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Incidence and severity of leaf spot and anthracnose diseases of *Rauwolfia serpentina* (L.) Benth. ex Kurz

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ABSTRACT: *Rauwolfia serpentina* (L.) Benth. ex Kurz is a medicinal plant. The plant is locally known as "Sarpagandha" also known as Indian snakeroot. During the tenure of 2007 to 2013 two types of symptoms viz. leaf spot and anthracnose were recorded from leaves of *R. serpentina.* Leaf spot disease incidence was started from February and gradually increased up to July. Leaf spot symptom showed lowest disease severity (1) in the month of February and the highest DS was (9) in the month of July. Whereas anthracnose disease incidence was started from March and gradually increased up to September. In case of anthracnose symptom, the lowest disease severity (2) was recorded in the month of March and the highest DS (9) was recorded in the month of September. Temperature shows noticeable effect on disease development. Rainfall and humidity also show slight effect on disease development. The fungi associated with the collected samples were isolated following 'Tissue planting' method on PDA (Potato Dextrose Agar) medium and 'Blotter method'
Pathogenecity test of the isolated fungi were done following 'Detached leaf technique' and 'Spraying of spore suspension' on healthy plants. Among the isolated fungi *Colletotrichum gloeosporioides* was found to be pathogenic to *R. serpentina.*

Keywords: Incidence, Severity, Leaf spot, Anthracnose, *Rauwolfia serpentina*

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INTRODUCTION

Rauwolfia serpentina (L.) Benth. ex Kurz is a medicinal shrub belongs to the family Apocynaceae. It is locally known as "Sarpagandha" as well 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. It is also found in the lower hills of Gangetic plains and Andamans. In Bangladesh it grows in Chittagong, Dhaka, Mymensingh and Sylhet. International Union for the Conservation of Nature and Natural Recourses (IUCN) has placed this plant under endangered status[1-3].Root of this shrub is mostly used as a good antidote for high blood pressure. Seventeen different alkaloids have been extracted from the bark of the root of this shrub. Leaf spot and anthracnose are common fungal diseases of *R. serpentina.* The other diseases of the plants include Target leaf blotch, Cercospora leaf spot, Die-back, Powdery mildew, Fusarium wilt, Root-knot disease, etc[4]. Most of the research work was carried out on Phytochemical, Pharmacological, Biochemical and Antimicrobial disciplines[5-10]. But research about Fungal Diseases of *Rauwolfia* is inadequate in Bangladesh[11].Thus, it is important to find out the etiology and identification of the associated fungi with the diseased plant. Present investigation was undertaken to detect the incidence and severity of leaf spot and anthracnose diseases of *R. serpentina.*

MATERIALS AND METHODS

Collection of Samples

Leaves of *R. serpentina* with leaf spot and anthracnose diseases were collected from Chittagong, Dhaka, Mymensingh and Sylhet during the period of 2007-2013. A total of 145 diseased samples were examined.

Measurement of disease severity: Disease severity was 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, Ghose t al. 2009)[11].

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.

Effect of temperature, humidity and rainfall on disease incidence and disease severity:

Disease incidence and disease severity were measured following Rahman and Rashid (2008)[12].

The incidence and severity of leaf spot and anthracnose of *R. serpentina* were recorded from the plants grown in gardens of Curzon Hall Campus, Dhaka University. Each plot was visit and data were recorded twice in a month. Data were expressed in percentage. The formula in calculating the disease incidence and severity is:

$$\% \text{ Leaf infection} = \frac{\text{Number of leaves / Plants infected} \times 100}{\text{Total number of leaves/plants counted}}$$

Temperature, humidity and rainfall data were recorded for the year of 2011-2013 (Collected from Bangladesh Meteorological Department, Agargaon, Dhaka).

Isolation of fungi

The fungi associated with the collected samples were isolated following 'Tissue planting' method on PDA (Potato Dextrose Agar) medium and 'Blotter method'[13].

