JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2010. Vol. 4(2), p. 565-570

Biocontrol of Stem Rot of Ground Nut Incited by *Sclerotium rolfsii* and *in vitro* Compatibility of Potential Native Antagonists with Fungicides

L. Vijaya Sai*, P. Anuradha, K. Vijayalakshmi and N.P. Eswar Reddy

Department of Applied Microbiology, Sri Padmavathi Women's University, Tirupati - 517 502 , India.

(Received: 14 January 2010; accepted: 18 February 2010)

Groundnut is an important oil seed crop of tropics and sub-tropical regions of the world. Among the fungal diseases, Stem rot of groundnut caused by *Sclerotium rolfsii* is potentially very destructive disease. A detailed study on isolation of pathogen, rhizosphere mycoflora, antagonism of rhizosphere against *S.rolfsii* and integrated management of the disease with biocontrol agent. Eight different mycoflora were *Aspergillus flavus*, *Rhizopus sp.*, *Pencillium*, *A.niger* and *Trichoderma* isolates (TG1, TG2, TG3, TG4) isolated consistently from the rhizosphere of groundnut. Among them TG_2 isolate was found to be significantly superior over others in inhibiting the mycelial growth of *S.rolfsii* to the extent of 67.83%.

Key words: *Trichoderma, Sclerotium rolfsii,* Ground nut, Rhizosphere mycoflora.

Groundnut (*Arachis hypogaea* L.) is a major legume and an important oilseed crop in India and in many Asian countries. In India groundnut is grown in an area of about 6.6 m.ha with a production of 5.9 m.t.[1]. In Andhra Pradesh it is grown in an area of about 1.84 m ha with a productivity of 891 kg/ha[2]. The productivity of groundnut crop in India is becoming low due to several factors among which diseases like leaf spot, collar rot, stem rot, bud necrosis etc. are very important.

The stem rot caused by *Sclerotium rolfsii* is the major problem with typical symptoms like dark brown lesion on the stem just below the soil surface followed by drooping and wilting of entire plant[3]. It is responsible for 25% seedling mortality in cultivar JL-24 at Parbhani[4] .

S. rolfsii is a polyphagous soil borne facultative parasite and induces root rot over 500 species of plants[5-8] .Though the fungus is seed and soil borne, soil borne inoculum is more important in causing infection and disease development. Biochemical investigation of *S.rolfsii* and their fungal activity[9]. The fungicides are the most common tools for controlling disease losses. In recent years, there has been growing concern in indiscriminate use of fungicides because they are potentially hazardous to environment. Genetic viability among mycelial compatibility groups of *S.rolfsii* in South Africa causing much damage during any stage of crop growth[10]. The chemical residues in the soil are adding to the pollution of land and water. These factors have led to the search for new and innovative approaches for plant disease management.

* To whom all correspondence should be addressed.
E-mail: Vijayasail@yahoo.co.in

Biological control has attained importance in modern agriculture to minimize the residual effects due to continuous and indiscriminate use of toxic chemicals for the disease control. Keeping this in view the present study has been carried to isolate potential native antagonists for integrated disease management.

MATERIAL AND METHODS

Isolation and identification of the pathogen

S. rolfsii was isolated from stem rot infected groundnut plants collected from S.V.Agricultural College Farm, Tirupati using tissues segment method[11].

Small pieces of tissues about 3 mm^2 from infected collar region with some healthy tissue were cut with sterile scalpel. Then the pieces were surface sterilized with one percent sodium hypochlorite solution for 30sec. The tissue pieces were subsequently washed in three changes of sterile distilled water to eliminate excess sodium hypochlorite and then pieces were transferred to PDA plates and were incubated at 28 ± 2°C and were observed periodically for growth of the fungus. The culture was purified by single hyphal tip method and maintained through out the present investigation by periodical transfer onto PDA. The pathogen was identified as *Sclerotium rolfsii* based on its mycelial and sclerotial characters[12].

Pathogenicity test

Koch's postulates were proved by artificial inoculation of groundnut plants with the pathogen in pot culture experiment. The pathogen was mass multiplied on sterilized sand maize meal medium (90 g sand, 10 g maize and 20 ml distilled water) in 250 ml conical flask[13]**.** This medium was inoculated with two discs of 10 mm diameter mycelial plug of three day old culture of *S. rolfsii* grown on PDA plate. The plates were incubated at 28 ± 2°C. After seven days of incubation the inoculum was mixed with sterilized soil in pots of 12 inches diameter @ 100 g/kg soil. Groundnut seeds were sown into the pathogen inoculated pots @ 5 seeds / pot. The plants were observed periodically for collar rot symptoms.

