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Original Article

Mycoflora associated with *Chrysanthemum morifolium* Ramat and their Pathogenic potentiality

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ABSTRACT: Two types of leaf blight symptom (Type-1and Type-2) were recorded on *C. morifolium*. Eleven species of fungi namely *Alternaria alternata* (Fr.) Keissler, *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* Van Tieghem, *Cladosporium cladosporioides* (Fresen). De Vries, *Colletotrichum gloeosporioides* (Penz.) Sacc., *Curvularia lunata* (Wakker) Boedijin, *Fusarium moniliforme* J. Sheld., *Penicillium* Link, *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill and *Trichoderma viride* Pers were found to be associated with the selected leaf samples. The fungi isolated from *C. morifolium* were tested for their pathogenic potentiality following "detached leaf technique". Among these fungi *C. lunata* and *F. moniliforme* were found to be pathogenic to *C. morifolium*.

Keywords: *Chrysanthemum morifolium*, Blight, Mycoflora, Pathogenic fungi

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INTRODUCTION

Chrysanthemum morifolium Ramat represents the second most important floricultural crop in the world belonging to Asteraceae. They are native to Asia and northeastern Europe. Chrysanthemum is one of the most leading commercial floriculture crop, grown for cut and loose flowers throughout the globe. Commercially, it is known as florist's Chrysanthemum or autumn queen[1]. *Chrysanthemum morifolium* Ramat, occupying a prominent place in ornamental horticulture is one of the commercially exploited flower crops. After rose, it is globally the most important floricultural crop.[2]. It is also used as a herb in both Japanese and Chinese traditional medicine. Extracts of *C. morifolium* have antioxidant, potent neuroprotective activity and therefore, might be a potential candidate in neurodegenerative diseases such as Parkinson's disease[3]. About 20,000 diverse varieties of Chrysanthemum are grown worldwide, out of which nearly 1000 varieties are cultivated in India. BARI has developed only two Chrysanthemum cultivars[4].

The climatic condition of Bangladesh is suitable for cultivation of Chrysanthemum but its commercial production is very low. One of the reasons behind the low yield is due to different kinds of fungal diseases. Out of all these fungal diseases, Fusarium wilt of Chrysanthemum caused by *Fusarium oxysporum* f. sp. *chrysanthemi* (Foc) is one of the most wide spread and destructive diseases[5]. It causes a severe losses of total production of Chrysanthemum. Present investigation was undertaken to find out the associated of fungi with *C. morifolium* leaves and to determine their pathogenic potentiality.

MATERIALS AND METHODS

Isolation of fungi

An investigation was carried out to find out the fungi associated with *C. morifolium* during April 2016 to May 2017. The diseased samples were collected from Agargaon Nursery , Dhaka and Botanical Garden , Curzon Hall Campus, University of Dhaka.

Fungi associated with the diseased leaves of *C. morifolium* were isolated following "Tissue planting" methods (CAB 1968).

Identification of the *C. morifolium* pathogens were confirmed following relevant literatures[6-13].

Pathogenecity test

Pathogenecity test of the fungi associated with *C. morifolium* was carried out following 'Detached leaf technique'[14].

Healthy matured leaves of the plant were thoroughly washed under running tap water and then surface disinfested in10% chlorox for 3 minutes. Excessive chlorox was removed by placing the leaves on two layers of sterile filter paper on Petri plate. Moist chamber was prepared by placing the small autoclaved cotton bar on Petri plates. All the isolated fungi were separately grown on PDA medium and incubated for seven days which were tested for their pathogenic potentiality. Leaves were placed on the autoclaved moist Petri plates and those were inoculated with mycelial block (5 mm) of the isolated fungi.