Microscopic observation of the fungi

Microscopic details of the associated fungi with *R. serpentina* were studied following standard techniques[14-15].

Identification of the fungi

Identification of the fungi were confirmed following relevant literatures [16-21].

RESULTS AND DISCUSSION

In Bangladesh *Rauwolfia serpentine* grows in Chittagong, Dhaka, Mymensingh and Sylhet. International Union for the Conservation of Nature and Natural Recourses (IUCN) has placed this plant under endangered status[22]. Fungal diseases are another constrain for growth of this valuable medicinal plant. Two types of symptoms were recorded from leaves of *R, serpentina* during the tenure of 2007-2013. The symptoms were leaf spot and anthracnose.

Fifteen species of fungi were isolated from the symptomatic leaves. Leaf spot and anthracnose symptoms were recorded on leaves of *R. serpentina* during the period of 2007-2013 (Plate 1 and 2).

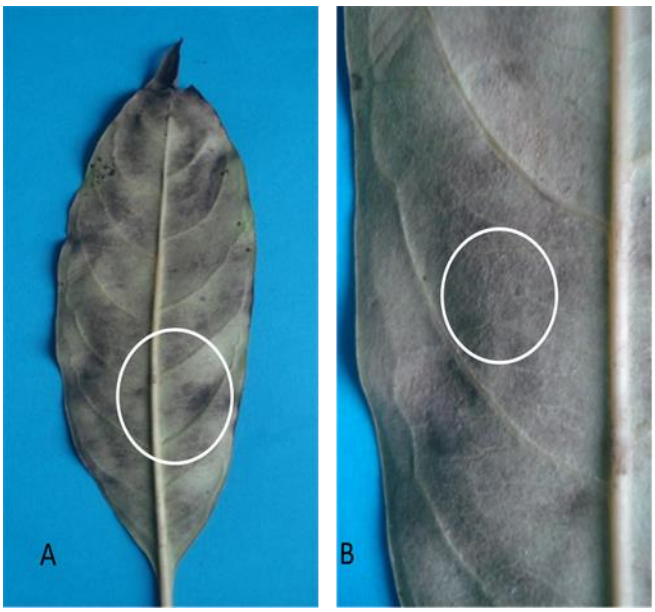

Fig. 1. *Rauwolfia serpentina*: A-B Leaf spot symptom.

Fig. 2. Anthracnose symptom of *Rauwolfia serpentina.*

Severe leaf spot symptom was noticed on leaves of *R. serpentina* during the period of February 2007 to July 2013. Disease severity was recorded at (0-9) DS scale in all the years studied. Highest disease severity 9 was recorded in the year 2008, 2009, 2010 and 2013, followed by DS 8 in 2007 and 2012. DS 7 was recorded in 2007, 2008, 2009, 2012 and 2013, DS 6 in 2007, 2008, 2010 and 2013. DS 5 was recorded in

2007, 2009, 2010, 2011 and 2012, DS 4 in 2008, 2010, 2011, 2012 and 2013. DS 3 was recorded in 2007, 2009, 2010, 2012 and 2013. DS 2 in 2007,2008, 2011, 2012 and 2013. Lowest DS was recorded 1 in the year 2009, 2010, 2011 and 2012 (Fig. 3 and 13).

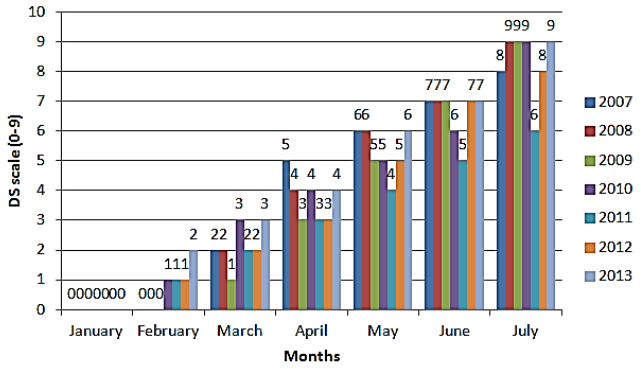

Fig. 3. Disease severity recorded at 0-9 scale on *Rauwolfia serpentina* owing to leaf spot caused by *Pseudocercospora liebenbergii* during the period of 2007 to 2013.

