

## FIRST REPORTS ON FUNGAL PATHOGENS OF IMPROVED JUJUBE IN BANGLADESH

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### ABSTRACT

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A survey was conducted in Bangladesh to identify the fungal diseases of jujube (*Ziziphus mauritiana* Lam.) and their causal pathogens occur in commercial orchards, planted with high yield varieties. The identified diseases and their causal fungi were leaf spots (*Alternaria alternata*, *Cercospora ziziphi*, *Curvularia lunata*, *Fusarium semitectum*, *Lasiodiplodia theobromae*, *Mitteriella ziziphina*, *Pestalotiopsis palmarum*), fruit spots and pre-harvest fruit rot (*A. alternata*, *Colletotrichum gloeosporioides*, *C. lunata*, *F. semitectum*, *L. theobromae*, *M. ziziphina*, *Nigrospora oryzae*, *P. palmarum*, *Phomopsis* sp.), post-harvest fruit rot (*A. alternata*, *Aspergillus*

spp., *C. gloeosporioides*, *C. lunata*, *F. semitectum*, *Nigrospora oryzae*, *Penicillium* spp., *Phomopsis* sp., *P. palmarum*, *Rhizopus* sp.), powdery mildew (*Oidium erysiphoides* f. sp. *ziziphi*), sooty mould or black mold or *Isariopsis* mold (*Isariopsis indica* var. *ziziphi*, *Capnodium* sp.) and stem bleeding or gummosis (*P. palmarum*, *C. lunata*). Among the fungal pathogens causing jujube diseases, *P. palmarum*, *C. gloeosporioides*, *A. alternata*, *F. semitectum*, *C. lunata*, *L. theobromae*, *C. ziziphi*, *Phomopsis* sp., *I. indica* var. *ziziphi*, *M. ziziphina* and *N. oryzae* were noted as new records in Bangladesh.

**Keywords:** Diseases, fungal pathogen, jujube

### INTRODUCTION

Jujube (*Ziziphus mauritiana* Lam.) is a nutritious indigenous fruit of Bangladesh cultivated in homestead since time immemorial. The fruits contain high protein, phosphorus, calcium, carotene and vitamins (Kuliev and Guseinova 1974). Available reports from China and India reveal that jujube is vulnerable to many diseases (Singh and Singh 1979, Rai *et al.* 1982, Sharma *et al.* 1993, Nallathambi *et al.* 1999, Quin and Tian 2004). *Alternaria alternata* causes pre-harvest infection on jujube fruits that result in 25% yield loss in India (Nallathambi 1999, 2001). Moreover, jujube fruits are highly vulnerable to post-harvest color fading, browning, decay, and water loss. Powdery mildew caused by *Oidium erysiphoides* f.sp. *ziziphi* is a major disease of jujube in India. Jamadar *et al.* (2009) found 50-60% loss in fruit yield due to powdery mildew of jujube and reduced market value of the produce. *Pseudocercospora jujubae* a fungal pathogen has been reported from Bangladesh by Khan *et al.* (1983).

In Bangladesh, many diseases appear on foliage and fruits of jujube. In many cases, disease occurrence is an important threat for commercial cultivation of jujube. However, reports on the occurrence of diseases in Bangladesh are scanty (Rahman *et al.* 2011).

Considering the above facts a study was undertaken to survey the occurrence of fungal diseases of jujube and their causal fungi in Bangladesh.

### MATERIALS AND METHODS

Commercial jujube orchards were selected in five jujube growing districts of Bangladesh namely Gazipur, Pabna, Rajshahi, Rangpur and Mymensingh. The orchards were planted with three improved jujube varieties viz. apple Kul, BAU Kul and BARI Kul-1. A total of 405 diseased samples representing 150 fruits, 135 leaves, 30 trunks and 90 inflorescences of jujube were collected from the selected areas during 2007-08, 2008-09 and 2009-10. Among 150 fruit samples 73 were collected from markets of those locations. The collected samples were examined carefully and symptoms of individual diseases were recorded and photographs were taken.

