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ORIGINAL ARTICLE

Evaluation of Biocontrol Potential of Seven Indigenous *Trichoderma* Species against Charcoal Rot Causing Fungus, *Macrophomina phaseolina*

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Abstract

Charcoal rot incited by *Macrophomina phaseolina* is one of the major diseases of green gram and black gram in Pakistan reducing yields up to 40%. As there are no long-term control strategies for this seed- and soil-borne pathogen, therefore, in the present study, seven indigenous species of *Trichoderma* were evaluated for their *in vitro* and *in vivo* effectiveness against *M. phaseolina* with the objective to identify alternatives to pernicious fungicides. All seven species of *Trichoderma* significantly retarded the growth of *M. phaseolina in vitro*. Maximum reduction (79.63%) was observed with *T. harzianum* followed by *T. hamatum* (76.3%) while *T. pseudokoningii* caused the minimum decrease (58.14%) in growth of the fungus. Similarly, *Trichoderma* species had significant effects on number and size of sclerotia. *M. phaseolina* produced the minimum number of sclerotia in the presence of *T. hamatum* followed by *T. harzianum* causing reductions of 69.5 and 66.84% over control, respectively. The maximum reduction in size of sclerotia was caused by *T. harzianum*. The maximum plant survival of green and black gram was obtained with *T. harzianum* followed by *T. hamatum* and *T. viride*. The maximum individual germination of 86.67% was achieved with *T. harzianum* at a concentration of 2×10^8 (propagules/ml), while the minimum (33.33%) was recorded with *T. pseudokoningii* at 2×10^4. *Trichoderma* concentrations also had significant effects on plant survival, being the maximum at the highest concentration. The plant survival decreased as the concentrations of the antagonists decreased showing a direct relationship between plant survival and concentrations.

Keywords *Trichoderma* species · Biocontrol · *Macrophomina phaseolina* · *Vigna radiata* · *Vigna mungo*

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Bewertung des biologischen Kontrollpotenzials von sieben einheimischen *Trichoderma*-Arten gegen den Holzkohlenfäule-verursachenden Pilz, *Macrophomina phaseolina*

Zusammenfassung

Die durch *Macrophomina phaseolina* hervorgerufene Holzkohlenfäule ist eine der Hauptkrankheiten bei der Mungbohne und der Urdbohne in Pakistan und reduziert die Erträge um bis zu 40 %. Da es keine langfristigen Bekämpfungsstrategien für diesen samen- und bodenbürtigen Erreger gibt, wurden in der vorliegenden Studie sieben einheimische *Trichoderma*-Arten auf ihre *in-vitro*- und *in-vivo*-Wirksamkeit gegen *M. phaseolina* mit dem Ziel untersucht, Alternativen zu schädlichen Fungiziden zu finden. Alle sieben *Trichoderma*-Arten verzögerten das Wachstum von *M. phaseolina in vitro* signifikant. Die maximale Reduktion (79,63 %) wurde bei *T. harzianum* beobachtet, gefolgt von *T. hamatum* (76,3 %), während *T. pseudokoningii* die geringste Abnahme (58,14 %) des Pilzwachstums verursachte. In ähnlicher Weise hatten die *Trichoderma*-Arten signifikante Auswirkungen auf Anzahl und Größe der Sklerotien. *M. phaseolina* produzierte die minimale Anzahl von Sklerotien in Anwesenheit von *T. hamatum*, gefolgt von *T. harzianum*, was zu einer Verringerung um 69,5 % bzw. 66,84 % gegenüber der Kontrolle führte. Die maximale Verringerung der Größe der Sklerotien wurde durch *T. harzianum* verursacht. Das maximale Überleben der Mungbohne und der Urdbohne wurde mit *T. harzianum* erreicht, gefolgt von *T. hamatum* und *T. viride*. Die maximale Keimung von 86,67 % wurde mit *T. harzianum* bei einer Konzentration von 2×10^8 (propagules/ml) erreicht, während das Minimum (33,33 %) mit *T. pseudokoningii* bei 2×10^4 erfasst wurde. Die verwendeten *Trichoderma*-Konzentrationen hatten ebenfalls signifikante Auswirkungen auf das Überleben der Pflanzen, wobei das Maximum bei der höchsten Konzentration erreicht wurde. Das Pflanzenüberleben nahm mit der Reduktion der Konzentrationen ab, was eine direkte Beziehung zwischen dem Pflanzenüberleben und den Konzentrationen zeigte.