Isolation of native antagonistic mycoflora from rhizosphere of groundnut

Serial dilution technique [14] was used to isolate mycoflora from rhizosphere soil of groundnut. Composite soil sample collected from rhizosphere of healthy plants and stem rot infected groundnut plants was shade dried and then used for serial dilution. Ten grams of this soil was dissolved in 100 ml of sterile distilled water to get 10^{-1} dilution. From this 1 ml of soil suspension was taken and added to 9 ml of sterile distilled water to get 10^{-2} dilution. This is repeated until a final dilution of 10^{-4} was obtained. Antagonistic mycoflora were isolated on Rose Bengal Agar medium by using a dilution of 10^{-4}. One ml of soil suspension was taken in sterilized Petri plates, then the melted and cooled medium was poured. Plates were rotated gently to get uniform distribution of soil suspension into the medium. Then the plates were incubated at 28 ± 2°C and observed at frequent intervals for the development of colonies. Three day old colonies of mycoflora were picked up and purified by single hyphal tip method.

Identification of native antagonistic rhizosphere mycoflora and their maintenance

Rhizosphere mycoflora were identified based on mycological keys[15]. Mycoflora was maintained by periodical transfer on PDA.

In vitro* screening of antagonistic mycoflora against *S. rolfsii

By using dual culture technique[16] antagonists were screened against the pathogen *invitro*.

Dual culture technique

20ml of sterilized PDA was plated in 9 cm Petri plates and allowed to solidify. Mycelial discs of 5 mm diameter of the antagonists as well as the test pathogen were cut with sterile cork borer from the periphery of actively growing three day old cultures and then placed on opposite sides of Petri plate. The distance between inoculum blocks was 7 cm. The inoculated Petri plates were incubated at 28 ± 2°C for three days. The Petri plates inoculated with pathogen alone served as control. Three replications were maintained per treatment.

The percent reduction in radial growth of the test pathogen was calculated using the following formula

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

Where,
I = Percent reduction in growth / sclerotial population of *S. rolfsii*
C = Radial growth (mm) / sclerotial population in control.
T = Radial growth (mm) / sclerotial population in treatment.

Percent inhibition of the growth of the antagonistic fungus was calculated using the following formula,

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

Where,
I = per cent reduction in growth of antagonistic fungus.
C = Radial growth of antagonistic fungus in control (mm).
T = Radial growth of antagonistic fungus in treatment (mm).

***Invitro* Compatibility of potential native antagonists with fungicides**

The compatibility of potential native antagonistic fungus i.e., T1 isolate (Identified as *Trichoderma viride* NO.5638 by Indian type Culture collection, NewDelhi) with the fungicides was determined using poisoned food technique. Mycelial disc of four day old antagonistic fungus was inoculated at the center of fungicide treated PDA .A control with out fungicide was maintained. Each treatment was replicated thrice. The plates were incubated at 28±2°C for four days

Percent of inhibition of growth of antagonistic fungus was calculated using this formula

RESULTS

The pathogen associated with the stem rot of groundnut plants collected from S.V. Agricultural College Farms based on mycological characters the pathogen was identified as *Sclerotium rolfsii*. The colony characters and morphological characters of mycelium and sclerotia were in agreement with earlier reports[17]. The native antagonistic mycoflora were identified based on colony and morphological characters[18]. The observations regarding the colour and number of colonies per gram of rhizosphere soil are presented (Table 1).

Table 1. List of antagonistic mycoflora isolated from rhizosphere of groundnut

S. No.	Antagonist	No. of colonies per gram of soil*	Colony colour
1.	*Pencillium* sp.	23	Dark green
2.	*Aspergillus flavus*	24	Yellowish green
3.	*A. niger*	7	Dark black
4.	*Rhizopus* sp.	11	Dull white
5.	*Trichoderma* isolate-1 (TG_1)	17	Dull green
6.	*Trichoderma* isolate-2 (TG_2)	24	Dull green
7.	*Trichoderma* isolate-3 (TG_3)	15	Dark green
8.	*Trichoderma* isolate-4 (TG_4)	13	Bright green

*Mean of 10 plates

The data reveals that the *Trichoderma* isolate-2 (TG_2) and *A. flavus* colonies were found to be maximum followed by *Pencillium sp.* A total of eight fungi *viz.,* TG_1, TG_2, TG_3, TG_4, *Aspergillus flavus, A.niger, Pencillium* sp. and *Rhizopus* sp. were isolated from rhizosphere samples of groundnut JL-24. The antagonistic effect of these isolates was assessed based on their ability to inhibit the pathogen growth (Table 2).

