BLIGHT OF TWO SPECIES OF MARIGOLD (*TAGETES*) CAUSED BY *ASPERGILLUS FUMIGATUS* FRESENIUS

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(A part of PhD thesis of first author)

ABSTRACT

An investigation was carried out to record the pathogenic fungi associated with foliage blight of two species of marigold (*Tagetes erecta* L. and *Tagetes patula* L.) under Bangladesh conditions during 2009 to 2014 crop seasons. Foliage blight symptom appeared under natural epipytotic conditions were recorded. Diseased samples of leaves, buds and flower were collected from Gazipur, Dhaka, Pabna, Rajshahi, Khulna, Comilla , Chittagong and Sylhet districts. Associated fungi with infected samples were isolated following "tissue planting" and "blotter" methods. *Aspergillus fumigatus* Fresenius was isolated frequently from the specimens. Pathogenicity test on detached leaves and seedlings confirmed *A. fumigatus* is a potential pathogen of foliage blight of both the species of *Tagetes.* This is the first report on *A. fumigatus* a pathogen of foliage blight of *T. erecta* and *T patula.*

Key words: Foliage blight, *Aspergillus fumigatus*, marigold.

INTRODUCTION

Two species of marigold *viz. Tagete erecta* and *Tagetes. patula* belong to Asteraceae (Compositae) family are native to North and South America. Presently, the species are naturalized around the world. They are sometimes known as American or African marigolds. *Tagetes patula* is bushy, somewhat smaller plant as compare to *T. erecta* and known as French marigold. Their flower color is brick red, orange red, yellowish or brownish yellow in color. French marigold is commonly planted in butterfly gardens as a nectar source. The floret of *Tagetes* spp. are rich in the orange, yellow carotenoid lutein and are used as a food colour. The essential oil of the flower contains antioxidants.

Tagetes spp. are important for their antifungal properties. Plant is also used against fever dysenteries, indigestions, ulcers and eczemas. Leaves are used as blood clotting agents in Ayurbedhic treatment. It is most effective against the nematode species *Pratylenchus penetrans* (Olabiyi and Oyedunmade 2000). Plant also has antimosquito potentiality (Rajasekaran *et al.*

2004). Seeds of *T. erecta* have pesticidal property. Marigold is a profitable crop in Bangladesh but its role in farm economy of Bangladesh is scanty. Hoque *et al.* (2012) reported that 95% farmers in some areas of Jessore and Jhenaidah districts of the country cultivate T-004 line and only 5% farmers cultivate T-003 line of marigold. They also reported that at least 2,650,447 flowers are produced from one hectare of land. The gross margin and net return may be Tk. 1,62,186 and Tk. 1,17,812 per hectare, respectively. The net return was 80% higher than lentil, 85% higher than mustard and 6% lower than potato cultivation.

Diseases are major problems for marigold cultivation. Leaf spot, foliage leaf blight, grey mold, powdery mildew and anthracnose are the common diseases of the plants (Dhilon and Arora 1990, Mukerji and Bhasin 1986) in India. Reports on the occurrence of diseases of marigold in Bangladesh are inadequate (Aktar and Shamsi 2014). The present paper reports the results of an experiment conducted to record the pathogenic fungi associated with foliage blight of marigold in Bangladesh.

MATERIALS AND METHODS

Isolation and identification of **pathogen**

Fungi associated with diseased leaves, buds and flowers of *Tagetes erecta* and *Tagetes patula* were isolated. For isolation, 135 diseased leaves, bud and flowers samples were collected from Gazipur, Dhaka, Pabna, Rajshahi, Khulna, Comilla , Chittagong and Sylhet during 2009 to 2014 during January to April 2009-2014. Fungi associated with healthy and infected samples were isolated following 'tissue planting' and 'blotter' methods. Experiment was conducted in the Laboratory of Mycology and Plant Pathology, Department of Botany, and in a nethouse and field plot of Botanic garden, Curzon Hall campus, University of Dhaka (DU), Dhaka.

Inocula of 2 mm x 2 mm were cut from a particular specimen' washed with sterile water and surface sterilized by dipping in 1.0% Chlorox for 3-5 minutes. Excess water on the surface sterilized inocula was soaked with sterilized blotter. In tissue planting method, 30 sterilized plant tissues were placed in ten glass Petri plates (90 mm) containing sterilized potato dextrose agar (PDA) medium having pH 6.0. Each plate received 3 incula. In blotter method, two layers of moist filter paper were placed in the bottom of 90 mm Petri plates and autoclaved at 120C under 1.0 kg/cm^2 pressure for 15 minutes. Thirty inocula were placed on filter paper in 10 glass Petri plates. All the inoculated plates were incubated at 25 to 28 C for 5-7 days.

