Bangladesh J. Bot. **45**(1): 167-173, 2016 (March)

REPORT ON BLIGHT OF *TAGETES* SPP. CAUSED BY *CURVULARIA LUNATA* (WAKKER) BOEDIJN

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Key words: Blight, *Curvularia lunata*, *T. erecta*, *T. patula*

Abstract

Blight symptom was recorded on two species of *Tagetes*, namely *T. erecta* L. and *T. patula* L. Fungi associated with symptomatic samples were isolated following "Tissue Planting" and "Blotter" methods. An anamorphic fungus *Curvularia lunata* (Wakker) Boedijn was frequently isolated from symptomatic plant parts of both the species. The pathogenicity of the isolated fungus was tested following modified "detached leaf technique" and "spraying of spore suspension methods". *Curvularia lunata* was found to be pathogenic to *Tagetes* spp. This is the first report on *Curvularia* blight of *T. erecta* and *T. patula.*

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Introduction

Tagetes erecta and *T. patula* belong to the family Asteraceae (Compositae). They are native to North and South America, but now become naturalized around the world. Though marigold is presently a profitable cultivated crop to the farmers in Bangladesh but socioeconomic data and information of this flower are very scare. Ninety five per cent farmers in Jessore and Jhenaidah districts cultivate marigold as commercial basis. The yield of marigold was 2,650,447 flowers per hectare. The gross margin and net return was Tk. 1,62,186 and 1,17,812 per hectare, respectively. The net return was 80% higher than lentil, 85% higher than mustard and 6% lower than potato cultivation (Hoque *et al.* 2012). Diseases were major constrain for marigold cultivation (Mukerji and Bhasin 1986). In Bangladesh, due to rapid expansion of commercial marigold cultivation many diseases appear on the plants. This plant has been used for medicinal purposes. *Tagetes erecta* is used by Cherokee as a skin wash and for yellow dye. Thiophenes, natural phytochemicals that include sulfur-containing rings, may be the active ingredients which has been established from scientific study. This ingredient have been shown to kill Gram negative and Gram positive bacteria *in vitro.* This plant may help protecting certain crop plants from nematode pests when planted in fields. It is most effective against the nematode species *Pratylenchus penetrans.* The oil of the flower may be added to perfumes to infuse an apple scent into them. Today, *T. erecta* is grown to extract lutein, a common yellow/orange food colour. The essential oil of the flower contains antioxidants (Schwartz 1999, Khachik *et al*. 1999). *Tagetes patula* used mainly as an edging plant on herbaceous borders. French marigolds are commonly planted in butterfly gardens as a nectar source. The essential oil from the plant is being investigated for antifungal activity, including treatment of candidiasis and treating fungal infections in plants. The plant is used in companion planting for many vegetable crops. Its root secretions are believed to kill nematodes in the soil and it is said to repel harmful insects, such as white flies on tomatoes (Mares *et al.* 2004, Romagnoli1 *et al*. 2005 and Datta *et al*. 2007). Both the species are used in Ayurvedic treatment.

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Plants have antifungal properties (Olabiyiand and Oyedunmade 2000). Plant has also mosqutocidal potentiality (Rajasekaran *et al.* 2004). However, reports on the occurrence of diseases of marigold in Bangladesh are scanty (Aktar and Shamsi 2014). Present study was undertaken to find out the presence of pathogenic fungi associated with marigold in Bangladesh.

Materials and Methods

Samples with characteristic symptoms were collected from BARI, Joydebpur, Gazipur, Dhaka, Chittagong, Comilla, Dhaka city, Khulna, Pabna, Rajshahi and Sylhet during 2009 to 2014 (January to June). Severe blight symptom on leaves, bud and flowers of two species of *Tagetes* were recorded. Fungi associated with infected leaves, buds and flowers of *Tagetes erecta* and *T. patula* were isolated following "Tissue Planting" and "Blotter" methods. From infected plant parts of both the species 139 samples were examined. Fungi associated with infected samples were isolated on PDA medium, pH of the medium maintained 6.0. Experiment was conducted in the Laboratory of Mycology and Plant Pathology, Department of Botany, University of Dhaka.

Identification of the isolates were determined by following Ellis (1971) and Ellis (1976). All the specimens were preserved in the Herbarium, Mycology and Plant Pathology section, Department of Botany, University of Dhaka, Bangladesh.

