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OCCURRENCE OF SOIL-BORNE PHYTOPATHOGENS IN CHICKPEA CROP AND THEIR BIOCONTROL MANAGEMENT

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ABSTRACT

Soil borne fungi, *Fusarium oxysporum, Macrophomina phaseolina* and *Rhizoctonia solani* causes soil-borne root-rot disease in different chickpea varieties and cause heavy losses annually due to rapidly increased by many factors such as the presence of moisture, access irrigation and rainfall. We isolated fourteen soil-borne fungi *Aspergillus flavus, A. niger, A. parasitica, A. terreus, Cladosporium cladosporioides, Curvularia lunata, Fusarium chlamydosporum, F. solani, F. oxysporum, Histoplasma capsulatum, Macrophomina phaseolina, Nigrospora sphaerica, Penicillium commune* and *Rhizoctonia solani* from three different soil samples including grass growing area, *Catharanthus roseus* growing area and *Aloe vera* growing area. Among all isolates, *A. flavus, N. sphaerica* and *C. cladosporioides* were found to be dominant species that were present in all soil samples. In Pathogenicity test, *F. oxysporum* and *R. solani* showed maximum (%) disease intensity in sterilized soil as compared to unsterilized soil. However, the infection (%) of *R. solani* and *M. phaseolina* were maximum (%) in both sterilized and unsterilized soil. In antagonistic test, all isolates inhibited the growth of *F. oxysporum, M. phaseolina* and *R. solani.* Among these isolates, *P. commune* was found to be the most potent antagonist inhibiting the growth of all fungi. In contrast treatments were less effective against *M. phaseolina* as compared to the antagonists used in *F. oxysporum* and *R. solani.*

Keywords: Chickpea, Pathogenicity test, Antagonism test, Soil-borne fungi.

INTRODUCTION

The chickpeas are essential food legumes that are cultivated worldwide. Because of their wide-spread cultivation chickpea crops are exposed to many fungal, viral, bacterial and other plant diseases (Harveson, 2011; Namvar and Sharifi, 2011; Arvayo-Ortiz *et al.,* 2012; Hameed *et al.*, 2012; Merkuz and Getachew, 2012; Moradi *et al.,* 2012). The wilt disease caused by fungi *Fusarium* sp. can result in substantial loss of produce (Khan *et al*., 2002; Dubey *et al*., 2007). General symptoms of chickpea wilt include drooping, yellowing, drying of the leaves and discoloration of vascular system. *R solani* alone can cause wet root rot disease (Singh, 2005), but its occurrence with *F. oxysporum* f. ssp. *ciceri* has been observed quite frequently (Blazier and Conway, 2004; De Curtis *et al*., 2010; Andrabi *et al.,* 2011). In addition, *M. phaseolina* may induce a wilting disease in chickpea plants grown under dryland conditions, but only where plants receive less than adequate water (Westerlund *et al.,* 1974). A number of management practices such as, development of resistance varieties, application of fungicides, biological practices and combination of approaches are employed to control wilt diseases of plant pathogens (Pawar *et al.,* 1985; Negron *et al.,* 1991 and Dhruj *et al*., 2000). Among these management practices chemical and the biological control have gained serious attention (Mahmood *et al.,* 2005). In recent years, management of plant diseases by biological controls has gained significant popularity (Mahmood and Khan, 2009). Biological control is one of the best low-cost and ecologically sustainable methods for managing plant diseases caused by soil-borne pathogens, *Fusarium* sp, *R. solani* and *Pythium* sp. (Dhruj *et al*., 2000; Mahmood and Khan, 2009).

In this study, we surveyed and identified various soil-borne and root fungi from field soil and investigated the antagonistic potential among them identifying those fungi which showed significant antagonistic activity and used them to investigate their potential for the control of important soil-borne fungal disease of chickpea crop.

