



## **Variability in *Sclerotium rolfsii* Sacc. causing Stem rot of groundnut**

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### **ABSTRACT**

Ground nut (*Arachis hypogea* L.) the king of oil seeds remain as the valuable source of all nutrients. The stem rot caused by *S. rolfsii* is becoming a major constraint to groundnut production. Hence the present study was taken up to assess the occurrence of stem rot in major growing areas of Tamil Nadu. The results of the survey revealed that stem rot incidence ranged from 7.88 to 32.02 %. The maximum incidence of 32.33 % was recorded in Adhivaraganallur village of Cuddalore district. The cultural characterisation of *S. rolfsii* on different solid medias revealed maximum mycelial growth with smooth margin and early sclerotia initiation in potato dextrose agar and Richard's agar medium. All the isolates of *S. rolfsii* varied in their ability to produce sclerotia on PDA medium. The maximum sclerotial number of 346 per nine mm culture disc were produced by the isolate SR<sub>1</sub> which was also found as the most virulent isolate.

**Key Words** Ground nut, *Sclerotium rolfsii* and Variability

### **INTRODUCTION**

Groundnut (*Arachis hypogea* L.) the king of oilseeds is popularly called as wonder nut, poor men's cashew nut, earthnuts, goober peas, monkey nuts and pig nuts. It belongs to the family of Fabaceae, subfamily *Papilionaceae* and it contains the valuable source of all nutrients. In India it's grown under rainfed as well as irrigated conditions. It is a legume which thrives best in tropical climate and requires 20°C to 30°C temperature, 50-75 cm rainfall. Well drained light sandy loams, red, yellow and black soils are well suited for its cultivation.

India is the second largest producer of groundnut after China. It is grown in 24.70 million hectares worldwide contributing 1.63 metric tonnes of pod yields. India, groundnut was cultivated in 4.56 Million hectares with a productivity of 0.98 metric tons and 4.47 Million metric tons per hectare of production in (2015-16) The major growing States are Gujarat, Andhra Pradesh, Tamil Nadu, Karnataka, Maharashtra, Rajasthan, Madhya Pradesh, Orissa, and Uttar Pradesh. According to Ministry of Agriculture, groundnut was cultivated in 2.89 lakh hectares with production of 9.31 lakh tonnes in Tamil Nadu during 2014-15. Major groundnut cultivating districts are Thiruvannamalai, Villupuram, Vellore, Kanchipuram, Thiruvallur, Cuddalore, Namakkal, Krishnagiri, Salem and Dharmapuri. In most of these districts, groundnut is sown during July to August (Adi pattam) and January to February (Thaipattam). In Thaipattam, it is grown under irrigated conditions.

Groundnut rich in energy (567 calories per 100 g), its seed contain 45-50% rich source of high-quality edible oil, 27-33% easily digestible protein as well as essential minerals and vitamins. Groundnut oil is composed of mixed glycerides and contains high proportion of unsaturated fatty acids, in particular, oleic (50-65%) and linoleic acids (18-30%) [12]. The flavonoids secreted by the ground nut root increase the growth of symbiotic and non-symbiotic nitrogen fixing bacteria, root nodules and nitrogen uptake by plants. Besides, the residual oilcake contains 7 to 8 per cent of N, 1.5 per cent of P<sub>2</sub>O<sub>5</sub> and 1.2 per cent of K<sub>2</sub>O and is used as organic fertilizer. Thus it helps maintain the fertility of the soil.

The groundnut oil has several uses but it is mainly used as cooking oil. It contains resveratrol, a polyphenol antioxidant, which has been found to have protective function against cancer, heart disease, degenerative nerve disease and viral infections. It is used in many preparations, like soap making, fuel, cosmetics, shaving cream, leather dressings etc., kernels, used for table purpose by frying, soaking,

roasting and boiling. Groundnut shell has great potential for commercial use. It is used as a fuel, filler in cattle feed, hard particleboard, cork substitute, activated carbon. Groundnut straw is mainly used as animal feed and fuel and in preparation of compost.

