



Original Research Article

Cultural and Morphological Variability *Sclerotium rolfii* Isolates Infecting Groundnut and Its Reaction to Some Fungicidal

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A B S T R A C T

Keywords

Morphological variability, fungicide mixtures, *Sclerotium rolfii*, sclerotia, stem rot,

Morphological and cultural variability of eight isolates of *Sclerotium rolfii* were studied based on their growth rate, colony colour, mycelial dispersion and appearance and sclerotium formation, colour, weight and number of sclerotia, arrangement and maturity days of sclerotia using two solid media viz; PDA and CDA. Significant variability with reference to mycelial and sclerotial characters across isolates of *S.rolfsii*, isolated from different locations of Southern zone of Andhra Pradesh was observed. *In vitro* evaluation of new fungicide mixtures revealed that, carbendazim + Mancozeb and Hexaconazole + Zineb could control the test pathogen even at 250 ppm.

Introduction

Groundnut (*Arachis hypogea* L.) an important oilseed crop grown in India, China, Nigeria, Senegal, Sudan, Burma and the United States of America. Peanuts are rich in energy (567 calories per 100 g) and contain health benefiting nutrients, minerals, antioxidants and vitamins that are essential for optimum health. The kernels are a good source of dietary protein; compose fine quality amino acids that are essential for growth and development. They compose sufficient levels of mono-unsaturated fatty acids especially oleic acid. It helps lower LDL or "bad cholesterol" and increase HDL or "good cholesterol" level in the blood.

Globally India ranks first in area and second in production. India, accounts for 31% of the total groundnut area in the world (24.6 m ha) and 22% of the total production (35.7 mt , 2012-13.(Proceedings of ZREAC,2014). In Andhra Pradesh, it is mainly cultivated in Rayalaseema districts viz; Ananthapur, Cuddapah, Kurnool and Chittoor districts followed by Telangana and coastal districts. In Andhra Pradesh, it is cultivated in an area of around 13.45 lakh ha with a production of about 11.1 lakh tones and a productivity of 829 kg/ha (2012-13) (Proceedings of ZREAC,2014).

The crop groundnut is affected by many diseases at different growth stages. Among these diseases stem rot of groundnut caused by *Sclerotium rolfsii* is one of the important diseases. The typical symptoms of this disease include yellowing and wilting of branches, presence of white mycelial growth at collar region and production of mustard seed like sclerotia (Asghari and Mayee, 1991). The fungus *S.rolfsii* has wide host range of 500 species in about 100 families including groundnut, green bean, lima bean, onion, pepper, potato, sweet potato, tomato and water melon (Aycock, 1966).Stem rot disease is a potential threat to groundnut production and is of considerable economic significance for groundnut grown under irrigated conditions. The disease causes severe damage to the crop, at any stage of crop growth and yield losses of over 25 per cent have been reported (Mayee and Datur, 1988). There are several reports of *S.rolfsii* which was significant variations in morphological behavior (Sharma *et al.*, 2002). Variability is the property of an organism to change its characters from one generation to the other. This pathogen is host specific and major hindrance to Groundnut production in tropics and sub-tropics needs an information base on the fungal features and variability in culture and pathogenicity. There is need to study and characterize the fungus in culture, isolated from different districts of Southern zone of Andhra Pradesh where Groundnut is grown on a large scale. The information on cultural, morphological and pathological variability among the isolates *S. rolfsii* is limiting.

The control practices of stem rot disease include cultural methods such as plant rotation, deep soil processing and weed control as well as soil solarization, using antagonistic microorganisms or fungicides treatments after sowing on the plant rows

(Damicone & Jackson, 1994). In some cases, some fungicides used for the control of leaf spots can control these diseases while the occurrence of soil-borne diseases can be increased. The information on efficacy of certain newly released fungicides mixtures into the market is scarce. Hence, in this present investigation an attempt was made to study the cultural and morphological variability along with fungicides for management of stem rot of groundnut incited by *S. rolfsii*.

