www.ThePharmaJournal.com

# The Pharma Innovation



ISSN (E): 2277- 7695 ISSN (P): 2349-8242 NAAS Rating: 5.23 TPI 2021; 10(5): 374-379 © 2021 TPI www.thepharmajournal.com Received: 02-03-2021

Accepted: 06-04-2021

#### P Ramanjineyulu

Department of Plant Pathology, S.V. Agricultural College, Tirupati, ANGRAU, Andhra Pradesh, India

#### K Viswanath

Department of Plant Pathology, Institute of Frontier Technology, RARS, Tirupati, ANGRAU, Andhra Pradesh, India

#### P Nagamani

Department of Plant Pathology, KVK- Nellore, Andhra Pradesh, India

#### N Kiran Kumar

Department of Plant Pathology, Agricultural College, Bapatla, ANGRAU, Andhra Pradesh, India

Corresponding Author: P Ramanjineyulu Department of Plant Pathology, S.V. Agricultural College, Tirupati, ANGRAU, Andhra Pradesh, India

### Evaluation of rhizospheric antagonistic microorganisms and fungicides against pod rot associated pathogens of groundnut (Arachis hypogaea L.)

#### P Ramanjineyulu, K Viswanath, P Nagamani and N Kiran Kumar

#### Abstract

Pod rot is a serious disease of groundnut crop affecting the quality and yield potentiality to a large extent, it poses a great threat to the groundnut farming community. In the evaluation of the efficacy of four isolates of *Trichoderma* spp, and four isolates of rhizospheric bacteria against all the isolates of *Fusarium* spp, *Rhizoctonia bataticola, Sclerotium rolfsii*, TT4 isolate of *Trichoderma* has shown 41.3 per cent and TB3 isolate of rhizospheric bacteria bas shown 66.0 per cent highest mean inhibition. Among the fungicides tested against pathogen isolates which exhibited the lowest mean per cent inhibition in dual culture studies *viz., Fusarium sp* (TF1), *Rhizoctonia bataticola* (TRb8), *Sclerotium rolfsii* (TSr6), tebuconazole and tebuconazole + trifloxystrobin has shown cent per cent inhibition at all the three concentrations. In *in vivo* evaluation of integrated management components against the three isolates of pathogens, the treatment T6 i.e. seed treatment with tebuconazole @ 1g/kg of seed, soil application of potential fungal and bacterial bio agents accompanied by soil application of gypsum at 45 DAS has recorded the lowest pod rot incidence of 11.4, 20.7 and 6.0 per cent respectively. Management of the disease itself is difficult due to the complex of pathogen association, for which there is a need for the integration of cultural, biological and chemical methods in disease management.

Keywords: Groundnut, pod rot, rhizospheric antagonistic microorganisms, fungicides

#### Introduction

Groundnut (*Arachis hypogaea* L.) is a major oilseed, feed and food crop widely cultivated in tropical and subtropical regions of the world. It is the fourth important source of edible oil and third most source of vegetable protein. The kernels contain 44-56 per cent high quality edible oil, 22-30 per cent easily digestible protein, 10-20 per cent carbohydrates, vitamins (E, K and B complex), minerals (Ca, P, Mg, Zn and Fe) and digestible fiber (Bell, 2008)<sup>[4]</sup>. Anantapur, Chittoor, Kurnool and Kadapa are the major groundnut growing districts, occupying 97 per cent of total groundnut growing area of the state (IOPEPC Report, 2017)<sup>[15]</sup>.

Productivity levels of groundnut in most of the developing countries are low due to several production constraints which include biotic and abiotic stresses, lack of seed dispersal systems, technological knowhow, market accessibility, low input use etc.

Among soil borne diseases of groundnut, pod rot is a complex and economically important disease widespread in the Semi-Arid Tropics causing severe damage to groundnut production in a number of countries including India (Abdou and Khadr, 1974; Frank, 1972; Mercer, 1977; Porter *et al.*, 1975) <sup>[1, 11, 23, 28]</sup>. Depending upon the geographic location, the losses in yield due to pre-harvest pod rot may vary in the range of 5-50 per cent (Subrahmanyam *et al.*, 1980) <sup>[32]</sup>. In Andhra Pradesh the mean disease incidence was varied from 11.4 to 28.6 per cent and 8.5 to 30.0 per cent in Chittoor and Anantapuramu districts respectively (Ramanjineyulu *et al.*, 2018) <sup>[30]</sup>. Pod rot of groundnut is emerging as a serious disease in different agro-climatic zones of Andhra Pradesh due to change in soil moisture, pH, soil temperature and crop sequence facilitating rapid multiplication of different microflora in crop rhizosphere

Management of pod rot has been difficult in part because of the wide host range of the pathogens (Melouk and Backman, 1995)<sup>[22]</sup> and limited cultivar resistance (Besler *et al.*, 2003; Lewis and Filonow, 1990; Walker and Csinos, 1980)<sup>[5, 19, 35]</sup>. Hence, in the present study an attempt was made to manage this soil borne disease with some bio agents and chemicals.