The crop is affected by various diseases caused by fungi, bacteria and viruses. In India among the soil-borne fungal diseases of groundnut, stem rot caused by *S. rolfisii* is a potential threat to production and is of considerable economic significance for groundnut grown under irrigated conditions. This disease causes severe damage during any stage of crop growth, and yield losses over 25% have been reported by Mayee and Datar [16]. Stem-rot caused by *S. rolfisii* is sporadic in most of the groundnut growing areas like Tamil Nadu, Andhra Pradesh, and Karnataka [20]. *S. rolfisii* is found throughout groundnut producing areas of the world and causes the severe damage during any stage of the crop growth with greatest yield losses up to 80% in severe conditions [25].

*Sclerotium rolfisii* Sacc., is a saprophytic soil-borne fungus causes disease on wide range of agricultural and horticultural including different types of diseases like collar-rot, *Sclerotium* wilt, stem-rot, charcoal rot, seedling blight, damping-off, foot-rot, stem blight and root-rot in more than 500 plants species including tomato, chilli, sunflower, cucumber, brinjal, soybean, maize, groundnut, bean, watermelon etc [11].

The symptoms of stem rot produced by *S. rolfisii* on groundnut plants under field conditions were characterized by formation of deep brown lesion on the stem region of the plant just near the ground followed by yellowing of groundnut leaves than by loss of vigour and premature death. The infected plant showed poor root growth and rotting of the stem region. Soon after this, the lesion was covered by a radiating white mycelium with the rotting underneath it. In later stages of infection, light deep brown spherical or round sclerotial bodies were formed, which adhered around the infected stem region and such bodies were produced abundantly on stem. Death of the plant occurred more rapidly under dry conditions during which the necrosis instead of browning appeared. On young pods light brown lesions were noticed mycelium and sclerotia developed on even inside the pods. Kernels were infected in the advanced stage of plant growth; such kernels were small and shriveled in size [2].

With this background, the present study has been undertaken with the following objective. Isolation and identification of pathogen from major groundnut growing areas of Tamil Nadu and assess the cultural and pathogenic variability among *S. rolfisii* isolates.

## MATERIALS AND METHODS

### Survey on the stem rot incidence of groundnut in Cuddalore district [10]

A field survey was conducted to assess the extent of stem rot occurrence of groundnut in Cuddalore district of Tamil Nadu state. Fifteen locations representing both rainfed and irrigated situations were selected for the study. The per cent disease index was worked out using the following formula

$$\text{Per cent Disease Incidence (PDI)} = \frac{\text{No. of diseased plants}}{\text{No. of plants observed}} \times 100$$

Also, the infected plants showing the typical symptoms of root rot due to infection with *Sclerotium rolfisii* were collected along with rhizosphere soil for isolation of the pathogen. The other information's regarding the soil type in which the crop is grown and the variety of groundnut cultivated were also recorded in the respective survey fields.

### Isolation and maintenance of the pathogen

The stem rot pathogen *S. rolfisii* were collected from major groundnut growing locations from Cuddalore districts such as Adhivaram, Kammapuram, Killai, Kuppanatham, Meenachipattu, Nellikuppam, Pattampakkam, Periyapattu, Ponveli and Puthuchathiram.

Groundnut plants infected with stem rot pathogen, were collected. The infected plant materials were selected and used as source for the isolation of causative agent. Infected portion of stem was cut into small pieces with sterilized scalpel, cleaned with distilled water, then surface sterilized with 0.1% HgCl<sub>2</sub> solution for 30 second and again washed thrice with sterile distilled water. Small 1 to 2 pieces were transferred aseptically on Potato Dextrose Agar (PDA) plates containing Chloramphenicol (30 mg/100 ml) with the help of sterilized forceps under aseptic condition [21]. Inoculated Petri plates were incubated at 25°C for 7 days for growth of the pathogen.

