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Biocontrol potential of *Gliocladium virens* against fungal pathogens isolated from chickpea, lentil and black gram seeds

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The pathogenic fungi associated with chickpea (*Cicer arietinum*), lentil (*Lens culinaris*) and black gram (*Vigna mungo*) seeds were isolated and were evaluated against *Gliocladium virens*, a potential biocontrol agent. Biological control of fungal plant pathogens appears as an attractive and realistic approach, and numerous microorganisms have been identified as biocontrol agents. In the present study samples of chickpea, lentil and black gram seeds were collected from local districts of Rajasthan and analyzed for seed-borne fungi. The pathogenic fungi isolated from these seeds were *Alternaria alternata, Chaetomium* spp., *Penicillium citrinum, Aspergillus niger, A. flavus, Rhizopus nigricans* and *Fusarium oxysporum*. We found that *G. virens* significantly inhibited the radial growth of the pathogenic fungi tested.

Key words: *Cicer arietinum*, *Lens culinaris*, *Vigna mungo, Gliocladium virens,* pathogens

Introduction

In Indian agriculture, pulses play an important role in maintaining soil fertility and supplying protein to the large vegetarian population of the country The legumes or pulses belong to the family Fabaceae (Leguminous) characterized by having a special kind of fruit, a legume. Nearly 11,000 species of legumes are known and many are of importance as industrial, medicinal of food plants. Considering the nutritional, agronomical and industrial value of pulses and yield of legumes the present study is aimed to study the seed borne fungal pathogens of three important pulse crops of Rajasthan namely chick pea *Cicer arietinum* (L.), lentil (*Lens culinaris* Medik.) and black gram (*Vigna mungo* L.). Chickpea (*Cicer arietinum* L.) commonly known as 'gram' is the most important legume grown in India and grown over 6.66 m ha of land (Kochhar, 2009). It has been found to be attacked by 172 pathogens including 67 species of fungi (Nene *et al.,* 1996). Chickpea suffers from a large number

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of fungal diseases namely Ascochyta blight (*Ascochyta rabiei*), Fusarium wilt (*Fusarium oxysporum*), dry root rot (*Rhizoctonia bataticola*) Alternaria blight (*Alternaria alternata*), Colletotrichum blight (*Colletotrichum dematium*), Stemphylium blight (*Stemphylium sarciniforme*), powdery mildew (*Leveillula taurica*), Sclerotinia stem rot (*Sclerotinia sclerotiorum*), wet root rot (*Rhizoctonia solani*), and foot rot (*Operculella padwickii*) (Singh and Sharma, 2005; Dubey *et al.*, 2007).

In the same way lentil is also used for human consumption as a protein source in a diverse range of product and is an excellent source of vitamin A and provides fiber, potassium, B vitamins, and iron (Kochhar, 2009). Some important seed-borne fungal disease of lentil are Ascochyta blight (*Ascochyta lentis*), gray mould (*Botrytis cinerea*), collar rot (*Sclerotium rolfsii*) and Fusarium wilt (*Fusarium oxysporum* f. sp. *lentis*) (Barkley, 2008; Anonymous, 2010). Black gram (*Vigna mungo* (L.) Hepper) is also very nutritious and is recommended for diabetics, as are other pulses. Blackgram is a closely relative species of mungbean. All these varieties of pulses are excellent source of easily digestible protein.But as most of the pulses grown under rainfed conditions, hence less attention is paid for the management of these crops and protection against diseases gets least priority.

Plant diseases play a direct role in the destruction of natural resources in agriculture. Among them, diseases play an important role (Nine, 1986; Pal, 1996). Many fungal pathogens, some of which are seed transmitted, often reduce the germination ability or kill the infected plants or substantially reduce the productive capacity. Some of these fungi produce aflatoxins which damage the liver and induce carcinogenic, mutagenic and teratogenesis (Pereyra *et al.,* 2008). Therefore, control of seed-borne fungi is extremely important and the damaging effects can be relieved through integrated approaches (Vaidehi, 2002) Hence the study was undertaken to investigate percentage incidence of seed-borne fungi associated with chickpea, lentil and blackgram seeds and to evaluate antagonistic effect of *G.virens* in order to protect these seeds from fungal diseases.

