International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(5): 443-446

Received: 02-07-2017
Accepted: 03-08-2017

DRS Vineela
Department of Plant Pathology, OUAT, Bhubaneswar, Odisha, India

SK Beura
Associate Professor, Department of Plant Pathology, OUAT, Bhubaneswar, Odisha, India

A Dhal
Plant Pathologist, AICRP on Groundnut, OUAT, Bhubaneswar, Odisha, India

SK Swain
Agronomist, AICRP on Groundnut, OUAT, Bhubaneswar, Odisha, India

D Sethi
Ph.D. student, Department of Soil Science and Agricultural Chemistry, OUAT, Bhubaneswar, Odisha, India

Correspondence
DRS Vineela
Department of Plant Pathology, OUAT, Bhubaneswar, Odisha, India

Efficacy of chemicals, bio-agents and their compatibility in management of stem rot disease of groundnut

DRS Vineela, SK Beura, A Dhal, SK Swain and D Sethi

Abstract
Stem rot of groundnut (*Sclerotium rolfsii*) causes losses in yield upto 25% and the losses may be accounted for 40-50% in terms of mortality of crop, particularly in kharif groundnut when the climatic conditions are more favourable for pathogens. Management of soil borne pathogens were found difficult, uneconomical and harmful for the environment. Under *in vitro* investigation on management of groundnut stem rot, *Trichoderma hamatum* showed highest antagonistic acitivity (75.2%) in dual culture method followed by *Trichoderma harzianum* (74.7%). Carbendazim 12% + mancozeb 63% , carboxin 37.5% + thiram 37.5%, Triazole group fungicides such as tebuconazole 25.9% SL, hexaconazole 5% EC, difenoconazole 10% WP, propiconazole 25% EC, showed 100% pathogen inhibition in poison food technique followed by Azoxystobin 25%EC (87.26%). Compatibility of bioagents with fungicides reveled that all the three species viz. *T. vorode*, *T. harzianum* and *T. hamatum* were compatible with the chemical azoxystobin.

Keywords: Bioantagonists, fungicides, compatibility, *Sclerotium rolfsii*

1. Introduction
Peanut (*Arachis hypogaea L.*) is an important food and oil- seed crop worldwide. Diseases pose a major threat to the production of peanuts each year, and prevention of disease in peanut is a major concern for producers. Diseases caused by soil borne pathogens especially pose a threat to peanut production due to the similarity of symptoms, which leads to problems in diagnosis. In addition to direct losses, the management of soil borne diseases results in increased input costs. Peanut is susceptible to losses incited by soil borne pathogens due to the close association of the pods with the soil. Soil borne diseases are especially complicated to manage due to the difficulty of dispersing fungicides through the peanut canopy to the soil profile. Several important soil borne pathogens that affect peanut, includes *Sclerotium rolfsii, Aspergillus niger ,Verticillium dahliae. Botrytis cinerea, Pythium spp. Rhizoctonia solani, Sclerotinia minor and S. sclerotiorum.*
Sclerotium rolfsii, causing stem rot of groundnut is one of the serious pathogens reducing the yield during epidemics. The management of seed/soil borne diseases such as *Sclerotium* rots has been offering serious challenges to plant pathologists, since there has not been a single package perfected to required level and this is a serious impediment in the productivity of this oil seed crop. Decline in production has been noticed in recent years due to high incidence of stem rot caused by *Sclerotium rolfsii* nearing to harvest time. Intensification and monocropping of groundnut led to an increase in the incidence of stem rot. Management of this disease is difficult due to prolonged survival ability and wide host range of the pathogen. Management of soil borne diseases by chemical means is difficult and not economical and has already proved to be harmful to the environment. Notable success on disease control through the combined use of antagonistic microorganisms and fungicides have been experimented during recent years. In the present study, an effort is made to identify potential antagonists of *Sclerotium rolfsii*, efficiency of chemicals and their compatability, when applied simultaneously *in-vitro*.

2. Materials and Methods
2.1 Isolation and identification of pathogen
The pathogen was isolated from the groundnut plants showing typical symptoms of stem rot disease by tissue segment method [15]. Pure cultures of the pathogen were obtained by single

hyphal tip method. The pathogen was identified based on its mycelial and sclerotial characteristics and maintained on potato dextrose agar (PDA) [5] for further studies.

2.2 Screening of potential bioagents by dual culture technique

The antagonistic activity of three *Trichoderma* spp. against *S. rolfsii* was determined by dual culture technique [7]. Mycelial discs measuring 5 mm diameter from four days old cultures of both fungal antagonists and the test pathogen were placed at equidistant on sterile petriplate containing PDA medium. The petriplates were then incubated at 28 ± 2°C. Three replications were maintained in each treatment. Suitable controls were kept without antagonist. Growth of antagonists, pathogen and zone of inhibition were measured after recording full growth in control plate and percentage inhibition of mycelial growth of test pathogen was calculated. Antagonistic potential was determined by using parameters viz. degree of inhibition or intermingled zone between both the colonies.

