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In vitro Characterization of *Trichoderma viride* for Abiotic Stress Tolerance and Field Evaluation against Root Rot Disease in *Vigna mungo* L.

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Abstract

Soil-borne phytopathogenic fungi pose serious threats to yield of several crops. Biological control is an eco-friendly approach in the effective management of crop diseases. *Trichoderma viride* is an important soil-borne fungus, which play an important role in antagonism by secretion of different hydrolytic enzymes. Black gram is an important pulse crop world-wide and its yield is severely affected by *Macrophomina* root rot. Abiotic stresses greatly influence the performance of biocontrol agents. *T. virid*e was evaluated for its *in vitro* abiotic stress tolerance ability and its field bioefficacy against root rot disease in blackgram. Growth of *T. viride* decreased with increasing in salinity, temperature and drought. *T. viride* effectively inhibited the growth of *R. solani* (45%) and *M. phaseolina* (40%) under *in vitro* conditions. *T. viride* was compatible with 0.25% mancozeb, 1.0% copper oxy chloride and metalaxyl. Among three doses, plants treated with 6 g.kg^{-1} of *T. viride* showed highest yield of 1375 kg.ha^{-1} and lowest root rot incidence of 14.77% which were statistically on par with 4 g.kg^{-1} *T. viride* treated plants. To conclude, this study identified an abiotic stress tolerant *T. viride* for effective management of root rot disease and enhanced yield of *Vigna mungo* when applied as seed dresser at a concentration of 4g Kg^{-1} under field conditions.

Introduction

Diseases caused by soil-borne phytopathogenic fungi pose serious threats to yield of several crops world-wide [1-2]. Biological control, the use of specific microorganisms that interfere with plant pathogens and pests, is a nature-friendly, ecological approach to overcome the problems caused by standard chemical methods of plant protection [3-4]. *Trichoderma* spp. are fungi that are present in nearly all agricultural soils and in other environments such as decaying wood. Major mechanisms involved in the biocontrol activity of *Trichoderma* spp. are competition for space and nutrients, production of diffusible and/or volatile antibiotics and hydrolytic enzymes like chitinase and β-1,3-glucanase. These hydrolytic enzymes partially degrade the pathogen cell wall and leads to its parasitization [5]. This process (mycoparasitism) limits growth and activity of plant pathogenic fungi. Different species of *Trichoderma* have the potential to control soil-borne plant pathogens more effectively than chemicals [6] and they also exhibit plant-growth-promoting activity [7,9]. Use of these fungi is not as harmful to the environment as chemical pesticides. They are present in substantial quantity in nearly all agricultural soils and in other environments such as decaying wood and their use is only now being recognized world over as an alternative in plant disease control [4].

Blackgram (*Vigna mungo* L.) is one of the important pulse crops gaining importance all over the world in recent years. It is rich in proteins and contains amino acids in higher quantities than any other cereals and pulses. It is affected by number of diseases caused by fungi, bacteria and viruses. Among them the root rot caused by *Macrophomina phaseolina* is a major barricade that leads to severe crop loss. Biocontrol of black gram root rot disease by *Trichoderma* spp. has been an alternative to chemical control [10].

Like crops are affected by abiotic stresses, microbes are also known to be affected by these conditions. However, successful deployment of these organisms in stressed ecosystems depend on their ability to withstand and proliferate under adverse environments such as drought, high temperatures, salt stress, mineral deficiency, chemical and heavy metal toxicity which are major problems in rainfed agro-ecosystems. The principal stress factors in India are drought or soil moisture stress, which adversely affects nearly two third area of arid and semi arid ecosystems. High temperatures, soil salinity/alkalinity, low pH and metal toxicity have a significant influence on the performance of agriculturally important microorganisms (AIMS). The selection and deployment of aims in stressed ecosystems therefore requires concerted research and technology development.

In view of this, we laid out an experiment carried out a study on abiotic stress tolerance of *T. viride* and its field bioefficacy in controlling root rot in black gram under typical rainfed conditions.

