International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(4): 73-76

Received: 01-05-2017
Accepted: 02-06-2017

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Management of collar rot disease of groundnut (*Arachis hypogaea* L.) Caused by *Aspergillus niger* through bio-agents

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Abstract
All the bio agents were found significantly inhibitory to the fungal growth as compared to control *in vitro* and *in vivo*. Antagonistic effect of of 2 *Trichoderma* strains (*T. harzianum and Trichoderma viride*), *Pseudomonas fluorescens and T. harzianum + P. fluorescens* against the collar rot disease-causing fungus *A. niger*, were studied *in vitro and in vivo*. Studied of relative efficacy of organic amendments with bio-agents field conditions also. Of all the 2 fungal and 1 bacterial biocontrol agents screened by dual culture technique. It was observed that *T. harzianum* inhibited maximum (81.66%) growth of test fungus, followed by *T. viridae* (66.94%) *in vitro* and in feild conditions observed *T. harzianum + P. fluorescens* was most effective (52.21%) followed by *T. harzianum (45.73%)*. Thus, these findings indicated that biological control using bioagents can be exploited in the frame work of integrated disease management to manage the collar rot of groundnut incited by *Aspergillus niger*.

Keywords: *Aspergillus niger*, bio agents, *Trichoderma* spp. and *Pseuodomons floureces*

Introduction
Groundnut (*Arachis hypogaea* L.) is a leguminous oilseed crop and India occupies the first position, both with regard to area and production of groundnut in the world. Groundnut is a crop which is mainly cultivated under rain-fed conditions, thus, pathogens have more of a chance to attack the crop. The crop suffered from many divastating disease such as early leaf spot (*Phaeoisariopsis arichidicola)* late leaf spot (*Phaeoisariopsis personata*), rust (*Puccinia arichidis*), collar rot (*Aspergillus niger* van Tieghem), stem rot (*Sclerotium rolfsii* Sacc.), root rot (*Macrophomina phaseolina*), and afla root (*Aspergillus flavus*) are economically important in India. Among the soil borne diseases of groundnut, collar rot caused by *Aspergillus niger* is an important disease. The collar rot (*Aspergillus niger*) of groundnut is an important seed and soil borne disease. It was first reported by [4]. However, first reported the *Aspergillus* blight of groundnut caused by *A. niger* in India [5]. This disease appears in two phases viz, pre-emergence and post-emergence phase. In the pre-emergence phase, the seed may rot in the soil or be covered with sooty black masses of spore on germination, the emerging hypocotyls are rapidly killed by these spores. In the post-emergence phase, circular light brown lesions appear initially on the cotyledons and as they advance the hypocotyl tissue or stem lesion becomes water-soaked and shows light brown discoloration. Collar rot causes heavy losses in pod and fodder yield of groundnut. Many seed dressing fungicides are reported to be effective against collar rot of groundnut [3, 6]. The above method is very needed to keep the disease below the economic threshold level without damaging the agro-ecosystem in soil [13]. *Trichoderma* have been used as biological control agents against soil-borne plant pathological fungi [7]. The main objective of the present study was to find an, *in vitro Trichoderma* strain that will act as the best bio-control agent for effectively inhibiting the growth of *A. niger* (as all *Trichoderma* strains do not work equally against a specific disease). The second aim was to determine the overall efficacy of bio-agents to control collar rot disease in field condition.

Experimental
Materials and Methods
Isolation and maintenance of microbes
Groundnut seedlings which showed typical symptoms of collar rot, were cut into small bits using a sterilized blade. The pure pathogen culture (*A. niger*) was made by the hyphal tip isolation method on the solidified PDA medium in petri plates [17].

A typical black mycelium (conidia) growth of *A. niger* was observed after 72 h of incubation, at 25±1 °C, in an incubator. This was maintained throughout the study by periodical transfers on (PDA) medium under aseptic conditions, to keep the culture fresh and viable. All the bio-agents (*Trichoderma viride, T. harzianum* and *Pseudomonas fluorescens*) obtained from Department of Plant Pathology, RARI, Durgapura, Jaipur, Rajasthan and maintained throughout the study by periodical transfers on PDA media under aseptic conditions, to keep the culture fresh and viable.