1. The percentage inhibition of radial growth was calculated by using equation:

Percentage of inhibition I =C-T/C × 100
Where C= growth of pathogen in control T = growth of pathogen in treatment

2. Efficiency of species was identified by scoring on modified Bell's scale [2]. R1=I00% overgrowth; R2=75% overgrowth; R3=55% overgrowth; R4= blocked at point of contact; R5= pathogen overgrows antagonist.
3. The kind and degree of antagonism was determined according to classification by [16]. A=mutual intermingling growth, Bi=overgrowth by antagonists, Bii= Intermingling growth in which test fungus under observation has ceased growth and overgrown by another colony, C= Light inhibition, D= Not detected.

2.3 Screening of efficacy of fungicides by poision food technique

The efficacy of different chemicals was tested on PDA medium against *S. rolfsii* by poisoned food technique. Required concentrations were prepared by dissolving known quantity of fungicides in sterile distilled water separately under aseptic conditions. The poisoned medium was equally distributed into three Petri plates each comprising 10 treatments and 3 replications. The mycelia growth of the pathogen *S. rolfsii* was cut into 5 mm discs from the periphery of actively growing colony with sterilized cork borer and transferred to the centre of each plate containing poisoned medium. Control was maintained by placing fungal discs in plates containing untreated medium. All the inoculated Petriplates were incubated at 28+ 2ºC in BOD incubator. The diameters of fungal colonies in the treatments were measured when the growth in control plate was full. Per cent inhibition in the growth of the organism in different chemical treatments over the control was calculated. The percentage inhibition of radial growth was calculated by using equation:
Percentage of inhibition I =C-T/C × 100
Where C= growth of pathogen in control T = growth of pathogen in treatment

2.4 Efficacy and compatibility of potential antagonists with different fungicides tested *in-vitro*

The selected native potential biocontrol agents were tested for their compatibility with the recommended fungicides used against *S. rolfsii* viz., chlorothalonil (0.2%), carbendazim+ mancozeb (0.2%), carboxin +thiram(0.2%),tebuconazole (0.1%), and azoxystrobin(0.1%). Fungal isolates were tested for their compatibility by poisoned food technique [12].

3. Results and Discussion

From the *in vitro* findings (table-1) it can be suggested that antagonist *Trichoderma hamatum* is most efficacious with 72.2% mycelial growth inhibition, 89.02% sclerotial inhibition followed by *Trichoderma harzianum* with 74.7% mycelial growth inhibition, 59.84% sclerotial inhibition and *Trichoderma viride* with 71.9% mycelia growth inhibition, 59.99% sclerotial inhibition with R2 bell score and Bi type of antagonism. Similar findings also have been reported[1],where maximum percentage of inhibition on growth of *Sclerotium rolfsii* was observed with *Trichoderma harzianum* (77.39%) followed by *Trichoderma viride* (76.54%).However the present investigation includes *Trichoderma hamatum* which performed better in both inhibiting mycelial growth as well as suppressing the formation of sclerotial bodies. There are two ways by which biocontrol agents can suppress the plant pathogen: (i) production of antibiotics or (ii) production of hydrolytic enzymes. Antagonistic microorganisms reduce growth, survival or infections caused by the pathogens by different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions and enzyme secretion. Proposed mechanisms of this biocontrol agent are thought to be antibiosis [6], mycoparasitism, competition or fungicidal action because of the capacity of *Trichoderma* for production of antibiotics or hydrolytic enzymes [10]. The *Trichoderma* species are capable of production of β-xylosidase, α-glycosidase, β- glycosidase, cellobiohydrolase, trypsin-, chymotrypsin- and chymoelastase-like proteases and N-acetyl- β-glucosaminidase, which are extracellular enzymes important for the bio-control activity. Antagonistic microorganisms, such as *Trichoderma*, reduce growth, survival or infections caused by the pathogens by different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions, and enzyme secretion [14].

Table. 1: *In-vitro* efficacy of antagonistic mycoflora against *S.rolfsii* by dual culture technique

	Treatment	*Sclerotium rolfsii*				
		Radial growth (mm)	%inhibitoin	% inhibition of sclerotial bodies	Score (Bell scale)	Type of antagonism
T1	*Trichoderma viride*	25.00	71.9(57.99)*	84.07(66.96)	R2	Bi
T2	*Trichoderma harzianum*	22.50	74.7(59.84)	79.57(63.52)	R2	Bi
T3	*Trichoderma hamatum*	22.00	75.2(60.19)	89.02(71.130	R1	Bi
T4	control	89.25	0.00(0.00)	0.00(0.00)		
	CD	0.297	2.126	8.373		
	SE(m)±	0.095	0.682	2.688		
	CV%	4.811	3.066	10.664		

*figures in the parenthesis are arc sine transformed values.

