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Evaluation of antagonists against *Macrophomina phaseolina* causing root rot of groundnut

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Efficacy of antagonists isolated from rhizosphere was tested against different isolates of *Macrophomina phaseolina* collected from groundnut growing areas of Rajasthan and adjoining states. One isolate of *Trichoderma harzianum,* one isolate of *Trichoderma viride* and one isolate of *Bacillus subtilis* gave distinct antagonistic reactions against all the isolates of *M. phaseolina* in dual culture plate method. *T. viride, T. harzianum* and *Bacillus sp.* minimized root rot incidence in groundnut up to 73.34, 66.67 and 53.34%, respectively, when compared to control where 100% mortality was observed in pots. *T. viride* was most effective with organic amendments followed by *T. harzianum* and *B. subtilis. T. viride* along with neem (*Azadirachta indica*) cake minimized the disease up to 73.58% under field conditions.

Key words: Antagonists, biocontrol, groundnut, *Macrophomina phaseolina*, organic amendments.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a strategic crop for food security and cash earnings which occupies a pre-eminent position in the national edible oil economy of India, in parts of Asia and Africa. The major groundnut growing states in India are Gujarat, Rajasthan, Tamil Nadu, Andhra Pradesh, Maharashtra and Uttar Pradesh. Rots caused by a complex of soil-inhabiting fungi have been found to cause serious reduction both in yield and quality of groundnut. Soil and seed borne diseases like collar rot (*Aspergillus niger* Van Tieghem), root rot [*Macrophomina phaseolina* (Tassi) Goid. (Mp) (*Rhizoctonia bataticola* - imperfect stage)] and crown and stem rot (*Sclerotium rolfsii* Sacc.) cause severe seedling death resulting in 'Patchy' crop stand in sandy loam soils and reduced pod yields between 25 to 50% (Ghewande et al., 2002). The incidence of Mp has been reported 21% for the first time in Gujarat state (Anonymous, 1988). Due to high temperature and dry conditions prevailing in sandy soils of Rajasthan, soil-borne pathogens flourish well and cause a number of serious diseases. Root rot of groundnut induced by Mp has been observed to cause considerable loss to the crop in Rajasthan. The disease is of wide occurrence in sandy soil of Rajasthan where climatic conditions are dry and temperature remains high. The pathogen being soil-borne and its propagules distributed randomly in soil is difficult to be controlled by fungicide. Moreover, the fungicides are effective only on the active metabolic stage of the propagules and

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Table 1. *Macrophomina phaseaolina* isolates identification

Code	Place of collection of isolates
AJM	Agricultural Research Sub-Station, Tabiji (Ajmer)
AWR	Agricultural Research Station, Navgaon (Alwar)
BKN	Agricultural Research Station, Beechwal (Bikaner)
CUR	Farmer's field - Momasar (Churu)
DO	Farmer's field - Lalsot (Dausa)
DLI	Indian Type Culture Collection, New Delhi
HSR	CCS HAU, Hisar
HMH	Agricultural Research Sub-Station, Hanumangarh
JJN	Farmer's field - Kithana (Jhunjhunu)
NRNL	Farmer's field - Narnaul (Haryana)
NHR	Farmer's field - Nohar
SRDR	Farmer's field - Sardarshahar
SWM	Farmer's field - Swaimadhopur
UDZ	Rajasthan College of Agriculture, Udaipur

not on resting structure. Soil application of fungicides is an expensive and deleterious to non target microflora. Biological control has become a critical component of plant disease management and it is a practical and safe approach in various crops (Patel and Anahosur, 2001). Bioprotectants provide unique opportunity for crop production, since they grow, proliferate, colonize and protect the newly-formed plant parts to which they were not initially applied (Harman, 1991). Microorganisms isolated during the course of studies were tested for antagonism to *M. phaseolina.* The microorganisms having such properties were utilized for possible biological control of groundnut root rot by addition of the cultures of antagonists to infested soil along with certain organic amendments.

MATERIALS AND METHODS

Isolation of *M. phaseolina*

The diseased plants collected from different locations of Rajasthan and adjoining states were used for isolation. The fungal pathogen *M. phaseolina* (Mp) was isolated from infected roots of groundnut plants on potato dextrose agar (PDA) plates and incubated at 28°C for four to six days. Stock culture of *M. phaseolina* was maintained on PDA slants and stored at 4°C. All the isolates were identified during this research to avoid confusion (Table 1).

