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In vitro Evaluation of Various Bioagents against Detected Seed Mycoflora of Groundnut

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A B S T R A C T

Biotic and abiotic stresses are major constraints in the production of groundnut. Among biotic stresses apart from bacterial and viral diseases, many fungal diseases are of economic importance. The major fungal diseases are Early leaf spot (*Cercospora arachidicola*), Late leaf spot (*Cercosporidium personatum*), Rust (*Puccinia arachidis*), Botrytis blight (*Botrytis cinera*), Aflarot/Yellow mould (*Aspergillus flavus*), Damping off (*Pythium spp*.), Stem and pod rot (*Ralstonia solani*), collar rot (*Aspergillus niger*), Charcoal rot (*Macrophom inaphaseolina*), Anthracnose (*Colletotrichum arachidis*), Wilt (*Fusarium oxysporum*), Root rot (*Fusarium solani*), Stem rot (*Sclerotium rolfsii*), Damping off (*Pythium spp*.) and Leaf blight (*Alternaria alternata*). Among which Yellow mould, Collar rot, Wilt, Damping off and Leaf blight are seed borne which affect groundnut crop. For the management of different mycoflora, effect of nine bio-control agents *Alternaria alternate*, *Aspergillus flavus*, *Aspergillus niger* were observed *in vitro* condition. The biocontrol agents are *Trichoderma viride*, *T. harzianum*, *T. koningii*, *T. hamatum T. virens*, *T. longibrachitum* and *T. lignorum* along with two bacterial bio-agents *P. fluorescens* and *B. subtilis* evaluated in vitro were found antifungal to *A. alternata*. However, *T. viride* was found most effective with significantly least mycelial growth (5.33 mm) and its highest inhibition (94.07 %). The second and third best antagonists found were *T. hamatum* and *T. koningii*, with minimum mycelial growth of 13.33 mm and 17.12 mm and inhibition of 85.14 and 80.87 per cent, respectively.

Introduction

Groundnut (*Arachis hypogaea* L.), is an annual legume crop, known as peanut, earthnut or monkey-nut. It is one of the 4th most important oilseed crop and 13th most important food crop of the world, being cultivated in more than 100 countries in six continents. Groundnut kernels contain 40-50 per cent fat, 20-50 per cent protein and 10-20 per cent carbohydrates and also rich in vitamin E, niacin, riboflavin, thiamine, falacin, calcium, phosphorus, magnesium, zinc, iron and potassium.

The groundnut diseases like late leaf spot caused by *Cercosporidium personatum* and rust caused by *Puccinia arachidis* are the most serious fungal diseases worldwide which accounted more than 50 per cent yield losses.

The late leaf spot disease generally appears at 55 to 60 days after sowing and also causes more than 50 per cent loss in pod and haulm yields in groundnut (Jyosthna *et al.,* 2004). Among collar rot caused by *Aspergillus niger* was also reported to cause 55 per cent yield loss (Dasgupta *et al.,* 2000). *Sclerotinia* blight is an important disease of groundnut and the yield loss of 30 to 50 per cent due to *Sclerotinia* blight (Bowen *et al.,* 2000). During the investigations various experiments were conducted at the Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani.

Materials and Methods

Seven fungal antagonists *viz., Trichoderma viride, T. harzianum, T. koningii, T. hamatum, T. virens, T. longibrachitum, T. lignorum* and two bacterial *P. fluorescens* and *B. subtlis* were evaluated *in vitro* against *Alternaria alternata, Aspergillus flavus* and *A. niger* applying Dual Culture Technique (Dennis and Webster, 1971). Seven days old cultures of the test bio-agents and test fungus (*Alternaria alternata, Aspergillus flavus* and *A. niger*) grown on (PDA, NA) were used for the study. Discs (5 mm dia.) of culture growth of the test fungus and bio-agents were cut out with sterilized cork borer. Then two culture discs, one each of the test fungus and bio-agents were placed at equidistance and exactly opposite with each other on solidified PDA medium in Petri plates aseptically and plates were incubated at 26 ± 2 °C. PDA plates inoculated only with culture discs of the test fungus were maintained as untreated control and all the treatments were replicated thrice.