Fungal pathogens associated with the diseased samples were isolated from infected plant samples following standard tissue planting methods using potato dextrose agar (PDA) medium as described by Aneza (2003). Whenever necessary, infected samples were placed in moist chambers and incubate at room (25 ± 2 C) temperature for two days. Fungal mycelia grew from the samples were transferred directly to PDA plates.

Fungi isolated from the collected diseased specimens were purified following single spore culture and/or hyphal tip culture method (Aneza 2003). The isolated fungi were identified based on colony and morphological characters on PDA and consulting available literature (Barnett and Hunter 1972, Sutton 1980). Pathogenicity of different fungal pathogens isolated from diseased samples of jujube was performed following detached leaf and detached fruit inoculation method (Omar and Wahid 2001)

## RESULTS AND DISCUSSION

### Occurrence of diseases in Bangladesh

In Bangladesh, at least seven diseases were found to attack jujube in commercial orchard planted with high yielding varieties. These were leaf spots (*Alternaria alternata*, *Cercospora ziziphi*, *Curvularia lunata*, *Fusarium semitectum*, *Lasiodiplodia theobromae*, *Mitteriella ziziphina*, *Pestalotiopsis palmarum*), fruit spots and pre-harvest fruit rot (*A. alternata*, *Colletotrichum gloeosporioides*, *C. lunata*, *F. semitectum*, *L. theobromae*, *M. ziziphina*, *Nigrospora oryzae*, *P. palmarum*, *Phomopsis* sp.), post-harvest fruit rot (*A.*

*alternata*, *Aspergillus* spp. *C. gloeosporioides*, *C. lunata*,, *F. semitectum*, *Nigrospora oryzae*, *Penicillium* spp., *Phomopsis* sp. *P. palmarum*, *Rhizopus* sp.), powdery mildew (*Oidium erysiphoidess* f. sp. *ziziphi*), sooty mould or black mold or *Isariopsis* mold (*Isariopsis indica* var. *ziziphi*, *Capnodium* sp.) and stem bleeding or gummosis (*P. palmarum*, *C. lunata*). Harvested jujube fruits were found to be attacked by *A. alternata*, *Aspergillus* spp., *C. gloeosporioides*, *C. lunata*, *F. semitectum*, *L. theobromae*, *Nigrospora* sp., *Penicillium* spp., *Phomopsis* sp., *P. palmarum*, *Rhizopus* sp. causing post harvest fruit rot (Table 1).

Table 1. Diseases of jujube occur in Bangladesh and their causal fungi recorded during 2008 to 2010 crop seasons

Diseases	Pathogens
Powdery mildew	<i>Oidium erysiphoidess</i> f.sp <i>ziziphi</i>
Leaf spots	<i>Alternaria alternata</i> , <i>Cercospora ziziphi</i> , <i>Curvularia lunata</i> , <i>Fusarium semitectum</i> , <i>Lasiodiplodia theobromae</i> , <i>Mitteriella ziziphina</i> , <i>Pestalotiopsis palmarum</i>
Sooty, black or <i>Isariopsis</i> mold	<i>Isariopsis indica</i> var. <i>ziziphi</i> , <i>Capnodium</i> sp.
Rust disease	<i>Phakopsora zizyphi-vulgaris</i>
Stem bleeding or gummosis	<i>P. palmarum</i> , <i>F. semitectum</i> , <i>C. lunata</i>
Fruit spots and Pre-harvest fruit rot	<i>A.</i> , <i>Colletotrichum gloeosporioides</i> , <i>C. lunata</i> , <i>F. semitectum</i> , <i>L. theobromae</i> , <i>M. ziziphina</i> , <i>Nigrospora oryzae</i> , <i>P. palmarum</i> , <i>Phomopsis</i> sp.
Post-harvest fruit rot	<i>A. alternata</i> , <i>Aspergillus</i> spp., <i>C. gloeosporioides</i> , <i>C. lunata</i> , <i>F. semitectum</i> , <i>L. theobromae</i> , <i>F. semitectum</i> , <i>N. oryzae</i> , <i>Penicillium</i> spp., <i>Phomopsis</i> sp. <i>P. palmarum</i> , <i>Rhizopus</i> sp.

