Dhaka Univ. J. Biol. Sci. **27**(2): 155-162, 2018 (July)

FUNGI ASSOCIATED WITH LEAVES OF *SONNERATIA APETALA* BUCH. HAM AND *SONNERATIA CASEOLARIS* (L.) ENGLER FROM RANGABALI COASTAL ZONE OF BANGLADESH

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Key words: Fungi, *Sonneratia apetala, Sonneratia caseolaris,* Coastal zone

Abstract

A total of six species and one genus of fungi associated with black leaf spot of *Sonneratia apetala* Buch. Ham (Kewra); and anthracnose and small leaf spot of *S. caseolaris* (L.) Engler (Choila) were isolated following "Tissue planting" method. The fungi associated with black spot of *S. apetala* were *Aspergillus fumigatus* Fresenius, *Colletotrichum lindemuthianum* (Sacc. & Magn.) Br. & Cav., *Pestalotiopsis guepinii* (Desm.) Stey. and *Phoma betae* Frank. Anthracnose of *S. caseolaris* showed the association of *A. fumigatus* and *P. guepinii.* The fungi associated with leaf spot of *S. caseolaris* were *Curvularia fallax* Boedijn, *Fusarium* Link, *Penicillium digitatum* (Pers.) Sacc. and *P. betae*. Frequency percentage of association of *P. guepinii* was highest (74.10) in black spot of *S. apetala* whereas the same fungus showed highest frequency percentage (85.70) in case of anthracnose of *S. caseolaris*. *Phoma betae* showed highest frequency percentage (60.00) in leaf spot of *S. caseolaris*. *Phoma betae* is first time recorded from Bangladesh.

Introduction

Sonneratia is a medium large size columnar tree belonging to the Sonneratiaceae family[1]. *Sonneratia apetala* Buch. Ham (Kewra) and *S. caseolaris* (L.) Engler (Choila) are the most important species of *Sonneratia. Sonneratia apetala* is a mangrove plant and it grows as tree along seaward fringes and intertidal areas. It is one of the main, most successful and pioneer planting species of the coastal afforestation program along the coastal belt of Bangladesh. Nowhere else in the world, mangrove plantation has been raised with such a large scale kewra plantation along the coastal belt in Bangladesh[2]. *Sonneratia caseolaris* is a typical non-viviparous mangrove species and a key component of mangrove community in the Indo-West Pacific region[3].

Various biotic and abiotic stresses are responsible for the damage of *S. apetala* and *S. caseolaris.* Among them fungal diseases play an important role for the severe damage of these plants[4]. Most important fungal diseases of mangrove plants are leaf blight, dieback, prop root rot and seed rot, wilt, quick decline and lethal yellowing, leaf blight,

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bleeding disease, leaf necrosis, anthracnose, sooty mold, leaf and fruit rot, powdery mildew, rusts, etc.[4]. Little information is available about factors governing the distribution, incidence or effects of fungi that infect leaves of mangrove species[5]. Foliar diseases have been shown to have significant effects on plant survival, growth and fitness in natural ecosystems[6,7]. Sarma and Vittal[8] reported 88 fungi belonging to 9 mangrove plant species from Godavari and Krishna deltas (Andhra Pradesh), east coast of India. Like all other mangrove plants disease free *S. apetala* and *S. caseolaris* plants are essential for ecological, economical and management point of view. Data are scare on fungal diseases of *S. apetala* and *S. caseolaris.* Although some research works have been done on the ecological condition of the coastal zone of Bangladesh[9,10]. So far no report is available regarding fungal diseases of *S. apetala* and *S. caseolaris* in Bangladesh. The present investigation was undertaken to find out the fungi associated with infected leaves of *S. apetala* and *S. caseolaris* in Rangabali coastal zone of Bangladesh.

Materials and Methods

Leaves of *Sonneratia apetala* showing black spot and *S. caseolaris* showing anthracnose and small leaf spot symptoms (Fig. 1) were collected and examined from Rangabali, Patuakhali during the tenure of December, 2016 to May, 2017.