Materials and Methods

Collection and isolation of the pathogen

A survey was conducted during September, 2012 in groundnut growing areas in Southern zone of Andhra Pradesh and groundnut plants infected with stem rot pathogen, *S. rolfsii* were collected. The pathogen, *Sclerotium rolfsii* was isolated from the stems of infected groundnut plants by tissue segment method (Rangaswami and Mahadevan, 1999) on potato dextrose agar (PDA) medium. Small pieces of tissue of about 0.5 to 1 cm from infected collar region with some healthy tissue were cut with sterile scalpel. The pieces were surface sterilized with 1% sodium hypochlorite solution for 30 sec. The tissue pieces were subsequently washed in three changes of sterile distilled water to eliminate excess sodium hypochlorite and then the pieces were transferred onto PDA medium in Petri dishes. Plates were incubated at $27 \pm 1^{\circ}\text{C}$ and observed periodically for growth of the fungus. Axenic culture of the pathogen was obtained by single hyphal tip method and maintained on PDA throughout the present investigation. The pathogen was identified as *Sclerotium rolfsii* based on its mycelial and sclerotial characters (Barnett and Hunter, 1972).

Cultural and Morphological Variability

Different isolates of *S. rolfsii* isolated from Chittoor, Nellore and Kadapa districts of Rayalaseema were studied for their cultural, morphological characters, growth rate and sclerotia formation etc using two solid media viz; potato dextrose agar (PDA) and Czapek Dox Agar (CDA) medium. All isolates of *S. rolfsii* were grown on PDA and CDA medium in Petri plates. The mycelial disc of 4 mm diameter of each isolate was inoculated at the centre of plate and replicated thrice. The inoculated plates were incubated at $27 \pm 1^\circ\text{C}$ for 15 days. Radial growth of each colony in two directions at right angles was measured. Visual observations on sclerotial formation were recorded. A total of 8 morphological characters based on mycelial (mycelial growth, colony colour, mycelial dispersion and appearance) and sclerotial parameters (sclerotial colour, weight and shape, number of sclerotia and their arrangement on surface of media) were recorded at 7 and 15 days of incubation, respectively for each isolate.

Evaluation of fungicides *in vitro*

In vitro efficacy of fungicides against the pathogen was evaluated by poisoned food technique (Nene and Thapliyal, 1993). The fungicides used are three fungicide mixtures (Tricyclazole + Mancozeb, Carbendazim + Mancozeb, Hexaconazole + Zineb), three systemic fungicides (Azoxystrobin, Difenconazole, Hexaconazole) and one non-systemic fungicide (Blitox). 50ml of double strength PDA was mixed with 50 ml of double concentrated fungicidal solution to obtain final concentrations of 250, 500, 1000 and 1500 ppm. 20ml of this medium was plated in 9 cm Petriplates, a control was maintained without fungicide. A 7 mm mycelial disc of five days old pathogen was inoculated at the centre and incubated at 27

$\pm 1^\circ\text{C}$ until full growth was observed in control. Three replications were maintained for each treatment. Per cent reduction in radial growth over control was calculated using the formula: $I = (C-T)/C \times 100$ where, I = Per cent inhibition in growth of test pathogen, C = Radial growth of pathogen in control, T = Radial growth of pathogen in treatment (Nene and Thapliyal, 1993).

Results and Discussion

The isolated pathogen was identified as *Sclerotium rolfsii* Sacc. based on mycological characters, the fungal mycelium was first silky white in color later turned to dull white with radial spreading given fan like appearance. Microscopic examination of the fungal culture revealed the aerial hyaline, thin walled, septate hyphae with profusely branched mycelium showing clamp connections, when fungus attained maturity small mycelial knots were formed which later turned to mustard seed like sclerotia which were deep brown or brownish black, shiny, hard and spherical to irregular in shape. Similar, reports were given by Subramanian (1964), Barnett and Hunter (1972), Mahmood *et al.*, (1976), Singh (1987), Mirza and Aslam (1993), Mohan *et al.* (2000). Different morphological and cultural characters of 8 isolates of *S.rolfsii* were studied based on mycelial and sclerotial parameters. From the results, it is evident that, there is significant difference between isolates with reference to time and culture media. The two isolates ISR-1 and ISR-2 were considered to be very fast growing, isolated from RARS farm, Tirupati which covered entire Petriplate(9.0 cm)within 96 hrs of incubation (Table 1). The isolates ISR-3 and ISR-4 (8.7 cm) were considered to be fast growing isolated from Western parts of Chittoor dist. i.eKalakada mandal. While, the isolates ISR-6, ISR-7 and ISR-8 were considered to be moderately

growing (4.8 -5.4 cm), isolated from Ramatheertham area of Nellore dist. On the other hand, the isolate ISR-5 (2.8 cm) from Kadapa area were recorded to be slow growing, .