Severe anthracnose symptom was noticed on leaves of *R. serpentina* during the period of May 2007 to July 2013. Disease severity was recorded at (0-9) DS scale in all the year 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.4 and 14).

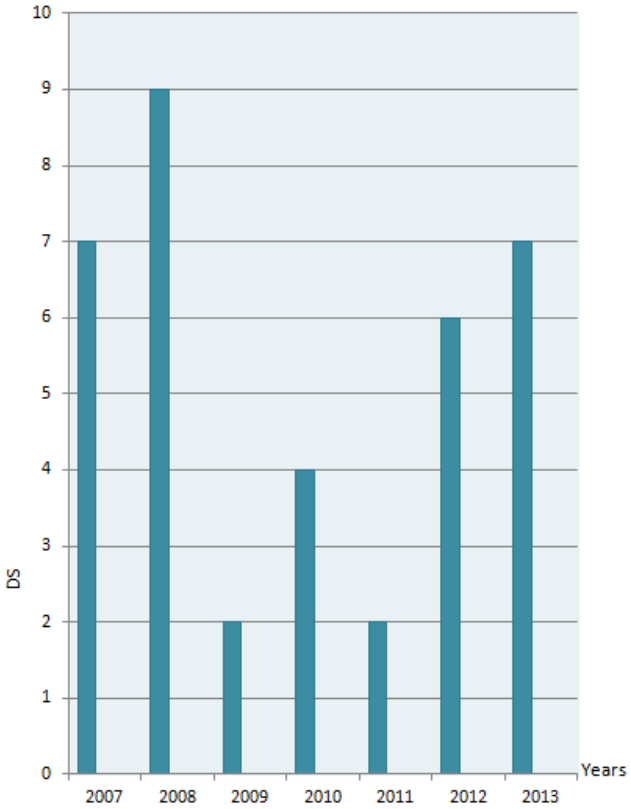

Fig.4. Disease severity (DS) recorded at 0-9 scale on *Rauwolfia serpentina* owing to anthracnose caused by *C. gloeosporioides* during the period of 2007 to 2013.

Effect rainfall, temperature and humidity on incidence of leaf spot and anthracnose of *R. serpentina* during 2011, 2012 and 2013 are presented in 5-11.

Leaf spot disease incidence was started from February and gradually increased up to July. Leaf spot symptom showed lowest disease severity (1) in the month of February and the highest DS was (9) in the month of July. Whereas anthracnose disease incidence was started from March and gradually increased up to September. In case of anthracnose symptom, the lowest disease severity (2) was recorded in the month of March and the highest DS (9) was recorded in the month of September. Temperature shows noticeable effect on disease development. Rainfall and humidity also show slight effect on disease development.

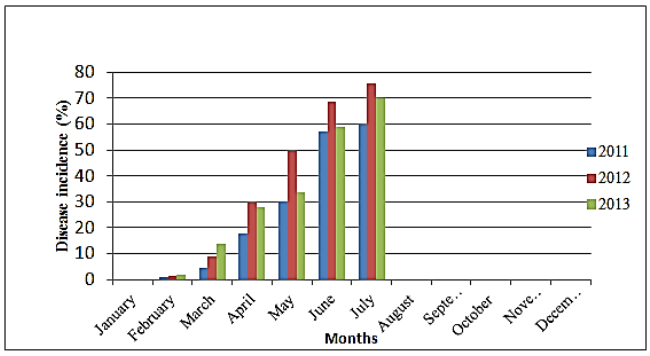

Fig.5. Disease incidence owing to leaf spot *of Rauwolfia serpentine* during 2011-2013.

