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Fungal Antagonists for the Biological Control of Ascochyta Blight of Chickpea

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Ascochyta blight *(Ascochyta rabiei)* is one of the most devastating diseases of chickpea. The biocontrol potential of fungal antagonists, *Chaetomium globosum, Trichoderma viride, Acremonium implicatum* were explored under *in vitro* and *in vivo*. *A. implicatum* isolate-1 overgrew the host mycelium and caused lysis, while *A. implicatum* isolate-2 produced inhibition zone. *C. globosum* profusely overgrew the mycelium of *A. rabiei* and *T. viride* showed overgrowth and profuse sporulation. Bioassay with culture filtrates of all the antagonists resulted in significant inhibition of pycnidiospore germination and reduction in colony development of *A. rabiei.* Syringe filtered culture filtrate when amended in liquid broth medium also significantly reduced the mycelial growth. Bioassay of culture filtrates under glass house conditions, although brought reduction in disease development in both pre- and post-inoculation sprays, but *C. globosum* was the most effective antagonist causing 73.12% reduction in disease index when used as post inoculation spray. Under *in vitro* conditions *C. globosum* caused 48.59% reduction in colony diameter and 70.86% reduction in pycnidiospore germination.

Keywords: Fungi, antagonists, biological control, Ascochyta blight.

Ascochyta blight caused by *Ascochyta rabiei* (Pass.) Lab. is one of the most devastating diseases and is widely prevalent in almost all chickpea growing countries of the world. In India, the disease was first reported in North-western frontier Province (now in Pakistan), in 1918 by Butler and since then it has been consistently appearing in several North-Indian states causing enormous losses (Nene et al., 1984).

The disease management strategies mainly include cultural practices, host resistance and use of chemicals for seed and foliar applications. Seed borne infection although is effectively controlled by treating the seeds with fungicides and secondary spread of the disease can be checked by foliar sprays, but these chemical control measures are not economical and eco-friendly. Therefore, biological control of this disease is being explored. So far there is no report on biological control of this disease although possibility of biological control of diseases of pulse crops has been reported (Vishwadhar et al., 2000). The present paper highlights the results obtained on the possible biological control of Ascochyta blight using fungal antagonists.

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Materials and Methods

Isolation and maintenance of pathogen and antagonist cultures

A highly virulent isolate of *Ascochyta rabiei* (ArD_{11}) was obtained from Pulse Pathology Laboratory, Division of Plant Pathology, IARI, New Delhi. The single spore culture was maintained on Potato Dextrose chickpea seed agar medium at 20 ± 1 °C.

Antagonistic fungi included *Chaetomium globosum* (isolate Cg2) and *Trichoderma viride* (TV-5-2) from Wheat Pathology Laboratory, Division of Plant Pathology, IARI, New Delhi, *Acremonium implicatum* isolates 1 and 2, isolated from chickpea leaves.

In vitro *antagonistic studies*

In vitro and *in vivo* tests were conducted to evaluate these fungal antagonists for their inhibitory effect on *A. rabiei.*

Dual culture studies

Discs (5 mm) of *A. rabiei* were placed in sterilized Petri plates containing PDA at one periphery and after 4 days of incubation, 5 mm disc of actively growing culture of each antagonist was placed at the opposite side of *A. rabiei.* The plates without antagonistic fungus served as control. The Petri plates after incubation at 25 ± 1 °C for 7 days were observed for colony diameter of *A. rabiei.*

Effect of culture filtrate on conidia germination

Culture filtrate of all the antagonists was obtained by growing them in 90 ml PDB taken in 250 ml conical flasks and subsequently incubating at 25 ± 1 °C for 21 days as static culture. The cultural filtrate was filtered through Whatmann filter paper no. 42 and made cell free and stored in McCartney's bottles at 4 °C. Effect of cultural filtrate on conidia germination was studied by cavity slide method. One drop of syringe filtered culture filtrate was mixed with pycnidial suspension of *A. rabiei* having a concentration of 10^3 pycnidia/ml. One slide with water and conidial suspension was kept as check. Experiment was replicated thrice. The cavity slides kept in moist chamber were incubated for 24 hrs at 25 ± 1 °C and germinated conidia were counted under compound microscope.

Effect of cultural filtrate on colony development

Effect of cultural filtrate on number and diameter of colonies of A. rabiei was tested by agar diffusion method. 0.5 ml conidial suspension of *A. rabiei* @ 10^3 conidia /ml, was uniformly spread in PDA poured Petri plates. Syringe filtered cultural filtrate of each antagonist was placed in the well made in the Petri plates with the help of sterilized cork borer. One set of plates with sterilized water served as check. The replicated plates (3) were incubated at 25 °C and observations on number and diameter of colony were recorded after 7 days of incubation.

