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Effect of Fungal Antagonists in Restriction of Radial Growth of French bean Isolates of *Sclerotinia sclerotiorum* (Lib.) de Bary in Dual Culture

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Abstract: ***In vitro*** **study of growth of French bean isolates of** ***Sclerotinia sclerotiorum*** **collected from different geographical isolates of NE India against fungal antagonists showed that** ***Trichoderma harzianum*** **caused maximum inhibition of radial growth of** ***S. sclerotiorum*** **followed by** ***Gliocladium virens*** **and** ***Trichoderma koningii*** **respectively. Maximum growth and least per cent inhibition of radial growth by different antagonists was observed in AS (Assam) isolate of** ***S. sclerotiorum.***

Keywords: Sclerotinia sclerotiorum**, antagonists,** ***T. harzianum G. virens, T. koningii.***

1. INTRODUCTION

French bean (*Phaseolus vulgaris* L.) is one of the most widely grown high yielding edible legume crop in different parts of NE India. The fresh pods and green leaves are used as vegetable. In Assam, the crop is generally grown in rabi season in an area of about 2250 ha with annual average production of 20593 tones (Anonymous, 1999). The crop is grown for green vegetables particularly for its tender fleshy pods, shelled green and dry beans. However, the crop is susceptible to white mold caused by *Sclerotinia sclerotiorum* affecting all stages of the crop even up to post-harvest storage. A number of microorganisms have been exploited and their use as potential antagonists against *S. sclerotiorum* has been well documented by several workers (Huang, 1980; Trutman *et al.*, 1982; Knudsen *et al.*, 1991). Among the several antagonists tested species of *Trichoderma, Gliocladium, Bacillus, Pseudomonas, Aspergillus* etc. have been widely used. Keeping in view the increasing use of chemical for management of such pathogens cause pollution of soil, air surface and ground water. Besides, the continuous use of chemical also has deleterious effect on the beneficial microorganism in soil, the present research work has been carried out to see the effect of fungal antagonists on restriction in radial growth of different French bean isolates of *S. sclerotiorum in vitro* for further application under field condition for management of soil borne plant pathogens.

2. MATERIALS AND METHODS

Sources of the antagonists:

Three widely accepted saprophytic fungal antagonists of the Plant Pathogens *viz., T. harzianum Rifai* (ITCC no. 7077*), T.koningii Oudems* (ITCC no. 4302) and *G. virens Miller* (ITCC no. 4177) respectively were used for the present investigation.

Growth of *S. sclerotiorum* against fungal antagonists in dual culture:

Radial growth of French bean isolates *of S. sclerotiorum* collected from different state of NE India [***viz.,*** Assam (AS), Nagaland (NL), Mizoram (MZ), Arunachal Pradesh (AP), Manipur (MP) and Meghalaya (ML)] in presence of fungal

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antagonists *viz.*, *T.harzianum*, *T. koningii* and *G. virens* were separately performed on PDA media by dual culture technique (Das, 1992). Mycelial discs (5mm dia.) of each antagonist was placed one opposite the other in separate petriplates (9cm dia.) near the periphery. In the centre of each petriplate, a disc of *S. sclerotiorum* was placed. The dual culture assays were replicated five times and incubated at 28 ±1°C. The zone of inhibition and restriction in the radial growth of the pathogen observed were measured after 24 h of inoculation.

The percentage of diameter of growth inhibition were estimated using the formula,

$$I = \frac{dc - dt}{dc} X\ 100$$

Where, I : mycelial growth inhibition of pathogen (%)

dc : colony diameter of pathogen in control dt : colony diameter of pathogen in treatment (Dennis and Webster, 1971, Mathur and Sarbhoy, 1978)