Results revealed that all the systemic fungicides were capable of inhibiting the growth of the test fungus at different concentrations as compared to check. Mixed fungicides carbendazim12%+ mancozeb 63% at 0.2%, carboxin 37.5% + thiram 37.5% at 0.2% and triazole group of fungicides *viz*., tebuconazole 25.9% EL, hexaconazole 5% SC, difenoconazole 10% WP and propiconazole 25% EC recorded inhibition of *Sclerotium rolfsii* growth by 100% followed by azoxystrobin 25% EC of 0.1% (87.26%), and thiophanate methyl 50%WP at 0.15% concentration (57.96%). Least antifungal property was shown by chlorothalonil 75%WP at 0.2% (10.73%). Similar findings also have been reported [8],where Seed treatment with tebuconazole resulted in minimum collar rot and stem rot (22.6%) incidence followed by hexaconazole + captan (10.1 and 26.1%) and carboxin + thiram (13.5 and 24.8%) compared to other treatments. The propiconazole was effective even at 250 ppm concentration against *S. rolfsii* [3]. Hexaconazole at a concentration of 1000, 1500 and 2000 ppm and propiconazole at a concentration of 500, 750 and 1000 ppm completely inhibited the growth of *S. rolfsii* [9] which were in agreement with present investigation.

Table 2: *in-vitro* efficacy of different fungicides against *Sclerotium rolfsii*

	Treatment	dosage	*Sclerotium rolfsii*	
			Radial growth (mm)	% inhibitoin
T1	Carbendazim 12%WP+Mancozeb63%WP	0.2%	0.88	100.00 (90.00)*
T2	Chlorothalonil 75%WP	0.2%	80.00	10.73 (19.00)
T3	Thiophanate methyl 50%WP	0.15%	40.33	54.96 (47.83)
T4	Tebuconazole 25.9%EL	0.1%	0.00	100.00 (90.00)
T5	Hexaconazole 5%SC	0.1%	0.00	100.00 (90.00)
T6	Difenconazole 10% WP	0.1%	0.00	100.00 (90.00)
T7	Azoxystrobin 25%EC	0.1%	11.33	87.26 (69.09)
T8	Propiconazole 25%EC	0.1%	0.00	100.00 (90.00)
T9	Carboxin 37.5%+Thiram 37.5%	0.2%	0.00	100.00 (90.00)
T10	Control		89.66	0(0.00)
	CD		2.029	1.793
	SE(m)±		0.683	0.603
	CV%		5.346	1.546

*figures in the parenthesis are arc sine transformed values.

A total of six fungicides were evaluated for their efficacy on growth of bioagents *Trichoderma viride, T.* Harzianum and *T. hamatum.* Out of six, three fungicides carboxin + thiram, tebuconazole, carbendazim + mancozeb recorded cent percent inhibition of 3 bioagents at recommended dosage. Chlorothalonil inhibited the bioagent by 88.75% of *Trichoderma viride* growth, 92.2% of *Trichoderma harzianum,* 84.4% of *T. hamatum.* Strobilurin group fungicide, azoxystrobin also inhibited growth of *T. viride* by 77%, *T. harzianum* by 82.5% and *T. hamatum* by 70.3%. Present result indicated that fungicide azoxystrobin was highly compatible with bioagents which was also effective against test pathogens (Table.3) and thus can be integrated with bioagent in soil borne disease management.

Earlier research on compatibility of fungicides with the bioagent *Trichoderma* indicated that the bioagent is compatible with certain fungicides. In case of groundnut, studies indicated that tebuconazole is highly inhibitory to the growth of fungal bioagent *Trichoderma* [11]. Though the fungicide tebuconazole is effective against stem rot at field level [4], its application along with bioagents is limited and is confined to experimental conditions. Similarly, *in-vitro* indicated that, there is 27.6 per cent inhibition of growth of *Trichoderma spp* by azoxystrobin [13]. Therefore, bioagents and fungicides are to be applied with time gap because of their incompatible nature.

Table 3: Compatibility of potential *Trichoderma spp.* with different fungicides by poison food technique *in vitro*.

