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Efficacy of Biocontrol Agents for the Control of Chickpea Stem Rot

B.K.SHARMA

HPKV, Regional Research Station,
Dhaulakuan, Distt. Sirmour (H.P) 173 001

ABSTRACT

The Antagonistic fungi, *viz., Trichoderma harzianum, T. viride, Gliocladium virens, Absidia cylindrospora Alternaria alternata* and *Fusarium solani* were evaluated against *Sclerotinia sclerotiorum* causing chickpea stem rot. *T.harzianum* and *A.cylindropora* were most effective in inhibiting the mycelial growth in dual cultures. Inhibitory activity of autoclaved culture filtrates was much less as compared to filter-sterilized culture filtrates. Culture filtrates of *T.harzianum, G.virens* and *T.viride* were particularly effective in inhibiting sclerotial germination. In pot culture experiments, wheat bran-saw-dust-tap water (1:1:6;W/W/v) mixture was most effective in reducing seedling mortality. Application of antagonists in *Sclerotinia* - sick plots reduced the disease incidence and increased yield over control. *T.harzianum* was most effective followed by *G. virens* and *T.viride*.

KEY WORDS : *Sclerotinia sclerotiorum, Trichoderma harzianum*, stem rot, chickpea, antagonist

The stem rot caused by *Sclerotinia sclerotiorum* (Lib) de Bary causes heavy losses in chickpea in Poanta valley of Himachal Pradesh. Since its control through host plant resistance/fungicides is difficult to achieve and not quite satisfactory, biological control can be an attractive alternative. Hence, the microflora isolated from soil were evaluated for their biocontrol potential *in vitro*, in the glass house and the field against chickpea stem rot.

MATERIALS AND METHODS

The sclerotia of *S.sclerotiorum* were mass multiplied on oat grains Sharma and Singh, 1990). Cultures of *Trichoderma harzianum* Rifai, *T. viride* Pers.fr., *Gliocladium virens, Alternavia alternata, Fusarium solani* and *Absidia cylindrospora* from ginger rhizosphere (IMI No. 343136) were used in the studies. Antagonistic activity of the microflora was tested in dual culture (Huang and Hoes, 1976). Growth inhibition of the test pathogen over control was calculated after 120 h of incubation at 25^0C. The culture filtrate of biocontrol agents was obtained by filtration through whatman No.1 filter paper. This was then sterlized by autoclaving at 1.05 kg/cm^2 for 15 min, and tested for its efficacy against the growth of *S.sclerotiorum* by well method (Singh and Webster, 1973). For filter - sterilized culture filtrates, the method adopted was similar except that the culture filtrate was sterilized using sintered glass filter (G-5). In another set of experiments, sclerotia were soaked for one 2h in the culture filtrates, washed 5-6 times with sterile water, dried on sterilized filter papers and plated on PDA. Germination of sclerotia was recorded after 7 days of incubation at 22± 2^0C. Each treatment was replicated thrice. Sclerotia soaked in sterilized water served as check. Early and late stage host parasite relationships were also examined by sampling of agar pieces from the zone of interaction (Huang and Hoes, 1976).

In glass house experiments (24-30^0C), different delivery systems were developed using seed coating with propagules of antagonists, seed and soil treatment with spore suspension, wheat bran-water (1:2), sawdust-tap water (1:2) and wheat bran-sawdust-tap water (1:1:6). Seeds were coated with mycelium and spores of antagonists by shaking them vigorously in test tubes containing sporulating

cultures of the antagonists. In another method, 50 seeds were dipped in 50 ml spore suspension for 10 minutes. In a third method, 50 ml of the spore suspension was mixed with upper 5 cm soil of each pot. The soil was inoculated with sclerotial bits (2 mm) and mycelia cultured on oat grains @ 250 mg/kg soil. The wheat bran and saw dust medium individually and in combination were mixed @ 5 g/kg soil in upper 5 cm layer of each pot. Chickpea seeds (10 seeds/pot)cv. C-235 were sown. Each treatment was replicated five times. Disease incidence was recorded 30 days after sowing and expressed on percentage mortality of plants.

A field experiment was conducted during 1992-93 crop season, in a Randomized Block Design .with a plot size of 2.0x1.8m and replicated thrice. Biocontrol agents multiplied on saw dust - wheatbran - tap water (1:1:6) were applied as soil application (200 g/m ^{2}) at the time of sowing. The plots were artifically inoculated with sclerotia of *S.sclerotiorum*. Two checks *viz.*, inoculations with and without *S.sclerotiorum* were also kept for comparison. The per cent infected plants were counted before harvesting.