3. RESULTS AND DISCUSSION

Table. 1 - 6 and **plate. 1** clearly showed that the fungal antagonists *viz.*, *T. harzianum*, *G. virens* and *T. koningii* could significantly inhibited the radial growth of all the isolates of *S. sclerotiorum.* The observations showed that the antagonists restricted the mycelial growth of the test fungus as evidenced by zone of inhibition. The antagonists were observed to overgrow *S. sclerotiorum* on PDA indicating the ability of these organisms as an ideal bio-control agents of the test fungus. Similar findings were reported by Tu (1980), Trutmann and Keanne (1990), Sharma (1994) and Das (2001) on *R.solani* , Anahosur and Patil (2001) on *S .rolfsii*. The results of the present investigation showed significant differences among the antagonists in inhibition of radial growth of *S. sclerotiorum.* Out of the antagonists used, *T. harzianum* caused maximum per cent inhibition of mycelial growth in all the isolates followed by *G. virens* and *T.koningii* respectively. The findings is in concurrence with Claydon *et al.* (1987), who identified volatile alkyl pyrons produced by *T. harzianum* that were inhibitory to a number of fungi in vitro, while Henis *et al.* (1984) observed *T. harzianum* secretes large amounts of chitinase and ß-(1,3)-glucanase

Table. 1. Growth of AS(Assam) isolate of *S. sclerotiorum* against fungal antagonists *in vitro*

Treatments	Radial growth (mm) at different hours of incubation			% Inhibition at different hours of incubation		
	24h	48h	72h	24h	48h	72h
T_1 = *S. sclerotiorum* (AS) + *T. harzianum*	26.03[d]	35.22[d]	37.15[d]	3.59	52.72	58.72
T_2 = *S. sclerotiorum* (AS) + *G. virens*	26.41[c]	38.70[c]	41.36[c]	2.18	48.05	54.04
T_3 = *S. sclerotiorum* (AS) + *T. koningii*	26.64[b]	41.31[b]	44.92[b]	1.33	44.50	50.08
T_4 = *S. sclerotiorum* (AS) alone	27.00[a]	74.50[a]	90.00[a]	-	-	-
SEd (±)	**0.05**	**0.07**	**0.09**	**-**	**-**	**-**
$CD_{0.05}$	**0.08**	**0.12**	**0.16**			

Table. 2. Growth of NL(Nagaland) isolate of *S. sclerotiorum* against fungal antagonists *in vitro*

Treatments	Radial growth (mm) at different hours of incubation			% Inhibition at different hours of incubation		
	24h	48h	72h	24h	48h	72h
T_1 = *S. sclerotiorum* (NL) + *T. harzianum*	21.22[d]	26.05[d]	29.08[d]	8.37	57.60	67.68
T_2 = *S. sclerotiorum* (NL) + *G. virens*	21.74[c]	28.26[c]	32.36[c]	6.13	54.01	64.04
T_3 = *S. sclerotiorum* (NL) + *T. koningii*	22.05[b]	31.50[b]	36.74[b]	4.79	48.73	59.17
T_4 = *S. sclerotiorum* (NL) alone	23.16[a]	61.45[a]	90.00[a]	-	-	-
SEd (±)	**0.02**	**0.04**	**0.05**	**-**	**-**	**-**
$CD_{0.05}$	**0.03**	**0.07**	**0.08**			

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Table. 3. Growth of MZ(Mizoram) isolate of *S. sclerotiorum* against fungal antagonists *in vitro*

Treatments	Radial growth (mm) at different hours of incubation			% Inhibition at different hours of incubation		
	24h	48h	72h	24h	48h	72h
T_1 = *S. sclerotiorum* (MZ) + *T. harzianum*	25.36[d]	34.26[d]	36.77[d]	4.01	53.37	59.14
T_2 = *S. sclerotiorum* (MZ) + *G. virens*	25.68[c]	36.37[c]	38.75[c]	2.80	50.50	56.94
T_3 = *S. sclerotiorum* (MZ) + *T. koningii*	25.90[b]	39.54[b]	42.56[b]	1.96	46.18	52.71
T_4 = *S. sclerotiorum* (MZ) alone	26.42[a]	73.48[a]	90.00[a]	-	-	-
SEd (±)	**0.04**	**0.06**	**0.08**	**-**	**-**	**-**
CD $_{0.05}$	**0.07**	**0.10**	**0.14**			