MATERIALS AND METHODS

Site selection and collection of soil samples

Three different soil samples including grass growing (*Lolium perenne*) area, *Catharanthus roseus* growing area and *Aloe vera* growing area were collected from different sites of Federal Urdu University of Arts, Science & Technology, Karachi. Approximately, 200 g soil samples at the depth of 1.5 to 3 inches were collected. The samples

were brought to the Dr. A.G. Laboratory of Aerobiology & Plant Pathology, FUUAST, Karachi and stored at 5 °C until needed.

Soil dilution Technique

One gram of soil was suspended in 9 mL of sterilized distilled water then we made serial dilutions of 1:100, 1:000 and 1:0000 from this stock solution. One mL aliquot sample was poured in sterilized Petri plates containing Potato Dextrose Agar (PDA). There were three replicates per sample. The Petri dishes were kept in incubator at 28 ± 2 °C. The fungal colonies that developed on the plates were counted (Singh *et al.,* 1991).

Germination Test

Chickpea seed germination test were performed in laboratory condition. In this test, we used five autoclaved sterilized Petri plates containing a double layered wet Whattman No. 1 filter paper. Then chickpea seeds were surface sterilized in 2% sodium hypochlorite solution for 1 minute. Seeds were washed with sterilized distilled water and blotted with filter paper. Then 5 seeds were placed in each Petri plates. Seeds were kept moist by adding water to the Petri plates. Seed germination rates were observed on regular basis (Khandakar and Bradbeer, 1983).

Pathogenicity Test

Pathogenicity test of *F. oxysporum, M. phaseolina* and *R. solani* were separately carried out in screen house. Seedlings (~ 12 cm in length) were planted in pots containing 250 g sterilized soil/fertilizer mixture (manure) (2:1 ratio). Spore suspensions of fungi including *F. oxysporum, R. solani* and *M. phaseolina* were prepared spore suspension containing 100 spores/mL by the help of haemocytometer. Then a suspension of 200 spores was inoculated in each pot. The experiment was conducted in the screen house at the Department of Botany, Federal Urdu University of Art, Science and Technology, Karachi. Two sets of experimental pots; one set s containing sterilized and the other set of pots containing unsterilized soil were separately kept on screen house bench for 20 days. Pathogenicity was observed after inoculation of fungal pathogens.

Antagonistic Test

Antagonistic fungi - *A. flavus, A. niger, A. parasitica, A. terreus, C. cladosporioides, C. lunata, F. chlamydosporum, F. solani, H. capsulatum, N. sphaerica* and *P. commune* were obtained from the rhizospheres soil of the samples. These fungi were multiplied on PDA for 5-6 days at 28°C± 30°C. Pathogenic fungi were isolated from roots of test plants (chickpea) using Potato Dextrose Agar (PDA). Petri plates containing PDA were inoculated with the pathogenic fungi (Odigie and Ikotun, 1982). Antagonist and pathogenic fungi were placed at the opposite ends. Each Petri dish contains 20 ml PDA. Three Petri dishes were used for replication of each antagonist and the same numbers were was also kept as control that contained fungal pathogen only The fungal inoculated Petri dishes were kept in incubator at 30°C for 6 days and its growth was recorded.

Dual culture Technique

The growth and inhibition of pathogen against the antagonist were performed on PDA media by using dual culture technique. In brief, 5-mm diameter of fungal mycelia plugs of each test antagonist were inoculated at the edge of three different culture plates and placed in incubator for 2 days at 30 ± 2°C (Event *et al*., 2003; Holmes *et al*., 2004). After two days each plate was -inoculated with another 5-mm diameter mycelial plug of the fungal pathogen were placed 5 cm from the test antagonist. The dual culture plates were incubated for additional 9 days at 30 ±2°C. . The growth of the pathogen in both the test and control experiments was recorded.

Identification of fungi

Isolated fungi were identified using standard literature (Ellis 1971; 1976; Barnett and Hunter 1972; Domsch *et al*., 1980).

Analysis of Data

Data were subjected to Analysis of variance (ANOVA). The follow up of ANOVA included least significant difference (LSD), Duncan's multiple range test was used to compare the treatment means.

