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In vitro antagonistic activity of *Trichoderma harzianum* against soilborne fungal pathogens

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Abstract
Groundnut (*Arachis hypogaea* L.) is an important oil seed crop. A large number of diseases attack on groundnut such as root rot, Stem rot, crown rot, pod rot, charcoal rot, black rot etc. These diseases cause the serious damage to this crop. In present investigation an attempt has been made to evaluate the *In vitro* antagonistic activity of *Trichoderma harzianum* against soilborne pathogens of groundnut like *Aspergillus flavus, Aspergillus niger, Fusarium roseum, Macrophomina phaseolina, Phythium myriotylum, Rhizoctonia solani,* and *Sclerotium rolfsii.* The results indicate that the application of *Trichoderma harzianum* reduces the growth of soilborne fungal pathogens as compared to control. The maximum inhibition was revealed against *Fusarium roseum (62.18%),* followed by *Phythium myriotylum (51.85%), Aspergillus flavus* (50.37%), *Aspergillus niger* (46.55%), *Rhizoctonia solani* (41.78%), *Macrophomina phaseolina* (30.58%) and minimum inhibition was shown against *Sclerotium rolfsii* (27.73%).

Keywords: antagonistic activity, groundnut, *Trichoderma harzianum* and soilborne fungal pathogens

Introduction
Groundnut (*Arachis hypogea* L.) is an important annual oil seed crop (Brown, 1999) [4]. It contains different kinds of vitamins and essential minerals. Incidences of diseases are most important obstacle for groundnut production. A large number of diseases attack on groundnut in India. The majority diseases are caused by fungi and several of them are yield reducers in certain regions and seasons. Near about 46 fungal diseases and approximate 67 fungi were recorded on groundnut. (Mayee and Datar 1988, Mayee 1995 and Shila and Shamn 2013) [11, 16].

Most of the fungal diseases of groundnut are soil borne. Soilborne pathogens have large host range. They persist for longer period in soil due to their resistant resting bodies like Sclerotia. Fungal Sclerotia are the first source for the infection which survives in soil for the several years in dormant stage. These Sclerotia infects suitable host and left over plant residues. (Faheem Amin *et al*. 2010 and Yalda vasebi *et al*. 2013) [7, 20].

The Soilborne diseases includes stem rot caused by *sclerotium rolfsii,* black root rot caused by *Macrophomina phaseolina,* A root rot caused by *Rhizoctonia solani* and root rot along with wilt is caused by *Fusarium* species. (P. Subrahmanyam *et al*.1980). Crown rot is characterized by wilt and it is caused by *Aspergillus niger*. (Anand. R and kulothungn S.). Also all these fungi with *Aspergillus flavus* and *Pythium species infects* the pod of groundnut. The *Pythium* species along with *pythium myriotylum* have been associated with pod rot and other groundnut diseases. (Lindsep and Jason, 2012 and sarah Rurak, 2017) [10, 15]. The *Aspergillus flavus* is responsible for aflaroot of groundnut such plants does not produce flowers and hence become infertile. These diseases of groundnut cause the declination in yield. (Vinod Kumar and P. P. Thirumalai, 2016) [19].

The chemical control or different fungicides against soil borne fungal pathogens feed somewhat control but they are very harmful, expensive, affecting on beneficial microorganisms, badly effects on environment and that develops the resistant strains. To overcome these problems the biological control is an alternative source for longtime sustainability. (Faheem Amin *et al*. 2010, Dhotre and Vanmare 2017) [7]. *Trichoderma* is the most commonly used fungi for biological control and it is recognized as antagonists against plant pathogenic fungi. Amongst the different species of *Trichoderma*, *Trichoderma harzianum* is considered to be the most effective agent (Vinale *et al.,* 2008) [18]. The present study aimed to evaluate the efficiency of *Trichoderma harzianum* against soilborne pathogenic fungi of groundnut. Near about all soilborne fungal pathogens which infect to groundnut were studied and these fungal pathogens were isolated from rhizosphere soil of groundnut collected from different locations of Marathwada region.

