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PATHOGENICITY OF SOME IMPORTANT ROOT ROT FUNGI TO THE CHILLI CROP AND THEIR BIOLOGICAL CONTROL

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ABSTRACT

A number of soil borne fungi are reported to cause diseases in chilli crop in Pakistan and induce heavy annual losses. During our survey conducted during July 2010 to August 2012 it was observed that in the chilli plants of lower areas of Sindh Province including Kunri, Kot Ghulam Muhammad, Mirpurkhas, Hyderabad, Tando Allahayar, Samaro, Umerkot and Digri show pathogenicity symptoms including wilting, stunted growth, chlorosis and blotch. Diseased plant specimens were collected and brought to laboratory. The soil borne fungi *Rhizoctonia solani*, *Macrophomina phaseolina*, *Fusarium oxysporum, F. solani* and *Pythium* sp. were isolated from specimens collected from these areas. Pathogenicity tests were carried out under greenhouse conditions using isolated fungi on chilli and colonization, and infection percentages were determined. During this study, *Pythium* sp., and *R. solani* severely affected plants compared to other fungi tested for their pathogenicity such as *F. oxysporum, F. solani* and *M. phaseolina*. In biological control, four antagonistic fungi *Aspergillus flavus, A. niger, Penicillium commune* and *Trichoderma harzianum* were used against the above mentioned plant pathogenic fungi which successfully suppressed the activity of pathogenic fungi. In addition, it disclosed that *T. harzianum* was highly antagonistic towards *R. solani, M. phaseolina, F. oxysporum, F. solani* and *Pythium* spp. as it showed a strong inhibitory effect on the growth and mycelial development.

Key-words:

INTRODUCTION

Chilli is affected by a number of plant pathogens including fungi which cause root and crown rot. These pathogenic fungi are recorded in almost all chilli growing areas of the world. In Pakistan, several plant parasitic pathogens which cause root rot and fungi including *Fusarium* spp. produce wilt, root rot while powdery mildew is caused by the fungus *Leveillula taurica*, damping off and wilting of seedling caused by *Pythium aphanidermatum* and root rot and wilting of chilli plant caused by *Macrophomina phaseolina* (Hafeez, 1986; Saleem *et al*., 1996; Mushtaq; Hashmi, 1997; Hussain and Abid, 2011). *Rhizoctonia* root rot caused by *R. solani* generally affects seedlings, but *R. solani* can also infect mature plants and induce root rot, which leads to wilting and death of chili plants. To date, there are no commercially acceptable chili cultivars that are resistant to *R. solani* (Muhyi and Bosland, 1992).

Isolates of *M. phaseolina* obtained from different plant species differed in cultural and morphological characters and pathogenicity (Dhingra and Sinclair, 1973). *Macrophomina* is a monotypic genus and efforts to divide *M. phaseolina* into sub-species were unsuccessful, based on the morphology and pathogenicty, there were extremely intraspecified variations (Dhingra and Sinclair, 1972; Echavez-Badel and Perdomo, 1991). The significant differences of morphological (Mayek-Perez *et al*., 2001), physiological (Mihali and Taylor, 1995), pathogenic (Mayek-Perez *et al*., 2001; Su *et al*., 2001) and genetic (Vandemark *et al*., 2000; Mayek-Perez *et al*., 2001; Su *et al*., 2001; Alvaro *et al*., 2003; Jana *et al*., 2003; Aboshosha *et al*., 2007) diversity have been reported. Many researchers have also found great variability in pathogenicity and morphology among isolates from the same host. That is affirmed that during the hyphal fusion, heterokaryosis could occur after mitotic segregation and recombination (Sinclair and Backman, 1986).

It is now widely recognized that biological control of plant pathogens using antagonistic fungi is a distinct possibility for the future and can be successfully utilized especially within the framework of integrated disease management system (Muthamilan and Jeyarajan, 1996). Use of antagonistic organisms against *Macrophomina* root rot has been well documented in several crops (Mukhopadhyay, 1987; Raguchander *et al*., 1995). Biological control offers an environmentally friendly approach to the management of plant disease and can be incorporated into cultural and physical controls and limited chemical usage for an effective integrated pest management (IPM) system (Monte, 2001). *Trichoderma* species have been extensively studied as biological control agents against fungal pathogens (Chet, 1990; Chet *et al*., 1998; Howell, 1998; Siddiqui *et al.,* 2001). In particular, *T. harzianum* has been demonstrated to be a very effective biocontrol agent (Zeilinger *et al.*, 1999; Siddiqui and Shaukat, 2004).

