WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 11, 662-672. Research Article ISSN 2277– 7105

IN VITRO SCREENING OF ANTAGONISTIC ACTIVITY OF FUNGAL BIOCONTROL AGENTS FOR THE MANAGEMENT OF *MACROPHOMINA PHASEOLINA* (TASSI) GOID IN *COLEUS FORSKHOLII* BRIQ.

***[1]Lily Trivedi and [2]Pravin Chandra Trivedi**

*[1]Assoc. Prof., Jayoti Vidyapeeth Women's University, Jaipur.

[2]Ex. Vice –Chancellor, D.D.U. Gorakhpur University.

Article Received on
11 April 2018,

Revised on 01 May 2018,
Accepted on 21 May 2018

DOI: 10.20959/wjpr201811-11324

***Corresponding Author**
Lily Trivedi
Assoc. Prof., Jayoti Vidyapeeth Women's University, Jaipur.

ABSTRACT

Macrophomina phaseolina (Tassi) Goid, isolated from diseased plants of *Coleus forskohlii* Briq *in vivo*, was evaluated by dual culture against antagonist fungal spp. Isolated from the rhizosphere of native fields of Rajasthan. All the 11 biocontrol fungi consisting of 9 *Trichoderma* sp. and *Gliocladium virens and Paecilomyces* significantly inhibited growth of *Macrophomina phaseolina* in dual culture experiments. The inhibiton ranged from 70-77% whereas other biocontrol fungi also showed good efficacy. Different isolates showed varying degrees of antagonism. Maximum growth inhibition of the test pathogen was caused by *Trichoderma harzianum* (T-2) and *Trichoderma hamatum* (T-8) followed by *Trichoderma aureoviride* (T-7) and *Trichoderma viride*(commercial) (T-11). The growth inhibition of *Trichoderma spp.* can be attributed to all the three modes of antagonism *in vitro* viz. competition, antibiosis and mycoparasitism.

KEYWORDS: *Macrophomina phaseolina* (Tassi) Goid, dual culture, biocontrol fungi, *Coleus forskohlii* Briq.

INTRODUCTION

Macrophomina phaseolina (Tassi) Goid., is an important root pathogen and causes dry root rot/stem canker, stalk rot or charcoal rot of over 500 plant species. *Coleus forskholii* Briq. (Syn *Coleus* barbatus Benth.) belonging to the family Lamiaceae is a well known medicinal plant grown throughout the country and is known as *Coleus*, Recently, Charcoal Rot of

Coleus caused by *Macrophomina bataticola* has been a disease of concern. Charcoal rot is an important disease during hot, dry weather or when unfavorable environmental conditions stress the plant. The incidence and severity of the disease is influenced by the antagonist interactions between the pathogenic fungi and the biocontrol fungi. *Trichoderma* have several modes of action which include direct and indirect effects. *Macrophomina phaseolina* (Tassi) Goid., is an important root pathogen and causes dry root rot/stem canker, stalk rot or charcoal rot of over 500 plant species.

The first report of the antagonist effect of *Trichoderma* against *Rhizoctonia solani* as a mycoparasite was given by Weindling.[1] Elad used *Trichoderma harzianum* in the biological control of *Macrophomina phaseolina* (Tassi.) Goid. and reported the growth inhibition in linear growth and microsclerotia production of *M. phaseolina* by four isolates of *T. harzianum invitro.*[2]

When hyphae of the antagonist *Trichoderma harzianum* approach those of the pathogenic fungus *Rhizoctonia solani*, they form branches which grow directly towards the host. Fibrillar material is deposited between the hyphae during the first two days of interaction. Vapour fixation and air drying of specimens enabled visualization of the delicate structures, without washing soluble material from the system. *T. harzianum* also produces hyphal coils over the interaction zone.[3]

