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Morphological and Pathogenic Variability of *Seclerotium rolfsii* Isolates Causing Stem Rot in Groundnut

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ABSTRACT

Variability among 10 isolates of Sclerotium rolfsii collected from various soil samples and localities in Chittoor district of Andhra Pradesh. The isolates varied in colony morphology, Colony colour, Appearance, mycelial growth rate, Site of production, Number of sclerotial per plate,Time taken for sclerotial production, Colour of selerotium. Mycelial incompatibility and computability among the isolates was also seen out of 100 combinations, Pathogenicity test was conducted for all the 10 isolates and isolate S.r-9 exhibited maximum disease incidence within 6 days and proved to be more virulent

Key words: *Sclerotium rolfsii, Variability, Pathogenicity*

INTRODUCTION

One of the important elements which restricted the productivity of groundnut is biotic stress. There are several soil-borne fungal diseases affecting yield of groundnut and among them stem rot is the most serious disease which significantly affects the growth and yield and wide spread under warm temperate and humid climate. Sclerotium stem rot is caused by *Sclerotium rolfsii* Sacc., a necrotropic soil borne fungus causes diseases on wide range of agricultural and horticultural crops including groundnut. *Sclerotium* stem rot, infecting about 500 plant species worldwide, causes considerable yield losses. Though this fungus is both seed as well soil borne, but soil-borne are instrumental in disease development. The pods, which are produced below the soil surface, come in contact with the fungus causing rotting of pods. This results in lowering of yield and quality of pods. Stem rot is also known as sclerotium blight, sclerotium rot, sclerotium wilt, southern blight, southern stem rot, root rot, white mould and pod rot.

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The yield loss up to 75-80 per cent has been reported in New Mexico[4]. 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[10]. About 20-60 per cent of pod yield reduction was observed due to pod rot in widely cultivated varieties, JL 24, KRG 1, Dh 40, TMV 2 in Karnataka and Andhra Pradesh[3].

MATERIALS AND METHODS

Collection and isolation of pathogen

Roving survey was conducted in ten mandals and five villages in each mandal of Chittoor district, Andhra Pradesh. The pathogen was isolated from the groundnut plants showing typical symptoms of stem rot disease by tissue segment method on potato dextrose agar medium. Small pieces of tissue measuring about 3mm size were cut off from the infected collar region with some healthy portions. Such pieces were surface sterilized in 1.0 per cent sodium hypochlorite solution for 30 seconds followed by three washings in sterile distilled water for 30 seconds each. These pieces were further transferred to sterile discs of blotting paper. The dried bits were subsequently transferred onto sterile potato dextrose agar medium in petriplates under aseptic conditions. The petriplates were incubated at 28 $\pm$ 2^0 C were observed periodically for growth of the fungus.

Identification and maintenance of the pathogen

The pathogen was identified based on its mycelial and sclerotial characteristics through standard mycological keys[5].

Cultural and morphological variability

A study was conducted to study the variation among the ten isolates of *S. rolfsii*in terms of morphological and cultural characters like colony growth, growth rate, number of sclerotia per plate, colour and arrangement of slerotia. To carry out this experiment, 15 ml of autoclaved and cooled medium was poured in each petriplate and allowed to solidify at room temperature. Later mycelia disc of 5 mm diameter was cut using sterilized cork borer from the periphery of actively growing three days old cultures of the fungus grown on PDA and transferred aseptically to the centre of each plate. Each treatment was replicated thrice and the plates were incubated at 28$\pm2^0$C.

Mycelial Compatibility Groups (MCG) among the isolates of S. rolfsii

For grouping of ten *S. rolfsii* isolates, based on mycelial compatibility, fresh mycelial discs (5 mm diameter) were cut off from the edge of an active colony (4-5 days old), transferred to new 85×15 mm petriplates with PDA and incubated at room temperature (24-28^{0}C). Three isolates per a plate were spaced 2 to 2.5 cm apart and visually examined after 5-8 days for the presence of an aversion or a barrage zone. Pairings were marked either as incompatible when an antagonistic barrage zone was observed between two paired isolates and were put into different groups; or compatible when mycelia from two isolates intermingled without a barrage zone between them, in which case they were placed into the same group.