Altogether 16 fungal pathogens were associated with diseased samples of jujube plants during the study. Of them, association of 11 fungi with jujube is new records in Bangladesh. They are *A. alternata*, *C. ziziphi*, *C. gloeosporioides*, *C. lunata*, *F. semitectum*, *I. indica* var. *ziziphi*, *L. theobromae*, *M. ziziphina*, *Nigrospora* sp., *P. palmarum* and *Phomopsis* sp. Results of their pathogenicity test on inoculated leaves and fruits, and their prevalence in collected samples are described below:

***Alternaria alternata*:** Among the tested samples, one hundred eighty five samples yielded *A. alternata*. Characteristic muriform spores in chain were observed on culture plate as well as on infected plant parts (Plate I A & B). The pathogen causes leaf spot (Plate I C), fruit spot (Plate I D & E) and fruit rot (Plate I F). Symptoms of leaf spots are observed on the upper surface of leaves as small irregular, brown lesions. Dark brown to black spots are

found on the lower surface of leaves. Sometimes several spots coalesce together to form large lesions. On fruits, symptoms appear as slightly depressed, almost circular, brown to black lesions on the surface. Sometimes concentric rings are formed on some spots. Ultimately, whole infected fruits become rotted (Plate 1 F).

***Cercospora ziziphi*:** *Cercospora ziziphi* (Plate II A & B) was isolated from leaf samples. Sometimes it was found on leaves as mixed infection with other fungi like *Pestalotiopsis*. Symptoms appeared on leaves as circular to oval lesions with yellow halo. Initially, spots are yellow and later turn into brown. Finally, spots are surrounded by a dark brown margin. In course of time, spots become larger and visible on both sides of the leaves (Plate II C).

***Colletotrichum gloeosporioides*:** *Colletotrichum gloeosporioides* was isolated from fruit and leaf samples of jujube. The fungus produces disc-shaped or cushion-

shaped and waxy acervuli (Plate III A) containing simple conidiophores bearing conidia. The conidia are hyaline, 1-celled (Plate III B). Under natural conditions, it infects immature fruits causing anthracnose symptoms (Plate III C). Initial symptom of the disease appears as black sunken lesions. On inoculated fruits, the pathogen develops characteristic symptoms of anthracnose (Plate III D).

***Curvularia lunata*:** *Curvularia lunata* was isolated from fruit, leaf, stem and inflorescence samples (Plate IV A). It was found on fruits as mixed infection with other fungi. Due to its infection, dark brown to black irregular lesions are observed on lower surface of leaves (Plate IV B). The upper surface of infected leaves become yellow. Black depressed spots are found on fruits (Plate IV C).

***Isariopsis indica* var. *ziziphi*:** The fungus was isolated from only leaf samples. It causes black leaf spot or *Isariopsis* mold on leaf (Plate VI A). The characteristic symptoms of the disease are sooty tuft-like circular to irregular black spots on ventral surface of leaves. Later, it covers the entire lower surface of leaves giving a sooty appearance (Plate VI B). Septate, dark mycelium and dark synnemata bearing dark conidia form sooty mold (Plate VI C).

***Lasiodiplodia theobromae*:** The pathogen was isolated from fruit samples and leaf samples and identified based on characteristics of mycelia and spore (Plate VII A, B & C). This was found on infected fruits as mixed infection with *P. plamarum*, *C. gloeosporioides*, *F. semitectum* and *A. alternata*. *L. theobromae*. The pathogen causes spots on leaves and fruits (Plate VII D & E) under natural condition as well as inoculated condition (Plate VII F).