Schlüsselwörter *Trichoderma*-Arten · Biologische Kontrolle · *Macrophomina phaseolina* · *Vigna radiata* · *Vigna mungo*

Introduction

Green gram (*Vigna radiata* L.) Wilczek and black gram (*Vigna mungo* L.) Hepper are two important summer pulse crops of Pakistan, cultivated on an area of 245.9 and 32.5 thousand hectares with a total production of 177.7 and 17.3 thousand tons, respectively, (Anonymous 2018) under a wide range of agro-ecological zones. The average yields of these pulses in Pakistan are very low as compared to their potential yields obtained in many other countries which can be imputed to legions of biotic and abiotic constraints. Among biotic factors, diseases are the most destructive (Aslam et al. 2019a, 2019b; Hussain and Mukhtar 2019; Mukhtar and Hussain 2019; Mukhtar and Kayani 2019; Tariq-Khan et al. 2020). The losses due to diseases to pulse crops have been estimated up to 44%, depending upon the crop variety (Bashir and Malik 1988). Green gram and black gram are invaded by about 26 diseases in the world (Charles 1978); charcoal rot incited by *Macrophomina phaseolina* (Tassi) Goid, is of prime significance in reducing crop yield especially in arid regions of the world (Hoes 1985). The pathogen is distributed in diverse climatic conditions from arid to tropical regions and has a broad host range. There are more than 500 hosts of the fungus including legumes and cereal plants. In Pakistan, it has 67 important economic host plant species including mungbean, mashbean, soybean, sunflower, sorghum, maize, linseed, chickpea and alfalfa (Javaid et al. 2017). *M. phaseolina* is a soil- and seed-borne pathogenic fungus. It produces cushion shaped black sclerotia and disease severity is correlated with viable sclerotia present in the soil (Iqbal and Mukhtar 2014).

All the growth stages of plants are infected by charcoal rot. The disease manifests in the form of dark lesions on the epicotyls and hypocotyls resulting in seedling death due to obstruction of xylem vessels. The pathogen causes red to brown lesions on roots and stems, produces dark mycelia and black microsclerotia. Severe infections cause defoliation and wilting resulting in 100% yield losses (Bashir and Malik 1988). The fungus is fairly distributed in all the agro-ecological zones of Pakistan. Significant morphological and pathogenic variations have been observed among different isolates of the fungus from these agro-ecological zones suggesting that these variations may be considered in disease management and breeding programs of green gram and black gram against charcoal rot (Iqbal and Mukhtar 2014).

The ever-increasing need for incessant supply of food to the bourgeoning human population of the world demands for the management of phytopathogens responsible for considerable reduction in crop yields (Javed et al. 2019a, 2019b; Khan et al. 2019; Nazir et al. 2019; Mukhtar and Kayani 2020). Currently, the management of charcoal rot is mainly relied on synthetic fungicides and resistant cultivars (Mukhtar et al. 2017; Iqbal and Mukhtar 2020). The commonly used fungicides against *M. phaseolina* in Pak-

istan are Propineb, Copper + Mancozeb, Mancozeb, Carbendazim, Captan, Benomyl, Mtalaxyl + Mancozeb, Copper oxychloride, Chlorothalonil. Synthetic fungicides give an instant and effective control of plant pathogens but their use is inimical to humans, livestock and environment. Moreover, the effectiveness of these fungicides as seed treatment is not long term and their soil application is expensive and causes imbalance in soil microbial communities which affect normal activities of beneficial organisms. In addition, continuous use of the same fungicides for the same pathogen results in the development of resistant strains of the pathogens (Iqbal et al. 2014). As no resistant cultivars of green gram and black gram are available against *M. phaseolina* in the country, and the use of fungicides is costly and hazardous, therefore, deployment of biocontrol agents could be one of the feasible and best alternative strategies to abate yield losses by the fungus. Among fungal biocontrol agents, *Trichoderma* species have been most widely studied against different phytopathogens. *Trichoderma* species are naturally occurring soil fungi that colonize roots and stimulate plant growth. Such fungi have been applied to a wide range of plant species for the purpose of growth enhancement, with a positive effect on plant weight, crop yields, and disease control. The large scale production of potential *Trichoderma* spp. would be economical and ecofriendly. In Pakistan, very little work has been done on the biocontrol potential of indigenous *Trichoderma* species for the management of charcoal rot of green gram and black gram. For these reasons, in the present study, seven indigenous species of *Trichoderma* were evaluated for their *in vitro* and *in vivo* effectiveness against *M. phaseolina* with the objective to identify alternatives to pernicious fungicides and to update disease management strategies.

