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# Efficacy of antagonists against *Macrophomina phaseolina* inciting dry root rot disease of groundnut

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## Abstract
Groundnut (*Arachis hypogaea* L.) is an important oilseed crop in Indian continent. Groundnut is being infected by several fungal, bacterial and viral diseases but dry root rot caused by *Macrophomina phaseolina* (Tassi) Goid. is considered as the most devastating disease in all the groundnut growing areas of Rajasthan. *Trichoderma viride, T. harzianum, T. atroviride, Pseudomonas fluorescens* and *Bacillus subtilis* gave distinct antagonistic reactions, showing stunting of *Macrophomina phaseolina* colony and clear zone of inhibition between colonies of antagonist and the pathogen was developed. Maximum growth inhibition was recorded in *Trichoderma viride* followed by *T. harzianum. Trichoderma viride* and *T. harzianum* in combination as seed treatment and soil application were most effective in minimising the root rot incidence followed by *T. viride* in combination with *Pseudomonas fluorescens*

**Keywords:** *Macrophomina phaseolina*, dry root rot, groundnut

## Introduction
Groundnut is an important oilseed and pulse crop. It contains 48-50% oil, 26-28% protein and 11-27% carbohydrate, minerals and vitamin (Mukhtar, 2009) [11]. Groundnut is called as the 'King' of oilseeds. It is one of the most important food and cash crops of our country. It is grown on 24.6 million hectares worldwide with a total production of 41.26 million metric tonnes and productivity of 1676 kg/ha (Anonymous, 2013) [1]. India occupies the first place, both in regard to the area and the production in the world. About 4.9 million hectares are put under it annually and the production is about 5.77 million tonnes (Anonymous, 2013) [1]. In Rajasthan, groundnut is cultivated in about 4.25 lakh hectares, with an annual production of 6.97 lakh tonnes and productivity of 1637 kg/ha (Anonymous, 2014-15). The pathogen may infect almost all parts of plants *i.e*. root, stem, branches, petioles, leaves and pods. 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 pathogen and 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) [12].

## Materials and methods
### Testing of antagonists against *M. phaseolina in vitro*:
Microorganisms isolated during the course of studies were tested for their antagonism to *Macrophomina phaseolina* on Czapek's sucrose nitrate agar medium in Petri dishes (Conn. 1921) [3].

### Dual culture method
The antagonistic potential of each antagonist was studied. A 5 mm diameter disc of antagonist was placed individually at one end of the Petri dish containing Czapek's sucrose nitrate agar medium and just opposite to that a 5 mm diameter disc of the pathogen was placed. Three replications were maintained for each antagonist. In control, the pathogen alone was inoculated. The Petri dishes were incubated at 28 ± 1$^0$C for seven days in a BOD incubator and observations were recorded. Microorganisms which inhibited growth of *M. phaseolina* in above method were termed as antagonistic microorganisms.

### Paper disc plate method
For bacterium *Bacillus* sp. paper disc plate method (Loo *et al*., 1945) [8] was followed. Circular disc (5 mm dia.) of whatman filter (No. 42) were cut and after dipping in suspension of

*Bacillus* sp. 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+10C for a week and observations were recorded. Microorganisms which inhibited the growth of *M. phaseolina* in above method were termed as antagonistic microorganisms.

Radial growth of *M. phaseolina* was recorded and inhibition percentage was calculated using formula as under:

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

C = Radial growth of *M. phaseolina* in control (mm)

T= Radial growth of *M. phaseolina* in presence of antagonist (mm)

**Evaluation of antagonists under field conditions**

In this experiment three bioagents *viz., Trichoderma harzianum, T. viride* and *Pseudomonas fluorescens* were used individually and combined for soil application and seed treatment both. For soil application bioagents used at 10 and (5+5) kg ha-1 alone and in combination, respectively, while for seed treatment used at 8 and (4+4) g kg-1 seed in individual and combined bioagent, respectively. In case of control, seeds were sown in *Macrophomina* inoculated soil without any bioagents. Observation on disease incidence and yield were recorded.