#### **Material and Methods**

### Isolation and maintenance of pod rot associated pathogens and rhizospheric antagonistic microorganisms

Pod rot associated pathogens which were isolated during the previous study (Ramanjineyulu *et al.*, 2018) <sup>[30]</sup> *viz.*, *Fusarium* spp., *Rhizoctonia bataticola* and *Sclerotium rolfsii* were used in the present investigation. Native rhizospheric soil samples collected during the survey (Ramanjineyulu *et al.*, 2018) <sup>[30]</sup> were used for isolation of rhizospheric antagonistic mycoflora. Rhizosphere antagonistic fungi were isolated on *Trichoderma* selective medium and rhizosphere antagonistic bacteria were isolated on Kings B medium by following serial dilution technique (Johnson and Curl, 1977) <sup>[16]</sup>.

### In vitro evaluation of bio-control agents against pod rot associated pathogens

Dual culture technique was used to identify the potential rhizospheric antagonists of groundnut (Dennis and Webster, 1971)<sup>[8]</sup>. Per cent inhibition of growth of the fungus over the control was calculated (Vincent, 1927)<sup>[34]</sup>.

Per cent inhibition (PI) (%) = 
$$\frac{C-T}{C} \times 100$$

Where, PI = Per cent inhibition in growth of test pathogen, C = Radial growth (mm) of test pathogen in control, T = Radial growth (mm) of test pathogen in treatment

Among all the rhizosphere antagonistic mycoflora and bacteria tested against pod rot pathogens, one of each

potential fungus, bacteria and each one virulent pathogen were selected and used for further studies.

### *In vitro* evaluation of the efficacy of fungicides against pod rot associated pathogens

Five fungicides *viz.*, Hexaconazole 5% EC, Tebuconazole 25.9% EC, Mancozeb + Carbendazim 63%) + (12%) WP, Metiram + Pyraclostrobin (55%) + (5%) WG and Tebuconazole + Trifloxystrobin (50%) + (25%) WG were evaluated *In vitro* efficacy of fungicides against the pathogens was evaluated by poisoned food technique (Nene and Thapliyal, 1993) <sup>[24]</sup>. Per cent inhibition of the growth of the fungus over the control was calculated. (Vincent, 1927) <sup>[34]</sup>.

Per cent inhibition (PI) (%) = 
$$\frac{C-T}{C} \times 100$$

Where, PI = Per cent inhibition in growth of test pathogen, C = Radial growth (mm) of test pathogen in control, T = Radial growth (mm) of test pathogen in treatment.

### Evaluation of effective fungicide and bio-control agents against dominant pod rot fungi *in vivo*

This experiment was conducted under glasshouse conditions by randomized block design (RBD) on Dharani cultivar and maintained three replications of each treatment. In this experiment the effective fungicide and bio-control agents were evaluated against dominant pod rot associated fungi as per the treatments given below:

Treatment. No	Treatment
T1	Seed treatment with Tebuconazole 2% DS @ 1 g/kg of seed
T2	T1+ Soil application with potential rhizospheric fungal bio-agent (RFx) at 45 DAS
T3	T1+ Soil application with potential rhizospheric bacterial bio-agent (RBx) at 45 DAS
T4	T1 + Foliar spray of effective fungicide at 60 and 75 DAS
T5	T1+ Soil application of Gypsum @ 500 kg/ha at 45 DAS
T6	T1+ Soil application of potential fungal and potential bacterial bio-agent + soil application of Gypsum at 45 DAS
T7	T1 + Soil application of Gypsum at 45 DAS + foliar spray of effective fungicide at 60 and 75 DAS
T8	Control

#### Mass multiplication of pod rot associated fungi and preparation of talc based formulation of potential fungal and bacterial bio control agent

Mass multiplication of pod rot associated fungi was carried out on substrate sorghum grains as per the method suggested by Patil et. al. (2017)<sup>[25]</sup>. Potential fungal biocontrol agent was mass multiplied on PDB and formulations prepared as per method suggested by Raguchander et al., 1997 [29]. Antagonistic bacterium was mass multiplied on nutrient broth and talc formulations are prepared by the method suggested by Vidhyasekaran and Muthamilan 1995 [33]. Seed treatment was done with Tebuconazole 2% DS (Raxil) @ 1 g kg<sup>-1</sup> of seed. Seeds are properly mixed with the required quantity of fungicide and shade dried. Earthen pots (30 cm in diameter) were filled with sterilized soil and pathogen inoculum was mixed at a proportion of 30 g kg<sup>-1</sup> to the soil then the seeds were sowed, later the treatments were imposed. Control pots were also maintained. The disease reduction percentage of pod rot diseases was calculated as follow:

```
Per \ cent \ reduction \ over \ control = \frac{disease \ reduction \ in \ control}{disease \ reduction \ in \ treatment} \times 100
```

#### **Results and Discussion**

### Isolation of bio-control agents from rhizosphere of groundnut

From the rhizosphere soil samples two isolates each of *Trichoderma* spp. and bacteria were isolated from Madibaka village (TT1; TB1) of Yerpedu mandal and TMV Kandriga (TT2; TB2) of Srikalahasti Mandal of Chittoor district. Similarly from the Anantapur district two isolates each of *Trichoderma* spp and bacteria from Siddampeta (TT3; TB3) of Bukkarayasamudram mandal and Jonnalakothapalle (TT4; TB4) of Mudigubba mandal were isolated (Plate 1, 2.