Effect of cultural filtrate on growth of A. rabiei *in liquid medium*

The effect of culture filtrate of all the antagonists on growth of *A. rabiei* was studied in potato dextrose broth (PDB). Syringe filtered culture filtrates from each antagonist was amended in the medium (in 2 percent). The flasks were inoculated with mycelial discs of *A. rabiei* and incubated at 25 °C in three replications. The observations on fresh mycelial weight were recorded and subjected to statistical analysis.

In vivo *testing of cultural filtrate*

In a three replicated pot experiment, 21 days old seedlings of chickpea variety Pusa-256 were sprayed with culture filtrate of all the antagonists as pre- and post- inoculation treatments.

Pre-inoculation test

For pre-inoculation test, seedlings were first sprayed with concentrated culture filtrate, till drenched and after 24 hrs, onidial suspension of *A. rabiei* @ 10^4 conidia/ml was applied on chickpea seedlings. The pots were kept in humidity chamber for another 24 hrs and then incubated in shade.

Post-inoculation test

Seedlings were first inoculated with conidial suspension of *A. rabiei* @ 10^4 conidia/ml and provided humidity for 24 hrs in moist chamber and thereafter sprayed with culture filtrate. Subsequently, the seedlings were kept outside the humidity chamber in shade for incubation till symptom development.

During the course of these experiments, suitable controls were kept, i.e. in one case seedlings were sprayed only with conidial suspension of *A. rabiei,* in second case seedlings were sprayed with water and in third case seedlings were pre- or post-sprayed with fungicide, Kavach at 2% concentration. After 8 days of incubation, observations on formation of lesions on stem and leaves and other visible symptoms were recorded and disease rating was given according to 1–9 scale of Singh et al. (1981).

Results and Discussion

Dual culture

The observation on suppression of radial growth of *A. rabiei* in dual culture with *A. implicatum* isolates 1 and 2, *C. globosum* and *T. viride* made after 6, 8, 10 and 12 days of incubation are presented in *Table 1.* Inhibition in radial growth began after 6 days in all the antagonists. *C. globosum* produced maximum reduction in growth (35.25%) at the 6th day, and minimum reduction in radial growth was observed in dual culturing of *A. implicatum*-2. *T. viride* reduced the growth of *A. rabiei* from 4.4 cm in check to 3.3 cm after

Table 1

Effect of different antagonists on radial growth of *Ascochyta rabiei* in dual culture

Antagonist	6 days of incubation		8 days of incubation		10 days of incubation		12 days of incubation	
	Colony diameter (cm)	% reduction	Colony diameter (cm)	% reduction	Colony diameter (cm)	% reduction	Colony diameter (cm)	% reduction
Acremonium implicatum-1	3.53[b]	19.77	4.03[b]	24.99	4.2[c]	30.8	4.33[b]	33.07
Acremonium implicatum-2	3.57[b]	18.86	3.83[b]	28.14	4.1[b]	32.45	4.2[b]	35.08
Chaetomium globosum	2.85[a]	35.27	3.13[a]	41.12	3.27[a]	46.12	3.33[a]	48.59
Trichoderma viride (TV5-2)	3.3[a]	25.00	3.73[b]	30.01	3.9[b]	35.74	4.03[b]	37.71
Control	4.4[c]	–	5.33	–	6.07	–	63.47	–
CD (P=0.05)	0.66	–	0.31	–	0.25	–	0.33	–

Values superscripted with same letter are not significantly different at (p=0.05) according to Duncan Multiple Range Test (DMRT).

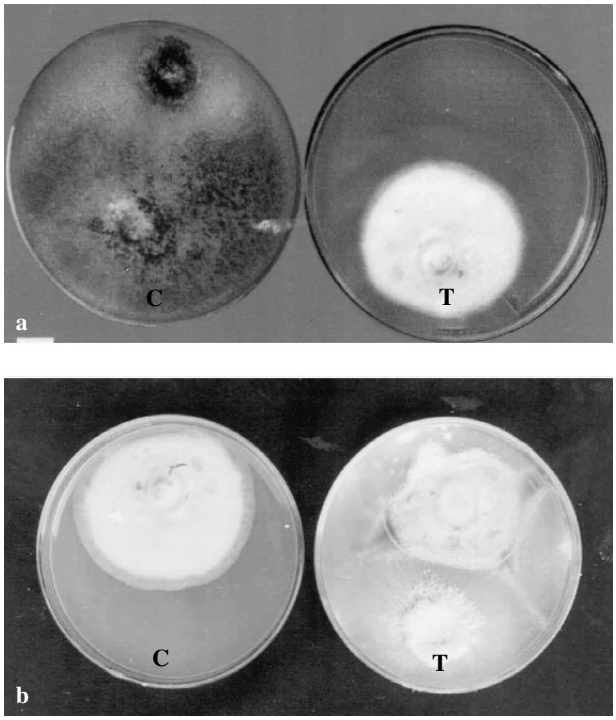

Fig. 1. Dual culture of *Ascochyta rabiei* with *Chaetomium globosum* isolate Cg2 (a), and *Acremonium implicatum* isolate-1 (b).
C – Control, T – Dual culture