Material and Methods

Fungal culture

Trichoderma viride culture was procured from Centre for Plant Protection Studies, Tamilnadu Agricultural University (TNAU), Coimbatore, Tamilnadu, India and maintained by sub-culturing in Elad and Chat medium (g. Lit^{-1}) (mycological peptone-5.0; dextrose-10.0; KH2PO4-1.0; MgSO4-0.5; rose Bengal-0.5; chloramphenicol-0.1; agar-agar-20; ph-5.2). It was commercialized under the trade name 'Trikoraksha' 1% WP.

Screening for abiotic stress tolerance of *T. Viride*

High temperature tolerance: A 5mm disc of *T. Viride* was inoculated on the potato dextrose agar (PDA) (peeled potatoes-200gm, dextrose-10gm, distilled water-1000 ml with ph-6.5) and incubated at

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30°C, 45°C and 50°C. The results were recorded based on growth and sporulation pattern compared to control plates which were incubated at 30°C.

Salinity tolerance: A 5mm disc of *T. viride* was placed on 0.1, 0.5, 0.75, 1.25, 1.5, and 2.0 M NaCl amended in PDA medium and incubated at 30°C for 4 days. Percentage reduction of growth in salt amended media was calculated by using the formula (100 x A-B /A), where A is radial growth in control plate in 'mm' of the isolate and B is radial growth in salt amended plate.

Drought tolerance: A 5 mm disc of *T. viride* was transferred to the PDA media containing 10%, 20%, 30%, 35% and 40% of polyethylene glycol (PEG 6000 Da) and incubated at 30°C for 4 days. Percentage reduction of growth in PEG amended media was calculated as described above.

Agrochemicals

Poison food method [11] was adopted to study the compatibility of *T. viride* with chemical fungicides. 0.1%, 0.25%, 0.5% & 1% solutions of mancozeb, carbendazim, copper oxy chloride and metalaxyl were prepared filter sterilized and incorporated into PDA medium. A 5 mm disc of actively growing *T. viride* was transferred to each plate and incubated at 30°C for 4 days. Results were recorded by the percent reduction of growth compared to fungicide free inoculated plates.

Screening for antagonistic activity of *T. viride*

To evaluate the *in vitro* antagonistic activity against selected phytopathogens *viz., Botrytis ricini, Fusarium oxysporum, Macrophomina phaseolina, Sclerotium rolfsii and Pyricularia oryzae*. A 5 mm disc of actively growing culture was placed on the periphery of the malt-dextrose agar (g. Lit^{-1}) (peptone-2.0, malt extract-20.0, yeast extract-2.0, dextrose-5.0, agar-agar-20.0, ph-5.8) plate. Similarly test pathogen was placed on the opposite side of the plate and incubated at 30°C for 5 days. Antagonistic activity was measured as zone inhibition and growth reduction.

M. phaseolina production and soil inoculation

Rice grains were soaked in water for 30min, air dried and filled in 500 ml Erlenmeyer flasks @ 50g/ flask. The mouth of each flask was plugged with cotton wool and wrapped in aluminium foil before autoclaving at 121°C for 1 h for two consecutive days. After cooling, the flasks were inoculated with a 2cm mycelial plug cut from the periphery of a 7-day-old culture of *M. Phaseolina*. The inoculated flasks were incubated at 28±2°C for 15 days for the rice seeds to be completely colonized by the pathogen. The inoculum was stored at 4°C before use in the field. One hundred colonized rice seed were placed on PDA and incubated at 28±2°C for five days and cultures were examined under microscope for the presence of pathogen. The number of seeds showing the mycelial presence of the pathogen and percentage recovery of *M. Phaseolina* from the inoculant was calculated. Ten colonized rice seeds were introduced per planting hole to induce *M. Phaseolina* infection in field.

Field experimental design and seed treatment

A field trial was conducted in randomized block design (5x5 m^2) with one cultivar (*V. mungo* var. T9) in two different plots at Hayathnagar research farm (N 17.34 and E 78.59) of Central Research Institute for Dryland Agriculture, Hyderabad, India. The plot was contaminated with *M. phaseolina* as described, in which negative control of disease, three different dosages of *T. viride* (2.0, 4.0 and 6.0 g.kg^{-1} seed) as seed dresser were applied with 4 replications. Positive control of disease was maintained in healthy plot with 4 replications. Seeds were sown in plots with 45x15 cm spacing. Germination was recorded 10 days after sowing (DAS). The root rot incidence was recorded at 15 days interval till harvest. After maturity, dry weight of the plants and grain yield (kg/ha) were recorded.