RESULTS

Fungi isolated from soil

Fourteen fungal species, *Aspergillus flavus, A. niger, A. parasitica, A. terreus, Cladosporium cladosporioides, Curvularia lunata, Fusarium chlamydosporum, F. solani, F. oxysporum, Histoplasma capsulatum, Macrophomina phaseolina, Nigrospora sphaerica, Penicillium commune* and *Rhizoctonia solani* from soil samples collected from three different localities of Federal Urdu University, Karachi. Among these isolate, *N. sphaerica, A. flavus* and *C. cladosporioides* were the dominant species with mean values of 82, 78 and 66% respectively over the other species such as *A. niger, A. parasitica, A. terreus, C. lunata, F. chlamydosporum, F. solani, H. capsulatum,* and *P. commune*. We also found the highest occurrence of these three fungi (*N. sphaerica, A. flavus* and *C. cladosporioides*) in samples that were collected from grass growing area (40%) but the minimum (37.72%) occurrence in *Aloe vera* growing area (Fig 1). The results of ANOVA for the fungal occurrence % on soil samples were collected from three localities. Ten fungal species *A. flavus* (F=22.34), *A. niger* (F=3.17), *A. parasitia* (F=13.16), *A. terreus* (F=4.40), *F. chlamydosporum* (F=37.38), *F. solani* (F=24.99), *H. capsulatum* (F=13.89), *N. sphaerica* (F=15.35), *C. cladosporioides* (F=53.89) and *C. lunata* (F=15.13) showed most significant differences (P<0.001) among localities. However, only *P. commune* showed non-significant difference (F=0.70, P<0.06) as compared to other species.

Germination Percentage Test

The germination % of chickpea was maximum (92%) in the 6th day of incubation. However, 39 % germination was observed after 2nd day (Fig. 2).

Pathogenicity Test

All fungal species used in the experiment were found to be pathogenic. *F. oxysporum* and *R. solani* showed the highest 22.4 and 22.17% disease intensity against fungi *A. flavus* and *N. sphaerica* in Chickpea plant in sterilized soil as compared to other treated fungi. However, the infection (%) caused by *R. solani* and *M. phaseolina* were maximum 26.57 and 24.4% against *A. flavus* in chickpea plants in both sterilized (17.95 to 22.4%) and unsterilized soil (18.4 to 26.57%) (Fig. 3). We also found *F. oyxsporum, M. phaseolina* and *R. solani* showing highly significant differences regarding pathogenicity (F=21.08, P<0.001). There was a significant difference in pathogenicity of chickpea in sterilized and unsterilized soils (F=2.561, P < 0.06). However, the interaction of soil-borne fungi × isolated fungi, soil-borne fungi × sterilized and unsterilized soil, isolated fungi × sterilized and unsterilized soil and soil-borne fungi × isolated fungi × sterilized and unsterilized were found to be non-significant. The infection of tested fungi against soil-borne pathogens was observed after the pathogenicity in sterilized and unsterilized soil (Fig. 3). The highest infection percentage in sterilized soil was 98% from the isolates *A. flavus*, *F. chlamydosporum* and *N. sphaerica* as compared to other tested species. However, minimum infection percentage was recorded 66.67% in *A. terreus*. Highest colonization percentages (89.68%) of in sterilized soil were recorded in *P. commune* and 87.78% in *H. capsulatum* and the, minimum 62.7% colonization percentage was recorded in *A. parasitica*.