6 days of incubation *(Table 1).* Similarly, 8 DAI, *C. globosum* reduced the growth of pathogen to the maximum (41.12%) followed by *T. viride* (30.01%), *A. implicatum*-2 (28.14%) and *A. implicatum*-1 (24.99%). *C. globosum* significantly reduced the growth of pathogen and rest of the antagonists were at par. After 10 days of inoculation also, maximum percent reduction in growth (46.12%) was observed in dual culturing with *C. globosum,* which also differed significantly from *A. implicatum*-1 and -2 and *T. viride (Table 1).* At 12 DAI, *C. globosum* showed overgrowth and lysis of the host pathogen, reducing the radial growth by 48.53% *(Fig. 1a),* and *A. implicatum*-1 showed mycoparasitism *(Fig. 1b),* whereas *A. implicatum*-2 lysed the host pathogen and reduced its growth to 35.68% producing inhibition zone *(Fig. 2a)* and *T. viride* showed complete overgrowth and sporulation on the test pathogen *(Fig. 2b).*

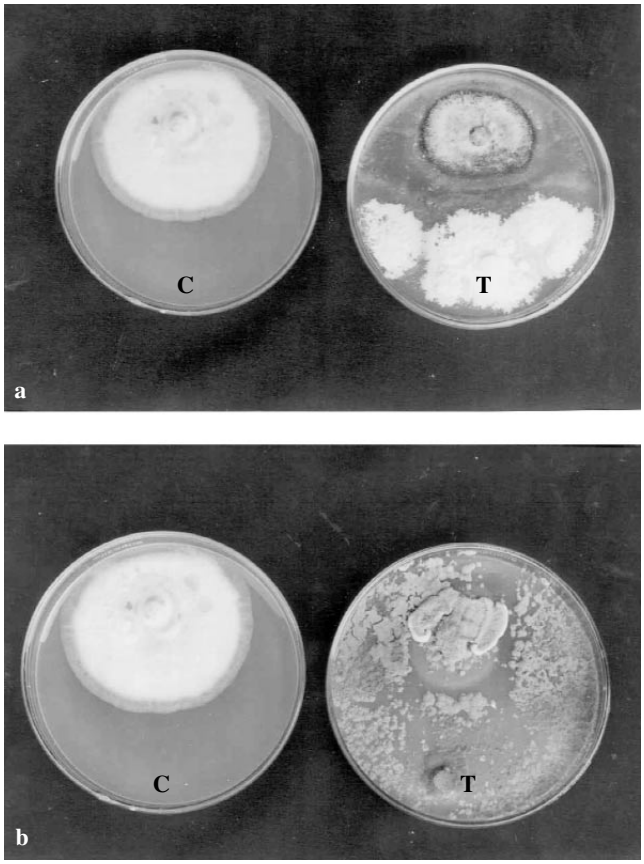

Fig. 2. Dual culture of *Ascochyta rabiei* with *Acremonium implicatum* isolate-2 (a), and *Tricoderma viride* isolate TV5-2 (b).
C – Control, T – Dual culture

Effect of culture filterate on conidia germination

The observations on pycnidiospore germination revealed that culture filtrate of *C. globosum* proved highly inhibitory to pycnidial germination showing 20.73% germination registering 78.86% reduction over check followed by *T. viride* isolate (TV5-2) which reduced germination upto 55%. Least inhibition in germination was seen in cultural filtrate of *A. implicatum* isolate 1 *(Table 2).*

Table 2

Effect of culture filtrate of different antagonists on pycnidiospore germination and colony development of *Ascochyta rabiei*

Antagonist	Pycnidiospore germination		Colony development					
	% spore germination	% reduction	Colony No.	% reduction	Colony diameter (mm)	% reduction	Fresh mycelial wt.	% reduction
Acremonium implicatum-1	43.3	38.85	14	36.36	5.33	70.76	4.53	22.82
Acremonium implicatum-2	36.86	48.18	10	54.54	14.67	29.52	4.4	25.04
Chaetomium globosum	20.73	70.86	4	81.81	4.61	74.71	3.4	42.07
Trichoderma viride (TV5-2)	32.11	54.86	8	63.63	8.27	54.63	3.87	39.07
Control	71.14	–	22	–	18.23	–	5.87	–
CD (P=0.05)	15.48	–	9.95	–	9.45	–	0.74	–

Effect of culture filtrate on colony development

In vitro test on the number of colonies formed in the medium amended with culture filtrate revealed that, in comparison to control where number of colonies was 22, *C. globosum* proved highly effective in reducing the colony number to 4 showing 81.8% reduction over check. This decline in percent reduction in colony number by *C. globosum* was followed by *T. viride* (63.3%), *A. implicatum*-2 (54.54%) and *A. implicatum*-1 (36.36%). *C. globosum* also proved highly effective in reducing colony diameter of *A. rabiei t*o 4.61 mm as compared to check (18 mm) registering 74.7% reduction.