Several *Trichoderma* species reduce the incidence of soil borne plant pathogenic fungi under natural conditions (Sivan and Chet, 1986; Calvet *et al*., 1990; Spiegel and Chet, 1998; Elad, 2000); nevertheless the effectiveness of this depends mainly on the physical, chemical and biological conditions of the soil. Antagonistic interactions have been recognized as one of the excellent mechanisms for biological control of pathogenic fungi (Khara and Hadwan, 1990). *Trichoderma harzianum* is an efficient bio-controlling agent commercially produced to thwart the development of several soil born pathogenic fungi (Shalini *et al.,* 2006).

The objectives of the present investigation were 1) to test the pathogenicity of some important root fungi to the chilli plant and 2) to investigate the biocontrol potential of some of the organisms, in particular *Trichoderma harzianum*.

MATERIALS AND METHODS:

Isolation of Pathogens:

The infected plants with roots were collected from the lower region of Sindh province including Mirpurkhas, Umerkot, Kunri, Samaro and Kot Ghulam Muhammad during the period of July 2010 to August 2012. These roots were placed in Petri dishes which contained Potato Dextrose agar medium and Czapek's agar medium after surface sterilization by 1 % Ca $(OCI)_2$ for 1 min. These Petri dishes were further incubated for 5 days at 28°C for the isolation of fungi.

Identification of fungi:

Isolated fungi were identified using the standard manuals or refernces including Ellis (1971;1976), Barnett and Hunter (1972), Nelson *et al.* (1983), Singh *et al*., (1991) and Sutton (1980).

Pathogenicity test:

The pathogenicity tests were conducted at the Department of Botany, Federal Urdu University of Art, Science and Technology Karachi in the greenhouse. Pathogenicity tests of *Rhizoctonia solani*, *Macrophomina phaseolina*, *Fusarium oxysporum, F. solani* and *Pythium* spp. were carried out separately in screen house using isolated fungi. Colonization and infection % were recorded after every five days. For pathogenicity test, seedlings (having average 10 cm in length) were taken and planted in pots containing 400g of sterilized and unsterilized soil/fertilizer mixture (2:1 ratio) separately. Pots were kept in two sets containing sterilized (Fig.1) and unsterilized soil (Fig.2) separately for 15 days. Expressions of pathogenicity were observed after inoculation of different fungi (Table 1). The spore suspension of different fungi including *Rhizoctonia solani*, *M. phaseolina*, *F. oxysporum, F. solani* and *Pythium* sp. were prepared in the laboratory. The spore suspension containing 100 spores/ ml were prepared and inoculated in pots. Hundred ml of a fungal spore suspension was applied by pouring suspension in three holes made around the plant in each pot.

Antagonism test:

In order to select some suitable antagonistic micro-organism against the pathogens were evaluated in the laboratory on PDA. Both antagonist and the pathogen were simultaneously inoculated at the opposite ends of the Petri dishes containing about 15 to 20ml PDA. Three Petri dishes were used for each antagonist and the same number was kept as control with the pathogen alone plated on one side of the Petri dish at the periphery. The inoculated Petri dishes were incubated at 30°C for 6 days and the final observations were recorded. The interaction was determined by the growth of the two interacting microorganisms. The colony diameter of the antagonist toward 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:

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

Some of the antagonists caused inhibitions at a noticeable distance whereas the others inhibited the pathogen on contact and continued to grow over the inhibited colony.

Preparation of inocula of Antagonistic fungi:

Native potential fungal antagonists were isolated from chilli rhizosphere and rhizoplane in chilli crop fields. The affected leaves, stems and roots of chilli plants were collected and surface-sterilized with 1% sodium hypochlorite. Isolations were made from these chilli plant parts in Potato Dextrose agar (PDA) and Czapex dox agar at room

temperature (28 ± 2°C) for 5 days, to allow for the growth of all organisms. Isolations were also made from the soil and infected plants. Transfers were made unto potato dextrose agar (PDA) plates (Odigie and Ikotun, 1982). Stock cultures of the isolates were maintained at 4° C in bottle slants for subsequent studies. Biocontrol agents including *Rhizoctonia solani*, *M. phaseolina*, *F. oxysporum*, *F. solani* and *Pythium* spp. were obtained from the crop of chilli samples collected from various locations of lower region of Sindh Province. These fungi were multiplied on Potato Dextrose agar and Czapex dox agar for 5 days at 28°C.

