Plant Archives
Journal home page: www.plantarchives.org
DOI Url: https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no1.047

DISEASE INCIDENCE AND SEVERITY OF *SCLEROTIUM ROLFSII* ON *ARACHIS HYPOGEA* L

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(Date of Receiving-11-10-2020; Date of Acceptance-07-01-2021)

ABSTRACT

Groundnut or peanut (*Arachis hypogea* L.) is an important oilseed and edible crops of the world. It is affected by many diseases, among which stem rot disease caused by *Sclerotium rolfsii* is major fungal disease that cause severe yield losses up to 80%. An experiment wasconducted in Tamil Nadu during 2019-2020, to study the cultural, morphological and pathogenicity of different isolates of S. rolfsii infecting groundnut in major cultivating areas of Tamil Nadu. The overall disease severity ranges from 9.82 to 36.83 percent. The highest disease incidence 36.83 per cent was noticed in the Kalpadai village and the least incidence 9.82 per cent was recorded in Pattukotttai village. All the isolates varied their ability to produce abundant mycelium and sclerotial production. The pathogenicity test was conducted to all the twenty isolates and the isolate Sr6 exhibited the maximum disease incidence and proved to be more virulent.

Keywords: Groundnut, Stem rot, *Sclerotium rolfsii*

INTRODUCTION

Groundnut or peanut (*Arachis hypogea L.*) is an important food and oil seed crop cultivated throughout the world in tropical, sub - tropical and temperate climates, considered as "King of oil seeds".Globally, India ranks first in area and second in production of 8.94 million tonnes from 4.89 million hectares with the productivity of 1825 kg/ha (India Stat, 2019). In India, the major growing states are Gujarat, Andhra Pradesh, Tamil Nadu, Karnataka, Maharastra, Rajasthan, Madhya Pradesh, Odissa and Uttar Pradesh. In Tamil Nadu,major groundnut cultivating districts are Tiruvannamalai, Cuddalore, Kallakurichy, Villupuram, Vellore, Kanchipuram, Ariyalur, Thanjavur, Salem and Dharmapuri. Groundnut kernel is rich source of energy (567 calories per 100g), because of its oil content (44-48%), protein content (25-36%) and contain health benefiting nutrients, minerals, antioxidants and vitamins that are essential for growth and development (Rashmi *et al*., 2017). The groundnut roots are secreted the flavonoid compounds that increase the growth of symbiotic and non-symbiotic nitrogen fixing bacteria, root nodules and nitrogen uptake by plants (Solaiman *et al*., 2014).

Groundnut plant suffers with many fungal, bacterial and viral diseases resulting in high yield losses. Nearly 67 fungal diseases were reported, among the fungal diseases stem rot disease caused by *Sclerotium rolfsii* Sacc. is one of the major concerns causing huge economic losses in groundnut (Deepthi and Reddy, 2013). Stem rot is also known as *Sclerotium* blight, *Sclerotium* rot, *Sclerotium* wilt, southern blight, southern stem rot, root rot, white mould and pod rot. The yield losses usually range from 10 to 25 per cent in India, Thailand, Indonesia, Taiwan, and the Philippines but may reach 80 per cent in severely infested fields (Doley K and Kaur JITE, 2013).

S. rolfsii is a necrotrophic soil borne pathogen, it possesses the ability to attack a large number of monocot and dicot plant species, belonging to about 100 families. Over 500 plant species are affected by *S. rolfsii*, due to the production of phytotoxin (oxalic acid), cell wall degrading enzymes and polysaccharides degrading multiplex enzymes in host system. Humid weather with the temperature ranges between 25°C to 30°C is favorable to mycelial growth and sclerotial germination of this pathogen. Development of *S. rolfsii* in host plants leads to production of profuse white mycelia and formation of appressoria with tissue necrosis and later on advanced mycelial growth causes tissue death (Tang *et al*., 2015).The melanized outer membrane of sclerotia enables the fungus to survive in harsh conditionin soil for more than three years in dormant stagewhich survives as first source for the infection (Punja, 1985).