#### *In vitro* evaluation of the antagonistic activity of biocontrol agents against pod rot associated pathogens *In vitro* evaluation of the efficacy of *Trichoderma* spp.

### *In vitro* evaluation of the efficacy of *Trichoderma* spp. against *Fusarium* spp.

The efficacy of four isolates of *Trichoderma* spp. was evaluated by dual culture against all the ten isolates of *Fusarium* spp. (Table 1). In the dual culture of the ten isolates of *Fusarium* spp. with TT1 *Trichoderma* sp., the highest per cent inhibition (35.8%) was recorded on TF2 *Fusarium* sp. which was at par with TF7 (34.4%), TF8 (33.6%), TF1

(32.3%), TF9 (31.9%) isolates and significantly differed with the remaining isolates. Whereas with TT2 isolate of *Trichoderma sp.* the highest per cent inhibition of 34.5 per cent was recorded on TF5 isolate of *Fusarium sp.*, which significantly differed with rest of the isolates. Similarly with TT3 isolate of *Trichoderma sp.*, the highest per cent inhibition of 67.6 per cent was recorded on TF2 *Fusarium* isolate which significantly differed with rest of the isolates. With TT4 isolate of *Trichoderma sp.* the highest per cent inhibition of 41.0 per cent recorded on TF8 isolate of *Fusarium sp* which significantly differed with the remaining isolates.

### In vitro evaluation of the efficacy of Trichoderma spp. against Rhizoctonia bataticola isolates

In the dual culture of TT1 isolate of *Trichoderma sp* against all the ten isolates of *R. bataticola*, the highest inhibition of 67.7 per cent was recorded with TRb5 isolate which significantly differed with other isolates. While with TT2 isolate of *Trichoderma sp.*, the highest per cent inhibition of 60.0 per cent was observed with TRb3 which significantly differed in inhibition with other isolates. Similarly, with TT3 isolate of *Trichoderma sp.*, the highest inhibition of 47.1 per cent was recorded with TRb4 which significantly differed with rest of the isolates. Finally with TT4 isolate of *Trichoderma sp.*, the highest inhibition (74.9%) was observed on TRb3 which significantly differed with rest of the *R. bataticola* isolates (Table 1).

### *In vitro* evaluation of the efficacy of *Trichoderma* spp. against *Sclerotium rolfsii* isolates

Interaction effects between ten isolates of *S. rolfsii* and TT1 isolate of *Trichoderma* sp. in dual culture revealed that the highest inhibition (57.2%) was observed on TSr5 which was on par with TSr4 (54.3%) and significantly differed with rest of the isolates (Table 1). While with TT2 isolate of *Trichoderma sp.*, the highest inhibition (50.3%) was observed on TSr5 which differed significantly with rest of the isolates. Similarly with TT3 isolate of *Trichoderma sp.*, the highest inhibition (45.5%) was observed on TSr7 which was on par with TSr5 (45.2%) and significantly differed with rest of the isolates. Finally with TT4 isolate of *Trichoderma sp.*, the highest inhibition (51.3%) was observed on TSr9 which was on par with TSr10 (50.0%) and differed significantly with rest of the isolates.

### *In vitro* evaluation of the efficacy of rhizospheric bacteria against *Fusarium* spp.

Interaction effects between ten isolates of *Fusarium* spp. and TB1 isolate of bacteria in dual culture revealed that the TB1 isolate of bacteria exhibited the highest inhibition (56.6%) on TF3 isolate of *Fusarium sp.* which was on par with TF9 (53.9%) and differed significantly with the rest (Table 1). With TB2 bacterial isolate, the highest inhibition of 55.5 per cent was observed on TF4 and TF6 which differed significantly with rest of the isolates. Similarly with TB3 bacterial isolate, the highest inhibition (62.2%) was observed on TF4 which differed significantly with rest of the isolates. Finally with TB4 bacterial isolate, the highest inhibition (69.8%) was observed on TF4 isolate of *Fusarium sp.* which differed significantly with other isolates.

## *In vitro* evaluation of the efficacy of rhizospheric bacteria against *Rhizoctonia bataticola* isolates

Interaction effects between ten isolates of R. bataticola and

TB1 isolate of rhizospheric bacteria in dual culture revealed that TB1 isolate of bacteria showed, the highest inhibition (64.4%) on TRb10 which was on par with TRb5 (62.2%) and significantly differed with remaining isolates (Table 1). With TB2 isolate of bacteria, the highest inhibition (63.5%) was recorded on TRb9 which was on par with TRb4 (58.4%) and significantly differed with remaining isolates. Similarly with TB3 isolate of bacteria, the highest inhibition (78.9%) was observed on TRb10 which was on par with TRb2 (73.3%) and differed significantly with remaining isolates. Finally with TB4 bacterial isolate, the highest inhibition (56.9%) was observed on TRb10 which was on par with TRb1 (55.0%), TRb6 (52.2%), TRb5 (51.2%) and significantly differed with remaining isolates of *R. bataticola*.