The isolated fungi were tested for their pathogenic potentiality following modified "detached leaf technique" (Azad and Shamsi 2011). Six treatments with three replications for each fungi was used as follows: T_1 = (control) dorsally uninoculated leaflets, T_2 = (control) 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.

The pathogenic fungus screened from "Detached leaf technique" was selected for net house experiment. Required amount of soil was sterilized with 10% formaldehyde and covered with polythene sheet for 3 days then it was exposed in air for 3 days to avoid smell of the chemical and then poured on clean pots. Healthy seedlings of *Tagetes erecta* and *T. patula* were separately transplanted in earthen pots (12 inch diameter) containing sterilized soil at three seedlings per pot and allowed to grow for one month in net house providing necessary water and nutrients. Test fungus was grown on PDA medium for 7 - 10 days. Identified fungus was purified and its pathogenecity was performed by inoculating fresh healthy plants following inoculation of spore suspension method. Spore suspension of the pathogenic fungus at the rate of 10^4 concentrations was sprayed on healthy plants using hand sprayer. Three replication was made for each treatment. Ten pots were used for each replication. Inoculated plants were covered by perforated bags to avoid contamination and to maintain humidity. The inoculated and control plants were placed in net house following completely randomized design.

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

Results and Discussion

Blight symptom in severe form was noticed on leaves, buds and flowers of *Tagetes erecta* and *Tagetes patula* during January to June, 2009 to 2014. Samples were collected from BARI, Joydebpur, Gazipur, Dhaka, Pabna, Rajshahi, Khulna, Comilla, Chittagong and Sylhet. Infected plants of *T. erecta* and *T. patula* are presented in (Figs 1A-B).

A study was conducted to identify the fungi associated with different parts of *Tagetes erecta* and *T. patula*. During the period of this study *Curvularia lunata*, was frequently isolated from different parts of *Tagetes* spp. Morphological characters of the fungi were recorded on PDA medium.

Fig. 1. A. *Tagetes erecta*: Infected plants. B. *Tagetes patula* : Infected plants.

Colonies effuse, dark black. *Conidiophores* solitary, mostly unbranched, straight or slightly undulating, often geniculate, pale to dark brown, septate, 20.0 - 64.4 (83.6) µm long, 3.0 - 5.4 µm thick, often swollen at the base. *Conidia* 3 septate, olivaceous black to dark brown, almost always curved at the third cell from the base which is larger and darker than the others, end cells subhyaline or pale brown, smooth, 20 - 34 × 9 - 15 µm (Figs 2A-B).

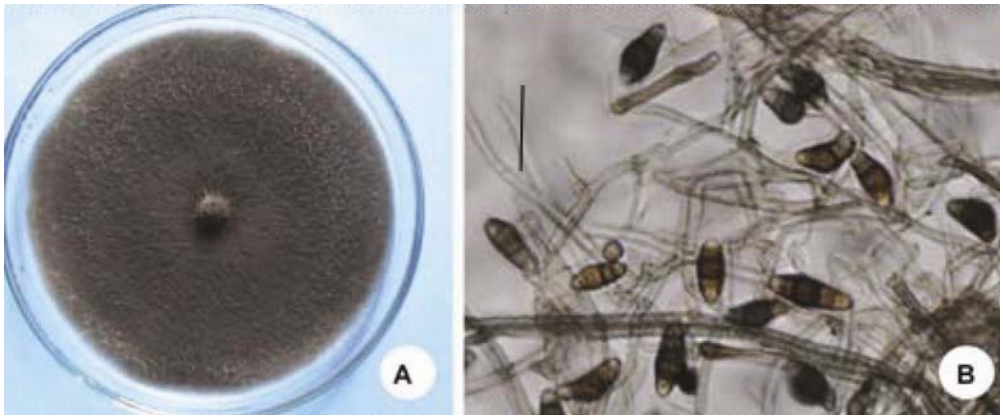

Fig. 2. *Curvularia lunata*: A. 12 days culture on PDA medium, B. Mycelia, conidiophores and conidia. (Bar = 50 µm).

The morphological characters recorded in the present study were compared with those reported by Ellis 1971.