Fungi grew from the incubated inocula were transferred to separate plates containing PDA and PDA slants for storage and identification. Isolated fungi were identified based on morphological characteristics recorded through light microscopic studies. Temporary mounts of the isolated fungi were prepared, observed under binocular compound light microscope and morphology of hyphae, conidiophores and conidia was recorded. Colony characters were recorded from PDA plates. A standard key book was consulted (Thom *et al*. 1945) for identification. All the specimens collected from different locations were preserved in the Herbarium of Mycology and Plant pathology section, Department of Botany, DU, Dhaka, Bangladesh.

Pathogenicity test of identified fungi

Detached leaf inoculation method: Modified detached leaf technique was used to determine pathogenicity of the isolated fungi (Azad and Shamsi 2011). Moist chamber was prepared by placing small cotton ball at the center of Petri plates and autoclaved at 120C under 1.0 kg/cm^2 pressure for 15 minutes. Young and healthy leaves of marigold were collected and washed with sterilized water. Leaflets in moist chamber were inoculated with individual fungus ventrally or dorsally with or without pricking with needles maintaining 6 treatments viz.T_1 = (control-1) dorsally uninoculated leaflets, T_2 = (control-2) ventrally uninoculated leaflets, T_3 = dorsally unpricked inoculated leaflets; T_4 = ventrally unpricked inoculated leaflets, T_5 = dorsally pricked inoculated leaflets and T_6 = ventrally pricked inoculated leaflets.

Seedling inoculation method: Five days old healthy seedlings of *Tagetes erecta* and *T. patula* were transplanted separately in earthen pots (25 cm diameter) containing sterilized soil. Each pot received three seedlings. The pots were placed in the nethouse and seedlings were allowed to grow providing necessary water and nutrients. Identified fungus was purified following single spore technique and multiplied on PDA in Petri plates. Conidia were harvested from 7 days old culture of individual test fungus by scraping with a sterilized glass slides and suspended in sterilized water in 250 ml conical flasks. The concentration of conidia per milliliter of water was adjusted to 10^4. The inocula were sprayed on healthy potted plants with a hand sprayer. Control plants received only sterilized water without fungal inoculum. Five pots were inoculated for each treatment. The pots having inoculated and uninoculated seedlings were placed on benches in the nethouse following completely randomized design. Experiment was repeated twice.

The plants were examined daily and continued for 10 days to record the development of symptoms. Symptom produced on artificially inoculated plants were recorded and compared with those observed on naturally infected plants. The fungus was reisolated from the inoculated plants of *Tagetes* spp. to fulfill Koch's postulates.

RESULTS AND DISCUSSION

Severe blight symptoms were noticed on leaves, buds, calyx and petals of two species of marigold (*T. erecta*, *T. patula*) under natural conditions. The disease was identified as foliage blight (Plate I). During the period of study, *Aspergillus fumigatus* Frsenius was frequently isolated from different parts of *Tagetes* spp. collected from the fields as diseased samples. The fungus was identified as the causal agent of the disease based on its morphological characteristics. Colonies of the fungus on PDA plates were grey-green, cottony and reverse side was off white. Conidiophores are aseptate, smooth, greenish up to 500 µm in length and 2-8 µm width. Vesicles flask shaped, 20-30 µm, typically fertile over the upper half. Sterigmata in one series are crowed. Conidia are grey-green, one celled, globose, echinulate, catenate, 2-3 µm diameter (Plate 1I).

Under natural conditions, the highest frequency of the fungus was 100% on calyx and bud, 60% on leaf and 50% on petal of *erecta* during 2014. In 2009, 80% petals yielded the fungus. The lowest frequency of 33.33% was found in 2011. In case of *T. patula*, the frequency of the fungal pathogen was 100% on leaves and petals in 2010. Frequency of association 60% on bud and 50% on calyx during 2013. The lowest incidence was 3.33% on calyx during 2009. During 2012, symptoms did not appear on sampling areas (Table 1).