### *In vitro* evaluation of the efficacy of rhizospheric bacteria against *Sclerotium rolfsii* isolates

Interaction effects between the ten isolates of S. rolfsii and TB1 rhizospheric bacteria in dual culture revealed that the TB1 bacterial isolate showed the highest inhibition of 69.1 per cent on TSr2, which was on par with TSr10 (67.1%), TSr1 (65.4%), TSr5 (65.4%) and TSr7 (64.5%) and significantly differed with the remaining isolates of S. rolfsii (Table 1). While, with TB2 isolate of bacteria, the highest inhibition of 89.9 per cent was observed on TSr8 which was on par with TSr4 (88.5%), TSr2 (87.4%), TSr5 (86.3%) and TSr3 (85.6%) and differed significantly with remaining isolates. Similarly with TB3 bacterial isolate the highest inhibition (92.4%) was observed against TSr7 which was on par with TSr10 (92.3%), TSr4 (90.6%), TSr1 (89.2%), TSr5 (88.2%) and TSr8 (87.8%) and significantly different with rest of the isolates. Finally with TB4 isolate of bacteria, the highest inhibition was observed on TSr5 (81.9%) which differed significantly with remaining isolates of S. rolfsii.

There were significant differences in average responses to antagonism by the four isolates each of *Trichoderma* spp and rhizospheric bacteria between the different genera of pathogens viz., *Fusarium, Rhizoctonia and Sclerotium*. Also there were significant differences in average antagonistic abilities of the isolates of *Trichoderma* and rhizospheric bacteria when averaged over pathogens. On comparing the overall per cent inhibition of the isolates of *Trichoderma* and rhizospheric bacteria on the isolates of *Fusarium* spp, *R. bataticola* and *S. rolfsii*, TT4 isolate of *Trichoderma*, TB3 isolate of rhizospheric bacteria has shown the highest mean per cent inhibition. Whereas, TF1 isolate of *Fusarium sp*, TRb8 isolate of *R. bataticola* and TSr6 isolate of *S. rolfsii* has exhibited the lowest mean per cent inhibition as shown in table 1.

## *In vitro* evaluation of the efficacy of fungicides against pod rot associated pathogens

Five fungicides were evaluated under *in vitro* for their overall efficacy on the isolates with the lowest degree of growth inhibition in dual culture *viz., Fusarium sp.* (TF1), *R. bataticola* (TRb8), *S. rolfsii* (TSr6). Among the fungicides tested against TF1 isolate of *Fusarium sp.*; tebuconazole, mancozeb + carbendazim and tebuconazole + trifloxystrobin have shown cent per cent inhibition of the mycelial growth at all the three concentrations. The mycelial growth inhibition increased with increase in the concentrations of hexaconazole (51.1 to 67.7%) and metiram + pyraclostrobin (61.9 to 64.4%) from 1500 to 2500 ppm (Table 2).

In case of fungicides tested against TRb8 isolate of R.

*bataticola*, tebuconazole, mancozeb + carbendazim, metiram + pyraclostrobin and tebuconazole + trifloxystrobin showed cent per cent inhibition at all the three concentrations. Whereas, hexaconazole showed increased inhibition from 78.3 to 88.3 per cent with the increase in concentration from 1500 ppm to 2500 ppm. In case of fungicides tested against TSr6 isolate of *S. rolfsii*, hexaconazole, tebuconazole, metiram + pyraclostrobin and tebuconazole + trifloxystrobin

showed cent per cent inhibition at all the three concentrations. Whereas, the combination product of mancozeb + carbendazim showed increased inhibition from 59.1 to 100 per cent with the increase in concentration from 2500 ppm to 3500 ppm (Table 2). The data revealed that all the fungicides at all the three concentrations reduced the mycelial growth of pathogens when compared to control.

Table 1: Summarized per cent inhi	bition of pod rot associate	ed pathogens by the funga	al and bacterial antagonists
-----------------------------------	-----------------------------	---------------------------	------------------------------

