



Short Communication

Report on endophytic fungi associated with *Rauwolfia Serpentina* (L.) Benth. Ex Kurz – a herbal medicinal plant

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ABSTRACT: During the tenure of May 2007 to July 2013 healthy leaves of *Rauwolfia serpentina* (L.) Benth. Ex Kurz were collected and associated fungi with the leaves were isolated following “Tissue planting” methods on PDA medium. A total of 12 fungal species namely, *Alternaria alternata* (Fr.) Keissler, *Aspergillus flavus* Link., *A. niger* van Tieghem, *Pseudocercospora* sp., *Colletotrichum gloeosporioides* (Penz.) Sacc., *Fusarium* sp., *Macrophoma* sp., *Nigrospora sphaerica* (Sacc.) Mason, *Penicillium* sp., *Pestalotiaopsis* sp., *Rhizopus stolonifer* (Ehrenb. ex Fr.) Lind, and *Trichoderma viride* Pers. were isolated from healthy leaves of *R. serpentina*. Present paper is the first report of endophytic fungi of *R. serpentina* from Bangladesh.

KEYWORDS: Endophytic Fungi, *Rauwolfia serpentina*, Herbal, Medicinal plant

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INTRODUCTION

Rauwolfia serpentina (L.) Benth. ex Kurz is a medicinal shrub belonging to the family Apocynaceae. It is an evergreen trees and shrubs. The genus is named to honor Leonhard Rauwolf. The approximately 85 species in the genus can mainly be found in tropical regions. The plant is a small erect glabrous shrub about 1 to 3 feet in height, bearing white or pinkish flowers. It grows fairly wild in the United Provinces, also in Bihar and Eastern and Western Ghats. The roots, the leaves and the juice have been considered of medicinal importance from the very early times and have attracted the attention of the practitioners of the indigenous system of medicine. It has been used as an anthelmintic, as an antidote against snake bite and bites of other poisonous insects, in diarrhoea, dysentery, cholera and also as an ecobolic. In recent years interest has been stimulated in this drug, because of its well marked hypnotic and sedative properties. 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 Bhutan¹. It is also found in the lower hills of Gangetic plains, and Andamans. It is mostly found in moist

deciduous forests at altitudes ranging from sea level to an altitude of 1,200 m high. In the Deccan, it is associated with bamboo forests. In Bangladesh it grows in Chittagong, Sylhet and Mymensingh². International Union for Conservation of Nature (IUCN) has placed this plant under endangered status. Root of this shrub is mostly used³. The plant contains more than 50 different alkaloids which belong to the monoterpenoid indole alkaloid family. The leaves and roots of *Rauwolfia serpentina* contain alkaloids which are secondary metabolites. Major alkaloids identified are Reserpine, Rauwolfine, Serpentine, Sarpagine, Ajmaline, Yohimbine and Ajmalicine. *Rauwolfia serpentina* contains a number of bioactive chemicals, including yohimbine, reserpine, ajmaline, deserpidine, rescinnamine, serpentinine⁴⁻⁶. Endophytes are microbes that inhabit plant tissues in their life cycle without causing any apparent harm to their host. Plant use of endophytic fungi in defense occurs when endophytic fungi, which live symbiotically with the majority of plants by entering their cells, are utilized as an indirect defense against herbivores. In exchange for carbohydrate energy resources, the fungus provides benefits to the plant which can include increased water or nutrient uptake and protection from phytophagous insects,

birds or mammals. Once associated, the fungi alter nutrient content of the plant and enhance or begin production of secondary metabolites. The change in chemical composition acts to deter herbivore by insects, grazing by ungulates and/or oviposition by adult insects. Endophyte-mediated defense can also be effective against pathogens and non-herbivore damage⁷⁻⁹.

This study was conducted to characterize and explore the endophytic fungi of selected plants from the different region of Bangladesh.

MATERIALS AND METHODS

Collection of samples

Samples were collected from field plot of Botanical garden, Curzon Hall Campus, Dhaka University, Lawachara, Sylhet, Botanic garden, Chittagong University campus and Mymensingh BAU campus during the period of April 2007 to August 2013.

Isolation of fungi

Fungi associated with the samples were isolated following "Tissue Planting" method. Experiment was conducted in the Laboratory of Mycology and Plant Pathology, Department of Botany, University of Dhaka. Thirty

inocula each measuring 2 mm². 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 28⁰ 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 was determined following the standard literatures¹⁰⁻¹⁵. All the specimens were preserved in the Herbarium, Mycology and Plant pathology section, Department of Botany, University of Dhaka, Bangladesh.

RESULTS AND DISCUSSION

Healthy leaves of *R. serpentina* were examined during the period of May 2007 to July 2013. Samples were collected from Joydebpur, Gazipur, Dhaka, Chittagong and Sylhet and Mymensingh. Healthy plants of *R. serpentina* are presented in Figure 1.