*Values are means of five replications
Means followed by same letter shown in superscript(s) are not significantly different

Table. 4. Growth of AP(Arunachal P.) isolate of *S. sclerotiorum* against fungal antagonists *in vitro*

Treatments	Radial growth (mm) at different hours of incubation			% Inhibition at different hours of incubation		
	24h	48h	72h	24h	48h	72h
T_1 = *S. sclerotiorum* (AP) + *T. harzianum*	23.86[d]	32.45[d]	35.47[d]	6.21	55.56	60.58
T_2 = *S. sclerotiorum* (AP) + *G. virens*	24.07[c]	34.52[c]	37.65[c]	5.38	52.72	58.16
T_3 = *S. sclerotiorum* (AP) + *T. koningii*	24.58[b]	37.97[b]	41.26[b]	3.38	48.00	54.15
T_4 = *S. sclerotiorum* (AP) alone	25.44[a]	73.02[a]	90.00[a]	-	-	-
SEd (±)	**0.03**	**0.04**	**0.06**	**-**	**-**	**-**
CD $_{0.05}$	**0.05**	**0.07**	**0.10**			

Table. 5. Growth of MP(Manipur) isolate of *S. sclerotiorum* against fungal antagonists *in vitro*

Treatments	Radial growth (mm) at different hours of incubation			% Inhibition at different hours of incubation		
	24h	48h	72h	24h	48h	72h
T_1 = *S. sclerotiorum* (MP) + *T. harzianum*	20.61[d]	25.33[d]	28.56[d]	8.31	57.95	68.26
T_2 = *S. sclerotiorum* (MP) + *G. virens*	20.93[c]	27.70[c]	30.27[c]	6.90	54.02	66.36
T_3 = *S. sclerotiorum* (MP) + *T. koningii*	21.56[b]	30.49[b]	34.55[b]	4.09	49.39	61.61
T_4 = *S. sclerotiorum* (MP) alone	22.48[a]	60.25[a]	90.00[a]	-	-	-
SEd (±)	**0.02**	**0.03**	**0.05**	**-**	**-**	**-**
CD $_{0.05}$	**0.03**	**0.05**	**0.08**			

Table. 6. Growth of ML (Meghalaya) isolate of *S. sclerotiorum* against fungal antagonists *in vitro*

Treatments	Radial growth (mm) at different hours of incubation			% Inhibition at different hours of incubation		
	24h	48h	72h	24h	48h	72h

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T_1 = *S. sclerotiorum* (ML) + *T. harzianum*	23.82[d]	32.55[d]	33.56[d]	5.40	55.16	62.71
T_2 = *S. sclerotiorum* (ML) + *G. virens*	23.96[c]	34.72[c]	36.28[c]	4.84	52.17	59.68
T_3 = *S. sclerotiorum* (ML) + *T. koningii*	24.33[b]	37.84[b]	40.24[b]	3.37	47.87	55.28
T_4 = *S. sclerotiorum* (ML) alone	25.18[a]	72.60[a]	90.00[a]	-	-	-
SEd (±)	**0.03**	**0.05**	**0.08**	-	-	-
CD $_{0.05}$	**0.05**	**0.08**	**0.14**			

*Values are means of five replications

Means followed by same letter shown in superscript(s) are not significantly different

