62) in the samples collected from Sham bazaar. In the sample collected from Polashi bazaar, the per cent frequency of *C. gloeosporioides* was highest (26.67) and *C. lunata* was lowest (1.67).

Out of 19 fungal species isolated from papaya fruits three fungi namely, *C. gloeosporioides*, *F. nivale* and *Fusarium* sp. developed characteristic symptoms on the inoculated fruit surface after 7 - 10 days of incubation. They were successfully re-isolated and fulfill Koch's postulate (Fig. 1).

Chowdhury *et al.* (2014) reported that the frequently occurring diseases of papaya in storage condition were caused by *Colletotrichum gloeosporioides*, *Botryodiplodia theobromae*, *Fusarium* sp. and *R. stolonifer* in Bangladesh. Rahman *et al.* (2008) reported a total of seven fungi, namely *Botryodiplodia theobromae*, *Colletotrichum capsici*, *C. gloeosporioides*, *Fusarium* sp., *Phomopsis* sp., *R. stolonifer* and *Stemphylium* sp. from the surface of fully matured papaya fruits in Malaysia. Among the diseases, the highest incidence ranged from 90 to 98% and severity of 25 to 38% was recorded for anthracnose caused by *C. gloeosporioides* followed by stem-end-rot caused by *Botryodiplodia theobromae*. Lim and Tang (1984) reported that *C. dematium* was the cause for 5% of anthracnose of papaya in Singapore. Awoite *et al.* (2013) reported some fungi viz., *Aspergillus niger*, *A. flavus*, *Rhizopus stolonifer*, *Penicillium italicum* and *Neurospora sitophila* associated with the spoilage of papaya fruits during their post-harvest period in Nigeria. Akinro *et al.* (2015) and Baiyewu *et al.* (2007) reported that *Aspergillus* sp. and *Rhizopus* sp. were

responsible for the soft rots of pawpaw in Nigeria. Gupta and Pathak (1986) reported that *Aspergillus niger*, *A. flavus*, *Rhizopus nigricans*, *Curvularia lanata*, *Rhizopus oryzae*, *Fusarium equiseti* and *F. moniliforme* were responsible for post-harvest losses of pawpaw in south western

Table 2. Per cent frequency of fungi associated with diseased red Shahi papaya collected from different markets of Dhaka city.

Name of fungi	Segunbagicha bazaar	Shantinagar bazaar	Karwan bazaar	Sham bazaar	Polashi bazaar
<i>Alternaria alternata</i>	-	5.00	3.46	-	2.34
<i>Aspergillus flavus</i>	9.19	5.00	-	5.63	2.76
<i>A. fumigatus</i>	7.50	4.16	7.77	6.57	5.00
<i>A. niger</i>	4.07	11.42	8.61	5.00	5.00
<i>Colletotrichum gloeosporioides</i>	37.49	24.70	27.08	27.88	26.67
<i>Corynespora citricola</i>	9.37	-	10.45	-	-
<i>Curvularia lunata</i>	9.60	2.49	7.21	-	1.67
<i>Fusarium nivale</i>	-	16.05	12.04	19.66	12.50
<i>Fusarium</i> sp.	-	7.88	16.52	17.33	17.45
<i>Lasiodiplodia theobromae</i>	11.10	12.08	-	-	18.27
<i>Monilia</i> sp.	-	1.05	-	3.62	-
<i>Mucor</i> sp.	-	1.52	-	-	-
<i>Penicillium</i> sp.	5.01	-	5.89	8.45	-
<i>Pestalotiopsis guepinii</i>	-	-	7.15	5.83	-
<i>Rhizoctonia solani</i>	6.67	-	-	-	3.34
<i>Rhizopus stolonifer</i>	-	2.00	3.67	-	8.05
<i>Syncephalastrum</i> sp.	-	1.57	4.09	-	1.93

- = No fungal growth.

Nigeria. Oke and Banjoko (1991) have also reported the presence of *Penicillium digitatum* and *Fusarium oxysporium* on pawpaw. Ewekeye *et al.* (2013) reported the occurrence of *Rhizopus stolonifer*, *Aspergillus flavus*, *A. niger*, *Mucor* sp., *Penicillium* sp. and *Fusarium acuminatum* from diseased papaya fruits. Pathak *et al.* (1976) reported that *Fusarium acuminatum* is responsible for the soft rot of papaya in their post-harvest condition. Present results revealed that out of 19 fungal species three were pathogenic to Shahi papaya. From the above discussion it could be said that the association of *Curvularia lunata*, *F. nivale*, *P. guepinii* and *Syncephalastrum* sp. with post-harvest disease of *C. papaya* is a new record. The fungi *Corynespora citricola* and *Fusarium flocciferum* are new record for Bangladesh. Their taxonomic details are given below.

1. *Corynespora citricola* M.B. Ellis, Z. Pflkrankh. 76:10-13, (1906) (Fig. 2)

Colonies effuse, grayish, cottony. Mycelium immersed or superficial. Conidiophores brown, straight or flexuous, unbranched, conidia mostly solitary, simple, cylindrical, smooth, straw colored, pseudoseptate, 48-350 × 4.5-8 µm.

Material studied: Isolated from diseased *Carica papaya*, Shambazaar, Soarighat, Dhaka, RB Helal 8, 4 July 2016.