RESULT AND DISCUSSION

The results obtained are given in the Table 2. All the fungi used in the experiment proved to be pathogenic. In case of soil inoculations with *Rhizoctonia solani,* the maximum disease intensity on chilli plant was 36% in sterilized soil compared with 39% in unsterilized soil. In soil inoculations with *Pythium* sp., the disease intensity was 29% in sterilized soil and 32% in unsterilized soil. The minimum disease intensity resulted following soil inoculation with *Fusarium oxysporum, F. solani* and *Macrophomina phaseolina i.e.* 14%, 13.33% and 8% in sterilized soil and 21%, 19%, 10.33% in unsterilized soil respectively (Table 2.).

Table 1. Effect of Pathogenicity test after inoculation of different fungi.

Pathogens	**Effect of pathogenicity after inoculation**		
	5th day	**10th day**	**15th Day**
Control	--	--	--
Fusarium oxysprorum	--	+	++
F. solani	--	+	++
Macrophomina phaseolina	--	--	+
Rhizoctonia solani	++	+++	++++
Pythium sp.	+	++	+++

No effect (--), Effect(+), Less Effect (++), More effect (+++) and highly effect (++++)

Table 2. Chilli plants inoculated with various fungi showing mean and S.E of disease intensity.

Treatments	**Average percent disease intensity**	
	Sterilized soil	**Unsterilized soil**
Control	0.00	0.00
Soil+ *Fusarium oxysporum*	14±2.64	21±5.50
Soil+ *F. solani*	13.33±3.52	19±7.76
Soil+ *Macrophomina phaseolina*	8±3.21	10.33±4.91
Soil+ *Rhizoctonia solani*	36±12.12	39±11.84
Soil+ *Pythium* sp.	29±9.53	32±8.38

Table 3. Effect of different antagonists on selected fungal pathogens. Mean are followed by ± standard error.

Antagonists	**Different fungi pathogens**					**Inhibiton%**
	Fusarium oxysporum	***Fusarium solani***	***Rhizoctonia solani***	***Macrophomina phaseolina***	***Pythium* spp.**	
Aspergillus niger	7.2±0.72	6.3±0.76	4.7±1.04	2.8±0.9	7.3±0.34	56.6
A. flavus	4.5±1.08	5.3±0.49	5.6±1.18	4.8±0.81	4.2±0.9	48.8
P. communes	4.9±1.43	4.2±1.12	3.4±1.07	2.8±0.86	5.3±0.61	41.2
T. harzianum	7.4±0.47	6.8±0.38	5.8±0.82	6.8±0.73	6.5±0.75	66.6

Fig.1. Chilli plants in sterilized soil before the inoculation of the diseases.

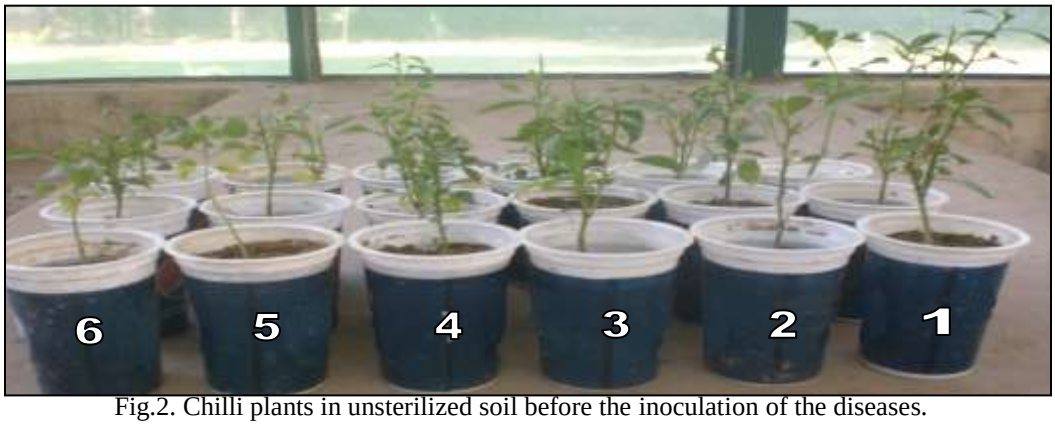

Fig.2. Chilli plants in unsterilized soil before the inoculation of the diseases.