Samples were collected in separate sterile polyethylene bags, labeled properly and then brought to the laboratory for isolation of fungi. The fungi associated with leaves were isolated following "Tissue planting method" on PDA medium[11]. One hundred inocula, each measuring 2 mm^2 long were cut with a pair of sterilized scissors from the infected leaves 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 in an incubator (25 ± 2°C) for 7 days. Percentage frequency of the occurrence of the fungal isolate was calculated by adopting the formula of Spurr and Wetly (1972)[12]:

$$\% \text{ frequency} = \frac{\text{Total number of inocula from which a fungal isolate was observed}}{\text{Total number of inocula}} \times 100$$

The microscopic studies of the associated fungi were done following Khan and Shamsi[13]. Identification of the isolates was determined following the standard literatures[14-18].

Fig. 1. A. Black leaf spot of *Sonneratia apetala*. B. leaf spot (a) and anthracnose (b) of *S. caseolaris*.

Results and Discussion

Six species and one genus of fungi associated with black spot of *S. apetala*; and anthracnose and small leaf spot of *S. caseolaris* were isolated. The fungi associated with black spot of *S. apetala* were *Aspergillus fumigatus, Colletotrichum lindemuthianum*, *Pestalotiopsis guepinii* and *Phoma betae*. Leaves of *S. caseolaris* with anthracnose symptom showed the association of *A. fumigatus* and *P. guepinii*. The fungi associated with small leaf spot of *S. caseolaris* were *Curvularia fallax*, *Fusarium* sp., *Penicillium digitatum* and

Phoma betae. Table 1 showed that for black spot of *S. apetala* the per cent frequency of *Pestalotiopsis guepinii* was highest (74.10) followed by *Colletotrichum lindemuthianum* (14.80), *Phoma betae* (7.40) and *A. fumigatus* (3.70). Table 1 also showed that for anthracnose of *S. caseolaris* the per cent frequency of *A. fumigatus* and *Pestalotiopsis guepinii* was 14.30 and 85.70, respectively. Per cent frequency of *Phoma betae* was highest (60) in *S. caseolaris* with small leaf spot symptom followed by *Curvularia fallux* (20.00), *Fusarium* sp. (13.33) and *Penicillium digitatum* (6.67).

Xing *et al.*[19] identified a total of 391 fungal isolates and recovered 39 distinct endophytic species, of which *Cytospora*, *Diaporthe*, *Fusarium*, *Glomerella*, *Mycosphaerella*, *Phoma*, *Phomopsis* and *Stemphylium* were the dominant fungal taxa from five mangrove species *viz*., *Sonneratia apetala, S. caseolaris, S. hainanensis, S. ovata, S. paracaseolaris* in the south coast of China.

Table 1. Per cent frequency of association of fungi with infected leaves of *Sonneratia apetala* and *S. caseolaris*.

Name of the fungi	Per cent frequency of isolated fungi		
	Sonneratia apetala	*Sonneratia caseolaris*	
	Black spot	Anthracnose	Small leaf spot
Aspergillus fumigatus	3.70	14.30	-
Colletotrichum lindemuthianum	14.80	-	-
Curvularia fallax	-	-	20.00
Fusarium sp.	-	-	13.33
Penicillium digitatum	-	-	6.67
Pestalotiopsis guepinii	74.10	85.70	-
Phoma betae	7.40	-	60.00

- = Respective fungus did not show mycelia growth.

Anamorphic fungus *Phoma* belongs to the class Coelomycetes comprises of more than 3000 species[20]. Morphological structures were done in detail with the aid of Camera Lucida. The genus is characterized by its hyaline or brown septate mycelium; unilocular, brown, globose, separate or aggregated pycnidia; hyaline, aseptate or occasionally 1 sepatate, ellipsoid, cylindrical, fusiform, pyriform or globose conidia. After critical observation the fungus was identified as *Phoma betae* Frank. A detailed survey of literatures revealed that *Phoma betae* has not been reported in any relevant literatures in Bangladesh[21,22]. Hence, *Phoma betae* Frank. is reported here as first time from Bangladesh.