Assessment of phytotoxicity and disease incidence

Observations on phytotoxicity were recorded on 1, 3, 7 and 14 days after seed treatment by recording the necrosis of leaf and stem region. The root rot incidence in black gram was assessed by morphological observation of the dead plant roots, followed by the isolation of pathogen under *in vitro* conditions. The percent of disease incidence was calculated based on the formula

$$\frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Results

Abiotic stress tolerance

High temperature tolerance pronounced effectively in the test strain. At 30°C 1.2x10^8 cfu/g were observed whereas at 45°C cfu were 3.8x10^7/ g and decreased further at 50°C (10^{7}cfu/g) (Figure 1).

In case of salinity, growth decreased significantly with increase in salt concentration in the medium. When compared to control plate, 0.1 M salt added medium had 92.9% growth followed by 0.5 M (85.6%), 0.75 M (69.4%), 1.25 M (35.2%), 1.5 M (21.1%) growth was observed and at 2 M salt concentration growth was completely inhibited (Figure 2).

Drought tolerance of the test isolate appeared to be more or less similar to other stresses (Figure 3). At 10% of PEG concentration growth was higher with only 1% decrease than control. Whereas, at 20% PEG concentration, 94.3% growth was recorded which, gradually decreased thereafter to 79.5% in 25% PEG and 68% in 30% and in 35% PEG 63% growth was observed (Figure 3). Profuse sporulation was observed in presence of 10 and 25% PEG concentration whereas, 25 and 30% PEG concentration showed scanty sporulation and at 35% PEG concentration sporulation was completely absent.

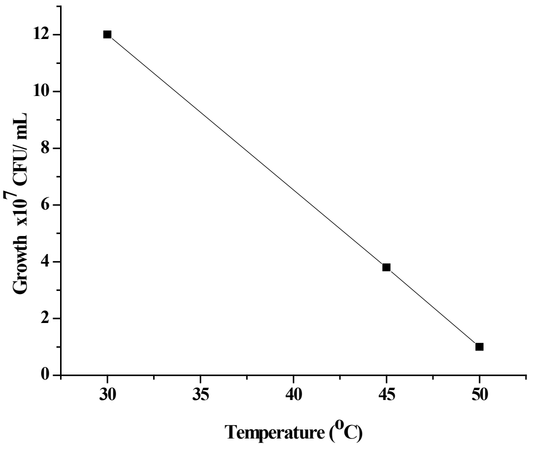

Figure 1: Temperature tolerance of *T. viride*.

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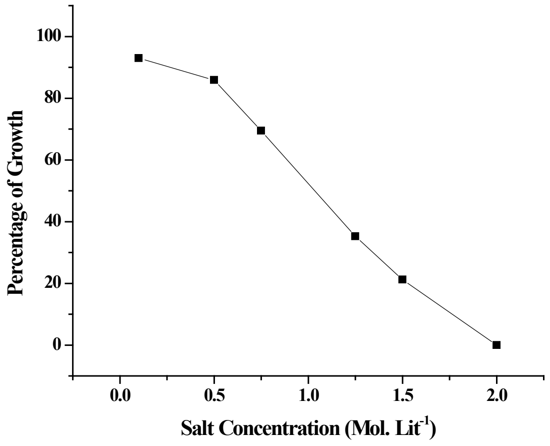

Figure 2: Salinity tolerance of *T. viride*.