All the isolates of *S.rolfsii* produced dull/light white to extra white colonies except in ISR-5, which produced slight pinkish colonies on both media. All isolates of *S.rolfsii* differed in their mycelial dispersion and appearance in Petri plates. All isolates showed dispersed growth all over the plate to aggregated fashion and their appearance was loose to dense cottony with sparse or fluffy mycelium. Isolates ISR-2,6,8 produced aggregated dispersion of mycelium on both media (Table 2). Similar result was observed by Rakholiya *et al.* (2011) studied variability of 30 isolates of *S.rolfsii* and reported considerable variability in mycelial and sclerotial dimensions. Sclerotial formation followed mycelial aggregation with in 7 to 15 days (Table 3). Sclerotia were light brown to dark brown in colour. The sclerotia of isolates ISR-1,4 and ISR-6 were light/dull brown, whereas ISR-2,3,7,8 produced dark brown, but in isolate ISR-5, sclerotia were not produced on both media. The number of sclerotia formed was differed in all the isolates on both media. The number was comparatively more on PDA medium than on CDA medium. The sclerotial weight also differed across isolates ranging from 2.3 to 12 mg/10 sclerotia on PDA medium while, it was 2.4 to 17 mg/10 sclerotia on CDA medium, except for the isolate ISR-5. The sclerotia were scattered all over the plate singly or joined together, preferably at the periphery and/or centre of the Petri plate. Sclerotia maturity on both media ranged between 7 to 15 days. Variations in sclerotial colour, shape and size and their ability to infect plants have been reported by different scientists on various hosts and

media (Sharma *et al.*, 2002; Palaiah and Adiver 2006).

The morphological characters of *S.rolfsii* isolates tested were highly variable. The variability among isolates observed in the present study could be attributed to physio-metabolic differences among isolates arising from different crop production systems and also some biochemical variability to adapt to their ecological and environmental conditions. Geographical variability among *S.rolfsii* populations was demonstrated by earlier workers (Harlton *et al.* 1995; Okabe *et al.* 1998). In India, Sharma *et al.* (2002) studied variability among 26 isolates of *S.rolfsii* collected from various hosts/soil samples and localities. Studies of variability within the population in a geographical region are important because these also document the changes occurring in the population. The significant variation in culture characteristics, mycelial morphology and pathogenicity amongst test isolates indicated that *S.rolfsii* can best be characterized by a combination of culture characteristics, morphology and virulence on host plants. The differences in sclerotial forming capacity among isolates could be a useful parameter for characterizing isolates, due to the fact that number of sclerotia formed among fungal isolates was significant.

Reaction of *S. rolfsii* with fungicides *in vitro*

Variable results were obtained from the *in vitro* evaluation of fungicides to inhibit the growth of *S. rolfsii*. From the results, it is evident that among the three fungicide mixtures, three systemic fungicides and one contact fungicide, Carbendazim + Mancozeb and Hexaconazole + Zineb were found highly effective at low dose i.e.250 ppm. However, Tricyclazole + Mancozeb and

Hexaconazole were effective at 15000 ppm. While the two systemic fungicides, Azoxystrobin and Difenconazole and the contact fungicide, Copper oxychloride were found ineffective even at 1500 ppm. (Table 4)

Results revealed that among the three fungicide mixtures, Carbendazim + Mancozeb and Hexaconazole + Zinebinhibited the mycelial growth completely at 250 ppm concentration. Shepard *et al.* (1986) reported that hexaconazole at 10 mg l⁻¹ completely inhibited the *S. rolfsii*. Other systemic fungicides Azoxystrobinand Difenconazolewere found very less effective against *S. rolfsii* in per cent inhibition of mycelial growth. Copper oxychloridedid not show any effect on per cent inhibition of mycelium even upto 2000 ppm. In the present investigation the pathogen *S. rolfsii* showed resistance to Azoxystrobin and Difenconazole, here the per cent inhibition of mycelial growth very less. The tolerance of this fungi towards the fungicides is correlated with increasing ability to synthesize extracellular melanin under fungicidal stress (Amany *et al.*, 2003). In *S.rolfsii* the outer layer of sclerotia contain two to four layers of melanized rind. Melanin may be the inhibitory factor playing the role, natural occurrence or the induction of melanin pigment secretion may be a mode of pathogen defence against the toxic effect of the fungicide. Melanized cells posses increased resistance to environmental stress (Fogarty and Tobin, 1996).

