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In-vitro Efficacy of different Bioagents against Dry Root Rot Disease of Groundnut

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A B S T R A C T

Groundnut (*Arachis hypogaea* L.) is a cultivated annually belonging to the plant family *Leguminosae* and sub family *Papillionaceae*. In groundnut several soil borne disease is occurred. Among them dry root rot is major which caused by *Macrophomina phaseolina* (Tassi) Goid has been having worldwide importance devastating disease causes severe losses in yield. *In-vitro*, the effectively colony growth of fungus *M. phaseolina* was inhibited through different bioagent. Among the seven biological control agents against *Macrophomina phaseolina* (Tassi) Goid, maximum inhibition was recorded in *Trichoderma viride* (75.42%) followed by *Trichoderma harzianum* (73.61%) with no sclerotial formation found of *M. phaseolina* have been identified as potent antagonistic agent.

Introduction

Groundnut (*Arachis hypogaea* L.) is a cultivated annually belonging to the plant family *Leguminosae* and sub family *Papillionaceae*. It is an important oilseed crop of the semi arid tropics (SAT). A variety of stresses affect groundnut production from planting to storage. Among these diseases are the major causes of losses in production. The majority of diseases are caused by fungi and several of them caused reduction in yield (Mayee, 1995). Among these, soil borne fungal pathogens causing serious losses and have prime importance (Mathur and Cunfer, 1993). Generally, these pathogens infect underground parts of the plant and reduce yield (Wisniewska and Chelkowski, 1999). Due to this disease production and quality of groundnut is hampered.

The groundnut & other legumes in India often suffer from various type of root rot & wilt. Among these the dry root rot caused by *Macrophomina phaseolina* (Tassi) Goid has been noticed to cause 33.33 % seed rotting

and 23.80 % post emergence mortality (Gupta and Kolte, 1982). *Macrophomina phaseolina* is a soil borne fungus causing the root rot disease on groundnut and is one of the cosmopolitan fungi. It has also been observed in Saurashtra region of gujarat during summer or *kharif*.

Due to several side effect of chemicals used in plant disease management has diverted plant pathologists or mankind to find out the other alternative methods for plant disease control with antagonistic microorganism. An experiment was undertaken to determine the antagonistic effect of different biocontrol agent against *M. phaseolina* fungus causes of root rot disease of groundnut.

Materials and Methods

Evaluation of biocontrol agents *in vitro*

Effect of fungal biocontrol agents on the growth of *M. phaseolina*

The antagonistic actions of five different *Trichoderma* Spp. were tested against *M. phaseolina* by using dual culture technique (Morton and Stroube, 1955) in CRD with three repetitions.

Twenty milliliters of sterilized melted PDA were poured aseptically in each 90 mm Petri plates and were allowed to solidify. Mycelial disc of four millimeter diameter of each antagonist and test fungus was cut with the help of sterilized cork borer from the edges of actively growing culture and were placed by keeping 1 cm distance from distal ends of PDA containing Petri plates. The plates were then incubated at 28 ± 2°C for five days.

After incubation the growth of antagonist and test fungus was measured by linear measurement. Per cent growth inhibition of test fungus by antagonist was calculated.

Effect of bacterial biocontrol agents on the growth of *M. phaseolina*

The antagonistic action of *Pseudomonas fluorescens* and *Bacillus* Spp. were tested against *M. phaseolina* by using dual culture technique. Twenty milliliters of NA were poured aseptically in each Petri plates and allowed to solidify. Mycelial disc of four mm diameter of test fungus was placed at both distal end of Petri dish by keeping 1 cm distance from the edges of the Petri dish and bacterial antagonist inoculated at the center with inoculating wire loop by streaking method. Each treatment was repeated thrice and arranged in CRD. The plates were then incubated at 28 ± 2 oC for five days. After incubation the growth of antagonist and test fungus was measured by linear measurement.

Per cent growth inhibition of test fungus by antagonist was calculated by using formula given by Vincent (1947).

$$\text{Per cent growth inhibition (PGI)} = \frac{C - T}{C} \times 100$$

Where,

C = Average diameter of mycelial colony in control treatment (mm) T = Average diameter of mycelial colony in treated plate (mm)

Sclerotial formation

Sclerotial formation was counted in fungal and bacterial culture suspensions under the microscope at low power (10X).