Experimental details

Design: CRD (Completely Randomized Design)
Replications: Three
Treatments: Ten

Treatment details

T_1: *Trichoderma viride*
T_2: *T. harzianum*
T_3: *T. hamatum*
T_4: *T. koningii*
T_5: *T. longibrachiatum*
T_6: *T. virens*
T_7: *T. lignorum*
T_8: *Pseudomonas fluorescens*
T_9: *Bacillus subtilis*
T_{10}: Control (untreated)

Observations on linear mycelial growth of the test pathogen and test bio-agent were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth of the test pathogen. Per cent inhibition of the test pathogen with the test bio-agent over untreated control was calculated by applying following formula (Arora and Upaddhyay, 1978).

$$\text{Per cent Inhibition} = \frac{\text{Colony growth in control plate} - \text{Colony growth in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

Results and Discussion

Radial mycelial growth / colony diameter of *A. alternata*

Results (Table 1, PLATE I and Fig. 1) indicated that all the bio-agents evaluated exhibited antifungal activity against *A. alternata* and significantly inhibited its mycelial growth, over control. Radial mycelial growth of *A. alternata* was ranged from 5.33 mm to 55.53 mm as against control (90.00 mm). However, *T. viride* was found most effective with significantly least mycelial growth (5.33 mm), these were followed by *T. hamatum* (13.33 mm), *T. koningii* (17.12 mm), *T. lignorum* (23.33 mm), *T.longibrachitum* (27.94 mm), *T. virens* (34.42 mm), *T.*

harzianum (34.66 mm), *P. fluorescens* (46.26 mm) and *B. subtilis* (55.53 mm), respectively.

Mycelial growth and inhibition of *A. alternata*

Results (Table 1, PLATE I and Fig. 1) indicated that mycelial growth inhibition of *A. alternata* was ranged from 38.07 per cent to 94.07 per cent as against control (90.00 mm). However, *T. viride* was found most effective with significantly highest mycelial growth inhibition (94.07 %), these were followed by *T. hamatum* (85.14 %), *T. koningii* (80.87 %), *T. lignorum* (74.07 %), *T. longibrachitum* (68.95 %), *T. virens* (62.86 %), *T. harzianum* (61.48 %), *P. fluorescens* (48.60 %) and *B. subtilis* (38.07 %).

Radial mycelial growth / colony diameter of *A.flavus*

Results (Table 2, PLATE II and Fig. 2) indicated that all the bio-agents evaluated exhibited antifungal activity against *A. flavus* and significantly inhibited its mycelial growth over control. Radial mycelial growth of *A. flavus* was ranged from 19.99 mm to 55.75 mm as against control (90.00 mm).

However, *T. harzianum* was found most effective with significantly least mycelial growth (19.22 mm), these were followed by *T. viride* (26.40 mm), *T. virens* (30.75 mm), *T. longibrachitum* (31.91 mm), *T. lignorum* (40.56 mm), *T. hamatum* (43.76 mm), *T. koningii* (41.93 mm), *P. fluorescens* (51.52 mm) and *B. subtilis* (55.75 mm).

Mycelial growth and inhibition of *A.flavus*

Results (Table 2, PLATE II and Fig. 2) indicated that mycelial growth inhibition of *A. flavus* was ranged from 38.05 per cent to 77.78 per cent as against control (90.00 mm). However, *T. harzianum* was found most effective with significantly highest mycelial growth inhibition (77.78 %), these were followed by *T. viride* (70.66 %), *T. virens* (65.83 %), *T. longibrachitum* (64.54 %), *T. lignorum* (54.93 %), *T. hamatum* (51.37 %), *T. koningii* (53.41 %), *P. fluorescens* (42.75 %) and *B. subtilis* (38.05 %).

Radial mycelial growth / colony diameter of *A. niger*

Results (Table 3, PLATE III and Fig. 3) indicated that all the bio-agents evaluated exhibited antifungal activity against *A. niger* and significantly inhibited its mycelial growth over control. Radial mycelial growth of *A. niger* was ranged from 21.85 mm to 55.55 mm as against control (90.00 mm). However, *T. harzianum* was found most effective with significantly least mycelial growth (21.85 mm), these were followed by *T. viride* (28.12 mm), *T. virens* (31.42 mm), *T. longibrachitum* (32.40), *T. lignorum* (41.05 mm), *T. hamatum* (44.62 mm), *T. koningii* (44.99 mm), *P. fluorescens* (52.35 mm) and *B. subtilis* (55.55 mm).

