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SCREENING OF TRICHODERMA AND PSEUDOMONAS ISOLATES AGAINST CORYNESPORA SP. AND ALTERNARIA SP. IN SARPAGANDHA AND THANKUNI UNDER IN VITRO CONDITION

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Abstract

Biocontrol is an important aspect of disease management for plant pathogens, among which *Trichoderma* and *Pseudomonas* species is the most exploited biocontrol agent in recent years. The soil specific nature of *Trichoderma* and *Pseudomonas* species is a well-known fact and hence native isolates should be more emphasized for control of plant pathogens in medicinal plants as these plants are important source of drugs throughout the world. In the present research work three isolates of *Trichoderma* and *Pseudomonas* are used against *corynespora sp.* and *Alternaris sp.* under *in vitro* condition where it is observed that generally isolates T1 and T2 was moderately antagonistic against *Corynespora sp.* and T3 isolate showed less virulence. While all the three isolates of *Trichoderma* were highly antagonistic against *Alternaria sp*. whereas isolate P1 of *Pseudomonas* was highly antagonistic against both the pathogen whereas, Isolate P3 was weakly antagonistic against both the pathogen.

Keywords: Trichodrma, Pseudomonas, Alternaria, Corymnespora.

Introduction

Indian subcontinent has known to be rich repository of medicinal plants. Medicinal plants yield variety of complex plant chemical substances such as glycosides, alkaloids and essential oils (Kumar and Dwivedi, 2018a; Kumar *et al*., 2018b; Kumar *et al*., 2018c). According to Harison-Masih *et al*. (2008) Sarpagandha (*Rauvolfia serpentine*) is perhaps the most useful traditional medicinal plant in India while According to Orhan 2010 *Centella asiatica* (L.) is a tropical medicinal plant native to India which has been widely used as Ayurvedic medicine for different purposes (Kumar and Dwivedi, 2018d; Kumar and Purnima *et al*., 2018e; Kumar and Pathak, 2019f; Kumar *et al*., 2019g). Leaf spotting and blight of Rauvolfia serpentina by Rhizoctonia solani was reported for the first time from India by Mehrotra and Thapar (1990), while Reddy 1975 observed leaf spot disease in Thankuni caused by *Cercospora sp.* Another disease in observed in Thankuni leaf spot caused by *Alterneria sp*. by Chowdhury 2011 while in Sarpagandha leaf spot by *Corynespora cassicola* by Momin 2010. A very less work has been done on management of both the medicinal plants. In the present work attempts have been made on suitable management practices with biocontrol agents like *Trichoderma* and *Pseudomonas* which is safe for our environment (Siddique and Kumar, 2018h; Siddique *et al*., 2018i; Pathak *et al.*, 2017j; Prakash *et al.*, 2014L; Kumar *et al.* 2014n).

Materials and Method

Collection of antagonist from the Soil

Three different isolates of *Trichoderma viride* and *Trichoderma harzianum* were collected from Deptt. Of Plant Pathology, Kalyani (Nadia) and three isolates of Pseudomonas fluorescens were collected from AICRP on MAPB. All the isolated were maintained on PDA slants at 50C.

Antagonistic Potential of Trichoderma Isolate

The antagonistic properties of Trichoderma isolate was tested on PDA medium by Dual Culture Plate Technique. 5 days old culture of the fungi under study was plated aseptically at the edge of Petri plates 2 days before the placement of *Trichoderma viride*. Paired cultures were observed for a total of 9 days before being discarded. All the ratings were done after contacts between pathogens and antagonist using a modified Bell's (Bell *et al*., 1982) scale (1-5) developed as follows:

Class I (R1) – The antagonist completely overgrew the pathogen (100% overgrowth).

Class II (R2) – The antagonist overgrew at least 2/3 rd of pathogen surface (75% overgrowth).

Class III (R3) – The antagonist colonized on half the growth of the pathogen (50% overgrowth).

Class IV (R4) – The pathogen and antagonist locked at the point of contact; and

Class V (R5) – The pathogen overgrew the mycoparasite.