***Mitteriella ziziphina*:** *Mitteriella ziziphina* (Plate VIII A & B) was isolated from fruit and leaf samples. The fungus develops almost round and black spots on leaves (Plate VIII C & C<sub>(a)</sub>) and fruits under natural (Plate VIII

D & E) as well as inoculated conditions (Plate VIII F). At advanced stage of infection, severely infected fruits become black and superficial black fungal growth is found on leaves and fruits (Plate VIII C, D & E).

***Nigrospora oryzae*:** The pathogen was isolated from infected fruits samples. It was found on fruits as mixed infection with *Alternaria*, *Lasiodiplodia*, *Fusarium* and *Colletotrichum*. The pathogen causes black discoloration on both young and mature fruits (Plate IX A & B). Apex of the immature fruits becomes shriveled, rotted and ultimately fruit dropping occurs.

***Pestalotiopsis palmarum*:** *Pestalotiopsis palmarum* was isolated from infected leaf, fruit and stem samples and it was identified based on conidia with appendages (Plate X B). The fungus causes leaf spot (Plate X C) and gummosis or stem bleeding diseases (Plate X D). Under natural conditions, irregular brown to dark brown lesions with black dot of acervuli were observed on infected leaves (Plate X C). Fruit spot and fruit rot were also observed (Plate XI D). Spots are brown to dark brown in color, raised and corky. Infected stem becomes rotted and gum like substance comes out from the infected area causing gummosis (Plate X E). Inoculated fruits also formed symptoms similar to natural symptoms (Plate XI C).

***Phomopsis* sp.:** *Phomopsis* sp. was isolated from diseased fruit samples mixed infection with *P. palmarum*, *C. gloeosporioides* and *A. alternata*. Due to infection of *Phomopsis*, initially black dot like water soaked small spots appear on fruits. Later, the spot size increases gradually and several spots coalesce together and form large lesion (Plate XI A). At later stage, fruits become soft and rotted and fruit color turns into pale brown. Pycnidia develop on PDA culture (Plate XI B). Sometimes dot like black pycnidia are found on lesions. Characteristic alpha and beta conidia were observed under microscope (Plate XI C).



A



B



C



D

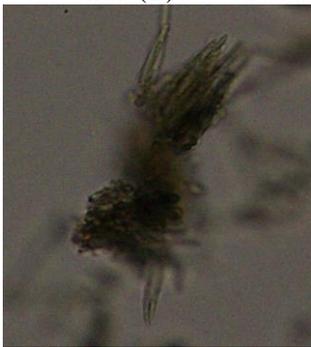


E



F

Plate I. Spore of *Alternaria alternata* (A&B) and *Alternaria* spots on naturally infected leaves (C) and naturally infected fruit (D) as well as inoculated (E) fruits and rotted and mummified fruits of jujube.



A



B



C

Plate II. Conidiophore (A) and conidia (B) of *Cercospora ziziphi* isolated from infected jujube plant samples and leaf spot symptoms developed on jujube leaf (A) after inoculation



A



B

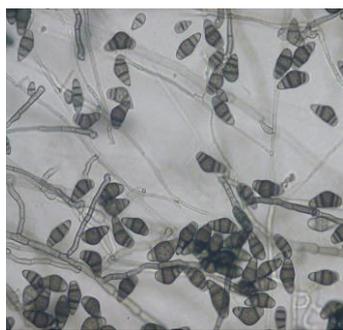


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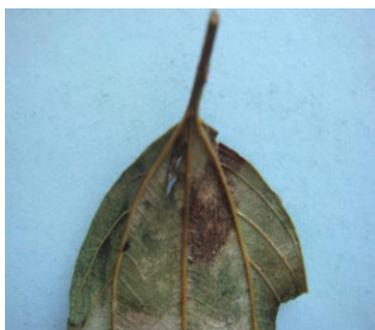


