Research Article

[Agrawal et al., 2(7): July, 2011]

ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Isolation of seed borne mycoflora of chickpea and its *in vitro* evaluation by some known bioagents

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Abstract

Due to hazardous effect of chemical fungicides, search for safer alternative to control the pathogen is better choice. This led to trials on the use of bioagents to control the pathogen. In the present study *in vitro* antagonistic activity using the dual culture technique was undertaken to assess the potential of six *Trichoderma* species .It showed inhibitory effects on some common pathogens of chickpea namely *Aspergillus flavus*, *Aspergillus fumigatus.* The test antagonists grow faster than the pathogen and produced inhibition zones thereby limiting the growth of the pathogen. Maximum growth inhibition of *A. flavus* (74.53%), *A. fumigatus* (77.77%) were found with the use of *Trichoderma harzianum* (T2) in dual culture. In *in vitro* tests, all *Trichoderma* isolates showed significant differences in inhibiting the colony growth of the fungal pathogens.

Key-Words: Antagonistic activity, *Trichoderma harzianum*, pathogens

Introduction

Chickpea (*Cicer arietinum*.) is an important legume crops grown extensively in India. Mainly two types of chickpea are grown, brown seeded types called "Desi" and white seeded called "Kabuli". Chickpea after dehulling is valued for its nutritive seeds with high protein content (12.3-31.5%). Chickpea seed has 58.9% carbohydrate, 3% fiber, 5.2% oil, 3% ash, 0.2% calcium, and 0.3% phosphorus. Digestibility of protein varies from 76-78% and its carbohydrate from 57-60%. Among the food legumes, chickpea is the most nutritive pulse extensively used as protein adjunct to starchy diet[1]. Many fungal species viz., *Alternaria porri, A. alternata, Aspergillus amstelodami, A. flavus, A. fumigatus, A. nidulans, A. niger, A. sydowi*, *A.wentii, Botrytis cinerea, Cladosporium macrocarpum, Curvularia lunata, Fusarium equiseti, F. moniliforme, F. oxysporum, F. semitectum, Macrophomina phaseolina, Myrothecium roridum, Penicillium notatum, Rhizoctonia* sp., and *Rhizopus arrhizus* have been reported from chickpea[2]. These fungal diseases can kill chickpea crops and is difficult to remove once it sets in.

After the crop is established, fungicides are required early in the season to reduce early infection and disease spread, additional fungicide sprays will be required depending on the resistance of the variety and the frequency of rain during the growing season.

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This disease management is critical, and relies heavily on an integrated management package involving variety choice, fungicide management and crop hygiene. The hazardous effects of chemicals used in plant disease management have diverted plant pathologists to find out the alternative techniques of plant disease control which may cause little or no adverse effect on environment. Hence a biological treatment applied specifically to the seed surface could provide an effective alternative to fungicides. *Trichoderma* spp. are now the most common fungal biological control agents that have been comprehensively researched and deployed throughout the world. Several fungal cell wall degrading enzymes, amongst them chitinase and glucanase, which seem to play an important role in the antagonistic action of *Trichoderma* against a wide range of fungal plant pathogens[3]. Antagonism of *Trichoderma* species against several pathogens has been reported by several workers[4-9]. The present study aimed to find out the efficiency of *Trichoderma* species against some seed borne pathogens isolated from Chickpea.

Material and Methods

Isolation of mycoflora

In present research study, detection of seed mycoflora associated with the seed samples was done (ISTA, 1996). 10 seeds per pre-sterilized petri-plates were equispaced aseptically on the plates having three layers of blotter paper. The plates were then allowed to

incubate at room temperature. Detail observations of fungal characters were done under the binocular microscope and their identification was confirmed with standard literature[10-11]. Two fungi namely *Aspergillus flavus* and *Aspergillus fumigatus* were isolated from Chickpea by Agar plate methods on Potato Dextrose medium.

