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Case report

Report on mycoflora associated with *Clitoria ternatea* L.: A herbal medicinal plant in Bangladesh

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Abstract. *Clitoria ternatea* L. is an excellent herbal medicinal plant. Severe anthracnose symptom was noticed on leaves and pods of the plant during the tenure of January to March 2014. Nine species of fungi representing 7 genera and a sterile fungus were found to be associated with *C. ternatea.* The isolated fungi were *Alternaria alternata* (Fr.) Keissler, *Aspergillus flavus* Link*., A. fumigates* Fresenius, *A. niger* Van Tiegh., *Cladosporium oxysporum* (Harz) Nannf., *Colletotrichum gloeosporoides* (Penz) Sacc., *Curvularia lunata* Boedijn*., Fusarium* oxysporum Schlecht., *Penicillium* sp. and a sterile fungus. Prevalence of the fungi varied with healthy and diseased leaves and pods. Pathogenicity test of the fungi following detached leaf technique revealed that isolated fungi were non pathogenic to *C. ternatea*.

Keywords: Mycoflora*, Clitoria ternatea*, medicinal plant, Bangladesh.

INTRODUCTION

Clitoria ternatea, common names including butterfly-pea, blue-pea, and cordo fan-pea, is a plant species belonging to the Fabaceae family, (Synonym: *Clitoris principissae*). This plant is native to tropical equatorial Asia, but has been introduced to Africa, Australia, Egypt, Syria, Mesopotamia, Iraq, Persia, Arabia and Afghanistan. In India, the plant is grown wild or cultivated. It is a perennial herbaceous plant, with elliptic, obtuse leaves. It grows as a vine or creeper, doing well in moist, neutral soil. The most striking feature about this plant is its vivid deep blue flowers; solitary, with light yellow markings. They are about 4 cm long and 3 cm wide. There are some varieties that yield white flowers. The fruits are 5 to 7 cm long, flat pods with 6 to 10 seeds in each pod. They are edible when tender (Ahmed et al., 2009). It is grown as an ornamental plant and as a revegetation species (e.g., in coal mines in Australia), requiring little care when cultivated. As a legume, its roots form a symbiotic association with soil bacteria known as rhizobia, which transform atmospheric N_2 into a plant usable form, therefore, this plant is also used to improve soil quality through the decomposition of N-rich tissue.

In traditional Ayurvedic medicine, it has been used for centuries as a memory enhancer, nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilizing and sedative agent. In Burmese and Thai cuisine the flowers are also dipped in batter and fried (Ghani, 1998).

The active constituents include tannins, resins, starch, taraxerol and taraxerone. Recently, several biologically active peptides called cliotides have been isolated from the heat-stable fraction of *Clitoria ternatea* extract. Cliotides belong to the cyclotides family and activities studies show that cliotides display potent antimicrobial activity against *E. coli, K. pneumonia, P. aeruginosa* and cytotoxicity against Hela cells. These peptides have potential to be lead compound for the development of novel antimicrobial and anti-cancer agents. Methanol extract of *C. ternatea* roots demonstrated nootropic, anxiolytic, antidepressant, anticonvulsant and antistress activity. Decoction is used for gargling in stomatitis and for cleaning wounds. It prevents pus formation. It has tranquillizing effect on the brain hence it is used in symptoms like syncope, vertigo and brain weakness. It is also used in common cold, cough and reduces the irritation of respiratory organs. Besides this, whole plant is used for smoking. Decoction is used for gargling in throat manifestations. It alleviates swelling and pain. It has haemostatic action hence it is used in piles specially bleeding piles. Piles are cleaned with the decoction and the paste of whole plant is applied over it. Leaf juice is

used as nasal drops in headache. Oil boiled with dhamasa is used for massage in rheumatoid arthritis (Gupta et al., 2010).

Aims of the study

A lot of research has been done on phytochemical, pharmacological and biochemical aspects of *C. ternatea* in home and abroad. But information about its fungal disease is inadequate (Yusuf et al., 2009; Joshi, 2000).

Fungal disease is one of the constraints for production of healthy plants and fruits. To protect the plant from diseases and disorders, it is a routine work for the Plant Pathologists to search the causal entities of the economically important plants of the country. Keeping this in mind the present study was undertaken (i) to identify the fungi associated with infected leaves and pods of *C. ternatea* and (ii) to determine the pathogenic potentiality of the fungi associated with the infected leaves and pods.

Collection of samples

Healthy and infected leave and fruit samples of *C. ternatea* were collected from Botanical garden in the campus of Dhaka University and a roadside vegetable garden of Mohakhali area Dhaka, Bangladesh during January to March 2014. Symptom was recorded and photograph was taken by Nikon D 5100, DSLR Camera (Plate 1).

