

MYCOFLORA ASSOCIATED WITH INFECTED FRUITS OF *MOMORDICA COCHINCHINENSIS* (LOUR.) SPRENG.

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Abstract

Eleven species of anamorphic fungi associated with anthracnose and fruit rot symptoms of *Momordica cochinchinensis* (Lour.) Spreng. (*Ban Kakrol*) have been described. The associated fungi were *Aspergillus niger* Van Tieghem, *Colletotrichum gloeosporioides* (Penz.) Sacc., *C. orbiculare* (Berk. & Mont.) Arx., *Corynespora cassiicola* (Berk. & Curt.) Wei, *Curvularia clavata* Jain, *Dendryphiella vinosa* (Berk. & Curt.) Reisinger, *Fusarium moniliforme* J. Sheld., *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl., *Pestalotiopsis guepinii* (Desm.) Stey., *Penicillium digitatum* Sacc. and *Xylohypha pinicola* D. Hawksw. *Xylohypha pinicola* is a new record for Bangladesh.

Introduction

Momordica cochinchinensis (Lour.) Spreng. (*Ban Kakrol*) belongs to the family Cucurbitaceae (Ahmed *et al.*, 2008). Its roots, leaves and fruits are important for its nutritional and medicinal properties. The plant is native to South East Asia. In Bangladesh, it grows mainly in the Chittagong Hill Tracts in wild condition. It is known as *Ban Kakrol* in Bangladesh, *Gac* in Vietnam and variously red melon, babyjackfruit, spiny bitter gourd or cochinchin gourd in English. *Momordica cochinchinensis* fruit is traditionally used in Asia to provide red colour for cuisines and enhance visional health. Recently, *M. cochinchinensis* fruit has emerged as a potential source of carotenoids, especially lycopene and β -carotene. Carotenoids and other identified bioactives from this fruit including phenolics, flavonoids and trypsin inhibitors are associated with many beneficial bioactivities such as antioxidant, anticancer and provitamin A activities. In addition to the traditional utilization, commercial products like *M. cochinchinensis* powder and oil is manufactured as natural colourants and medicinal supplements (Chuyenet *et al.*, 2015). In Vietnam, *gac* is prized by natives for promoting longevity and vitality. In a supplementation trial among Vietnamese children, *gac* increased serum vitamin A levels more than synthetic beta-carotene (Burke *et al.*, 2005). From the above discussion it is clear that diseases free *M. cochinchinensis* fruits are carrying need for nutritional and medicinal point of view. From India Mukerji and Bhasin (1986) reported eight fungal diseases of two species of *Momordica* L. viz., *M. charantia* L. and *M. dioica* Roxb. ex Willd. They exclusively reported only fruit rot disease on *M. cochinchinensis* species. So far there is no report is available regarding fungal diseases of *Momordica* spp. in Bangladesh. Recently fruit rot and anthracnose symptoms showing severe damage of *M. cochinchinensis* fruits was recorded on the plants grown in Botanical garden, Curzon Hall, Dhaka University. Present investigation was conducted to find out the fungi associated with infected fruits of *M. cochinchinensis*.

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Materials and Methods

Infected fruits of *Momordica cochinchinensis* (Lour.) Spreng. having characteristic symptoms were collected from selected fields of the Botanical Garden of Dhaka University to record the prevalence of the diseases and association of fungi during the tenure of December 2015 to January 2016. Samples were collected in separate sterile polyethylene bags, labeled properly and then brought to the laboratory for isolating associated pathogenic fungi following “Tissue planting method” on PDA medium. From infected fruits of *Ban Kakrol* showing anthracnose and soft rot symptoms, each measuring 2 mm² sized were cut separately with a pair of sterilize scissors and kept in a separate sterilized Petri plate. The inocula were washed with sterile water and then surface sterilized by dipping in 10% chlorox solution for three minutes. The inocula were again washed with sterile water. For fruit rot symptoms a total of 100 inocula were placed separately on 30 sterilized Petri plates containing 15 ml of PDA medium with an addition of 1 drop (ca 0.03 ml) of lactic acid to check the bacterial growth and incubated in an incubator (25 ± 2°C) for 7 days.