Materials and method

Survey, Isolation and Identification of pathogenic fungi isolated from the seeds

Seed samples were collected from different localities of Rajasthan viz Ajmer, Alwar, Bharatpur, Jaipur and Kota. From each sample, 200 seeds were tested. Two methods blotter and potato dextrose agar were used as recommendation of ISTA (1966). For the standard blotter method, untreated

seeds and seeds after treatment with 0.1% sodium hypochlorite for 10 minutes were placed on three layers of moistened blotter paper, 10 seeds per Petri dish. The plates were then incubated in B.O.D incubator at 25± 2˚C for 8 days under 12 hrs alternating cycles of light and darkness. Same way for Agar plate method, the treated and untreated seed components was plated on PDA amended with streptomycin to eliminate bacterial contamination. Thus, the exposed seeds were examined on the 9th day under stereo binocular microscope for the presence of seed borne pathogens (if any). The method suggested by Mathur *et al*. (1975) was used to detect the location of seedborne fungi with slight modification. The isolated fungal strains were purified and identified, according to Burgess *et al.* (1988); Domsch, *et al*. (1980); Ellis (1976).

In vitro evaluation of the antagonist *G.virens* against the pathogens isolated

One agar disc (5mm) with the active mycelium of the bioagent (*G.virens*) was taken from 5-7days-old culture grew on PDA medium and transferred to one side of a Petri plate. In the same way the other side of the Petri plate was inoculated with active mycelium plug taken from the outer margin of a few days old culture of the causal pathogens (El-Kafrawy *et al*., 2002). This experiment was conducted in 3 replicates per each bioagent and plates were incubated at 25+3 °C for 5 days. Plates containing only pathogen were used as a control. Inhibition percentages of the pathogen were calculated just after overlapping of the two tested fungi according to the following equation

$$100x \frac{(r1 - r2)}{r1}$$

Where, r1 = diameter of fungal colony in control, r2 = diameter of fungal colony in dual inoculationInteractions were assayed by giving ranking accordingto Bell's ranking scale (Bell *et al.,* 1982) which is; R_1= complete overgrowth; R_2 = 75 % overgrowth; R_3 = 50% overgrowth; R_4 = growth inhibition at line of contact; R_5 = pathogen overgrowing antagonist.Microscopic examinations of hyphae from the interaction zone was also carried out to find out the events of hyphal interactions.

Table 1. Seed samples of Chickpea in Dry seed examination and incubation tests (SBM and PDA) in various districts of Rajasthan

Districts	No. of samples collected	No. of samples Studied		
		Dry seed examination	SBM	PDA
Ajmer	7	7	7	2
Alwar	13	13	13	7
Bharatpur	9	9	9	4
Jaipur	15	15	15	8
Sikar	5	5	5	2
Total	49	49	49	23

Table 2. Seed samples of Lentil in Dry seed examination and incubation tests (SBM and PDA) in various districts of Rajasthan

Districts	No. of samples collected	No. of samples Studied		
		Dry seed examination	SBM	PDA
Ajmer	7	7	7	3
Alwar	10	10	10	5
Bharatpur	4	4	4	4
Jaipur	9	9	9	4
Sikar	7	7	7	4
Total	37	37	37	20

Table 3. Seed samples of Black gram in Dry seed examination and incubation tests (SBM and PDA) in various districts of Rajasthan

Districts	No. of samples collected	No. of samples Studied		
		Dry seed examination	SBM	PDA
Ajmer	5	5	5	2
Alwar	10	10	10	6
Bharatpur	11	11	11	6
Jaipur	10	10	10	4
Sikar	13	13	13	3
Total	49	49	49	21

Table 4. Seed borne fungi isolated through blotter method

Fungi isolated	Chickpea				Lentil				Black Gram			
	US	R.P.O	SS	R.P.O	US	R.P.O	SS	R.P.O	US	R.P.O	SS	R.P.O
A.niger	+	62.85	+	49.32	+	54.36	-	-	+	47.58	+	3.57
A.flavus	+	30.35	+	7.14	+	21.65	-	-	+	58.69	+	7.14
A.fumigatus	-	-	-	-	+	50.23	+	19.56	-	-	-	-
A. alternata	+	32.14	+	14.28	-	-	-	-	+	11.35	-	-
Penicillium sp.	+	28.57	+	10.85	+	11.25	-	-	-	-	+	-
Chaetomium	+	26.92	+	7.98	+	26.78	+	3.29	-	-	-	-
F.oxysporum	+	48.51	-	-	-	-	-	-	-	-	-	-
Rhizopus sp.	+	61.86	+	19.64	+	21.78	+	6.21	+	40.23	+	8.92