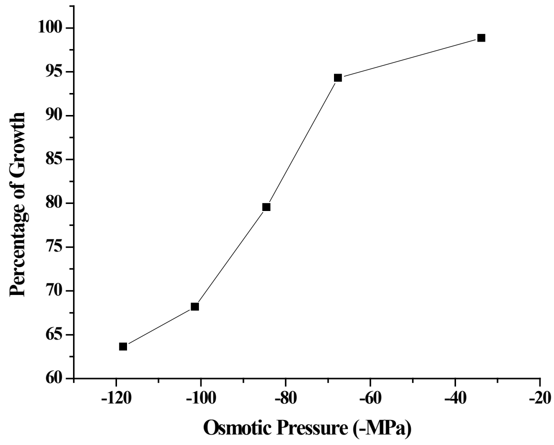

Figure 3: Drought tolerance of *T. viride*.

The test *T. viride* strain was also more tolerant to chemical fungicides. Among the tested fungicides, 0.5% mancozeb completely inhibited the growth of *T. viride*, whereas, 100% and 56% growth was recorded with 0.1% and 0.25% mancozeb respectively compared to control. Test strain was more compatible with copper oxy chloride (COC) than any other chemicals tested. *T. viride* was not inhibited at 0.1-0.5% of COC tested whereas, at 1% of COC the growth was reduced to 45%. In case of metalaxyl, 47% and 13.5% growth was recorded at 0.5% and 1% concentration respectively (Table 1).

In vitro antagonistic activity of *T. Viride*

The test isolate was also effective in inhibiting the growth of potential phytopathogenic fungi (Figure 4). *Botrytis ricini* growth was inhibited by 27% compared to control whereas, 31% inhibition of *Fusarium oxysporum* f. Sp. *ciceri*, *Macrophomina phaseolina* (40%), *Rhizoctonia solani* (45%), *Sclerotium rolfsii* (36%) were recorded whereas, in case of *Pyricularia oryzae* only 12% growth was inhibited (Figure 4).

Root rot disease control

Treatments	Percentage germination	Plant dry mass (gm)	Percent root rot incidence	Total yield (Kg/ha)
Trikoraksha (*T.viride*) 2 g/kg	91.48^{b}	17.23^{a}	18.32^{b}	1260^{b}
Trikoraksha (*T.viride*) 4 g/kg	97.30a	18.75^{a}	14.91^{a}	1370^{a}
Trikoraksha (*T.viride*) 6 g/kg	97.16^{a}	18.88^{a}	14.77^{a}	1375^{a}
Negative check (sick plot-control)	84.66^{c}	14.6^{b}	39.06d	870^{d}
Positive check (healthy plot)	90.20^{b}	16.98^{a}	21.73^{c}	1140^{c}
LSD	2.30	2.26	2.71	90.05
CV%	5.86	15.50	43.65	14.50

Values superscripted by same alphabet are not significantly different according to Fisher's least significance difference test (P<0.05).

Table1: Effects of different dosages of Trikoraksha (*Trichoderma viride*) on root rot management in black gram.

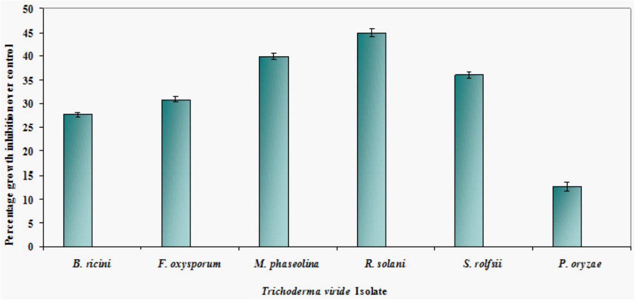

Figure 4: Antagonistic activity of *T. viride* test isolate against major phytopahtogens in vitro (bars indicate±SD).

Inorganic fungicides	Compatibility of *T. viride* with chemical fungicides in %			
	0.1%	0.25%	0.5%	1%
Mancozeb 75%	100	56.4	0.0	0.0
Carbendazim 50%	0.0	0.0	0.0	0.0
Copper oxy chloride 50 %	100	100	100	45.0
Metalaxyl 20%	100	100	47.2	13.5

Table 2: Compatibility of *T. viride* with chemical fungicides.