A total ten (SR<sub>1</sub> to SR<sub>10</sub>) isolates causing stem rot was isolated from infected plant samples collected from different tracts of Cuddalore districts. The fungal growth on 5<sup>th</sup> day, which arose through the sclerotial bodies was cut by inoculation loop and transferred aseptically to the PDA slants and allowed to grow at room temperature to obtain the pure culture of fungus. The culture thus obtained was stored in refrigerator at 5°C for further studies and was sub culture periodically.

### Cultural and Morphological Variability

Fifteen ml of the sterilized PDA medium was poured into sterile Petri dishes and allowed to solidify. A nine mm culture disc of *S. rolfsii* obtained from actively growing region was aseptically placed at the center of the dish and incubated at room temperature (28 ± 2°C). The radial growth of the isolates (in mm) was measured five days after inoculation. 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 sclerotial character (sclerotial colour, weight and shape, number of sclerotia and their arrangement on surface of media) were recorded at 7 and 15 days of incubation.

#### **Mass multiplication of *Sclerotium rolfsii* isolates [6]**

A total of ten isolates were multiplied on sorghum grains (200 g) soaked overnight in water for pot experiment. About 100 g of soaked sorghum grains were taken in 500 ml capacity saline bottles tightly plugged. The bottles were then sterilized for 20 min at 121°C. After sterilization the sorghum seeds in saline bottles were inoculated with 5 mm mycelial disc from 7-day-old pure culture of *S. rolfsii* at each bottle and bottles were incubated for a 15 days at 27°C ± 2°C for proper mycelial growth.

#### **Assessing the virulence of *S. rolfsii* isolates**

The potting mixture was prepared by thoroughly mixing clay loam soil, sand and farm yard manure at 1:1:1 ratio. The sorghum grain based medium inoculum of each isolate of *S. rolfsii* collected from different locations were separately mixed at five per cent level (w/w) with the sterilized soil filled in 30 cm earthen pots ten days before sowing. Surface sterilized (using 0.1 % HgCl<sub>2</sub> solution for 30 sec. followed by two washings in sterile water) groundnut seeds were sown @ 5 seeds pot<sup>-1</sup> without inoculum served as control. Soil moisture was maintained at moisture holding capacity of soil by adding sterilized water on weight basis throughout the period. After 20 day of inoculation, the plant showing the typical wilting symptoms were observed. Re isolation was made from such affected portion of plant tissue and compared with that of original isolates for conformity.

#### **Effect of different level of inoculum of *S.rolfsii* (SR<sub>1</sub>) on the incidence of stem rot of groundnut**

The soil, sand and farmyard manure were sieved by passing through 2mm mesh and sterilized separately and then mixed in 1:1:1 per cent proportion, respectively. After mixing. It is filled in 15 × 30 cm surface sterilized earthen pots *S.rolfsii* culture grown on sorghum grain based medium for 20 days was mixed to each pot so as to get different inoculum levels viz., 0,1,2,3,4 and 5 per cent. The pots filled with sterilized soil without inoculum served as control (uninoculated). Each treatment was replicated three times. Groundnut seeds were sown @ 5 seeds pot<sup>-1</sup>. Water was added to the pots at the regular intervals to maintain the soil moisture. The observation on the incidence of stem rot was recorded.

#### **Identification of susceptible stage of the crop to stem rot of groundnut**

To know the susceptible stage of the crop, an experiment was conducted under glasshouse condition. Five stages of the groundnut crop 0, 15, 30, 45 and 60 DAS of the groundnut plants were taken for their susceptible reaction against stem rot causal pathogen *S. rolfsii*. These stages of plants were maintained in the eighteen pots of 15 × 30 cm diameter replicated three times and filled with sterilized soil. In each pot 10 seeds of groundnut (VRI-2) was shown and fertilizer dose applied as per recommended. After raising all the respective stages, the sorghum grain inoculums were added at near the stem up to 4-5 grain on each plants of groundnut. Inoculated pots were kept in open place for observation and the pots were irrigated as when required. Stem rot disease severity was made at 15, 30, 45, 60 and 75 days after inoculation at respective stages, number of plants showed typical symptoms i.e. stem rot, lesion of stem, weathering of leaf and dead plants due to *S. rolfsii* was observed and per cent disease incidence was calculated using formula (Table 1)

$$\text{Disease Incidence (PDI)} = \frac{\text{No. of diseased plants}}{\text{No. of plants observed}} \times 100$$

