

FUNGI ASSOCIATED WITH TWO SPECIES OF *OXALIS*

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Abstract

The present piece of research work was conducted to determine fungi associated with healthy and diseased leaves of *Oxalis* species. Tissue planting methods using potato dextrose agar medium was followed for isolation of fungi. Twenty two species of fungi representing 13 genera were found to be associated with *Oxalis corymbosa* (DC.) Lourteig and *O. latifolia* L. The isolated fungi were *Alternaria alternata* (Fr.) Keissler, *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire, *Aspergillus flavus* Link., *A. niger* Van Tiegh., *Chaetomella raphigera* Fekl., *Chaetomium* sp. Kunze, *Cladosporium oxysporum* (Harz) Nannf., *Cladosporium* sp., *Colletotrichum coffeanum* Noack, *C. dematium* (Pres. Ex Fr.) Grove, *C. falcatum* Went, *C. gloeosporoides* (Penz) Sacc., *Curvularia brachyspora* Boedijn, *C. lunata* Boedijn., *C. pallescens* Boedijn., *Gloeosporium* Besm and Mont, *Nigrospora oryzae* Hudson, *Penicillium* sp., *Pestalotiopsis guepinii* (Desm.) Stey., *Pestalotiopsis* sp., *Pestalozziella subsessilis* Sacc. and Ellis and *Rhizopus stolonifer* (Ehrenb. Ex. Fr.) Lind. Prevalence of the fungi varied with species of *Oxalis* as well as healthy and diseased leaves. Pathogenicity of the fungi was tested following detached leaf method. None of the fungi was pathogenic to *Oxalis* spp. *Chaetomella raphigera* Swift, *Gloeosporium* sp., *Pestalotiopsis* Stey., *Pestalozziella subsessilis* Sacc. & Ellis, are new records from Bangladesh.

Key words: Fungal species, *Oxalis corymbosa*, *Oxalis latifolia*, pathogenicity

Introduction

Oxalis is the largest genus in the woodsorrel family Oxalidaceae. Species diversity is particularly rich in tropical Brazil, Mexico and South Africa. *Oxalis corniculata* (yellow flower,) is found throughout Bangladesh as a common weed. *Oxalis corymbosa* (pink flower) is native of South America and *Oxalis latifolia* (white flower) is indigenous to central and tropical South America. Among them *Oxalis corymbosa* and *Oxalis latifolia* show severe disease symptoms. *Oxalis corymbosa* is used as an ornamental plant. *Oxalis latifolia* can be used as a green manure as it contains a sufficient quantity of nitrogen and can also be used as an ornamental plant (Ahmed *et al.* 2009).

A lot of research has been done on phytochemical, pharmacological and biochemical aspects of *Oxalis* spp. But information about its fungal disease is inadequate

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(Bais *et al.* 2002). The disease grey mould caused by *Botrytis cinerea* was reported from abroad (Soares 2008). From Bangladesh, brown spot caused by *Alternaria alternata* was reported by Akhter (2000).

The present study was undertaken to identify the fungi associated with two species of *Oxalis* plants and to determine their prevalence and pathogenicity of the identified fungi.

Materials and Methods

Infected leaves and petiole of *Oxalis corymbosa* and *O. latifolia* (Figures 1 and 2) were collected from a Botanical Garden in the campus of Dhaka University during March, 2010 to August, 2011. Fungi associated with the specimens were isolated following tissue planting methods using potato dextrose Agar medium (PDA) (Tuite 1969).

The specimens were cut into small pieces (2 mm × 2 mm) and surface sterilized by dipping in 10% chlorox for 3 - 5 minutes followed by rinsing in sterilized water. Surface sterilized plant pieces were placed on PDA plants (Tuite 1969). From each sample 50 inocula consisted of leaf and petiole pieces were taken and placed on solidified PDA in Petri dishes at three pieces per plate. The plates were incubated for 5 - 7 days at 25 ± 1°C. Fungi grew from the inocula were transferred to separate PDA plates and PDA slants for further studies and preservation. The isolated fungi were identified based on morphological characteristics observed under a compound microscope following standard keys (Barnett and Hunter 1972, Ellis 1971, 1976, Ellis and Ellis 1997 and Sutton 1980). Prevalence (%) of fungi in different specimens was also recorded.