Vigna mungo treated with *Trichoderma viride* was able to effectively combat root rot disease in the conducted field trial, denoting the importance of *T. viride* in disease management. Highest seed germination of 97.3% was observed when *T. viride* was used at the concentration of 4g.Kg^{-1}, whereas at 6g.Kg^{-1} seed coating showed 97.1% germination. In case of plant dry mass, 6g.Kg-1 seeds showed highest dry mass of 18.88 gm followed by 4g. Kg^{-1} *T. viride* treated seeds. Highest disease incidence was observed in un-treated control plants with 39% root rot incidence whereas, lowest disease was observed where *T. viride*@6g.Kg^{-1} was applied. Highest yield of 1375 kg.Ha^{-1} followed by 1370 kg. Ha^{-1} was observed in the plots treated with 6g.Kg^{-1} and 4g.Kg^{-1} *Trichoderma* respectively. In case of plants sown in sick plot the yield was only 870 kg. Ha^{-1} (Table 2) compared to the seeds sown in healthy plot (1140 kg. Ha^{-1}). None of the treatments showed any adverse effect on the plant in terms of necrosis on leaves and stem which are common symptoms of phytotoxicity. Hence, the products could be safely recommended as seed dressers.

Discussion

The test *T. viride* isolate has the ability to withstand different abiotic stresses suggesting that the inoculant has better survival, efficacy, adaptability and thereby improving plant productivity under rainfed conditions. Widden and Hsu [12] observed that the ability of different species of *Trichoderma* to colonize pine or maple litter differed with temperatures. The reason behind evaluation of abiotic stress tolerance in the current strain was that the stress tolerant strains can be efficiently deployed in extreme environments where they can show better rhizosphere competence and saprophytic competitive ability. Interestingly, some of the abiotic stress tolerant microbes also protected plants from abiotic stresses like drought [13], chilling injury [14], high temperature [15], and salinity [16]. A few recent reports demonstrated that these fungi alleviate abiotic stresses. Field data indicates that they may confer tolerance to drought stress at least in part through promotion of deeper root penetration into the soil profile [17]. In a recent report, *T.hamatum* increased tolerance of cocoa plants to water deficit through increasing root growth that provided greater water resources to treated plants and delayed the onset of water deficit in these plants [18].

Results showed that *T. viride* could restrict the growth of potential phytopathogens in dual culture which prove its efficacy in management of crop diseases. The growth inhibition of tested pathogens may be due to antibiotic secretion of like trichodermin, dermadin, trichovirdin and sesquiterpene heptalic acid [19], nutrient impoverishment and ph alteration in the medium [20]. Hence*, T. viride* has a potential to develop as a biological agent to control the common post harvest diseases. The growth inhibition of postharvest 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 glucanases, endochitinases and chitinases and mycoparasitism [19,21,22].

There is scanty information about interactions between pesticides and those fungi which are antagonistic to various plant pathogens. Our experiments with routinely used chemical fungicides have confirmed the published reports and also have assessed the activity of different chemical fungicides on *T. viride*. This concluded that this bio-agent can be used in combination with COC, metalaxyl and mancozeb at determined dosages. This study also suggests that, even in chemical fungicides contaminated fields the organism has a better survival for enhancing crop productivity. There have been few reports on the effect of some pesticides on naturally occurring strains of *T. viride*: for example, Pribela et al. [23] have reported that benomyl and dichlofluanid are highly active against this fungal biocontrol agent. Baicu [24] identified some pesticides which can be used along with *T. viride* in integrated control of plant pathogens.

Results obtained from this study showed that *Trichoderma* species could be used effectively to control the root rot disease. This ability of the *Trichoderma* as a biocontrol agent was also reported by Upadhyay and Mukhopadhyay [25]. They reported that *T. harzianum* isolate IMI 238493 lyses the mycelia and sclerotia of *Sclerotium rolfsii*. Inbar et al. [26] also observed hyphal interaction between the mycoparasite, *T. harzianum* and the soilborne pathogen, *Sclerotinia sclerotiorum*. Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods [27]. *Trichoderma* spp. are now the most common fungal biological control agents that have been comprehensively researched and deployed throughout the world.

The inhibitory activity of *T. viride* against soil borne fungal pathogens found here were similar to the findings of [28,29]. The inhibitory effects observed here were mainly attributed to competition for space, nutrition between the pathogens and antagonists. Antagonists may also affect growth of pathogen either through antibiosis or mycoparasitism. Besides, they may also produce antifungal phenolic compounds [30].

