In vitro biological control of *Colletotrichum gloeosporioides*, causal organism of anthracnose of sarpagandha (*Roulvolfia serpentina*)

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ABSTRACT

Five isolates of *Trichoderma viride* and two isolates of *Beauveria bassiana* were screened against *Colletotrichum gloeosporioides* for their antagonistic potentiality by dual culture plate method. Three isolates of *T. viride* and one isolate of *Beauveria bassiana* were class –1 antagonists. Among the class -1 antagonists ,T1 isolate of *T. viride* showed best hyperparasitic activity(3.30 cm) followed by T10 (3.10cm) ,T12 (3.00 cm) and BB1 of *B.bassiana*(!.20 cm). The selected cultures from pairing of mycoparasitized pathogen were observed under microscope (Leitz Laborlux K , Germany) to study of hyphal interactions between the antagonist and the pathogen and photographed when required. Therefore, this *in vitro* study strongly suggested that *T. viride* can be applied in field trial (*in vivo*) as effective biocontrol agents against anthracnose of sarpagandha (C. O. *Colletotrichum gloeosporioides*).

Keywords: Anthracnose, Antagonism, Trichoderma viride, Beauveria bassiana

INTRODUCTION

Sarpagandha (Roulvolfia serpentina Benth.) is universally considered one of the most important medicinal crop in India. This plant is growing wildly in different parts of India and neigbouring countries . Its roots have a chief source of serpentine and have been used commonly in the Ayurvedic system of medicine from ancient times . At present ,it is cultivated commercially in different parts of the country. Recently, the commercial field of sarpagandha have been severely affected by anthracnose disease. The incidence of the disease appears after the on set of Monsoon in the month of August and become epidemic in October . (Alam et al, 2007). Disease incidence caused more than 60 % death and drying in severely affected fields (Manjusha,2005).

Flower blight, fruit rot, and leaf spots are among the symptoms of this disease (Alam *et al*, 2007). Generally to combat this disease, fungicides (eg bavistin , blitox -50) are applied but fungicides are environmental pollutant. The non chemical or eco-friendly methods are now popularized in developed countries (America , U.K. etc). cultivation of disease resistant variety and biocontrol method are eco-friendly strategies to manage diseases .

Therefore, the main objective of this work is to note antagonistic efficacy of biocontrol agents -*Trichoderma viride*, and *Beauveria bassiana* against Colletotrichum gloeosporioides ,causal organism of anthracnose of sarpagandha *in vitro*.

MATERIAL AND METHODS

a) Study of symptoms of anthracnose :The infected leaves, petioles, twigs, panicles, flower and fruits were collected separately in sterilized biodegradable polythene bags and carried in laboratory and the symptoms caused by anthracnose were studied with the help of simple microscope.

b) Isolation and purification of pathogen from diseased parts : The infected leaves , petioles , twigs , panicles , flower and fruits were collected separately in sterilized biodegradable polythene sheets ,carried in laboratory ,and isolation of the pathogen was done in PDA medium in Petri dishes at 28° C by the method presented by Dhingra & Sinclair(1994).

For purification of isolated pathogen, single hyphal tip method was taken.

c) Identification or Characterization of the pathogen :The identification of the pathogen was done by cultural and microscopical characteristics with the help of published fungal Key and books (Nagamani *et al*, 2006; Domsch *et al* 1980; Bailey and Jeger, 1992; Freeman *et al*, 1998).

d) Pathogenecity test of the pathogen: It was done by koch's postulates .

e) Isolation and characterization of antagonistic fungi :Isolation of fungi from different soils from different geographical regions of west Bengal, were done in the laboratory by dilution Plate Method followed by Dhingra & Sinclair(1994).

Fungal colonies, were isolated and sub-cultured repeatedly for getting pure colonies and then preserved in slant tubes for further identification. The fungal strains were identified after staining them with cotton blue, by following the keys of Domsch et.al 1980. Nagamani *et al*,(2006) and www.mycobank.org .The identifications of isolates of *T. viride* were done by IARI, Delhi, India.

f) Antagonistic potentiality test or rating of mycoparasitism of isolated antagonistic fungi: The mycoparasites were rated for their antagonistic property following Bell's test (Bell *et al*, 1982) in pair culture plate. Five mm diameter of mycelial colony from the margin of actively growing colony of *C gloeosporioides* and that of antagonist were incubated simultaneously at opposite ends of a Petri dish containing 25 ml of sterilized PDA medium. The plates containing the paired culture were incubated at $28^0 \pm 1^0$ C for 9 days in a B.O.D. incubator and were subsequently scored for degrees of antagonism on a 1—5 scale (Bell *et al*, 1982).

Class -1 : The mycoparasites completely over grows the pathogen and covered the entire surface of the medium .

Class-2 : The mycoparasite over grows at least 2/3 portion of the medium surface .

Class -3: The mycoparasite and the pathogen each colonized approximately one half of the medium surface and neither of the organism appeared to dominate over the other .

Class –4: The pathogen colonized at least two –third of the medium surface and appeared to withstand the encroachment by the mycoparasite

Class -5 : The pathogen completely over grows the mycoparasite and occupied the entire medium surface .

An isolate of the mycoparasite was considered highly antagonistic to the pathogen when the mean score for a given comparison (when rounded to the nearest whole class number) was ≤ 2 , but not highly antagonistic if the number was ≥ 3 .