Recording disease severity

For visual estimation of severity, 0 to 9 point scale were used for rating of all foliar diseases studied.

No infection – 0, 0 to 10% leaf area infected – 1, 10 to 20% leaf area infected – 2, 20 to 30% leaf area infected – 3, 30 to 40% leaf area infected – 4, 40 to 50% leaf area infected – 5, 50 to 60% leaf area infected – 6, 60 to 70% leaf area infected – 7, 70 to 80% leaf area infected – 8, 80 to 90% or more leaf area infected – 9.

For visual estimation of severity of infected fruits 0-6 point scale has been designed following 0 to 9 point scale used for rating of all foliar diseases studied (Ghosh et al., 2009).

No infection – 0, 0 to 10% fruit area infected – 1, 10 to 20% fruit area infected – 2, 20 to 30% fruit area infected – 3, 30 to 40% fruit area infected – 4, 40 to 50% fruit area infected – 5, 50 to 60% fruit area infected – 6, 60 to 70% fruit area infected.

Isolation of the fungi

Fungi associated with the specimens were isolated following “tissue planting” methods using potato dextrose agar medium (PDA) (Tuite, 1969). Thirty samples were examined in search of fugal associates of the leave and fruit samples of the plant. From each sample 50 inocula were prepared. Healthy and infected leave and fruit samples were cut into 2 square mm in size and placed in separate autoclaved petri dishes and surface sterilized by dipping in 10% chlorox for 3 to 5 min followed by rinsing in sterilized water. Surface sterilized plant pieces were placed on solidified PDA in Petri dishes at 3 pieces per plate. Thus thirty inocula were incubated for each sample.

The plates were incubated for 5 to 7 days at 25 ± 1°C. Fungal mycelia 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; Booth, 1972; Ellis, 1971; 1976; Ellis and Ellis, 1997; Sutton, 1980). Prevalence (%) of fungi in different specimens was also recorded. Percentage of frequency of the occurrence of the fungal isolates was calculated by adopting the following formula (Spurr and Welty, 1972):

$$\% \text{ frequency} = \frac{\text{No. of inocula from which a fungal isolate was obtained}}{\text{No. of inocla cultured}} \times 100$$

Pathogenicity test of the isolated fungi

In total, nine fungal species and a sterile fungus was isolated from *C. ternatea.* The isolated fungi were tested for their pathogenic potentiality following modified “Detached leaf technique” (Azad and Shamsi, 2011).

Healthy matured leaves o of the plant were thoroughly washed under running tap water and then surface disinfested in 10% Chlorox for 2 min. 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 wet cotton bar on petri plates. Then leaves were placed on the autoclaved moist petri plates and those were inoculated with 5 mm (diam.) mycelila block that were previously grown on PDA medium and incubated for seven days. All the fungi were tested to find out their pathogenic potentiality. Six treatments with three replications for each fungi was made as follows: T_1 = dorsally inoculated leaf with PDA block (control), T_2 = ventrally inoculated leaf with PDA block (control), T3 = Unpricked dorsally inoculated leaf, T_4 = Unpricked ventrally inoculated leaf, T_5 = dorsally pricked inoculated leaf and T_6 = ventrally pricked inoculated leaf. The inoculated plates were incubated at 26 to 28°C. After 3 days of inoculation, examination of leaves under pathogenicity test was started and continued for 7 to 10 days for disease development after 10 days of inoculation lesion size was recorded.

