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REPORT ON ANTHRACNOSE OF RAUWOLFIA SERPENTINA (L.) BENTH. EX KURZ CAUSED BY COLLETOTRICHUM GLOEOSPORIOIDES (PENZ.) SACC. FROM BANGLADESH

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Abstract

During the tenure of May 2007 to July 2013 severe anthracnose symptom was recorded on leaves of *Rauwolfia serpentina* (L.) Benth. Ex Kurz. The frequency percentage of association of *Colletotrichum gloeosporioides*, was higher than any other fungi associated with symptomatic leaves. The frequently isolated fungus *C. gloeosporioides* was found to be pathogenic to respective plants when pathogenicity of the isolated fungus was tested following modified "detached leaf technique" and "spraying of spore suspension" methods. Present paper is the first report of anthracnose of *R. serpentine* from Bangladesh.

Key words: Anthracnose, *Rauwolfia serpentina*, Bangladesh

Introduction

Rauwolfia serpentina (L.) Benth. ex Kurz is a medicinal shrub belonging to the family Apocynaceae. The shrub is locally known as "Sarpagandha" also known as Indian snakeroot. It grows in India, Thailand and other parts of Asia, South America and Africa. It is widely distributed in the Sub – Himalayan tract from Punjab to Nepal, Sikkim and Bhutan (Ahmed *et al.* 2008). It is also found in the lower hills of Gangetic plains, and Andamans. In the Deccan, it is associated with bamboo forests. In Bangladesh it grows in Chittagong, Sylhet and Mymensingh (Chowdhury 1995). International Union for Conservation of Nature (IUCN) has placed this plant under endangered status (Mabberley 2008). Root of this shrub is mostly used. Seventeen different alkaloids have been extracted from the bark of the root of this shrub (Panda 2004). Serpentine is one of those alkaloids. Barks are collected in winter because they contain more alkaloids during that time. It is a good antidote for high blood pressure. This plant is occurring naturally in India and Bangladesh and is found to grow wild in the Asian continent. It has been reported to contain 50 indole alkaloids that are mainly localized in the root bark (Yusuf *et al.* 2009).

Rauwolfia serpentine contain the alkaloid reserpine chief use of which is as a sedative and hypnotic and for reducing blood pressure. This alkaloid stimulates the central nervous system. Reserpine is more suitable for cases of mild anxiety or patients of chronic mental illness. It also reduces cardiac output and the total peripheral resistance.

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As such, it is useful in lowering blood pressure. Reserpine is principally advocated in case of mild to moderate hypertension, insomnia and in psychiatric disorder but with extreme precaution. Indian snakeroot reduces fever, stop diarrhoea and dysentery (Kapoor 1990).

Anthracnose and leaf spot are common fungal diseases of *R. serpentina*. The other diseases include Target leaf blotch, Cercospora leaf spot, Die-back, Powdery mildew and Fusarium wilt, and Root–knot disease Mukerji and Bhasin 1986. Most of the research work carried out on it falls under Phytochemical, Pharmacological, Biochemical and Antimicrobial disciplines. But research about its fungal diseases is inadequate so far and under Bangladesh condition no report in this regard has so far been available. Thus, it is important to find out the etiology and identification of the associated fungi with the diseased plant. The main purpose of the present research is to investigate the association of fungi with diseased leaves and their identification and to test the pathogenic potentiality of the isolates.

Materials and Methods

Collection of samples: Samples were collected from field of Botanical garden, Curzon Hall Campus, Dhaka University, Lawachara, Sylhet, Botanic garden, Chittagong University campus and Mymensingh during the period of April 2007 to August 2013. (Samples examined : voucher specimen No. BOT. ZY - 01 to ZY -150).

Measurement of disease severity: Disease severity were estimated by the following formula:

$$\text{Severity} = \frac{\text{Sum of all Ratings}}{\text{No. of observation} \times \text{Highest Rating}} \times 100$$

For visual estimation of severity, 0 – 9 point scale were used for rating of all foliar diseases studied (PDI=McKinney's Index, Ghos *et al.* 2009).

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

Isolation of fungi: Fungi associated with infected samples were isolated following "Tissue Planting" and Blotter" methods. Experiment was conducted in the Laboratory of Mycology and Plant Pathology, Department of Botany, University of Dhaka. In case of Tissue Planting" method, 30 inocula each measuring 2 square mm. were cut with a sterilized scalpel from a particular specimen and kept in a sterile Petri plate. The inocula were washed in sterile water and then surface sterilized by dipping in 10% Clorox for 3-5 minutes. Three inocula were placed in each plate containing sterilized Potato Dextrose Agar (PDA) medium in pH 6 and incubated for 5-7 days at temperature 25 to 28^0 C. In

"Blotter" method, moist chambers were made by placing two layers of filter paper on the bottom of the Petri plates, moistened with sterilized water, covered with upper lids and were sterilized (autoclaved at 15 lbs pressure and 120°C temperature). Four square mm. sized inocula were prepared from the samples, surfacesterilized with 10% chlorox for 3-5 minutes. A total number of 30 inocula were transferred in 10 Petri plates and incubated for 5-7 days at temperature 25 to 28^0 C. The fungi growing out of the inocula were transferred to separate plates and slants for further studies and storage. Percentage association of the fungi was also recorded.

