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RESEARCH ARTICLE

A COMPREHENSIVE ACCOUNT OF ANTHRACNOSE DISEASE SYMPTOMS, OCCURRENCE AND INTENSITY ON *RAUVOLFIA SERPENTINA* (SARPAGANDHA) IN AND ADJOINING AREAS OF NORTH 24 PARGANAS, WEST BENGAL, INDIA

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ABSTRACT

A destructive anthracnose disease of *Rauvolfia serpentina* L. Benth. Ex Kurz. is found in West Bengal. Its causal organism was isolated and phenotypically identified as *Colletotrichum gloeosporoides*. Once in a week survey for two consecutive years of 2013 and 2014 in ten places of 24 Parganas (N) district, West Bengal establishes the presence of disease in all ten places. PDI was recorded by monitoring its cultivation at 4 selected areas in and adjoining North 24 Parganas, West Bengal for the same time period. Maximum PDI (66.66%-89.90%) was during September and minimum (17.64-39.20 %) was recorded during December – January in all four studied areas. There was only few reports of anthracnose diseases on *Rauvolfia serpentina* but also this is the first approach to the study of disease intensity of anthracnose disease of *Rauvolfia serpentina* in this state. So our work is an entirely new one in this regard.

INTRODUCTION

Rauvolfia serpentina L. Benth. Ex Kurz. (Sarpagandha) is an evergreen, woody, glabrous and perennial shrub belongs to family Apocynaceae and occurs in habitats of tropical and subtropical regions, distributed worldwide in Himalayas, Indian peninsula, Burma, Indonesia and Sri Lanka (Ghani, 1998; Singh *et al.*, 2009). The roots, leaves and juice are of medicinal importance and have attracted the attention of indigenous system of medicine practitioners, as it contains a large number of secondary metabolites (N- containing indole alkaloids) localized mainly in the roots and rhizomes (Mittal *et al.*, 2013; Poonam *et al.*, 2013). In Ayurvedic medicines, the roots of *R. serpentina* are used as a remedy for curing hypertension, insomnia, mental agitation, gastrointestinal disorders, excitement, epilepsy, traumas, anxiety, excitement, schizophrenia, sedative insomnia and insanity (Poonam *et al.*, 2013; Meena *et al.*, 2009). According to Rajendran and Agarwal (2007), fruits and seeds have also been used for its medicinal or ethnobotanical purposes by the ethnic tribes of Virudhngar district Tamil Nadu, India. Recently the plant *Rauvolfia serpentina* is frequently reported with anthracnose disease in West Bengal, India.

There are few available reports about such disease on this plant. As the medicinal plants are subjected to production of many important pharmaceutical drugs, fungal invasion to them with the production of mycotoxin are potent enough to interfere with the active principles of these plants. Some fungal pathogens and non-pathogens produce mycotoxins in their infected hosts and substrates on which they grow (Anthony *et al.*, 2009). WHO (1979) reported that mycotoxins are hazardous to human and animal health. The main objectives of this work are to report the anthracnose disease of *Rauvolfia serpentina*, to identify the causal organism phenotypically and record occurrence and intensity of this disease in and around 24 – Parganas (N) district, West Bengal.

MATERIALS AND METHODS

Study of Symptoms

The infected leaves were carried to the laboratory in sterilized biodegradable polythene bags and the symptoms studied under hand lens and simple microscope.

Isolation and purification of pathogen from diseased plant parts

The collected leaf samples were washed in sterile distilled water and soaked in alcohol to remove the surface impurities.

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After that the leaf samples were cut into small pieces of 3-5 mm in size from the diseased portion. Then they are passed through 0.1% of HgCl₂ solution for one minute for surface sterilization and washed in three changes of sterile distilled water. These leaf cuttings were blotted between sterile filter papers and aseptically plated on Potato Dextrose Agar (PDA). In each plate a single piece was placed and incubated at BOD (28± 1 °C) for 7 days. After appearance of mycelial growth it was transferred on to fresh PDA slant. For purification of isolated pathogen, single hyphal tip method was taken. The entire procedure for isolation of the disease was done under laminar air flow. The purification was done by single hyphal tip method.