Name of the pathogens	Name of the isolate		richode	rma sp	p.	Rhizospheric bacteria				Moon nor cont inhibition	
Name of the pathogens	Name of the isolate	TT1	TT2	TT3	TT4	TB1	TB2	TB3	TB4	Mean per cent inhibition	
	TF1	32.3	16.4	19.9	31.6	46.3	34.1	42.0	44.4	33.4	
	TF2	35.8	12.8	62.6	33.7	42.5	44.0	46.0	43.0	40.0	
	TF3	30.5	24.2	38.0	31.7	56.6	45.8	45.8	51.5	40.5	
	TF4	24.6	23.0	21.5	24.9	45.6	55.5	62.2	69.8	40.9	
Eugenium ann	TF5	23.7	30.2	34.4	23.1	46.3	42.1	45.5	41.5	35.8	
Fusarium spp	TF6	25.0	14.4	26.8	14.8	43.2	55.5	55.6	55.5	36.4	
	TF7	34.4	22.8	47.2	33.3	45.1	47.0	55.0	53.0	42.2	
	TF8	31.2	21.1	19.4	34.3	41.6	47.0	41.6	45.2	35.2	
	TF9	31.9	13.6	28.1	27.6	53.8	35.6	47.7	47.8	35.8	
	TF10	24.4	10.3	26.0	32.0	39.1	47.6	46.7	45.7	34.0	
	TRb1	57.6	49.0	34.8	53.7	57.5	38.1	48.9	55.0	49.3	
	TRb2	48.1	44.0	30.0	70.2	51.9	49.6	73.3	34.3	50.2	
	TRb3	49.2	60.0	25.4	74.9	51.9	38.1	50.8	40.0	48.8	
	TRb4	25.0	42.1	47.1	62.4	59.3	58.4	55.2	37.1	48.3	
Rhizoctonia bataticola	TRb5	67.6	45.0	26.1	33.1	62.2	52.9	71.1	51.2	51.2	
Knizocionia balancola	TRb6	38.1	45.5	28.1	51.7	59.6	50.0	68.2	52.2	49.2	
	TRb7	48.4	36.0	38.5	47.7	55.6	50.6	65.4	40.8	47.9	
	TRb8	34.4	46.9	27.9	48.3	55.6	45.2	62.6	47.0	46.0	
	TRb9	23.1	47.6	31.6	57.4	54.2	63.5	60.6	34.4	46.6	
	TRb10	37.3	47.8	25.9	56.3	64.4	50.4	78.9	56.9	52.2	
	TSr1	50.5	34.3	34.3	42.2	65.4	65.2	89.2	66.3	55.9	
	TSr2	49.5	38.6	37.0	41.1	69.1	87.4	75.8	54.8	56.7	
	TSr3	50.0	42.0	29.3	38.0	55.5	85.6	82.2	61.7	55.5	
	TSr4	54.3	34.7	26.8	31.7	58.8	88.5	90.6	67.0	56.5	
Salanatium nalfaii	TSr5	57.2	50.3	45.2	36.5	65.4	86.3	88.5	81.9	63.9	
Sclerotium rolfsii	TSr6	31.9	29.1	24.1	22.1	37.1	65.9	73.9	17.7	37.7	
	TSr7	49.0	37.5	45.5	46.7	64.5	78.8	92.4	54.3	58.6	
	TSr8	51.6	34.0	30.1	36.8	63.1	89.9	87.8	71.9	58.2	
	TSr9	52.4	34.4	30.4	51.3	51.6	78.4	85.1	69.6	56.6	
	TSr10	50.7	31.7	31.5	50.0	67.1	75.6	92.3	73.3	59.0	
Mean per cent inhibition		40.7	34.0	32.4	41.3	54.3	58.4	66.0	52.2		

 Table 2: In vitro evaluation of fungicides on the mycelial growth of virulent isolates of Fusarium spp. Rhizoctonia bataticola and Scleortium rolfsii

		Fusarium spp.			Rhizocto	nia bataticola	Sclerotium rolfsii		
S. No	Fungicides	concentration (ppm)	Mycelial growth (cm)	Per cent inhibition over control	Mycelial growth (cm)	Per cent inhibition over control	Mycelial growth (cm)	Per cent inhibition over control	
		1500	4.4	51.1 (45.6)	2.0	78.3 (62.2)	0.0	100.0 (90.0)	
1	Hexaconazole (5% EC)	2000	4.2	53.1 (46.7)	1.8	80.6 (63.8)	0.0	100.0 (90.0)	
		2500	2.9	67.8 (55.4)	1.1	88.3 (70.0)	0.0	100.0 (90.0)	
	T-h1- (25.00/	500	0.0	100.0 (90.0)	0.0	100.0 (90.0)	0.0	100.0 (90.0)	
2	Tebuconazole (25.9% EC)	1000	0.0	100.0 (90.0)	0.0	100.0 (90.0)	0.0	100.0 (90.0)	
		1500	0.0	100.0 (90.0)	0.0	100.0 (90.0)	0.0	100.0 (90.0)	
	Mancozeb (63%) + Carbendazim (12%) WP	2500	0.0	100.0 (90.0)	0.0	100.0 (90.0)	3.68	59.2 (50.2)	
3		3000	0.0	100.0 (90.0)	0.0	100.0 (90.0)	1.63	81.9 (64.8)	
		3500	0.0	100.0 (90.0)	0.0	100.0 (90.0)	0.00	100.0 (90.0)	
	Metiram (55%) +	1500	3.4	61.9 (51.9)	0.0	100.0 (90.0)	0.0	100.0 (90.0)	
4	Pyraclostrobin (5%) WG	2000	3.2	64.4 (53.4)	0.0	100.0 (90.0)	0.0	100.0 (90.0)	
		2500	3.4	62.8 (52.4)	0.0	100.0 (90.0)	0.0	100.0 (90.0)	
	Tebuconazole (50%) +	660	0.0	100.0 (90.0)	0.0	100.0 (90.0)	0.0	100.0 (90.0)	
5	Trifloxystrobin (25%)	1320	0.0	100.0 (90.0)	0.	100.0 (90.0)	0.0	100.0 (90.0)	
	WG	1980	0.0	100.0 (90.0)	0.0	100.0 (90.0)	0.0	100.0 (90.0)	
6	Control	-	9.0	-	9.0	-	9.0		
	C.D		0.14	1.68	0.06	1.05	0.06	0.51	