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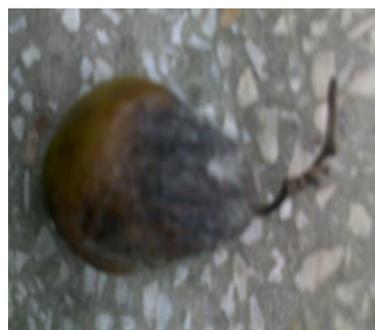
Plate III. Acervulus (A) and conidiophores bearing conidia (B) of *Colletotrichum gloeosporioides* isolated from jujube fruits infected with anthracnose (C) and symptoms on inoculated fruits (D)



A



B



C

Plate IV. Mycelia and conidiophores bearing conidia of *Curvularia lunata* (A) isolated from diseased samples and symptoms of leaf spot (B) and fruit rot (C) of jujube



A



B



C

Plate V. Characteristic conidia (A) of *Fusarium semitectum* isolated from diseased samples of jujube and fruit rot under natural (B) and inoculated conditions (C)



A



B



C

Plate VI. Symptoms of black leaf spot or *Isariopsis* mould on jujube leaves and conidia of *I. indica* var. *ziziphi* [A. Black leaf spot symptoms, B. Severely infected leaves C. Conidia of *I. indica* var. *ziziphi*]

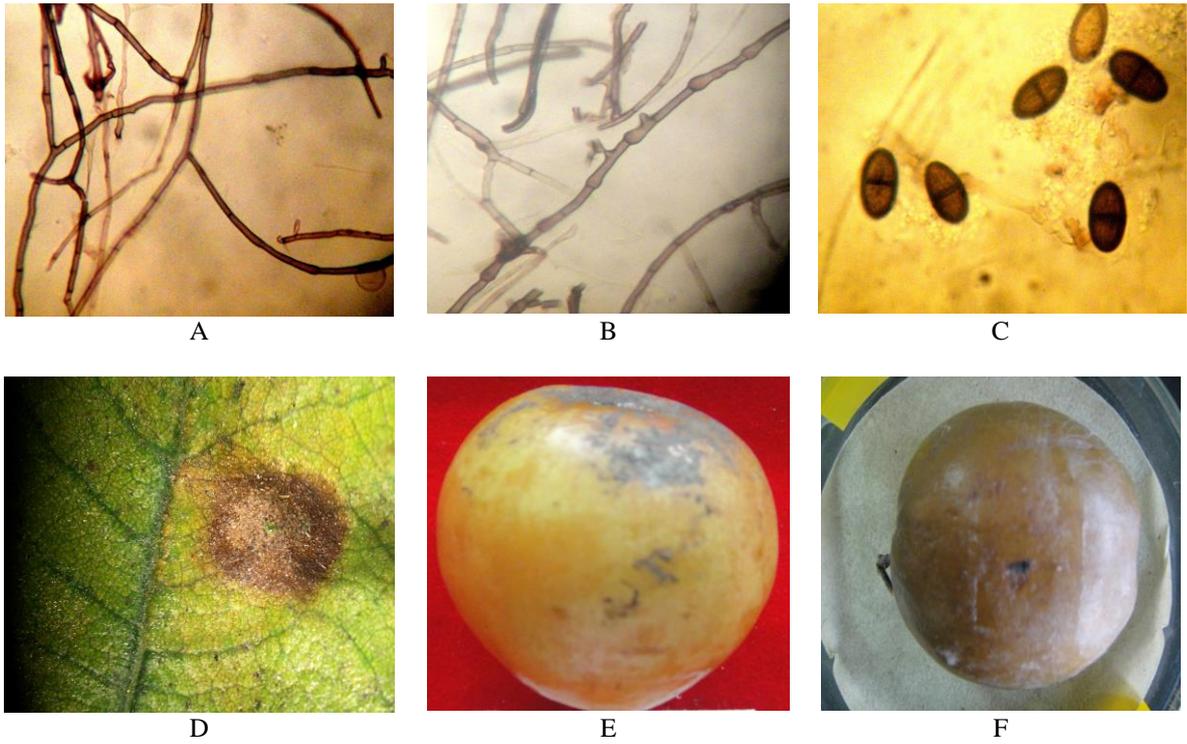


Plate VII. *Lasiodiplodia theobromae* isolated from diseased specimens of jujube [A. Characteristic mycelium, B. Enlarge view of mycelium, C. Characteristic 2-celled conidia, D. Leaf spot symptoms, E. Fruit spot symptoms under natural conditions and F. fruit spot symptoms under inoculated condition]