Detail morphological studies of the fungal isolates were made in order to determine their identification. The microscopic structural characters of the isolated fungi were recorded under a digital camera. Species identification was done by Camera Lucida drawing. All specimens, included in the present study were preserved in Mycology and Plant Pathology section and Salar Khan Herbarium, Department of Botany, University of Dhaka, Bangladesh. Identities of the isolates were determined following the standard literature (Booth, 1971; Ellis, 1971; Barnet and Hunter, 1972; Sutton, 1980).

Results and Discussion

A total of eleven species of fungi were associated with two types of symptoms *viz.*, anthracnose and fruit rot were recorded on infected fruits of *Momordica cochinchinensis* (Lour.) Spreng. (Fig. 1). The fungi associated with anthracnose symptoms were *Colletotrichum gloeosporioides*, *C. orbiculare*, *Curvularia clavata*, *Fusarium moniliforme* and *Penicillium digitatum*. Fruit rot infected samples showed the association of *Aspergillus niger*, *Colletotrichum gloeosporioides*, *Corynespora cassiicola*, *Curvularia clavata*, *Dendryphiella vinosa*, *Fusarium moniliforme*, *Lasiodiplodia theobromae*, *Pestalotiopsis guepinii* and *Xylohypha pinicola*. Of these *X. pinicola* is a new record for Bangladesh.

Taxonomic treatment of fungal taxa

1. *Aspergillus niger* Van Tieghem, Ann. Sci. Nat. Bot., Ser. 5, **8**: 240 (1867). **(Fig. 2A).**

Colonies effuse, black. Mycelium well-developed, septate, profusely branched and brownish. Cells are multinucleate. Conidiophores brown 200–400 × 7–10 µm. Vesicles globose or subglobose, thick walled, commonly 20–50 µm, occasionally up to 100 µm in diameter. Sterigmata 20–30 × 6–8 µm. Conidia dark brown, one celled globose, spinose 2–4 (5) µm in diameter. Catenulate.

Specimen examined: Isolated from infected fruits of *M. cochinchinensis*, Botanical Garden, University of Dhaka, Dhaka, S. Hosen 12, 6 December 2015.

2. *Colletotrichum gloeosporioides* (Penz.) Sacc., Fung. Agrum. **2**: 6 (1882). **(Fig. 2B).**

Colonies greyish. Acervuli black, sub-epidermal but later the epidermis are ruptured and expose them. Hyphae septate, hyaline, both inter and intra-cellular. Conidia hyaline, straight, obtuse at the apex, 11.2–25.2 × 3.6–5.0 µm.

Specimen examined: Isolated from infected fruits of *M. cochinchinensis*, Botanical Garden, University of Dhaka, Dhaka, S. Shamsi 3077, 17 December 2015.