Antagonistic effects of *Trichoderma* against isolated fungi

Antagonism of *Trichoderma viride* on post harvest pathogens was studied by dual culture technique[12]. A mycelial disc (5mm) obtained from the peripheral region of 5-7-day-old cultures of *Aspergillus flavus and Aspergillus fumigatus* on PDA, was placed on a fresh PDA plate (3 cm from the center) and then a 5mm-mycelial disc, obtained from the periphery of a 5-7-day-old culture of *Trichoderma* species was placed 3 cm away from the inoculum of the pathogen, the plates were incubated at 28˚C and measurements were taken after 7 days. In the control experiment a sterile agar disc was placed in the dish. At the end of the incubation period, radial growth was measured. The percentage inhibition growth of tested pathogens in presence of *Trichoderma* strains was calculated over control. The growth inhibition was calculated by using the formula:

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

Where, r1 = diameter of fungal colony in control, r2 = diameter of fungal colony in dual inoculation

Interactions were assayed by giving ranking according to Bell's ranking scale [5] which is ; R1= complete overgrowth ;R2 = 75 % overgrowth ; R3 = 50% overgrowth ; R4 = growth inhibition at line of contact; R5 = pathogen overgrowing antagonist.Microscopic examinations of hyphae from the interaction zone was also carried out to find out the events of hyphal interactions.

Results and Conclusion

The variety of chickpea used here showed the incidence of seed-born fungi. Among the other pathogens two fungi were purely recorded. Such type of work was earlier supported by several workers [13-15]. Result showed that *Trichoderma spp.* could restrict growth of pathogens on Potato Dextrose Agar medium in the dual culture (Table 1 and Table 2).

The per cent inhibition of radial growth of tested A. *flavus* (74.53%), *A. fumigatus* (77.77%) were reduced by *T. harzianum*(T2). *T.harzianum* (T2) showed visible inhibition zone. Previous studies have demonstrated that before mycelia of fungi interact, *Trichoderma* sp. produces low quantities of extracellular exochitinases [16-17]. The growth inhibition of pathogenic fungi by dual culture in this study could be due to its fast growing nature, secretions of harmful extra-cellular compounds like antibiotics, cell wall degrading enzymes such as gluconases, endochitinases and chitinases and mycoparasitism in dual culture as found with other fungi[18-20]. Interaction with *T.harzianum* (T1, T7) and *Trichoderma viride* (T.v) growth was inhibited at the line of contact. *T.harzianum* (T8) did not produce any demarcation zone.

Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods [21]. In the present study a significant decrease of both growth and conidia production of the pathogen was obtained compared to the control. The highest percentages of diameter colony reduction and conidial production were obtained with T2, causing 77.77% reduction in colony diameter. Microscopic examination at the point of contact of two fungi revealed that the overgrowing mycelium of the antagonist penetrated the mycelium of the pathogen and the tip of the pathogen swelled and curled. Thus, it is well known that all isolates collected from different samples of soil are not equally antagonistic to pathogen and searching of effective isolate to locally suit the purpose is important.

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ISSN: 0976-7126

Table 1: Antagonistic activity of *Trichoderma spp.* against *A.flavus* by dual culture method

S/No.	Test Mycoflora	Colony growth (mm)of *A.flavus*		Growth Inhibition *A.flavus* (%)	Grade
		Control (mm)	Interaction (mm)		
1	*Trichoderma harzianum(T_1)*	45	13.33	70.37	R2
2	*Trichoderma harzianum(T_2)*	45	11.46	74.53	R1
3	*Trichoderma harzianum(T_3)*	45	13	71.11	R2
4	*Trichoderma harzianum (T_7)*	45	10.22	77.28	E1
5	*Trichoderma harzianum (T_8)*	45	17.46	61	R2
6	*Trichoderma viride (T.V.)*	45	19	57.77	R3

Table 2: Antagonistic activity of *Trichoderma spp.* against *A.flavus* by dual culture method

S/No.	Test Mycoflora	Colony growth (mm)of *A.fumigatus*		Growth Inhibition *A.fumigatus* (%)	Grade
		Control (mm)	Interaction (mm)		
1	*Trichoderma harzianum(T_1)*	45	18.66	58.53	R3
2	*Trichoderma harzianum(T_2)*	45	10	77.77	R1
3	*Trichoderma harzianum(T_3)*	45	16	64.44	R2
4	*Trichoderma harzianum (T_7)*	45	13	71.11	R2
5	*Trichoderma harzianum (T_8)*	45	30.33	32.6	R4
6	*Trichoderma viride (T.V.)*	45	29	35.55	R4