

PREVALENCE AND PATHOGENIC POTENTIALITY OF FUNGI ASSOCIATED WITH LEAF SPOT OF *BASELLA ALBA* AND *B. RUBRA*

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Abstract

Two major species of Indian spinach, namely *Basella alba* L. and *B. rubra* L. were selected to find out associated mycoflora and their pathogenic potentiality. Ten fungi viz., *Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Colletotrichum dematium*, *C. lindemuthianum*, *Curvularia lunata*, *Drechslera sacchari*, *Fusarium semitectum* and *Penicillium* sp. were found to be associated with the selected leaves of *Basella* spp. Among the isolated fungi *C. lindemuthianum*, *D. sacchari* and *F. semitectum* were found to be pathogenic to *Basella* spp. Association of *C. lindemuthianum* and *D. sacchari* with *Basella* spp. are recorded first time from Bangladesh.

Introduction

Basella alba L. and *B. rubra* L. commonly called Indian spinach belong to Basellaceae⁽¹⁾. Both the species are widely grown in Bangladesh and are considered to be excellent sources of essential nutrients⁽²⁾. *Basella alba* is the green variety while *B. rubra* has red stalk and lightly reddish purple on the lower sides of its leaves⁽³⁾. Due to easy adaptation to a variety of soils and climates *B. alba* is considered one of the best tropical spinach throughout the tropical world⁽⁴⁾. As *B. alba* is a succulent crop that contains 90.50% moisture, it is highly vulnerable to microbial infection, resulting leading to spoilage and loss of quality⁽⁵⁾. *Basella* spp. are severely attacked by fungi causing leaf spot, anthracnose, powdery mildew and leaf rot disease. Out of these diseases, leaf spot is a major disease of *Basella* spp. which causes red spots in leaves.

Fusarium spp. and *Cladosporium* spp. can greatly damage the *Basella alba* leaves⁽⁶⁾. Sixteen different diseases of Indian spinach have so far been reported from different parts of the world⁽⁷⁻⁸⁾. A good number of work have been done to identify the fungi associated with leaf spot abroad but little work has been done on *Basella* spp. in Bangladesh⁽⁹⁻¹⁰⁾. So, the present investigation was undertaken to identify the fungi associated with leaf spot of *Basella alba* and *B. rubra* and to screen out their pathogenic potentiality.

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Materials and Methods

The samples were collected from three different markets of Dhaka city *viz.*, 'Karwan bazar', 'Anando bazar' and 'Polashi bazar' during April to November, 2017. Fungi associated with the leaf spot of *Basella* spp. were isolated separately following Tissue planting method⁽¹¹⁾. The fungi were isolated from the infected surface sterilized inocula and their microscopic structural view was taken by a digital camera. Identities of the isolates were determined following previous reports⁽¹²⁻¹⁷⁾. Percentage frequency of occurrence of the fungal isolates was calculated by adopting the formula of Spurr and Wetly⁽¹⁸⁾.

All the isolated fungi were tested for their pathogenic potentiality has been done on detached leaf assay followed by Azad and Shamsi⁽¹⁹⁾ with slight modification. Healthy mature leaves of *Basella* spp. were thoroughly washed under running tap water and then surface was disinfected in 10% Chlorox for 3 minutes. Excessive chlorox was removed by placing leaves on two layers of sterile autoclaved filter paper on Petri plate. The leaves were inoculated with 5 mm mycelial block that were previously grown on PDA medium and incubated for seven days at $25 \pm 2^\circ\text{C}$. Disease symptoms were recorded after 5 days of inoculation. Re-isolation of the pathogen was made from the artificially infected *Basella* leaf following the usual procedure. The morphological characters of the re-isolated fungi were compared with the original isolates by which they were inoculated.

Results and Discussion

The fungi associated with the leaf spot of *Basella* spp. and their per cent frequency of occurrence are shown in Tables 1 & 2. Ten fungi were isolated from *Basella alba* (Table 1) and nine from *B. rubra* (Table 2). The fungi were *Alternaria alternata* (Fr.) Keissler, *Aspergillus flavus* Link., *A. fumigatus* Fresineus, *A. niger* van Tieg., *Colletotrichum dematium* (Pers.) Kx. Fr., *C. lindemuthianum* (Sacc. & Magn) , *Curvularia lunata* Wakker, *Drechslera sacchari* Butler) Subram. & Jain, *Fusarium semitectum* Berk. & Rav. and a genus of *Penicillium* Link.

The highest mean per cent frequency of fungi associated with infected leaves of *Basella alba* was *Colletotrichum dematium* (18.24) which was followed by *C. lindemuthianum* (10.03), *Alternaria alternata* (9.89), *Penicillium* sp. (8.14), *A. flavus* (7.89), *Fusarium semitectum* (7.18), *A. niger* (6.61), *Drechslera sacchari* (5.27), *A. fumigatus* (4.66) and *Curvularia lunata* (2.00) (Table 1).