Trichoderma viride causes loops, coiling and rupture of cell walls of the pathogen. *G. virens* causes loops, coiling and rupture of cell walls of the pathogens hyphae while *Trichoderma harzianum* causes twisting, air bubbling and disintegration of the pathogen hyphae while *Trichoderma harzianum* caused severe vacuolation, shrinkage and co-agulation of the cytoplasm of the pathogen hyphae. Microscopic examination at the point of contact of two fungi revealed that the overgrowing mycelium on the antagonist penetrated the mycelium of the pathogen and the tip of the hyphae of the pathogen swelled and curled.[4] Biological antagonists evaluated *Trichoderma viride, Trichoderma harzianum* and *Gliocladium virens* against *Rhizoctonia solani invitro* dual culture technique and found *Gliocladium virens* the most effective followed by *Trichoderma viride*, in inhibiting mycelia growth and sclerotial production.[5]

The fungus antagonists like *Trichoderma viride, Trichoderma harzianum, Trichoderma hamatum, Trichoderma koningii, Trichoderma pseudokoningii, Trichoderma*

longibrachiarum and *Gliocladium virens* were evaluated against black gram root rot fungus *Macrophomina phaseolina. Trichoderma harzianum* and *Trichoderma longibrachiarum* were on par in controlling *Macrophomina phaseolina*.[6]

The effects of volatile and diffusible compounds of two *Trichoderma* spp. (*Trichoderma harzianum* and *Trichoderma viride*) isolates against coleus root rot pathogens like *R. solani* and *Macrophomina phaseolina* were studied and were found to effectively inhibit the mycelial growth and sclerotial production of *R. solani* and *Macrophomina phaseolina*.[7]

Twenty-six local isolates of *Trichoderma* spp from Kerala were evaluated for their antagonistic activity *in vitro* against *R. solani*. Different isolates showed varying degrees of antagonism. The two most antagonistic isolates against *R. solani* were *T. pseudokoningii* TR17 and *T. harzianum* TR20.[8] *Trichoderm. viride* was best in inhibiting *R. bataticola* found by Ramaprasad Shresty.[9]

The efficacy of four fungal bioagents viz., *Trichoderma hamatum*, *T. harzianum*, *T. polysporum* and *T. viride* were evaluated *in vitro* condition against the Eggplant root-rot pathogen, *Macrophomina phaseolina*. Among the bioagents, *T. harzianum* produced the maximum inhibition zone of 18.20 percent compared to the minimum of 7.30 per cent by *T. hamatum*.[10]

Six isolates of *Trichoderma harzianum* were tested as potential bio-control agents under dual culture plate technique and inhibition through volatile substances for their efficacy against *Macrophomina phaseolina*.[11] The efficacy of microbial antagonist *Trichoderma viride* Pers. was evaluated for its effect on plant growth promotion and against *Macrophomina phaseolina* (Tassi) Goid and in dual culture assays they inhibited the growth of *M. phaseolina*.[12]

According to Monteiro, *Trichoderma harzianum* ALL42 were capable of overgrowing and degrading *Rhizoctonia solani* and *Macrophomina phaseolina* mycelia, coiling around the hyphae with formation of appressoria and hook-like structures. Hyphae of *T. harzianum* ALL42 did not show any coiling around *Fusarium* sp. hyphae suggesting that mycoparasitism may be different among the plant pathogens.[13]

A commercial formulation of *Trichoderma viride* and *Pseudomonas fluorescens* under *in vitro* conditions inhibited the mycelial growth of *M. phaseolina* isolates in *Gloriosa superba*.[14]

In vitro potentialities of seven species of *Trichoderma* were evaluated against phytopathogen *Macrophomina phaseolina* by dual culture techniques. Microscopic study showed that these two antagonists were capable of overgrowing and degrading *M. phaseolina* mycelia, coiling around the hyphae with appressoria and hook-like structures.[15]

Various experiments on dry root rot of black gram and their management were carried out by using *Trichoderma sp.*[16] Biological control capability of 11 *Trichoderma* spp. isolates, against *M. phaseolina* showed inhibition of 20.22 - 58.67% in dual culture tests *in vitro.*[17] The efficacy of various biocontrol agents against *Macrophomina phaseolina* (Tassi.) Goid causing root rot of greengram were studied The antagonism studies revealed that among six biocontrol agents *T. harzianum*-2 showed maximum inhibition and superiority over the other biocontrol agents followed by *T. harzianum*-3 and *T. koningii.*[18]