Fig. 3. Chilli plants inoculated with different fungi in sterilized soil after the appearance of the disease.
1. Control; 2. Soil+ *Fusarium oxysporum;* 3. Soil+ *F. solani;* 4. Soil+ *Macrophomina phaseolina;*
5. Soil+ *Rhizoctonia solani;* 6. Soil+ *Pythium* sp.

Table 4. F- ratios derived from ANOVA for pathogenicity of sterilized and unsterilized soil by fungal species.

Source	**F**	**P-value**	**$LSD_{0.05}$**
All five Fungi	40.56	.0000***	1.54
Sterilized & Unsterilized soil	8.36	.0062**	0.97
Fungi × Sterilized and Unsterilized soil	1.31	.2813ns	

F= F-ratio was obtained from ANOVA tables, **LSD**=Least significant difference at P=0.05

Fig. 4. Chilli plants inoculated with different fungi in unsterilized soil after the appearance of the disease.
1. Control; 2. Soil+ *Fusarium oxysporum;* 3. Soil+ *F. solani;* 4. Soil+ *Macrophomina phaseolina;*
5. Soil+ *Rhizoctonia solani;* 6. Soil+ *Pythium* sp.

Table 4 shows the results of ANOVA for pathogenicity of sterilized and unsterilized soil by fungal species on chilli plants. Five fungal species including *Fusarium oyxsporum, F. solani, Macrophomina phaseolina, Rhizoctonia solani* and *Pythium* spp. showed significant differences regarding pathogenicity (P<0.001). As expected there was a significant difference in pathogenicity in sterilized and unsterilized soils (P<0.001). However, the interaction of species X sterilized/unsterilized soil was found to be non-significant.

Table 5. F- ratios derived from ANOVA for antagonist effect on different pathogen fungi.

Source	**F-ratio**	**P-value**	**$LSD_{0.05}$**
All five Pathogen fungi	4.22	.0061**	0.90
Antagonist fungi (4 spp.)	7.60	.0004***	0.80
Pathogen fungi × Antagonist fungi	1.86	.0705ns	

F= F-ratio was obtained from ANOVA tables, **LSD**=Least significant difference at P=0.05

Table 5 shows the results of ANOVA for antagonist effect on different pathogen fungi. Five fungal species including *Fusarium oyxsporum, F. solani, Macrophomina phaseolina, Rhizoctonia solani* and *Pythium* spp. showed significant differences and inhibited by antagonist including *Trichoderma harzianum, Aspergillus niger, A. flavus* and *Penicillium communes* and. All five species proved as being antagonistic to at least some of the fungal pathogens.

Chilli crop is vulnerable to be attacked by several soil-borne pathogenic fungi which are responsible for considerable plant mortality and consequently high losses in yield and quality in many parts of the world (Abada, 1994; Lu *et al.*, 1984; Mushtaq and Hashmin, 1997). Hafeez (1986) while conducting 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.

Siddiqui *et al.*, (2001) investigated the biocontrol potential of five species of *Trichoderma* which significantly reduced the nematode population and root knot development in okra and mungbean. The highest activity was expressed by *T. harzianum* which suppressed the root knot nematode population and elevated plant height and fresh weight of shoot. Siddiqui and Shaukat (2004) reported the influence of soil-borne fungus *Trichoderma harzianum* on the biocontrol potential of *Pseudomonas fluorescens* against plant parasitic nematode *Meloidogyne javanica* and concluded the mixture of compatible *T. harzianum* and *P. fluorescens* are effective against plant parasitic nematodes and could be controlled by the mixture of both microognisms. The molecular mechanism of antagonistic activity of *T. harzianum* involves the formation of hydrolytic enzymes and peptaibol antibiotics (Schirmböck *et al.*, 1994) and possibly 6-pentyl-α-pyrone metabolite (Scarselletti and Faull, 1994; Cooney *et al.*, 1997).

The result of pathogenicity test indicated that among the selected fungi, *Macrophomina phaseolina, Fusarium oxysporum* and *F. solani* exhibited lower pathogenicity symptoms on chilli, while two soil pathogens *Rhizoctonia solani* and *Pythium* spp. were found to show high pathogenicity effect on the chilli plant (Fig. 4; Table 1). Interestingly, *Macrophomina phaseolina* showed much lower pathogenicity effect on chilli compared to the other pathogens tested.