Antagonistic Test

Fungi isolated from soil were tested for their antagonistic properties against common soil-borne plant pathogenic fungi (*F. oxysporum*, *M. phaseolina* and *R. solani*) *in vitro* experiment. The interaction was determined by the growth of the two interacting microorganisms. The colony diameter of the antagonist towards the pathogen was recorded. The colony diameter of the pathogen alone (control) and in combination (dual culture) were measured. Percentage decrease over the control was calculated by the following formula:

$$\text{Percent decrease over control} = \frac{\text{Average colony diameter} - \text{Average colony diameter of the pathogen against the antagonist in the treatment}}{\text{Average colony diameter of the pathogen in the treatment}}$$

We recorded the pathogens growth and development after six days of incubation. We found that *F. oxysporum* inhibited the growth of *A. flavus* and *A. terreus* with mean percentage of 4.23 and 3.37% respectively. However, the effect of *F. chlamydosporum* and *A. flavus* was the maximum with the mean percentage of 2.43% and 1.91% against the growth of *Macrophomina phaseolina* as compared to other species of fungi. During this test, *A. parasitica, A. flavus* and *N. sphaerica* was relatively more effective in inhibiting the growth of selected soil-borne pathogens by 2.57, 2.02 and 2.02% against *R. solani* as compared to other fungi. All eleven isolated fungal species showed significant differences (F=4.22, P<0.001) and their growth and development was inhibited by soil-borne fungi *F. oxysporum*, *M. phaseolina* and *R. solani*. All isolated antagonistic fungi inhibited the growth of *F. oxysporum, M. phaseolina* and *R. solani* (Fig. 4). Among these isolates, *P. commune* resulted as effective antagonist inhibiting the

growth of pathogenic fungi by 89%, while minimum growth percentage of the pathogen was 44.2%. In contrast, treatments were less effective against *M. phaseolina* as compared to the other antagonists. We found a little antagonistic effect against *M. phaseolina* which inhibited the mycelia growth of the pathogen by only 44.2%. All the isolated fungi inhibited the growth of *F. oxysporum* and *R. solani* used in the experiment (Fig. 4).