To understand the ecology, pathogenicity and evolutionary potential aspects of the *S. rolfsii*, it is important to study about the cultural, morphological and pathogenic variability of the *S. rolfsii*. Therefore, the present study was undertaken with a view to study the morphological, cultural and pathogenic variability's among isolates of *S. rolfsii* collected from groundnut plants of different locations.

MATERIALS AND METHODS

Survey of groundnut stem rot from different districts of Tamil Nadu

A field survey was carried to assess the stem rot incidence of groundnut from different districts of Tamil Nadu such as Ariyalur, Cuddalore, Kallakurichy, Perambalur,Thanjavur, Tiruvannamalai, Tiruchirappalli and Villupuram districts. Among these districts, twenty villages were selected for this study. In each village, four

Table 1 Survey on the disease incidence of groundnut stem rot in different localities of Tamil Nadu

Sl.No	Districts	Locality	Soil type	Variety	Disease Incidence (%)
1.	Ariyalur	T. Melur	Red loam	VRI 2	21.92hi (27.90)
2.		Vadugapalayam	Red loam	VRI 2	30.74de (33.65)
3.	Cuddalore	Sivapuri	Clay loam	LOCAL	20.22i (26.71)
4.		Kurinjipadi	Red loam	VRI 2	33.01bc (35.06)
5.		Kammapuram	Red loam	LOCAL	30.94de (33.77)
6.	Kallakurichy	Kalpadai	Red loam	TMV 7	36.83a (37.35)
7.		Paranginatham	Red loam	LOCAL	28.36f (32.20)
8.		Malligaipadai	Red loam	LOCAL	34.43b (35.91)
9.		Pottiyam	Clay loam	TMV 2	30.57e (33.58)
10.	Perambalur	Padalur	Black soil	VRI 2	26.68f (31.11)
11.		Sridevimangalam	Red loam	VRI 2	22.77gh (28.52)
12.	Thanjavur	Peravurani	Red loam	JL 24	15.62j (23.26)
13.		Pattukottai	Clayey loam	VRI 2	09.82l (18.24)
14.	Thiruvannamalai	Polur	Red loam	TMV 2	33.36bc (35.30)
15.		Vaanapuram	Sandy loam	TMV 2	24.56g (29.73)
16.		Kalasappakkam	Sandy loam	LOCAL	32.45cd (34.76)
17.		Thanipadi	Red loam	VRI2	23.12gh (28.73)
18.	Tiruchirappalli	Pholurpatti	Sandy loam	TMV 7	26.65f (31.11)
19.	Villupuram	Valavanur	Red Sandy loam	JL 24	12.69k (20.88)
20.		Sengadu	Red sandy loam	JL 24	23.68gh (29.13)

Mean of three replications

Values in the column followed by common letters do not differ significantly at 5% level by Duncan's multiple range test (DMRT)

Table 2 Morphological identification of *Sclerotium rolfsii* isolates in different localities of Tamil Nadu

Isolates number	Locality	Mycelial characters			Sclerotial character		
		Colony character	Mycelial growth (5DAI)	No of Sclerotia (15DAI)	Color of Sclerotia	Shape of Sclerotia	Sclerotial Arrangement
Sr_1	T. Melur	Fluffy white mycelium	80	92	Brown	Spherical	central
Sr_2	Vadugapalayam	Dense cottony white mycelium	85	238	Chocolate brown	Oval	Scattered
Sr_3	Sivapuri	Dense cottony white mycelium	79	85	Brown	oval	Scattered
Sr_4	Kurinjipadi	Light cottony white mycelium	87	286	Dark Brown	Spherical	Central
Sr_5	Kammapuram	Dull white profused mycelium	86	253	brown	Round	Peripheral
Sr_6	Kalpadai	Profused cottony white mycelium	90	330	Dark brown	Round	Scattered
Sr_7	Paranginatham	Fluffy white mycelium	85	206	Chocolate brown	Round	Peripheral
Sr_8	Malligaipadai	Dense cottony white mycelium	89	317	Chocolate brown	Spherical	Central