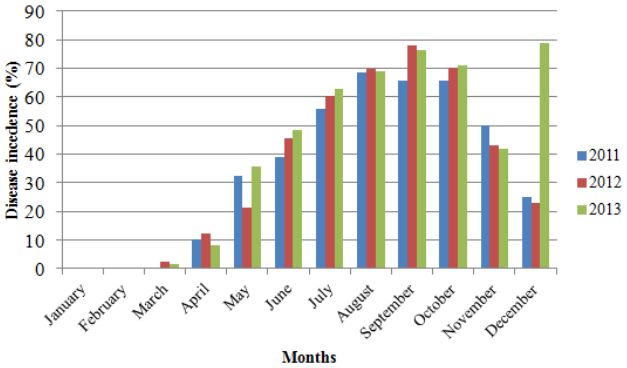

Fig. 6. Disease incidence owing to anthracnose of *Rauwolfia serpertina* during 2011-2013.

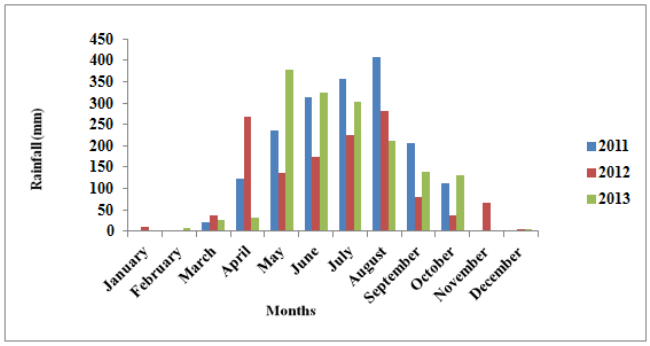

Fig. 7. Rainfall from January-December (2011-2013) in Dhaka city.

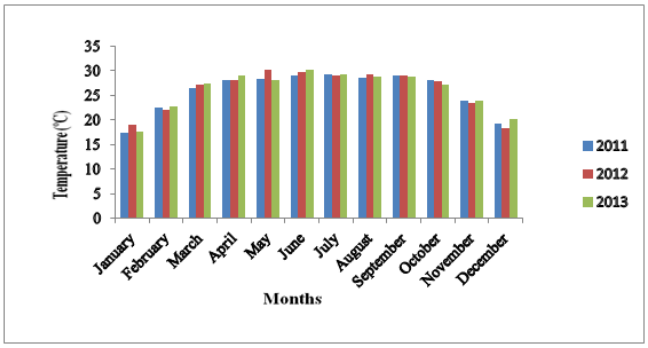

Fig.8. Temperature from January-December (2011-2013) in Dhaka city.

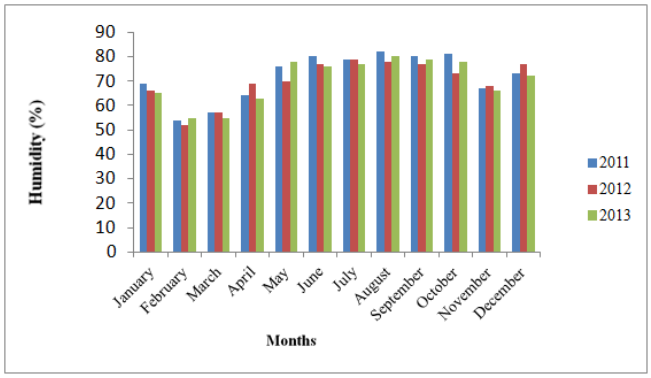

Fig. 9. Humidity from January-December (2011-2013) in Dhaka city.