Pathogenicity test of the pathogen

Pathogenicity test was done following the Koch postulates.

Characterization and Identification of the pathogen

The characterization and identification of the pathogen were done phenotypically by following Domsch *et al.* (1980), Dhingra, and Sinclair (1986), Nagamani *et al.* (2006).

Study of the disease occurrence and intensity (PDI)

An extensive survey was carried out in few selected areas of North 24 Parganas to record disease occurrence of this novel medicinal plant from January 2013 to December 2014. We have also carried out an intensive survey for the same time period in order to record the Percentage of Disease Intensity (PDI) by monitoring cultivation of *Rauvolfia serpentina* at 4 selected areas of West Bengal namely Kalyani (BCKV), Nilgunj, North 24-Parganas, State Pharmacopical Laboratory and Pharmacy for Medicine, Govt. of W.B., Nadia and The Agri Horticultural Society of India, Alipore The fields were visited once in a week regularly for the entire time duration to study the Percentage of Disease Intensity (PDI). For the study of PDI, three plots from each of the four areas were taken and per plot 50 plants were randomly selected and tagged. Tagged plants were visited regularly, once in a week from January 2013 to December 2014. The disease rating was done on the scale: 0= 1%, 1=10; 2=25%; 3= 40% 4 =60%, 5=70, 6=90 % and 7=95%. The PDI was calculated by using the following formula.

$$PDI=A/B \times 100$$

A=Total Number of infected plant; B=Total Number of selected plants.

The temperature (Minimum & maximum) and humidity (minimum & maximum) of every day were recorded in the study area, they were averaged month wise to find the correlation of disease occurrence and percentage of disease incidence with seasonal weather change (Banerjee and Ghosh, 2015).

RESULTS

Symptom of the disease

On the leaf lamina the symptoms initially appear as necrotic, circular or oval shaped irregular spots surrounded by halo zone, variable in size (2-6 mm), predominantly towards the tips (Fig. 1). Under conducive condition humidity >70% and temperature >30°C with advancement of disease the spots increase in size and coalesce together to form big necrotic lesions leading to premature drying and defoliation of infected leaf. The anthracnose symptoms are also found on the petiole and flowers.

Phenotypical characterization and identification

The pure culture was analyzed macroscopically and microscopically. The cultures on PDA grew well with growth rate from 2-3 mm per day. Colonies at first dense white in appearance, gradually turns into dark grey (Fig 2A), reverse plate shows dark brown to black colouration (Fig 2B). Slide was prepared from the margin of the colony. The mycelium was hyaline, brown, sometimes abundant, at times sparse with floccose, loose or compact growth. The conidia were cylindrical, single celled and varying in size, with both apices rounded. Acervuli was found with round black bristly heads (Fig 3A). Conidia fusiform, apices acute, 18-23×3.5-4µm. appressoria abundant, medium brown, clavate to circular, edge usually entire, 9-14×6.5-11.5µm (Fig 3B). On the basis of all these phenotypical characters the pathogen was identified as *Colletotrichum gloeosporoides* Penz.. The data presented in the Table -1 showed that this disease was found to present in all studied zones (Naihati, Halishahar, Hasnabad, Hingalganj, Badu, Nilganj, Duttafulia, Gopalnagar, Rajarhat, and Mohishbathan) all through the year for 2013 & 14. The disease was predominant in hot and humid weather from March to September during 2013 and 2014 where as the occurrence is getting attenuated during the winter from November to February.

The data presented in Table 2 showed that the disease intensity have it's maximum effect during the month of September in all studied areas.

Table 1. Occurrence of anthracnose disease *Rauvolfia serpentina* in different zones of North 24 Parganas during 2013 & 14