Materials and Methods

Isolation, Purification and Identification of *Macrophomina phaseolina*

The fungus, *Macrophomina phaseolina*, used in the study was isolated from stem bark tissues of black gram bearing fungal sclerotia and characteristic charcoal rot symptoms. The samples were cut into small pieces (5–10 mm long), surface sterilized with 1% sodium hypochlorite for 2 min and then rinsed thrice in sterilized distilled water. The pieces were placed on Chloroneb Mercury Rose Bengal Agar (CMRA) medium (Meyer et al. 1973) in petri dishes and incubated in dark at 25 ± 1 °C for 7 d. A small portion of the actively growing colony of *M. phaseolina* was taken from the periphery of 90 mm diameter petri dish and spread onto petri dishes containing glucose agar medium (glucose, 20 g; agar, 20 g and water, 1000 ml) and incubated in dark at 25 ± 1 °C for 7 d. A small portion of the colony having sclerotia was taken into a drop of sterilized water and agitated with a sterilized needle to separate the sclerotia from the mycelia. Sclerotia were then transferred to 90 mm diameter petri dishes containing CMRA medium. Colonies appearing from single sclerotium were again transferred to CMRA medium in 90 mm petri plates, incubated as mentioned above and identified on the basis of standard key (Barnett and Hunter 1972).

Multiplication of *M. phaseolina* for Pot Assay

Sorghum seeds were water soaked overnight, air dried under room temperature and placed in conical flasks. The mouth of each flask was plugged with cotton wool, wrapped in aluminum foil and autoclaved at 15 psi (121 °C) for 20 min. After cooling, the seeds in flasks were inoculated with 4 mm mycelial plugs from a 7-day-old culture of *M. phaseolina* and incubated at 25 ± 1 °C for 15 d. The flasks were shaken at alternate days for uniform colonization of the grains. The inoculum thus produced was used in pot experiments.

Biomass Production of *Trichoderma* Species

Seven indigenous *Trichoderma* species viz. *Trichoderma harzianum, T. hamatum, T. koningii, T. pseudokoningii, T. viride, T. aureoviride* and *T. virens* used in the experiment were collected from the First Fungal Culture Bank, The University of Punjab, Lahore. The biocontrol agents were grown on potato dextrose agar (PDA). For biomass production of antagonists, Richard's medium was used. The medium (100 ml) was poured in 250 ml Erlenmeyer flasks, autoclaved at 15 psi for 30 min, inoculated with two scoops of 10 mm diameter taken from a 7-day-old culture of each *Trichoderma* species on PDA and incubated at 25 ± 1 °C for 15 d. The biomass of each antagonist was centrifuged at 11,000 rpm for 30 s and their slurries were made. The propagules of each fungal slurry were enumerated using a haemocytometer. Based on these counts, the concentrations (2×10^4, 2×10^6 and 2×10^8 propagules/ml) were made by the addition of requisite amount of distilled water to the slurry of fungal antagonist. Two ml of gelatin as an adhesive was added to the fungal suspensions of each concentration of each antagonist. The seeds each of green gram (cv. NM-92) and black gram (cv. Mash-98) were surface sterilized and primed with different concentrations of biocontrol agents. Seeds treated with sterilized distilled water served as controls.