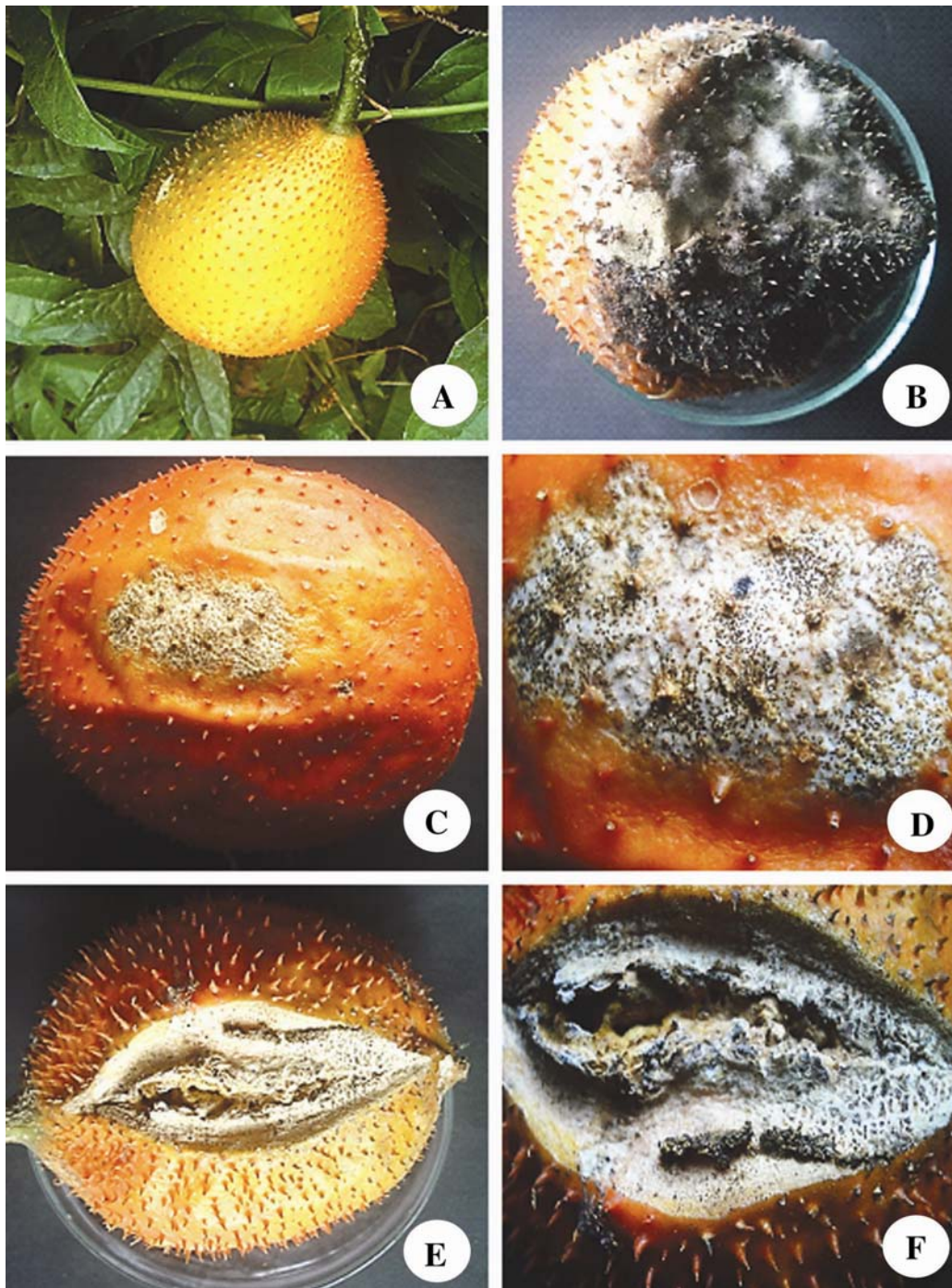


Fig. 1. *Momordica cochinchinensis*. A. Healthy fruit, B. Rotten fruit, C-F. Fruit showing different stages of anthracnose symptom.

3. *Colletotrichum orbiculare* (Berk. & Mont.) Arx., Verh. Akad. Wet. Amst. **51**(3): 112(1957).

(Fig. 2C, D).

Colonies with abundant white greyish aerial mycelium, reverse dark brown, conidial masses salmon pink. Setae present, conidia straight, cylindrical obtuse at the apices, $11.7\text{--}14.4 \times 4.5\text{--}6 \mu\text{m}$.

Specimen examined: Isolated from infected fruits of *M. cochinchinensis*, Botanical Garden, University of Dhaka, Dhaka, S. Shamsi 3078, 1 January 2016.

4. *Corynespora cassiicola* (Berk. & Curt.) Wei, Mycol. Pap. **34**: 5 (1950). (Fig. 2E).

Colonies effuse, grey, thinly hairy. Mycelium mostly immersed; no stroma. Conidiophores pale to mid brown, with up to 9 successive cylindrical proliferations. Conidia solitary or in chains of 2–6, very variable in shape. Obclavate to cylindrical, straight or curved, subhyaline to rather pale olivaceous brown or brown, smooth, with 4–20 pseudosepta, $48.6\text{--}131.4 \times 12\text{--}23 \mu\text{m}$.

Specimen examined: Isolated from infected fruits of *M. cochinchinensis*, Botanical Garden, University of Dhaka, Dhaka, S. Hosen 13, 6 December 2015.

5. *Curvularia clavata* Jain, Trans. Brit. Mycol. Soc. **45**(4): 542 (1962). (Fig. 2F).

Colonies effuse, brown, grey or black, hairy, cottony or velvety. Mycelium brown, septate, branched. Conidiophores dark brown, paler towards the apex, septate, mostly geniculate. Conidia solitary, simple, often curved, clavate, ellipsoidal, broadly fusiform with 3 septate, dark brown, end cells paler than the others, smooth, $16.5\text{--}25 \times 7\text{--}11 \mu\text{m}$.

Specimen examined: Isolated from infected fruits of *M. cochinchinensis*, Botanical Garden, University of Dhaka, Dhaka, S. Hosen 14, 17 December 2015.