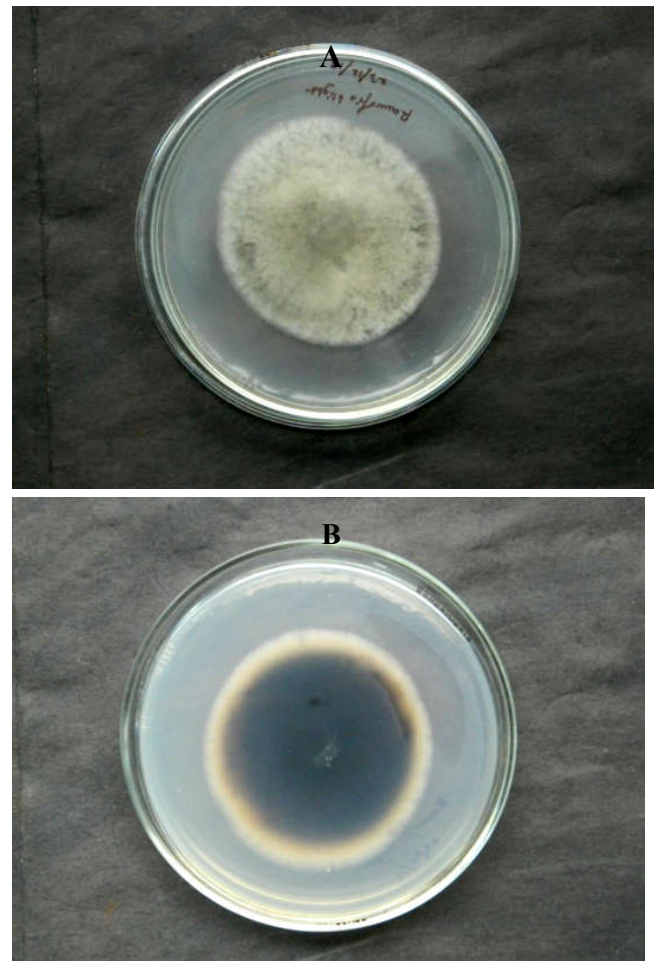
Places	Occurrence of anthracnose disease (<i>Colletotrichum gloeosporoides</i>) in 2013 & 14																							
	Jan		Feb		Mar		Apr		May		Jun		Jul		Aug		Sep		Oct		Nov		Dec	
	13	14	13	14	13	14	13	14	13	14	13	14	13	14	13	14	13	14	13	14	13	14	13	14
Naihati	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Halishahar	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Hasnabad	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
Hingalganj	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	
Badu	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	
Nilganj	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	
Duttafulia	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	
Gopalnagar	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	
Rajarhat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	
Mohishbathan	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	

*(+) indicates occurrence of disease and (-) indicates absence of disease.

Table 2. Percentage of disease incidence (PDI) of Rauvolfia serpentina at the selected areas