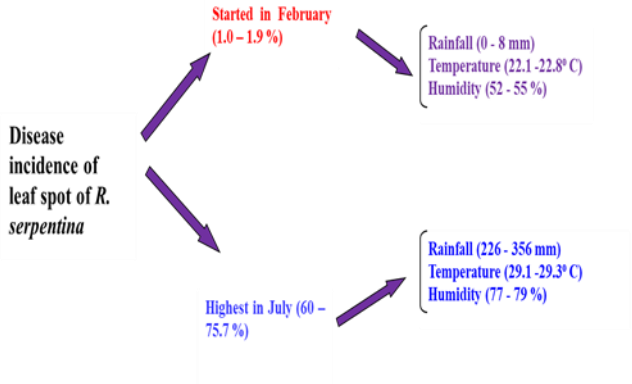

Fig. 10. Disease incidence of leaf spot of *Rauwolfia serpentina*during the tenure of 2011- 2013.

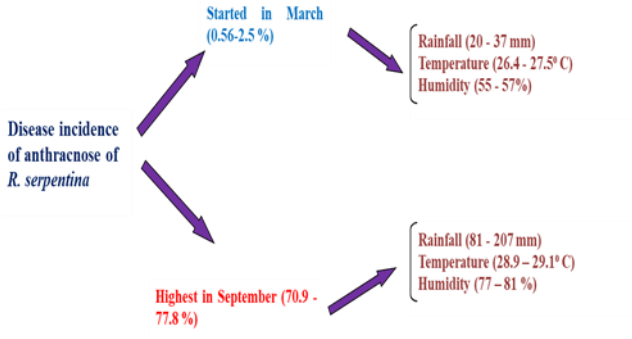

Fig. 11. Disease incidence of anthracnose of *Rauwolfia serpentine* during the tenure of 2011- 2013.

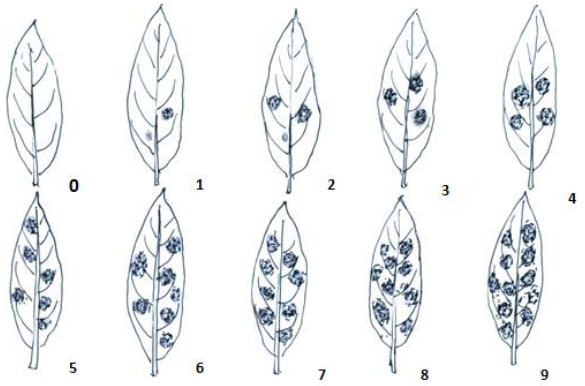

Fig.13. Diagrammatic presentation of disease severity scale 0-9 for leaf spot symptom.

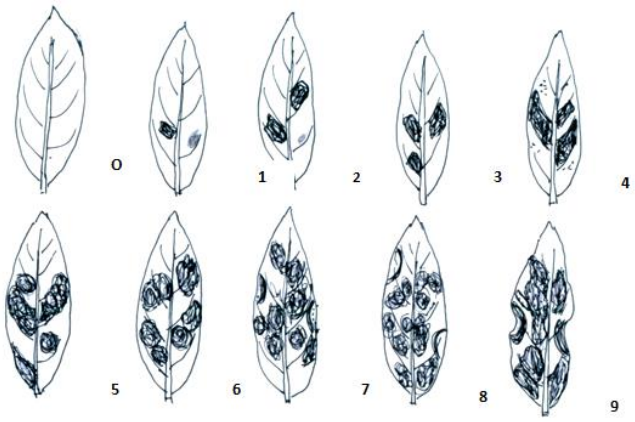

Fig.14. Diagrammatic presentation of disease severity scale 0-9 for anthracnose symptom.

A total of 15 species of fungi were isolated from. infected leaves of *R. serpentina*.The isolated fungi were presented in Table 1.