6. *Dendryphiella vinosa* (Berk. & Curt) Reisinger, Bull. trimest. Soc. Mycol. Fr. **84**: 27–39 (1968). (Fig. 3A).

Colonies effuse, rust-colored, brown or black, hairy or velvety. Mycelium mostly immersed. Stroma none. Setae and hyphopodia absent. Conidiophores reddish brown, verruculose. Conidia 3septate, distinctly verruculose when mature, pale clear brown to burnt sienna, darker at the hilum, $16.2\text{--}27 \times 3.6\text{--}7.2 \mu\text{m}$.

Specimen examined: Isolated from infected fruits of *M. cochinchinensis*, Botanical Garden, University of Dhaka, Dhaka, S. Hosen 15, 6 December 2015.

7. *Fusarium moniliforme* J. Sheld., Annual Rep. Neb. Agric. Exp. Sta. **17**: 23–32 (1904).

(Fig. 3B).

Colony white, cottony, reverse violet in PDA medium. Microconidia are formed in chains under optimum growing conditions and these can readily be observed *in situ* under the low power of the microscope. Microconidia measure $8\text{--}11 \times 1.8\text{--}2.25 \mu\text{m}$ and are fusiform to clavate with a slightly flattened base; they occasionally become 1 septate. Macroconidial formation is rare in many strains, where present they develop from conidiophores formed as lateral branches on the hyphae. The conidiophore consists of a single basal cell bearing 2–3 apical phialides or it may form 2–3 metulae which in turn bear simple doliform to obclavate phialides, $17\text{--}23.4 \times 1.8\text{--}2.7 \mu\text{m}$.

Specimen examined: Isolated from infected fruits of *M. cochinchinensis*, Botanical Garden, University of Dhaka, Dhaka, S. Hosen 16, 6 December 2015.