Figs 1. Symptoms of foliage blight of marigold appeared under natural condition: [A. infected plants of *Tagetes erecta* B. Infected plants of *Tagetes patula*].

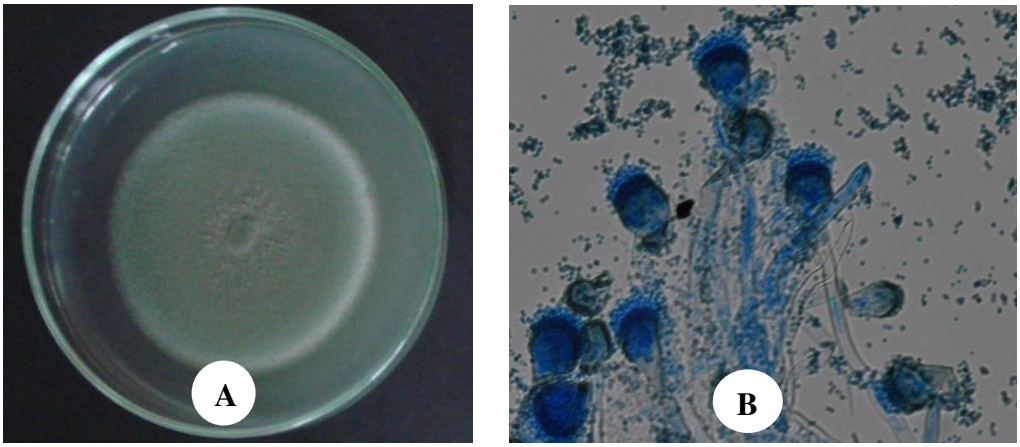

Plate II. Morphology of *Aspergillus fumigatus:* [A. 7 days old culture on PDA culture, B. Light microscopic photograph of mycelia, conidiophores and conidia].

Under natural field conditions, In case of *T. erecta* the highest incidence of the fungus was 100% on calyx and bud, 60% on leaf and 50% on petal during 2014. In 2009, 80% petals yielded the fungus. The lowest frequency of 33.33% was found in 2011. In case of *T. patula*, the frequency of the fungal pathogen was 100% on leaves and petals in 2010. Frequency of association 60% on bud and 50% on calyx during 2013. The lowest incidence was 3.33% on calyx during 2009. During 2012, symptoms did not appear on sampling areas (Table 1).

Table 1. Frequency (%) of association of *Aspergillus fumigatus* with symptomatic plant parts of *Tagetes erecta* and *T. patula* during 2009-2014 under field conditions.

Years	*T. etrecta*				*T. patula*			
	Bud	Leaf	Calyx	Petal	Bud	Leaf	Calyx	Petal
2009	-	-	-	80	-	100	-	3.33
2010	-	-	-	-	-	-	-	100
2011	-	33.33	-	-	-	-	-	-
2012	-	-	-	-	-	-	-	-
2013	-	-	-	-	60	-	50	-
2014	100	60	100	50	-	-	-	-

"- " = plants did not show symptom in sampling area

In pathogenicity test, *A. fumigatus* developed characteristics symptoms on all the inoculated leaflets of two species of marigold *in vitro* pathogenicity tests (Plate-III and IV) and on inoculated plants *in vivo* pathogenicity test (on inoculated plants) as observed on naturally infected plants (Plate V). The fungus was reisolated from inoculated leaflets in moist chambers as well as on potted plants, which was the confirmatory test for identification of *A. fumigatus* as the causal fungus blight.

