Plant Archives
Journal home page: www.plantarchives.org
DOI Url: https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no1.053

CULTURAL, MORPHOLOGICAL AND PATHOGENICITY CHARACTERS OF *SCLEROTIUM ROLFSII* CAUSING STEM ROT IN GROUNDNUT

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(Date of Receiving-02-11-2020; Date of Acceptance-12-01-2021)

ABSTRACT

The present studies were undertaken to investigate the cultural, morphological and pathogenicity characters of *Sclerotium rolfsii*. Maximum per cent disease incidence was recorded in Nadiyappattu followed by Sivapuri, Kammapuram, Killai, Kurinjipadi, Parangipettai, Chathiram and Puthuchathiram in the decreasing order of merit of Cuddalore district, Tamil Nadu. The native isolates of S. rolfsii were isolated from the respective locations and designated as (SR1 to SR9). All the isolated showed variations with respect to colony character, mycelial growth, no of sclerotia, colour of sclerotia, shape and arrangement of sclerotia. Among the nine isolates of S. rolfsii collected from different groundnut growing areas of Cuddalore district, the isolate (SR5) collected from Nadiyappattu was found to be more virulent and recorded the maximum incidence followed by SR8 collected from Sivapuri. Maximum susceptibility was recorded when the plants are about 45 days showing disease incidence of upto 79.86 per cent.

Keywords: Groundnut, Pathogenicity, Susceptible stage, *Sclerotium rolfsii*

INTRODUCTION

Groundnut (*Arachis hypogaea* L.), also known as peanut, earth nut, wonder nut, monkey nut, goobers, is an annual leguminous plant. It is called as king of oil seed. Today groundnut is widely distributed and is cultivated in more than eighty countries in tropical and sub tropical regions of the world (Madhusudhana, 2013). Groundnut crop is affected by several fungal, bacterial and viral diseases. In India among the soil-borne fungal diseases stem rot caused by *Sclerotium rolfsii* Sacc. is a potential threat to production and is of considerable economic significance for groundnut grown under irrigated conditions. *Sclerotium rolfsii* the incitant of groundnut stem rot is a soil inhabitant, polyphagous, facultative parasite. The pathogen has a wide host range of over 500 plant species in 100 families, throughout the world (Kuldhar and Suryawanshi, 2017). The disease is more serious in tropical and sub-tropical regions where warm temp and high relative humidity exists. This fungus survives in the soil for many years by producing sclerotial bodies and causing the disease either in the form of stem rot or foot rot or root rot or collar rot in addition to leaf blight on several of its hosts (Rashmi *et al.,* 2017). The present studies were undertaken to investigate the cultural, morphological characters of *Sclerotium rolfsii.*

MATERIALS AND METHODS

Survey on the stem rot incidence of groundnut in Cuddalore district (Sivakumar *et al.,* 2016)

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

$$\text{Disease incidence } \frac{\text{Number of plants infected}}{\text{Total number of plants observed}}(\%) = \times 100$$

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

Cultural and morphological characteristics of the isolates (Reddi Kumar *et al.,* 2014)

Fifteen ml of the sterilized PDA medium was poured into sterile petri dishes and allowed to solidify. A 9 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 observation on sclerotial formation were recorded. a total of 6 morphological characters based on mycelial (mycelial growth, colony colour,) and sclerotial character (sclerotial colour, shape, number of sclerotia and their arrangement on surface of media) were recorded at 7 and 15 days of incubation.

Virulence of stem rot *S. rolfsii* isolates under pot culture condition

The sand, maize medium based inoculums were inoculated nearer to the collar region of the stem @ 5 gm pot^{-1} at 30 DAS. Inoculated plants were observed regularly and irrigated 6 to 7 days interval depends on the requirement. Typical stem rot incidence were observed and calculated

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

Disease rating	Treatment (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

Table 2. Survey of disease incidence of groundnut stem rot in different localities of Cuddalore district, Tamil Nadu