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

**Results and Discussion**

**Testing of antagonists against *Macrophomina phaseolina* isolate *in vitro***

The antagonistic actions of three spp. of *Trichoderma* and two spp. of bacteria were evaluated against the test fungus by dual culture and paper disc technique, respectively. Based on observations of radial growth of antagonist and test fungus, per cent inhibition was calculated. The results are expressed in table (1) and depicted in fig. (1).The results revealed that all biocontrol agents were significantly superior in inhibiting the growth of test fungus over the control. *Trichoderma* spp. inhibited above 60% growth of the test fungus. Maximum growth inhibition was recorded in *T. viride* (74.72%) strain followed by *T. harzianum* (71.54%) and *T. atroviride* (62.55%). After these in descending order of inhibition *Pseudomonas fluorescens* (48.32 %), this was at par with *Bacillus subtilis* (47.51%). Majumdar *et al*.(1996) [9], Sindhan *et al*. (2002) [13], Kaswate *et al*. (2003) [6], Kaur *et al*. (2004) [7], Mathur and Srivastava (2005) [10], Karthikeyan *et al*. (2006) [5], Sreedevi and Devi (2012) [14] found *Trichoderma* spp., *Bacillus* spp., *Pseudomonas* spp. and other microbes antagonistic to *M. phaseolina* in their studies *in vitro* confirming the present findings.

**Table 1:** Effect of antagonists on the growth of *Macrophomina phaseolina* on Czapek's sucrose nitrate agar medium

| S. N. | Antagonist | Per cent inhibition of *Macrophomina phaseolina* |
|---|---|---|
| 1. | *Trichoderma harzianum* | 71.54 (57.74) |
| 2. | *T. viride* | 74.72 (59.79) |
| 3. | *T. atroviride* | 62.55 (52.25) |
| 4. | *Pseudomonas fluorescens* | 48.32 (44.02) |
| 5. | *Bacillus subtilis* | 47.51 (43.56) |
| 6. | Control | ----- |
| | S.Em.± CD (P=0.05) | 0.31 0.96 |

*values in parenthesis are angular transformed values

**Evaluation of antagonists under field conditions**

The effect of three bioagents *i.e. T. harzianum, T. viride* and *P. fluorescens* were taken as sole or in combination against *Macrophomina phaseolina* incitant of dry root rot disease in groundnut under field conditions.

**Per cent root rot incidence**

During *kharif* 2014, biocontrol agents minimized the root rot incidence in groundnut significantly as compared to control [Table-2 and Fig.2]. *Trichoderma viride* in combination with *T. harzianum* (9.45%) was most effective in minimizing the root rot incidence followed by *Trichoderma viride* in combination with *P. fluorescens* (11.51%) and *Trichoderma harzianum* in combination with *P. fluorescens* (14.19 %) as compared to other treatments. *T. viride* alone also effective in minimizing the disease incidence followed by *T. harzianum* and *P. fluorescens*. Conclusively, the bioagents in combination reduced the disease incidence more effectively as compared to sole application of bioagents.

**Per cent inhibition**

The effect of three bioagents i.e. *T. harzianum, T. viride* and *P. fluorescens* were taken as sole or in combination to study the per cent inhibition of *Macrophomina phaseolina* causing dry root rot disease in groundnut under field conditions. Biocontrol agents increased the per cent inhibition in groundnut significantly as compared to control [Table-2 and Fig.2]. Maximum per cent inhibition of dry root rot disease of groundnut was found with *Trichoderma viride* in combination with *T. harzianum* (77.30%) followed by *Trichoderma viride* in combination with *P. fluorescens* (72.35%) and *Trichoderma harzianum* in combination with *P. fluorescens* (65.91%), while in case of sole treatment of bioagents *T. viride* also inhibited the dry root rot disease (59.36%) followed by *T. harzianum* (55.44%) and *P.fluorescens* (39.25%). Conclusively, the bioagents in combination reduced the per cent inhibition more effectively as compared to sole application of bioagents.

**Pod yield**

All the treatments in the field trial were found significantly increased the pod yield as compared to control. Highest pod yield of 1681 kg/ha was obtained in the treatment of *Trichoderma viride* in combination with *T. harzianum* and remained significantly superior to all treatments. The pod yield increase in this treatment was 70.65 per cent higher than control. The combination of *Trichoderma viride + P. fluorescens* (1640 kg/ha) as well as *Trichoderma harzianum* in combination with *P. fluorescens* (1571 kg/ha) also produced higher pod yield. The sole treatment of three bioagents also increased pod yield. Conclusively, the bioagents in combination enhanced the pod yield more effectively as compared to sole application of bioagents. Karthikeyan *et al.* (2006) [5] confirmed the present findings while studying disease incidence of *M. phaseolina* in groundnut. Gaikwad *et al.* (2002) [4] found that the effectiveness of bio-control agents *viz*., *T. harzianum* and *T. viride* against charcoal rot of sorghum in the greatest reduction of per cent incidence of charcoal rot.