Taxonomic descriptions of fungal taxa associated with infected leaves of *Sonneratia apetala* and *S. caseolaris*

1. **Aspergillus fumigatus** Fresenius (1863) (**Fig. 2A**)

Colonies greenish. Mycelia profusely branched, septate. Cells multinucleate. Conidiophores long. Conidia catenulate, dry, usually globose, smooth, brown. Colonies of the fungus produced thousands of minute gray green conidia (2 - 3 µm).

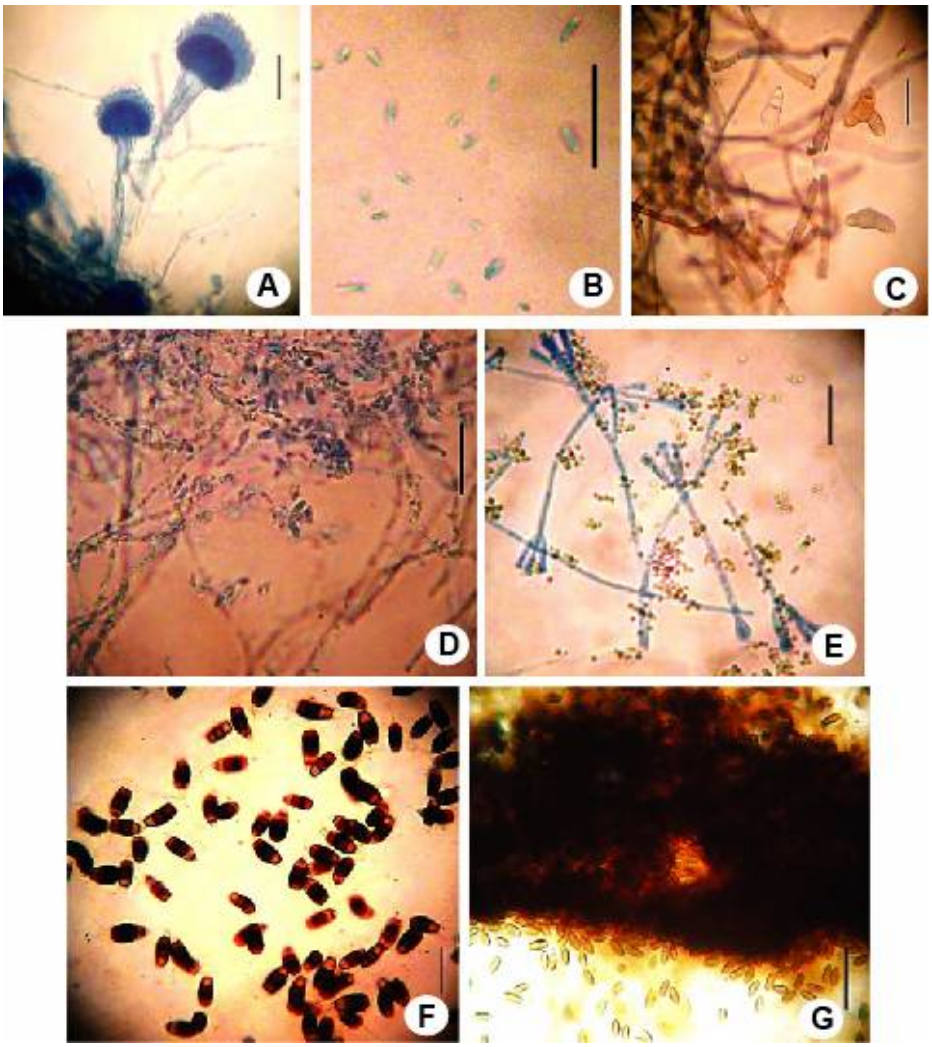

Fig. 2: A. Mycelium, conidiophore, vesicle and conidia of *Aspergillus fumigatus*; B. Conidia of *Colletotrichum lindemuthianum*; C. Mycelium, conidiophore and conidia of *Curvularia fallax*; D. Mycelium and conidia of *Fusarium* sp.; E. Conidiophore, sterigmata and conidia of *Penicillium digitatum*; F. Mycelium and conidia of *Pestalotiopsis guepinii* and G. Conidia and pycnidia of *Phoma betae*. (Bar = 50 µm).