Isolation of antagonists from rhizospheric soil

Rhizospheric soil from healthy plants of groundnut was collected in poly-ethylene bags and brought to the laboratory. Appropriate dilutions (10^{-4} for fungi and 10^{-6} for bacteria) of these soil suspensions were plated on PDA (potato dextrose agar) and NA (nutrient agar) for the isolation of fungi and bacteria respectively. The plates were incubated for 72 h at 28±2°C. The isolated colonies developed were then purified on nutrient agar slants and used for screening against the pathogen for biocontrol ability.

Testing of antagonists to *M. phaseolina in vitro*

Trichoderma sp. and *Bacillus subtilis* isolated from rhizosphere were tested for their antagonism to *M. phaseolina* on Czapek's sucrose nitrate agar medium (Agar-agar 15.0 g, sodium nitrate 2.0 g, dipotassium hydrogen phosphate 1.0 g, magnesium sulphate 0.50 g, potassium chloride 0.50 g, ferrous sulphate 0.01 g, sucrose 30.0 g and distilled water 1000 ml) in Petri dishes.

Dual culture method

The antagonistic potential of each fungal antagonist was studied by dual culture method (Dennis and Webster, 1971). A 5 mm diameter disc of antagonist was placed individually at one end of the petridish containing Czapek's sucrose nitrate agar medium and just opposite to that a 5 mm diameter disc of the pathogen (*M. phaseolina*) was placed. Three replications were maintained for each antagonist. In control, the pathogen alone was inoculated. The Petri dishes were incubated at 28+1°C for seven days in a biological oxygen demand (BOD) incubator and observations were recorded.

Paper disc plate method

For bacterium *B. subtilis,* Paper disc plate method (Loo et al., 1945) was followed. Circular disc (5 mm diameter) of Whatman filter (No. 42) were cut and after dipping in bacterial suspension were placed 1 cm inward from the periphery of Petri dishes at four equidistance places, having in the centre the inoculum of pathogen (*M. phaseolina*). The inoculated dishes were placed in incubator at 28+1°C for a week.

Radial growth of Mp was recorded and inhibition percentage was calculated using formula:

$$\text{Percent growth inhibition} = \frac{C - T}{C}$$

Where C = Radial growth of *M. phaseolina* in the control (mm); T= Radial growth of *M. phaseolina* in presence of antagonist (mm).

Antagonism in green house conditions

The highly pathogenic isolate (BKN) of Mp was multiplied on sand maize medium (10 g maize flour, 90 g sand and 20 ml distilled water in each flask and was autoclaved in 250 ml Erlenmeyer's flasks) for 15 days. The antagonistic microorganisms *T. harzianum, T. viride* and *B. subtilis* were also multiplied on overnight soaked sterilized sorghum grains side by side. The separate media for pathogen and antagonists were taken with the view that they will not utilize the media of each other for growth and multiplication in pots. After multiplication of both organisms on their respective media, Mp and antagonist were added in equal quantity in pots filled with sterilized soil. Seeds of highly susceptible variety RSB-87 after surface sterilization with 0.1% mercuric chloride were sown in each pot and five plants in each pot were maintained after germination. A control with only the pathogen was maintained. The disease was recorded till there was 100% mortality in control pots. Soil sample for isolation of antagonists and pathogen were also taken at two stages; pre sowing in unsterilized soil and after 100% mortality in control in sterilized soil.

For enumeration and isolation of fungi, Martin's peptone dextrose agar medium (Martin, 1950) (Agar agar, 20.0 g; potassium dihydrogen phosphate (KH_2PO_4), 1.0 g; magnesium sulphate

Table 2. Effect of antagonists on the growth of *Macrophomina phaseolina in vitro*