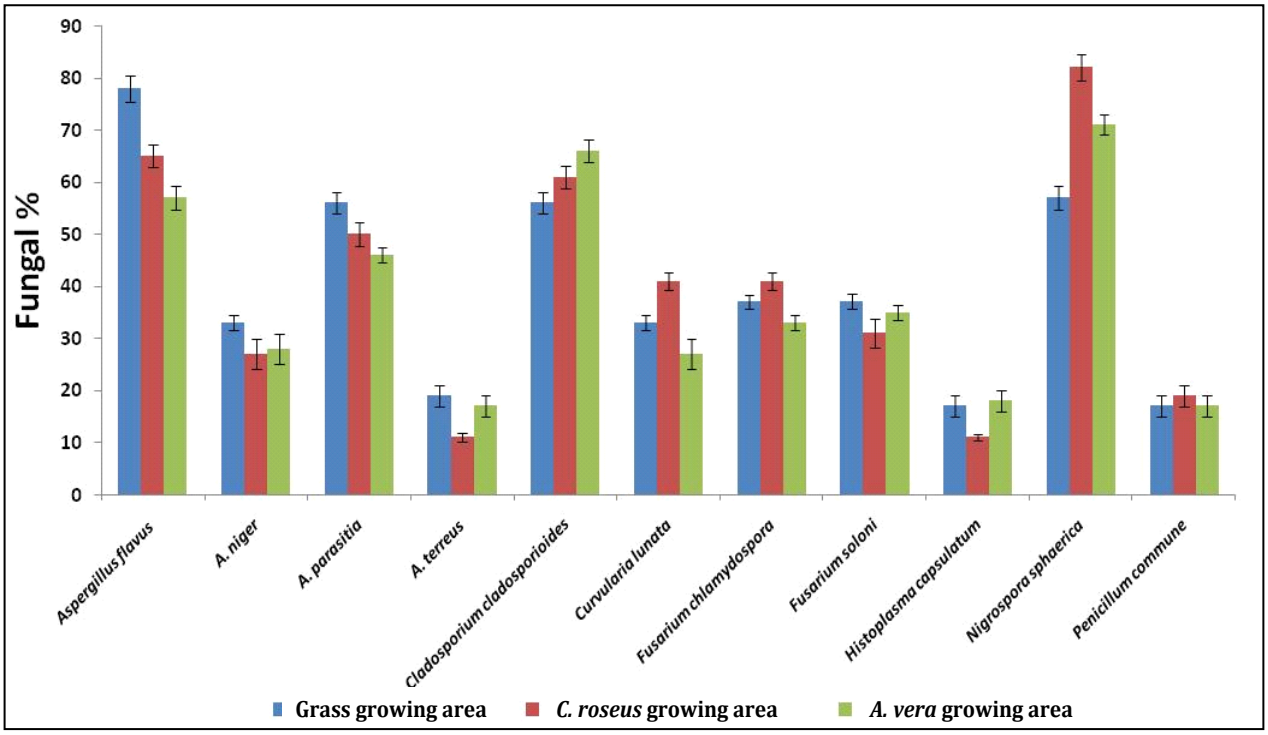

Fig.1. Occurrence (%) of different fungi isolated from three different soil samples.

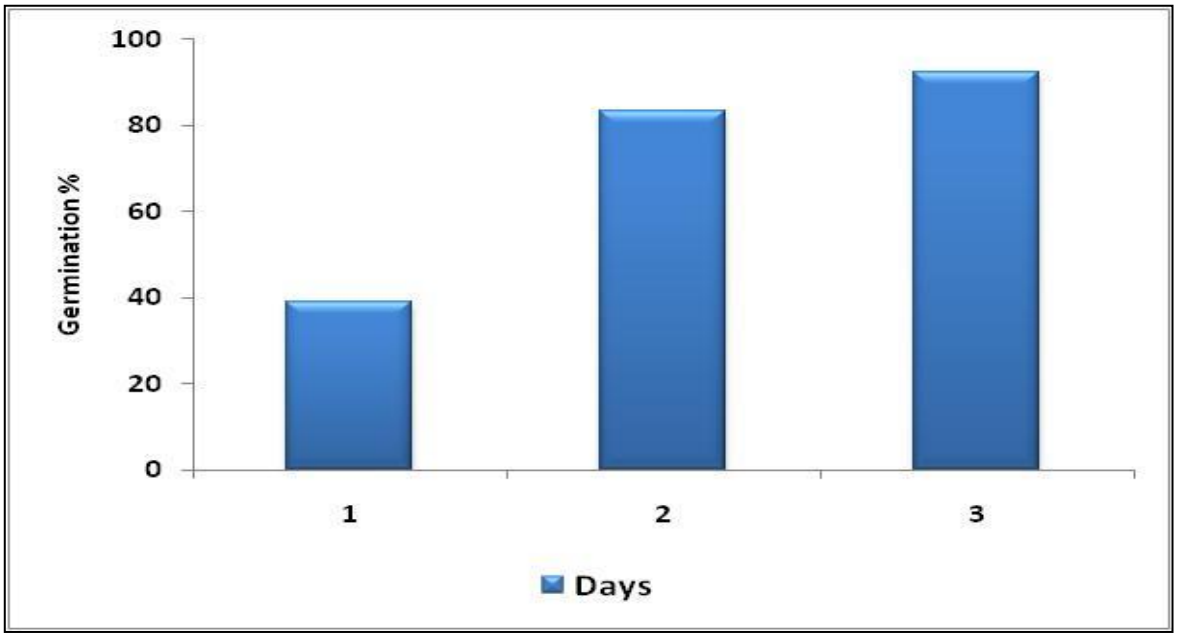

Fig. 2. Germination (%) of chickpea seeds.