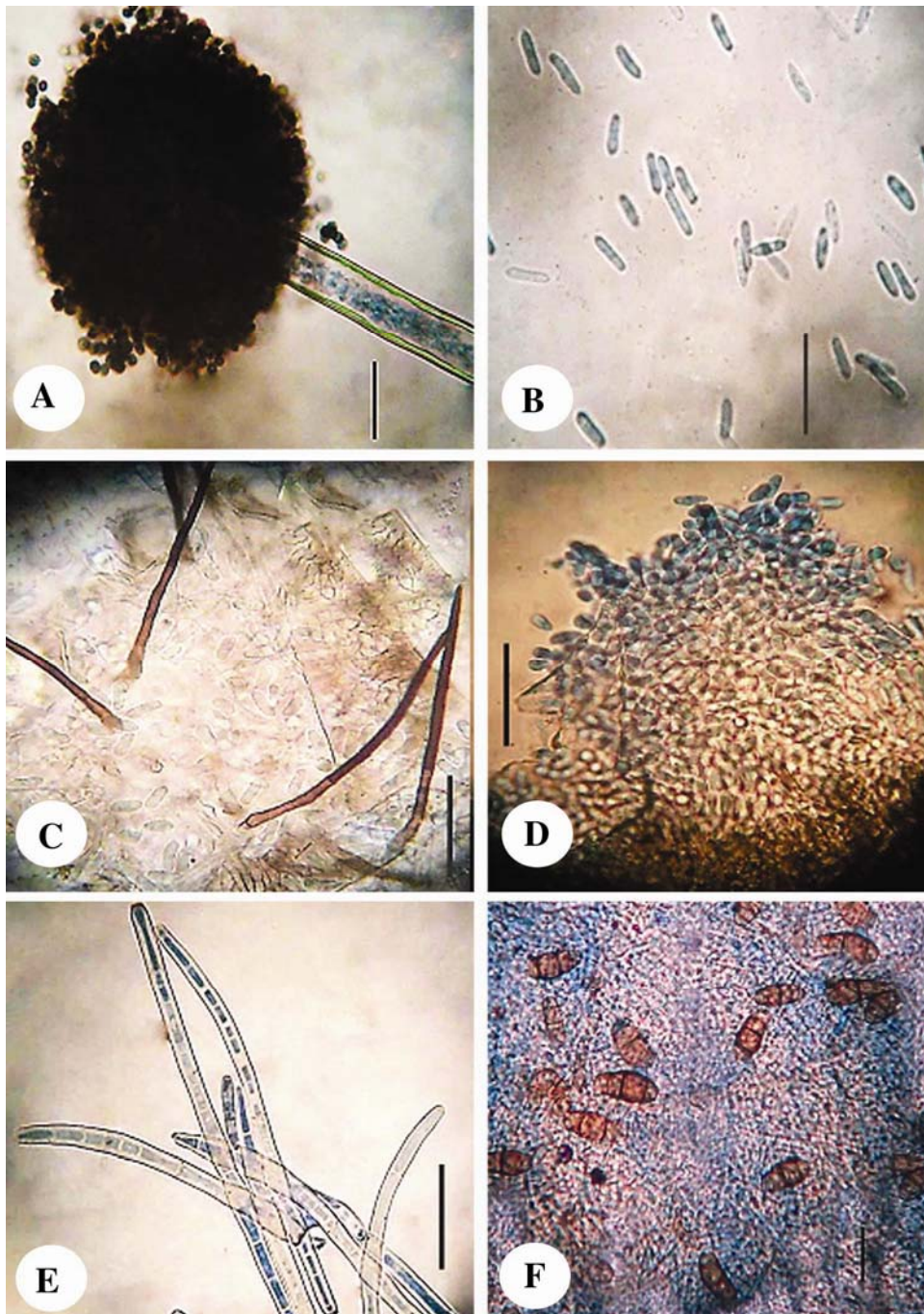


Fig. 2. A. *Aspergillus niger*, B. *Colletotrichum gloeosporioides*, C-D. *C. orbiculare*, E. *Corynespora cassicola* and F. *Curvularia clavata*. (Bar = 50 μ m).