Isolate	Mycelial growth inhibition (%)			
	T. harzianum	***T. viride***	***Bacillus* sp.**	**Mean**
AJM	75.38 (60.26)	70.83 (57.32)	38.46 (38.33)	61.56 (51.97)
AWR	71.01 (57.43)	68.84 (56.07)	54.35 (47.50)	64.73 (53.67)
BKN	80.00 (63.44)	74.98 (59.99)	47.50 (43.57)	67.49 (55.67)
CUR	72.92 (58.64)	76.25 (60.84)	45.00 (42.13)	64.72 (53.87)
DO	71.67 (57.84)	63.33 (52.74)	51.67 (45.96)	62.22 (52.18)
DLI	81.25 (64.35)	78.33 (62.26)	48.75 (44.28)	69.44 (56.96)
HSR	75.83 (60.56)	72.92 (58.64)	62.50 (52.24)	70.42 (57.15)
HMH	74.79 (59.86)	81.62 (64.62)	60.25 (50.92)	72.22 (58.47)
JJN	71.43 (57.70)	70.75 (57.26)	57.82 (49.50)	66.67 (54.82)
NNL	77.08 (61.40)	73.75 (59.18)	47.50 (43.57)	66.11 (54.72)
NHR	77.23 (61.50)	73.17 (58.81)	47.56 (43.60)	65.99 (54.64)
SRDR	68.79 (56.4)	74.47 (59.66)	48.94 (44.39)	64.07 (53.48)
SWM	65.64 (54.11)	71.28 (57.61)	52.30 (46.32)	63.07 (52.68)
UDZ	75.86 (60.15)	68.96 (56.15)	47.12 (43.35)	63.98 (53.22)
Mean	74.21 (59.55)	72.82 (58.55)	50.69 (45.40)	
	S. Em±	CD (P=0.05)	CD (P=0.01)	
Isolate	0.23	(0.63)	(0.84)	
Antagonist	0.10	(0.29)	(0.39)	
Isolate x Antagonist	0.39	(1.10)	(1.45)	
C.V. (%)	1.24			
General Mean	54.95			

*Figures in parentheses are mean angles corresponding to percentage

($MgSO_4.7H_2O$), 0.5 g; Peptone, 5.0 g; Dextrose, 10.0 g; Rose Bengal, 1 : 30,000; Streptomycin, 30 μg/ml; distilled water (1000 ml) was used. For enumeration and isolation of bacteria, Thornton's standardized medium (Thornton, 1922) (Agar agar, 20.0 g; Dipotassium hydrogen phosphate (K_2HPO_4),1.0 g; Magnesium sulphate ($MgSO_4.7H_2O$), 0.2 g; Calcium chloride ($CaCl_2$), 0.1 g; Sodium chloride (NaCl), 0.1 g; Ferric chloride ($FeCl_3$), Trace; Potassium nitrate (KNO_3), 0.5 g; Mannitol 1.0 g; Asparagine 0.5 g; Distilled water (1000 ml) was used. Population of pathogen (*M. phaseolina*) and antagonists was calculated on the basis of number of colonies (cfu) per 'g' soil.

Percent root rot incidence and disease control in various experiments were calculated by using the following formulae:

$$\text{Root rot incidence (\%)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

$$\text{Disease control (\%)} = \frac{\text{Root rot incidence in inoculated control (\%)} - \text{Root rot incidence in treatments (\%)}}{\text{Root rot incidence in inoculated control (\%)}} \times 100$$

Evaluation of antagonists under field conditions

Farm yard manure (FYM) (10 t ha^{-1}), vermicompost (10 t ha^{-1}) and neem cake (0.5 t ha^{-1}) were used as organic amendments. FYM, vermicompost and neem cake were mixed with the soil at required proportion and moistened giving light irrigation. The sorghum grain based formulation of three bioagents *viz., T. harzianum, T. viride* and *B. subtilis* were used as soil application (1:200). After 24 h of organic amendment, pathogen inocula and biocontrol agents were added to soil and allowed to stabilize for 48 h. Three bioagents; *T. harzianum, T. viride, B. subtilis* and in combination with organic amendment *viz.* FYM, vermicompost and neem cake were tested in this experiment. In case of control, seeds were sown in Mp inoculated soil without any organic amendment. Observation on disease incidence and yield were recorded.