Table 2 showed that the highest mean per cent frequency of fungi associated with the leaves of *B. rubra* was *Alternaria alternata* (16.49), which was followed by *Penicillium* sp. (13.02), *A. fumigatus* (8.61), *Colletotrichum lindemuthianum* (8.50), *A. niger* (8.85), and *Curvularia lunata* (7.52), *Fusarium semitectum* (6.67), *A. flavus* (6.19) and *C. dematium* (2.39).

Table 1. Per cent frequency of fungi associated with leaf spot of *Basella alba* collected from different markets of Dhaka city at different intervals.

Fungi	Anando	Karwan	Anando	Anando	Polashi	Mean
	bazar	bazar	bazar	bazar	bazar	
	(26-03-17)	(06-04-17)	(10-04-17)	(21-05-17)	(24-05-17)	
<i>Alternaria alternata</i>	6.45	13.04	16.67	3.33	10	9.89
<i>Aspergillus flavus</i>	16.13	-	10	6.67	6.66	7.89
<i>A. fumigatus</i>	-	-	3.33	3.33	16.66	4.66
<i>A. niger</i>	-	13.04	-	20	-	6.61
<i>Colletotrichum dematium</i>	16.13	21.74		40	13.33	18.24
<i>C. lindemuthianum</i>	29.03	-	10	-	11.11	10.03
<i>Curvularia lunata</i>	-	-	-	-	10	2.00
<i>Drechslera sacchari</i>	9.67	-	6.67	10		5.27
<i>Fusarium semitectum</i>	12.90	13.04	10	-	-	7.18
<i>Penicillium sp.</i>	-	17.39	3.33	-	20	8.14

- = represents no growth of respective fungus.

Table 2. Per cent frequency of fungi associated with leaf spot of *Basella rubric* collected from different markets of Dhaka city at different intervals.

Fungi	Anando	Karwan	Anando	Anando	Polashi	Mean
	bazar	bazar	bazar	bazar	bazar	
	(18-04-17)	(28-05-17)	(06-06-17)	(11-06-17)	(12-07-17)	
<i>Alternaria alternata</i>	34.61	4.76	11.63	17.95	13.51	16.49
<i>Aspergillus flavus</i>	-	-	4.65	12.82	13.51	6.19
<i>A. fumigatus</i>	3.84	-	4.65	10.26	24.32	8.61
<i>A. niger</i>	-	9.52	13.95	7.69	8.11	7.85
<i>Colletotrichum dematium</i>	3.84	-	-	-	8.11	2.39
<i>C. lindemuthianum</i>	3.84	-	25.58	7.69	5.40	8.50
<i>Curvularia lunata</i>	-	4.76	11.63	7.69	13.51	7.52
<i>Fusarium semitectum</i>	7.69	7.14	-	7.69	10.81	6.67
<i>Penicillium sp.</i>	7.89	11.90	16.28	12.82	16.22	13.02

- = represents no growth of respective fungus.

Out of ten fungal species isolated from *Basella* spp., *Colletotrichum lindemuthianum*, *Drechslera sacchari* and *Fusarium semitectum* developed characteristic leaf spot symptoms on the inoculated leaf surface after 10 - 15 days of incubation in pathogenicity test. They were successfully re-isolated and fulfilled Koch's postulates.

Taxonomic descriptions of the pathogenic fungi associated with leaf spot of *Basella* spp. are given below.

Colletotrichum lindemuthianum Sacc. & Magn.) Br. & Cav. *FunghiParass.* **50**(1889).

(Fig. 1A-C)

Colonies slow growing, dark brown to black, with abundant brown aerial mycelium, margin regular and even. Setae present. Conidia straight, cylindrical, obtuse at the apices, 9.5-11.5 × 3.5- 4.5 μm. Appressoria sparse, pale to dark brown, clavate or circular in outline, regular, 8 × 6-7 μm, not becoming complex.

Material studied: Isolated from leaf spot of *Basella alba* and *B. rubra*, collected from Anando bazar, Dhaka. NJ Shova 01, 26 March 2017.

Drechslera sacchari (Butler) Subram. & Jain, *Curr. Sci.*, **35**: 354(1966).

(Fig. 1D-F)

Colonies grey, brown or blackish brown. Conidiophores arising singly or in small fascicles, often from groups of dark cells which form loose stromata, straight or flexuous, mid to dark brown, paler towards the apex, up to 200 μm long, 5 - 8 μm thick (up to 700 × 10 μm in culture). Conidia slightly curved, occasionally straight, cylindrical or narrowly ellipsoidal, mid pale to mid golden brown, with 5-9 (mostly 8) pseudosepta, 35 - 96 (65) × 9 - 17 (13.8) μm, hilum 2 - 3 μm wide.

Material studied: Isolated from leaf spot of *Basella alba* and *B. rubra*, collected from Anando bazar, Dhaka. NJ Shova 3, 26 March 2017.

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

(Fig. 1G-I)

Cultures at first white peach tinge and peach coloured from below. Aerial mycelium floccose, peach gradually changing buff brown (14 - 21 days). Sporodochia absent. Macroconidia formed in aerial mycelium from loosely branched conidiophores. Each branch terminates in a conidiogenous cell 19 - 24 × 2 - 4 μm. The conidia mostly from 3 to 5 septate and are curved with a wedge-shaped., 3 septate 17- 28 × 2.5 - 4 μm and 5 septate 22 - 40 × 3.7 - 4 μm.