#### **Symptoms on groundnut plants were observed as per 1-5 rating scale (Shokes *et al.*, 1996).**

Disease rating	Treatments (Days)	Description
1	0	Healthy
2	15	Lesions on stem only
3	30	Up to 25% of the plant symptom (wilt, dead or dying)
4	45	26% to 50% of the plant symptom
5	60	>50% of the plant symptom

#### **Effect of different solid media on growth and formation of sclerotia of *S.rolfsii* (SR<sub>1</sub>)**

Variation in the growth of *S. rolfsii* in different solid media viz., Potato dextrose agar, Czapek's Dox agar, Richard's agar, Yeast extract agar, Coon's agar and Carrot agar was studied. Fifteen ml of molten media were dispense into each of 90 mm sterile Petri plates mycelial discs taken from the advancing margins of seven days old culture of *S.rolfsii* by the aid of a cork borer were separately placed each at the center of

the plate containing the above mentioned medium. The inoculated plates were incubated at room temperature (28±2°C) for nine day and the diameter of the mycelial growth of pathogen was measured in each case at five days after incubation. Further, the plates were examined for culture characteristics like growth of mycelium, mycelium pigmentation, type of colony, Degree of sclerotia formation after 15 days.

## RESULTS AND DISCUSSION

### Survey on the stem rot incidence of groundnut in Cuddalore district of Tamil Nadu

**Table 1. Survey on the incidence of groundnut stem rot disease in Cuddalore district of Tamil Nadu**

S. No	Village	Soil type	Variety	Irrigated/ Rain fed	Stem rot incidence (%)
1	Adhivaraganallur	Sandy loam	Local	Irrigated	32.02
2	Killai	Sandy loam	JL-24	Rain fed	25.02
3	Kuppanaththam	Red sandy	JL-24	Irrigated	11.76
4	Meenachipattu	Red sandy	Local	Irrigated	21.22
5	Pattampakkm	Clay loam	VRI-2	Rain fed	22.45
6	Periyapattu	Clay loam	VRI-2	Rain fed	27.66
7	Ponveli	Sandy loam	VRI-2	Irrigated	29.56
8	Puthuchathiram	Clay loam	JL-24	Irrigated	18.45
9	Rajakuppam	Red sandy	VRI-2	Irrigated	7.88
10	Sivapuri	Clay loam	Local	Irrigated	13.00

The roving survey conducted during the year 2015 - 2016 in different locations of cuddalore district revealed the endemic nature of the stem rot disease incidence and the results are presented in table 1. Among the different locations of Cuddalore districts surveyed for stem rot incidence, Adhivaraganallur village registered the maximum incidence of 32.0 percent followed by Ponveli (29.56%), Periyapattu (27.66%), Killai (25.02%), Pattampakkm (22.45%), Meenachipattu (21.22%), Puthuchathiram (18.45%), Sivapuri (13.00%) and Kuppanatham (11.76%) in the decreasing order of merit. The minimum stem rot incidence was recorded in Rajakuppam (7.88%). The native isolates of *S. Rolfsii* were isolated from the respective locations and designated as (SR<sub>1</sub> to SR<sub>10</sub>).

The variation observed in the disease incidence might be due to the prevalence of the strains of *S. rolfsii* with varied virulence and the environmental factors in the respective areas. Likewise, the survey conducted by Divya Rani *et al.* (2016) revealed that the stem rot incidence ranged from 4% (Lingala mandal of Mahaboobnagar district) to 12.8% (Ramachandrapur mandal of Chittoor district). Similarly, in Anantapur stem rot incidence ranged from 6% (Singanamala mandal) to 11.1% (Mudigubba mandal) in 24 villages spread over in six mandals of the district in Andhra Pradesh. The stem rot incidence ranged from 8.96 per cent in Chandragiri mandal to 12.8 per cent in Ramachandrapuram mandal in 16 villages spread over in four mandals of the Chittoor district. Similarly in Mahaboobnagar the disease incidence ranged from 4 % (Lingalamandal) to 10 % (Balmoor mandal) in 20 villages of five surveyed mandals, whereas in, Warangal district the stem rot disease ranged from 5% (Torrur) to 9.1 % (Sangem mandal) in 16 villages across the four mandals.