Evaluation of *Trichoderma* Species for their Efficacy Against *M. phaseolina*

In Vitro Evaluation of *Trichoderma* Species

The *in vitro* antagonistic activities of *Trichoderma* species were tested against *M. phaseolina* by seeing their effects on different parameters viz. radial growth, number and size of sclerotia produced by the pathogen. The effects of *Trichoderma* species on radial growth of *M. phaseolina* were tested by dual culture technique (Naik et al. 2009). Petri dishes (90 mm dia.) containing 15 ml of autoclaved PDA were inoculated with 5-mm-diameter mycelial discs each of 7-day-old culture of the pathogen and antagonists at equi-distance from the periphery and incubated for 6 d at 25 ± 1 °C in an incubator. Petri plates without antagonists served as control. Each treatment was replicated five times. After 6 d, the diameters of the colonies of both the bio-control agents and the pathogenic fungus were measured in four directions, their averages were calculated and percent inhibition of radial growth of *M. phaseolina* was calculated (Iqbal et al. 2014). The effect of *Trichoderma* species on sclerotia production was studied by counting sclerotia per microscopic field (under 10×). The size of sclerotia was measured with the help of an ocular micrometer from control and treated plates. To calculate the size, the averages of 50 sclerotia from each treatment were taken.

Greenhouse Assay

To evaluate the efficacy of antagonists in greenhouse, the cornmeal sand inoculum was thoroughly mixed with sterilized potting soil at 2.5 g per kg of potting soil. The infested potting soil was filled in plastic greenhouse flats. Plastic greenhouse flats filled with sterilized un-infested potting soil served as controls. Twenty seeds each of green gram (cv. NM-92) and black gram (cv. Mash-98) treated with different concentrations of *Trichoderma* species were sown in infested potting soil contained in greenhouse flats in four rows of five seeds. The seeds treated with sterilized distilled water sown in infested flats served as control. Each treatment was replicated five times. The flats were placed in a glasshouse at 25 °C in completely randomized design. Data on percentage germination were recorded after 20 d, and percentage increase or decrease over control was calculated.

Statistical Analysis

The experiment was conducted twice. Percent reduction in mycelial growth, number and size of sclerotia of the pathogenic fungus and increase in seedling emergence were calculated over controls prior to statistical analysis (Mukhtar 2018). All the data were subjected to Analysis of Variance (ANOVA) using GenStat package 2009, (12th edition) version 12.1.0.3278 (www.vsni.co.uk). The differences among means were compared by Fisher's protected least significant difference test at ($P \leq 0.05$). No significant interaction was observed between the data of both the experiments, so the two sets of data were combined for analysis. Standard errors of differences of means were calculated in Microsoft Excel 2010. Taking concentrations of antagonistic fungi as independent variables (X) and plant survival as dependent variable (Y), linear relationships were established and regression equations and correlation coefficients (R^2) were calculated in Microsoft Excel 2010. The closer the R^2 is to 1.00, the better the fit.

Results

Inhibitory Effects of *Trichoderma* Species Against *M. phaseolina*

All the seven species of *Trichoderma* significantly retarded the growth of *M. phaseolina in vitro*. Maximum reduction (79.63%) was observed with *T. harzianum* followed by *T. hamatum* (76.3%) while *T. pseudokoningii* caused the minimum decrease (58.14%) in growth of the fungus. The inhibitions in growths caused by other *Trichoderma* species were found to be intermediate as shown in Fig. 1. Similarly, *Trichoderma* species had significant effects on number of sclerotia. *M. phaseolina* produced the minimum number of sclerotia in the presence of *T. hamatum* followed by *T. harzianum* causing reductions of 69.5 and 66.84%, respectively, over control. The reductions caused by these two fungi were statistically similar. On the other hand, *T. virens* caused the minimum reductions in number of sclerotia produced by *M. phaseolina* followed by *T. pseudokoningii* giv-