Material and Methods
Isolation of fungi
The fungi were isolated from Rhizosphere soil of groundnut collected from different locations of Marathwada region. The Rhizosphere soil fungi were isolated by using soil dilution method and it was maintained on PDA slants. A total seven pathogenic fungi of groundnut were studied. Through detail observations of fungal characters their identification was done and it was confirmed with standard literature (Barnett, 1972 and C.V. Subhramaniyan, 1971) [8, 5].

In vitro antagonistic activity by dual culture
Trichoderma harzianum was isolated from rhizosphere soil of groundnut on PDA along with pathogens and screened for their antagonistic activity against soilborne fungal pathogens

i.e. *Aspergillus flavus, Aspergillus niger, Fusarium roseum, Macrophomina phaseolina, Phythium myriotylum, Rhizoctonia solani,* and *Sclerotium rolfsii* of groundnut by dual culture technique. Seven day old cultures of both pathogenic and antagonistic fungi were inoculated on PDA plates at periphery. In control only pathogenic fungi were inoculated. Three replications were kept for each treatment. Plates were incubated at 28^0c. Observations of colony growth were recorded. Diameter of colony was measured in mm and percent inhibition was calculated by using following formula.

$$T = \frac{C - T}{C} \times 100$$

Where,
C = Radial growth of the pathogen (mm) in the control,
T = Radial growth of the pathogen (mm) in the treatment and
I = Inhibition of radial growth of pathogen.

Results and Discussion

In dual culture *In vitro* antagonistic activity of *Trichoderma harzianum* was studied against soilborne fungal pathogens isolated from rhizosphere soil of groundnut *viz*. *Aspergillus flavus, Aspergillus niger, Fusarium roseum, Macrophomina phaseolina, Phythium myriotylum, Rhizoctonia solani* and *Sclerotium rolfsii*. *Trichoderma harzianum* had been marked a significant inhibition of selected fungal pathogens as compared to their respective controls. The different workers checked these pathogens against *Trichoderma harzianum* from different hosts. During the present investigation all these pathogens were firstly tested against *Trichoderma harzianum* isolated from rhizosphere soil of groundnut from Marathwada region.

The maximum percentage of inhibition were revealed by *Trichoderma harzianum* against *Fusarium roseum (62.18%),* followed by *Phythium (51.85%), Aspergillus flavus* (50.37%), *Aspergillus niger* (46.55%), *Rhizoctonia solani* (41.78%), *Macrophomina phaseolina* (30.58 %) and minimum inhibition was shown against *Sclerotium rolfsii* (27.73%) as shown in Table no. I. Dhotre and Wanmare (2017) [6] were got the equal interaction of *Trichoderma* against root rot causing fungus of soybean *i.e. Fusarium moniliforme* (85 mm) followed by *Rhizoctonia solani* (81.5 mm), *Fusarium oxysporium* (80 mm), *Fusarium solani* (79 mm), *Fusarium roseum* (79 mm), *Phytopthora sojae* (76 mm), *Macrophomina phaseolina* (73.5 mm). Also the Mahamune and Kakde (2011) [17] were found that the *Trichoderma harzianum* inhibit the growth of *Macrophomina phaseolina* followed by *Aspergillus niger* and *Fusarium oxysporum* pathogens isolated from seeds.

The *Trichoderma* reached the pathogen within 3- 4 days and overgrew them in 8-10 days. The occurrence of an inhibition zone in dual culture characterizes the secretion of some diffusible non-volatile substances. The zone of inhibition was found against two pathogens i.e. *Macrophomina phaseolina* and *Rhizoctonia solani*. (Fig 1.) This zone of inhibition lasts for 3 to 7 days and then this antagonist was overgrowing on them. Similar kind of results was found by Hajigharari *et al*. (2008) [9] for different species of *Trichoderma* including *Trichoderma harzianum* against *Rhizoctonia solani* and *Macrophomina phaseolina*.