**Table 2. Morphological characters of *Sclerotium rolfsii* isolates on potato dextrose agar**

Isolate number	Mycelial characters			Sclerotial characters				Wt (mg)
	Colony characters	Mycelial growth (mm)	No of sclerotia (15 days)	Colour of Sclerotia	Shape of Sclerotia	Maturity (day)	Arrangement	
SR1	Light cottony white mycelia	90	346.	Light brown	Spherical	10	Scattered all over plate	14.0
SR2	Cottony profused mycelia	85	222	Chocolate	Oval	15	Peripheral	7.3
SR <sub>3</sub>	Dull white profused mycelia	89	178	Chocolate	Spherical	10	Scattered	4.8
SR4	Profused cottony mycelia	86	121	Brown	Round	10	Scattered	4.9
SR5	Cottony white mycelia	80	150	Brown	Oval	12	Scattered all over plate	6.7

SR6	Profused cottony mycelia	84	193	Dark brown	Spherical	09	Scattered	8.2
SR7	Cottony white mycelia	87	155	Dark brown	Round	11	Central	7.0
SR8	Profused cottony mycelia	83	190	Brown	Spherical	14	Central	6.0
SR9	Cottony white mycelia	81	239	Brown	Round	09	Scattered	11.0
SR <sub>10</sub>	Profused cottony mycelia	88	234	Brown	Pear	10	Peripheral	10.3
	S.Ed CD (0.05)	0.76 1.59	0.81 1.70					

**Table 3. Pathogenicity of *S. rolfii* isolates**

S. No	Isolates	Stem rot incidence (%)				Mean
		30 DAS	60 DAS	90DAS	At harvest	
1	SR1	29.10	46.35	49.10	55.70	45.06
2	SR2	18.80	27.30	36.70	43.40	31.55
3	SR <sub>3</sub>	20.40	31.50	40.40	49.35	35.41
4	SR4	19.30	26.80	38.30	46.50	32.72
5	SR5	12.55	18.25	22.90	30.60	21.07
6	SR6	14.75	20.30	26.80	38.90	25.18
7	SR7	15.86	23.50	29.70	36.70	26.44
8	SR8	14.40	26.30	35.10	42.70	29.62
9	SR9	18.47	27.37	37.10	45.45	32.097
10	SR10	17.50	26.87	35.80	42.90	30.767
	S.Ed CD(0.05)	0.25 0.52	0.26 0.55	0.31 0.66	0.86 1.89	

**Table 4. Identification of susceptible stages of the crop**

Tr. No	Treatments	Disease incidence
1	Zero stage	25.71
2	15 days old crop	69.36
3	30 days old crop	74.45
4	45 days old crop	79.04
5	60 days old crop	49.68
6	Control	0.00
	S.Ed CD(0.05)	0.29 0.96

**Table 5. Effect of different solid media on mycelial growth and sclerotia formation of *S. rolfii* (SR<sub>1</sub>)**  
**Cultural characteristics of *S. rolfii* isolates**

S. No	Name of the medium	Mycelial growth (mm)			Type of colony	Pigmentation	Degree of Sclerotia formation (After 15 days)
		72h	96 h	120 h			
1	Potato dextrose agar	43	65.00	90.00	Appressed	White	Good
2	Czapek's Dox agar	35	50.50	75.30	Fluffy	Dull white	Poor
3	Richard's agar	40	56.16	80.50	Fluffy	Dull white	Poor
4	Yeast extract agar	29	37.00	50.83	Appressed	White	Fair
5	Coon's agar	25	32.16	40.66	Fluffy	White	Fair
6	Carrot agar	30	40.83	70.00	Fluffy	White	Fair
	S.Ed CD(0.05)	1.21 2.43	1.30 2.81	1.49 3.21			

#### **Mycelial growth**

All the ten isolates of *S. rolfii* produced profuse mycelium with radial spread giving fan like appearance on Potato Dextrose Agar (PDA) medium which was first silky white in color later turned to dull white.