In vitro* antagonism between bio-agents and pathogen – *A. niger

The dual culture technique was used to test the antagonistic effect of of 2 *Trichoderma* stains (*T. viride* and *T. harzanium*) and P. fluorescens against *A. niger* on PDA media. A 5 mm dia mycelial disc, from each *Trichoderma* isolate and test fungus (*A. niger*) were placed on PDA medium in the same petri plate, approximately 4 cm away from each other. The experiment was conducted in four replications for each antagonist. All the inoculated plates were incubated at a temperature of 25±1°C. After six days, the plates were observed for growth of antagonist and test fungus. Index of antagonism as per cent growth inhibition of *A. niger*, was calculated using formula as suggested by [1].

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

Where,

I = Per cent mycelial growth inhibition

C = Mycelial growth of fungal plant pathogen in control (mm)

T = Mycelial growth of fungal plant pathogen in treatment (mm)

In vivo* antagonism between bio-agents and pathogen – *A. niger

The fungus *A. niger* multiplied on sorghum grain at 25 $\pm$ 1 ^{0}C for one week used as soil inoculum. Seeds of groundnut were treated with *T. harzianum*, *T. viride* @ 4g/kg seed and *Pseudomonas fluorescens* @ 8g/kg seed separately and fungus inoculated pots without treatment served as control. Each treatment was taken with four replication and Observed the disease incidence recorded up to 45 days. Percent collar rot incidence was calculated by following formula:

$$\% \text{ disease incidence} = \frac{\text{Number of rotted plants}}{\text{Total number of plants}} \times 100$$

Results and Discussion

Antagonism between bio-agents and pathogen – *A. niger in vitro* and *in vivo*

1. Groundnut is an economically important crop but the collar rot disease was affecting its growth. Growth inhibition of *A. niger* during *in vitro* interaction with bio-control agents *Trichoderma and Pseudomonas* at 6 days after inoculation (DAI), was depicted in (Table-1., Fig. 1 and Plate-1). Per cent growth inhibition of pathogen (*A. niger*) was significantly higher in Maximum (81.66%) mycelial growth inhibition of pathogen was recorded in *T. harzianum* followed by (66.94%) in *T. Viride*. Non significant differences were observed between antagonists *T. harzianum* (81.66%) and *P. fluorenscens (43.14%)*.
2. However, other antagonists were recorded with a below 40 % growth inhibition of fungal pathogen. Thus, it was observed that *T. harzianum* antagonist (*i.e.* interaction between *Trichoderma harzianum* and pathogen *A. niger*) have a better growth inhibition of test fungus *A. niger*, compared to the other bio-control agents. Percent disease incidence of collar rot of groundnut during *in vivo* interaction with bio-control agents *Trichoderma and Pseudomonas* at 10-45 day after sowing (DAS) was depicted in (Table- 2., Fig. 2). Efficacy of bio-agents viz. *T. harzianum + P. fluorenscens* was found significantly superior with (52.21%) disease control followed by *T. harzianum (45.73*%). *P. fluorensens (34.40%)* and *T. viride (38.28%)* were least effective against *A. niger*. Thus, it was observed that *T. harzianum + P. fluorenscens* bioagent have minimum percent disease incidence compared to the other bio-control agents.
3. The present experiment was initiated to study the comparative efficacy of the bio-control agents *Trichoderma harzianum, T. viridae and P. fluresecence* against *A. niger* causing collar rot at the pre emergence post emergence phase. An antagonistic effect of fungal bio-control agents against the test pathogen fungus (*A. niger*) was observed. *T. harzianum* showed maximum reduction in growth of test fungus followed by *T. harzianum T. viride and P. fluresence*. These results are in confirmation with the finding of [9], who reported that the *T. viride* and *T. harzianum* were found to be effective in reducing the radial growth of *A. niger in vitro*. [14 12] also documented that *Trichoderma* isolates significantly inhibited the growth of *A. niger*. [2, 11, 10] they reported that *T. harzianum* was found significantly superior in reducing mycelial growth of *A. niger*.
4. Amongst different bio-agents used tested under pot conditions. *T. harzianum + P. fluoresens* was found more effective disease control followed by *T. harzianum and T. viride*. *P. fluoresens* were found least effective. The mycoparasitism inhibitory effects of *T. harzianum*, on the growth of collar rot in groundnut (*A. niger)* under field condition [8 15] reported that seed dressing with *T. harzianum* was found to be more effective in reducing collar rot incidence in groundnut.