Results and Discussion

The hazardous effect of chemicals used in the plants disease management has diverted plant pathologists to find out the alternative

methods having little or no adverse effect on environment. There is a possibility of developing biological control agent for management of plant disease under field condition. The commercial formulation of bio agents are already available in the market. An experiment was conducted to determine the antagonistic action of five fungal bio agents *viz, Trichoderma harzianum, T. viride, T. virens, T. hamatum, and T. koningii* and two bacterial bioagents *Pseudomonas fluorescens* and *Bacillus subtilis* against the test fungus by dual culture technique. Based on observation on radial growth of antagonist and test fungus, per cent inhibition was calculated. The results presented in table 3, plate-1 and depicted in Fig.1. makes it clear that, all the antagonists are tested against *M. phaseolina* were effective in checking the growth of the pathogen. Out of seven antagonists tested, maximum inhibition over control was recorded in *Trichoderma viride* (75.42 %) which are statically at par with *T. harzianum* (73.61 %). While *Pseudomonas fluorescens* (68.17%) was found next best followed by *T. virens* (64.64 %) and *T.hamatum* (63.66%) were moderately effective to inhibit fungal growth. Least inhibition was recorded in *T. koningii* (54.38 %) and *B. subtilis* (47.28 %).

Moderate sclerotial formation were observed in *T. koningii* and *B. subtilis* whereas sclerotial formation was absent in rest of the treatments. It is evident from these studies that among all the antagonists evaluated by dual culture method, *T. viride* and *T. harzianum* consistently showed strong antagonistic property against *M. phaseolina* compared to the other antagonists tested hence considered as potential antagonists.

Table.1 List of different bio-control agents tested against *M. phaseolina in vitro*

Sr. No.	Name of the antagonist
1	*Trichoderma harzianum*
2	*Trichoderma virens*
3	*Trichoderma viride*
4	*Trichoderma hamatum*
5	*Trichoderma koningi*
6	*Pseudomonas fluorescens*
7	*Bacillus subtilis*
8	Control

Table.2 Sclerotial formation

No. of sclerotia per microscopic field	Grade	Sign
0	Absent	_
1-4	Scanty	+
5-8	Moderate	++
9-15	Good	+++
>15	**Abundant**	++++

Table.3 Effect of different bio-agents against *M. phaseolina in vitro* condition

Sr. No	Bio control agents	Percent inhibition over control* (%)	Sclerotial formation
1.	*Trichoderma harzianum*	59.09 (73.61)	-
2.	*Trichoderma virens*	53.33 (64.34)	-
3.	*Trichoderma viride*	60.23 (75.42)	-
4.	*Trichoderma hamatum*	52.93 (63.66)	-
5.	*Trichoderma koningi*	47.51 (54.38)	++
6.	*Pseudomonas fluorescens*	55.65 (68.17)	-
7.	*Bacillus subtilis*	43.44 (47.28)	+
	S.Em. ±	0.71	
	C. D. at 5%	2.06	
	C.V. %	2.28	

Sclerotial formation:
++++ = Abundant; +++ = good; ++ = moderate; + = scanty; - = no sclerotial formation.
*Average of three replications
Values in parentheses are re-transformed values while outside were transformed with arcsine transformation before analysis

Plate.1 Effect of different bio-agents against *M. phaseolina in vitro*

1. *Trichoderma harzianum*
2. *Trichoderma hamatum*
3. *Trichoderma viride*
4. *Trichoderma virens*
5. *Trichoderma koningii*
6. *Pseudomonas fluorescens*
7. *Bacillus subtilis*
8. Control