RESULTS AND DISCUSSION

A.cylindrospora and *T.harzianum* were most effective in vitro and inhibited mycelial growth of *S.sclerotiorum* by 82.9 and 80.4 percent respectively (Table 1). Inhibitory activity of autoclaved culture filtrates was much less as compared to filter - sterilized culture filtrates indicating that the substances reposible for inhibitory activity are heat labile. Sclerotial germination was reduced considerably in culture filtrates of *T.harzianum, G.virens* and *T.viride*. Similar results were also reported by Kansal *et al.* (1990) and Dohroo *et al.* (1990). The inhibitory activity of antagonists might be due to diffusible metabolites secreted by them since, host - parasite relationship revealed hyphal breakage and swelling of *S.sclerotiorum*. Lee and Wu (1984) showed that *G.virens* and *T.viride* produced antibiotics that inhibited the mycelial growth of *S.sclerotiorum* completely and induced swelling and plasmolysis of affected cells.

The effect of different delivery systems of biocontrol agents in pot culture experiments revealed that *T.harzianum, T.viride,* and *G.virens* grown on wheat bran-saw-dust -tap water (1:1:6 w/w/v) mixture increased the seed germination and reduced the mortality of seedlings due to *S.sclerotiorum* (Table 2). Although wheatbran -tap-water (1:2 w/v) individually gave good disease control, their combination was most effective resulting in maximum seedling stand. Other methods of applications of biocontrol agents were much inferior to the wheat bran-saw dust mixture. The same food base was used in field experiments as soil application. *T.harzianum, G.virens* and *T.viride* were most effective in reducing disease incidence in the field giving 93.7, 92.1 and 91.3 per cent disease control respectively over the control and also increased the yield (Table 3). *A.cylindrospora* was not so effective in the field.

Table 1. Inhibition of mycelial and sclerotial growth of *S. sclerotiorum* by different antagonists

Antagonist	Mycelial growth inhibition (%)			Sclerotial germination (%) in culture filtrate
	Dual Culture	Filter - Sterilized	Heat - sterilized	
T. harzianum	80.4	46.2	2.8	16.6
T. viride	76.0	35.0	4.2	18.0
G. virens	76.3	44.0	3.7	17.3
A. cylindrospora	82.9	49.2	5.0	24.6
A. alternata	53.9	15.2	2.5	41.3
F. solani	35.4	19.2	4.1	28.6
Control	-	-	-	66.3
CD (P=0.05)	6.2	5.6	1.6	5.33

Table 2. Effect of methods of application of biocontrol agent on seedling mortality of chickpea

Treatment	*T. harzianum*	*T. viride*	*G. virens*
Propagules to seed	14.2 (21.8)	20.8 (26.9)	25.4 (29.7)
Spore suspension to seed	30.7 (35.5)	21.7 (27.1)	26.7 (32.1)
Spore suspension to soil	20.5 (29.7)	29.1 (32.9)	20.5 (27.1)
Wheat bran inoculum to soil	6.21 (13.9)	1.6 (8.1)	0 (6.52)
Sawdust inoculum to soil	10.5 (18.1)	5.8 (13.9)	6.1 (14.2)
Wheat bran+saw dust to soil	0.0 (6.3)	0.0 (7.1)	0.0 (6.5)
Untreated check	52.8 (41.1)	54.6 (46.8)	50.7 (44.5)
C.D (P=0.05)	(5.8)	(4.4)	(5.1)

Figures in parenthesis are arc sine values

Table 3. Biological control of stem rot of chickpea in the field

Biocontrol agent	Infected plants (%)	Yield (kg/ha) of grain
T. harzianum	3.2	2628
T. viride	4.5	2582
G. virens	4.1	2472
A. cylindrospora	31.7	1370
A. alternata	49.7	1342
F. solani	40.8	1129
Check (Inoculation with *S. sclerotiorum* only)	52.3	851
Control (No inoculation)	30.7	1342
C.D (P=0.05)	6.8	354

T.harzianum, T.viride and *G. virens* containing young actively growing hyphae, embedded in the food base i.e. wheat bran + saw dust were more effective in reducing chickpea stem rot. Lewis and Papavizas(1984) have also shown that mycelial preparations were more effective than conidial preparations of antagonists. The activity of wheat bran-saw dust medium in enhancing the biocontrol potential of the antagonists resulting in suppression of pathogen and prevention of stem rot may be explained by the principle of substrate possession (Bruehl, 1975).

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