RESULTS AND DISCUSSION

Testing of antagonists against *M. phaseolina* isolates *in vitro*

The biocontrol agents isolated from rhizosphere of groundnut were tested for their antagonistic reactions against the pathogen, Mp. The mycilial growth of all 14 isolates of *M. phaseolina* was significantly reduced by *T. harzianum, T. viride* and *B. subtilis*. Inhibition of mycelial growth (%) of pathogen by *T. harzianum, T. viride* and *B. subtilis* varied from 65.64 to 81.25, 63.33 to 81.62 and 38.46 to 62.50, respectively (Table 2). In present study, results indicate that *Trichoderma* sp. significantly reduced the growth area of pathogenic fungus under *in vitro* conditions. Such results concerning the inhibitory effect of

Table 3. Effect of antagonists on *Macrophomina phaseolina* soil population and on root rot of groundnut under greenhouse conditions

Antagonist	Mean population/g soil (pre-sowing		Mean population/g soil (After 100% mortality in control)		Disease incidence in percent	Disease control (%)
	M. phaseolina	Antagonist	*M. phaseolina*	Antagonist		
Control (Soil infected with *M. phaseolina* alone)	1904.76	-	7238.09	-	100.00 (90.00)	-
T. harzianum (Soil infected with *M. phaseolina* + *T. harzianum*)	1714.28	476.18	3238.09	3523.80	33.33 (35.26)	66.67
T. viride (Soil infected with *M. phaseolina* + *T. viride*)	1619.05	571.42	2666.66	3809.52	26.66 (31.09)	73.34
Bacillus subtilis (Soil infected with *M. phaseolina* + *Bacillus subtilis*)	1619.05	4095.24	4190.48	13333.33	46.66 (43.09)	53.34
S.Em ±			3.55			
CD at 5%			(11.58)			
CD at 1%			(16.85)			
CV (%)			12.34			
General Mean			49.86			

*Figures in parentheses are mean angles corresponding to percentage. Soil population/g was taken by counting colony forming unit (CFU).

various fungal antagonists on soil-borne plant pathogens were reported previously by many investigators (Abdel-Kader et al., 2013; El-Moughy et al., 2012). Bhagat and Pan (2010) screened 12 isolates of *Trichoderma* against *Rhizoctonia solani* causing root and collar rot of Frenchbean *(Phaseolus vulgaris* L.) *in vitro* by dual culture tests and found that all the isolates significantly inhibited the mycelial growth of *the pathogen*. Biswas and Sen (2000); Pandey et al. (2000); Patel and Anahosur, (2001); Lambhate et al., (2002); Sindhan et al. (2002); Kaswate et al. (2003); Kaur et al., (2004); Mathur and Srivastava, (2005) and Karthikeyan et al. (2006) also found *Trichoderma* sp., *Bacillus* sp*.,* actinomycetes and other microbes antagonistic to Mp in their studies *in vitro* confirming the present findings.

Antagonism in green house conditions

The biocontrol agents; *T. harzianum, T. viride* and *B. subtilis* were able to reduce the root rot incidence in susceptible RSB-87 groundnut cultivar. An apparent correlation between the decrease in disease incidence of root rot of groundnut and the magnitude of population/g soil of Mp and antagonists have been illustrated in Table 3. The mean population of Mp and antagonists was optimum in naturally infested unsterilized soil of groundnut field (Pre-sowing). Population of each antagonists assayed revealed its increase over Mp. The mean population of Mp observed with treatment of *T. harzianum, T. viride* and *B. subtilis* was 3238.09, 2666.66 and 4190.48, respectively, in sterilized soil. In control Mp population was 7238.09. A significant decrease in disease incidence by antagonists was found as compared to control. *T. viride, T. harzianum* and *B. subtilis* minimized root rot incidence (%) in groundnut up to 73.34, 66.67 and 53.34, respectively, as compared to control where 100% mortality was observed. *T. viride* was most effective among the antagonists trial in checking the disease followed by *T. harzianum* and *B. subtilis*.

Table 4. Effect of bio-control agents and organic amendments on root rot of groundnut and pod yield under field conditions