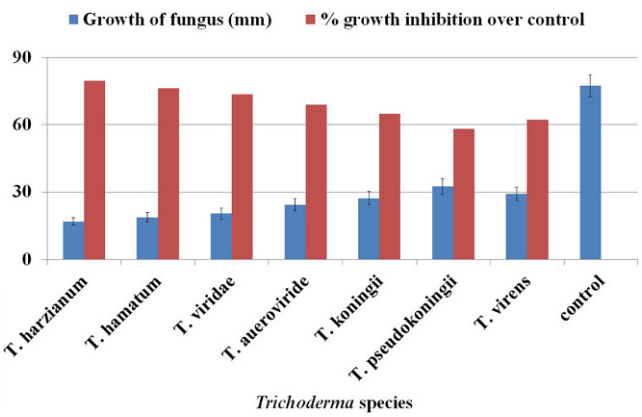

Fig. 1 Effect of *Trichoderma* species on growth of *Macrophomina phaseolina*

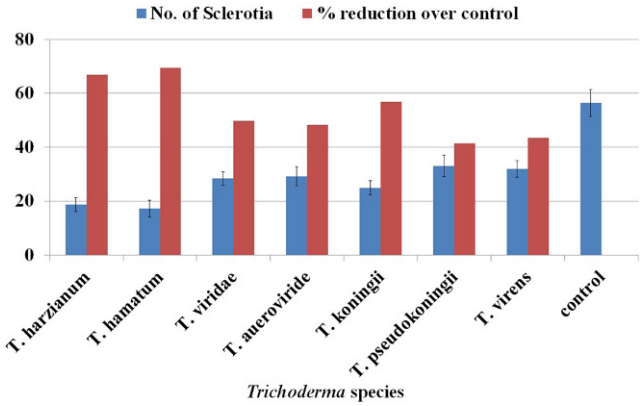

Fig. 2 Effect of *Trichoderma* species on number of sclerotia produced by *Macrophomina phaseolina*

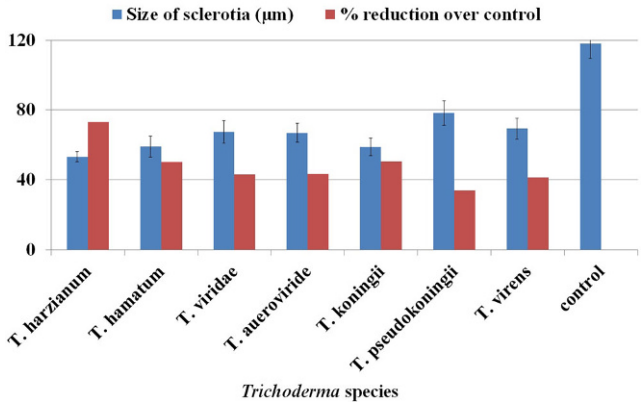

Fig. 3 Effect of *Trichoderma* species on size of sclerotia of *M. Macrophomina phaseolina*

ing 43.44 and 41.31% reductions, respectively, over control as shown in Fig. 2. Likewise, the size of sclerotia of *M. phaseolina* was also significantly affected by *Trichoderma* species. The maximum reduction in size of sclerotia was caused by *T. harzianum* while *T. pseudokoningii* caused the minimum reduction in size. The reductions in size caused by other *Trichoderma* species were in-between as shown in Fig. 3.

Effects of *Trichoderma* Species on Plant Survival of Green Gram and Black Gram

Antagonists exhibited significant variations in their efficacy on plant survival of green gram when used as seed treatment. Maximum average plant survival was obtained with *T. harzianum* followed by *T. hamatum* and *T. viride* while *T. pseudokoningii* gave the poorest germination of all the test biocontrol agents. Maximum individual germination of 86.67% was achieved with *T. harzianum* at a concentration of 2×10^8 (propagules/ml), while the minimum (33.33%) was recorded with *T. pseudokoningii* at 2×10^4. Individual germination of green gram with all the species at three concentrations is given in Table 1. Concentrations also had significant effects on plant survival being the maximum at the highest concentration. The plant survival decreased as the concentrations of the antagonists decreased showing a direct relationship between plat survival and concentrations and has been shown by regression equations in Table 1. The *Trichoderma* species gave almost the similar kind of results in case of black gram and are shown in Table 2.