Months	Nilganj			Agri Horti, Alipore			State pharmacopical, nadia			BCKV		
	2013	2014	Pull data	2013	2014	Pull data	2013	2014	Pull data	2013	2014	Pull data
Jan	17.64 (24.80)	22.05 (27.97)	19.84 (26.42)	29.33 (32.77)	26.66 (31.05)	27.99 (31.88)	20.58 (26.92)	35.29 (36.39)	27.93 (31.88)	31.2 (33.96)	39.20 (38.76)	35.20 (36.39)
Feb	23.52 (29.00)	27.94 (31.88)	25.72 (30.46)	34.66 (35.97)	32.00 (34.45)	33.33 (35.24)	27.94 (31.88)	38.23 (38.17)	33.08 (35.06)	38.04 (38.06)	40.80 (39.70)	39.42 (38.88)
Mar	27.94 (31.88)	20.58 (26.92)	24.26 (29.47)	38.66 (38.41)	36.00 (36.87)	37.33 (37.64)	30.88 (33.71)	39.70 (39.06)	35.29 (36.39)	39.20 (38.76)	42.40 (40.63)	40.80 (39.70)
Apr	38.23 (38.17)	29.41 (32.83)	33.82 (35.55)	48.00 (43.85)	40.00 (39.23)	44.00 (41.55)	38.23 (38.17)	42.64 (40.74)	40.43 (39.47)	44.80 (42.02)	45.60 (42.48)	45.20 (42.25)
May	42.64 (40.74)	35.29 (36.39)	38.96 (38.59)	52.00 (46.15)	45.33 (42.30)	48.66 (44.20)	41.17 (39.87)	44.11 (41.61)	42.64 (40.74)	52.80 (46.61)	48.00 (43.85)	50.40 (45.23)
Jun	55.88 (48.33)	45.58 (42.42)	50.73 (45.40)	50.66 (45.34)	49.33 (44.60)	49.99 (44.94)	57.35 (49.20)	48.52 (44.14)	52.93 (46.66)	55.20 (47.98)	50.40 (45.23)	52.80 (46.61)
Jul	66.17 (54.39)	54.41 (47.52)	60.29 (50.89)	57.33 (49.20)	53.33 (46.89)	55.33 (48.04)	70.58 (57.10)	58.82 (50.07)	64.70 (53.55)	62.40 (52.18)	54.40 (47.52)	58.40 (49.84)
Aug	77.94 (61.96)	67.64 (55.30)	72.79 (58.50)	69.33 (56.35)	60.00 (50.77)	64.66 (53.49)	75.00 (60.00)	67.64 (55.30)	71.32 (57.61)	68.80 (56.04)	60.80 (51.24)	64.80 (53.61)
Sep	89.90 (71.47)	75.00 (60.00)	82.45 (65.20)	80.00 (63.44)	66.66 (54.70)	73.00 (58.69)	77.94 (61.96)	72.05 (58.05)	74.99 (59.93)	76.00 (60.67)	68.00 (55.55)	72.00 (58.05)
Oct	55.88 (48.33)	51.47 (45.80)	53.63 (47.06)	57.33 (49.20)	60.00 (50.77)	58.66 (49.95)	70.58 (57.10)	63.23 (52.65)	66.90 (54.88)	54.40 (47.52)	56.00 (48.45)	55.20 (47.98)
Nov	47.17 (43.34)	58.82 (50.07)	52.99 (46.66)	42.66 (40.74)	50.66 (45.34)	46.66 (43.05)	60.29 (50.89)	57.35 (49.20)	58.82 (50.07)	52.80 (46.61)	44.80 (42.02)	48.80 (44.51)
Dec	37.35 (37.64)	47.05 (43.28)	42.20 (40.51)	26.66 (31.05)	38.66 (38.41)	32.66 (34.82)	48.52 (44.14)	44.11 (41.61)	46.31 (42.88)	44.00 (42.02)	40.00 (39.23)	42.00 (40.40)
CD(p=0.05)	8.40	7.96	22.57	5.90	6.22	17.30	4.93	4.94	20.90	7.05	5.01	12.99
SE±	4.06	3.84	10.90	2.85	3.00	8.35	2.38	2.38	10.09	3.40	2.42	6.27

It was recorded maximum in Nilganj during the month of September (89.90%) in 2013 and the percentage reaches upto 75.00% in the next year. After Nilganj the effect of the disease intensity was recorded most at Agri horticultural society, Alipore. It was 80.00% for the month of September (2013) which goes down to 66.66% in 2014 at the same time of the next year. State pharmacopical laboratory, Nadia was recorded with 77.94 % and 72.05% PDI during Spetember at 2013 & 2014 respectively. In BCKV the disease intensity was recorded maximum as 76.00% and 68.00% again during the month of September for 2013 & 14. Whereas the intensity of disease was recorded lowest during the month of December-January. It was recorded as 17.64% & 22.05% for the month of December (2013) and January (2014) respectively in Nilganj. At Agri-Horticultural Society Alipore, Alipore 26.66% in December (2013) and the data exactly repeats in January 2014. In State pharmacopical laboratory, Nadia the PDI was 20.58% during January, 2013 & 35.29 % during January 2014.

**Fig. 1. Symptom of anthracnose disease on Rauvolfia leaf****Fig. 2. Culture plate of Colletotrichum gleosporoides. Front view (2A) reverse view (2B)**