All the isolates in the study inhibited the growth of *Fusarium oxysporum, Fusarium solani, Rhizoctonia solani, Macrophomina phaseolina,* and *Pythium* spp. as shown in Fig. 5 (Table 3). Among these isolates, *Trichoderma harzanium, Aspergillus niger* and *A. flavus* resulted as effective antagonist inhibiting the growth of the pathogen by 66.6%, 56.6% and 48.8% respectively. The least antagonist was *Penicillium communes* which inhibited the growth of the pathogen by 41.2% only. *Macrophomina phaseolina* and *Rhizoctonia solani* were also inhibited by all the isolates used in the experiment as shown in Fig. 5 (Table 3).

Windham *et al.*, (1986) and Malik and Dawar (2003) indicated that *Trichoderma* species produces plant growth promoting factors and various species of *Trichoderma* gained considerable success against pathogenic fungi particularly *T. harzianum* protects the root system against *F. solani, R. solani* and *M. phaseolina* infection on a number of crops.

It is concluded that biological treatment with *Trichoderma harzianum* and *Aspergillus niger* as antagonists are effective for management of root rot fungi to the chilli crop.

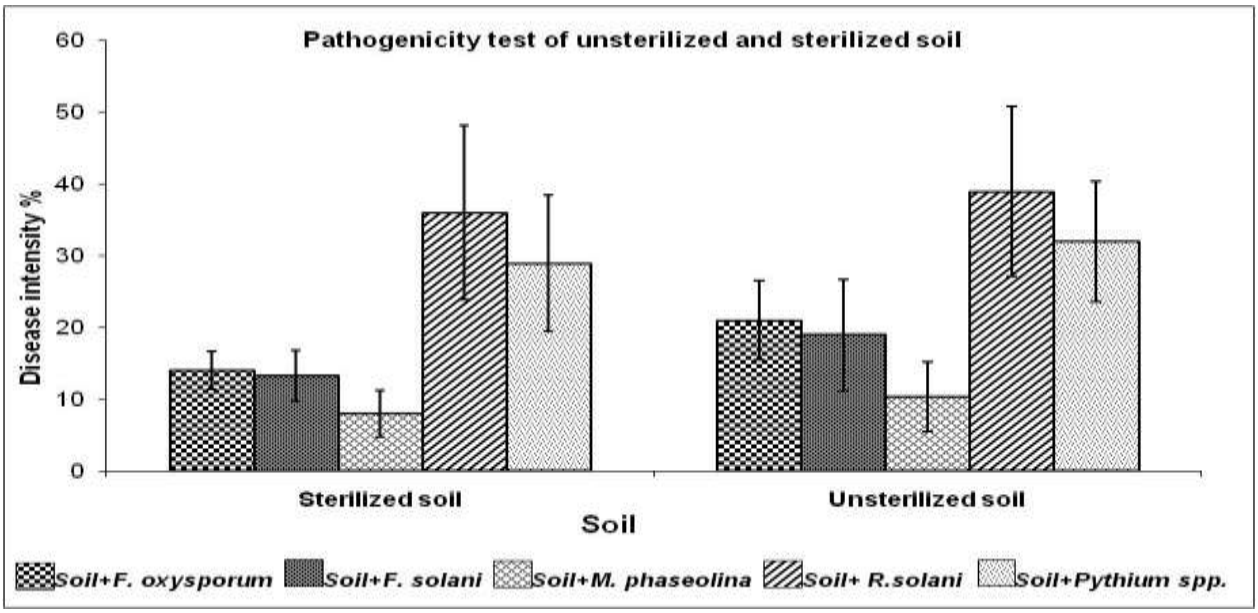

Fig. 4 Graph showing sterilized soil and unsterilized disease intensity % with Mean and S.E.