Specimen examined: Isolated from infected leaves of *S. caseolaris*, Rangabali, S. Hosen 20, 15 January, 2017.

2. **Colletotrichum lindemuthianum** (Sacc. & Magn.) Br. & Cav., Fung. Parass. **50** (1889) (**Fig. 2B**)

Colonies slow growing, dark brown to black, margin regular, immersed. Hyphae septate, sub-hyaline. Conidia straight, cylindrical, obtuse at the apices 9.5 - 11.5 × 3.5 - 4 μm.

Specimen examined: Isolated from infected leaves of *S. apetala*, Rangabali, S. Shamsi 3086, 15 January, 2017.

3. **Curvularia fallax** Boedijn (**Fig. 2C**)

Colonies effuse grayish black. Conidiophores brown, straight or flexuous, septate, unbranched, often geniculate, smooth. Conidia olivaceous brown, solitary, simple, 3-4 septate, mostly curved, clavate, ellipsoidal, obovoid or pyriform, occasional triradiate stauroconidia at the same time as normal conidia, smooth, 20 - 34× 9 - 16 μm.

Specimen examined: Isolated from infected leaves of *S. caseolaris*, Rangabali, S. Shamsi 3087, 25 January, 2017.

4**. Fusarium** Link (**Fig. 2D**)

Colonies white, cottony, reverse light pink. Mycelium hyaline, septate, profusely branched. Conidiophores hyaline, short, bearing whorl of phialides. The conidiophores may consist of a single basal cell bearing 2 - 3 apical phialides. Macroconidia absent. Microconidia were thin-walled, with an elongated, often sharply curved apical cell and pedicellate basal cell, aseptate. Chlamydospores absent both in mycelium and conidia.

Specimen examined: Isolated from infected leaves of *S. caseolaris*, Rangabali, S. Hosen 21, 25 January, 2017.

5. **Penicillium digitatum** Sacc., Bur. Anim. Ind., Bul. **118**: 31-33 **(**1910) (**Fig. 2E**)

Colony small, cottony, greenish, reverse creamy. Hyphaeseptate, branched, hyaline. Conidiophores hyaline, septate. Sterigmata equally variable, 15 - 28 μm long and 3.5 - 5.0 μm width. Conidia elliptical to subglobose, smooth aceptate with greenish, tinge commonly 3.5 - 5.0 μm and occasionally up to 10 - 12 μm in diameter. Cattenulate.

Specimen examined: Isolated from infected leaves of *S. caseolaris*, Rangabali, S. Hosen 22, 25 January, 2017.

6. **Pestalotiopsis guepinii** (Desm.) Stey., Bull. Jard. Bot. État Brux. **19**(3): 312 **(**1949) (**Fig. 2F**)

Colonies white, cottony, reverse white. Hyphae septate, branched, hyaline. Acervuli black, small, shining. Conidiophores septate, sub-hyaline. 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, spathulate or espathulate appendages: median cells brown, thicker-walled, smooth, 14 - 23 × 5 - 7.5 μm.

Specimen examined: Isolated from infected leaves of *S. apetala*, Rangabali, S. Hosen 22, 14 March, 2017.

7. Phoma betae Frank, Z. Ver. Rubenz. Indus. Dtsch. Reichs**. 42**: 904 (1892) (**Fig. 2G**)

Colonies olivaceous brown, reverse greenish brown. Pycnidia abundant in areas lacking aerial mycelim. Conidia abundant, straight, ellipsoid, 6 - 10 × 3.5 - 5.0 μm.

Specimen examined: Isolated from infected leaves of *S. caseolaris*, Rangabali, S. Shamsi 3088, 20 March, 2017.

Coastal afforestation is very important for Bangladesh. *Sonneratia apetala* and *S. caseolaris* are most important mangrove plant species in coastal region like Rangabali, Patuakhali. Fungal diseases of the plants are one of the major constraints for the development of mangrove forest. This is the first report of fungi associated with aforesaid plants. Present investigation will be helpful for the production of healthy plants as well as seeds to maintain harmony and management of the ecosystem.

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(Manuscript received on 12 February, 2018; revised on 2 May, 2018)