Sr_9	Pottiyam	Cottony profused mycelium	85	211	Brown	Oval	Central
Sr_{10}	Padalur	Fluffy white mycelium	84	188	Dark brown	Round	Peripheral
Sr_{11}	Sridevimanga-lam	Light cottony white mycelium	80	103	Light brown	Oval	Scattered
Sr_{12}	Peravurani	Fluffy white mycelium	78	82	brown	oval	Central
Sr_{13}	Pattukottai	Dull white profused mycelium	76	78	Light brown	Round	Peripheral
Sr_{14}	Polur	Profused cottony white mycelium	88	302	Light brown	Spherical	Peripheral
Sr_{15}	Vaanapuram	Profused cottony white mycelium	83	157	Chocolate brown	Spherical	Scattered
Sr_{16}	Kalasappakkam	Fluffy white mycelium	87	270	Dark brown	Oval	Scattered
Sr_{17}	Thanipadi	Cottony profused mycelium	81	108	Brown	Round	Central
Sr_{18}	Pholurpatti	Light cottony white mtcelium	84	169	Brown	Oval	Central
Sr_{19}	Valavanur	Cottony profused mycelium	77	80	Chocolate brown	Oval	Peripheral
Sr_{20}	Sengadu	Cottony profused mycelium	82	130	Dark brown	Spherical	Scattered

Table 3 Pathogenicity of *Sclerotium rolfsii* isolates under pot culture condition

S. No	Isolates	Stem rot incidence (%)				Mean
		30 DAS	60 DAS	90 DAS	At harvest (105 DAS)	
1	Sr1	15.76lm (23.42)	20.70l (27.06)	21.42m (27.56)	33.01q (35.06)	22.72
2	Sr2	24.14ef (29.40)	31.16fg (33.96)	33.62f (37.23)	44.19g (41.67)	34.03
3	Sr3	14.49mn (22.38)	19.09m (25.91)	20.06n (26.57)	31.56r (34.20)	21.30
4	Sr4	27.47cd (31.63)	34.61cd (36.03)	40.56d (39.58)	47.77d (43.74)	37.60
5	Sr5	25.85de (30.59)	32.41ef (34.70)	38.24e (38.17)	45.39f (42.36)	35.47
6	Sr6	32.10a (34.51)	37.26a (37.64)	46.51a (42.99)	52.31a (46.32)	42.05
7	Sr7	21.83g (27.83)	29.97g (33.21)	32.95h (35.06)	41.90i (40.34)	31.66
8	Sr8	30.03b (33.21)	36.41ab (37.11)	44.69b (41.96)	50.90b (45.52)	40.51
9	Sr9	22.82fg (28.52)	30.61g (33.58)	34.61g (36.03)	42.83h (40.86)	32.72

10	Sr10	21.44gh (27.56)	28.26h (32.13)	31.38i (34.08)	40.18j (39.35)	30.32
11	Sr11	16.36kl (23.89)	21.91kl (27.90)	23.89l (29.27)	34.15op (35.79)	24.08
12	Sr12	13.22nop (21.30)	18.01mn (25.10)	19.00op (25.84)	30.02s (33.21)	20.06
13	Sr13	09.71q (18.15)	14.49op (22.38)	15.32r (23.03)	27.06u (31.31)	16.65
14	Sr14	28.56bc (32.33)	35.65bc (36.63)	42.36c (40.63)	49.45c (44.71)	39.01
15	Sr15	18.39ij (25.40)	26.12i (30.72)	28.85j (32.46)	37.70l (37.88)	27.77
16	Sr16	26.61d (31.05)	33.47de (35.37)	39.47d (38.94)	46.47e (42.99)	36.51
17	Sr17	17.15jkl (24.43)	23.18jk (28.79)	25.83k (30.53)	35.16n (36.39)	29.12
18	Sr18	19.71hi (26.35)	27.00hi (31.31)	30.61i (33.58)	39.16k (38.76)	29.12
19	Sr19	12.02op (20.27)	17.40n (24.65)	18.28q (25.33)	28.74t (32.39)	19.11
20	Sr20	18.07ijk (25.18)	24.11j (29.40)	26.83k (31.18)	36.58m (37.17)	26.37