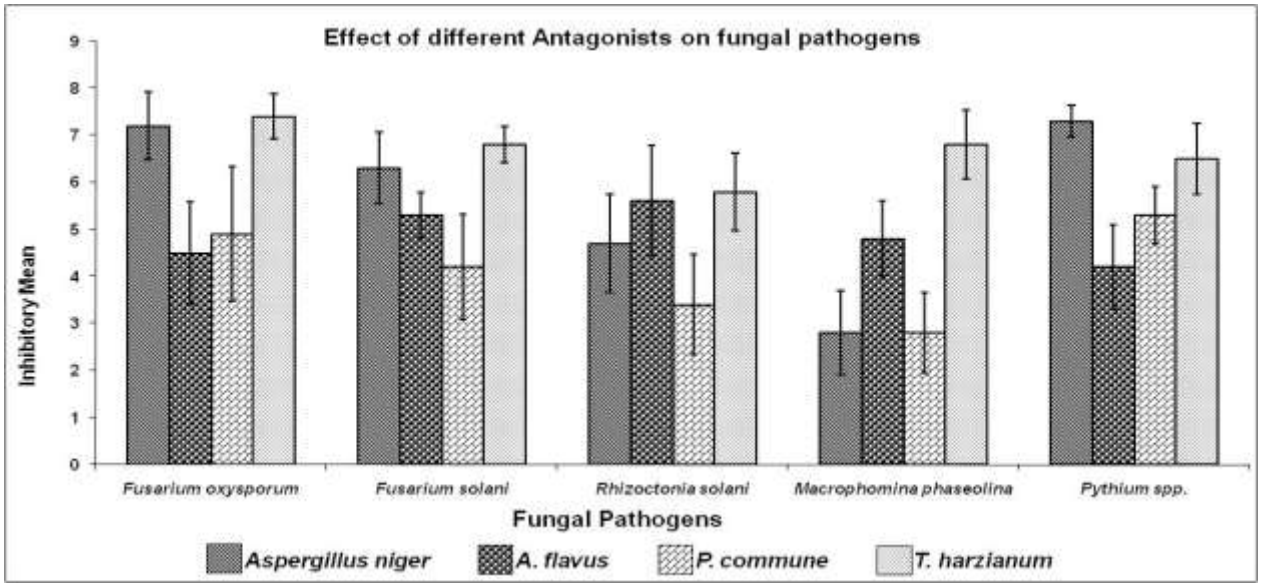

Fig. 5 The effect of different antagonists on selected fungal pathogens.

REFERENCES

Abada, K. A. (1994). Fungi associated with root rot of pepper and some factors affecting disease incidence. *7th Congress of Phytopathology*, Giza 220-226.

Aboshosha, S.S., S.I. Attaalla, A.E. EL-Korany and E. El-Argawy (2007). Characterization of *Macrophomina phaseolina* isolates affecting sunflower growth in El-Behera governorate, Egypt. *Internat. J. Agric. & Biol.,* 6: 807 − 815.

Alvaro, M.R.A., V.A. Ricardo, A.A.A. Carlos, P.C. Valdemar, S.J.F. David, R.R.M. Silvana, C.B. Luis, C.P. Mauro and G.P.C. Claudio (2003). Genotypic diversity among Brazilian isolates of *Macrophomina phaseolina* revealed by RAPD. *Fitopatologia Brasileira*, 28: 279 – 285.

Barnett, H. and B. B. Hunter (1972). *Illustrated Genera of Imperfect Fungi*. Burgess Publishing Co., Minneapolis, Minnesota. 241p.

Calvet, C., J. Pera and J. M. Bera (1990). Interaction of *Trichoderma* spp. with *Glomus mossaeae* and two wilt pathogenic fungi. *Agric. Ecosyst. Environ*., 9: 59-65.

Chet, I., N. Benhamou and S. Haran (1998). Mycoparasitism and lytic enzymes. In: G.E. Harman, and C.P. Kubicek. (Eds.) *Trichoderma and Gliocladium. Vol 2. Enzymes, biological control, and commercial applications*. Taylor & Francis, London. 153–172.

Chet, J. (1990). Mycoparasitism recognition, physiology and ecology. In: R.R. Baker and P.E. Dunn. (Eds) *New Directions in Biological Control: Alternatives for Suppressing Agricultural Pests and Diseases*. Alan Liss, New York. 725–733.

Cooney, J.M., J.L.Vannests, D.R. Lauren and R.A. Hill (1997). Quantitative determination of the antifungal compound 6-pentyl-α-pyrone (6PAP) using a simple plate bioassay. *Letters in Appl. Microbiol.,* 24: 47–50.

Dhingra, O. D., and J. B. Sinclair (1972). Variation among isolates of *Macrophomina phaseolina* (*Rhizoctonia bataticola*) from the same soybean plant. *Phytopathology*,62, 511- 518.