Among the isolates SR<sub>1</sub> recorded the maximum (90 mm) mycelial growth which was followed by SR<sub>10</sub>, SR<sub>7</sub>, SR<sub>4</sub>, and SR<sub>2</sub> in the decreasing order of merit while it was the minimum (80.00 mm) in the case of SR<sub>5</sub>. (Table 2).

Similar result was observed by Rakholiya *et al.* [23] who studied variability of 30 isolates of *S. rolfsii* and reported considerable variability in mycelial and sclerotial dimensions. Also, similar such variation in the cultural characteristics of *S. rolfsii* on PDA was reported by Madiya Waskale [15].

The isolated pathogen was identified as *S. rolfsii* based on mycological characters, the fungal mycelium was first silky white in colour 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 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 Mohan *et al.* [17], Savita Ekka *et al.* [27].

#### **Sclerotial number**

All the isolates of *S. rolfsii* varied in their ability to produce sclerotia on PDA medium. The maximum sclerotial number of 346 per nine mm culture disc was obtained from SR<sub>1</sub> which was also the most virulent isolate. This was followed by the isolates SR<sub>9</sub>, SR<sub>10</sub>, SR<sub>2</sub> and SR<sub>6</sub>, which produced 239, 234, 222 and 193 numbers of sclerotia, respectively. The minimum number of sclerotia of 121 was recorded by SR<sub>5</sub> the least virulent isolate (Table 2).

In the present study, all the isolates of *S. rolfsii* varied in their ability to produce sclerotia on PDA media. The maximum sclerotial number was obtained from the most virulent isolate SR<sub>1</sub>. The minimum number of sclerotial production was recorded by the least virulent isolate SR<sub>4</sub> (Table 3). It was further observed in our studies that isolates with heavy mycelial growth produced more number of sclerotia. These findings were consistent with the earlier reports [1, 4, 29].

#### **Sclerotial Weight**

The isolates of *S. rolfsii* produced varying sizes of sclerotia on PDA. The most virulent isolate SR<sub>1</sub> produced the biggest sclerotia with a size of 14.0mg (Table-2) and the smallest sclerotial size of 4.8 mg was recorded with SR<sub>3</sub>, which was the least virulent isolate. This was followed by other isolates *viz.*, SR<sub>9</sub>, SR<sub>10</sub>, SR<sub>6</sub> and SR<sub>7</sub>, which produced sclerotia with the size of 11.0, 10.0, 8.2 and 7.3 mg respectively. Similar result was observed by Rakholiya *et al.* (2011)

#### **Sclerotia colour**

The isolates of *S. rolfsii* produced different colour of sclerotia on PDA. SR<sub>1</sub> produced light brown colour, SR<sub>2</sub>, SR<sub>3</sub> produced chocolate colour, SR<sub>4</sub>, SR<sub>5</sub>, SR<sub>8</sub> and SR<sub>9</sub> produced brown colour and SR<sub>6</sub> and SR<sub>7</sub> produced dark brown colour. Initially white colour sclerotia were formed, then the colour changed from white to light brown or chocolate, dark brown or brown as they attained maturity after utilization of nutrients, the plates become dry. However, dark brown coloured sclerotia survived for long period. The change in colour of sclerotia might also be due to utilization or exhaustion of nutrients [9] Similar such colour change was reported earlier [30, 24]. (Table-2)

#### **Pathogenicity of *S. rolfsii* isolates on groundnut**

The result depicted in table 3 revealed varied levels of pathogenicity with difference in isolates. Among the ten isolates of *S. rolfsii* collected from different conventional groundnut growing areas of Cuddalore district, the isolate (SR<sub>1</sub>) collected from Adhivaraganallur was found to be more virulent and recorded the maximum incidence of 55.70 per cent (at harvest) followed by SR<sub>3</sub> (49.35%) collected from Meenachipattu. The isolates SR<sub>4</sub> and SR<sub>9</sub> showed 46.50 and 45.45 per cent of disease incidence and were on par. The isolate SR<sub>5</sub> collected from Pattampakkam was the least virulent which recorded the minimum (30.60%) stem rot disease incidence.