	Treatment	Dose	*Trichoderma viride*		*Trichoderma harzianum*		*Trichoderma hamatum*	
			Radial growth (mm)	% inhibition	Radial growth(mm)	% inhibition	Radial growth	% inhibition
T1	Carboxin 37.5% +Thiram 37.5%	0.2%	0.00	100.00(90.00)*	0.00	100.00(90.00)*	0.00	100.00(90.00)*
T2	Chlorothalonil 75%WP	0.2%	10.00	88.75(70.44)	7.00	92.20(73.85)	14.00	84.40(66.77)
T3	Tebuconazole 25.9%EL	0.1%	0.00	100.00(90.00)	0.00	100.00(90.00)	0.00	100.00(90.00)
T4	Carbendazim 12%WP+Mancozeb63%WP	0.2%	0.00	100.00(90.00)	0.00	100.00(90.00)	0.00	100.00(90.00)
T5	Azoxystrobin 23%EC	0.1%	20.00	77.56(61.71)	15.66	82.53(65.33)	26.66	70.33(56.98)
T6	Control		89.33	0.00(0.00)	90.00	0.00(0.00)	90.00	0.00(0.00)
	C.D.		2.243	1.878	2.681	2.561	2.472	2.033
	SE(m)±		0.72	0.603	0.861	0.822	0.793	0.653
	C.V.%		6.271	1.558	7.939	2.088	6.311	1.722

*figures in the parenthesis are arc sine transformed values.

The bioagent *Trichoderma hamatum* has showed 75.20% inhibition against the test pathogen. Carbendazim + manczeb, carboxin + thiram and triazole group fungicides has showed 100% inhibition on growth of *Sclerotium rolfsii*. But these chemicals are not compatible with bioagents, only except Azoxystobin which inhibits pathogen by 87.26% as well as expressed compatibility with all the three species of *Trichoderma.*

4. Reference

1. Basumatary M, Dutta BK, Singha D M, and Das N. Some *in-vitro* observations on the biological control of *Sclerotium rolfsii*, a serious pathogen of various agricultural crop plants. IOSR-JAVS. 2015; 8:87-94.
2. Bell DK, Wells HD and Markham C R. *In vitro* antagonism of Trichodrema species against six fungal plant pathogens. Phytopathology, 1982; 72:379-382.
3. Bhat N and Srivastava, LS. "Evaluation of Some Fungicides and Neem Formulations Against Six Soil-borne Pathogens and Three *Trichoderma spp*.", *In vitro* Plant Disease Research, 2003; 18: 56-59.
4. Brenneman TB, Mixon JA, Sills PA, and Mullis KL. Evaluation of various fungicides for the control of peanut soil borne diseases, Fungicides Nematiides Tests, 1999; 54:376-377.
5. Dhingra O D and Sinclair J B. Basic Plant Pathology Methods, 2nd Edition. Lewis Publishers, CRC Press, London. 1995.
6. Ghisalberti EL, Narbey MJ, Dewan MM and Sivasin K. Varability among strains of Trichoderma harzianum in their ability to reduce take-all and to produce pyrones. Plant Soil, 1990; 121: 287-291.
7. Johnson LF, Curl EA, Bond JH and Fribourg HA. Methods for studying soil microflora. Plant Disease Relationships. Burgess Publishing Company, Minneapolis, USA. 1959.
8. Johnson M and Subramanyam K. "*In vitro* Efficiency of Fungicides Against Stem Rot Pathogen of Groundnut", Ann Pl Prot Sci, 2000; 8:255-257.
9. Johnson M, Reddy PN and Reddy D R. "Comparative Efficacy of Rhizosphere Mycoflora, Fungicides, Insecticides and Herbicides against Groundnut Stem Rot Caused By Sclerotium Rolfsii", Annals of Plant Protection Sciences, 2008; 16(2):414-418.
10. Lorito M, Peterbauer CK, Hayes CK and Herman GE. Synergistic interaction between fungal cell-wall degrading enzymes and different antifungal compounds on spore germination. Microbiol. 1994; 140:623-629.
11. Mc Lean KL, Hunt J, Stewart A and Zydenbos SM. Compatibility of the biocontrol agent *Trichoderma harzianum* C-52 with selected fungicides. Newzealand Plant Protection 2001; 54: 84-88.
12. Nene YL and Thapliyal PN. Fungicides in plant disease control, 3rd edition. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi. 1993; 526-531.
13. Pandey KK, Pandey PK and Mishra KK. Bio-efficacy of fungicides against different fungal bioagents for tolerance level and fungistatic behavour. Indian Phytopathology. , 2006; 59 (1): 68-71.
14. Ponnusamykonar P, Venkitasamy K and Varatharajan P. *In vitro* study of antagonistic effect of Trichoderma sp., on tea plant pathogen, *Phomopsis theae*. Archives of Applied Science Research. 2011; 3 (4):352-358.
15. Rangaswami G and Mahadevan A. Diseases of crop plants in India, 4th edition. Prentice Hall of India Pvt. Ltd., New Delhi. 1999; 65-66.
16. Skidmore AM and Dickinson CH. Colony interaction and hypheal interference between *Septoria nodorum* and Phylloplane fungi. Transactions of the British Mycological Society, 1976; 66(1):57-64.