S. No	Village	Soil type	Variety	Irrigated/ Rain fed	Stem rot incidence (%)
1	Chathiram	Red sandy	JL-24	Irrigated	12.47 [f]
2	Kammapuram	Clay loam	VRI-2	Rain fed	19.74 [c]
3	Killai	Sandy loam	VRI-2	Irrigated	17.23 [d]
4	Kurinjipadi	Clay loam	JL-24	Rain fed	15.14 [e]
5	Nadiyappattu	Clay loam	VRI-2	Irrigated	24.63 [a]
6	Parangipettai	Sandy loam	JL-24	Rain fed	13.11 [f]
7	Puthuchathiram	Clay loam	Local	Rain fed	7.92 [g]
8	Sivapuri	Clay loam	Local	Irrigated	22.82 [b]
9	Vadavadi	Sandy loam	VRI-2	Rain fed	7.43 [g]

* Values in the column followed by same letters not differ significantly by DMRT(P=0.05)

Table 3. Culture variability among the isolates of S. rolfsii causing stem rot in groundnut

Mycelial characters			Sclerotial character			
Isolate number	Colony characters	Mycelial growth (mm) (5 days)	No of sclerotia (15 days)	Colour of sclerotia	Shape of sclerotia	Arrangement
SR_1	Cottony profuse mycelia	79.42[f]	153	Light brown	Oval	Peripheral
SR_2	Cottony white mycelia	85.49[c]	185	Chocolate	Round	Scattered all over plate
SR_3	Profused cottony mycelia	83.17[d]	121	Dark Brown	Spherical	Central
SR_4	Dull white profused mycelia	85.42[c]	168	Brown	Spherical	Scattered
SR_5	Light cottony white mycelia	90.00[a]	205	Brown	Round	Scattered
SR_6	Cottony white mycelia	83.43[d]	115	Dark Brown	Spherical	Peripheral
SR_7	Profuse cottony mycelia	81.38[e]	94	Brown	Round	Scattered
SR_8	Cottony white mycelia	88.72[b]	196	Chocolate	Pear	Central
SR_9	Cottony white mycelia	79.46[f]	82	Dark Brown	Oval	Scattered

* Values in the column followed by same letters not differ significantly by DMRT (P=0.05)

Table 4. Evaluation of virulence and pathogenicity of S. rolfsii isolates on groundnut (pot culture)

S. No	Isolates	Stem rot incidence (%)
1	SR_1	30.87 [f]
2	SR_2	43.71 [c]
3	SR_3	38.21 [d]
4	SR_4	43.45 [c]
5	SR_5	52.76 [a]
6	SR_6	35.42 [e]
7	SR_7	29.15 [g]
8	SR_8	48.56 [b]
9	SR_9	24.82 [h]

* Values in the column followed by same letters not differ significantly by DMRT (P=0.05)

Table 5. Identification of susceptible stages of the crop

Tr. No	Treatments	Disease incidence (%)
1	Zero stage	26.17 [e]
2	15 days old crop	69.32 [c]
3	30 days old crop	75.45 [b]
4	45 days old crop	79.86 [a]
5	60 days old crop	48.63 [d]
6	Control	0.00

* Values in the column followed by same letters not differ significantly by DMRT(P=0.05)

using formula (Kokalis-Burelle *et al.,* 1997).

$$\text{Disease incidence } \frac{\text{Number of plants infected}}{\text{Total number of plants observed}}(\%) = \times 100$$

Inoculation of *S. rolfsii* on the incidence of stem rots of groundnut (Saraswathi and Reddy, 2012)

Ten days old sand maize medium culture of *S. rolfsii* was thoroughly mixed with sterilized soil at 10 per cent . This inoculum – soil mixture was then distributed in 12 diameter earthenware pots and left undistributed for two days. After this period, one week old seedlings in seed pans were lifted carefully without causing much damage to the root system and transplanted into the pots. They were watered on alternate days and kept in an open atmosphere.

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 fifteen 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 fertilized dose applied as per recommended. After raising all the respective stages, the maize grains 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, and 60 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

$$\text{Disease incidence } \frac{\text{Number of plants infected}}{\text{Total number of plants observed}}(\%) = \times 100$$

RESULTS AND DISCUSSION

Survey of disease incidence of groundnut stem rot in different localities of Cuddalore district, Tamil Nadu

The data presented in table 2 on the roving survey conducted in groundnut growing areas of Cuddalore district of Tamil Nadu revealed the prevalence of stem rot disease in all the villages surveyed. Maximum per cent disease incidence (24.63%) was recorded in Nadiyappattu followed by Sivapuri (22.82%), Kammapuram (19.74%), Killai (17.23%), Kurinjipadi (15.14%), Parangipettai (13.11%), Chathiram (12.47%) and Puthuchathiram (7.92%) in the decreasing order of merit. The minimum stem rot incidence was recorded in Vadavadi (7.43%). The native isolates of *S. rolfsii* were isolated from the respective locations and designated as (SR_1 to SR_9).