Antagonistic Potential of *Pseudomonas fluorescens* Isolate

The antagonistic properties of Pseudomonas fluorescens isolate were tested on PDA medium by Dual Culture Plate Technique. Five days old culture of antagonist under study was inoculated by making long streak on the two sides of the plate keeping 1 cm away from the periphery aseptically containing PDA medium (Kumar 2013o; Kumar and Dwivedi, 2015p; Gogia *et al*., 2014q; Kumar, 2014r; Kumar *et al*., 2012s; Mishra *et al*., 2012t; Kumar *et al*., 2011u; Kumar *et al*., 2011v). At the centre of Petri plates, 2 days after the inoculation of antagonist, 5 days old culture of the fungi under study in PDA were placed aseptically in the centre of the plate. In control treatment, only 5 days old culture of the fungi under study in PDA was placed aseptically in the center of the Petri plate containing PDA medium. When full plate fungal growth in control treatment was noticed, the growth of the fungus in treated plate was recorded. The zone of inhibition between fungal growth and antagonist growth was also recorded. Percent inhibition due to antagonist was recorded following the formula:

$$\text{Percent Inhibition} = \frac{C - T}{T} \times 100$$

C= Full growth of the test pathogen in control treatment

T= Growth of the test pathogen in treated

Results and Discussion

Collection of *Trichoderma*

Two isolates of *Trichoderma viride* and one isolate of *Trichoderma harzianum* were collected from the lab of Plant Pathology, BCKV and were maintained in PDA medium and the three isolates were designated as T1 and T2 for *Trichoderma viride* and T3 for *Trichoderma harzianum.*

Screening of *Trichoderma* Isolates Against *Corynespora cassicola* and *Alternaria sp.*

The three isolates of Trichoderma were tested against Corynespora cassicola and Alternaria sp by dual plate technique, Rating of antagonism (Table. 1 and Fig., 1, 2, 3, 4, 5, 6) was recorded according to the modified Bell's ranking stated earlier.

Table 1: Screening of *Trichoderma* isolates against *Corynespora cassicola* and *Alternaria sp*

Pathogens	Isolates of *Trichoderma* sp.	Point of contact (day)	Bell's ranking (modified)
Corynespora cassicola	T_1	3	R1-R2
	T_2	3	R1-R2
	T_3	3	R1-R3
Alternaria sp	T_1	3	R1
	T_2	3	R1
	T_3	3	R1

Bell's ranking: R_1 = The antagonist completely over grew the pathogen and cover the entire medium surface;

R_2 = the antagonist over grew at least $^2/_3$ of the pathogen's surface;

R_3 = the antagonist colonized on ½ of the growth of pathogen;

R_4 = the pathogen and antagonist locked at the point of contact and

R_5 = the pathogen over grew the mycoparasite.

The results i.e., the rating chart (Table 1 and Fig.1 and 2.) showed that generally, isolates T_1 and T_2 of *T. viride* against *Corynespora cassicola* was moderately antagonistic (R_1-R_2) and T_3 isolates of *Trichoderma harzianum* showed less virulence (R_3). While all the three isolates of *Trichoderma* (Fig. 4, 5, 6) were highly antagonistic (R_1) against *Alternaria* sp.

Screening of *Pseudomonas fluorescens* Isolates Against *Corynespora cassicola* and *Alternaria* sp.

Three isolates of *Pseudomonas fluorescens* were tested against *Corynespora cassicola* and *Alternaria* sp. by Dual Culture Plate Technique. Rating of antagonism (Table 2) was recorded from the formula. The results (Table 2 and Fig. 7, 8, 9) revealed that isolate P_1 of *Pseudomonas fluoroscens* was highly antagonistic against both the pathogen as higher percent inhibition and zone of inhibition was recorded against both the pathogen. Whereas, Isolate P_3 was weakly antagonistic against both the pathogen as revealed from percent inhibition and zone of inhibition.