S.Em	0.05	0.59	0.02	0.37	0.02	0.18
C.V	5.24	1.58	5.00	0.86	4.98	0.42

#### Evaluation of effective fungicide and potential bio-control agents against pod rot associated pathogens under pot culture conditions

In the present study, TF1 isolate of *Fusarium sp*, TRb8 isolate of *R. bataticola*, TSr6 isolate of *S. rolfsii* recorded the lowest degree of growth inhibition when compared to other isolates of the respective fungi in dual culture with each of the four fungal and bacterial antagonists. The fungicide tebuconazole, TT4 isolate of *Trichoderma sp* and TB3 isolate of bacteria showed significant efficacy under *in vitro* against all the isolates of *Fusarium sp.*, *R. bataticola* and *S. rolfsii*. Hence, in order to evaluate the same under *in vivo*, the treatments were designed by integrating effective antagonists, fungicide, and isolates of the pathogen

Results observed from the table 3 revealed that, against the three isolates of pathogen *viz.*, TF1 isolate of *Fusarium sp*, TRb8 isolate of *R. bataticola*, TSr6 isolate of *S. rolfsii*, treatment T6 i.e. seed treatment with tebuconazole @ 1g/kg of seed + soil application of potential fungal and bacterial bio agents + soil application of gypsum at 45 DAS has recorded the lowest pod rot incidence of 11.4, 20.7 and 6.0 per cent respectively with 73.3, 64.4, 87.6 per cent reduction over

untreated control in the pot culture studies. The dry weight of the plants in pots inoculated with *Fusarium sp* (31.1 and 30.3 g), *R. bataticola* (31.8 and 29.8 g) and *S. rolfsii* (33.2 and 32.5 g) was found to be on par in T6 and T7 treatments.

The in vitro screening with arbitrary rating system for biological antagonists effective against soil borne plant pathogens is a simplistic approach to understanding a small sector of biological systems in disease control (Bell et al., 1982) [3]. Trichoderma spp. are effective in control of soil/seed borne fungal diseases in several crop plants (Kubicek et al., 2001) [18], including groundnut (Podile and Kishore, 2002). Antagonists like T. harzianum and T. viride could inhibit the pathogens A. niger, F. solani, Rhizopus sp, M. phaseolina causing pod rot disease of groundnut (Elad et al., 1982; Meher, 1997) <sup>[9, 21]</sup>. The evaluation of the antagonistic activities of T. harzianum strain T100, T. viride and T. haematum against F. oxysporum and F. proliferatum, showed that T. harzianum T100 strain lysed the confronting mycelia and produced volatile metabolites exhibiting the highest inhibition (Perveen and Bokhari, 2012; Ghanbarzadeh et al., 2014) [26, 12].

Table 3: Evaluation of effective fungicide and potential bio control agents against virulent isolate of <i>Fusarium</i> spp. under pot	er pot culture conditions
--	---------------------------

		<i>Fusarium</i> sp	р	R	Rhizoctonia bata	ıticola	Sclerotium rolfsii			
Treatment	Plant dry	Pod rot	% reduction	Plant dry	Pod rot	% reduction	Plant dry	Pod rot	% reduction	
Treatment	wt/pot (g)	incidence (%)	over control	wt/pot (g)	incidence (%)	over control	wt/pot (g)	incidence (%)	over control	
T1	22.1	36.5 (37.1)	14.7 (22.49)	22.1	36.5 (37.1)	14.7 (22.49)	22.1	36.5 (37.1)	14.7 (22.49)	
T2	27.8	27.0 (31.3)	36.9 (37.39)	27.8	27.0 (31.3)	36.9 (37.39)	27.8	27.0 (31.3)	36.9 (37.39)	
T3	28.1	27.0 (31.3)	36.9 (37.38)	28.1	27.0 (31.3)	36.9 (37.38)	28.1	27.0 (31.3)	36.9 (37.38)	
T4	29.0	18.2 (25.2)	57.4 (49.26)	29.0	18.2 (25.2)	57.4(49.26)	29.0	18.2 (25.2)	57.4 (49.26)	
T5	28.0	26.8 (31.1)	37.3 (37.46)	28.0	26.8 (31.1)	37.3 (37.46)	28.0	26.8 (31.1)	37.3 (37.46)	
T6	31.1	11.4 (19.7)	73.3 (58.87)	31.1	11.4 (19.7)	73.3 (58.87)	31.1	11.4 (19.7)	73.3 (58.87)	
T7	30.3	14.6 (22.3)	65.8 (54.21)	30.3	14.6 (22.3)	65.8 (54.21)	30.3	14.6 (22.3)	65.8 (54.21)	
T8	20.6	42.8 (40.8)		20.6	42.8 (40.8)		20.6	42.8 (40.8)		
CD	3.62	2.69	1.72	3.62	2.69	1.72	3.62	2.69	1.72	
S.Em	1.20	0.89	0.56	1.20	0.89	0.56	1.20	0.89	0.56	
CV	7.60	5.16	2.29	7.60	5.16	2.29	7.60	5.16	2.29	