Ramaraju Cherkupally *et al.* (2017) [14] studied the seven fungal antagonists for their efficiency, the *Trichoderma harzianum* showed maximum extent of inhibition, followed by *Trichoderma koningii, Trichoderma pseudokoningii and Trichoderma viride, Trichoderma virens, Trichoderma atroviride* and *Trichoderma reesei* against *Fusarium* species. Similar effects of antagonists were reported in numerous studies (Zhang Ru and Wang Di 2012, Ashwini tapwal 2011, Yaldavasebi *et al.* 2013, Anupama sonawane *et al*. 2015) [21, 3, 20, 2]. Hence, The *Trichoderma harzianum* has a potential to develop as a biological agent to control the soilborne fungal pathogens. If the percentage of *Trichoderma harzianum* increased in soil were definitely controls soil borne fungal diseases of groundnut.

Increased percentage of *Trichoderma harzianum* suppress the growth of the population of *Aspergillus flavus, Aspergillus niger, Fusarium roseum, Macrophomina phaseolina, Phythium myriotylum, Rhizoctonia solani* and *Sclerotium rolfsii* in the rhizosphere soil through competition and thus reduce disease development. The antagonist *Trichoderma harzianum* hyphae developed along with host or pathogen secretes different enzymes that are definitely effect on growth of pathogen.

Table 1: Antagonistic activity of *Trichoderma harzianum* against soil borne fungal pathogens of groundnut

Sr. No.	Fungal Pathogen	Radial growth of pathogen in mm		Percent (%) of Inhibition
		Control	Treatment	
1	*Aspergillus flavus*	66.5 mm	33.0 mm	50.37 %
2	*Aspergillus niger*	55.2 mm	29.5 mm	46.55 %
3	*Fusarium roseum*	71.4 mm	27.0 mm	62.18 %
4	*Macrophomina phaseolina*	85.0 mm	59.0 mm	30.58 %
5	*Phythium myriotylum*	59.2 mm	28.5 mm	51.85 %
6	*Rhizoctonia solani*	73.0 mm	42.5 mm	41.78 %
7	*Sclerotium rolfsii*	75.0 mm	54.5 mm	27.73 %

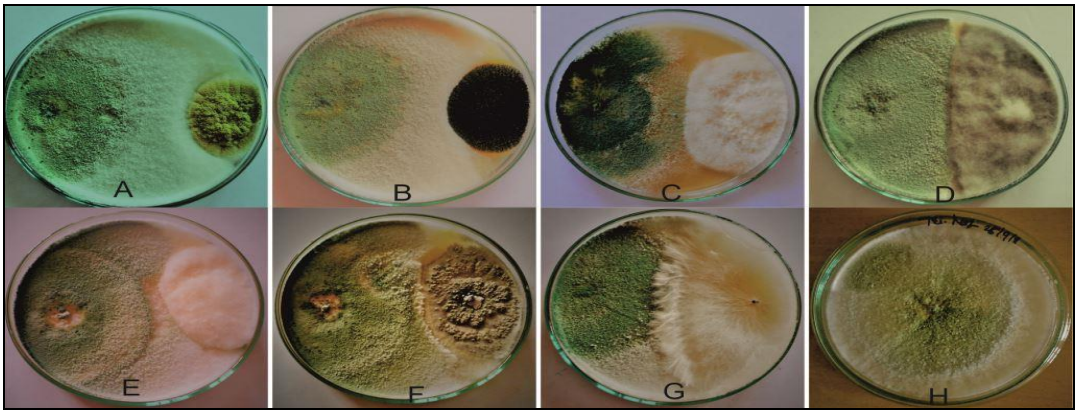

Fig 1: *In vitro* antagonistic activity of *Trichoderma harzianum* against A. *Aspergillus flavus,* B. *Aspergillus niger,* C. *Fusarium roseum,* D. *Macrophomina phaseolina* E. *Phythium myriotylum* F. *Rhizoctonia solani* G. *Sclerotium rolfsii* H. *Trichoderma harzianum*

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