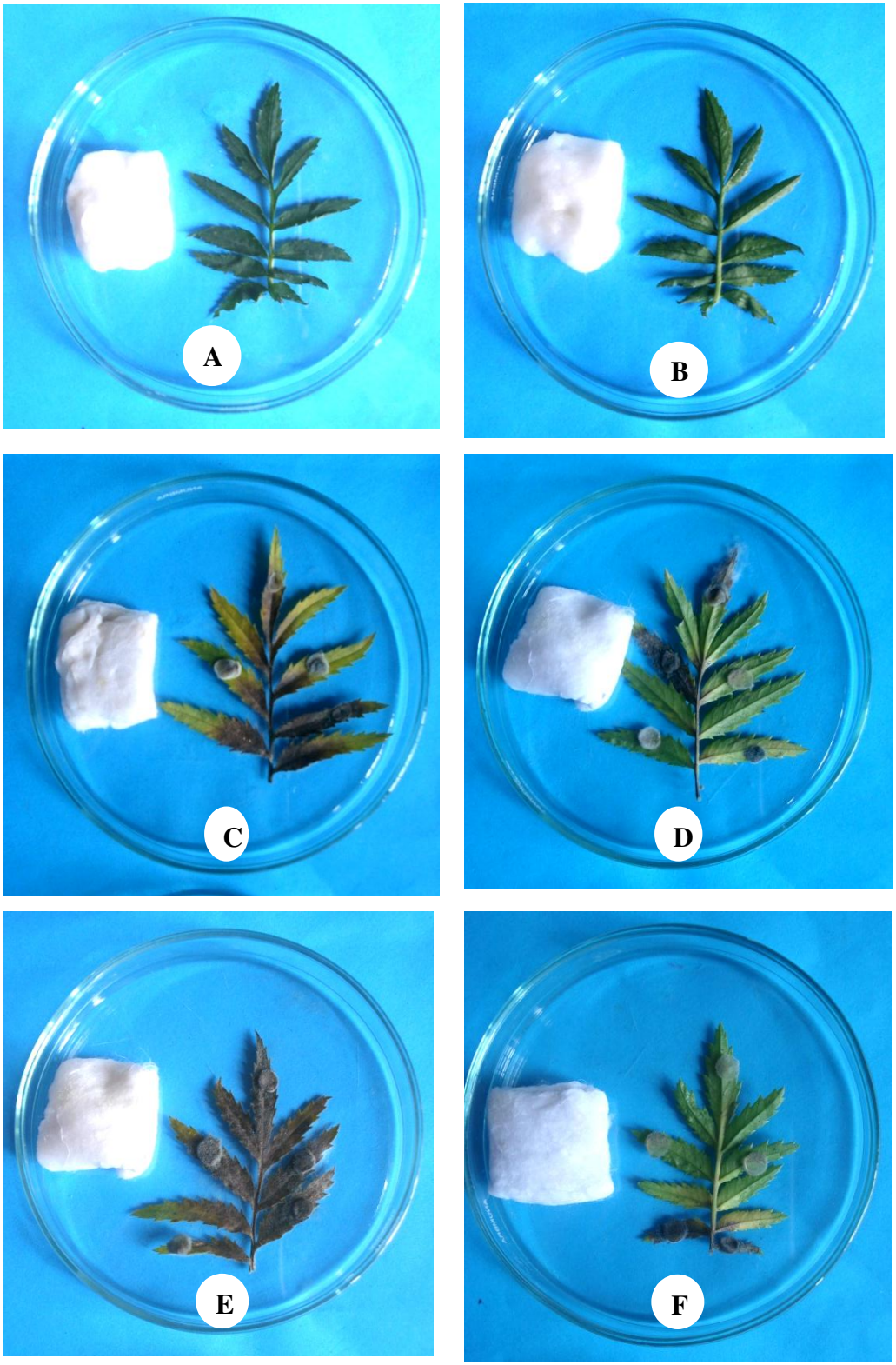

Plate III. Blight symptoms appeared on inoculated leaflets of *Tagetes erecta* [dorsally (A) as well as ventrally (B) uninoculated leaflets showing no symptoms, dorsally (C) as well as ventrally (D) unpricked inoculated leaflets showing symptoms, dorsally (E) as well ventrally (F) pricked inoculated leaflets showing severe symptoms of the disease].