Dhingra, O.D. and J.B. Sinclair (1973). Variation among isolates of *Macrophomina phaseolina* from different regions. *Phytopathol. Z.,* 76: 200-204.

Echavez-Badel, R., and A. Perdomo (1991). Characterization and comparative pathogenicity of two *Macrophomina phaseolina* isolates from Puerto Rico. *J. Agric. Univ. P. R*., 75: 419 − 421.

Elad, Y. (2000). Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. *Crop Prot*., 19: 709-714.

Ellis, E. B. (1976). *More Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, UK: 507p.

Ellis, M. B. (1971). *Dematiacicious Hyphomycetes*. CMI., Kew Surrey, England, 608 p.

Ghaffar, A. (1988). Soilborne disease research center. Final research report. Dept. of Botany, Univ. of Karachi, Karachi-75270, Pakistan.111pp.

Hafeez, A. (1986). Plant disease. *Pak. J. Agric. Res.,* Council. Islamabad. 552 P.

Howell, C.R. (1998). The role of antibiosis. In: G.E. Harman and C.P. Kubicek. (Eds.) *Trichoderma and Gliocladium. Vol 2. Enzymes, biological control, and commercial applications*. Taylor & Francis, London. 173–184.

Hussain, F. and M. Abid (2011). Pest and diseases of chilli crop in Pakistan: A review. *Int. J. Biol. Biotech.,* 8: 325-332.

Imran, A.S., A. Zareen, M. J. Zaki and S.S. Shaukat (2001). Use of *Trichoderma* species in the control of *Meloidogyne javanica*, root knot nematode in Okra and Mungbean. *Pak.J. Biol. Sci*., 4: 846-848.

Jana, T, T.R. Sharma, R.D. Prasad, and D.K. Arora (2003). Molecular characterization of *Macrophomina phaseolina* and *Fusarium* species by a single primer RAPD technique. *Microbiological Research,* 158: 249 −257.

Khara, H. S. and H. A. Hadwan (1990). *In vitro* studies on antagonism of *Trichoderma* spp. against *Rhizoctonia solani*, The casual agent of Damping off of Tomato. *Plant Dis. Res.,* 2:144 -147.

Lu, J. Y., C.F. Yu and L.H. Ju (1984). Studies on differentiation of the virulence of *Verticillium dahlia* on pepper. *Acta Phytopathologia Scientia,* 14:213-18.

Malik, G. and S. Dawar (2003). Biological control of root infecting fungi with *Trichoderma harzianum*. *Pak. J. Bot.,* 35: 971-975.

Mayek-Perez, N., C. Lopez-Castaneda, M. Gonzalez-Chavira, R. Garcia-Espinosa, J. A. Acosta-Gallegos, D. I. Martinez, O. Vega and J. Simpson (2001). Variability of Mexican isolates of *Macrophomina phaseolina*, based on the pathogenesis and AFLP genotype. *Physiological and Molecular Plant Pathology,* 59: 257 − 263.

Mihali, J.D. and S.J. Taylor (1995). Interpreting variability among isolates of *Macrophomina phaseolina* in pathogenicity, pycnidium production and chlorate utilization. *Canadian Journal of Botany*. 73: 1596-1603.

Monte, E., 2001. Understanding *Trichoderma*: Between biotechnology and microbial ecology, *Int. Microbiol.,* 4: 1-4.

Muhyi, R. and P.W. Bosland (1992). Evaluation of *Capsicum* germplasm for sources of resistance to *Rhizoctonia solani*. *Hort*. *Science*, 30: 341-342.

Mukhopadhyay, A.N. (1987). Biological control of soil borne plant pathogens by *Trichoderma* spp. *Indian Journal of Mycology and Plant Pathology*, 17: 1-9.

Mushtaq, M. and M. H. Hashmi. 1997. Fungi associated with wilt disease of *Capsicum* in Sindh, Pakistan. *Pak.J.Bot.*, 29: 217-222

Muthamilan, M. and R. Jeyarajan (1996). Integrated management of *Sclerotium* root rot of groundnut involving *T. harzianum, Rhizobium* and *carbendazim*. *Ind. J. Mycol. & Plant Pathol.*, 26: 204-209.

Nelson, P. E., T.A. Toussoun, and W.F.O. Marasas (1983). *Fusarium* species: an illustrated manual for identification, The Pennsylvania State University Press, 193p.