Aggarwal et al. [31] reported isolates of *T. viride* improved growth of wheat crop such as shoot length, root length and 1000 gram weight. Singh and Singh [32] observed maximum disease reduction in pyrite treatment followed by neem cake. Similar type of results were recorded by Basu and Maiti [33] who reported that stem rot of potato was reduced by the amendments of NPK+FYM. The increase in yield in *Trichoderma* treated plots could be due to control of root rot and its plant growth promoting activities. Plant growth promoting activity of *T. harzianum* was well documented for its phosphate and micronutrient solubilization [34]. Highest yield was recorded in 6g.Kg^{-1} *T. viride* treatment which was statistically ($P<0.05$) on par to 4g.Kg^{-1} treatment. So, 4g.Kg^{-1} *T. viride* as seed treatment in *Vigna mungo* is optimum dosage for attaining disease control and maximum yield.

Conclusion

This study has identified a potential strain of abiotic stress, chemical fungicides tolerant *Trichoderma viride* capable of effectively controlling root rot disease in *Vigna mungo* L. And attaining maximum yield under field conditions when applied as seed dresser at a concentration of 4g.Kg^{-1}. This research opens a new way in disease management of rainfed crops for enhancing crop productivity in rainfed agro-ecosystems for the benefit of small and marginal farmers.

References

1. Punja ZK (1988) *Sclerotium* (Athelia) *rolfsii*, a pathogen of many plant species. Advances in Plant Pathology. Academic Press, San Diego, CA, USA.
2. Willetts HJ, Wong JAL (1980) The biology of *Sclerotinia sclerotiorum*, *S. Trifolium*, *S. Minor*, with emphasis on specific nomenclature. Bot Rev 46: 101–165.
3. Harman GE, Jin X, Stasz TE, Peruzzotti G, Leopold AC, et al. (1994) Method of increasing the percentage of viable dried spores of a fungus.
4. Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004) *Trichoderma* species-opportunistic, avirulent plant symbionts. Nat Rev Microbiol 2: 43-56.
5. Kubicek CP, Mach RL, Peterbauer CK, Lorito M (2001) *Trichoderma*: From genes to biocontrol. J Plant Pathol 83: 11-23.
6. Papavizas GC (1985) *Trichoderma* and *Gliocladium*: Biology, ecology and potential for biocontrol. Ann Rev Phytopathol 23: 23-54.
7. Harman GE, Bjorkman T (1998) Potential and existing uses of *Trichoderma* and *Gliocladium* for plant disease control and plant growth enhancement. *Trichoderma* and *Gliocladium*. Taylor and Francis, London, United Kingdom.
8. Kleifeld O, Chet I (1992) *Trichoderma harzianum*-interaction with plants and effect on growth response. Plant Soil 144:267–272.
9. Duffy BK, Simon A, Weller DM (1996) Combination of *Trichoderma koningii* with fluorescent pseudomonads for control of take-all on wheat. Phytopathology 86: 188-194.
10. Indra N, Gayathri S (2003) Management of blackgram root rot caused by *Macrophomina phaseolina* by antagonistic microorganisms. Madras Agric J 90: 490-494.
11. Das K, Tiwari RKS, Shrivastava DK (2010) Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. J Med Plant Res 4: 104-111.
12. Widden P and Hsu D (1987) Competition between *Trichoderma* species: effects of temperature and litter type. Soil Biol Biochem 19: 89-93.
13. Timmusk S, Wagner EGH (1999) The plant growth-promoting rhizobacterium *Paenibacillus polymyxa* induces changes in *Arabidopsis thaliana* gene expression: a possible connection between biotic and abiotic stress responses. Mol Plant Microbe Interact 12: 951-959.
14. Ait Barka E, Nowak J, Clement C (2006) Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth promoting rhizobacterium; *Burkholderia phytofirmans* strain psjn. Appl Environ Microbiol 72: 7246-7252.
15. Ali skz, Sandhya V, Minakshi G, Kishore N, Venkateswar Rao L, et al. (2009)

Pseudomonas sp. Strain AKM-P6 enhances tolerance of sorghum seedlings to elevated temperatures. Biol Fertil Soil 46: 45-55.