The culture filtrate of the antagonists also reduced the growth of *A. rabiei* in liquid medium. The flasks inoculated with culture filtrate of *C. globosum* showed 3.4 g fresh mycelial weight as compared to check showing 5.87 g, registering 42.07% reduction over check. Next best isolate was *T. viride* (TV5-2), followed by *A. implicatum*-2 and *A. implicatum*-1 *(Table 2).*

The bioefficacy of culture filtrate of all the antagonists tested under glass house conditions as foliar sprays indicated that post-inoculation sprays were more effective than pre-inoculation sprays *(Table 3).* In pre-inoculation spray experiment, an infection index of 3.3 was obtained with spray of culture filtrate of *C. globosum* showing 61.59% reduction in disease severity as compared to 8.67 disease severity attained in check II. The dis-

ease severity in case of *A. implicatum*-2 was 5.33 followed by *A. implicatum*-1 (6.67) and *T. viride* (7.67). Post-inoculation spray of culture filtrate of *C. globosum* caused 73.12% reduction in disease severity. The percent reduction in disease severity in case of *A. implicatum*-2, *A. implicatum*-1 and *T. viride* was 46.3, 46.13 and 11.53%, respectively.

Table 3

Bioefficacy of culture filtrate of antagonists on *Ascochyta rabiei* infection in chickpea under glass house conditions

Antagonists	Pre-inoculation spray		Post-inoculation spray	
	infection index	% reduction in disease over check II	infection index	% reduction in disease over check II
Acremonium implicatum-1	6.67[b]	23.06	4.67[b]	46.13
Acremonium implicatum-2	5.33[a]	38.52	4.00[b]	53.86
Chaetomium globosum	3.33	61.59	2.33[a]	73.12
Trichoderma viride (TV5-2)	7.67	11.53	7.67[b]	11.53
Check I (Fungicide 0.2%)	0.00	–	1.00[a]	–
Check II (Pathogen inoculated)	8.67	–	8.67	–
CD (P=0.05)	2.56	–	2.42	–

Biocontrol is fundamentally applied ecology. More specifically, the goal is to manage a microbial community to favour biocontrol agents and disfavour the pathogen. Therefore, in the present study efforts were made to identify potential biocontrol agents against *A. rabiei.* The comparative bioefficacy studies of *C. globosum, T. viride* and *A. implicatum,* have shown that *C. globosum* is the best antagonist of *A. rabiei,* followed by *T. viride* (TV5-2) and *A. implicatum* (isolate 2).

The bioefficacy of antagonists when compared with fungicide (check I) indicated that culture filtrate of *C. globosum* was at par with fungicide treatment, and significantly superior to *A. implicatum* isolates and *T. viride.*

Although, antagonistic effect of microorganisms on *A. rabiei* has not been studied earlier, but limited information is available on biological control of wilt and root rot diseases of chickpea and lentil (Vishwadhar et al., 2000). In our present studies, *C. globosum* has been observed to be a potential antagonist of *A. rabiei,* reducing the mycelial growth up to 50% and controlling the disease up to 73% under glass house. Earlier research findings of different workers have also indicated inhibitory property of *Chaetomium* spp. against a number of plant pathogens (Vannacci and Pecchia, 1986; Hinton and Parry, 1993). Recent studies have also indicated that *C. globosum* is a potential antagonist of *Bipolaris sorokiniana,* causing spot blotch of wheat (Biswas et al., 2000; Aggarwal et al., 2004). In our findings, *Acremonium implicatum* has also been identified as a mycopara-

site of *A. rabiei. A. strictum* has been reported to be antagonistic to *Alternaria solani* (Ahmed and Saleh, 1987) and *Alternaria alternata* as hyperparasite of cucurbit powdery mildew pathogen, *Sphaerotheca fulginea* (Malatharkis, 1985). In the present investigations, we observed that syringe filtered culture filtrate of *C. globosum* reduced the size and number of colonies of *A. rabiei* on PDA and also inhibited the growth of the pathogen in liquid broth culture. The culture filtrate also resulted in significant inhibition of conidia germination. These results were further strengthened by efficient disease control under glass house conditions by spraying with culture filtrates as pre- and post-inoculation spray. These results have indicated that culture filtrate might contain antifungal metabolites, which inhibit the growth of pathogen *in vitro* and *in vivo.*

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