Odigie, E.E. and T. Ikotun (1982). *In vitro* and *in vivo* inhibition of growth of *Phytophthora palmivora* by antagonistic microorganisms. *Fitopatologia Brasileira.,* 7: 157 -167.

Raguchander, T., K. Rajappan and K. Prabhakar (1995). Evaluation of talc based product of *T. viride* for the control of urd bean root rot. *J. Biol. Cont.*, 9: 63-64.

Saleem, A., M. H. Bokhari, K. Hamad and M. Ansar (1996). Mycoflora associated with root and collar rot disease of chillies in different districts of the Punjab (Pakistan). *Pak. J. Bot*., 9: 80-84.

Scarselletti, R. and J.L. Faull (1994). *In vitro* activity of 6-pentyl-α-pyrone, a metabolite of *Trichoderma harzianum*, in the inhibition of *Rhizoctonia solani* and *Fusarium oxysporum* f sp. *lycopersici. Mycol. Res*., 98: 1207–1209.

Schirmböck, M., M. Lorito, C.K. Hayes, I. Arisan-Atac, F. Scla, G.E. Harman and C.P. Kubicek (1994). Parallel formation and synergism of hydrolytic enzymes and peptaibol antibiotics, molecular mechanisms involved in the antagonistic action of *Trichoderma harzianum* against phytopathogenic fungi. *Appl. Environ. Microbiol*., 60: 4364–4370.

Shalini, S., K. P. Narayan, Lata and A. S. Kotasthane (2006). Genetic relatedness among *Trichoderma* Isolates inhibiting a pathogenic fungi *Rhizoctonia solani*. *African J. Biotechnology*, 5: 580-584.

Siddiqui, I. A., A. Zareen, M. J. Zaki and S. S. Shaukat (2001). Use of *Trichoderma* species in the control of *Meloidogyne javanica*, root knot nematode in Okra and Mungbean. *Pak. J. Biol. Sci.*, 4: 846-848.

Siddiqui, I.A. and S.S. Shaukat (2004). *Trichoderma harzianum* enhances the production of nematicidal compounds *in vitro* and improves biocontrol of *Meloidogyne javanica* by *Pseudomonas fluorescens* in tomato. *Lett Appl Microbiol.* 38:169-75.

Sinclair, J.B. and P.A. Backman (1986). *Compendium of soybean diseases*. Third edition. – The American Phytopathological Society. 30-33p.

Singh, K., J.C. Frisvad, U. Thrane and S.B. Mathur (1991). *An Illustrated Manual of Identification of Some Seed-borne Aspergilli, Fusaria, Penicillia and their Mycotoxins*. Danish Govt. Inst. Seed Path. for Dev. Count., Ryvangs Allc 78, DK-2900 Hellerup, Denmark. 133 p.

Sivan, A. and I. Chet (1986). Biological control of *Fusarium* spp. in cotton, wheat and muskmelon by *Trichoderma harzianum*. *J. Phytopathol.*, 116: 39-47.

Spiegel, Y. and I. Chet (1998). Evaluation of *Trichoderma* spp. as biocontrol agent against soil-borne fungi and plant parasitic nematodes in Israel. *Integr. Pest Manage. Rev*., 3: 169-175.

Su, G., S.O. Suh, R.W. Schneider and J.S. Russin (2001). Host specialization in the charcoal rot fungus *Macrophomina phaseolina*. *Phytopathology*. 91: 120 – 126.

Sutton, B. C. (1980). *The Coelomycetes* (CAB, IMI) Kew, Surrey, U.K. 696 p.

Vandemark, G., O. Martinez, V. Pecina, and M. J. Alvarado (2000). Assessment of genetic relationships among isolates of *Macrophomina phaseolina*, using a simplified AFLP technique and two different methods of analyses. *Mycologia,* 92: 656 – 664.

Windham, M.T., Y. Elad and R. Baker (1986). A mechanism for increase plant growth induced by *Trichoderma* spp. *Phytopathology*, 76: 518-521.

Zeilinger, S., C. Galhaup, K. Payer, S.L. Woo, R.L. Mach, C. Fekete, M. Lorito and C.P. Kubicek (1999). Chitinase gene expression during mycoparasitic interaction of *Trichoderma harzianum* with its host. *Fungal Genet. Biol*., 26: 131–140.

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