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Efficacy of bio agents against *Aspergillus niger* causing collar rot of groundnut *in vitro*

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Abstract
In vitro studies of fungal and bacterial antagonists, *viz.*, *Trichoderma* spp., *Pseudomonas fluorescens* and *Bacillus subtillis* indicated that *T. viride* was more effective in inhibiting *A. niger*. Among the ten different isolates of *Trichoderma*, *Tv*- 3 has shown maximum inhibition of 77.7 per cent followed by *Tv* - 18 (75.6%) and *T*-29 (69.6%). Whereas, *T. asperullum* was found to be least effective in inhibiting mycelial growth with per cent inhibition of (50.83%). Out of eight different isolates of *P. fluorescens, P. fluorescens* strain pf-7 showed maximum inhibition of 66.94 per cent followed by *P. fluorescence.* strain pf-2 (66.23%) and *P. fluorescens* strain pf-26 (64.46%). Whereas, *P. fluorescence* strain pf-1 recorded least mycelial inhibition (44.4%). Eight strains of *B. subtillis* were tested for their antagonistic activity against *A. niger*. Among them, *B. subtillis* strain Bs-10 showed maximum inhibition of 64.10 per cent followed by *B. subtillis* strains Bs-29 and Bs-9 (61.46%). Whereas, *B. subtillis* strain Bs-4 was found to be least effective in inhibiting mycelila growth (45.36%).

Keywords: *Aspergillus niger*, causing collar rot, groundnut, *in vitro*

Introduction
Groundnut or peanut (*Arachis hypogaea* L.), is a very important legume crop of tropical and sub tropical areas of the world, described in 1753 by Linnaeus (Pattee and Young, 1982) [11]. It is originated from Brazil in South America and was introduced in India by the Portuguese traders in the middle of sixteenth century. In India, groundnut occupies an area of 4.15 million hectare with an annual production of 7.07 million tonnes with an average productivity level of 1704 kg per hectare (Anon, 2017-2018) [1]. In Karnataka groundnut covers, about 3.78 lakh hectare and production is 3.30 million tones and productivity is 874 kg per hectare (Anon, 2017-18**)** [1].

Groundnut is infected by several soil borne pathogens causing diseases like collar rot, *Sclerotium* wilt and dry root rot etc., which limit the yield considerably. These diseases largely account for the death of the seedlings. Of these, pre and post emergence damping off and collar rot caused by *A.niger* van Tieghem is the most prevalent disease causing seedling losses up to 50 per cent (Chohan 1969) [2]. Many seed dressing fungicides are reported to be effective against collar rot of groundnut. (Gangopadhayay *et al.,* 1996; Karthikeyan 1996) [5, 6]. But limited work has been done on successful exploitation of bio-control agents, for the management of collar rot. In recent years, biological control of plant diseases has attributed more attention and awareness. Of these, potential species of *Trichoderma, Pseudomonas fluorescens* and *Bacillus subtilis* have been extensively exploited due to their high efficacy, broad spectrum, ease in cultivation and mass multiplication. Keeping this in view, different strains of the bio agents were screened *in vitro* to find out the best strain for effectively inhibiting the growth of *A. niger*.

Material and Methods
Bio-agents were evaluated for their efficacy through dual culture technique. Twenty ml of PDA was poured in 90 mm diameter Petri plates and allowed to solidify. For evaluation of fungal bio control agents, mycelial disc (5 mm dia.) of test fungus was inoculated at one end of the Petri plate and antagonistic fungus was placed opposite to it on the other end. In case of evaluation of bacterial antagonists the bacterium was streaked both sides and mycelial disc of the test fungus was placed at the centre. Each treatment was replicated three times and incubated he plates were incubated at 27 ± 1 ºC.

The activity of antagonistic organisms were recorded by measuring the colony diameter of *A. niger* in each treatment and compared with control.

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

Where;
I= Per cent mycelial inhibition
C= Radial mycelial growth of fungus in control
T= Radial mycelial growth of fungus in treatment

Results and Discussion

Bio efficacy of different isolates of *Trichoderma* against *A. niger*

It is evident from the data of Table 1and Fig 1, revealed that all the isolates showed impressive results. Among the ten *Trichoderma* isolates, strain Tv-3 recorded maximum inhibition of 77.70 per cent of target pathogen and showed severe antagonism followed by strain Tv-18 and Tv-29 which inhibited mycelial growth of 75.60 and 69.60 per cent respectively. Least inhibition was recorded with *T. asperullum* (50.83 per cent). The present findings are in close conformity with the findings of Rohtas *et al.* (2016) [13], Gajera., *et al*. (2011) [4] and Devi and Prasad (2009) [3]. The inhibition of growth of *A. niger* could be attributed mainly due to antibiosis or hyperparasitism. Most fungi have chitin and ß- 1,3 glucanase an essential constituent in their cell wall. *Trichoderma* spp. produce chitinase and ß- 1,3 glucanase, which degrades the cell wall leading to lysis of pathogens as reported by Wue *et al.* (1986).