From Bangladesh, *C. lunata* (perfect stage *Cochliobolus lunatus* Nelson and Haasis) was commonly recorded as a seed borne fungus of various crop plants like cereals *Oryza sativa* L.

(rice), *Triticum aestivum* L. (wheat), pulses *Phaseolus mungo* L. (black gram), *Cicer arietinum* L. (gram), *Lathyrus sativus* L. (khesari), *Lens culinaris* L. (lentil), *Phaseolus aureus* Roxb. (mung bean), *Cajanus cajan* L. (pigeon pea). Oil yielding plant like *Glycine max* L. (soybean), *Sesamum indicum* L. (sesame) and fiber yielding plants like *Corchorus capsularis* L. (white jute) and *C. olitorius* L. (tossa jute). The fungus was also recorded from garden and field soils of Bangladesh. The presence of *C. lunata* with seeds of *Lycopersicum esculentum* Mill. (tomato) and *Capsicum annum* L. (chilli). The species was reported as responsible for leaf and grain spot of rice, seed rot and seedling blight of *Setaria italica* (L.) P. Beauv (kaon). *Curvularia lunata* was found to be associated with affected parts of *Andropogon sorghum* Brot. *Panicum* sp. (Shama grass), *Saccharum spontaneum* L. (Kan grass), *Typhanum trilobatum* (L.) Schrott., *Vigna catjag* Walp. (cow pea), *Helianthus debli* L., *Mangifera indica* L. (mango), *Solanum nigrum* L. and *Lagenaria siceraria* (Mol.) Stan. Akhter (1993) recorded the fungus from fallen leaves of *Artocarpus lakoocha* Roxb. (Moraceae). This fungus was also found associated with infected parts of *Abelmoscus esculentus* (L.) Moen, *Eleusine indica* (L.) Gaertn, *Lycopersicon lycopersicum* (L.) Karst (Akhter 2001). This species was also reported on rice grain 2003 (Shamsi *et al*. 2003), *Zea mays* L. (Yasmin 2007), Groundnut (Sharmin 2012), *Gerbera* spp. (Yasmin 2014) and *Oxalis* spp. (Fatema 2012).