Since *Trichoderma* isolate (TG2) was used in subsequent integrated management of *S.rolfsii* ,its compatibility with fungicides was tested using poisoned food technique.

The fungicides used were captan (captaf 50 wp), thophanate methyl (roko 50 wp), Mancozeb, Blitox (coco50 wp) at the concentrations viz 50,100,250,500,1000ppm the data are presented.

Table 2. *Invitro* evaluation of efficacy of antagonistic mycoflora against *S. rolfsii* at 7 days after inoculation by dual culture technique

Fungal antagonist	Radial growth (mm)	Per cent inhibition of mycelial growth
Pencillium sp.	33.6	60.39 (50.94)
Aspergillus flavus	39.6	53.33 (46.89)
Aspergillus niger	53.6	36.86 (37.35)
Rhizopus sp.	55.0	35.29 (36.39)
Trichoderma isolate-1 (TG_1)	30.6	63.91 (53.07)
Trichoderma isolate-2 (TG_2)	27.3	67.83 (55.43)
Trichoderma isolate-3 (TG_3)	35.3	58.42 (49.84)
Trichoderma isolate-4 (TG_4)	42.3	50.19 (45.06)
Control	85.0	

SEm ± 0.45, CD at 5%, 1.35; All the figures are means of 3 replications. Figures in parenthesis are angular transformed values.

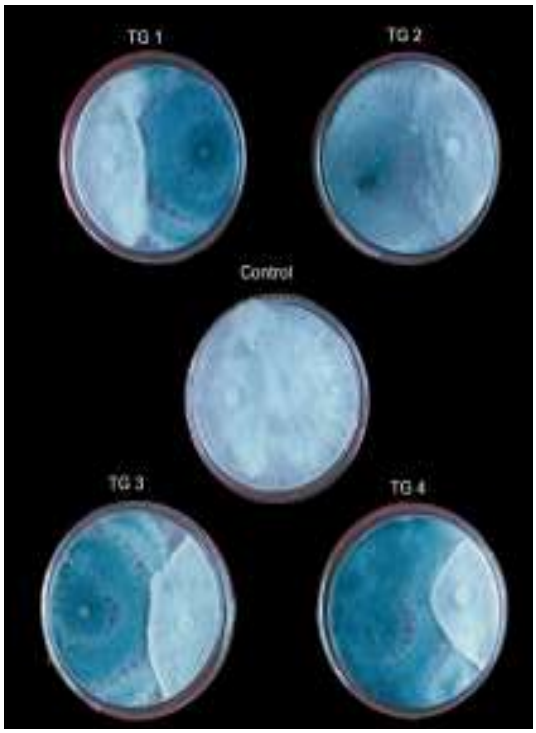

Fig. 1. Effect of *Trichoderma* isolates (TG_1, TG_2, TG_3, and TG_4) with *S.rolfsii* in dual culture technique

Table 3. *In vitro* compatibility of potential native antagonists with fungicides

Fungicides	Percentage inhibition of growth of TG 2 Concentration (ppm)					
	50	100	250	500	1000	Mean
Captan (captaf 50WP)	5.87 (13.4)	41.56 (29.30)	41.56 (40.11)	80.67 (63.65)	85.09 (07.21)	47.43 (43.5)
Thiophanate Methyl	65.84 (53.97	81.56 (64.53)	93.32 (75.00)	100.00 (90.00)	100.00 (90.00)	88.07 (69.73)
Mancozeb	3.52 (10.78)	6.66 (14.89)	21.56 (27.62)	59.60 (50.53)	68.61 (55.920)	31.99 (34.39)
Blitox	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)

Mean of Replications		SEM ±	Cdat 5%
Fungicide		0.2291	0.6636
Fungicide ×	concentration	0.2562	0.7491
		0.5123	0.4838