Bio efficacy of different isolates of *P. fluorescens* against *A. niger*

Eleven *P. fluorescens* strains were evaluated for their antagonistic activity against *A. niger*. Among them *P. fluorescens* strain pf-7 proved suprior over all the treatment and showed maximum inhibition of 66.96 per cent followed by *P. flourescens* strain pf-2 (66.23%) and *P. fluorescens* strain pf-26 (64.46%). Whereas, *P. flourescens* strain pf-1 was found to be least effective in inhibiting mycelial growth of *A.niger* with per cent inhibition of 44.40 per cent (Table 2 and Fig. 2). The antifungal activity of *P. fluorescens* was attributed to the production of iron chelating agent siderophore, hydrocyanic acid, indole acetic acid, wide variety of secondary metabolites such as fluorescent pigment, antibiotics, enzymes, phytoharmones associated with microbial antagonism reducing phytopathogenic fungi (Kloepper *et al.*, 1988) [7].

Bio efficacy of different isolates of *B. subtillis* against *A. niger*

Among the Eleven *B. subtilis* strains evaluated against *A. niger*, strain Bs-10 recorded maximum inhibition of 64.10 per cent followed by *B. subtillis* strains Bs-2 and Bs-9 with a per cent inhibition of 62.33 and 61.46 per cent, respectively. Whereas, *B. subtillis* strain Bs-4 was found to be least effective in inhibiting the mycelial growth with percent inhibition of 45.36 (Table 3 and Fig. 3). The antifungal nature of *B. subtillis* was supposed to be because of biosurfectant, iturin and fengycin as reported by Mnif and Ghribi (2015) [8] and Ongena and Jacques (2008) [9]. The antagonistic activity was reported by Prabhakaran and Ravimycin (2012) [12].

Table 1: Bio efficacy of different strains of *Trichoderma* spp. against *Aspergillus niger*

Sl. No.	Strains	Mycelial inhibition (%)
1	*Tv*-1	66.90* (54.89)**
2	*Tv* -4	53.66 (47.10)
3	*Tv* -10	56.23 (48.57)
4	*Tv* -18	75.60 (60.40)
5	*Tv* -29	69.60 (56.53)
6	*Tv* -25	57.43 (49.27)
7	*Tv* -27	61.46 (51.62)
8	*Tv* -3	77.70 (61.81)
9	*Tv* -12	66.23 (54.48)
10	*T. asperullum*	50.83 (45.47)
	S. Em±	0.65
	CD at 1%	2.61

* Original value ** Arc sine transformed value

Table 2: Bio efficacy of different strains of *Pseudomonas fluorescens* against *Aspergillus niger*

Sl. No.	Strains	Mycelial inhibition (%)
1	*Pf*-1	44.40* (41.78) **
2	*Pf* -2	66.23 (54.47)
3	*Pf* -3	54.40 (47.52)
4	*Pf* -7	66.96 (54.92)
5	*Pf* -26	64.46 (53.40)
6	*Pf* -32	56.60 (48.79)
7	*Pf* -31	55.50 (48.15)
8	*Pf* -39	50.00 (44.99)
	S. Em±	0.52
	CD at 1%	2.14

*Original value ** Arc sine transformed value

Table 3: Antagonistic activity of *Bacillus subtilis* strains on the mycelial growth of *Aspergillus niger*

Sl. No.	Strains	Mycelial inhibition (%)
1	*Bs*-1	54.86* (47.79)**
2	*Bs*-2	62.33 (52.14)
3	*Bs*-3	56.60 (48.79)
4	*Bs*-4	45.36 (42.34)
5	*Bs*-6	57.43 (49.27)
6	*Bs*-9	61.46 (51.62)
7	*Bs*-10	64.10 (53.18)
8	*Bs*-20	56.23 (48.57)
9	*Bs*-21	60.06 (50.80)
10	*Bs*-22	50.00(44.99)
11	*Bs*-29	53.66 (47.10)
	S. Em±	0.44
	CD at 1%	1.78