Mean of three replications
Values in the column followed by common letters do not differ significantly at 5% level by Duncan's multiple range test (DMRT)

fields were selected and mean disease incidence was assessed from four plots in each field. Percentage of disease incidence was calculated using the following formula:

$$PDI = \frac{\text{Number of plants affected}}{\text{Total number of plants observed}} \text{ X } 100$$

Isolation and identification of pathogen

The pathogen was isolated from the infected groundnut plants showing typical symptoms of stem rot disease by tissue segment method (Rangaswami, 1958). The infected portion of the stem was cut into small bits, surface sterilized in 0.1 percent NaOH solution for 30 sec., washed in repeated changes of sterile distilled water and plated onto Potato Dextrose Agar medium in sterilized petridishes. The plates were incubated in room temperature 28±2°C for five days and were observed for the fungal growth. The fungal isolates were purified by single hyphal tip method. The purified isolates were identified as *Sclerotium rolfsii* based on morphological and colony characteristics such as mycelial growth, colony colour, mycelial dispersion, shape, colour, number of sclerotia and sclerotial arrangement on surface media.

Mass multiplication of *Sclerotium rolfsii*

A total of twenty isolates (Sr_1 to Sr_{20}) were multiplied in sand maize medium. Maize and river sand were added proportionately ratio(1:20) and thoroughly mixed with desired quantity of water, then it was transferred to open mouthed bottles (500 ml capacity saline bottles) and closed with a cotton wool plug. The bottles were sterilized at 15 lbs pressure for 2 h for 2 successive days. After sterilization, the bottles were inoculated with 9 mm size mycelial discs were taken from the seven days old culture of *Sclerotium rolfsii* and the bottles were incubated for 15 days at 28±2°C for proper mycelial growth.

Assessing the virulence of *Sclerotium rolfsii* isolates

The pot mixture was prepared by thoroughly mixing sand, clay loam soil and farm yard manure at the ratio of 1:1:1respectively. The inoculum of each isolate of *Sclerotium rolfsii* collected from different locations were separately mixed with soil in pots @ 50 g Kg-1 of soiland filled in 15×30 cm dia. earthen pots ten days before sowing. The groundnut seeds were surface sterilized with 0.1% $NaOCl_2$ solution for 30 sec. followed by two washings in sterile water. Plants in pots without inoculum served as control.Three replications were maintained and the groundnut cultivarVRI-2was used in this study. Soil moisture was sustained at 25 per cent moisture holding capacity of soil by adding sterilized water on weight basis throughout the period. After 30 days of inoculation, observe the typical wilting symptoms on the plants. Re-isolation was made from such affected portion of the plant tissue and compared with that of original isolate for conformity.

RESULTS AND DISCUSSION

Survey of groundnut stem rot from different districts of Tamil Nadu

A field survey was conducted during 2019 – 2020 in major groundnut growing districts of Tamil Nadu viz., Ariyalur, Cuddalore, Kallakurichy, Perambalur, Thanjavur,

Tiruvannamalai, Tiruchirappalli and Villupuram districts to measure the stem rot incidence of groundnut plants and the date werepresented in Table 1. The incidence was recorded on local and improved groundnut cultivars. The stem rot incidence ranged from 9.82 to 36.83 percent. Among the different locations, Kalpadai village registered maximum disease incidence of 36.83 per cent followed by Malligaipadai village reported 34.43 per cent of disease incidence and the minimum disease incidence was reported in Pattukottai village of 9.82 percent.

