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Standardization of inoculation technique to identify the sources of resistance against stem and pod rot of groundnut

■ Poornima*[1] **and** Gururaj Sunkad[2]

[1] Department of Plant Pathology, AICRP (Sunflower), MARS, **Raichur (Karnataka) India**
[2] Department of Plant Pathology, University of Agricultural Sciences, **Raichur (Karnataka) India**

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***Corresponding author:**
Email : poornimaagri@gmail.com

ABSTRACT

A technique was standardized to screen for resistance to stem rot (*Sclerotium rolfsii* Sacc.) in groundnut (*Arachis hypogaea* L.). A total of seven different inoculation techniques were screened by using susceptible genotype TMV-2. The techniques involved inoculation of 10 day old groundnut plants raised in pots by spreading mycelial propagules of *S. rolfsii* grown on sorghum grain medium (SGM) on soil surface, Inoculum spread on the soil surface and covered with groundnut leaf debris, Inoculum placed around the collar region, Inoculum placed around the collar region and covered with groundnut leaf debris, Inoculum mixed in the soil, Inoculum mixed in the soil and covered with groundnut leaf debris, Agar disc method. Among these techniques inoculum spread on the soil surface and covered with groundnut leaf debris was found to be most efficient in getting highest per cent incidence of stem rot (84.86%) and pod rot (70.48%).

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INTRODUCTION

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 tons 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 tons production and with a productivity of 908 kg ha^{-1} (Anonymous, 2014). The crop groundnut is affected by many diseases at different growth stages. Among these diseases stem and pod 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 per cent (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. Shew *et al*. (1987) evaluated groundnuts for resistance to stem rot, but high levels of resistance have not been identified. 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). It is, therefore, desirable to develop a simple greenhouse screening technique for evaluation of groundnuts for stem rot resistance.

MATERIAL AND METHODS

The experiment was carried out in a Randomized Complete Block Design (RCBD) with 3 replications. Seeds of susceptible variety TMV-2 were sown in plastic pots of 5 inch diameter filled with sterilized soil. Five seeds per pot were sown equi-distantly at a depth of 4 cm. The pots were watered for two consecutive days and maintained on a glasshouse at 26 ± 2^0 C and at RH 90 per cent until harvest.

Giant culture of *S. rolfsii*:

In order to get gaint culture of *S. rolfsii*, 200 g sorghum grains were soaked for 16 hr in 2 per cent sucrose solution, these sorghum grains were filled in 1000 ml conical flasks and autoclaved at 121°C for 45 min. Each flask was seeded with a mycelial plug (1cm) from a 10 day old culture of *S. rolfsii* grown on PDA, and incubated at 25 ± 1° C for 20 days.

Inoculation techniques:

Five seedlings were retained in each pot per treatment at the time of inoculation and a total of three replications were maintained for the experiment. One month old groundnut seedlings were inoculated with 20 days old inoculum by different inoculation techniques as mentioned below.

Inoculum spread on the soil surface:

The inoculum of 15 g was spread on the soil surface in the pots.

Inoculum spread on the soil surface and covered with groundnut leaf debris:

The inoculum of 15 g was spread on the soil surface and covered with 10 g of dried groundnut leaves.

Inoculum placed around the collar region:

S. rolfsii infested sorghum grains of 15 g were kept around the collar region of the plant.

Inoculum placed around the collar region and covered with groundnut leaf debris:

S. rolfsii infested sorghum grains of 15 g were kept around the collar region of the plant and covered with 10 g dried groundnut leaves.

Inoculum mixed in the soil:

The inoculum of 15 g was mixed in the soil before sowing.

Inoculum mixed in the soil and covered with groundnut leaf debris:

The inoculum of 15 g was mixed in the soil and covered with 10 g of dried groundnut leaves.

Agar disc method:

A germinating sclerotia on a 1 cm diameter agar disk (potato dextrose agar (PDA) appressed to the base of each central stem.

Control :

Untreated

The plants were observed at 30-40 days after inoculation for stem rot development. Observations on number of plants infected by stem rot were recorded.