In vitro efficacy of some *Trichoderma* isolates against the pathogenic fungi, *Alternaria alternata* (Fr.) Keissl., *Penicillium* sp *and Aspergillus niger* Van Tiegh known to infect tomato fruits were studied in Kashmir. The maximum growth inhibition of *Alternaria alternata* and *Aspergillus niger* was due to *Trichoderma* isolate, PPT3 followed by isolate PPT1 and isolate PPT2 respectively.[19] Twenty four fungal biocontrol agents and twelve bacterial biocontrol agents were screened for their efficacy against phytopathogenic fungi *Rhizoctonia solani* through dual culture technique.*Trichoderma harzianum*-1 and *Pseudomonas fluorescence*-2 were found effective in inhibition of mycelium of *Rhizoctonia solani* under *in vitro* conditions.[20]

MATERIALS AND METHOD

Macrophomina phaseolina was isolated from diseased plants of *Coleus forskohlii* Briq *in vivo*. Antagonist fungal spp. was isolated from the rhizosphere of native fields of Jaipur and Udaipur. Isolations were done in medium specific for fungi[21] and *Trichoderma*[22] by soil plate technique.[23] All cultures were maintained on PDA at 28±2^{0}C.

T-1 = *Trichoderma harzianum* 1 (Jaipur)

T-2 = *Trichoderma harzianum* 2 (Jaipur)

T-3 = *Trichoderma harzianum* 3 (Jaipur)

T-4 = *Trichoderma harzianum* 4 (Jaipur)

T-5 = *Trichoderma harzianum* 5 (Jaipur)

T-6 = *Trichoderma harzianum* (Udaipur)

T-7 = *Trichoderma aureoviride*

T-8 = *Trichoderma hamatum*

T-9 = *Gliocladium virens*

T-10 = *Paecilomyces*

T-11 = *Trichoderma viride* (commercial)

Dual culture method- Method given by Wood was followed for examining the interactions 3mm discs of *Macrophomina phaseolina* and test fungus in triplicate Petri plates on PDA from their 7 days old cultures.[24] The plates were then incubated for 7 days at 28±2^{0}C. Control plates were kept simultaneously. The colony grows on both sides i.e. towards and opposing each other from loci was measured. The parameters used for the assessment of colony interaction were width of inhibition or intermingled zone between both colonies. The per-cent inhibition of radial growth was calculated by using Fokkema[25] formula:-

% Inhibition = 100 x r_1 - r_2 / r_1

r_1= radial growth of *Macrophomina phaseolina* in control.

r_2 = radial growth of *Macrophomina phaseolina* in dual inoculation.

Visual Ranking of growth was done according to Bell's ranking scale.[26]

R_1 = complete overgrowth.

R_2 = 75% overgrowth.

R_3 = 50% overgrowth.

R_4 = blocked at point of contact.

R_5 = pathogen overgrowing antagonist.

Table- *Invitro* antagonism of biocontrol fungi. against *Macrophomina phaseolina*

		Pathogenic Fungi	Test Fungi	Interaction	% Growth Inhibition	Grade
S.no	Biocontrol Fungi	Control	Average	Average	Average	
1	T_1	90	39.33	25.00	72.22	R3
2	T_2	90	40.67	20.00	77.78	R4
3	T_3	90	36.67	26.67	70.37	R3
4	T_4	90	27.33	40.00	55.56	R2
5	T_5	90	38.33	23.33	74.07	R4
6	T_6	90	42.67	23.67	73.70	R2
7	T_7	90	40.00	20.67	77.04	R2
8	T_8	90	45.67	20.00	77.78	R1
9	T_9	90	38.67	23.33	74.07	R2
10	T_{10}	90	13.00	43.33	51.85	R4
11	T_{11}	90	39.33	21.00	76.67	R1
CD at 5%			1.023175	1.371541	1.52422	

R1= complete overgrowth.