Mycelial growth and inhibition of *A.niger*

Results (Table 3, PLATE III and Fig. 3) indicated that mycelial growth inhibition of *A. niger* was ranged from 38.28 per cent to 75.72 per cent as against control (90.00 mm). However, *T. harzianum* was found most effective with significantly highest mycelial growth inhibition (75.72 %), these were followed by *T. viride* (68.76 %), *T. virens* (67.31 %), *T. longibrachitum* (64.00 %), *T. lignorum* (54.39 %), *T. hamatum* (50.42 %), *T. koningii* (50.01 %), *P. fluorescens* (45.17 %) and *B. subtilis* (38.28 %).

Result (Table 1) revealed that bioagents tested were found effective against *A. alternata*and significantly inhibited its mycelial growth, over control.

Table.1 *In vitro* efficacy of bioagents against *A. alternate*

Tr. No.	Treatments	Colony Dia.of test pathogen * (mm)	Per cent Inhibition
T_1	*Trichoderma viride*	5.33	**94.07 (75.90)**
T_2	*T. harzianum*	34.66	**61.48 (51.63)**
T_3	*T. koningii*	17.12	**80.87 (64.06)**
T_4	*T. hamatum*	13.33	**85.14 (67.32)**
T_5	*T. virens*	34.42	**62.86 (52.45)**
T_6	*T. longibrachiatum*	27.94	**68.95 (56.13)**
T_7	*T. lignorum*	23.33	**74.07 (59.38)**
T_8	*Pseudomonas fluorescence*	46.26	**48.60 (43.89)**
T_9	*Bacillus subtilis*	55.53	**38.07 (38.09)**
T_{10}	Control	90.00	**0.00 (0.00)**
	SEm±	**0.38**	**0.41**
	C.D. @ 1%	**1.26**	**1.35**

*Mean of three replication, figures in Parentheses are arc sine transformed values

Table.2 *In vitro* efficacy of bioagents against *A.flavus*

Tr. No.	Treatments	Colony Dia. of test pathogen * (mm)	% mycelial Inhibition
T_1	***T. viride***	26.40	**70.66 (57.20)**
T_2	***T. harzianum***	19.99	**77.78 (61.88)**
T_3	***T. koningii***	41.93	**53.41 (46.96)**
T_4	***T. hamatum***	43.76	**51.37 (45.79)**
T_5	***T. virens***	30.75	**65.83 (54.23)**
T_6	***T. longibrachiatum***	31.91	**64.54 (53.45)**
T_7	***T. lignorum***	40.56	**54.93 (47.83)**
T_8	***Psudomonas fluorescens***	51.52	**42.75 (40.83)**
T_9	***Bacillus subtilis***	55.75	**38.05 (38.09)**
T_{10}	**Control**	90.00	**0.00 (0.00)**
	SEm±	**0.37**	**0.41**
	C.D. @ 1%	**1.21**	**1.36**

*Mean of three replications, figures in Parentheses are arc sine transformed values

Table.3 *In vitro* efficacy of bioagents against *A. niger*

Tr. No.	Treatments	Colony Dia.of test pathogen * (mm)	% mycelial Inhibition
T_1	***T. viride***	28.12	**68.76 (56.01)**
T_2	***T. harzianum***	21.85	**75.72 (60.48)**
T_3	***T. koningii***	44.99	**50.01 (45.01)**
T_4	***T. hamatum***	44.62	**50.42 (45.24)**
T_5	***T. virens***	31.42	**67.31 (55.13)**
T_6	***T. longibrachiatum***	32.40	**64.00 (53.13)**
T_7	***T. lignorum***	41.05	**54.39 (47.52)**
T_8	***Psudomonas fluorescens***	52.35	**45.17 (42.22)**
T_9	***Bacillus subtilis***	55.55	**38.28 (38.22)**
T_{10}	**Control**	90.00	**0.00 (0.00)**
	SEm±	**0.32**	**0.36**
	C.D. @ 1%	**1.08**	**1.20**

*Mean of three replications, figures in parenthesis arc sine transformed values.