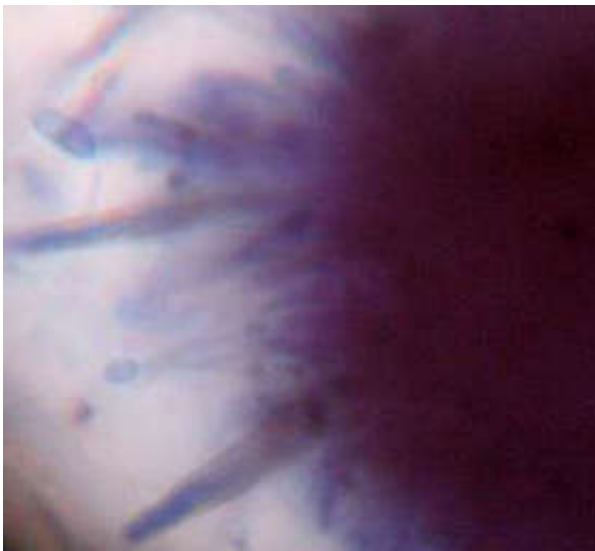


Fig. 3A. Acervuli of *Colletotrichum gloeosporoides* showing setae and conidiophore



Fig. 3B. Conidia of *Colletotrichum gloeosporoides*

BCKV also exhibited minimum disease intensity during January, recorded as 31.20% (2013) & 39.20% (2014). This study indicated that maximum disease intensity range (66.66%-89.90%) was during September in all four studied areas. It was probably due to high temperature and moisture (25-35°C & humidity 85-95%) as per record obtained from Alipore Metereological Data Station (West Bengal). Moreover minimum percentage of disease intensity (17.64-39.20 %) was recorded during December – January in all four studied areas. During this time temperature and moisture are low (10-26°C & humidity 65-84%) and not so favorable to the fungal pathogen.

DISCUSSION

The symptoms found by us resemble the work of Varadarajan, (1964) and Ghosh and Chakraborty (2012) on *Rauvolfia serpentina*. The occurrence of anthracnose disease on *Rauvolfia* caused by *Colletotrichum gloeosporoides* was first reported in India by Varadarajan (1964). Recently the fields of Sarpagandha have been severely affected by anthracnose disease in between the months of August and October just after the onset of monsoon. Disease incidence causes more than 60% death and drying in severely infected fields in northern

India (Alam *et al.*, 2007). In our survey we have also found similar kind of Disease incidence during the month of September. Ghosh and Chakraborty (2012) recorded the anthracnose of sarpagandha in West Bengal and tried to control it by biological agents. Leaf Blight disease of *Rauvolfia serpentina* (sarpagandha) caused by *Macrophomina phaseolina* was reported by Mahrotra (1976). *Colletotrichum* blight (bloom blight) caused by *C. capsici* was recorded by Shukla *et al.* (2006). *Curvularia* leaf spot of sarpagandha has been reported from Jammu and Kashmir by Thakur *et al.* (1974). Wilt is one of the most important and serious diseases of Sarpagandha. The disease was first reported from jammu & Kashmir (Janardhanan *et al.*, 1964). *Alternaria* leaf blight of ashwagandha (*W. somnifera*), caused by *A. alternata* and root rot wilt is one of the serious diseases of ashwagandha in the nursery as well as in the commercial fields at Lucknow and its adjoining areas in North India (Gupta *et al.*, 2004). Although there are a number of available disease reports on *Rauvolfia serpentina* but there was no significant report on the intensity of the disease all through the world. So our work is an entirely new one in this regard. The pathogen *Colletotrichum gloeosporoides* is causing enormous crop loss by anthracnose disease but how much they minimizing the medicinal properties of this important crop should be analyzed further. In this perspective it is urgent to control this disease immediately.

Conclusion

Our work establishes that even the plant *Rauvolfia serpentina*, inspite of having enormous medical importance, getting invaded by the pathogen *Colletotrichum gloeosporoides* very frequently and exhibiting devastating effect. This work indicates that anthracnose of *Rauvolfia serpentina* occurs in few places of 24 Parganas, West Bengal throughout the two consecutive years 2013 & 14 where as in other areas of our survey they are reported mainly during the rainy season. The disease intensity was at it's peak in September (66.66%-89.90%) in four places of our extensive survey. Where as the minimum percentage of disease was recorded in December-January (17.64-39.20 %) in all four study areas. This is the first approach to the study of disease intensity of anthracnose disease of *Rauvolfia serpentina* in this state. It is expected that this work may encourage other workers to study this disease, its severity, crop loss and it's proper management to combat it. Infection of *Rauvolfia serpentina* by *Colletotrichum gloeosporoides* not only reduces the crop loss and market value but it may reduce medicinal efficacy of this shrub.

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