Six treatments with three replications for each fungus were used as follows:

T1= Dorsally uninoculated leaf (Control), T2= Ventrally uninoculated leaf (Control) T3= Dorsally unpricked inoculated leaf with test fungus, T4= Ventrally unpricked inoculated leaf with test fungus, T5= Dorsally pricked inoculated leaf with test fungus and T6= Ventrally pricked inoculated leaf with test fungus. The inoculated plates were incubated at 25-28º C. After five days of inoculation lesion size were recorded. The fungus was reisolated from the inoculated leaves to fulfill Koch's postulates.

RESULTS AND DISCUSSION

Chrysanthemum morifolium with beautiful colours are grown in Bangladesh (Fig.1. A-D). Blight is one of the major diseases of the plant . Two types of leaf blight symptom found in *C. morifolium* (Type-1 showed sub circular to rectangular brown necrotic lesions on leaves . Type -2 showed light brown irregular large lesions with yellowish surrounding (Fig . 1. E and F). Total 11 species of fungi were isolated from the diseased leaves of *C. morifolium* . The isolated fungi were *Alternaria alternata , Aspergillus flavus, A. fumigatus, A. niger, Cladosporium cladosporioides, Colletotrichum gloeosporioides , Curvularia lunata , Fusarium moniliforme , Penicillium sp., Rhizopus stolonifer and Trichoderma viride (Table 1).*

Table 1. List of Fungi isolated from two types of leaf blight symptom of *Chrysanthemum morifolium*

Serial no.	Name of Fungi	Type-1	Type-2
1	*Alternaria alternata*	+	+
2	*Aspergillus flavus*	+	+
3	*A. fumigatus*	+	+
4	*A. niger*	+	+
5	*Cladosporium cladosporioides*	+	+
6	*Colletotrichum gloeosporioides*	+	+
7	*Curvularia lunata*	+	+
8	*Fusarium moniliforme*	+	+
9	*Penicillium sp.*	+	+
10.	*Rhizopus stolonifer*	+	+
11.	*Trichoderma viride*	+	+

+ represents the presence of fungi.

In the present investigation *A. alternata, A. flavus, C. gloeosporioides, C. lunata* and *F. moniliforme*, were the predominatingfungi associated with *C. morifolium*.

Table 2 Showed that among the isolated fungi frequency percentage of association of *Alternaria alternata* was highest (34.08) in the sample showing Type 1 symptom collected from botanical garden,

Curzon Hall Campus, DU. Frequency percentage of the fungus was lowest (7.42) in the sample showing Type 2 symptom collected from the same location.

Frequency percentage of association of *Aspergillus flavus* was highest (19.16) in both the symptom types collected from Agargaon Nursery. Frequency percentage of the fungus was lowest (5.83) in both the symptom types collected from botanical garden, Curzon Hall Campus, DU.

Frequency percentage of association of *Curvularia lunata* was highest (16.67) in the sample showing Type 2 symptom collected from botanical garden, Curzon Hall Campus, DU. Frequency percentage of the fungus was lowest (13.33) in the sample showing Type 1 symptom collected from the same location (Table 2).

Frequency percentage of association of *Fusarium moniliforme* was highest (21.67) in the sample showing Type 2 symptom collected from Agargaon Nursery. Frequency percentage of the fungus as lowest (4.99) in the sample showing Type 1 symptom collected from the same location (Table 2).

Table 2. Per cent frequency of fungi associated with two types of blight symptoms of *C. morifolium*

	Frequency per cent of fungi isolated from diseased leaves of *C. morifolium*			
Name of Fungi	Type-1 Agargaon Nursery	Type- 1 Botanical Garden	Type-2 Agargaon Nursery	Type-2 Botanical Garden
Alternaria alternata	29.99	34.08	15.83	7.42
Aspergillus flavus	19.16	5.83	19.16	5.83
A. fumigatus	3.33	0.84	3.33	3.33
A. niger	10.83	26.67	10.83	26.67
Cladosporium cladosporioides	0.83	0.85	1.67	0.83
Colletotrichum gloeosporioides	16.67	29.17	19.17	29.17
Curvularia lunata	16.67	13.33	14.17	19.17
Fusarium moniliforme	4.99	10.83	21.67	19.17
***Penicilluim* sp.**	5	2.5	5.0	2.5
Rhizopus stolonifer	7.5	0.83	7.5	0.84
Trichodermma viride	2.5	2.5	2.5	–