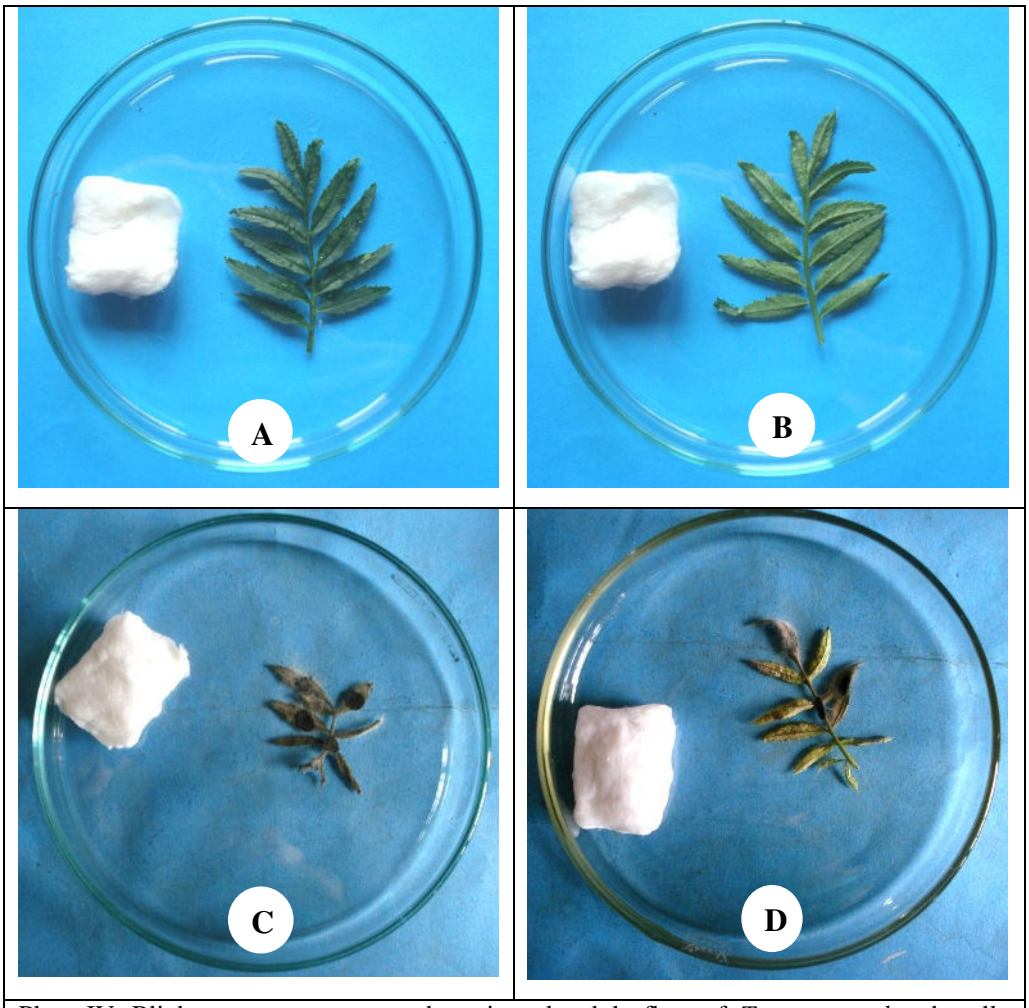

Plate IV. Blight symptoms appeared on inoculated leaflets of *Tagetes patula*: dorsally uninoculated leaflets (A), ventrally uninoculated leaflets (B), dorsally pricked inoculated leaflets (C) and ventrally pricked inoculated leaflets (D).

Leaf spot and foliage blight are two common diseases of *T. erecta* and *T. patula* caused by *Alternaria alternata* (Fr.) Keissler, *A. tagetica* (Shome & Mustaffe) and *A. tenuissima* (Kze. Ex Pers) Wilt.). Flower and bud rot (*A. dienthii* Stevens & Hae), leaf and inflorescence blight (*A. zinnia* Pape), and head blight and grey mold (*Botrytis cinerea* Pers.) are also common diseases of *T. erecta* and *T. patula* India (Mukerji and Bhasin 1986). From Bangladesh, Sultana and Shamsi (2011) reported gray mold of *T. erecta* caused by *Botrytis cinerea.* Dhiman and Arora (1990) reported leaf spot and flower blight of marigold (*T. erecta* L.) caused by *A. tagetica* in Punjab.

The morphological characters recorded in the present study were found to be identical as described by Thom *et al*. 1945. It is also associated with healthy tissues of the plant as endophytes as well as saprophytes (Yasmin and Shamsi 2013 and Shamsi and Saha 2015).

Available literature reveal that foliage blight of marigold caused by *A. fumigates* is a new disease in Bangladesh.

Plate V. Foliage blight symptoms developed on marigold plants due to inoculation with *A. fumigatus* in pathogenicity test invivo: [uninoculated plans showing no symptoms (A) & inoculated plants showing symptoms (B) on *Tagetes patula*, and C. uninoculated plants showing no symptom (C) & inoculated plant showing characteristic symptoms on *Tagetes erecta*].

AKNOWLEDGEMENT

The authors expresses their gratitude and thanks to "Research and Higher Education Fund" of the Prime Minister Office, Govt. of peoples Republic of Bangladesh" for their financial support in the form of Scholarship during the tenure of research work.

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