Pathogenicity of the isolated fungi was tested following modified 'detached leaf technique' (Azad and Shamsi 2011). Healthy and young leaves of *Oxalis* spp. were collected. Leaves were washed with distilled water, surface sterilized with 10% Clorox for five minutes and rinsed in sterilized water. Both ventral and dorsal sides of the leaves with and without pricking were inoculated with spores of the isolated fungi. Another set of leaves with and without pricking and without inoculation were maintained, which served as controls. The inoculated leaves were placed in Petri dishes containing water soaked cotton bar to maintain sufficient humidity to initiate infection. The plates were incubated at 25 - 28° C. After 3 days of inoculation, examination of leaves under pathogenicity test was started and continued for 7 - 10 days for disease development.

Results and Discussions

Altogether 8 species of fungi were isolated from healthy leaves of *Oxalis corymbosa*. In order of their prevalence they were *Gloeosporium* sp., *Aspergillus niger*, *A. flavus*,

Colletotrichum coffeanum, *Pestalotiopsis guepinii*, *Cladosporium* sp, *Curvularia pallescens* and *Rhizopus stolonifer*. Their prevalence was 20.00, 14.40, 11.70, 8.30, 6.70, 1.70, 1.70 and 0.33%, respectively. Leaves of *O. corymbosa* infected with anthracnose symptom yielded 14 species of fungi. Prevalence of *Alternaria tenuissima* was the highest followed by *Gloeosporium* sp., *Pestalotiopsis guepinii*, *C. coffeanum* and *Pestalotiopsis* sp. showing the prevalence of 33.30, 32.80, 31.70, 30.50 and 23.30%, respectively. Prevalence of other fungi ranged 1.67 - 11.10%. The lowest prevalence was found in *Alternaria alternata*, *Pestalozziella subsessiles* and *R. stolonifer*, which were followed by *C. pallescens* (5.00%), *C. falcatum* (5.55%) and *Cladosporium oxysporum* (8.33%). The diseased leaves of *O. corymbosa* gave 10.00% of *C. dematium* and *C. gloeosporioides* and 11.10% of *Cladosporium* sp. (Table 1).

Table 1. Prevalence of different fungi associated with healthy and anthracnose infected leaves of *Oxalis corymbosa* and *O. latifolia*.

Name of isolates	Healthy leaves		Leaves with anthracnose	
	<i>O. corymbosa</i>	<i>O. latifolia</i>	<i>O. corymbosa</i>	<i>O. latifolia</i>
<i>Alternaria alternata</i>	-	-	1.67	3.32
<i>A. tenuissima</i>	-	-	33.30	-
<i>Aspergillus flavus</i>	11.70	0.07	-	3.33
<i>A. niger</i>	14.40	0.14	-	10.46
<i>Chaetomella raphigera</i>	-	-	-	9.52
<i>Chaetomium</i> sp.	-	-	-	0.47
<i>Cladosporium oxysporum</i>	-	-	8.33	0.32
<i>Cladosporium</i> sp.	1.70	-	11.10	-
<i>Colletotrichum coffeanum</i>	8.30	10.50	30.50	19.10
<i>C. dematium</i>	-	14.30	10.00	15.60
<i>C. falcatum</i>	-	-	5.55	3.02
<i>C. gloeosporioides</i>	-	-	10.00	3.17
<i>Curvularia brachyspora</i>	-	-	-	0.95
<i>C. lunata</i>	-	-	-	0.95
<i>C. pallescens</i>	1.70	-	5.00	-
<i>Gloeosporium</i> sp.	20.0	-	32.80	5.39
<i>Nigrospora oryzae</i>	-	0.95	-	0.95
<i>Pestalotiopsis guepinii</i>	6.70	-	31.70	-
<i>Pestalotiopsis</i> sp.	-	-	23.30	0.43
<i>Pestalozziella subsessiles</i>	-	-	1.67	-
<i>Penicillium</i> sp.	-	1.67	-	-
<i>Rhizopus stolonifer</i>	0.33	-	1.67	0.43

- = Not isolated.