'No fungal isolate'

Frequency percentage of association of *Colletotrichum gloeosporioides* was highest (29.17) in both the symptom types collected from botanical garden, Curzon Hall Campus, DU. Frequency percentage of the fungus was lowest (16.67) in the sample showing Type 1 symptom collected from Agargaon Nursery (Table 2).

Among the isolated fungi *C. lunata* and *F. moniliforme* showed positive result in pathogenicity test. The symptoms produced by *Curvularia lunata* and *Fusarium moniliforme* were similar to those symptoms that were observed in nature. The pathogens were re-isolated from the inoculated leaves of *C. morifolium* and confirmed Koch's postulates (Figure 1).

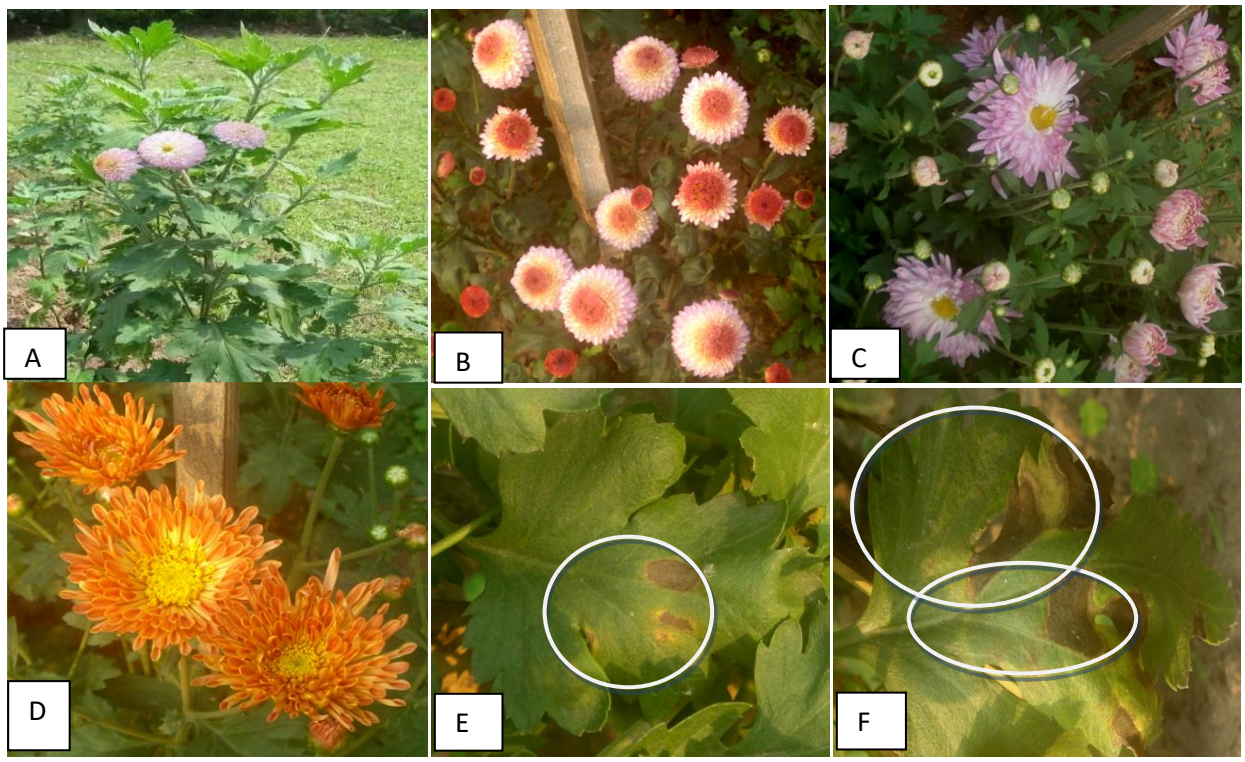

Figure 1. *Chrysanthemum morifolium*: A-D. Healthy plants. E. Blight Type 1 and F. Blight Type 1