Identification of the isolates were determined following the standard literatures (Booth 1971, Barnett and Hunter 1972, Ellis 1971, 1976 Ellis and Ellis 1997 and Sutton 1980). (All the specimens were preserved in the Herbarium, Mycology and Plant pathology section, Department of Botany, University of Dhaka, Bangladesh, Isolate No. 00ZY- 01 to 00ZY-180).

Pathogenicity test following modified "Detached leaf technique" : In total 16 fungal species were isolated from the anthracnose infected *Rauwolfia serpentina.* The isolated fungi were tested for their pathogenic potentiality following modified "Detached leaf technique" (Azad and Shamsi, 2011) and "spraying of spore suspension technique" (Azad and Shamsi 2011).

Healthy matured leaves of the plant were thoroughly washed under running tap water and then surface disinfested in 10% 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. Then 25^2 mm leaf pieces were placed on the autoclaved moist petri plates and those were inoculated with 5 mm (diam.) mycelia 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 fungus was used as follows: T_1 = (control) dorsally inoculated leaf pieces with PDA block, T_2 = (control) ventrally inoculated leaf pieces with PDA block, T_3 = dorsally unpricked inoculated leaf pieces with test fungus; T_4 = ventrally unpricked inoculated leaf pieces with test fungus, T_5 = dorsally pricked inoculated leaf pieces with test fungus and T_6 = ventrally pricked inoculated leaf pieces with test fungus. The inoculated plates were incubated at 25-28°C. After 5 days of inoculation lesions size were recorded.

Pathogenicity test following spore inoculation test: The pathogenic fungus screened from "Detached leaf assay" were selected for net house experiment. Healthy seedlings of *R. serpentina* was transplanted in earthen pots (10 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. Soil was sterilized with 10% formalin and covered with polythene sheet for three days and then polythene sheet was removed for three gays then soil was poured on pots. Identified fungus was purified and its pathogenicity was examined

by inoculating fresh healthy plants following spraying of spore suspension method. Conidia from seven days old culture of test fungus was taken in 250 ml conical flask with sterilized water. Ten ml water suspension of test fungus at 10^4 ml conc. was prepared and taken in a hand sprayer and sprayed on healthy potted plant. Control received only sterilized water without fungal inocula. Five pots were inoculated for each treatment. The inoculated and control plants were placed in a clean bench in net house following completely randomized design.

The plants were examined daily and continued for 10 days to record the development of symptoms Inoculated plants were covered by perforated polythene bags to avoid contamination and to maintain humidity. Symptom produced on artificial inoculated plants were recorded and compared with those observed on naturally infected plants. Inoculated fungus was re-isolated to fulfill Koch's postulate.

Results and Discussion

Severe anthracnose symptom was noticed on leaves of *R. serpentina* during the period of May 2007 to July 2013. Samples were collected from Joydebpur, Gazipur, Dhaka, Chittagong and Sylhet and Mymensingh. Healthy and infected plants of *R. serpentine* are presented in Plate I A and B.

Plate 1. *Rawulfia serpentina*: A. Healthy plants, B. Plants with anthracnose symptom.

Disease severity was recorded at (0-9) DS scale in all the years studied. Highest disease severity 9 was recorded in the year 2008 followed by DS 7 in 2007 and 2013, DS 6 in 2012, DS 4 in 2010. Lowest DS was recorded 2 in the year 2009 and 2011 (Fig. 1).

A total of 12 fungal species namely, *Alternaria alternata* (Fr.) Keissler, ex Fr., *Aspergillus niger* van Tieghme, *Colletotrichum gloeosporioides* (Penz.) Sacc., *Fusarium* spp., *Macrophoma* sp., *Nigrospora* sp., *Penicillium* spp., *Pestalotia* guepini., *Rhizopus stolonifer* (Ehrenb. ex. Fr.) Lind, and *Trichoderma viride* was isolated from infected leaves of *R. serpentina* (Table 1).

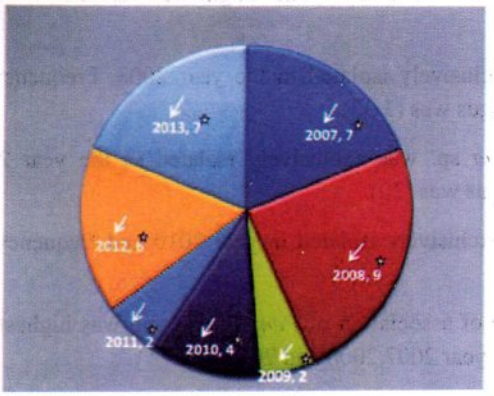

Fig. 1. Disease severity recorded at 0-9 scale on *Rauwolfia serpentina* owing to anthracnose caused by C. *gloeosporioides* during the period of 2007 to 2013 (Arrows = sampling years, * = DS scale).