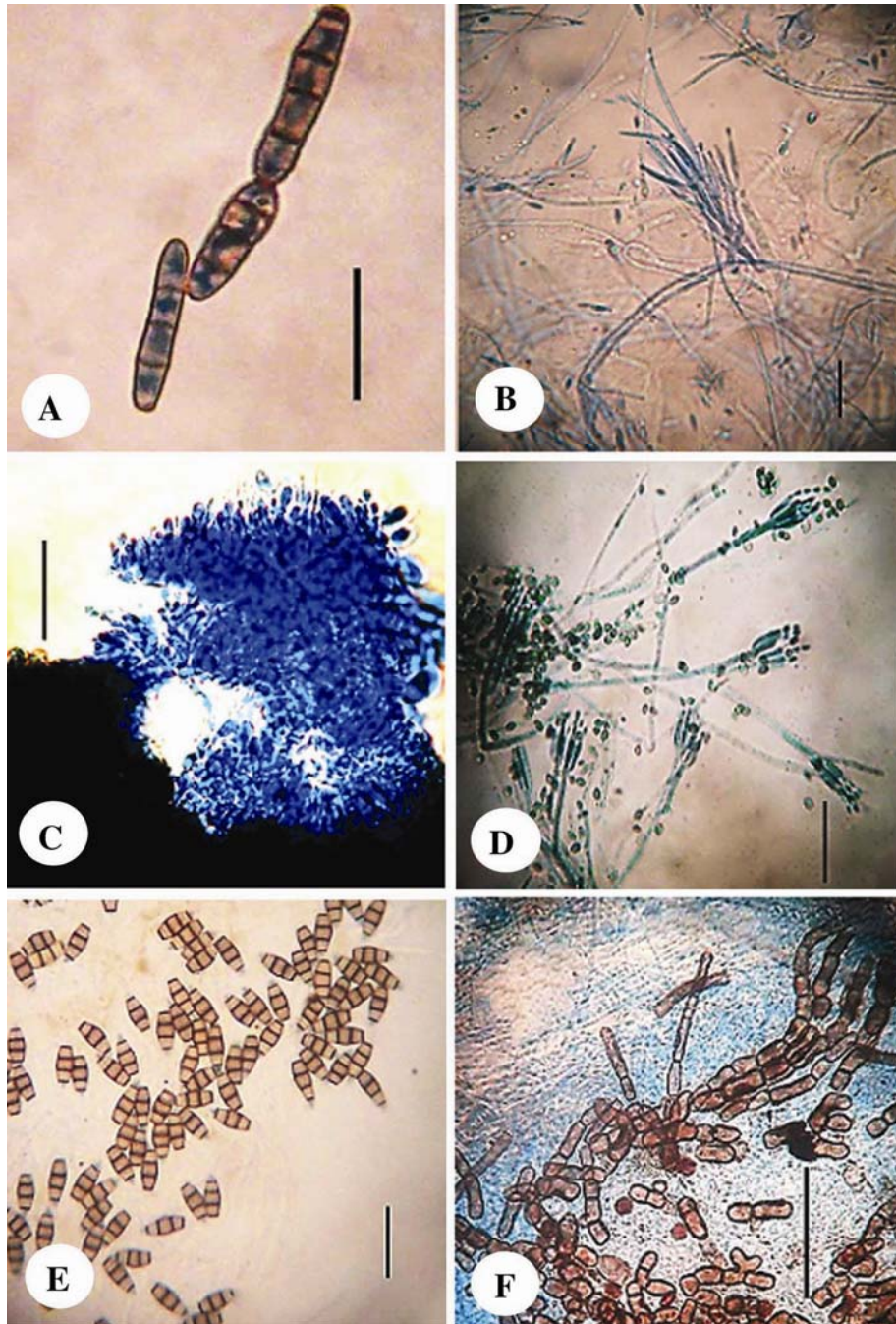


Fig. 3. A. *Dendryphiella vinosa*, B. *Fusarium moniliforme*, C. *Lasiodiplodia theobromae*, D. *Penicillium digitatum*, E. *Pestalotiopsis guepinii*, F. *Xylohypha pinicola*. (Bar = 50 μ m).

8. *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl, Bull. Trimest. Soc. Mycol. Fr. **8**:136 (1892). (Fig. 3C).

Colonies greyish brown, cottony, reverse brownish black. Hyphae septate, branched, dark chocolate brown. Pycnidia globose, dark brown, ostiolate. Conidiophore short, hyaline. Conidia dark brown, two-celled, ellipsoidal, $16-22 \times 8-12 \mu\text{m}$.

Specimen examined: Isolated from infected fruits of *M. cochinchinensis*, Botanical Garden, University of Dhaka, Dhaka, S. Hosen 17, 1 January 2016.

9. *Penicillium digitatum* Sacc., Bur. Anim. Ind., Bul. **118**: 31–33 (1910). (Fig. 3D).

Colony small, cottony, greenish, reverse creamy. Hyphae septate, branched, hyaline. Conidiophores hyaline, septate. Sterigmata equally variable, $15-28 \mu\text{m}$ long and $3.5-5.0 \mu\text{m}$ width. Conidia elliptical to subglobose, smooth aseptate with greenish, tinge commonly $3.5-5.0 \mu\text{m}$ and occasionally up to $10-12 \mu\text{m}$ in diameter. Catenulate.

Specimen examined: Isolated from infected fruits of *M. cochinchinensis*, Botanical Garden, University of Dhaka, Dhaka, S. Hosen 18, 17 December 2015.

10. *Pestalotiopsis guepinii* (Desm.) Stey., Bull. Jard. Bot. État Brux. **19**(3): 312 (1949). (Fig. 3E).

Colonies white, cottony, reverse white. Hyphae septate, branched, hyaline. Acervuli black, small, shining. Conidiophores septate, branched, dark brown, cylindrical or lageniform, formed from the upper cells of the pseudoparenchymata. Conidia fusiform, straight or slightly curved, mostly 3 euseptate: basal cells hyaline, truncate, with an endogenous, cellular, appendage: apical cell conic, hyaline, with 2 or more apica, simple or branched, spatulate or espatulate appendages: median cells brown, sometimes versicoloured, thicker-walled, smooth, $14-23 \times 5-7.5 \mu\text{m}$.

Specimen examined: Isolated from infected fruits of *M. cochinchinensis*, Botanical Garden, University of Dhaka, Dhaka, S. Hosen 19, 6 December 2015.

11. *Xylohypha pinicola* D. Hawksw., Trans. Brit. Mycol. Soc. **64**: 89–99 (1975). (Fig. 3F).

Colonies effuse, dark brown to black. Conidiophores septate, simple or branched, straight or flexuous, mid to dark brown, thick-walled, smooth, up to $160 \mu\text{m}$ long, $4-7 \mu\text{m}$ thick. Conidia in simple or branched acropetal chains ellipsoidal, pale to mid brown, smooth, $4-10 \times 3.5-5 \mu\text{m}$.

Specimen examined: Isolated from infected fruits of *M. cochinchinensis*, Botanical Garden, University of Dhaka, Dhaka, S. Shamsi 3079, 6 December 2015.

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