Pathogenic variability

Mass Multiplication of Pathogen

The pathogen was further purified and multiplied on sorghum grains to carry out pathogenicity test. Sorghum grains were pre-soaked in 2 per cent sucrose solution overnight, boiled in fresh water for 30 minutes and drained excess water. This was transferred into 1000 ml flasks @ 400 g and autoclaved. The flasks were allowed to cool at room temperature and inoculated with 5 mm culture discs of 5 days old *S. rolfsii* grown on PDA. Seven discs per flask were added and the flasks were incubated for two weeks at 28 ± 1^0C.

Pathogenic variability among the ten isolates of *S. rolfsii* was conducted in pot culture in the glass house and the pathogenic potential in terms of time taken for disease expression of each isolate of *S. rolfsii* and percent disease incidence (PDI) was recorded on groundnut variety TMV 2.

Pathogenicity Test

Soil inoculation method was followed to prove the pathogenicity of test *S. rolfsii* isolate. The

Plastic pots of 15 cm diameter each containing 3kgs of soil were used for raising the host plants. The two weeks old mass cultured of *S. rolfsii* was mixed with soil in pots @ 50 g Kg^{-1} soil. The seeds of groundnut (var. TMV 2) were sown in the pathogen inoculated soil @ 10 seeds per pot. The observations were recorded up to 15 DAI by maintaining three replications in each treatment. Per cent disease incidence was calculated by using the following formula.

$$\text{PDI} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

RESULTS

Total radial growth and growth rate of S. rolfsii isolates on PDA

There was a considerable variation among the *S. rolfsii* isolates in their total radial growth and growth rate. The maximum radial growth and growth rate recorded in the isolates S.r-1, S.r-2, S.r-3, S.r-5, S.r-6, S.r-7, S.r-8 and S.r-9 as 90.00 and 18.00mm respectively followed by isolates S.r-10 (85.55 and 17.11mm). Among all the isolates the least radial growth and growth rate was observed in S. r-4 (83.33 and 16.66mm). The average growth rate was 17.77 mm/day.

Geographical variability among *S. rolfsii* populations was demonstrated by earlier workers[9,12]. In India, Sharma *et al*[16]., studied variability among 26 isolates of *S. rolfsii* collected from various hosts/soil samples and localities.

Cultural characters of S. rolfsii isolates

Colony colour and appearance were recorded and presented in (Table - 2 & fig - 1). These results indicated that S.r-1, S.r-2, S.r-3 and S.r-9 isolates showed cottony white colour mycelium with pluffy growth at centre and upright in position. S.r-4 and S.r-10 isolates shown extra white colour with upright growth habit and pluffy aggregated. S.r-5and S.r-6 isolates observed as white in colour, cottony with sparse appearance. S.r-7 isolate was dirty white in colour with cottony dense at centre aggregated and S.r-8 isolate was light white in colour with suppressed thin strands.

Table 1: Colony characters of different isolates of *S. rolfsii* on PDA medium

S. No	Isolate	Colony Colour	Colony type (Appearance)
1	S.r-1	Cottony white	Pluffy Growth at centre, Upright
2	S.r-2	Cottony white	Pluffy Growth at centre, Upright
3	S.r-3	Cottony white	Pluffy Growth at centre, Upright
4	S.r-4	Extra White	Upright growth Habit, pluffy Aggregated
5	S.r-5	White	Cottony, sparse
6	S.r-6	White	Cottony, sparse
7	S.r-7	Dirty white	Cottony, dense at centre, aggregated
8	S.r-8	Light white	Suppressed, thin Strands
9	S.r-9	Cottony white	Upright growth habit, pluffy aggregated
10	S.r-10	Extra White	Upright growth Habit, pluffy Aggregated

