

Standardization of inoculation techniques for resistance evaluation against stem rot in groundnut caused by *Sclerotium rolfsii* Sacc.

Sadia Ameen, M Suresh, K Vemana and G V Suneel Kumar
Department of Plant Pathology, Acharya N G Ranga Agricultural University,
Agricultural College, Bapatla-522101, Andhra Pradesh, India.

ABSTRACT

Sclerotium rolfsii Sacc. (Teleomorph: *Athelia rolfsii*) is a necrotrophic, soil-borne fungus that causes stem rot in groundnut. The pathogen survives as sclerotia in the soil for several years and causes infection by the germination of sclerotia when the conditions are congenial. Persistence of the pathogen in soil and wide host range (about 500 species) often limits the effectiveness of chemical and cultural control of stem rot disease. Developing and planting resistant germplasm has been recognized as an effective disease management strategy. To quickly identify resistance in groundnut accessions against the stem rot pathogen, it is essential to standardize the technique. Considering its significance, an experiment was carried out under greenhouse conditions using susceptible check (K6). Different inoculation methods were imposed among which modified slurry method identified and standardized in this study has proven to be efficient, resulting in highest disease incidence (99.07%). These standardized inoculation methods can be utilized in breeding programs aimed at developing stem rot-resistant groundnut cultivars.

Keywords: Groundnut, Necrotrophic Sclerotia and Stem off

Groundnut (*Arachis hypogaea* L.) is a major legume and important oil seed crop in India which is grown over an area of 52.50 lakh ha. with an annual production and productivity of 94.72 lakh and 1804 kg ha⁻¹ respectively (Anonymous, 2014). In Karnataka, it is grown to the extent of 7.25 lakh ha with 6.58 lakh production and with a productivity of 908 kg ha⁻¹ (Anonymous, 2014). The groundnut crop 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 disease. *Sclerotium rolfsii* Sacc. is a serious soil borne pathogen common in tropical and sub-tropical regions of the world where high temperature coupled with high humidity is prevalent during the rainy season causing severe damage to the crop with yield losses of over 27% (Ghewande *et al.*, 2002). Host-plant resistance offers the most economical means of controlling plant diseases but progress on transferring resistance into high yielding genotypes depends on the availability of an effective technique to identify resistant genotypes. Various groundnut germplasm for their resistance to stem rot were evaluated, but high levels of resistance have not been identified (Shew *et*

al., 1987). Earlier, several methods of inoculation have been tried to develop high levels of disease severity under greenhouse conditions (Patil and Rane, 1983; Patil *et al.*, 1977 and Shew *et al.*, 1987). But, no technique has yet produced consistently high levels of disease in repeated tests (Patil and Rane, 1983; Patil *et al.*, 1977 and Shew *et al.*, 1987). Hence, the present experiment is laid out to develop a simple greenhouse screening technique for evaluation of groundnut genotypes for stem rot resistance. With this background, the present study was aimed towards identifying and standardizing an effective technique for inoculating *Sclerotium rolfsii* the stem rot pathogen, on groundnut plants in order to screen for disease resistance and susceptibility in germplasm.

MATERIALS AND METHODS

The experiment was conducted under greenhouse conditions at Agricultural College, Bapatla (Kharif, 2024). Susceptible Kadiri-6 genotype planted in polybags was used for the experiment. The test plants were raised in poly bags filled with sterilized soil under greenhouse conditions. The experiment was conducted by using a Completely Randomized

Design, with 3 replications of each treatment. Inoculation was done at four weeks stage in the test genotype. Disease score (0-4) was taken based on visual symptoms as given vide Table 1 (Subedi *et al.*, 2020). Seven different inoculation techniques were screened as given in Table 2.

Further disease index was calculated based on Townsend heuberger formula (Tinivella *et al.*, 2009).

$$DI = \frac{[\sum (\text{number of disease scale} \times \text{corresponding number of plants})]}{(\text{total no. of plants} \times \text{maximum number of scale})} \times 100$$

Table 1. Disease rating scale used in present experiment (Subedi *et al.*, 2020)

Score	Symptoms
0	No disease symptoms (lesions)
1	Disease symptoms without visible outgrowth of the pathogen (only lesions)
2	Disease symptoms along with visible growth of the pathogen
3	Partial wilting of the plants
4	Complete wilting and plant death