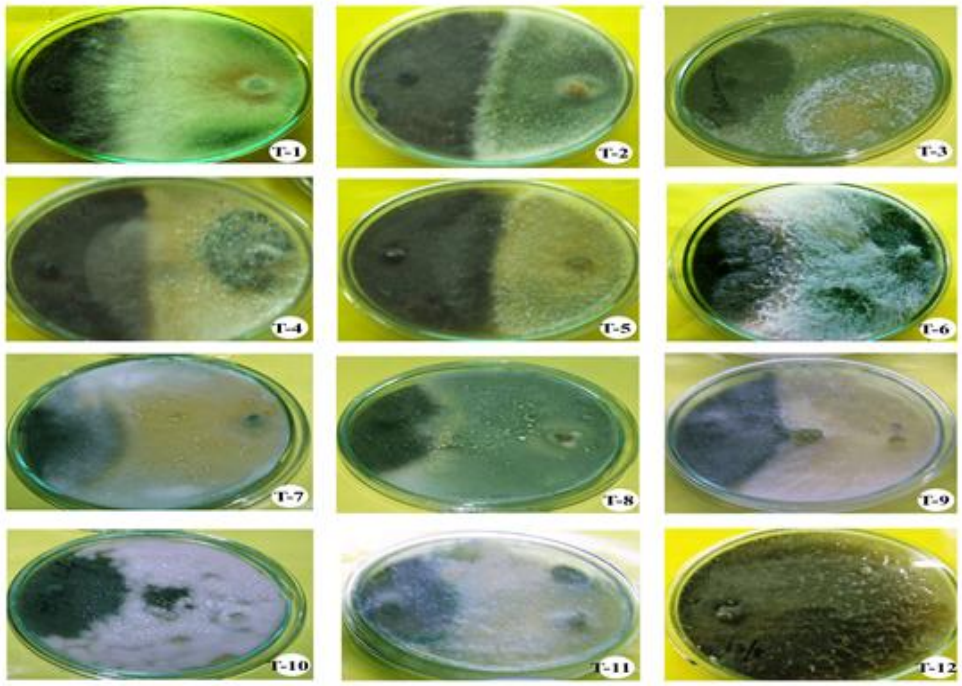
R2= 75% overgrowth.

R3= 50% overgrowth.

R4= blocked at point of contact.

R5= pathogen overgrowing antagonist

T-1=*Trichoderma harzianum* 1 (Jaipur)
T-2=*Trichoderma harzianum* 2 (Jaipur))
T-3=*Trichoderma harzianum* 3(Jaipur)
T-4=*Trichoderma harzianum* 4(Jaipur)
T-5=*Trichoderma harzianum* 5(Jaipur)
T-6=*Trichoderma harzianum* (Udaipur
T-7=*Trichoderma aureoviride*
T-8=*Trichoderma hamatum*
T-9= *Gliocladium virens*
T-10=*Paecilomyces*
T-11=*Trichoderma viride*(commercial)



T-1 = *Trichoderma harzianum* 1 (Jaipur) T-2 = *Trichoderma harzianum* 2 (Jaipur))
T-3 = *Trichoderma harzianum* 3 (Jaipur) T-4 = *Trichoderma harzianum* 4 (Jaipur)
T-5 = *Trichoderma harzianum* 5 (Jaipur) T-6 = *Trichoderma harzianum* (Udaipur)
T-7 = *Trichoderma aureoviride* T-8 = *Trichoderma hamatum*
T-9 = *Gliocladium virens* T-10 = *Paecilomyces*
T-11 = *Trichoderma viride* (commercial) T-12 = *Macrophomina phaseolina*

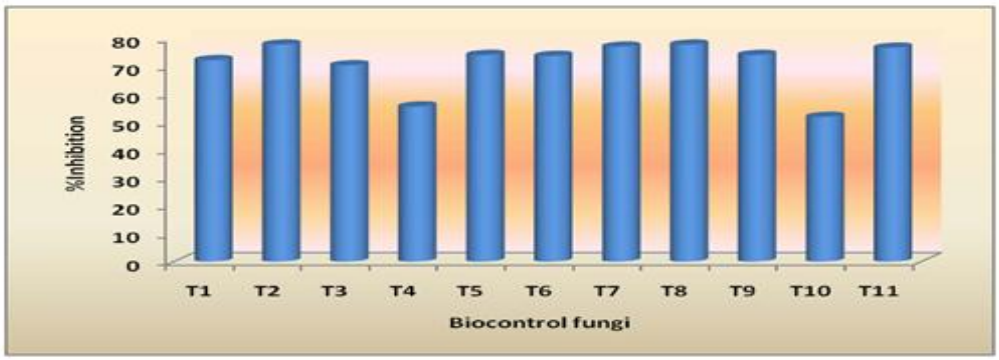
In vitro **antagonism of biocontrol fungi against** ***Macrophomina phaseolina***