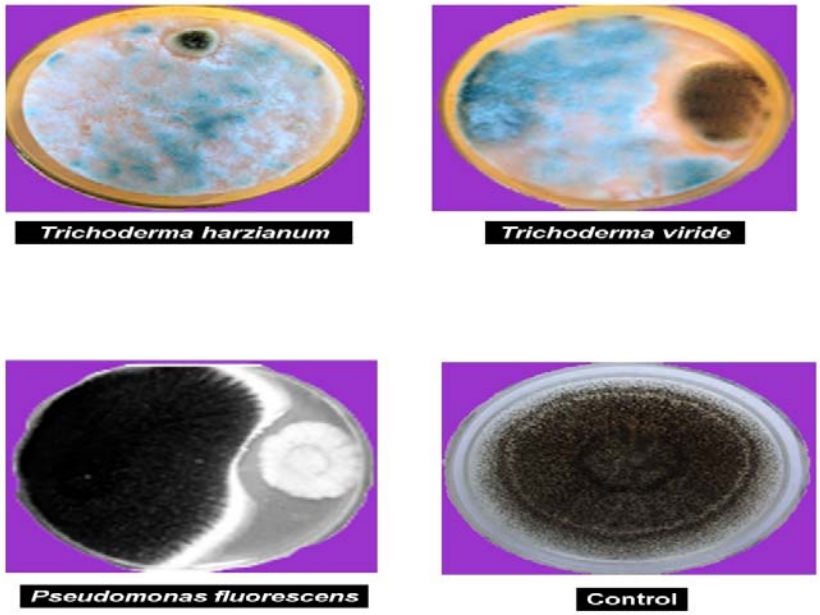

Plate : 4.6 Efficacy of bio-agents on mycelial growth of *Aspergillus niger*

Table 1: Efficacy of bio-agents against growth of *Aspergillus niger* by dual culture *(in vitro)*

Bio-agents	Growth inhibition (%)
Trichoderma harzianum	81.66
	(65.00)
Trichoderma viridae	66.94
	(54.90)
Pseudomanas fluorescens	43.14
	(41.06)
Control	0.00
	(0.00)
SEm+	2.11
CD (p = 0.05)	6.51

*Average of four replications
Figures in parentheses are angular transformed values

Table 2: Efficacy bio-agents against collar rot incidence caused by *Aspergillus niger (in vivo)*

Treatment	Dose	Germination (%)	PDI*	Disease control (%)
T. harzianum	4g/kg	90.75	33.24	45.73
		(75.52)	(35.21)	
T. viride	4g/kg	84.00	36.8	38.28
		(66.42)	(37.35)	
P. fluoresens	8g/kg	81.50	40.18	34.40
		(64.53)	(39.34)	
T. harzianum + *P. fluoresens*	4g + 8g/kg	87.50	29.31	52.21
		(69.30)	(32.78)	
Control		72.00	60.9	0.00
		(58.05)	(51.30)	
SEm+		0.04	0.06	
CD (p = 0.05)		0.12	0.19	

Average of four replications
Figures in parentheses are angular transformed values
PDI = Percent disease incidence