Treatment		Root rot incidence (%)			Disease control (%)	Yield (kg ha^{-1})		
		I year	II year	Pooled		I year	II year	Pooled
T_1	*Trichoderma harzianum* (1:200)	18.46(25.44)	17.22(24.52)	17.84(24.98)	55.69	1322	1372	1347.00
T_2	*Trichoderma viride* (1:200)	17.21(24.50)	15.87(23.48)	16.54(23.99)	58.93	1422	1525	1473.50
T_3	*Bacillus subtilis* (1:200)	23.29(28.85)	25.00(30.00)	24.14(29.43)	40.04	1250	1181	1215.50
T_4	*T. harzianum* + FYM (10 t ha^{-1})	15.88(23.48)	13.45(21.52)	14.67(22.50)	63.58	1372	1489	1430.50
T_5	*T. harzianum* + Vermi compost (10 t ha^{-1})	18.46(25.44)	14.77(22.60)	16.62(24.03)	58.73	1414	1567	1490.50
T_6	*T. harzianum* + Neem cake (0.5 t ha^{-1})	12.46(20.66)	14.25(22.18)	13.36(21.43)	66.83	1531	1572	1551.50
T_7	*T. viride*+ FYM (10 t ha^{-1})	14.23(22.13)	13.77(21.78)	14.00(21.97)	65.24	1575	1606	1590.50
T_8	*T. viride*+ Vermi compost (10 t ha^{-1})	11.77(20.05)	12.46(20.67)	12.12(20.37)	69.91	1653	1619	1636.00
T_9	*T. viride*+ Neem cake (0.5 t ha^{-1})	9.51(17.95)	11.77(20.06)	10.64(19.01)	73.58	1672	1694	1683.00
T_{10}	*B. subtilis* + FYM (10 t ha^{-1})	19.86(26.46)	18.16(25.23)	19.01(25.84)	52.79	1219	1381	1300.00
T_{11}	*B. subtilis* + Vermi compost (10 t ha^{-1})	22.78(28.51)	22.48(28.30)	22.63(28.41)	43.80	1472	1256	1364.00
T_{12}	*B. subtilis* + Neem cake (0.5 t ha^{-1})	21.47(27.60)	20.13(26.66)	20.80(27.13)	48.35	1289	1406	1347.50
T_{13}	Control (without bioagents and soil amendments)	38.00(38.05)	42.53(40.70)	40.27(39.38)	-	989	781	885.00
	S.Em. ±	0.49	0.46	0.33		68.45	78.07	51.91
	CD (P=0.05)	(1.42)	(1.34)	(0.96)		199.8	227.8	149.20
	CD (P=0.01)	(1.93)	(1.81)	(1.29)		270.7	308.8	0.00
	CV (%)	3.34	3.15	4.39		8.48	9.53	9.03

*Figures in parentheses are mean angles corresponding to percentage

A direct correlation was found between the population of pathogen and percent mortality of plants which was inversely proportionate to the population of antagonists. *T. viride* was most effective among the antagonists in checking the disease followed by *T. harzianum* and *B. subtilis.* Gurjar et al., (2004); Ramezani, (2008) and Jaiman et al., (2009) tested biocontrol agents including *T. harzianum, T. viride* and *Bacillus sp*. against Mp in different crops and found the reduction of disease incidence with an increase of grain yields of tested crops.

Evaluation of antagonists under field conditions

The efficacy of three organic amendments *viz.* farm yard manure, vermicompost and neem (*Azadirachta indica*) cake and three bioagents, that is, *T. harzianum, T. viride* and *B. subtilis* were taken as sole or in combination were studied against Mp under field conditions.

Data (Table 4) reveals that bioagents along with organic amendments reduced the disease incidence more effectively as compared to control rather used alone. The efficacy of bioagents *viz. T. harzianum, T. viride* and *B. subtilis* along with FYM, vermicompost and neem cake had the same trend in both the *Kharif* seasons that is 2007 and 2008. The incidence of root rot disease during *Kharif* 2008 was slightly higher. *T. viride* was most effective with organic amendments followed by *T. harzianum* and *B. subtilis. T. viride* along with neem cake minimized the disease upto 73.58%. *T. harzianum* minimized the disease incidence upto 66.83% with neem cake while *B. subtilis* minimized the disease up to 52.79% with FYM. During both years *T. viride* was most effective in minimizing the disease followed by *T. harzianum* and *B. subtilis* along with organic amendments. The sole use of bioagents was least effective in

reducing the disease incidence. The pod yield of groundnut (kg ha^{-1}) was also increased over control in all the treatments during both years (Table 4).

Karthikeyan et al. (2006) confirmed the present findings while studying disease incidence of *M. phaseolina* in groundnut. Sudha and Prabhu (2008) found that organic soil amendments with FYM at 12.5 t ha^{-1} and neem cake 250 kg ha^{-1} reduced the inoculum levels of Mp, thereby, reducing the incidence of charcoal rot of sunflower up to 13.3 and 15.0%.

Conflict of interests

The author(s) have not declared any conflict of interest.