16. Han HS, Lee KD (2005) Plant growth promoting rhizobacteria effect on antioxidant status, photosynthesis, mineral uptake and growth of lettuce under soil salinity. Res J Agric Biol Sci 1: 210-215.
17. Harman GE (2000) Myths and dogmas of biocontrol: changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Dis 84: 377-393.
18. Bae H, Sicher RC, Kim MS, Kim SH, Strem MD, et al. (2009) The beneficial endophyte *Trichoderma hamatum* isolate DIS 219b promotes growth and delays the onset of the drought response in *Theobroma cacao*. J Exp Bot 60: 3279-3295.
19. Nakkeeran S, Krishnamoorthy AS, Ramamoorthy V, Renukadevi (2002) Microbial inoculants in plant disease control. J Ecobiol 14: 83-94.
20. Maheshwari DK, Dubey RC, Sharma VK (2001) Biocontrol effects of *Trichoderma virens* on *Macrophomina phaseolina* causing charcoal rot of peanut. Ind J Microbiol 41: 251-256.
21. Ramesh Sundar A, Das ND, Krishnaveni (1995) *In vitro* antagonism of *Trichoderma* spp. against two fungal pathogens of castor. Ind J Plant Prot 23: 152-155.
22. Thirumala Rao SK, Sitaramaiah K (2000) Management of collar rot disease (*Aspergillus niger*) in Groundnut with *Trichoderma* spp. J Mycol Plant Pathol 30: 221-224.
23. Pribela A, Kovac J, Savillova J (1976) Vyber fungicidov proti niekotorym hubam produkcjucim horke latky rajcin, jablek a hrusiek. Ochrana roslin 12: 37-44.
24. Baicu T (1982) Toxicity of some pesticide to *Trichoderma viride* pers. Crop Prot 1: 349-358.
25. Upadhyay JP, Mukhopadhyay AN (1986) Biological control of Sclerotium rolfsii by *Trichoderma harzianum* in sugar beet. Trop. Pest Manag 32: 215-220.
26. Inbar J, Menendez A, Chet I (1996) Hyphal interaction between *Trichoderma harzianum* and *Sclerotinia sclerotiorum* and its role in biological control. Soil Biol Biochem 28: 757-763.
27. Baker R, Paulitz TC (1996) Theoretical basis for microbial interactions leading to biological control of soil borne plant pathogens In: Principles and practice of managing soil borne plant pathogens. The American Phytopathol Soc St. Paul, MN.
28. Robert R, Ghisellini L, Pisi A, Flori P, Filippini G (1993) Efficacy of two species of *Trichoderma* as a biological control against *R. Solani* isolated from bean root rot in Italy. Adv Hort Sci 7: 19-25.
29. Abdollahzadeh J, Goltapeh EM, Rouhani H (2003) Evaluation of antagonistic effect of *Trichoderma* species in biological control of causal agents of crown and root rot of sunflower (*Sclerotinia minor*) *in vitro*. Agricultural Sciences Tabriz 13: 13-23.
30. Saba Banday, Dar GH, Ghani MY, Sagar V, Nasreen F (2008) *In vitro* interaction of bioagents against *Dematophora necatrix* and *Pythium ultimum* causing apple root rot in Jammu and Kashmir. SKUAST 10: 341-350.
31. Aggarwal R, Srivastava KD, Singh DV (2001) Biological control of loose smut of wheat: Seed treatment with *Trichoderma viride* and its influence on plant growth. Ann Plant Protec Sci 9: 63-67.
32. Singh R, Singh LB (2007) Evaluation of different soil amendments against *Sclerotinia* blight of brinjal. Ann Plant Protec Sci 15: 265-266.
33. Basu A, Maiti MK (2006) Role of host nutrition and varieties on the development of stem rot of potato. Ann Plant Protec Sci 14: 479-480.
34. Altomare C, Norvell WA, Bjorkman T, Harman GE (1999) Solubilization of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* rifai. Appl Environ Microbiol 65: 2926-2933.