Figure 1. *Rauwolfia serpentina*: Healthy plant

A total of 12 fungal species namely, *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Pseudocercospora* sp., *Colletotrichum gloeosporioides*., *Fusarium* sp., *Macrophoma* sp., *Nigrospora sphaerica*, *Penicillium* sp., *Pestalotiaopsis* sp., *Rhizopus stolonifer* and *Trichoderma viride* were isolated from healthy leaves of *R. serpentina* (Table 1).

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

Name of the isolates	2007	2008	2009	2010	2011	2012	2013	Total	Mean
<i>Alternaria alternata</i>	6.66	-	-	76.66	-	3.33	6.66	83.32	41.66
<i>Aspergillus flavus</i>	3.33	-	-	-	-	-	3.33	3.33	3.33
<i>Aspergillus niger</i>	96.66	-	99.88	10	-	6.66	-	206.54	60.84
<i>Pseudoercospora</i> sp.	-	36.66	-	-	6.66	-	-	43.32	6.18
<i>Colletotrichum gleosporioides</i>	79.99	6.66	73.33	3.33	-	-	-	163.31	40.82
<i>Fusarium</i> sp.	16.66	-	-	-	-	3.33	-	16.66	16.66
<i>Macrophoma</i> sp.	20	-	-	-	-	-	-	20	20
<i>Nigrospora sphaerica</i>	-	-	-	20	-	3.33	-	20	20
<i>Penicillium</i> sp.	6.66	6.66	-	-	-	-	-	13.32	6.66
<i>Pestalotiopsis</i> sp.	-	-	6.66	6.66	-	-	-	13.32	6.66
<i>Rhizopus stolonifer</i>	10	-	-	-	-	-	3.33	10	10
<i>Trichoderma viride</i>	16.66	3.33	-	-	-	-	-	19.99	9.99

- = No fungal growth

Table 1 showed that *A. niger* was the predominating fungus with healthy leaves of *R. serpentina*. Frequency percentage of association of the fungus was highest (99.88) in 2009 followed by (96.66) in 2007. Frequency percentage of association of the fungus was lowest (10) in 2010.

Frequency percentage of association of *A. alternata* was highest (76.66) in the year 2010 and it was lowest (3.33) in the year 2012. The fungus was not detected in 2008, 2009 and 2011.

Frequency percentage of *Colletotrichum gleosporioides* was highest (79.99) in 2007 followed by (73.33) in 2009 and it was lowest (3.33) in the year of 2010.

Frequency percentage of association of *Fusarium* sp. was highest 16.66 in 2007 and it was lowest 3.33 in 2012.

Aspergillus flavus and a species of *Macrophoma* were exclusively isolated from healthy leaves in 2007. Frequency percentage of the fungi were 3.33 and 20.

Frequency percentage of association of *Pseudocercospora* sp. was highest (36.66) in the year 2008 and lowest in 6.66 in 2011. The fungus was not detected in 2007, 2009, 2010, 2012 and 2013.

Nigrospora sphaerica was exclusively isolated in the year 2010. Frequency percentage of association of the fungus was 20%.

Frequency percentage of *Penicillium* sp. was 6.66% in the year 2007 and 2008.

Frequency percentage of association of *Pestalotiopsis* sp. was 6.66% in the year 2009 and 2010. The fungus was not detected in rest of the years.

Frequency percentage of association of *Trichoderma viride* was highest 16.66 in 2007 and it was the lowest 3.33 in the year 2008.

Frequency percentage of association of *Pestalotiopsis* sp. was the highest 16.66 in the year 2007 and it was the lowest 6.66 in the year 2009.

Frequency percentage of association of *Rhizopus stolonifer* was highest 10 in the year 2007 and it was the lowest 3.33 in the year 2013.

Rauwolfia serpentina is an ethno medicinal plant used as anti-hypertensive remedy by the ethnic tribes of northeast India. The plant was screened for the endophytic fungi and their associated biological activities to assess if these fungi have any significant hypocholesterolemic, antioxidant and antimicrobial activities. The endophytes were characterized as *Colletotrichum gloeosporioides*, *Penicillium* sp. and *Aspergillus awamori*, from a total 15 isolates, *C. gloeosporioides* was found to be the dominant fungus with an average colonization frequency of 6.8 % followed by *Penicillium* sp. (3.8 %) and *A. awamori* (2.1 %). *Alternaria* and *Fusarium* species were the dominant taxa obtained from *Withania somnifera*, *Artemisia annua*, *Platanus orientalis* and *Rauwolfia serpentina*, as compared to the other host plant.¹⁶

From this investigation it has been found that *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Pseudocercospora* sp., *Macrophoma* sp., *Nigrospora sphaerica*, *Pestalotiopsis* sp., *Rhizopus stolonifer* and *Trichoderma viride* are the newly recorded endophytic fungi of *R. serpentina*.

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