PLATE-18

DUAL CULTURE GRAPH

Graph-9

In vitro* antagonism of biocontrol fungi against *Macrophomina phaseolina



T-1 = *Trichoderma harzianum* 1 (Jaipur)
T-2 = *Trichoderma harzianum* 2 (Jaipur)
T-3 = *Trichoderma harzianum* 3 (Jaipur)
T-4 = *Trichoderma harzianum* 4 (Jaipur)
T-5 = *Trichoderma harzianum* 5 (Jaipur)
T-6 = *Trichoderma harzianum* (Udaipur
T-7 = *Trichoderma aureoviride*
T-8 = *Trichoderma hamatum*
T-9 = *Gliocladium virens*
T-10 = *Paecilomyces*
T-11 = *Trichoderma viride* (commercial)

RESULTS AND DISCUSSION

All the 11 biocontrol fungi consisting of 9 *Trichoderma* sp. of which six were *T. harzianum*, significantly inhibited growth of *Macrophomina phaseolina* in dual culture experiments. All isolates showed varying degree of antagonism. Different species of *Trichoderma* sp. and *T. harzianum* gained considerable success *M. phasoelina* infection on a number of crops.[6,7,17,20]

Trichoderma spp. are among the most promising biocontrol agent. It is also reported to be one of the most widely distributed soil fungi and the biocontrol potential has been extensively studied. Screening of potential *Trichoderma* strains was done by many workers[14,16,18,20] against major root pathogens.

Results showed maximum growth inhibition by *Trichoderma harzianum* (T-2) and *Trichoderma hamatum* (T-8) followed by *Trichoderma aureoviride* (T-7) and *Trichoderma viride*(commercial) (T-11). *T. harzianum* & *T. hamatum* gave maximum inhibition and *Trichoderma harzianum* is a good biocontrol antagonist against *Macrophomina phaseolina*[6,10], [Fig and Table] Biological antagonists *Trichoderma viride, Trichoderma harzianum* and *Gliocladium virens* against *Rhizoctonia solani in vitro* in dual culture technique used by[5] found *Gliocladium virens* the most effective followed by *Trichoderma viride*, in inhibiting mycelia growth and sclerotial production. Anis also obtained best results

with *Trichoderma viride*.[12] Many recent workers have also found *Trichoderma harzianum* as a good biocontrol agent.[15,20]

The growth of pathogenic fungi was arrested on coming in contact with biocontrol fungi. Some stopped at line of contact and produced showed visible zone of inhibition. Others showed overgrowth in which some biocontrol fungi were fastgrowing (masking the growth of pathogenic fungi) whereas some were slow growing. This overgrowth may be due to its fast growing nature, rapid sporulation or secretion of cell wall lytic enzymes in dual culture. *Trichoderma harzianum* T2 showed visible zone of inhibition while there was a distinct change in colour at the zone of contact which became light yellowish after sometime and also gave good inhibition. Coiling of antagonist hyphae and lysis in different species of *Trichoderma* was present. *T. harzianum* secretes substances into the growth medium, which inhibited growth of micro- identified by producing zones of inhibition. [Plate].