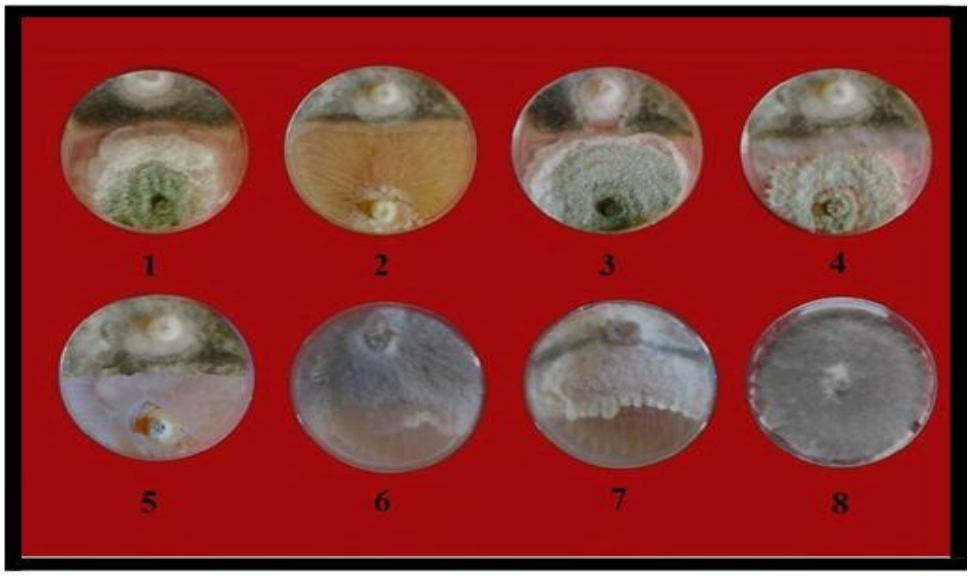
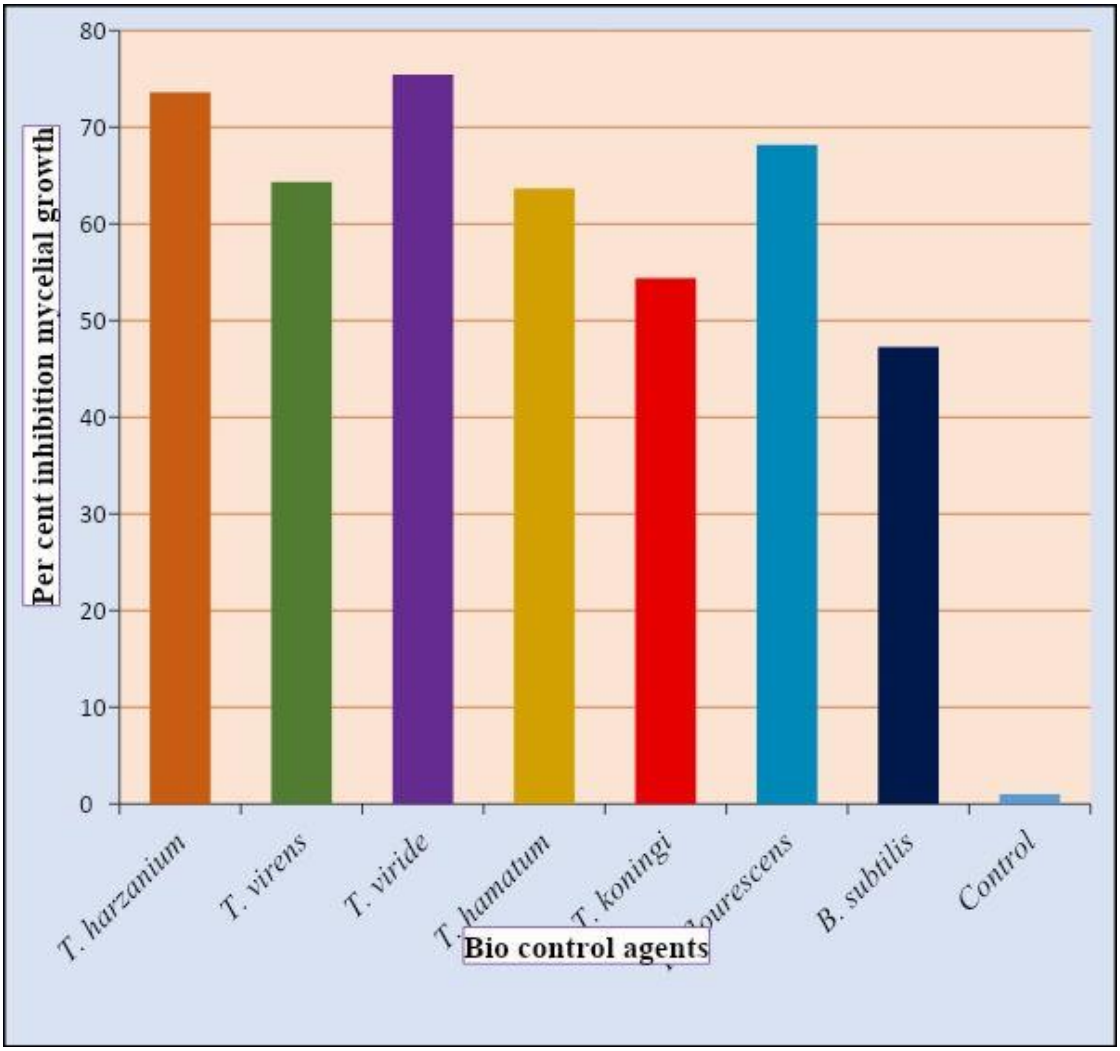


Fig.1 Effect of different biocontrol agents against *M. phaseolina in vitro*



This results are congruent with Rajeshwari *et al.*, 1998; Ahmad and Shrivastava, 2000; Indra and Tribhuvanmala, 2002; Malathi and Sabitha, 2004; Suriachandra selvan *et al.,* 2004; Rani *et al.,* 2009; Chaudhary *et al.,* 2010; Sreedevi *et al.,* 2015; Dhingani and Kelaiya, 2015; Kumar *et al.,* 2015; Meena and Pandey, 2015 and Gojiya *et al.,* 2016 who reported *T. harzianum* and *T. viride* as a strong antagonist against *M. phaseolina* in dual culture technique.

Pseudomonas fluorescens was found effective against *M. phaseolina* (dry root rot of chickpea) under dual culture technique have been earlier reported by Ahmad and Shrivastava (2000). Kumar *et al.,* (2007), Lokesha and Benagi (2007), Manjutha *et al.,* (2013) and Mallaiah and Krishna Rao (2016) were also reported *P. fluorescence* was found effective in inhibiting the growth of *M. phaseolina*.

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