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, the susceptibility of the host and the environmental factors in the respective areas (Hossain *et al.,* 2010). Also, the results of the present study are similar to the findings of Divya Rani *et al.,* (2016) who reported maximum disease incidence in Ramachandrapur mandal of Chittoor district and attributed growing of susceptible variety as the reason.

Culture variability among the isolates of *S. rolfsii* causing stem rot in groundnut

Groundnut showing typical stem rot symptoms were collected from nine conventional groundnut growing areas of Cuddalore district of Tamil Nadu their variability with respect to colony character, mycelial growth, no of sclerotia, colour of sclerotia, shape and arrangement of sclerotia were compared. Maximum mycelial growth was recorded by SR_5 (90.00 mm) isolate, followed by SR_8 (88.72 mm), SR_2 (85.49mm), SR_4 (85.42mm), SR_6 (83.43mm), SR_3 (83.17 mm), SR_7 (81.38 mm), SR_1 (79.42 mm) and SR_9 (79.46 mm). All the isolates of *S. rolfsii* varied in their ability to produce sclerotia on PDA medium. On the 15th day of inoculation maximum number of sclerotia (205 nos.) per nine mm culture disc was obtained from SR_5 which was also the most virulent isolate. This was followed by the isolates SR_8, SR_2, SR_4 and SR_1, producing 196, 185, 168 and 153 numbers of sclerotia, respectively. The minimum number of sclerotia of 82 was recorded by SR_9 the least virulent isolate. Different isolates of *S. rolfsii* produced different coloured sclerotia on PDA. The isolate SR_5, SR_4 and SR_7 produced brown colour, SR_2 and SR_8 produced chocolate colour, SR_3, SR_6 and SR_9 produce dark brown colour and SR_1 produced light brown coloured sclerotia (Table 3).

This variability may be due to variation in environment and biotypes, or strains of the pathogen (Cannon *et al.,* 2000). Santha Lakshmi Prasad *et al.,* (2012) identified seventeen isolates of *S. rolfsii* based on cultural variability. Rakholiya and Jadeja (2011) who studied variability of 30

isolates of *S. rolfsii* and reported considerable variability in mycelial and sclerotial dimensions. The change in colour of sclerotia might also be due to utilization or exhaustion of nutrients (Darkshanda *et al.,* 2007). Similar such colour change was reported earlier reports (Venkatesh *et al.,* 2014; Savita Ekka *et al.,* 2016).

Evaluation of virulence and pathogenicity of *S. rolfsii* isolates on groundnut (pot culture)

The result presented in table 4 revealed varied levels of pathogenicity with difference in isolates. Among the nine isolates of *S. rolfsii* collected from different groundnut growing areas of Cuddalore district, the isolate (SR_5) collected from Nadiyappattu was found to be more virulent and recorded the maximum incidence of 52.76 per cent followed by SR_8 (48.56%) collected from Sivapuri. The isolates SR_2 and SR_4 showed 43.71 per cent and 43.45% per cent of disease incidence respectively and were found to be on par with each other. The isolate SR_9 collected from Vadavadi was the least virulent which recorded the minimum 24.82% stem rot disease incidence.

Ali *et al.,* (2002) reported that virulence of pathogen differed from locality to locality with the change of temperature, humidity and rainfall. The plant which shows disease resistance or susceptibility depends on genotypes of variety. The resistance to disease should be based on knowledge of infection and pathogenicity of the fungus (Karthik Pandi *et al.,* 2017). All these earlier reports corroborated and lend support to the present findings.

Identification of susceptible stages of the crop

To find out the most 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. Maximum susceptibility was recorded when the plants are about 45 days showing disease incidence of upto 79.86 per cent whereas 30 & 15 days old plant recorded 75.45 per cent and 69.32% per cent incidence, respectively.

Similar finding was reported by Bekriwala, (2016) who reported that, groundnut plants were found most susceptible to the attack of *S. rolfsii* during 45 days of the growth and the per cent infection of the plant reduced with ageing. Similar such observation was made by several workers (Kulkarni *et al.,* 1994; Vinod, 2006; Muthukumar and Venkatesh, 2013).

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