Since most of the fungi have chitin and a (1-3) glucanase enzymes that degrade the cell wall, lysis of hyphae was observed when hyphae of the antagonist *Trichoderma harzianum* approached those of the pathogenic fungus, they form branches which grow directly towards the host. Fibrillar material was deposited between the hyphae during the first two days of interaction. *T. harzianum* also produces hyphal coils over the interaction zone. *Trichoderma viride* causes loops, coiling and rupture of cell walls of the pathogen. Same were also observed in case of *Trichoderma viride* and *T. harzianum*.[3,4]

Gliocladium virens (T-9) and *Trichoderma harzianum* (T-5) along with *Trichoderma harzianum* T-6 and *Paecilomyces* (T-10) also gave good inhibition. [Table and Chart] *G. virens* causes loops, coiling and rupture of cell walls of the pathogens hyphae while *Trichoderma harzianum* causes twisting, air bubbling and disintegration of the pathogen hyphae along severe vacuolation, shrinkage and co-agulation of the cytoplasm of the pathogen hyphae. Microscopic examination at the point of contact of two fungi revealed that the overgrowing mycelium on the antagonist penetrated the mycelium of the pathogen and the tip of the hyphae swelled with formation of appressoria and hook-like structures. The hyphal coiling and production of inhibitory substances by pathogen. Coiling of antagonist hyphae and lysis was also observed. Similar results were obtained with *T. koningi* and *T. harzianum* against phytopathogen *Macrophomina phaseolina*.[15]

Anis[12] evaluated the efficacy of microbial antagonists viz., *Paecilomyces variotii* Bainier, *Trichoderma viride* Pers., and found all antagonists inhibited the growth of *M. phaseolina*.. The combination of hydrolytic enzymes and antibiotics results in a higher level of antagonism than that obtained by either mechanism alone. Thus, various isolates showed varying degree of antagonism. The growth inhibition of presence of *Trichoderma* spp. could be attributed to all the three modes of antagonism *in vitro* viz. competition, antibiosis and mycoparasitism.[Table and Graph] Similar findings have been observed by other researchers.[8,10,11,14,15,20]

CONCLUSION

All the biocontrol fungi, significantly inhibited growth and *Trichoderma harzianum* 2 (Jaipur) and *Trichoderma hamatum* gave best results and was equally effective to their screened commercial market formulations. *Trichoderma harzianum* 2 (Jaipur), *Trichoderma hamatum, Trichoderma aureoviride* and *Trichoderma viride* (commercial) can be considered in use for biocontrol of *Macrophomina phaseolina*.

ACKNOWLEDGEMENT

The author Lily Trivedi is thankful to Botany Deptt, Univ. of Rajasthan for providing Departmental Scholarship during the research work.

REFERENCES

1. Weindling, R Trichoderma lignorum as a parasite of other soil fungi. Phytopathology, 1932; 22: 837-845.
2. Elad Y, Zvieli Y, Chet I. Biological control of Macrophomina phaseolina (Tassi) Goid By Trichoderma harzianum. Crop Protection, 1986; 5: 288–292.
3. Elad Y, Sadowsky Z, Chet I. Scanning electron microscopic observations of early stages of interaction of Trichoderma harzianum and Rhizoctonia solani. Transactions of the British Mycological Society, 1987; 88: 259-263.
4. Pandey, KK, Upadhyay, JP. Microbial population from rhizosphere and non-rhizosphere soil of pigeonpea: Screening for residents antagonists and mode of mycoparasitism. Indian J Mycol Pl Path, 2000; 30: 7-10.
5. Dubey, S C. Biological management of web-blight of ground (Rhizoctonia solani). J Mycol Pl Path, 2000; 30: 89-90.
6. Indra, N, Chitra, K, Kamalakannan, A. In vitro screening of fungal antagonists against Macrophomina phaseolina in blackgram Annual Meeting and Symposium on Recent

Developments in the Diagnosis and Management of Plant Diseases for Meeting Global Challenges, 18-20 December 2003, held at Department of Plant Pathology, University of Agricultural Sciences, Dharwad, p 28.