Discussion

In the present study, seven indigenous *Trichoderma* species were tested against the devastating fungus *M. phaseolina*. All the species significantly reduced the growth of *M. phaseolina*, number and size of sclerotia, and improved the survival and germination of green gram and black gram. *Trichoderma* species have been considered effective against a plethora of pathogens. Alice et al. (1996) found *T. harzianum* and *T. viride* effective against *M. phaseolina* infecting jasmine. Similarly, Etebarian (2006) found that *T. harzianum* (M), *T. harzianum* (T39) and *T. virens* (DAR 74290) completely retarded growth of *M. phaseolina*

Table 1 Effect of *Trichoderma* species on plant survival of green gram

Trichoderma species	Increase in plant survival over control at				Regression equation	R^2
	2×10^4	2×10^6	2×10^8	Average		
T. harzianum	63.33 ± 3.33 a	73.33 ± 3.33 a	86.67 ± 3.33 a	74.44 A	y = 11.67x + 51.103	0.9932
T. hamatum	60.00 ± 0.00 ab	70.00 ± 0.00 ab	76.67 ± 3.33 b	68.89 AB	y = 8.335x + 52.22	0.9869
T. viride	56.67 ± 3.33 bc	63.33 ± 3.33 c	70.00 ± 0.00 c	63.33 BC	y = 6.665x + 50.003	1.0000
T. aureoviride	53.33 ± 3.33 c	60.00 ± 0.00 cd	66.67 ± 3.33 c	60.00 C	y = 6.67x + 46.66	1.0000
T. koningii	43.33 ± 3.33 de	53.33 ± 3.33 e	56.67 ± 3.33 d	51.11 D	y = 6.67x + 37.77	0.9233
T. pseudokoningii	33.33 ± 3.33 f	40.00 ± 5.77 g	43.33 ± 3.33 f	38.86 F	y = 5x + 28.887	0.9641
T. virens	40.00 ± 5.77 de	43.33 ± 3.33 fg	50.00 ± 5.77 e	44.44 EF	y = 5x + 34.443	0.9641

Values are means of five replicates. Figures following ± are standard errors. Means sharing common letters in each column do not differ significantly at ($P \leq 0.05$). Fisher's protected least significant difference test was used for comparison of means

Table 2 Effect of *Trichoderma* species on plant survival of black gram

Trichoderma species	Increase in plant survival over control at				Regression equation	R^2
	2×10^4	2×10^6	2×10^8	Average		
T. harzianum	63.33 ± 3.33 a	70.00 ± 5.77 a	80.00 ± 5.77 a	71.11 A	y = 8.335x + 54.44	0.9869
T. hamatum	60.00 ± 5.77 ab	66.67 ± 3.33 a	73.33 ± 3.33 a	66.66 A	y = 6.665x + 53.337	1.0000
T. viride	56.67 ± 3.33 b	60.00 ± 0.00 bc	66.67 ± 3.33 bc	61.11 B	y = 5x + 51.113	0.9641
T. aureoviride	46.67 ± 3.33 cd	56.67 ± 3.33 c	63.33 ± 3.33 c	55.56 C	y = 8.33x + 38.897	0.9868
T. koningii	43.33 ± 3.33 d	46.67 ± 3.33 de	56.67 ± 3.33 de	48.89 DE	y = 6.67x + 35.55	0.9233
T. pseudokoningii	30.00 ± 0.00 f	43.33 ± 3.33 f	50.00 ± 0.00 f	41.11 F	y = 10x + 21.11	0.9644
T. virens	33.33 ± 3.33 ef	46.67 ± 3.33 def	53.33 ± 3.33 ef	44.44 EF	y = 10x + 24.443	0.9641

Values are means of five replicates. Figures following ± are standard errors. Means sharing common letters in each column do not differ significantly at ($P \leq 0.05$). Fisher's protected least significant difference test was used for comparison of means