REFERENCES

Abdel-Kader MM, Abdel-Kareem F, El-Mougy NS, El-Mohamady RS (2013). Integration between compost, *Trichoderma harzianum* and essential oils for controlling peanut crown rot under field conditions. J. Mycol. 262130: 1-7 pages. http://dx.doi.org/10.1155/2013/262130

Anonymous (1988). International *Arachis* Newsletter, Legumes programme ICRISAT, Patancheru, A.P. 3 : p. 5

Bhagat S, Pan S (2010). Biological management of root and collar rot (*Rhizoctonia solani*) of Frenchbean (*Phaseolus vulgaris*). Indian J. Agric. Res. 80 (1):42-50

Biswas KK, Sen C (2000). Identification of effective isolates of *Trichoderma harzianum* Rifai for bio-control of *Macrophomina phaseolina* (Tassi) Goid. J. Mycol. Plant Pathol. 30(3):408-410.

Dennis C, Webster J (1971). Antagonistic properties of species group of *Trichoderma.* I. hyphal interactions. Trans. Br. Mycol. Soc. 57:367-369.

El-Mougy NS, Abdel-Kader MM, Lashin SM, Aly MDA (2012). Evaluating survival and antagonistic activity of introduced bio-agents to the soil under greenhouse conditions. J. Appl. Res. 8(4):2401-2411.

Ghewande MP, Desai S, Basu MS (2002). Diagnosis and management of major diseases of groundnut. NRCG Bull. 8-9.

Gurjar KL, Singh SD, Rawal P (2004). Management of seed borne pathogens of okra with bio-agents. Plant Dis. Res. 19(1):44-46.

Harman GC (1991). Seed treatment for biological control of plant diseases. Crop. Prot. 10:166-176.

Jaiman RK, Jain SC, Sharma P (2009). Field evaluation of fungicides, bioagents and soil amendments against root rot caused by *Macrophomina phaseolina* in clusterbean. J. Mycol. Plant Pathol. 39(1):74-76.

Karthikeyan V, Sankarlingam A, Nakkeeran S (2006). Management of groundnut root rot with biocontrol agents and organic amendments. Arch. Phytopathol. Plant Prot. 39(3):215-223.

Kaswate NS, Shinde SS, Rathod RR (2003). Effect of biological agents against different isolates of *Rhizoctonia bataticola* (Taub.) Butler *in vitro.* Ann. Plant Physiol. 17(2):167-168.

Kaur P, Singh, RS, Kaur, J, Munshi, GD and Grewal, HS (2004). Efficacy of fungal and bacterial antagonist against charcoal rot of chilli caused by *Macrophomina phaseolina* (Tassi) Goid. Plant Dis. Res. 19(2): 159-162.

Lambhate SS, Chaudhari GK, Mehetre SS, Zanjare SR (2002). Biological control of root rot of cotton caused by *Macrophomina phaseolina.* J. Maharashtra Agric. Univ. 27(1): 98-98.

Loo YL, Skell PS, Thornberry HH, Ehrlich J, McGuire JM, Savage GM, Sylvester C (1945). Assay of streptomycin by the paper disc plate method. J. Bacteriol. 50:701-709.

Martin JP (1950). Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. Soil Sci. 69:215-232.

Mathur AC, Srivastava MP (2005). *In vitro* inhibition studies of *Macrophomina phaseolina* by local *Trichoderma* spp. - the cause of charcoal rot of cowpea. Ann. Appl. Biol. 21(1): 79-81.

Pandey KK, Pandey PK, Upadhyay JP (2000). Selection of potential isolate of biocontrol agents based on biomass production, growth rate and antagonistic capability. Vegetable Sci. 27(2):194-196.

Patel ST, Anahosur KH (2001). Potential antagonism of *Trichoderma harzianum* against *Fusarium* sp., *Macrophomina phaseolina* and *Sclerotium rolfsii.* J. Mycol. Plant Pathol. 31:365-366.

Ramezani H (2008). Biological control of root rot of egg plant caused by *Macrophomina phaseolina.* Am. Eurasian J. Agric. Environ. Sci. 4(2):218-220.

Sindhan GS, Hooda I, Karwasra SS (2002). Biological control of dry root rot of chickpea caused by *Rhizoctonia bataticola*. Plant Dis. Res. 17(1):68-71.

Sudha A, Prabhu S (2008). Evaluation of organic soil amendments against *Macrophomina phaseolina* (Tassi). J. Biopestic. 1(2):143-145.