Table 1. List of fungi associated with infected leaves of *Rauwolfia serpentina.*

1. *Alternaria alternata*(Fr.) Keissler	9. *Nigrospora sphaerica* (Sacc.) Mason
2. *Aspergillus flavus* Link ex Fr.	10.*Penicillium digitatum* Saccardo
3. *Aspergillus niger* van Tieghm	11.*Penicillium italicum* Whemer
4. *Colletotrichum gloeosporioides* (Penz.) Sacc.	12.*Pestalotiopsis guepinii* (Desm.) Stay
5. *Curvularia lunata* (Wakker) Boedijn	13. *Pseudocercospora liebenbergii* (Syd.) Deighton
6. *Fusarium* sp. 1	14.*Rhizopus stolonifer* Bull. Toney
7. *Fusarium* sp. 2	15.*Trichoderma viride* Pers.
8. *Macrophoma* sp.	

Thirteen species of fungi were associated with leaf spot of *Rauwolfia serpentina.*

Table 2 showed that frequency percentage of association of *Alternaria alternate* was highest 73.32 in the year 2009 followed by 70 in 2008 and it was lowest 3.33 in the year 2010 and 2013.

Frequency percentage of association of *Aspergillus niger* was highest (99.99) in the year 2009 and it was lowest 3.33 in the year 2008.

Frequency percentage of *Colletotrichum gloeosporioides* was highest 43.33 in the year 2007 and it was lowest 3.33 in the year 2008, 2009 and 2011. The fungus was not detected in the year 2010.

Frequency percentage of *Fusarium* sp.1. was 3.33 and *Fusarium* sp. 2. was 6.66. The fungi were exclusively isolated in 2008 and 2007 respectively.

Frequency percentage of association of *Macrophoma* sp. was 20 and it was exclusively isolated in the year 2007.

Frequency percentage of association of *Nigrospora sphaerica* was 23.33 and it was exclusively isolated in the year 2010. Frequency percentage of association of *P. digitatum* was the highest 6.66 in the year 2008 and it was the lowest 3.33 in the year 2009 and 2011.

Penicillium italicum was exclusively isolated in 2008.

Frequency percentage of association of the fungi was 3.33. Frequency percentage of association of *P. liebenbergii* was highest (59.99) in the year 2007 followed by 43.33 in 2009, 33.33 in 2008, 26.33 in 2010, 23.66 in 2011. Lowest frequency percentage of association of the fungus was (16.66) in 2012 and 2013.

Frequency percentage of association of *P. guepinii* was the highest 16.66 in the year 2007 and 2010. Lowest count of the fungus was 6.66 in the year 2009.

Frequency percentage of association of *Rhizopus stolonifer* was highest 45.0 in the year 2007 and it was lowest 3.33 in the year 2010.

Frequency percentage of association of *Trichoderma viride* was highest 6.66 in 2009 and it was lowest 3.33 in the year 2007 (Table 2).

Table 2. Frequency (%) of association of different fungi with leaf spot of *Rauwolfia serpentina.*

Name of the isolates	2007	2008	2009	2010	2011	2012	2013
Alternaria alternata	**70**	**6.66**	**73.32**	**3.33**	**6.66**	**-**	**3.33**
Aspergillus niger	**9.99**	**3.33**	**99.99**	**-**	**6.66**	**-**	**-**
Colletotrichum gloeosporioides	**43.33**	**3.33**	**3.33**	**-**	**3.33**	**33.33**	**26.33**
***Fusarium* sp. 1**	**-**	**3.33**	**-**	**-**	**-**	**-**	**-**
***Fusarium* sp. 2**	**6.66**	**-**	**-**	**-**	**-**	**-**	**-**
***Macrophoma* sp.**	**20**	**-**	**-**	**-**	**-**	**-**	**-**
Nigrospora sphaerica	**-**	**-**	**-**	**23.33**	**-**	**-**	**-**
Psedocercospora liebenbergii	59.99	**33.33**	**43.33**	**26.33**	**23.66**	16.66	16.66
Penicillium digitatum	**-**	**6.66**	**3.33**	**-**	**3.33**	**-**	**-**
Penicillium italicum	**-**	**3.33**	**-**	**-**	**-**	**-**	**-**
Pestalotiopsis guepinii	**16.66**	**-**	**6.66**	**16.66**	**-**	**-**	**-**
Rhizopus stolonifer	**45**	**-**	**-**	**3.33**	**-**	**-**	**-**
Trichoderma viride	**3.33**	**-**	**6.66**	**-**	**-**	**-**	**-**