In India, stem rot incidence occurs in all groundnut growing states and most severe in Andhra Pradesh, Maharashtra, Gujarat, Madhya Pradesh, Karnataka, Orissa and Tamil Nadu and the yield losses range from 10 to 25% annually (Kumar *et al*., 2013). Variations observed in disease incidence and intensities of the stem rot infection occurring in the conventional groundnut growing areas of Mathur and Sivapuri areas of Cuddalore district recorded the maximum disease incidence of 34.43 % and 32.73 % respectively (Manikandan, 2018).The variation in the extent of the disease incidence might be due to the prevalence of the isolates of the pathogen differing in their virulence and environmental factors as observed in the present study.

Morphological characteristics of different *Sclerotium rolfsii* isolate (Sr_1 to Sr_{20})

Mycelial characteristics

All the twenty isolates of *Sclerotium rolfsii* pathogen grown on potato dextrose agar medium exhibited variation in their colony characteristics as fluffy white mycelium, profused cottony white mycelium, dense cottony white mycelium, dull white profused mycelium and white cottony mycelium (Table 2). Among the twenty isolates, Sr_6 showed profused cottony white mycelium with maximum mycelial growth of 90mm, whereas least mycelial growth of 76mm was observed in the isolate of Sr_{13}.

The variation in the morphological characters observed in this study mainly due to the different strains of *S. rolfsii*. Asish Mahato and Mohan Kumar Biswas, 2017 stated that isolates of *S. rolfsii* varied in their mycelia dispersion and appearance in Petri plates and showed dispersed growth all over the plate to aggregated fashion and their appearance was loose to dense cottony with sparse or fluffy mycelium.

Sclerotial characteristics:

Sclerotial characters of twenty *Sclerotium rolfsii* isolates were observed and presented in table 2. All the twenty isolates produced sclerotial bodies and its color was grouped into different categories viz., brown, dark brown, chocolate brown and light brown. The maximum sclerotial number of 330, 317, 301was obtained from isolate Sr_6, Sr_8,Sr_{14}respectively, while at least sclerotial number of 78 was observed by isolate Sr_{13}. Among the morphological characters sclerotial shape were differs from round, oval and spherical, which were arranged in scattered, central and peripheral of 90 mm petridish.

Karthik Pandi *et al*., 2017 stated that the sclerotial productions and color of eight different isolates of *S. rolfsii.* The number of sclerotia which varied from 274 to 360 sclerotia /plate. The size and color of sclerotia varied in different isolates, mostly in light brown to reddish brown at maturity. Savita ekka *et al*., 2016 reported the sclerotial characters of *S. rolfsii*, mustard seed like sclerotia produced which were deep brown or brownish black, shiny, hard and spherical and irregular in shape.

Pathogenicity of *Sclerotium rolfsii* isolates under pot culture condition

A pot culture experiment was conducted to test the virulence of isolates collected from different villages of major groundnut growing areas of Tamil Nadu. The result represented in the table 3 revealed varies levels of pathogenicity with difference in isolates. Among the twenty isolates of*S. rolfsii*, the isolate Sr_6collected from Kalpadai reported the maximum disease incidence of 42.05 per cent under artificial inoculation and was identified as virulent culture followed by Sr_8which recorded 40.51 per cent from Malligaipadai. The isolate Sr_{13} collected from Pattukottai was the least virulent culture which recorded the minimum stem rot incidence of 16.65 per cent.

Yrlania *et al*., (2015) proved virulence of *S. rolfsii* on groundnut plants. The first symptoms were tested 48 h after inoculation, which evolved to stem bottleneck and plant wilting with the presence of white mycelium under greenhouse condition. Chandra Sekhar *et al*., 2017 reported the pathogenicity reactions for all the 10 isolates *S. rolfsii*, the isolate Sr9 exhibited 100% disease incidence followed by 90.67% disease incidence in the isolate of Sr7. The lowest disease incidence of 46.33% was recorded in Sr6isolate under pot culture experiments. The disease intensity of different *S. rolfsii* isolates upon artificial inoculation exhibited the virulence nature of isolates to determine the disease intensity in their host.

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