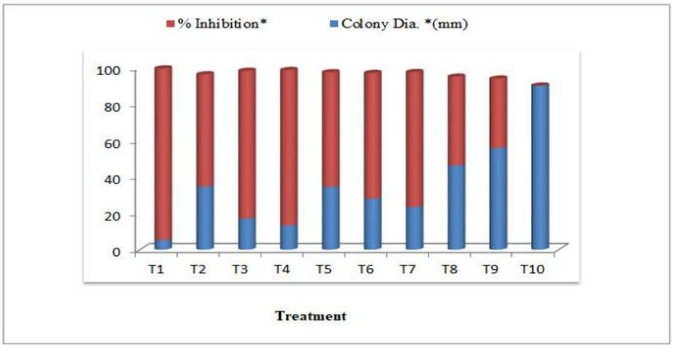

Fig. 1 ***In vitro*** **efficacy of bio-agents (*A. alternata*)**

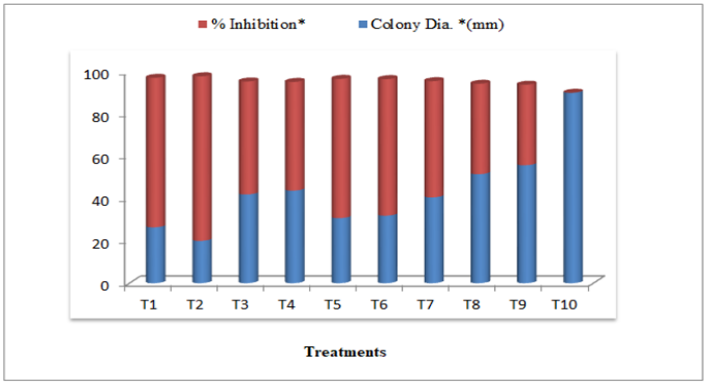

Fig. 2 ***In vitro*** **efficacy of bio-agents (*A. flavus*)**

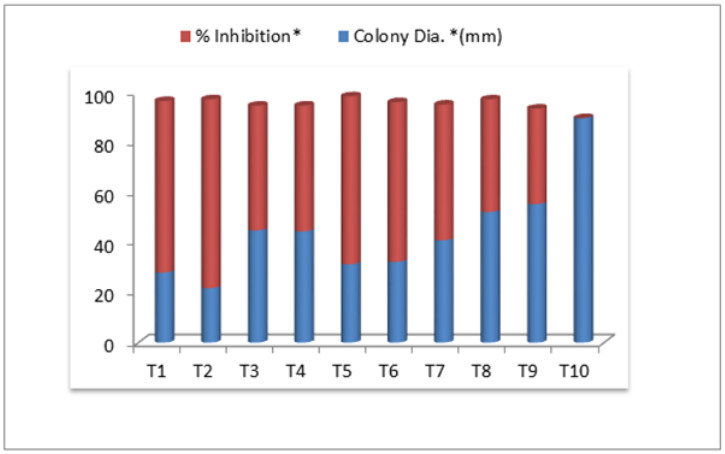

Fig. 3 *In vitro* efficacy of bio-agents (*A. niger*)

In vitro efficacy of bioagents against mycelial growth and inhibition of *A. alternata*

In vitro efficacy of bioagents against mycelial growth and inhibition of *A. flavus*

In vitro efficacy of bioagents against mycelial growth and inhibition of *A. niger*

Fungal bioagents *viz*., *T. viride, T. hamatum* and *T. koningii* were reported efficient antagonists against *A. alternata,* these results were in agreement with the finding of several workers. Similar result were reported on other crops like onion by Wanggikar *et al.,* (2014) and Mishra *et al.,* (2012), in safflower by Taware *et al.,* (2014), in sesame by Lubaina *et al.,* (2014) and Bharathi *et al.,* (2013) and in cotton by Ramegowda *et al.,* (2007).

Result (Table 2) revealed that all bioagent tested were found effective against *A. flavus* and significantly inhibited its mycelial growth, over control. Fungal bioagents *viz*., *T. harzianum, T. viride* and *T. virens*were reported efficient antagonists against *A. flavus* these results were in agreement with the finding of several workers. Studies on similar line were reported on groundnut and other crops like rice, black gram, *Pennisetum americanum*, sesame and cowpea by Johnson *et al.,* (2008); Halgekar *et al.,* (2014); Bhushan *et al.,* (2014); Ashwini *et al.,* (2014); Bharathi *et al.,* (2013) and by Agarwal *et al.,* (2011).

Result (Table 3) revealed that all the bioagents tested were found effective against *A. niger* and significantly inhibited its mycelial growth, over control. Fungal bioagents *viz*., *T. harzianum, T. viride* and *T. virens* were reported efficient antagonists against *A. niger* and these results were in agreement with the finding of several workers *viz.,* Johnson *et al.,* (2008), Agarwal *et al.,* (2011), Gajera *et al.,* (2011) and Rakholiya *et al.,* (2010) and in other crops like rice reported by Halgekar *et al.,* (2014); *Pennisetum americanum* by Bhushan *et al.,* (2014); black gram by Ashwini *et al.,* (2014) and sesame by Bharathi *et al.,* (2013).

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