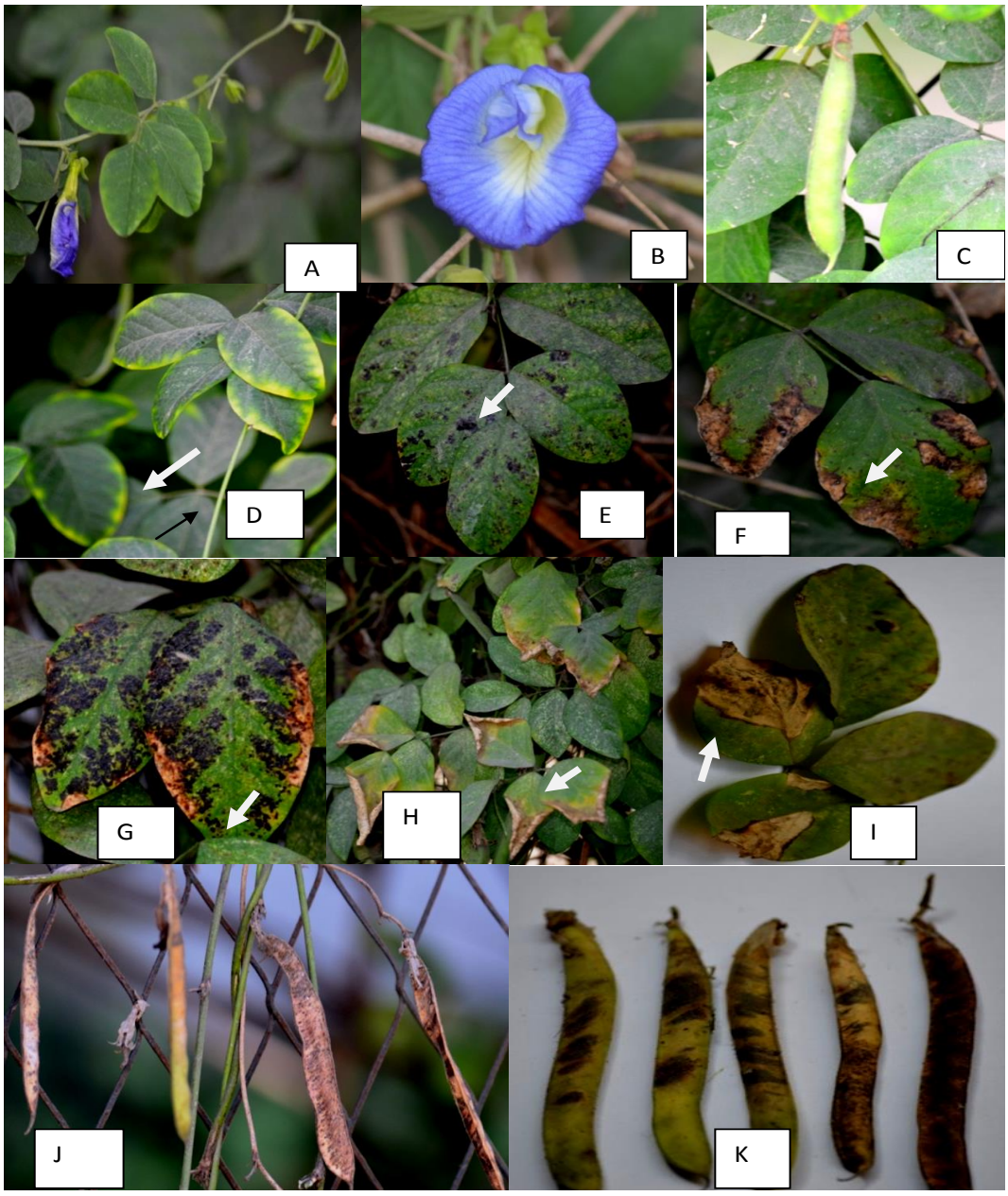

Plate 1. *Clitoria ternatea*: A-C: Healthy twig with flower and fruit; D-I: Developmental stages of anthracnose symptom on leaves; J- K: Infected fruits.

Diagnosis of disease symptom

Severe anthracnose symptom was noticed on leaves and pods of the plant during the tenure of January to March 2014.

Anthracnose

Anthracnose was found in all leaf samples collected starting from early to late stages of disease development (Plate 1D to I). The disease also appeared on pod samples also (Plate 1J to K). Infection started from first week of January, 2014 and gradually increased 1 to 6 DS in the last week of the month. Highest DS was recorded 9 in the first and second week of February. In the last week of March due to shower older leaves gradually falls l and severity reduced to 4-6 DS scale. Disease severity of the infected pods was recorded at DS 0-6 Scale. Infection started from the last week of January which was recorded at DS 2-3 Highest DS was recorded at DS 6 in last week of February.

Colletotrichum gloeosporioides was associated with leaf and pod samples showing anthracnose (Plate 2A and B).