Out of four combi products viz., vitavax power (Thiram 37.5% + Carboxin 37.5%), avatar (Hexaconazole 4% + Zineb 68%),

merger (Tricyclazole 18% + Mancozeb 62%) and nativo (Tebuconazole + Trifloxystrobin) tested except merger (Tricyclazole 18% + Mancozeb 62%) all other combi products showed cent per cent inhibition at all the five concentrations tested (Table 3). Merger is also an effective fungicide as it showed complete inhibition of the pathogenic growth at 1000ppm concentration, but the inhibition was only up to 57.44, 73.00, 84.88 and 87.11 per cent at 125, 250, 500 and 750ppm concentration respectively. These results are in agreement with Virupaksha Prabhu and Hiremath (2003); and Arunasri *et al.* (2011); who reported that the combi products containing triazoles viz., Avatar, Merger and Nativo were highly inhibitive to the growth of *Sclerotium rolfsii*. Vyas and Joshi (1977) and Sujatha (1991) reported that carboxin was highly effective on *Sclerotium rolfsii*. In our studies also carboxin and triazole containing fungicides showed the complete inhibition of mycelial growth of *Sclerotium rolfsii*. Manu et al (2012) reported that systemic fungicides like hexaconazole, propiconazole, difenconazole and combi products, Avatar (Hexaconazole 4% + Zineb 68%), Nativo (Tebuconazole 50% + Trifloxystrobin 25%) and Vitavax power (Thiram 37.5% + Carboxin 37.5%) showed complete inhibition of *S.rolfsii* at all the concentrations tested in finger millet. Whereas,the contact fungicide mancozeb was found inhibitive only at higher concentrations. By this study it was observed that the carbendazim + Mancozeb and Hexaconazole + Zineb containing combi products were found to be effective even at low concentrations under lab conditions against *Sclerotium rolfsii*.

Table.1 Growth rate of different isolates of *Sclerotium rolfsii* on different solid media

S.No.	Isolate	Growth rate on PDA (cm) **				Growth rate on CDA (cm) **			
		24 hr	48 hr	72 hr	96 hr	24 hr	48 hr	72 hr	96 hr
1	ISR-1 ^a	2.4	5.4	7.9	9.0	1.9	3.8	7.2	9.0
2	ISR-2 ^b	2.4	5.4	7.9	9.0	1.5	2.8	5.9	7.7
3	ISR-3 ^c	1.5	4.2	7.2	8.7	1.2	2.5	4.8	6.0
4	ISR-4 ^d	1.2	3.5	6.9	8.7	1.2	2.5	4.8	6.0
5	ISR-5 ^e	0.6	1.4	2.3	2.8	0.5	1.2	1.8	2.3
6	ISR-6 ^f	1.3	2.6	4.1	4.8	0.8	1.8	3.0	3.6
7	ISR-7 ^f	0.5	2.1	4.3	5.4	0.9	1.9	3.1	4.1
8	ISR-8 ^f	1.4	2.6	4.1	5.0	0.9	1.8	3.3	3.7

- Acc. To Dunnetts, ** indicates significant difference at 1% level with control
- Same alphabet indicates insignificant difference (DMR)

Table.2 Comparison of mycelial characters of isolates of *S.rolfsii* on different solid media

Isolate	Mycelial characters on PDA **			Mycelial characters on CDA **			Location of Isolate
	Growth	Colony colour	Appearance	Growth	Colony Colour	Appearance	
ISR-1	Very fast	Dull white	Cottony, pluffy at edges, dense at margins	Very fast	Dull white	Thead like, cottony	RARS farm, Tirupati
ISR-2	Very fast	Dirty white	Cottony, dense at centre, aggregated	fast	Extra white	Wavy like, condensed at margins, aggregated	RARS farm, Tirupati
ISR-3	Fast	Light white	Suppressed, thin strands	mode rate	white	Cottony, centralised	Kalakada, Chittoor
ISR-4	Fast	Cottony white	Pluffy at centre, upright growth habit	mode rate	Extra white	Centralized, thick strands	Bhakarapeta, Chittoor
ISR-5	Very slow	Slight pinkish	Sparse, thin strands	Very slow	Pinkish white	Sparse, restricted growth	Railway Koduru, Cuddapah
ISR-6	moderate	Dull white	Aggregated, dense cottony	Slow	Dull white	Sparse, cottony, aggregated	Ramatheertham, Nellore
ISR-7	moderate	white	Cottony, sparse	slow	Dull white	Sparse, cottony	Ramatheertham, Nellore
ISR-8	moderate	Extra white	Upright growth habit, cottony & pluffy aggregated	slow	Extra white	Aggregated, dense cottony	Ramatheertham, Nellore