Only 6 species of fungi, namely *A. flavus*, *A. niger*, *C. coffeanum*, *C. dematium*, *Nigrospora oryzae* and *Penicillium* sp. were associated with healthy leaves of *Oxalis latifolia*. Their prevalence was 0.07, 0.14, 10.50, 14.30, 0.95 and 1.67%, respectively. Maximum 16 species of fungi were associated with anthracnose infected leaves of *O. latifolia*. Their prevalence ranged 0.43-19.10%. Among the fungi *Chaetomella raphigera*, *Cheatomium* sp., *Curvularia brachyspora*, and *C. lunata* were found to be associated with only anthracnose infected leaves of *O. latifolia*. Prevalence of *C. coffeanum* was the highest (19.1%) and that of *C. oxysporum* was the lowest (0.32%) (Table 1).

Two fungal species were isolated from healthy petiole of *O. latifolia*. They were *A. alternata* (4.44%) and *C. coffeanum* (8.87%). Pathogenicity test of the isolated fungi showed that none of the fungi was pathogenic on *O. corymbosa* and *O. latifolia*.

This is the first record of *Chaetomella raphigera*, *Gloeosporium* sp., *Pestalotiopsis* sp. and *Pestalotziella subsessiles* from Bangladesh. At the same time this is the first record of association of these four fungi with *Oxalis* plants. The newly recorded fungi are described with illustrations and photomicrographs. Taxonomic enumeration of the newly recorded taxa is shown below:

Chaetomella raphigera Swift, Mycologia 22: 165 (1930). (Figure 3 A, B)

Conidiomata 150-450 × 100-200 μ, Setae 40-80 × 2-4 μ, pale brown, clavate, often hooked at the apices, conidia 5-6 × 1.5-2 μ.

Recorded from a wide variety of substrates throughout the world (Sutton 1980) but most commonly from soil. Represented in herb. IMI by 30 collections.

Specimen examined: Isolated from the infected petiole of *Oxalis latifolia*, Botanic Garden, Curzon Hall campus, Dhaka University, 8 June, 2011, Y. Fatema, 23.

Gloeosporium Desm. and Mont. (Figure 3 C, D)

Acervuli subepidermal erumpent, disc-shaped or cushion-shaped, waxy; conidiophores simple, variable in length; conidia hyaline, 1-celled, ovoid to oblong, sometimes curved; parasitic, chiefly on leaves or fruits; mostly conidial stages of *Glomerella* sp. Conidia 5-9 × 1-2 μ.

Specimen examined: Isolated from infected leaf and from the soil around the root of infected *Oxalis latifolia* L., Botanic Garden, Curzon Hall, Dhaka University, 31 March, 2010, Y. Fatema,

Pestalotiopsis sp. (Figure 3 E, F)

Conidia fusiform, straight or slightly curved, 4 euseptate; hyaline, truncate, with an endogenous, cellular, simple or rarely branched appendage; apical cell conic, hyaline, with

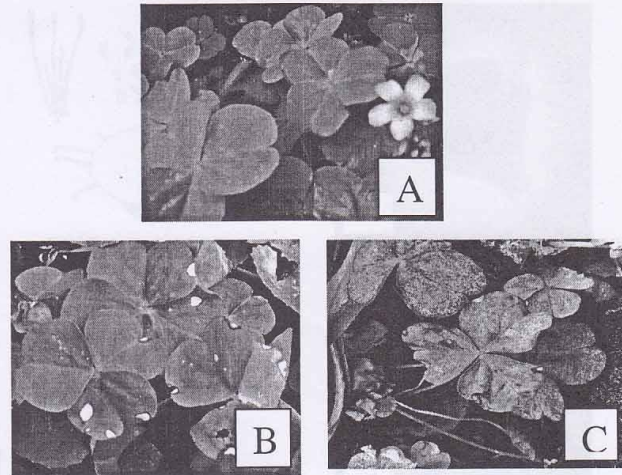


Figure 1. *Oxalis corymbosa*, (A) healthy plants, (B, C) infected plants.

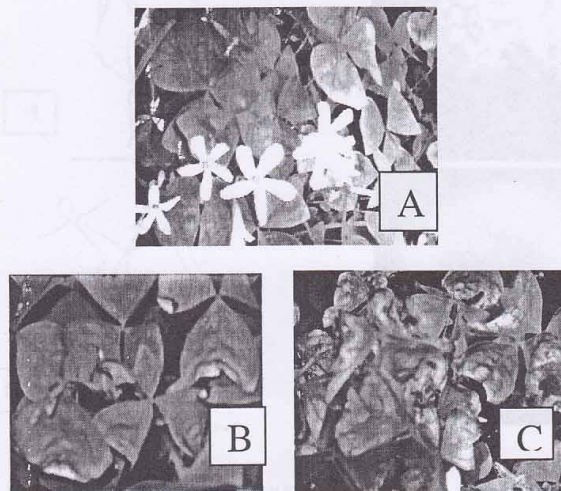


Figure 2. *Oxalis latifolia*, (A) healthy plants, (B, C) infected plants.