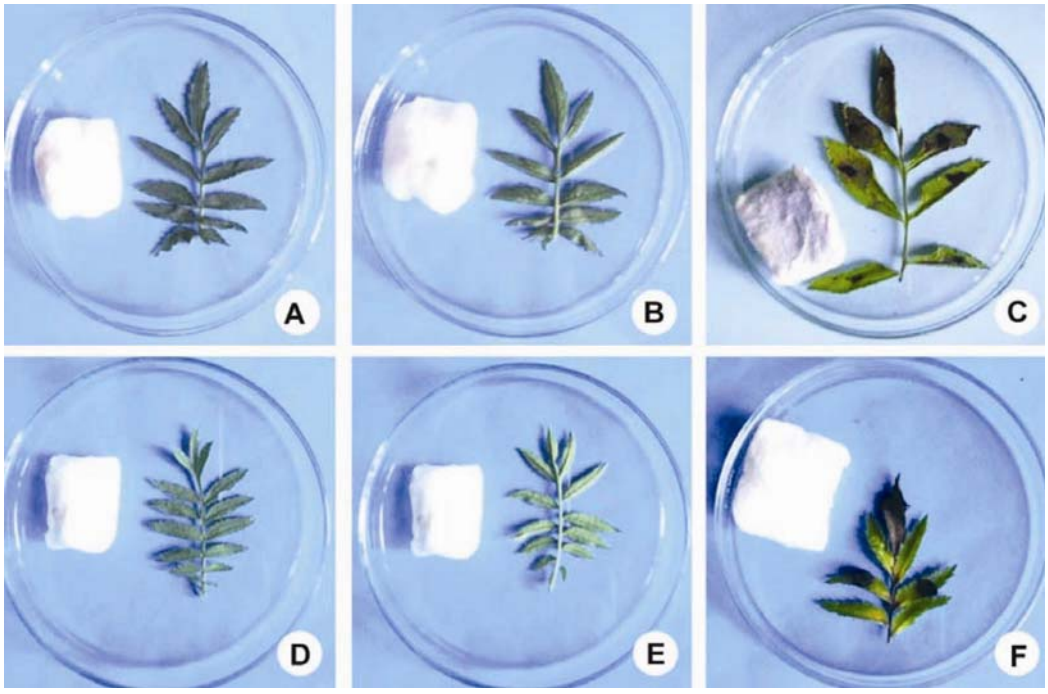

Fig. 3. *Tagetes erecta*: A. $T_{1\ =}$ (control) dorsally uninoculated leaflets, B. $T_{2\ =}$ (control) ventrally uninoculated leaflets, C. $T_{3\ =}$ dorsally unpricked inoculated leaflets, *Tagetes patula:* D. $T_{1\ =}$ (control) dorsally uninoculated leaflets, E. $T_{2\ =}$ (control) ventrally uninoculated leaflets, F. $T_{3\ =}$ dorsally unpricked inoculated leaflets.

Leaf spot and blight are two common diseases of *Tagetes erecta* and *T. patula*. From India Mukerji and Bhasin (1986) reported leaf spot caused by *Alternaria alternata* (Fr.) Keissler and *A. tenuissima* (Kze. Ex Pers) Wilt. and *A. tagetica* (Shome & Mustaffe), flower and bud rot caused

by *A. dienthii* Stevens & Hae, leaf and inflorescence blight caused by *A. zinniae* Pape and head blight and grey mold caused by *Botrytis cinerea* Pers., leaf spot, sometimes spot on stem and inflorescence caused by *Cercospora tageticola* Ell. & Ev., Blight on petiole, involucre, peduncle and branches caused by *Colletotrichum capsici* (Syd.) Butler & Bisby. Spot on petiole caused by *Diplodia tagetes-erectae* Rao, powdery mildew caused by *Leveillula taurica* (Lev.) Arnaud and damping off caused by *Rhizoctonia* Kuhn.

Fig. 4. *Tagetes erecta*: A. control, B. plant inoculated with *C. lunata, Tagetes patula*: C. control, D. plant inoculated with *C. lunata*.

Dhiman and Arora (1990) reported leaf spot and flower blight of marigold (*Tagetes erecta* L.) caused by *Alternaria tagetica* in Punjab. Due to disease average reduction of 28.2 and 53.53% in seed weight and germination, respectively. The seeds obtained from diseased flowers produced 2 - 5% sickly seedlings.

From Bangladesh Sultana and Shamsi (2011) reported gray mold of *T. erecta* caused by *Botrytis cinerea*. Aktar and Shamsi 2014 reported alternaria blight of *Tagetes* spp.

In the present study *Curvularia lunata* was the causal agent of blight disease of *Tagetes* spp. In case of *T. erecta* highest frequency percentage of association of the fungus was recorded (66.66) in 2009 on bud. Frequency percentage of association of the fungus was recorded 66.66 on petal in 2010 (*T. patula*). During 2011, 2012 and 2014 *T. patula* did not show any symptom in sampling areas (Table 1).

Table 1. Frequency (%) of association of *Curvularia lunata* with symptomatic plant parts of *Tagetes erecta* and *T. patula* during 2009-2014.

Year	*T. erecta*				*T. patula*			
	Leaf	Bud	Calyx	Petal	Leaf	Bud	Calyx	Petal
2009	-	66.66	16.66	-	16.66	-	3.33	-
2010	-	-	-	26.66	33.33	16.66	-	66.66
2011	-	-	-	-	-	-	-	-
2012	-	-	-	50	-	-	-	-
2013	33.33	-	-	-	16.66	-	-	-
2014	16.66	-	-	50	-	-	-	-

'- ' = Plants did not show symptom in sampling area.

Cuvularia lunata showed symptom on all the inoculated leaflets and plants of *Tagetes* spp. *in vitro* and *in vivo* except control leaflets and plants. The fungus was successfully reisolated from inoculated leaflets and plants (Figs 3 and 4 A-D). This is the first report of Curvularia blight of *Tagetes* spp. Present investigation will be helpful for designing successful control measures of the disease.

Acknowledgement

The authors express their gratitude to "Research and Higher Education Fund of the Prime Ministers Office, Govt. of the People's Republic of Bangladesh" for their financial support in the form of Scholarship during the period of research work.

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(Manuscript received on 10 August, 2015; revised on 14 October, 2015)