RESULTS AND DISCUSSION

A glasshouse experiment was conducted to screen the different inoculation techniques to identify the sources of resistance (Plate 1). A total of seven different inoculation techniques were screened by using susceptible genotype TMV-2 and the data were presented in Table 1. Inoculum spread on soil surface and covered with groundnut leaf debris resulted in significantly more stem rot (84.86%) and pod rot (70.48%) infected plants than rest of the methods tested. Next highest stem rot (82.27%) and pod rot (63.49%) was recorded in inoculum spread on the soil surface technique followed by inoculum placed around the collar

region and covered with groundnut leaf debris technique recorded stem rot (57.33%) and pod rot (53.17%). However, the method where in Agar disc method was found least effective in creating disease pressure of stem and pod rot by recording 26.19 per cent and 16.98 per cent stem and pod rot, respectively (Plate 2 and 3).

Host-plant resistance offers the most economical means of controlling plant diseases, but progress on

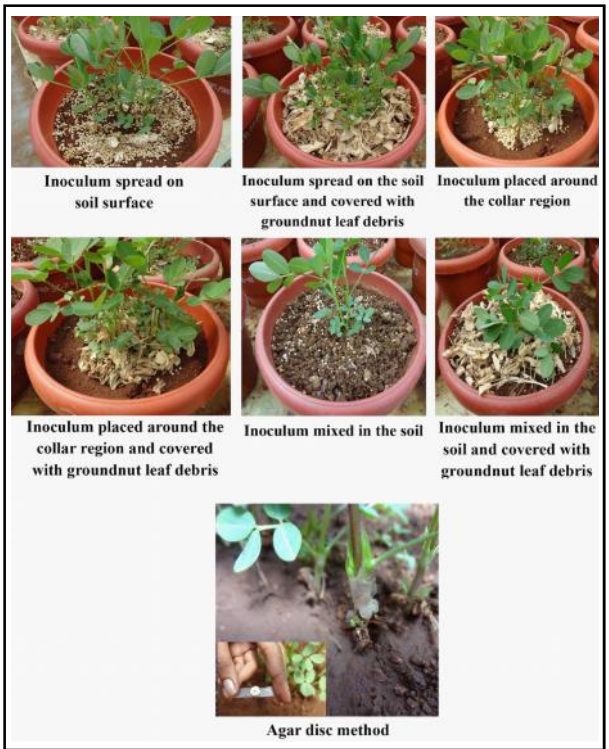

Plate 1: Standardization of inoculation techniques for *Sclerotium rolfsii*

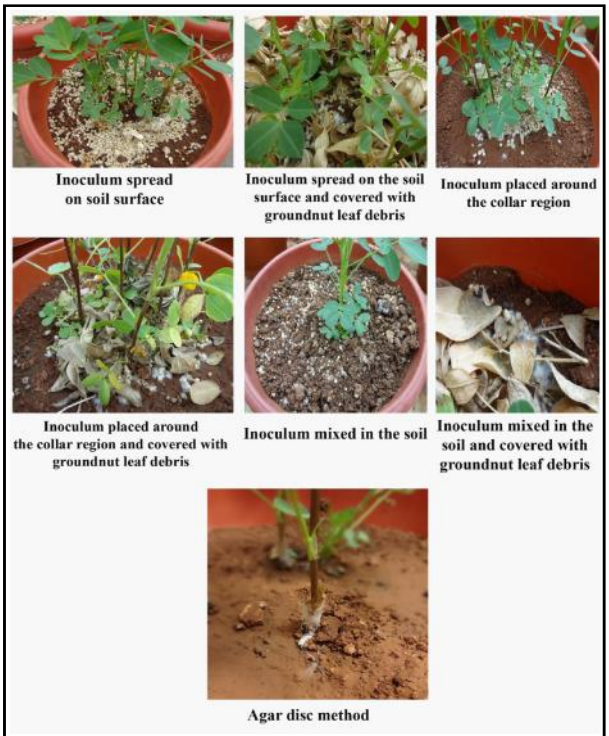

Plate 2: Development of inoculum of *Sclerotium rolfsii* at two days after inoculation