Table.3 Comparison of sclerotial characters of isolates of *S.rolfsii* on different solid media

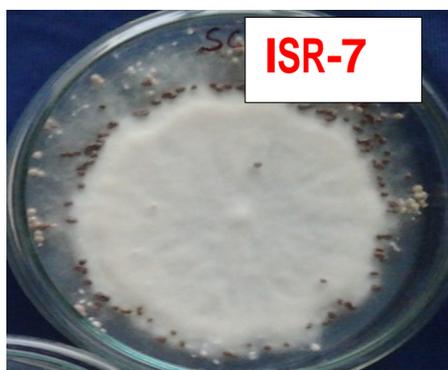
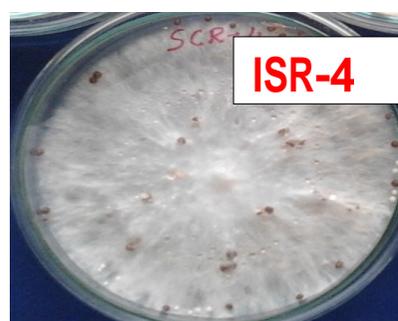
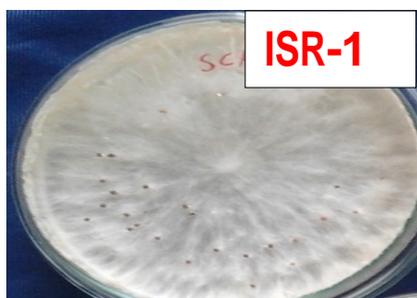
Isolate	Sclerotial characters on PDA *						Sclerotial characters on CDA *					
	No./plate (15 d)	colour	Shape	Wt (mg)	Arrangement	Maturity (d)	No./plate (15d)	colour	Shape	Wt (mg)	Arrangement	Maturity (d)
ISR-1	45.5	Light brown	spherical	4.8	Scattered all over plate	15	61.5	Dull brown	irregular	2.4	Peripheral	10
ISR-2	95.0	Dark brown	spherical	11.8	Central	11	40.0	Dark brown	irregular	12.9	Peripheral	09
ISR-3	488.0	Dark brown	Irregular	2.3	Peripheral	10	109.0	Dark brown	spherical	3.7	Peripheral	10
ISR-4	39.0	Dull brown	irregular	8.9	Scattered	10	309.0	Light brown	spherical	15.1	Centralised	12
ISR-5	0.0	No sclerotia	--	--	--	--	0.00	No sclerotia	--	0.0	---	--
ISR-6	67.5	Light brown	Spherical	12.0	Scattered	09	101.5	Dull brown	Irregular	17.0	Scattered	14
ISR-7	195.0	Dark brown	spherical	7.0	Peripheral	15	89.0	Dark brown	Spherical	9.0	scattered	15
ISR-8	139.0	Dark brown	irregular	5.0	central	14	209.0	Dark brown	spherical	10.0	peripheral	14

• **Average of three replication**

Table.4 *In vitro* evaluation of efficacy of fungicides on mycelial growth of *S. rolfsii*

S.No	Fungicide	Per cent inhibition of mycelial growth of <i>S. rolfsii</i> over control			
		Concentration (ppm)			
		250	500	1000	1500
1	Tricyclazole+ Mancozeb ^a	49.32	74.4	85.44	90.00
2	Carbendazim +Mancozeb ^b	90.00	90.00	90.00	90.00
3	Hexaconazole + Zineb ^b	90.00	90.00	90.00	90.00
4	Axoxystrobin ^c	36.14	41.79	49.07	50.95
5	Difenconazole ^d	56.89	69.27	75.76	83.86
6	Hexaconazole ^e	74.14	79.65	77.4	90.00
7	Copper oxy chloride ^f	0.00	0.00	0.00	0.00
8	Control ^f	0.00	0.00	0.00	0.00

- **Acc. To Dunnetts, ** indicates significant difference at 1% level with control**
- **Same alphabet indicates insignificant difference (DMRT)**



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