Material studied: Isolated from leaf spot of *Basella alba* and *B. rubra*, collected from Anando bazar, Dhaka. NJ Shova 5, 26 March 2017.

In contrast to the present study, Akter and Shamsi reported a total of 12 species of fungi with *Basella alba* and *B. rubra*⁽¹⁰⁾. The isolated fungi were *Alternaria alternata* (Fr.) Keissler, *Ascochyta* sp., *Aspergillus flavus* Link., *A. fumigatus* Fresenius, *A. niger* van Tieghem, *A. terreus* hom., *Colletotrichum dematium* (Pers. Ex Fr.) Grove, *Curvularia pallescens* Boedijn, *Fusarium oxysporum* Schlecht, *Macrophomina phaseolina* (Tassi) Goid and two species of *Penicillium*. Eboh and Okoh isolated *Fusarium* sp, *Aspergillus flavus*, *A. niger* and *Mucor* sp. from the leaves of *Basella alba* and *B. rubra* in Nigeria⁽²⁰⁾. Ogunbusola

et al.⁽²¹⁾ reported species of *Pullularia*, *Mucor*, *Rhizopus*, *Microsporium*, *Absidia* and *Aspergillus* associated with *Basella alba* and *B. rubra*. Dhar *et al.* (2015)⁽⁶⁾ observed that the pathogenic fungi namely, *Fusarium* spp. and *Cladosporium* spp. can greatly damage the *Basella alba* leaves.

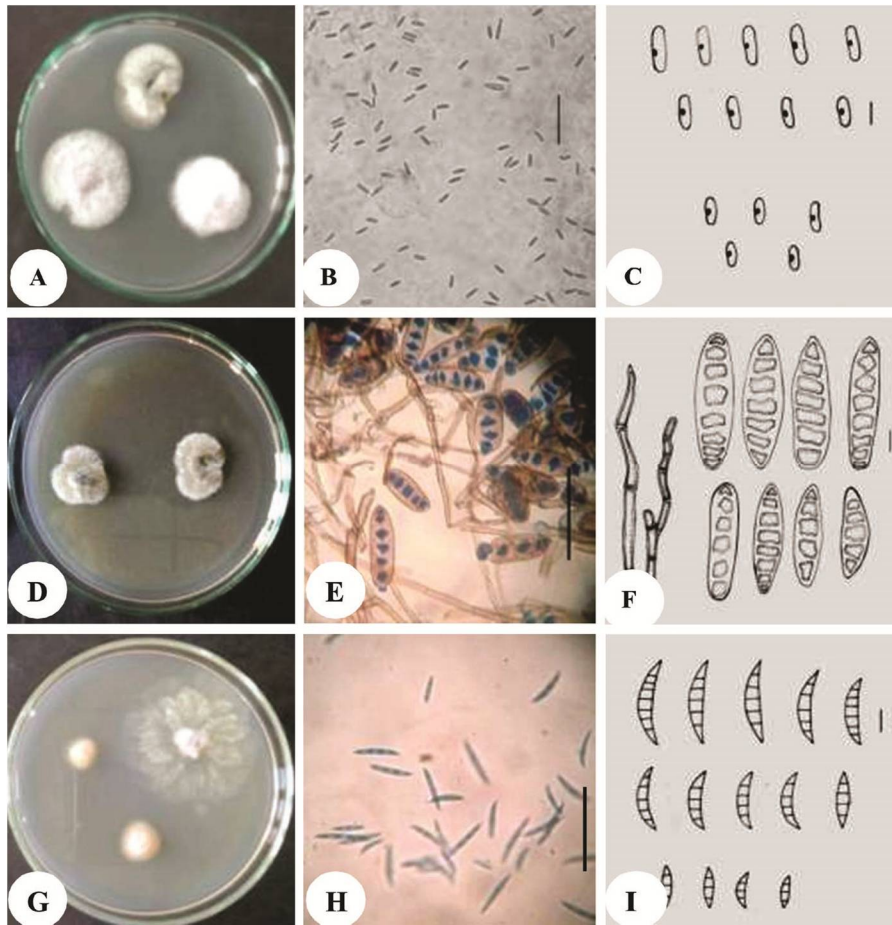


Fig. 1. Colonies, conidia (Bar = 50 μ m) and Camera Lucida (Bar = 11 μ m) of re-isolated fungi from *Basella* spp. A - C: *Colletotrichum lindemuthianum*, D - F: *Drechslera sacchari* and G - I: *Fusarium semitectum*.

Ten fungi were isolated from the leaf spot of *Basella*. Among them *C. lindemuthianum*, *D. sacchari* and *F. semitectum* were found to be pathogenic. Present findings will be helpful to develop the protocol of control measure of the pathogens. So far, association of *C. lindemuthianum* and *D. sacchari* with *Basella* spp. has not been reported in Bangladesh⁽²²⁻²⁴⁾. Therefore, association of the aforesaid fungi with *Basella* spp. is reported here as first time.

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