Table 1 : Standardization of inoculation technique to identify the sources of resistance against *S. rolfsii* in groundnut

Sr. No.	Treatments	Stem rot (%)	Pod rot (%)
1.	Inoculum spread on the soil surface	82.27	63.49
		(65.15)*	(52.85)
2.	Inoculum spread on the soil surface and covered with groundnut leaf debris	84.86	70.48
		(67.24)	(57.32)
3.	Inoculum placed around the collar region	48.33	50.00
		(44.03)	(45.00)
4.	Inoculum placed around the collar region and covered with groundnut leaf	57.33	53.17
	debris	(49.23)	(46.86)
5.	Inoculum mixed in the soil	32.77	42.06
		(34. 81)	(40.40)
6.	Inoculum mixed in the soil and covered with groundnut leaf debris	30.55	46.67
		(33.51)	(43.08)
7.	Agar disc method	26.19	16.98
		(30.77)	(24.29)
8.	Control	0	0
		(0.00)	(0.00)
	S.E.±	1.74	2.84
	C.D. (P=0.04)	5.29	8.62

*Figures in the paranthesis are arc sine values

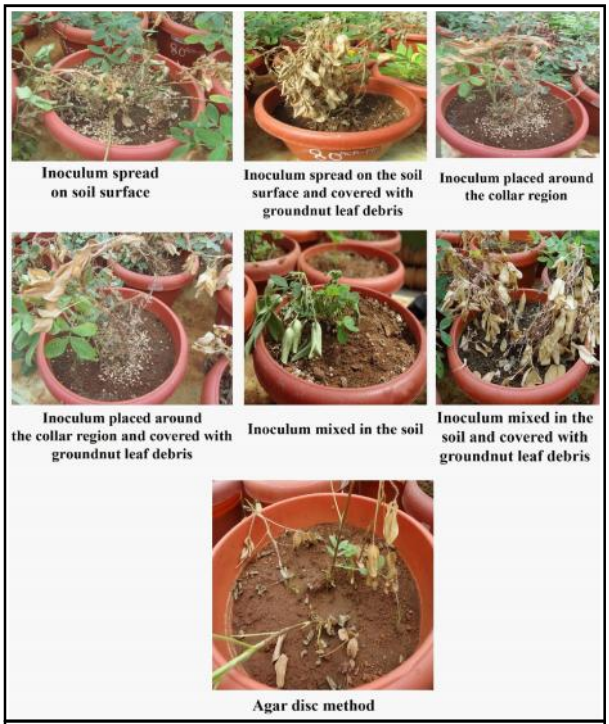

Plate 3 : Development of stem rot in different treatments for standardization of inoculation technique

transferring resistance into high yielding genotypes depends on the availability of an effective technique to identify resistant genotypes. Therefore, in the present investigation different inoculation techniques were screened to identify the source of resistance.

The results indicated that inoculum spread on the soil surface and covered with groundnut leaf debris was found to be most efficient in getting highest per cent incidence of stem rot (84.86%) and pod rot (70.48%) followed by inoculum spread on the soil surface which recorded 82.27 per cent of stem rot and pod rot (63.49%). These findings are in agreement with Shokes *et al.* (1996) and Pande *et al.* (1994). They reported that inoculum spread on the soil surface and covered with groundnut leaf debris and inoculum spread on the soil surface techniques were found to be most effective and convenient for screening of groundnut genotypes for stem and pod rot disease of groundnut. Several studies have shown that volatiles from dried and moistened plant tissue stimulate germination of sclerotia (Beute and Kabana, 1979 a and b). Mycelia from germinated sclerotia usually colonize dead or senescent plant tissue on soil surface, and bridge the distance between germinating sclerotia and the host. The results of the present study supported earlier observations (Pande *et al*., 1994 and Sennoi *et al.*, 2012) that mycelium applied to the soil surface infect the groundnut plants more effectively in the presence of organic matter.

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