Table-1. Frequency (%) of association of different fungi with leaves of *Rauwolfia sarpentina* during 2007-2013.

Name of Isolate	Frequency (%) of fungi in different years						
	2007	2008	2009	2010	2011	2012	2013
Alternaria alternata	-	-	60.00	3.33	-	3.33	-
Aspergillus niger	13.33	66.66	6.66	-	3.33	-	3.33
Colletotrichum Gloeosporioidis	73.33	93.33	16.66	43.33	20.00	60.00	79.99
Fusarium sp.	-	3.33	-	-	-	-	-
Macrophoma sp.p	20.00	-	-	-	-	-	-
Nigrospora sp.	-	-	-	3.33	-	-	-
Penicillium spp	3.33	3.33	-	6.66	-	3.33	
Pestalotiopsis guepinii.	16.66	-	-	-	3.33	-	-
Rhizopus stolonifer	43.33	6.66	20.00	3.33	-	-	-
Trichoderma viride	6.66	6.66	-	3.33	-	-	-

Table 1. showed that frequency percentage of association of *A. alternate* was highest (60.00) in the year 2009 and it was lowest (3.33) in the year 2010 and 2012. The fungi was not detected in the years 2007, 2008, 2011and 2013.

Frequency percentage of association of *A. niger* was highest (66.66) in the year 2008 followed by (13.33) in 2007 and (6.66) in 2009. Lowest frequency percentage of the fungus was (3.33) in 2011 and 2013. The fungi was not found in 2010 and 2012.

Frequency percentage of association of C. *gloeosporioidis* was highest (93.33) and it was lowest (16.66) in the year of 2009.The fungus was isolated from the sample studied during 2007 to 2013.

Fusarium sp. was exclusively isolated in the year 2008. Frequency percentage of the association of the fungus was (3.33).

Similarly *Macrophoma* sp. was exclusively isolated in the year 2007 and frequency percentage of the fungus was (20).

Nigrospora sp. was exclusively isolated in year 2010 and frequency percentage of the fungus was (3.33).

Frequency percentage of association of *Penicillium* spp was highest in (6.66) in 2010 and lowest 3.33 in the year 2007,2008 and 2012.

Frequency percentage of association of *P. guepinii* was highest (16.66) in year 2007 and it was lowest (3.33) in 2011.

Frequency percentage of association of *R. stolonifer* was highest (43.33) in the year 2007 and it was lowest (3.33) in the year 2010.

Frequency percentage of association of *T. viride* was highest (6.66) in the year 2007 and 2008 and it was lowest (3.33) in the year 2011.

Anthracnose caused by *Colletotrichum gloeosporioides* showed reddish brown irregular lesion on dorsal surface of the leaves, corresponding ventral surface was pale brown in colour. Symptoms appear as irregular to circular spots 1 to 10 mm in diameter, sharply defined, occasionally slightly depressed. In the present study *Colletotrichum gleosporioides* causes the disease and appears as numerous tiny spots of the acrevuli scattered all over but mainly confined to the upper leaf surface. Infected spots enlarge into circular patches and invades the surrounding tissues. Several such patches coalesce and cause drying of the lamina resulting in defoliation. Every infected spot is an acervulus packed with spores.

A study was conducted to identify the fungi associated with leaves of *R. serpentina.* During the period of this study *Colletotrichum gleosporioidis*, was frequently isolated from leaves of *R. serpentina.* Morphological characters of the fungi were recorded on PDA. *Colletotrichum gloeosporioides* is a facultative parasite belongs to the order *Melanconiales.* Colony cottony, white, grayish, reverse grayish black at maturity. Setae absent. The waxy acervuli, that are produced in infected tissue, are subepidermal, typically with setae, and simple, short, erect conidiophores Masses of conidia appear pink or salmon colored. The fungus produces hyaline, one- celled, ovoid to oblong conidia, comparatively large, straight, obtuse at the apex, 14.8-24.4 × 3.6-5.2 µm. Appressoria abundant. pale to medium brown, circular or slightly irregular (Plate II A and B).

Leaf spot and premature defoliation (*Curvularia lunata* (Wakker) Boedijn), Leaf sot (*Epicoccum nigrum* Link., *Fusarium oxysporum* Schlecht, powdery mildew (*Levillula taurica* (Lev.) Arnnaud), root rot (*Macrospomina phaseolina* (Tassi) Goid.) leaf spot (Mycosphaerella rauvolfiae Ramakr T.S. & Ramakr K. and Pellcularia filamentosa (Pat.) Rogers) and on leaf (*Phomopsis sethii* Mehrotra).

This is the first report of anthracnose of *R. serpentina.* from Bangladesh. Present investigation will be helpful for designing successful control measures of the disease.

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