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Studies on target spot of *Rauvolfia serpentina* caused by *Corynespora cassicola*

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Medicinal plants are important source of medicines throughout the world and particularly in developing countries. Janardhanan (2) claims that more than 25% of the prescriptions in USA contained one or more plant products. Organized cultivation of medicinal plants in West Bengal is yet to take a shape. State government has recommend some medicinal plants like Aswagandha, Sarpagandha, Senna, Tulsi etc for commercial cultivation in different zones. As one of the limitations pathogenic diseases caused significant loss of production and quality of the produce. *Rauvolfia serpentina* suffers from many fungal diseases like leaf spot and blight caused by *Rhizoctonia solani* (anamorph of *Thanatephorus cucumeris*), *Pseudocercospora rauwolfiae-serpentinae,* target spot caused by *Corynespora cassicola* (2,3,4), *Periconia macro-spinosa*, foliage and inflorescence diseases caused by *Macrophomina phaseolina*, *Phomopsis sethii* and *Mycosphaerella rouvolfiae*, viral diseases like severe mosaic and stunting, transmitted by plant sap on *Nicotiana tabacum cv. White Burley*, *N. rustica* and *N. glutinosa*. Very little work except those by AINP on Medicinal and Aromatic plants had been done on diseases of Sarpagandha (*Rauvolfia serpentina*). Present paper reports on target spot of *Rauvolfia serpentina* caused by *Corynespora cassicola.*

Observations on the crop grown plots was done at monthly intervals from May, 08 to April,09. Incidence and severity of the diseases on Sarpagandha was recorded. For percent disease incidence total no. of leaves infected in a plot were recorded. For percent disease index, number of leaves infected per 10 plants in each plot were recorded and rated on a 0-4 scale, where 0= healthy leaves; 1= 1 - 5% leaf area infected; 2= 6 - 12% leaf area infected; 3= 13 - 25% leaf area infected; 4= above 25% leaf area infected. Percent disease incidence and percent disease index were calculated. Isolation of the pathogen was made following standard procedure using 0.1% $HgCl_2$ for surface sterilization. Cultures obtained were taken in PDA slants and stored in refrigerator at 5°C. Culture was maintained by transferring at 15 days interval. For confirmation of the pathogen associated with the disease, pathogenicity test was done following Koch's postulate in spray inoculation method. Spore suspensions of the pathogen *Corynespora cassicola* ($1.15x10^5$spores/ml) was sprayed thoroughly on the potted plants after removing all the diseased leaves and control plants were sprayed only with distilled water. Inoculated plants were covered with polyethylene bags to create

favorable environmental conditions for disease development and kept on the laboratory benches providing water as and when required. The fungal culture obtained was studied under microscope, shape, size, septation and color of the spores noted and measurement was made using Stage and Ocular Micrometer. Sensitivity of the pathogen towards a common biocontrol fungus namely *Trichoderma* was also done.Two different isolates (*Trichoderma harzianum* and *Trichoderma viride*) of *Trichoderma* sp. were collected from Betelvine Research Laboratory, Bidhan Chandra Krishi Viswavidyalaya, Kalyani (Nadia). Sensitivity of the isolated pathogen was tested toward two isolates namely *harzianum* and *viride* on PDA medium by Dual Culture Plate Technique. Five day old cultures of the pathogen was plated aseptically at the edge of Petri plates 2 days before placement of *Trichoderma* sp. Antagonism was observed in paired culture plates for 9 days. Ratings were done after contact between the pathogen and the antagonist using a modified Bell's (1) scale (1-5).

Fixed plot survey results (Table 1) revealed that on *R. serpentina* lowest target spot disease, both in terms of percent Disease Index and percent Disease Incidence were recorded during May-August and highest was during March- April. Pre-monsoon summer has favoured the disease that declined with monsoon.

The leaves, during flowering, showed dark brown chocolate centered spots of different shapes and sizes from the margin and also on other parts of the lamina. Spots were isolated or coalesced to cause blighting. The lesions and the spots showed yellow diffusing haloes around. The mid-veins also had spots but without yellowing. Concentric rings were conspicuous on the spots. The affected tissue turned brittle at the centre of the spot. In some blighted leaves, green island formations were also observed. Both the surfaces of the leaves were equally damaged (Plate 1). Severely affected leaves dropped. Colony characters of the pathogen was studied for 5 days on PDA visually and also under Phase – Contrast Microscope by directly placing the plate under the microscope (Table 2) (Plate 2).

Table 1.
Fixed Plot Survey of Diseases

Month	Percent Disease Index	Percent Disease Incidence
May,08	13.03	19.14
June, 08	13.75	19.67
July,08	16.84	23.75
Aug,08	20.36	25.33
Sept,08	24.33	29.42
Oct,08	24.87	30.35
Nov,08	25.89	32.37
Dec,08	29.01	34.91
Jan,09	38.96	45.23
Feb,09	50.09	57.89
March,09	75.68	78.28
April,09	77.31	85.01

Characteristics of the fungal pathogen on PDA media are presented in Table 2 (Plate 3).

Table 2.
Colony character along with the length and breadth of the spores

Colony character of the pathogen	Length (µ)	Breadth (µ)
Olivaceous to grayish or white, dense, fluffy growth in the PDA medium. Back of the medium showed black pigmentation with white borders of mycelium	135 – 195	30 – 45

The result (Table 3) showed that the isolate *harzianum* and *viride* were less inhibitory (R_4 and R_5 respectively) against the pathogen (Plate 4 and 5).

Thus, it could be concluded that there is scope for using biocontrol agents like *Trichoderma* to check target spot disease of *Rauvolfia serpentina*. However, the bio-agents to be tested will have to be collected from the medicinal plant garden and the bio-agents should be tested under field conditions before recommendation to the farmers.

Literature Cited

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Table 3.
Inhibitory action of *Trichoderma harzianum* and *Trichoderma viride* against the pathogen

Isolates	Duration required for point of contact (days)	Bell's scale after (days)					
		4	5	6	7	8	9
Trichoderma harzianum	4	R_4 - R_3	R_4	R_4	R_4	R_4	R_4
Trichoderma viride	4	-	R_5	R_5	R_5	R_5	R_5

Photo 1. Target spot

Photo 2.
Colony of *Corynespora cassicola*

Photo 3. Spore of *Corynespora cassicola*

Photo 4. *T. harzianum* × *C.cassicola cassicola*

Photo 5.*T. viride*×*C. cassicola*