The variability in the pathogenicity among the isolates of *S. rolfsii* was reported by earlier worker [19, 5] investigated the pathogenicity of different isolates of *S. rolfsii* on groundnut. Observations revealed that all the isolates were found to be pathogenic towards groundnut but extent of their pathogenicity in respect of their diseases severity differed in some isolates. These earlier reports corroborate with the present findings.

#### **Identification of susceptible stages of the crop**

To find out the susceptible stage of the groundnut to stem rot disease development, an experiment was laid out in glasshouse conditions as explained in materials and methods and the results are presented in the table 4. The results revealed that, there was significant difference in wilting percentage among the different stages of the plant to stem rot disease development. Significantly higher per cent of wilting of 79.04 % was recorded in plants at 45 days of age after emergence and it was found significantly more susceptible compared to rest of the treatments. The 30 and 15 days old plants recorded 74.45% and 69.36% incidence, respectively. In the present study, it was observed that *S. rolfsii* can infect all the stages

of groundnut crop. However, forty five day old plant had maximum 79.04% disease severity followed by 30 and 15 days old plants. Similar finding was reported by Bekriwala [6], who reported that, groundnut plants were found most susceptible to the attack of *S. rolfisii* during 45 days of the growth and the per cent infection of the plant reduced with ageing.

Similarly the disease severity was decreased as the age of plant increased and maximum plant mortality due to *S. rolfisii* was recorded in 15 days old groundnut seedling followed by 30 days old plants [14]. According to Nathawat *et al.* [18] 10 days old plants were more susceptible to collar rot infection (80.00%) followed by 15 days old groundnut plants (75.00%). The per cent plant killing increased with increased in age up to (5 to 10days) but it was decreased beyond 15 days, also in chick pea plant [13] and peppermint [8].

### **Effect of different solid media on radial growth and sclerotia formation of *S. rolfisii***

#### **Effect on radial growth**

Maximum radial growth (90.0 mm) was recorded on PDA medium followed by Richard's agar medium and Czapek'sDox agar which recorded 80.50 mm and 75.30mm radial growth, respectively. Least radial growth (40.66 mm) of the test fungus was recorded on Coon's agar medium. The radial growth recorded on Carrot agar medium and Yeast extract agar were 70.00 and 50.83 mm respectively. The fungus produced appressed to fluffy type of growth and dull white to white pigmentation on all the media tested. This indicates that maximum growth of *S. rolfisii* was supported by PDA medium.

#### **Effect on sclerotia formation**

Data presented in table 5 clearly indicated that potato dextrose agar medium was best for radial growth and sclerotia production of *S. rolfisii*. The test fungus produced sclerotia on all the media tried but excellent sclerotia production was not observed in any medium. Good sclerotia production was observed on PDA, Czapek'sDox agar, Richard's agar supported poor sclerotia formation.

It is evident from the data presented in that out of seven media, *S. rolfisii* preferred Potato dextrose agar (PDA) medium for best growth. Colony diameter was observed significantly superior on Potato dextrose agar medium (90.00 mm) followed by Richard's agar medium and Czapek's Dox agar (80.50mm) after 5 days of inoculation.

Potato dextrose agar was best for the radial growth and sclerotial production of *S. rolfisii*, as stated by Akram *et al.* [3], Rajalakshmi *et al.* [21]. Chaurasia *et al.* [7] also reported that potato-dextrose medium was most suitable for mycelial growth and sclerotia production of *S. rolfisii*. Similar growth PDA medium of *S. rolfisii* was observed by several workers [31, 27]. These earlier reports add value to the present observations.

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