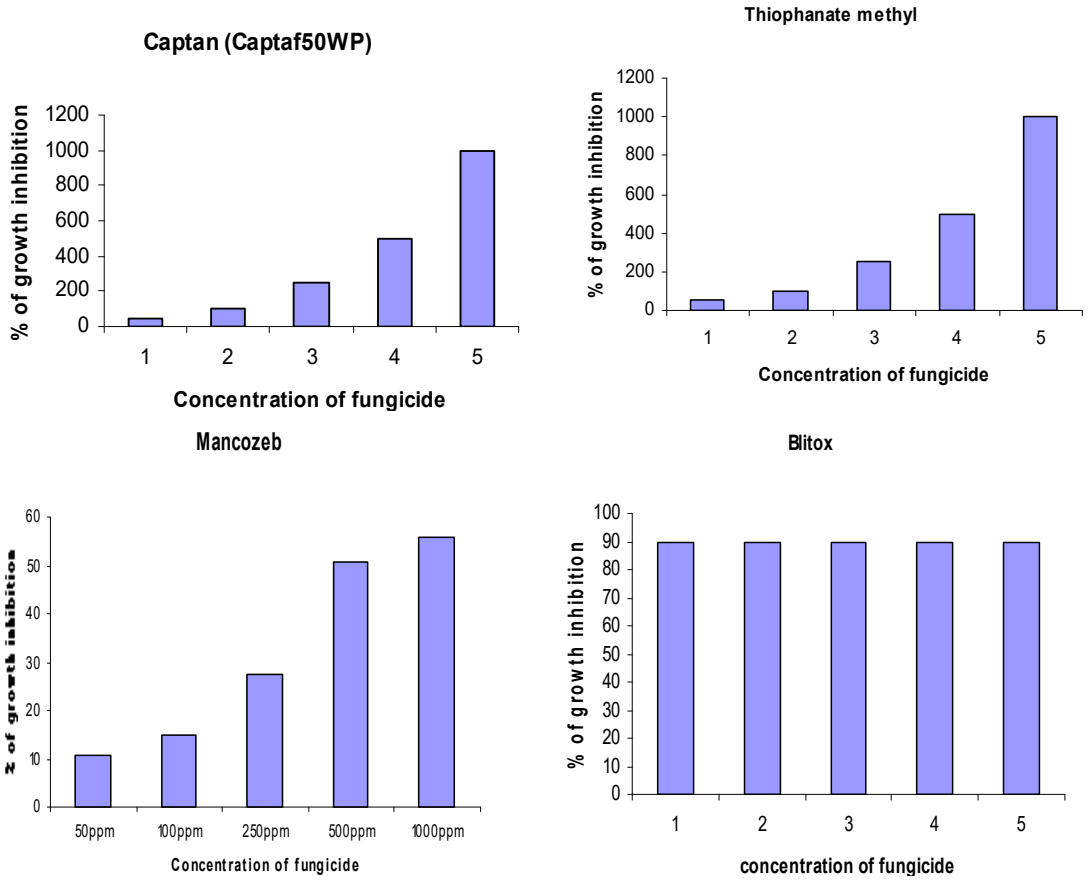

Fig. 2. *Invitro* compatibility of potential native antagonists with fungicides

DISCUSSION

Though India is the largest producer of groundnut in the world, the productivity of the crop is far below the optimum. Stem rot of groundnut caused by *Sclerotium rolfsii*[19] is resulting in considerable losses. The present investigation was undertaken to isolate native antagonistic mycoflora to test. The efficacy of mycoflora against Sclerotium rolfsii under glass house conditions. In modern agriculture, biological control has gained importance through the use of native and exotic antagonists[20].

In the present study, *Trichoderma* isolate-2 (TG_2) was found to be highly potential native antagonistic fungus against *S. rolfsii* and inhibited mycelial growth to an extent of 67.83 percent and sclerotial population by 92.60 percent. *Trichoderma harzanium*, reduced the mycelial growth of *S.rolfsii* of groundnut [21]. Although, all other fungal isolates showed some degree of antagonistic potential, they were less effective compared to TG_2. *Trichoderma sp*. have received considerable attention as possible, biocontrol agents of several soil borne pathogen and often used as means of *invitro* screening for the selection of best biocontrol agent [22]. Application of biocontrols agents as soil treatments increased germination and reduced the disease, Influence of organic amendments and biological component on stem rot of ground nut[23].

In the present investigation, *Trichoderma* isolate-2 (TG_2) hyphae coiled around the hyphae *S.rolfsii* and resulted in subsequent reduction in mycelial growth and sclerotial population and the data reveals that Thiophenate methyl inhibited growth of TG2 isolate completely at even 100 ppm, captan 5.87% growth at 50 ppm

concentration and at 1000 ppm Mancozeb found to be less effective and best compatable with TG2 isolate, since the percent of inhibition in its presence was least. Thus, *Trichoderma* isolate-2 (TG_2) was proved superior to all other antagonists and therefore selected for further glass house studies.