The results from experimentation shows that tebuconazole, a triazole fungicide having broad spectrum activity; might have inhibited the sterol demethylation in the cell wall of *Fusarium sp*, *R. bataticola and S. rolfsii* when tested alone (Baird *et al.*, 1991; Brenneman *et al.*, 1994) <sup>[2, 6]</sup> and also in combination with trifloxystrobin. Being an ubiquinol oxidase inhibitor molecule, trifloxystrobin intereferes with mitochondrial respiration pathway thereby, inhibits the growth of fungi (Kodandaram *et al.*, 2013) <sup>[17]</sup>. Similar results were obtained in controlling *R. bataticola* (Maruti *et al.*, 2017) <sup>[20]</sup>. Significant reduction in the *Rhizoctonia* pod rot of groundnut was observed on spraying with tebuconazole and azoxystrobin (Grichar *et al.*, 2000; Besler *et al.*, 2003) <sup>[14, 5]</sup>. In addition, increased calcium levels in plant tissues following applications of calcium nitrate or calcium sulfate provide

applications of calcium nitrate or calcium sulfate provide some disease control, especially under low inoculum pressure conditions. High levels of calcium in plant tissues perfectly offset the action of oxalic acid and the cell wall degrading enzymes of the pathogen (Grichar and Boswell, 1990). Soil application of gypsum reduces the severity of stem and pod rot of groundnut with the increase in the rate of gypsum application (Csinos and Gaines, 1986; Grichar and Boswell, 1990) <sup>[7, 13]</sup>. In the present study, the lowest per cent disease incidence was recorded in treatments consisting of gypsum application in combination with soil application of potential fungal and bacterial antagonists and also with foliar spray of tebuconazole. However, the disease incidence was found to be high in gypsum alone applied pots which may be attributed to the high inoculum potential in the soil as proposed by Filonow and Jackson, 1989<sup>[10]</sup>.

From the present study it can be concluded that the groundnut pod rot disease is complex in nature due to the association of more than one soil borne pathogens for the control of which there is a need for the integration of cultural, biological and chemical methods in disease management.

#### Acknowledgement

The authors expressing their gratitude to Department of Plant Pathology, S.V. Agricultural College and Regional Agricultural Research Station, Tirupati, for the valuable support rendered during the course of investigation.

#### References

1. Abdou YA, Khadr AS. Systemic control of seedling and pod rot disease of peanut (*Arachis hypogaea* L.). Plant Disease Reporter 1974;58:176-179.

- 2. Baird RE, Brenneman TB, Bell DK, Murphy AP. The effects of the fungicide propiconazole (Tilt®) on the groundnut shell mycobiota. Mycological Research 1991;95:571-576.
- 3. Bell DK, Well HD, Markham CR. *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. Phytopathology 1982;72:379-382.
- 4. Bell SL. Peanuts and their classification under the HTSUS. An informed compliance publication 2008,22.
- 5. Besler BA, Grichar WJ, Brewer KD, Baring MR. Assessment of six peanut cultivars for control of *Rhizoctonia* pod rot when sprayed with azoxystrobin or tebuconazole. Peanut Science 2003;30:49-52.
- 6. Brenneman TB, Sumner HR, Chandler LR, Hammond JM, Culbreath AK. Effect of Application Techniques on Performance of Propiconazole for Peanut Disease Control. Peanut Science 1994;21:134-138.
- 7. Csinos AS, Gaines TP. Peanut pod rot complex: a geocarposphere nutrient imbalance. Plant Diseases 1986;70:525-529.
- 8. Dennis C, Webster J. Antagonistic properties of speciesgroups of *Trichoderma*. III. Hyphal interaction. Transactions of British mycological Society 1971;57(3):363-369.
- 9. Elad Y, Hadar Y, Chet I, Henis Y. Prevention with *Trichoderma harzianum* Rifai aggr., of reinfestation by *Sclerotium rolfsii* Sacc. and *Rhizoctonia solani* Kuhn of soil fumigated with methyl bromide and improvement in disease control in tomato and peanuts. Crop Protection 1982;1:199-211.
- 10. Filonow AB, Jackson KE. Effect of Metalaxyl Plus PCNB or Metalaxyl Plus Tolclofos-methyl on Peanut Pod Rot and Soil Populations of *Pythium* spp. and *Rhizoctonia solani*. Peanut Science 1989;16:25-32.
- 11. Frank ZR. Notes on soil management in relation to *Pythium* rot of peanut pods. Plant Disease Reporter 1972;56:600-601.
- 12. Ghanbarzadeh B, Safaie N, Goltapeh EM. Antagonistic activity and hyphal interactions of *Trichoderma* spp. against *Fusarium proliferatum* and *F. oxysporum in vitro*. Archives of Phytopathology and Plant Protection 2014;47(16):1979-1987.
- 13. Grichar WJ, Boswell TE. Comparison of metalaxyl/PCNB with PCNB, gypsum, and metalaxyl for the control of pod rot organisms in peanuts. *Oleagineux* 1990;4:183-187.
- Grichar WJ, Besler BA, Jaks AJ. Use of azoxystrobin for disease control in Texas peanut. Peanut Science 2000;27:83-87.
- 15. IOPEPC. Kharif- Survey of Groundnut Crop 2017. Retrieved from www.iopepc.org
- Johnson LF, Curl EA. Methods for research on the ecology of soil borne plant pathogens. Burgess Publishing Company. Minneapolis. USA 1977,27-35.
- Kodandaram MH, Saha S, Rai AB, Naik PS. Compendium on pesticide use in vegetables. Indian Institute of Vegetable Research Extension Bulletin No. 50, Varanasi, 133 2013.
- Kubicek C, Mach R, Peterbauer CK, Lorito M. *Trichoderma*: From genes to biocontrol. Journal of Plant Pathology 2001;83(2):11-23.
- 19. Lewis PI, Filonow AB. Reaction of peanut cultivars to *Pythium* pod rot and their influence on populations of *Pythium* spp. in soil. Peanut Science 1990;17:90-95.