inciting charcoal rot in melon. Moreover, many soil-borne pathogens including *Rhizoctonia solani, Pythium ultimum, Fusarium moniliforme* and *Sclerotium rolfsii* have also been controlled by different *Trichoderma* species (Ghazanfar et al. 2018; Mukhtar 2018). In the present study, *Trichoderma* species showed variations in effectiveness in reducing fungal growth and number and size of *M. phaseolina*; *T. harzianum* and *T. hamatum* were found to be the most effective. The variations among different *Trichoderma* species in their ability to control the growth of phytopathogens have also been reported by many scientists (Laha et al. 1992; Naik et al. 2000; Upmanyu et al. 2002; Singh et al. 2008). This emphasizes the need to select the antagonistic fungi which give best results for the management of phytopathogens under specific agro-ecological conditions. The antagonistic activity of *Trichoderma* species is attributed to parasitism, production of lytic enzymes such as chitinases and glucanases (Chet, 1987; Woo et al. 2002; Compant et al. 2005) which degraded β-glucans, chitin and polysaccharides, responsible for fungal cell wall rigidness (Gupta et al. 1995; Howell 2003) or by antibiotic production such as glioviridin and gliotoxin (Di Pietro et al. 1993) or by competition or rhizosphere competence. It has also been reported that, in dual culture technique, *Trichoderma* hyphae hyper-parasitized the pathogen and coagulated its protoplasm leading to shrinkage, granulated and vacuolated protoplasm of the pathogen (Pandey and Upadhyay 2000). Suppression of fungal growth by antagonists might be due to overgrowth forming inhibition zone (Hashem 2004).

Dressing green gram and black gram seeds with three different concentrations of *Trichoderma* species in greenhouse decreased the incidence of charcoal rot caused by *M. phaseolina* and enhanced germination. Earlier, a number of researchers reported similar results by different *Trichoderma* species against different plant pathogens. Adekunle et al. (2001) showed that dressing of cowpea seeds with different *Trichoderma* spp. gave maximum plant stand. Dawar et al. (2008) observed a decrease in root rot infection and enhanced plant height and weight in *T. harzianum* coated seeds of okra and sunflower. Hussain et al. (1990) found that seed treatment with *T. harzianum, T. virens, Paecilomyces lilacinus* or *Streptomyces* spp. reduced *M. phaseolina* infection in sunflower and mungbean. Several fungi such as *M. phaseolina, F. semitecum, F. moniliforme* and *F. solani* infecting cowpea, horse gram, black and green gram have also been effectively controlled by seed treatment with conidial suspension of *T. harzianum* (Krishna et al. 2003). Rettinasbabady et al. (2000) found that *T. viride* treated black gram seeds decreased sclerotial formation of *M. phaseolina*. Similarly, the same fungus effectively controlled *R. bataticola in vitro* (Kaswate et al. 2003). Seed treatment and soil application with *T. virens* reduced root rot incidence in pigeon pea (Lokesha and Benagi 2007). Seed treatment with both *T. harzianum* and *T. viride* also reduced root rot incidence and increased growth in mungbean plants and hence regarded as a suitable method for controlling seed- and soil-borne fungi (Ashraf et al. 2006).

The improvement in germination was due to multiplication of antagonists on seed surface which prevented the fungal entry into seeds by instantly colonizing the roots (Chao et al. 1986; Raguchander et al. 1998). The enhanced germination might also be due to triggering of host plant cell by antagonists to synthesize growth hormones and toxic substances in large quantities which inhibited pathogenic fungi (Hendelsman and Eric 1996). Similar results by Pineda (2001) in sesame, Malik and Dawar (2003) in chickpea and mash bean also confirmed the present findings.

The application of *Trichoderma* species in soil as seed treatment could lead to the establishment and rapid build-up of these biocontrol agents in the soil which would assist in reducing the infectivity of soil-borne inoculum or suppression of the pathogen. This is particularly applicable for soil-borne pathogens such as *M. phaseolina* where soil drenching with chemicals is not only deleterious to the environment but is also practically not feasible due to high costs. Considering the cost of seed dressing chemicals which is about three times that of the indigenously pro-

duced biocontrol agents (like *Trichoderma* species tested in the present study), the use of these antagonistic fungi as seed treatment will not significantly affect the cost of production. Furthermore, the continuous application of these antagonistic fungi will enhance their rhizosphere population resulting in increase in disease suppressiveness of the soil. Therefore, the present study identified the antagonistic potential of indigenous *Trichoderma* species which can be used for the effective management of *M. phaseolina* in different agro-ecological zones of the country and can be incorporated in an integrated pest management strategy.

Conflict of interest U. Iqbal and T. Mukhtar declare that they have no competing interests.

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