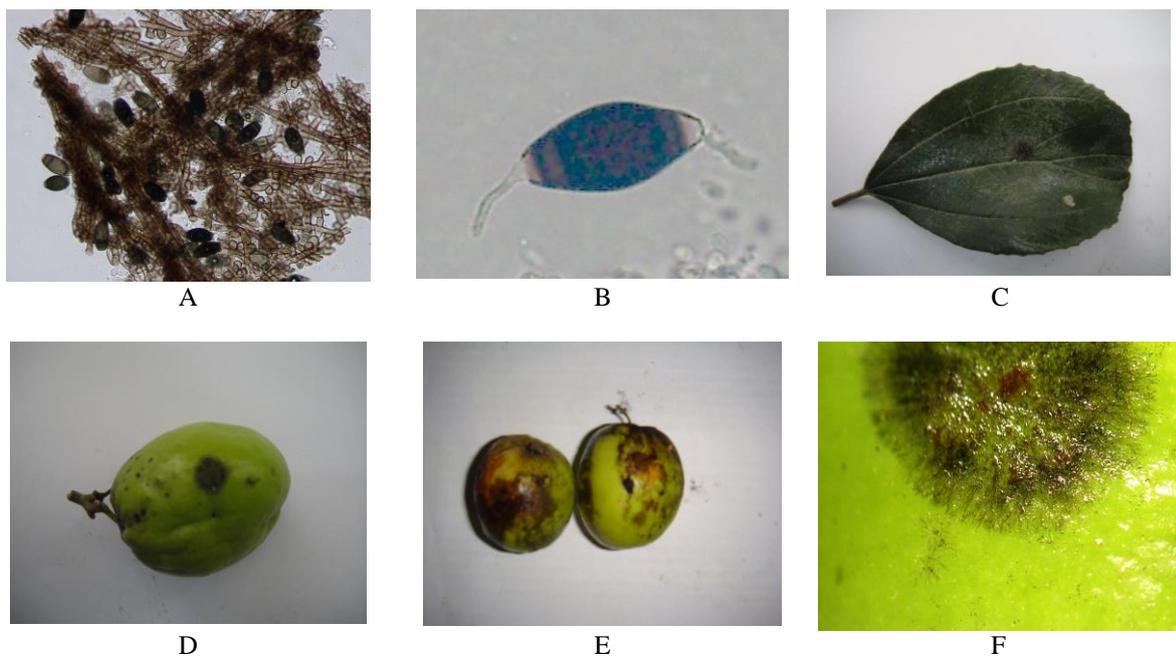
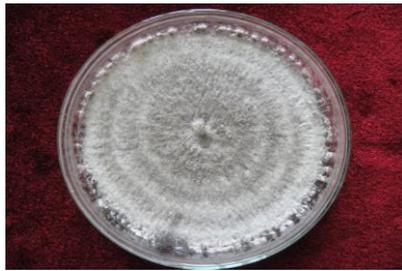
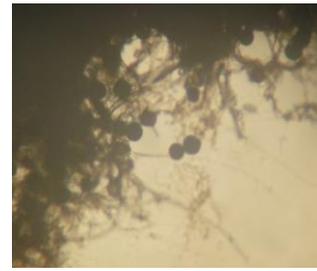


Plate VIII. Symptoms of leaf and fruit spots of Jujube caused by *Mitteriella ziziphina* [A. Dark mycelium, B. Black conidia with two polar germ tubes, C. Leaf spot, D. Black spot on fruit, E. Severely infected fruits, F. Enlarged view of spot on inoculated fruit.]