Table 3. Frequency (%) of association of different fungi with anthracnose infected leaves of *Rauwolfia sarpentina* during 2007-2013.

Name of the isolates	2007	2008	2009	2010	2011	2012	2013
Alternaria alternata	-	-	60	3.33	-	3.33	-
Aspergillus niger	13.33	66.66	6.66	-	3.33	-	-
Colletotrichum gloeosporioides	73.33	93.33	16.66	43.33	20	60	79.99
***Fusarium* sp. 1**	-	3.33	-	-	-	-	-
***Fusarium* sp. 2**	-	6.66	-	-	-	-	-
***Macrophoma* sp.**	20	-	-	-	-	-	-
Nigrospora sphaerica	-	-	-	3.33	-	-	-
Penicillium digitatum	3.33	3.33	-	6.66	-	3.33	-
Penicillium italicum		3.33					
Pestalotiopsis guepinii	16.66	-	-	-	3.33	-	-
Rhizopus stolonifer	43.33	6.66	20	3.33	-	-	-
Trichoderma viride	6.66	6.66	-	3.33	-	-	-

Table 3. showed that frequency percentage of association of *A. alternata* 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, 2011 and 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. The fungi were not found in 2010 and 2012 and 2013.

Frequency percentage of association of *C. gloeosporiodis* was highest (93.33) in the year 2008 and it was lowest (16.66) in the year of 2009.

Fusarium sp. 1 was exclusively isolated in the year 2008. Frequency percentage of the association of the fungus was (3.33).

Fusarium sp. 2 was exclusively isolated in the year 2008. Frequency percentage of the association of the fungus was (6.66).

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

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

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

Frequency percentage of *Penicillium italicum* was 3.33 in the year 2008.

Frequency percentage of association of *P. guepinii* was highest (16.66) in the year 2007and 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 2010.

A total of 15 species of fungi were isolated from *R. serpentina*. Pathogenecity test of the isolated fungi were done following 'Detached leaf technique' and 'Spraying of spore suspension' on healthy plants. Among the isolated fungi *Colletotrichum gloeosporioides* was found to be pathogenic to *R. serpentina*.

Leaf spot disease incidence was started from February and gradually increased up to July. The lowest disease severity (DS 1) was recorded in the month of February and the highest DS was (DS 9) in the month of July for leaf spot symptom.

Anthracnose disease incidence was started from March and gradually increased up to September. The lowest disease severity (DS 2) was recorded in the month of March and the highest DS was (DS 9) in the month of September for anthracnose symptom.

Temperature showed noticeable effect on disease development. Rainfall and humidity also showed slight effect on disease development.

CONCLUSION

Rauwolfia serpentina is one of the important herbal medicinal plant. All parts of the plant, including the stem and leaves, contain indole alkaloids, but they are found in highest concentration in the bark of the root. *Rauwolfia* contains many different phytochemicals, including alcohols, sugars and glycosides, fatty acids, flavonoids, phytosterols, oleoresins, steroids, tannins, and alkaloids. The most important alkaloids found in the plant are indole alkaloids, with more than 50 of those alkaloids having been isolated in the plant. Leaf spot and anthracnose disease of the plant drastically damaged the plant. Present investigation is the first approach of surveying and monitoring diseases of *R. serpentine* in Bangladesh. Finding of these research will be helpful for designing a proper management of leaf spot and anthracnose diseases of *R. serpentina*.

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