7. Kamalakannan, A, Mohan, L, Amutha, G, Chitra, K, Prathibha, VK, Rajinimala, N, Mareeswari, P, Angayarkanni, T. Effect of volatile and diffusible compounds of biocontrol agents against Coleus forskohlii root rot pathogens Annual Meeting and Symposium on Recent Developments in the Diagnosis and Management of Plant Diseases for Meeting Global Challenges, 18-20 December 2003, held at Department of Plant Pathology, University of Agricultural Sciences, Dharwad, pp 92-93.
8. Rini, CR, Sulochana, KK. Usefulness of Trichoderma and Pseudomonas against Rhizoctonia solani and Fusarium oxysporum infecting tomato. J Trop Agric., 2007; 45: 21–28.
9. Ramaprasad Shresti AY. Studies on collar rot complex of Coleus forskohlii (Wild) Briq. MSc ThesisUniversity of Agricultural Sciences, Dharwad, 2005.
10. Ramezani, H Biological Control of Root-Rot of Eggplant Caused by Macrophomina phaseolina. American-Eurasian J Agric & Environ Sci., 2008; 4: 218-220.
11. Shekhar, Meena, Kumar, Sangit. 'Potential biocontrol agents for the management of Macrophomina phaseolina, incitant of charcoal rot in maize', Archives Of Phytopathology And Plant Protection, 2010; 43: (4) 379 — 383, First published on: 07 February 2008 (iFirst).
12. Anis, M, Abbasi, M W, Zaki, M J. Bioefficacy of microbial antagonists against Macrophomina phaseolina on sunflower. Pak J Bot, 2010; 42: 2935-2940.
13. Monteiro, Valdirene; Nascimento Silva, Roberto; Steindorff, Andrei; Costa, Fabio; Noronha, Eliane; Ricart, Carlos; Sousa, Marcelo; Vainstein, Marilene and Ulhoa, Cirano. New Insights in Trichoderma harzianum Antagonism of Fungal Plant Pathogens by Secreted Protein Analysis. Current Microbiology, 2010; 61: 298-305.
14. Alice, D, Sundravadana, S. Effects of biocontrol agents and plant products Macrophomina phaseolina and colchicine content in Gloriosa superb. Plant Protect Sci, 2012; 48: 110–115.
15. Gajera HP, Bambharolia RP, Patel SV, Khatrani TJ, Goalkiya BA. Antagonism of Trichoderma spp against Macrophomina phaseolina: Evaluation of Coiling and Cell Wall Degrading Enzymatic Activities. J Plant Pathol Microb., 2012; 3: 149.

16. Karthikeyan, V, Brindha, S, Annadurai, B, Gangwar, S K. Biological control of Macrophomina phaseolina root rot in Vigna mungo with Trichoderma International Journal of Advanced Biological Research, 2015; 5: 118-127.
17. Khaledi, Nima, Parissa Taheri. Biocontrol mechanisms of Trichoderma harzianum against soybean charcoal rot caused by Macrophomina phaseolina. Journal of Plant Protection Research, 2016; 56: 21-30.
18. Gojiya S K, Akbari LF, Chudasama M K. Evaluation of Biocontrol Agents Against Macrophomina phaseolina in vitro. Advances in Life Sciences, 2016; 5: 2718-2720.
19. Jahangir A K, Abdul Hamid Wani, Mohd Y B, Shazia Parveen. In Vitro Efficacy Of Trichoderma Isolates Against Some Fungi Causing Fungal Rot Disease Of Tomato, 2017; Int J Adv Res., 5: 2050-2053.
20. Rajendraprasad M, Vidyasagar B, Uma Devi G, Koteswar Rao S R. In vitro Evaluation of Fungicides and Biocontrol Agents against Rhizoctonia solani in Tomato IJPSS, 2017;, 2017; 17: 1-9 .
21. Martin James P. Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. Soil Science, 1950; 69: 215-232.
22. Elad Y, Chet I. Improved selective media for isolation of Trichoderma spp or Fusarium spp. Phytoparasitica, 1983; 11: 55-58.
23. Parkinson, D. New methods for qualitative and quantitative study of fungi in the rhizosphere. Pedologie, 1957; 7: 146-154.
24. Wood, R K S. The control of diseases of lettuce by use of antagonistic organisms I The control of Botrytis cinerea pers Annl. Appl Biol., 1951; 38: 203.
25. Fokkema, N J. The role of saprophytic fungi in antagonism against Drechslera sorokiniana on agar plates and on rye leaves with pollen. Physiol Pl. Pathol., 1973; (3): 195-205.
26. Bell, DK, Wells, HD and Markham, CR. Invitro antagonism of Trichoderma spp against six fungal plant pathogens. Phytopathology, 1982; 72: 379–382.