Table 1. Mean and Standard Error of disease intensity of soil-borne pathogens after different antagonistic fungal treatments**.**

Antagonistic Fungi	Soil-borne pathogens	Disease intensity	
		Sterilized soil	Unsterilized soil
	Control	0.00	0.00
Aspergillus flavus	Soil+ *F. oxysporum*	22.4 ± 3.60	18.4 ± 4.60
	Soil+ *M. phaseolina*	19.55 ± 3.78	24.4 ± 6.60
	Soil+ *R. solani*	17.95 ± 2.05	26.57 ± 1.43
A. niger	Soil+ *F. oxysporum*	15.17 ± 0.50	9.72 ± 2.62
	Soil+ *M. phaseolina*	10.88 ± 1.55	16.02 ± 3.98
	Soil+ *R. solani*	13.22 ± 0.88	22.83 ± 5.50
A. parasitia	Soil+ *F. oxysporum*	14.57 ± 1.23	18.23 ± 0.23
	Soil+ *M. phaseolina*	11.87 ± 0.13	21.67 ± 0.33
	Soil+ *R. solani*	14.57 ± 1.77	17.47 ± 0.80
A. terreus	Soil+ *F. oxysporum*	18 ± 6	19.92 ± 4.75
	Soil+ *M. phaseolina*	14.65 ± 0.02	15.12 ± 3.55
	Soil+ *R. solani*	13.42 ± 0.42	10.03 ± 0.97
Cladosporium cladosporioides	Soil+ *F. oxysporum*	12.5 ± 1.17	19.53 ± 3.13
	Soil+ *M. phaseolina*	10.43 ± 1.57	18 ± 2
	Soil+ *R. solani*	12.9 ± 1.10	22.63 ± 3.03
Curvularia lunata	Soil+ *F. oxysporum*	9.25 ± 0.42	19.63 ± 4.37
	Soil+ *M. phaseolina*	14.75 ± 0.25	21.95 ± 7.05
	Soil+ *R. solani*	9.25 ± 0.75	27.28 ± 8.38
Fusarium chlamydospora	Soil+ *F. oxysporum*	7.73 ± 0.60	21.52 ± 0.82
	Soil+ *M. phaseolina*	13.17 ± 0.50	16.18 ± 2.18
	Soil+ *R. solani*	12.13 ± 1.13	14.2 ± 0.13
F. soloni	Soil+ *F. oxysporum*	15.78 ± 0.22	22.08 ± 4.58
	Soil+ *M. phaseolina*	17.98 ± 1.68	18.012 ± 5.32
	Soil+ *R. solani*	16.43 ± 0.23	21.08 ± 7.25
Histoplasma capsulatum	Soil+ *F. oxysporum*	11.5 ± 0.50	13.62 ± 2.38
	Soil+ *M. phaseolina*	11.17 ± 0.17	10.82 ± 1.52
	Soil+ *R. solani*	7.53 ± 0.47	11.47 ± 0.53
Nigrospora sphaerica	Soil+ *F. oxysporum*	17.77 ± 1.23	8.48 ± 1.48
	Soil+ *M. phaseolina*	16.67 ± 2.67	9.65 ± 2.35
	Soil+ *R. solani*	22.17 ± 0.83	21.12 ± 3.55
Penicillium commune	Soil+ *F. oxysporum*	10.65 ± 0.68	19.67 ± 3.33
	Soil+ *M. phaseolina*	11.67 ± 0.67	10.83 ± 1.17
	Soil+ *R. solani*	12.98 ± 0.65	22.83 ± 6.83

DISCUSSION

Soil-borne diseases are one of the most important factors limiting the productivity of vegetables in Pakistan (Hussain *et al.,* 2013a; Usman *et al.,* 2014). Hafiz (1986) reported that pathogenicity test of *Fusarium* sp. (root rot fungi) found brown discoloration of roots near soil line. Whereas Ghaffar (1988) observed severe colonization of cortical tissues of infected plants by root rot fungi. Hussain *et al.* (2013b) reported that *R. solani* and *Pythium* sp. were cause of root-rot, wilt symptoms, stunted seedling and reduction in growth of chilli plant. Some of the microorganisms such as *Penicillium* spp, and *Aspergillus* spp. also have been used to control the chickpea diseases (Kaiser and Hannan, 1984; Haral and Konde, 1986; Parakhia and Vaishnav, 1986). These findings with similar and confirm the results of Attia *et al.* (2003), Hussain *et al.* (2013a) and Usman *et al.* (2014).