Like all other ornamental plants, one of the major constraints of Chrysanthemum plants production is its frequent exposure to different biotic and abiotic stresses. Chrysanthemum plants can be infected by fungi, bacteria, viruses and nematodes. Among the various diseases, fungal diseases play a vital role for the severe yield loss of Chrysanthemum production. Major fungal diseases of Chrysanthemum are Alternaria leaf spot (*Alternaria* sp.), Bipolaris leaf spot (*Bipolaris setaria*), Cercospora leaf spot (*Cercospora chrysanthemi*), Charcoal stem rot (*Macrophomina phaseolina*), Cylindrosporium leaf spot (*Cylindrosporium chrysanthemi*), Fusarium wilt (*Fusarium oxysporum*), Gray mold (*Botrytis cinerea*), Itersonilia petal blight (*Itersonili aperplexans*), Phymatotrichum root rot (*Phymatotrichiopsis omnivore*), Powdery mildew (*Erysiphe cichoracearum*), Pythium root rot (*Pythium* spp.), Ray blight (*Didymella ligulicola*), Ray speck (*Stemphylium lycopersici*), Rhizoctonia stem rot (*Rhizoctonia solani*), Root rot (*Phoma chrysanthemicola*), Septoria leaf spot (Septoria rysanthemi), Sclerotinia rot (*Sclerotinia sclerotiorum*), Southern blight (*Sclerotium rolfsii*), Stem rot (*Fusarium solani*), Verticillium wilt (*Verticillium albo-atrum*) and White rust (*Puccini ahoriana*)[15]. Out of all these fungal diseases fusarium wilt of Chrysanthemum caused by *Fusarium oxysporum* f. sp. *chrysanthemi* (Foc) is one of the most wide spread and destructive disease, causing infection and loss from nursery to flowering stage[14]. This disease is difficult to control because of its persistence in the soil. Severe losses to Chrysanthemum crop caused by Foc are reported from various part of the world [16-18]. Foc are an unique ability to change its host range and it is reported that along with C*hrysanthemum*, it also infects *Gerbera jamesonii*, *Argyranthemum frutescens* (Paris daisy) and *Osteospermum* sp.

Arunakumar *et al.* (2013) reported Chrysanthemum leaf blight caused by *Alternaria alternata*[17]. In this investigation a total of 11 species of fungi were isolated from two types of blight symptoms of *C. morifolium*. Leaf blight of *C. morifolium* caused by *C. lunata* and *F. moniliforme* is new record.

CONCLUSION

C*hrysanthemum morifolium* with a high ornamental value is one of the ten most popular traditional flowers in China and one of the four most popular cut flowers in the world traded in the global flower market; therefore this flower occupies a very important position in the world Flower industries. Its herbal infusions are used in the treatment of bacterial and viral infections, sinusitis, blood pressure, digestive, skin problems, influenza virus PR3, leptospira, HIV-1, human colon cancer Colon205 cells, headache, dizziness, sore throat, hypertension, flu,cough etc *C. morifolium* extract (CME) has the protective effect on cardiovascular diseases. The plant is widely used as a dietary supplement or herbal tea and it exhibit an allelopathic activity. Chrysanthemum tea can help detoxify blood, regulate blood pressure and calm the nerves. Simultaneously, it is a good source of vitamins C and A, niacin, folic acid, pantothenic acid and also rich in calcium, magnesium, potassium, iron

and phosphorus. Leaf blight disease of the plant drastically damaged the plant. Finding of these research will be helpful for designing the management protocol of mycoflora of *C. morifolium.*

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