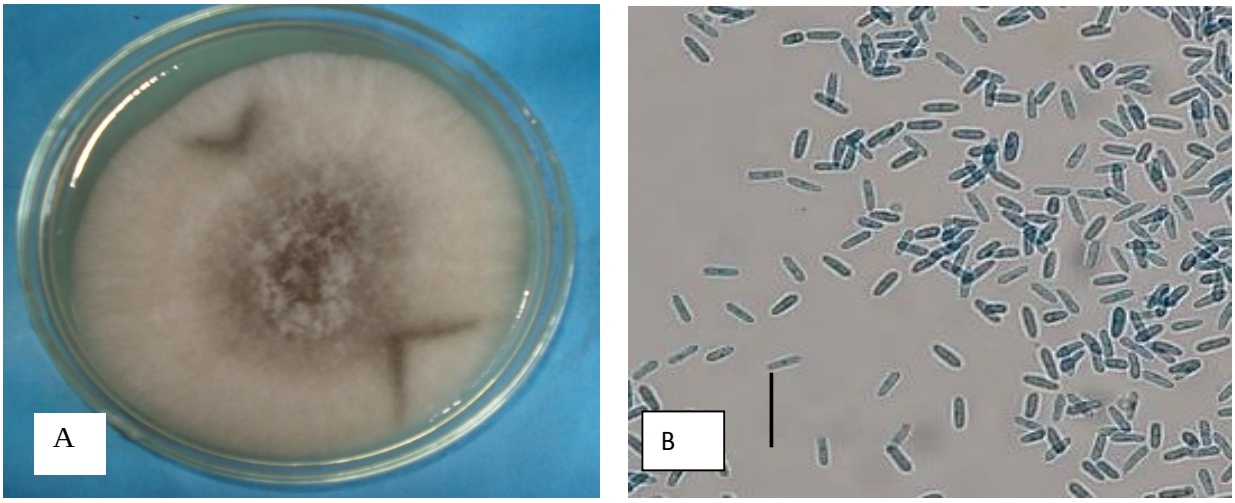

Plate 2. A. Culture plate and B. conidia and of *Colletotrichum gloeosporioides*. (Bar = 50 µm).

The fungus belongs to the class *Coelomycetes*.

Fungi associated with infected leaves and pods

Nine fungal species representing 7 genera and a sterile fungus were found to be associated with *C. ternatea.* The isolated fungi were *Alternaria alternata, Aspergillus flavus, A. fumigatus*, *A. niger*, *Cladosporium oxysporum*, *Colletotrichum gloeosporoides*, *Curvularia lunata, Fusarium* oxysporum, *Penicillium* sp. and a sterile fungus.

Three species of fungi were isolated from healthy leaves of *C. ternatea.* In order of their prevalence they were *Aspergillus niger, Claddosporium* oxysporum and *A. fumigatus.* Their prevalence was 33.33, 18.00 and 8.33%, respectively.

Five fungal species and a sterile fungus where isolated from infected leaves showing antracnose symptom. Prevalence of *Colletotrichum gloeosporoides* was the highest followed by *A*. *alternata*, sterile fungus, *Fusarium* sp., *C. lunata* and *A. flavus* showing the prevalence of 80.00, 46.18, 33.33, 14.33, 10.00 and 8.33, respectively.

Only 3 species of fungi, namely *A. flavus, A. niger* and *Penicillum* sp., were associated with infected pods. Their prevalence was 0.07, 0.14, 10.50, 14.30, 0.95 and 1.67%, respectively. Healthy pods were free from fungal contamination.

Leaf spot and blight are two common diseases of *C. ternatea.* Mukerji and Bhasin (1986) reported leaf spot caused by *Alternaria alternata* (Fr.) Keissler, *Cercospora pentaleuca* and *C. terateae* Petch.; anthracnose caused by *Colletotrichum dematium* (Pers. Ex Fr.) Grove, and *C. gloeosporioides* Penz., wilt caused by *Fusarium oxysporum* Schlecth ex Fr.; Spots on legume, caused by *Leptothyrium leguminum* (Cooke) Saac., spot on stem caused by *Macrophomaa clitocarpa* (Cooke) Beri. & Vogl and Powdery mildew caused by *Oidium clitoriae* Naarayanaswamy and Ramakris from India.

Fungal leaf diseases of *C. ternatea* is caused by *Cercospora*, *Colletotrichum*, *Oidium* and *Rhizoctonia* (Staples, 1992).

This is the first record of association of *A. niger*, *A. flavus*, *A. fumigatus*, C. *oxysporum*, *C. lunata* and *Penicillum* sp. with *C. ternatea* have been recorded in cool wet weather but rarely as a serious problem.

Pathogenicity test following 'detached leaf technique' revealed that the fungi isolated from the infected leaves were non pathogenic to *C*. *ternatea.* The isolated fungi even in saprophytic form severely damaged the leaves and fruits of the plant. Similar observation was reported by Fatema and Shamsi (2012) where *Oxalis* spp. showing severe anthracnose symptom was recorded by the authors but all the isolated fungi including *C. gloeosporioides* were non-pathogenic to the plants.

Green plants that contain chlorophyll in their leaves, stems and fruits are 'green power' of the living world. They are primary producer in food chain and also release fresh oxygen to environment for living entities. It is the exclusive mission of the Naturalists, Botanists, Mycologists and Plant Pathologists to defend the green resources of the earth from diseases, disorders and disasters.

Considering the economic values of the plant, present research is an initial step to the diagnosis of the fungal diseases of the plant in Bangladesh. Consequence of this study will be helpful for designing the proper management of fungal diseases of *C*. *ternatea*.

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