A

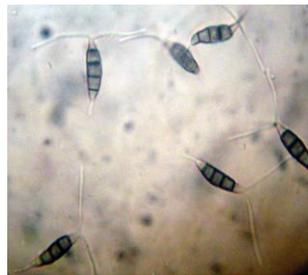


B

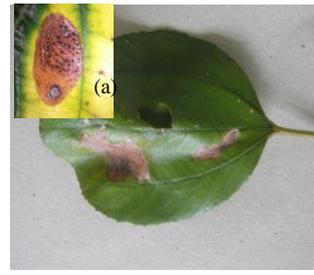
Plate IX. Mycelium, conidiophore and spore of *Nigrospora* sp. isolated from jujube fruits [A. Fungal colony on PDA plate, B. Mycelium and conidiophore bearing conidia]



A



B



C



D



E

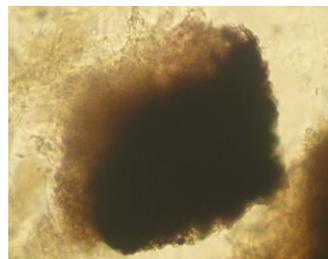


F

Plate X. *Pestalotiopsis palmarum* causing leaf spot, fruit spot, fruit rot and gummosis diseases of jujube [A. Acervuli on PDA, B. Conidia with appendages, C. Leaf lesion symptom, D. Fruit spot fruit rot, E. Stem gummosis diseases and F. Fruit spot on inoculated fruit].



A



B



C

Plate XI. Symptoms of fruit rot of jujube caused by *Phomopsis* sp. [A. fruit rot symptoms, B. Pycnidia and C.  $\alpha$  and  $\beta$  conidia of *Phomopsis* sp.]

Findings of the present investigation reveal that leaf spots, fruit spots and pre-harvest and post-harvest fruit rot, sooty mould or black mold or *Isariopsis* mold and stem bleeding or gummosis are common diseases of high yielding jujube varieties in Bangladesh. After

harvest jujube fruits are attacked by *A. alternata*, *Aspergillus* spp., *C. gloeosporioides*, *C. lunata*, *F. semitectum*, *L. theobromae*, *Nigrospora* sp., *Penicillium* spp., *Phomopsis* sp., *P. palmarum* and *Rhizopus* sp. causing post harvested fruit rot. The diseases and their

causal fungi recorded in Bangladesh also occur in other jujube growing countries of the world. In India, jujube plants are attacked by powdery mildew (*O. erysipoides* var. *ziziphi*), sooty mold (*I. indica* var. *ziziphi*), and leaf spots caused by *A. alternata*, *Cercospora*, *Septoria*, *Cladosporium*, *Pestalotiopsis* (Jamadar *et al.* 2009) and *P. palmarum* (Madan and Gupta 1976, Rai *et al.* 1982). From China, Yuan *et al.* (2009) reported that jujube trees are attacked by powdery mildew, anthracnose (*C. gloeosporioides*), black spot (*A. alternata*) and brown spot (*P. mauritiana*). Jujube fruits are attacked by *A. chartarum*, *A. nanus*, *A. parasiticus*, *H. atroolivaceum*, *P. hessarensis*, and *S. valparadisicum*. Fruit rots are caused by *Fusarium* spp., *N. oryzae*, *Epicoccum nigrum*, and *Glomerella cingulata*. Of the diseases only anthracnose (*C. gloeosporioides*) has been reported earlier from Bangladesh (Rahman *et al.* (2011).

Based on findings of the present investigation, it may be concluded that 16 fungal plant pathogens are associated with improved and high yielding varieties of jujube diseases in Bangladesh. They cause leaf spots, rust, fruit spots and fruit rot, powdery mildew, sooty mold, stem bleeding and post-harvest deterioration of the fruits. Among the fungal pathogens of jujube *A. alternata*, *C. ziziphi*, *C. gloeosporioides*, *C. lunata*, *F. semitectum*, *I. indica* var. *ziziphi*, *L. theobromae*, *M. ziziphina*, *Nigrospora* sp. and *Phomopsis* sp. are recorded as new pathogens of the fruit in Bangladesh

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