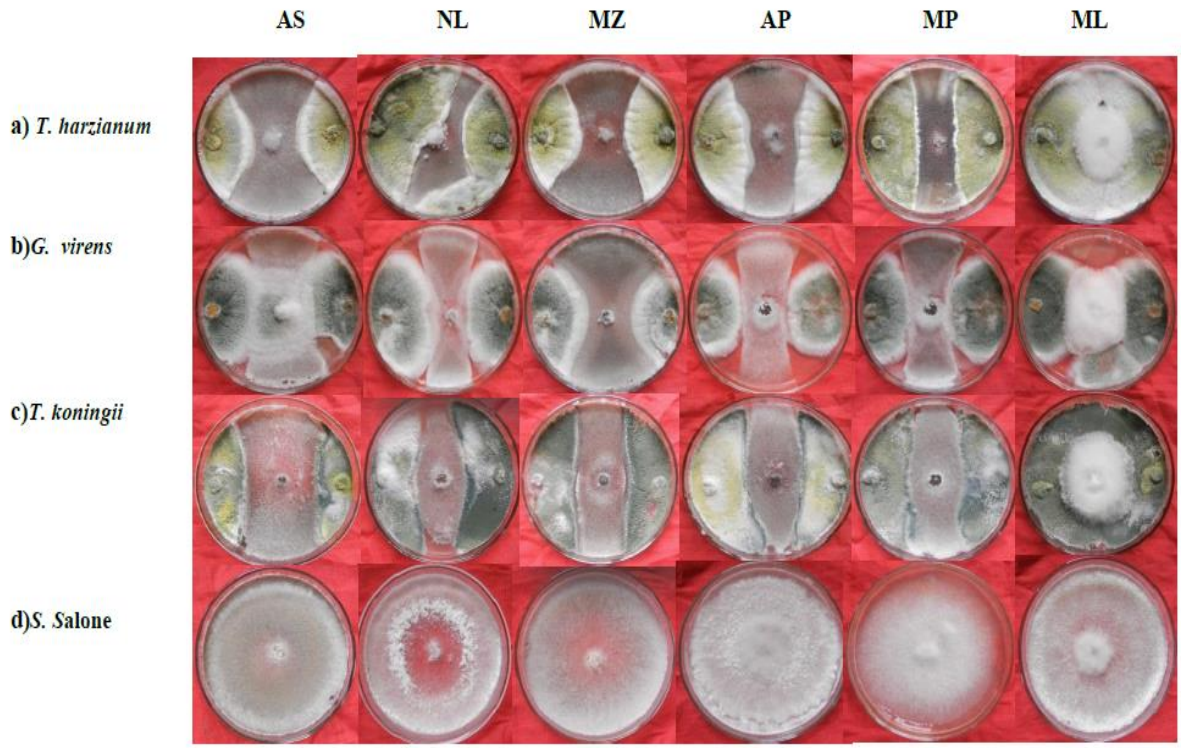

Plate.1. Growth of isolates of *Sclerotinia sclerotiorum* against fungal antagonists in dual culture

a. Growth of isolates of *S. sclerotiorum* (AS, NL, MZ, AP, MP and ML) in presence of *T. harzianum*
b. Growth of isolates of *S. sclerotiorum* (AS, NL, MZ, AP, MP and ML) in presence of *G. virens*
c. Growth of isolates of *S. sclerotiorum* (AS, NL, MZ, AP, MP and ML) in presence of *T. koningii*
d. Growth of isolates of *S. sclerotiorum* (AS, NL, MZ, AP, MP and ML) alone

Which have antibiotic properties. Mukherjee *et al.* (1995) also reported that *G. virens* effectively suppressed mycelial growth of *S. rolfsii* and *R. solani in vitro*. The high inhibitory activity of the antagonists observed on *S. sclerotiorum* in dual cultures and the frequency of mycoparasitic activities and antibiosis by the antagonists against *S. sclerotiorum* is probably due to the production of these toxic metabolites, antibiotics, volatile gases and cell wall degradating enzymes. From the results of the present investigation, it appears that maximum per cent inhibition of mycelial growth of *S. sclerotiorum* by different antagonists was found in MP(Manipur) isolate followed by NL(Nagaland) isolate, while AS(Assam) isolate of *S. sclerotiorum* was found to be least per cent inhibition of the isolates in relation to ability to synthesize phenolic acids for its self-defence and resistance against other microbes and unfavourable environmental conditions. Sarma *et al.* (2002) also reported that phenolic acids are believed to contribute resistance in fungus against certain pathogens and biological control agents.

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