REFERENCES

1. FAO Bulletin of statistics, Food and Agricultural Organization of the *United Nations, Rome.* 2005.
2. Statistical Abstract Statical Organization, Department of Statistics and programme implementation, Govt of India, New Delhi. 2005 .
3. Asghari M A andMayee C D, Comparative efficacy of management practices on stem and pods rots of groundnut. *Indian Phytopathology* 1991; **44**(3): 328-332.
4. Ingale R V and Mayee C D. Efficacy and economics of some management practices of fungal diseases of groundnut. *Journals of Oilseeds Research*, 1986, **3**: 201-204.
5. Punja Z K and Damiani A, Comparative growth morphology and physiology of three *Sclerotium* species. *Mycologia* 1996; **88**: 694-706
6. Sarma, B.K., Singh,U.P. and Singh,K.P. Variability in Indian isolates of *S. rolfsii Mycologia* 2002; **94**(6): 1051-1058.
7. Ganesan *et al,* S.,Ganesh,K.R. and Sehhar ,R. Integrated management of stem rot disease (*S. rolfsii)* of ground nut ,using Rhizobium and Trichoderma harzanium (ITCC).*Turk.J.Agric*. .2007; **31**: 103-108
8. Anahosur KH Integrated Management of potato sclerotium wilt caused by *S.rolfsii.Indian ported phytopathology* 2001; **54**: 158-166
9. Pandey, M.K. Sharma, B.K, Singh, D.P and Singh, U.P. Biochemical investigations of Sclerotial exudates of *Sclerotium rolfsii* and their antifungal activity. *Journal of Phytopathology*, 2007; **155**(2): 84-89.
10. Charitha Devi. M and and MN Reddy, Biological control of *S. rolfsii* the incitant of stem rot of groundnut (*Arachis hypogaea*) ISOR, National seminar : Stress Management in Oil Seeds, 2003; 28-30.
11. Rangaswami G and Mahadevan A,.*Diseases of crop plants in India*.,(Fourth edition). Prentice Hall of India Pvt. Ltd., New Delhi 1999 60-79.
12. Barnett H H and Hunter B B. Illustrated genera of imperfect fungi. Burgess Publishing Company, Minnesota., 1972.
13. Kajal Kumar B and Chitreswar Sen. Management of stem rot of groundnut caused by *Sclerotium rolfsii* through *Trichoderma harzianum*. *Indian Phytopathology*, 2000; **53**: 290-295.
14. Johnson L F and Curl F A. Methods for research on the ecology of soil borne plant pathogens. *Burgess Publishing Company, Minneapoli*, 1972; 247.
15. Morton D J and Stroube W H, Antagonistic and stimulatory effects of soil microorganism upon *Sclerotium rolfsii*. *Phytopathology* 1955; **45**: 417-420.
16. Mirza MS and; Anitha Chowdary. K, Rajaram Reddy. D, Chandrasekhar Rao.K, Bhupal Reddy. T and Prabhakar Reddy. I, 2000. Integrated Management of Sclerotial wilt disease of bell pepper. *Indian Journal of Plant Protection.* 1977; **28**: 15-18.
17. Mirza MS Aslam *Helianthus tuberoses*, a new host of *S. rolfsii* in Pakistan 1993; **16**: 85-88.
18. Ahmad Y, Mirza M S, Aslam M and Ahmad Y, Collar rot of maize caused by *Sclerotium rolfsii* in Pakistan. *Pakistan Journal of Agricultural Research* 1988; **9**: 604-605.
19. Cook R J Biological control of plant pathogens. Theory to application. *Phytopathology*, 1985; **75**: 25.
20. Chilliers. A.J., Herselman. L. and Pretorius.Z.A. Genetic viability with in and among mycelial compatability groups of *Sclerotium rolfsii* in South Africa. *Journal of Phytopathology* 2000; **90**(9): 1026-1031
21. Chet Trichoderma –application, Mode of action and potential as a biocontrol agent of soil borne plant pathogenic fungi in innovative approaches to plant disease control (ed.I Chet).*Jhon Wily and Sons New york* 1987; 137-160.
22. Vyas and mathur,K, Distribution of *Trichoderma sp* in rhizosphere and their potential in suppression of wilt. *Indian Phytopathology* 2002; **55**(4):451-457
23. Adiver S S. Influence of organic amendments and biological component on stem rot of groundnut. ISOR, National Seminar: *Stress Management in Oil Seeds*, 2003; **28-30**: 15-17.