Table 2 : Antagonistic Potential of *Pseudomonas fluorescens* Isolate

Pathogen	Isolates of *Pseudomonas fluoroscens*	Percent inhibition	Zone of inhibition (mm)
Corynespora cassicola	P_1	53	16mm
	P_2	44	13.6mm
	P_3	11	7mm
Alternaria sp.	P_1	49	13mm
	P_2	48	11.5mm
	P_3	21	9mm

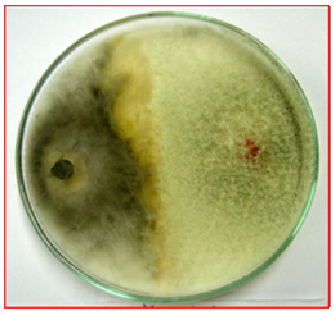

Fig: 1(Isolate T1)

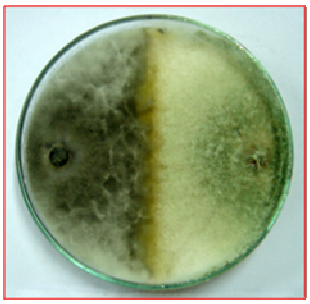

Fig:2 (Isolate T2)

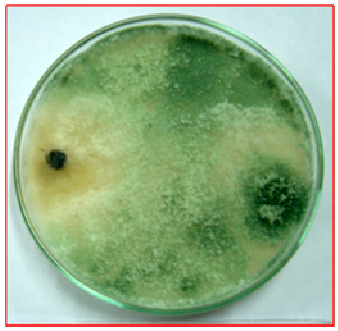

Fig: 3 (Isolate T3)

Corynespora sp. X Trichoderma

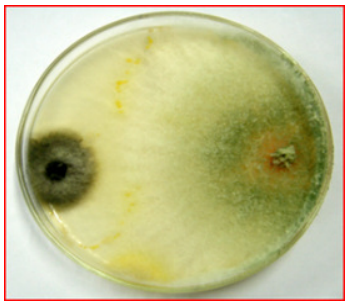

Fig: 4 (Isolate 1)

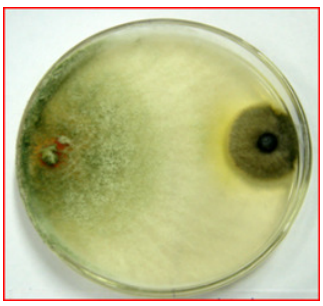

Fig: 5 (Isolate 2)

Fig: 6 (Isolate 3)

Alternaria sp. X Trichoderma

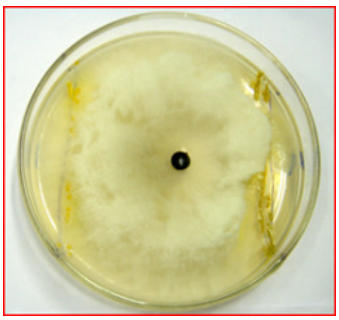

Fig. 7 : (Isolate 1)

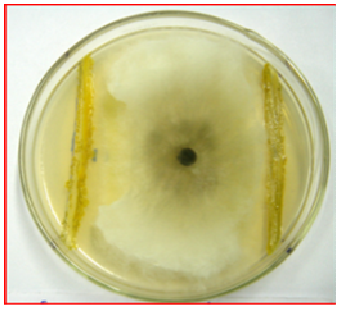

Fig. 8 : (Isolate 2)

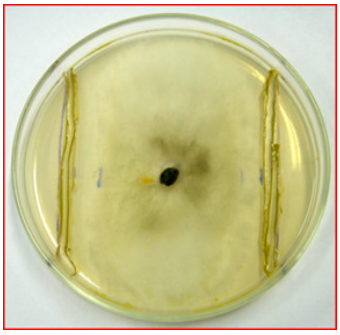

Fig: 9 (Isolate 3)

Corynespora sp. X Pseudomonas

Discussion

More Research works have to be done under field condition with other biocontrol agents for better results and molecular works have to be done so that the confusion become more clear that why the medicinal plants are being effected by the plant pathogens as these plants itself have medicinal properties itself (Kumar *et al.*, 2016x; Kumar *et al*., 2018y; Kumar *et al*., 2018z).

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