- Maruti, Savitha AS, Sunkad G, Amaresh YS. In Vitro Efficacy of Fungicides and Bioagents against Dry Root Rot of Pigeonpea Caused by *Rhizoctonia bataticola* (Taub.) Butler. International Journal of Pure & Applied Bioscience 2017;5(6):1341-1347.
- 21. Meher SK. Pod rot of groundnut and its management. M.Sc. (Ag) thesis submitted to Orissa University of Agriculture & Technology, Bhubaneswar, Odisha, India 1997.
- Melouk HA, Backman PA. Managemant of soil borne fungal pathogens. In H.A. Melouk and F.M. Shokes (ed.) Peanut Health Managemaent. APS Press, USA 1995,75-82.
- 23. Mercer PC. A pod rot of peanuts in Malawi. Plant Disease Reporter 1977;61:51-55.
- 24. Nene YL, Thapliyal PN. Fungicides in plant disease control. Oxford and IBH Publishing Company Private Limited. Calcutta 1993,531-550.
- 25. Patil VM, Patole KR, Paprikar MS, Rajput JC. Biological control of brinjal wilt caused by *Fusarium oxysporum* f. sp. *melongenae* using soluble powder formulation of *Aspergillus niger*. International Journal of Advanced Research in Biological Sciences 2017;4(11):66-71.
- Perveen K, Bokhari NA. Antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* isolated from soil of date palm field against *Fusarium oxysporum*. African Journal of Microbiology Research 2012;6(13):3348-3353.
- 27. Porter DM, Garren KH, Van schaik PH. Pod breakdown resistance in peanuts. Peanut Science 1975;2:15-18
- 28. Porter DM, Smith DH, Kabana RR. Peanut plant diseases. Peanut science and technology 1982,326-410.
- 29. Raghuchendar T, Rajappan K, Samiappan R. Evaluation methods of application of biocontrol agents in the control of mungbean root rot. Indian Phytopathology 1997;50:229-234.
- Ramanjineyulu P, Viswanath K, Kumar MR, Sumathi P. Incidence of pod rot of Groundnut (*Arachis hypogaea* L.) in Chittoor and Anantapur districts of Andhra Pradesh. Annals of Plant Protection Sciences 2018;26(2):328-331.
- 31. Sabet KK, Mostafa MA, El-Bana OHI, El-Sherif M. *Pseudomonas lindbergii* and *Coniothyrium minitans* as biocontrol agents effective against some soil fungi pathogenic to peanut. Egyptian Journal of Agricultural Research 1992;70:403-414.
- 32. Subrahmanyam P, Mehan VK, Nevill DJ, McDonald D. Research on Fungal Diseases of Groundnut at ICRISAT. Proceedings of the International Workshop on Groundnuts, 13- 17 October 1980. International Crop Research Institute for Semi- Arid Tropics, Patancheru, India 1980,193-198.
- Vidhyasekaran PP, Muthamilan M. Development of formulations of *Pseudomonasa fluorescens* for control of chick pea wilt. Plant Disease 1995;79(2):782-786.
- 34. Vincent JM. Distortion of fungal hyphae in presence of certain inhibitors. Nature 1927,159: 850.
- 35. Walker ME, Csinos AS. Effect of gypsum on yield, grade and incidence of pod rot in five peanut cultivars. Peanut Science 1980;7:109-113.