RESULTS AND DISCUSSION

All the inoculation techniques consistently resulted in considerable disease incidence, and two techniques resulted in significantly high disease severity (Table 2.) Final DI was found to be highest in two treatments viz; modified slurry method (99.07%), application of pathogen grown in PD broth alone (89.62%) (Figure 1 & 2). Lowest disease severity was observed in plug method and seed coating with pathogen mycelial mat, showing 27.67% and 28.42% PDI, respectively (Table 2). The results indicate that the use of the modified slurry method may come closer to simulate the natural infection process since it involves actively growing mycelia, few active sclerotia along with some food base (the PDA slurry) without wounding the host under the conditions that are favourable for infection with optimum temperature and high humidity thus, providing optimum conditions for the disease appearance. Additionally, in comparison to other techniques like the agar disk/plug method, which is less convenient to handle, the modified slurry method which is more suitable for large inoculations and is less time-consuming. Consequently, this method

can effectively screen large groundnut populations under controlled/semi-controlled conditions within a limited time frame. Further, Host-plant resistance offers the most economical means of controlling plant diseases. Since, no absolute resistant varieties have been identified against stem rot pathogen in groundnut, due to sensitivity of the pathogen to the environmental conditions, developing a reliable method with maximum disease pressure will facilitate a robust and efficient evaluation process in developing resistant genotypes against the disease, especially under green house/ semi-controlled conditions, for further utilization under breeding programmes.

Table 2. Mean Per cent disease index of treatments followed under greenhouse conditions

S. No.	Treatment	Mean PDI (%)
1	Control	0 *(0.00)
2	Modified slurry method (inoculation of mycelial and sclerotial slurry @ 5ml/plant having 2-3 sclerotia/ml)	99.07 -84.5
3	Plug method- mycelial disc (1cm diameter) placed around the collar region	27.67 -31.75
4	Inoculation of pathogen grown PD broth @ 5ml/plant at collar region	89.62 -71.24
5	Mycelial mat applied to the soil surface	77.43 -61.67
6	Inoculated peanut shells (@ 5g/pot) spread on the soil surface in pots	75.25 -60.2
7	Applying pathogen inoculated peanut shells before sowing + pathogen grown PDB at plant base on two week old seedlings	79.76 -63.3
8	Seed coating with pathogen mycelial mat	28.42 -32.23
SEm ₊		1.57
CD(p≤0.05)		4.71
C.V(%)		4.56

*Figures in parenthesis are arc sine transformed values



Figure 1. a, Modified slurry method (T2) b. Pathogen growth on PD broth (T4)

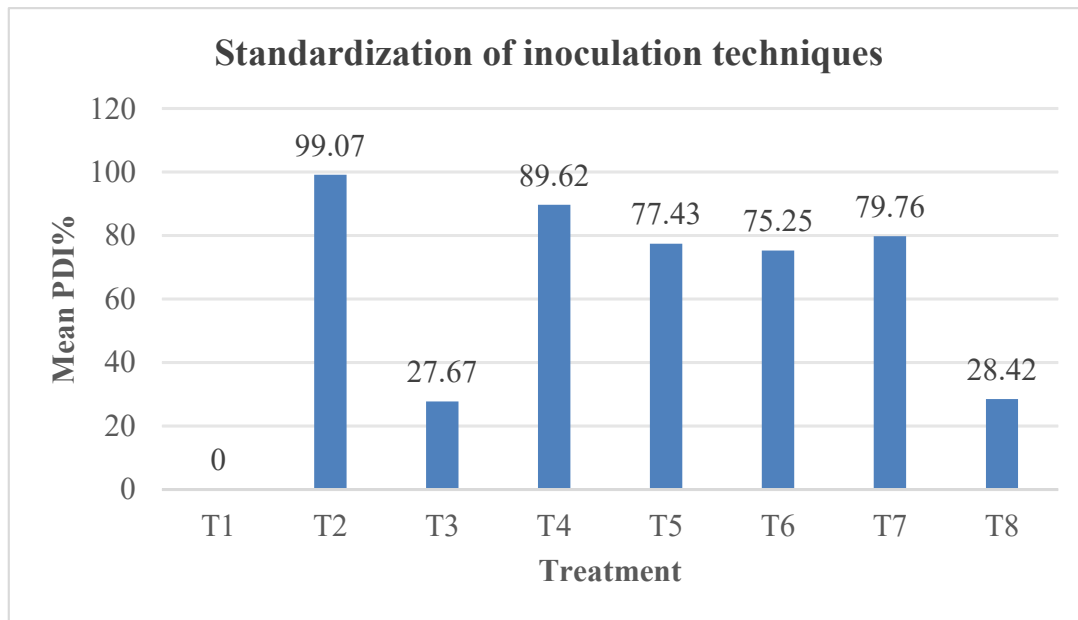


Figure 2. Disease Incidence (PDI %) for different methods of pathogen inoculation

CONCLUSION

The use of stem-rot-resistant cultivar is an effective and sustainable approach to manage the disease in groundnut, but identification of resistance sources is a challenging task, due to uneven disease pressure, and standardization of inoculation techniques for the stem rot pathogen is highly essential. The present study concluded that, inoculation using a modified slurry method can be used as a standardized technique for screening stem rot resistance germplasm/accessions for quick and extensive population screening under semi controlled/green house conditions.

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