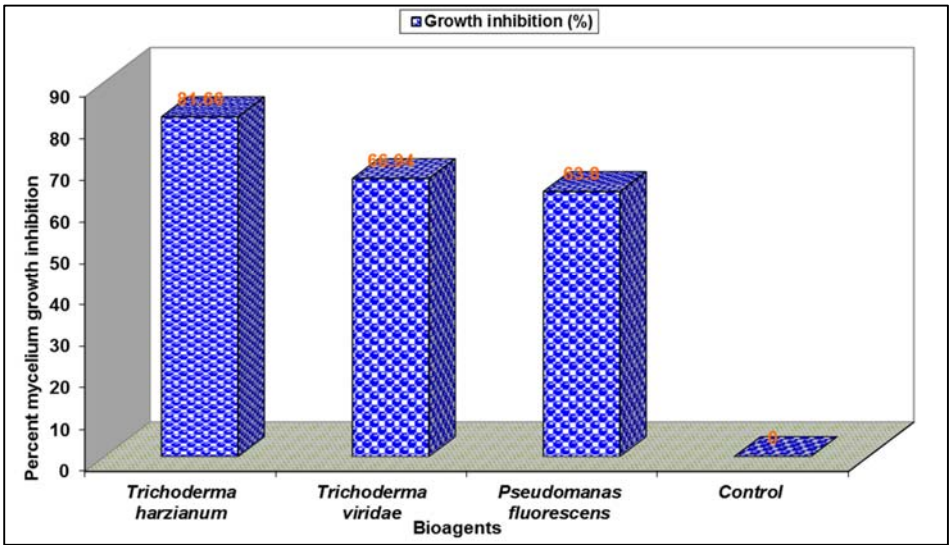

Fig 1: Efficacy of bioagents on mycelial growth of *Aspergillus niger in vitro*

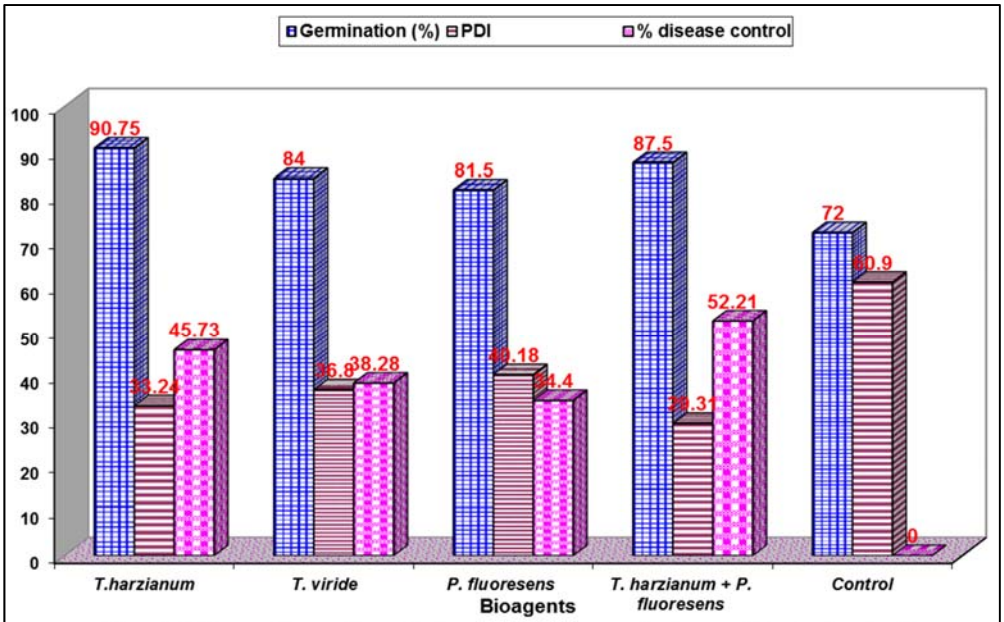

Fig. : 2 : Efficacy bioagents against collar rot of groundnut caused by *A. niger in vivo*

Acknowledgement

I would like to acknowledge the Major Advisor, Dean of college and lab technician for great support to conducted lab experiment and experimental field trial.

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