Among the Class—I antagonists ,a comparative analysis was done on the basis of ability of

antagonist to grow faster than other over *C. gloeosporioides.*

The selected cultures from pairing of mycoparasitized pathogen were observed under microscope (Leitz Laborlux K, Germany) to study of hyphal interactions between the antagonist and the pathogen and photographed when required.

RESULT AND DISCUSSION

Symptoms of the disease: i) On the leaf lamina the symptom starts as necrotic circular or oval shaped irregular spots variable in size (2-6 mm) (Fig.1).ii)Under conducive condition (Humidity >90 and temperature 30° C), the spot increases in size and disease tissues become rotted. During dry weather, the spot can not enlarge and it becomes dried and drops off . iii) The disease spots are observed on the petiole also. Iv)in some twigs and flowers bore anthracnose symptoms. The occurrence of anthracnose ,leaf blight and die back diseases on sarpagandha was first reported in India by Varadarajan (1964). The symptoms noted by him were similar to the symptoms recorded in this study. Similar symptoms of anthracnose of sarpagandha reported by Alam et al (2007) in Lucknow, India. This study first recorded the occurrence of this disease of sarpagandha in West Bengal.

Identification of the pathogen :The isolated pathogen was *Colletotrichum gloeosporioides* (Penz).The identification of the pathogen was done by cultural and microscopical characteristics with the help of published fungal Key and books (Nagamani *et al*, 2006; Domsch *et al.*, 1980; Bailey and Jeger, 1992; Freeman *et al*, 1998).

Antagonistic potentiality test or rating of mycoparasitism of isolated antagonistic fungi: A total of seven fungal isolates were screened against *C. gloeosporioides* under *in vitro* condition by Bell's test(Fig.2) for determining mycoparasitic activity. The data presented in the Table -1 indicate that four isolates were rated as class—I mycoparasites /antagonists .Out of them five isolates(T1,T2,T3,T10 &T12) identified by IARI as *Trichoderma viride* .Two isolates(BB& B2) were two strains of *Beauveria bassiana*.

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Serial No.	Organism	or	Mean scores				
	isolates						
1	Ti		1.22 (1.00)				
2	T2		3.60(3.00)				
3	T3		2.00(2.00)				
4	Tio		1.30(1.00)				
5	T12		1.40(1.00)				
6	BB1		1.80(1.00)				
7	BB2		3.30(3.00)				
I-Moon of t	brog replication		II -Data aro				

Table-1 Rating of mycoparasites in dual culture plate by Bell's method.

I=Mean of three replications II =Data are recorded 9 days after incubation

III =scale of classes described by Bell *et al* (1982) was followed. ; the figures in parenthesis indicate the whole class .

Further, the results from Table -2 revealed that out of five strains of *T. viride*, T1 showed maximum (3.30) mycoparasitism over *C. gloeosporioides* followed by T10(3.10),T12(3.00),T3(1.30) and T2 (1.00). Out of two strains of *B. bassiana*, BB1 was better than BB2.

Interaction between *Trichoderma viride* (T10) and *C. gloeosporioides* under microscope showed that spores of T10 adhered on the wall of *C. gloeosporioides* and shrinkage of protoplast of the latter were recorded (Fig 3).The Figure 4 depicted that the hyphae of *C. gloeosporioides* was malformed and swollen.

Table 2 Comparative mycoparasitic activity of different isolates of hyperparasites showing class -I activities against	
C. gloeosporioides	

Serial No.	IARI Herb. No.	Organism or isolate	Scientific name of mycoparasite	Radialgrowth(cm)ofmycoparasitesoverC.oloeosporioidesat 24 hr. interval		
				24	48	72
1	108	T1	Trichoderma viride	1.05*	2.30	3.30
	109	T2	Trichoderma viride	0.40	0.80	1.00
3	110	T3	Trichoderma viride	0.61	1.00	1.30
4	112	T10	Trichoderma viride	1.00	2.25	3.10
5	115	T12	Trichoderma viride	0.80	2.02	3.00
6		BB1	Beauveria bassiana	0.30	0.55	1.20
7		BB2	Beauveria bassiana	0.20	0.32	0`70
S. Em±					•	0.082

C.D.(P≤0.05)

* Each insertion is average of three replications

0.346



Fig. 1 Anthracnose of sarpagandha

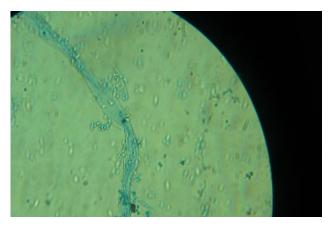


Fig. 3 Shrinkage of protoplast of *C. gloesporioides*

Similar phenomenons were reported in the interaction between mycoparasites (*Trichoderma viride*) and plant pathogens by other workers(Chat *et al*,1981; Pan and Ghosh,1997). Alam *et al* (2007) in their Book 'Healthy Plants for Health 'recorded some important diseases of some important medicinal plants and their management.

Application of *Trichoderma harzianum* and *Bacillus subtilis* on some medicinal crops showed effective biocontrol against some diseases (Alam *et al*, 2007).

CONCLUSION AND RECOMMENDATION:

Trichoderma viride strains are good biocontrol agents as tested *in vitro* and this experiment encourages other to apply this biocontrol agent in the field of Sarpagandha *in vivo*.



Fig. 2 in vitro control of C. gloeosporioides



Fig. 4 Malformation & swollen of hypha of *C. gloeosporioides*

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