In Pathogenicity test, *F. oxysporum* and *R. solani* showed high disease intensity against different fungi. However, the percent infection of *R. solani* and *M. phaseolina* were maximum (%) against *A. flavus* in both sterilized and unsterilized soil. However, %germination was maximum (92%) in the 6th day of planting. but it was

39 % after 2nd day of planting. Haral and Konde (1986) determined that culture filtrates of a *Bacillus subtilis* str. reduced the mycelial weight of the *F. oxysporum* f. sp. *ciceri* and *R. solani*. Parakhia and Vaishnav (1986) reported that when chickpea seeds were treated with *Trichoderma harzianum* before sowing in pots inoculated with *R. bataticola* (*M. phaseolina*), infection was reduced up to 18%. *R. solani* and *Fusarium* spp, isolated from chilli were found to cause damping off, root rot and wilt diseases (Hussain *et al.*, 2013b). These results confirm those reported by Attia *et al.* (2003) and Hussain *et al.* (2013b).

Table. 2 Inhibition percentages of isolated fungi against soil-borne pathogens

Fungi	Inhibition (%)		
	Fusarium oxysporum	***Macrophomina phaseolina***	***Rhizoctonia solani***
A. flavus	64	78.78	77.56
A. niger	75.56	47.78	79.78
A. parasitia	75.11	84.44	71.44
A. terreus	70.78	84.89	81.89
C. cladosporioides	82	90.33	87.89
C. lunata	72.56	78.89	83.44
F. chlamydospora	72.67	79.67	82.22
F. soloni	75.11	73	82.22
H. capsulatum	81.33	89.44	83.44
N. sphaerica	75.33	78.78	77.56
P. commune	85.22	93.33	90.44

In present study, we investigated the antagonism between fungal species. We found *A. flavus* and *A. terreus* be effective antagonists inhibiting the growth of *F. oxysporum* as compared to other isolated fungi. However, the growth inhibiting effect of *F. chlamydosporum* and *A. flavus* were the maximum (%) on *M. phaseolina* as compared to other fungi. We separately examined the growth inhibiting effect of *R. solani* on different isolated fungi after six days of incubation. During this test, *A. parasitica*, *A. flavus* and *N. sphaerica* was relatively found to be more effective in inhibiting the growth of *R. solani* as compared to other fungi. Biological control of plant pathogen by microorganisms has been considered more natural and environmentally acceptable alternative to the existing chemical methods (Baker and Paulitz, 1996). There have been considerable successes in utilizing antagonistic microorganism to control both pre-harvested and post-harvested diseases (Janisiewicz and Korsten, 2002). Use of antagonistic fungi against *Macrophomina* root rot has been well documented in several crops (Mukhopadhyay, 1987; Raguchander *et al*., 1995). Different isolates of bacteria and fungi displayed antagonistic activity against *M. phaseolina*, which showed growth inhibition in dual plate method (Meziane *et al.* 2005). Usman *et al.* (2013) and Hussain *et al.* (2013b) reported that several fungi including *Aspergillus* spp. *Penicillium* spp. and *Trichoderma* spp. can be utilized for the inhibition of *F. oxysporum, R. solani* and *M. phaseolina*. We found *A. flavus* and *P. commune* as most effective antagonists inhibiting the growth of the pathogens. *A. niger*, the antagonist also showed inhibitory activity against several pathogenic fungi. It is also reported that *A. niger* and *A. terreus* etc. were found to be antagonistic fungi against *R. solani* by inhibiting the growth of several fungi when tested under *in vitro*. We show that t some *Aspergillus* sp. have the activity of inhibition and effectively reduced the growth of *F. oxysporum*, *M. phaseolina* and *R. solani* mycelium and sclerotial bodies. The antagonistic activity of *Trichoderma* sp. has been elaborated by Howell (2003). Our results also confirmed the findings of Gokulapalan and Nair (1984); Gogoi and Roy (1993); Zhang and Wu (2011); Usman *et al.* (2013) and Hussain *et al.* (2013b).

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