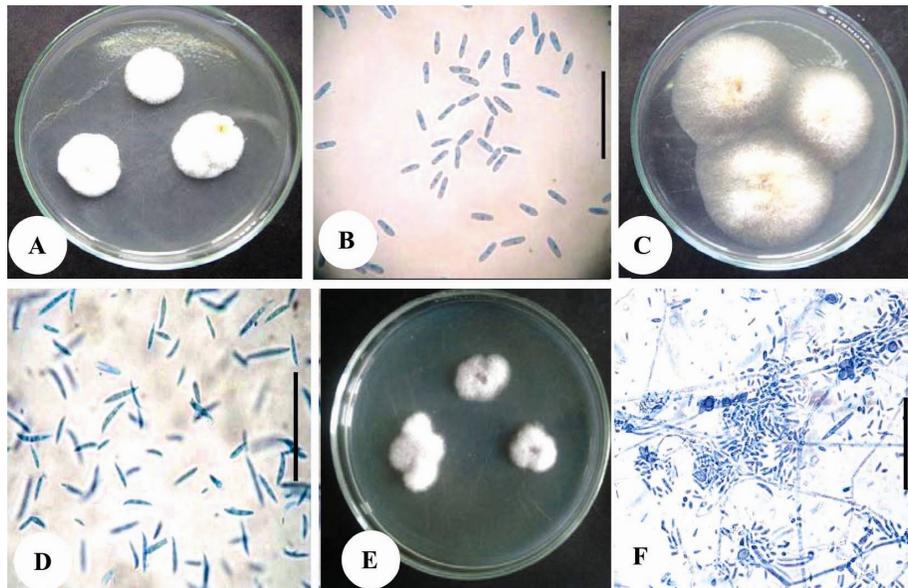


Fig. 1A-D. Colonies and conidia of re-isolated pathogens from *Carica papaya*. A-B. *Colletotrichum gloeosporioides*; C-D. *Fusarium nivale* and E-F. *Fusarium* sp. Bar = 50 µm.

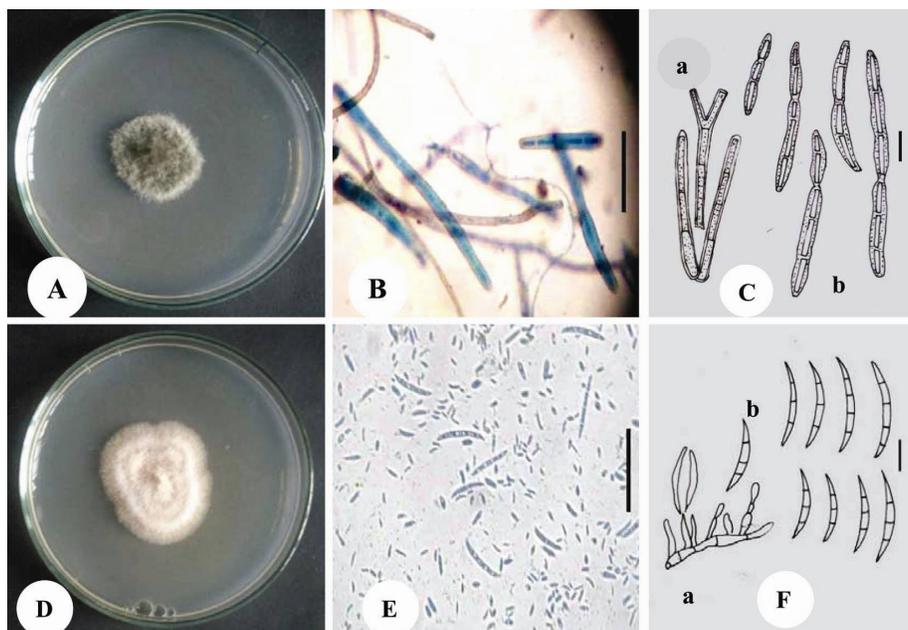


Fig. 2A-F. *Corynespora citricola*: A. Colony on PDA medium, B. Conidiophores with conidia and C. Camera lucida of conidiophores (Ca) and conidia (Cb); *Fusarium flocciferum*: D. Colony on PDA medium, E. Conidia and F. Camera lucida of conidiophores (Fa) and Conidia (Fb). Bar = 50 µm (B, E) and 11 µm (C,F).

2. *Fusarium flocciferum* Corda, Sturm's Deutschl. Fl. 2:17, (1828) **(Fig. 2)**

Colony light pinkish from both sides. Conidia variable in size, produced from lateral conidiophores in the aerial mycelium. Conidiophores rather loosely branched with 2 - 3 terminal phialides to each branch, 16 - 22 × 2 - 5 μm. Conidia hyaline straight to curved, indistinctly septate, 22 - 41 × 3.5 - 4.5 μm.

Material studied: Isolated from diseased *Carica papaya*, Karwan bazaar, Dhaka, RB Helal 9, 04 July, 2016.

Acknowledgements

The first author (RBH) expresses her gratitude to the Ministry of Science and Technology, Govt. of the People's Republic of Bangladesh for providing financial assistance to this research work through NST fellowship program.

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(Manuscript received on 14 October, 2017; revised on 15 July, 2018)