**Table 2:** Effect of bio-control agents on dry root rot of groundnut incited by *Macrophomina phaseolina* in field conditions

| Treatment | Root rot incidence (%) | Percent inhibition (%) | Yield (kg $ha^{-1}$) |
|---|---|---|---|
| *Trichoderma harzianum* ST 8 g $kg^{-1}$ + SA 10 kg $ha^{-1}$ | 18.55 (25.49) | 55.44 (48.01) | 1322 |
| *T. viride* ST 8 g $kg^{-1}$ + SA 10 kg $ha^{-1}$ | 16.92 (24.28) | 59.36 (50.37) | 1426 |
| *Pseudomonas fluorescens* ST 8 g $kg^{-1}$ + SA 10 kg $ha^{-1}$ | 25.29 (30.53) | 39.25 (38.78) | 1243 |
| *T. harzianum* + *P. fluorescens* ST(4+4)g $kg^{-1}$+SA(5+5) kg $ha^{-1}$ | 14.19 (22.12) | 65.91 (54.26) | 1571 |
| *T. harzianum* + *T. viride* ST (4+4) g $kg^{-1}$ + SA(5+5) kg $ha^{-1}$ | 9.45 (17.89) | 77.30 (61.53) | 1681 |
| *T. viride* + *P. fluorescens* ST (4+4) g $kg^{-1}$ + SA (5+5) kg ha-$^{1}$ | 11.51 (19.82) | 72.35 (58.24) | 1640 |
| Control (without bioagents) | 41.63 (40.16) | 0.00 (00.00) | 985 |
| S.Em. ± CD (P=0.05) | 0.72 2.25 | 0.37 1.16 | 64.77 201.81 |

* Values in parenthesis are angular transformed values

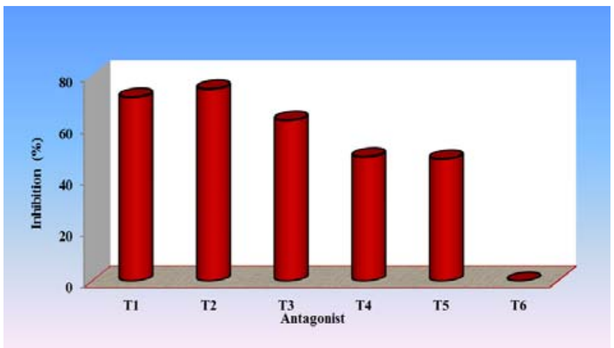


**Fig 1:** Effect of antagonists on the growth of *Macrophomina phaseolina* on Czapek's sucrose nitrate agar medium

| | | | |
|---|---|---|---|
| T1 | *Trichoderma harzianum* | T4 | *Pseudomonas fluorescens* |
| T2 | *Trichoderma viride* | T5 | *Bacillus subtilis* |
| T3 | *Trichoderma atroviride* | T6 | Control |

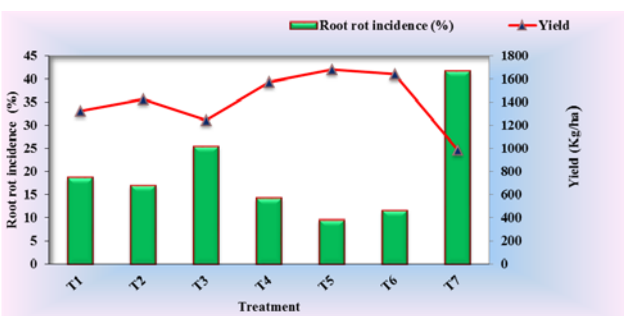


**Fig 2:** Effect of bio-control agents on dry root rot of groundnut incited by *M. phaseolina* in field conditions

| | | | |
|---|---|---|---|
| T1 | *Trichoderma harzianum* | T5 | *T. harzianum* + *T. viride* |
| T2 | *Trichoderma viride* | T6 | *T. viride* + *P. fluorescens* |
| T3 | *Pseudomonas fluorescens* | T7 | Control |
| T4 | *T. harzianum* + *P. fluorescens* | | |

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