* Original value ** Arc sine transformed value

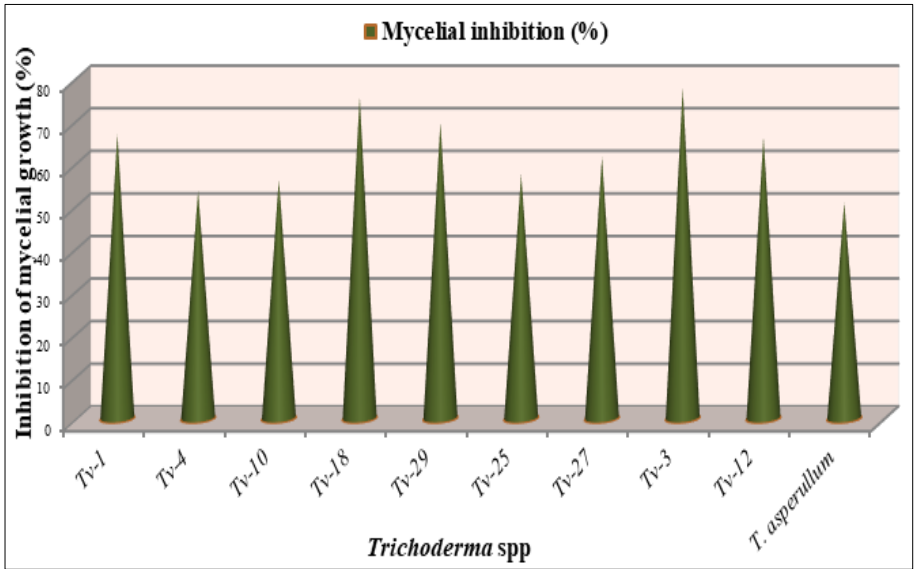

Fig 1: Bio efficacy of different strains of *Trichoderma* spp. against *Aspergillus niger*

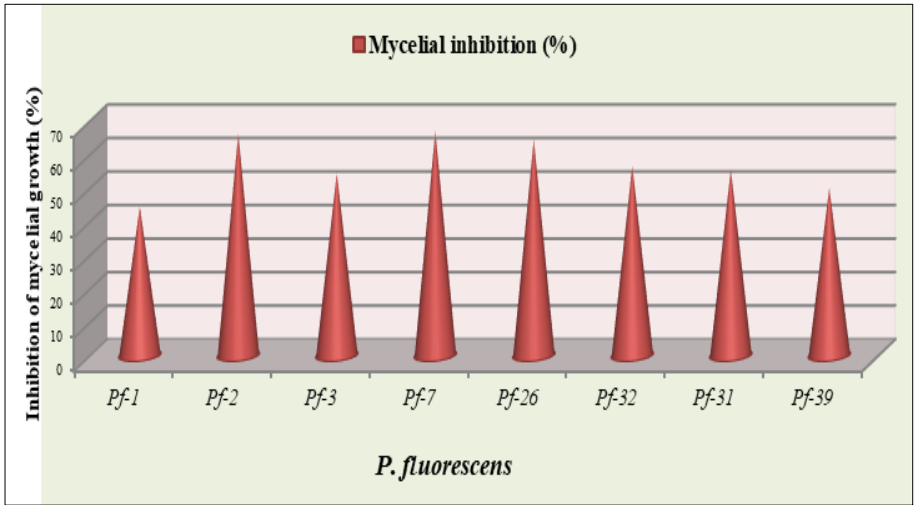

Fig 2: Bio efficay of *Psuedomonas fluorescens* strains on inhibition of mycelial growth of *A. niger*

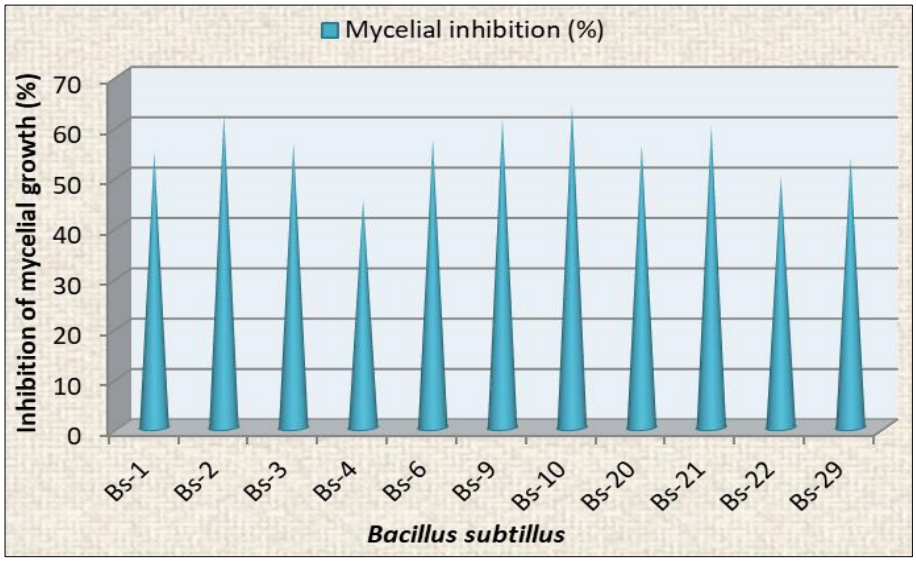

Fig 3: Antagonistic activity of *Bacillus subtilis* strains on the mycelial growth of *Aspergillus niger*

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