US –unsterilized;SS- sterlised

Table 5. Seed borne fungi isolated through Agar-plate method

Fungi isolated	Chickpea				Lentil				Black gram			
	US	R.P.O	SS	R.P.O	US	R.P.O	SS	R.P.O	US	R.P.O	SS	R.P.O
A.niger	+	60.52	-	-	+	61.29	+	5.80	+	50.28	+	10.71
A.flavus	+	31.58	-	-	+	28.34	-	-	+	32.54	+	12.95
A.fumigatus	+	15.52	-	-	+	62.58	+	17.98	-	-	-	-
A. alternata	+	28.62	+	9.85	-	11.54	-	-	-	-	-	-
Penicillium sp.	+	18.23	-	-	-	-	-	-	+	13.22	-	-
Chaetomium sp.	+	16.98	-	-	-	-	-	-	-	21.58	+	4.29
F.oxysporum	+	50.25	+		-	-	-	-	-	22.12	-	-
Rhizopus sp.	+	65.25	+	28.62	+	24.25	-	-	+	50.21	+	11

US –unsterilized; SS- sterlised

Table 6: Antagonistic activity of *G.virens* against fungal pathogens by dual culture method

Pathogens	Colony growth in Control (mm)	Growth with *G.virens* (mm)	Growth Inhibition of pathogen (%)	Grade
A.niger	45	13.32	70.40	R_2
A.flavus	45	16.33	63.71	R_2
A.fumigatus	45	14.63	67.48	R_2
A. alternata	45	15.44	65.68	R_2
Penicillium sp.	45	11.22	75.06	R_1
Chaetomium sp.	45	13.66	69.64	R_2
F.oxysporum	45	19.33	57.04	R_3
Rhizopus sp.	45	12.77	71.62	R_2

Results

Among the samples studied the fungi were found as follows:- *A.niger, A.fumigatus, A.alternata A.flavus Chaetomium* spp. *Penicillium* spp. *Rhizopus* spp., *Fusarium oxysporum* (Table 4 and 5). Biological control of these seedborne plant pathogens is a potential alternative to the use of chemical pesticides, which have already been proved to be harmful to the environment. In the dual culture study here G. *virens* exhibited a strong antagonistic behavior against *Penicillium sp.*that is 75.06% (Table 6). At five days after incubation, G. *virens* showed 71.62% inhibition of radial growth for *Rhizopus* sp. as compared with 70.4% for *A. niger*. Within seven days, *G. virens* was found to be completely overgrown over the colonies of *Penicillium* sp., *Rhizopus* sp., *A. niger* and *Chaetomium* sp. On microscopic observation it was found that G. *virens* produced several short branches which coiled compactly around the hyphae of the pathogens causing them to become granulated and malformed.On the other hand lowest zone of inhibition was observed for *F. oxysporum* (57.04%) which concludes that there could be a correlation between ability of the antagonist to parasitize individual fungal pathogen in its own way.In order to analyze it in better way further testing should be done in field conditions.

Discussion

The present study provided a strong evidence that identification and biocontrol of the seed borne pathogens can be used in crop production for the prevention of disease caused by these seed-borne pathogens. Seed-borne diseases have been found to affect the growth and productivity of crop plants (Kubiak and Korbas, 1999; Weber *et al*., 2001; Dawson and Bateman, 2001). Contaminated seeds results in poor germination, poor seedling vigor, and un-healthy crop. Healthy seed is the foundation of healthy plant; a necessary condition for good yields (Diaz *et al*., 1998). In the above study significant numbers of fungi was isolated from the seed samples and were evaluated against *G. virens*, a potential biocontrol agent. It has been demonstrated that *G. virens* have significantly inhibited the radial growth of almost all the pathogens tested, when compared with the control.Such type of inhibitory effects have also been reported by Suarez *et al.* (2004) among others, and are a likely indicator that the antagonistic fungi produce some metabolite(s) that inhibit pathogen growth.

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