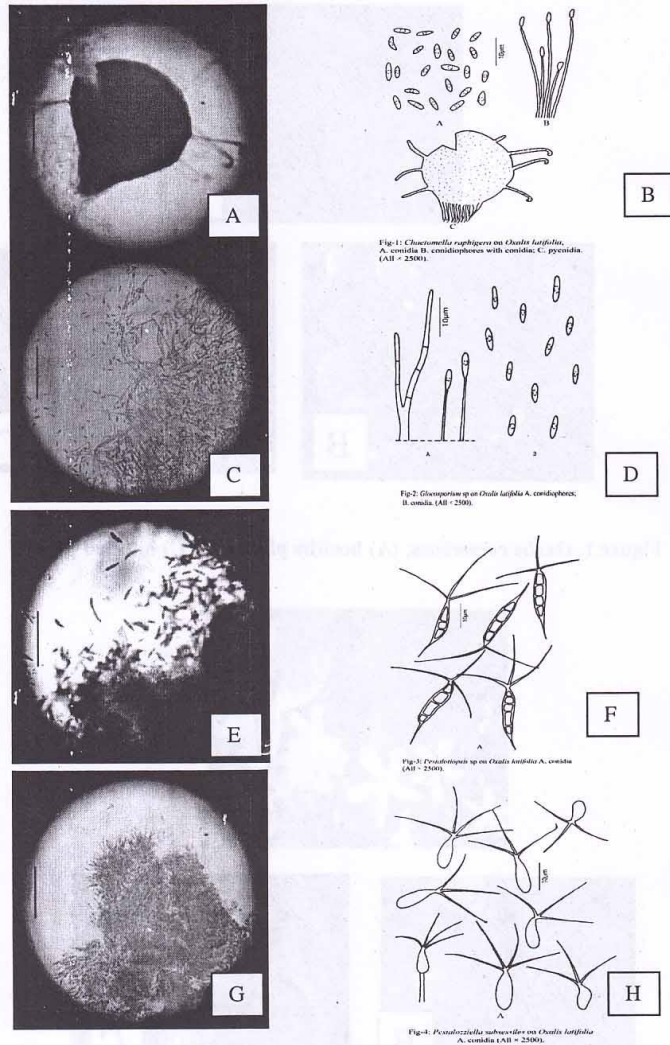


Figure 3. A. Pycnidia with conidia, B. Drawing figure of *Chaetomella raphigera*; C. Conidiophore with conidia, D. Drawing figure of *Gloeosporium* sp.; E. Conidiophore, conidia in acervulus, F. Drawing figure of *Pestalotiopsis* sp.; G. Conidiophore with conidia, H. Drawing figure of *Pestalozziella raphigera*.

2 or more apical, simple or branched, spathulate or espathulate appendages; sometimes versicoloured, thicker-walled, smooth or verruculose ($18-22 \times 4-5 \mu$).

Specimen examined: Isolated from the infected petiole of *Oxalis latifolia*, Botanic Garden, Curzon Hall, Dhaka University, 26 April 2011, Y. Fatema, 16.

Pestalozziella Sacc. and Ellis. (Figure 3 G, H)

Acervuli subcuticular, conidiophores slender, simple or branched; conidia hyaline, 1-celled, ovoid or oblong, with hyaline branched appendage at the apex; parasitic; conidia $8-15 \times 3-5 \mu$.

Specimen examined: Isolated from the infected leaves of *Oxalis latifolia*, Botanic Garden, Curzon Hall, Dhaka University, 29 December 2010, Y. Fatema, 18.

Twenty two fungal species representing 13 genera were found to be associated with *Oxalis* spp. Among these species, *Chaetomella raphigera*, *Gloeosporium* sp., *Pestalotiopsis* sp., *Pestalozziella subsessiles* are the new record for Bangladesh. All the isolated fungi were non-pathogenic. In the field of Mycology and Plant Pathology these findings were added latest information regarding the association of fungi with *Oxalis* plant.

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