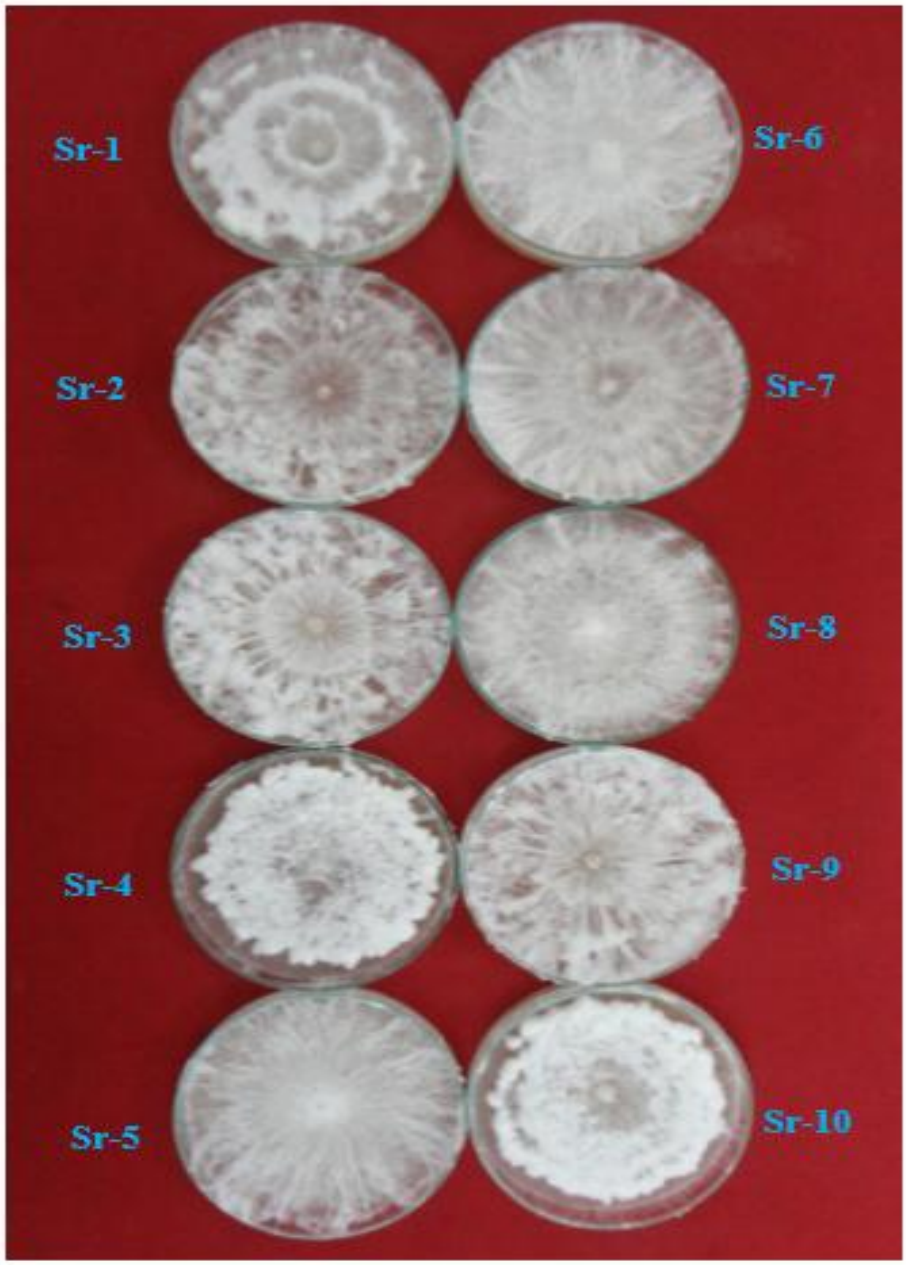

Fig. 1: Pure cultures of Sclerotium rolfsii isolates

Sclerotial characters of S. rolfsii isolates

Sclerotial characters of ten isolates of *S. rolfsii* were studied and presented in (Table 2 & fig-2) The isolates of S.r-4, S.r-9 and S.r-10 have taken maximum time of 14 days, whereas the isolates S.r-1and S.r-8 has taken only 8 days for sclerotia production. Remaining isolates took 12-13 days for sclerotial production. Most of the isolates produced reddish brown coloured sclerotia (S.r-1, S.r-2, S.r-3 and S.r-9), whereas (S.r-5, S.r-6, S.r-7 and S.r-8) isolates produced light brown sclerotia and the remaining two isolates (S.r-4 and S.r-10) produced dark brown sclerotial bodies. Among the isolates, S.r-4 produced 187 sclerotia/plate followed by S.r-10 (176/plate) and the least was observed in case of S.r-6 (47/plate). S.r-2, S.r-3, S.r-4, S.r-9 and S.r-10 produced sclerotial bodies on periphery and in case of S.r-1, S.r-5, S.r-6, S.r-7 and S.r-8 spread over the plate.

Similar reports were given by Reddi kumar *et al*[15]., and Durga Prasad *et al*[8]. Variability in cultural morphology, mycelial growth rate, sclerotium formation, sclerotial size and colour among *S. rolfsii* isolates were observed by many researchers[1,2,13].

Table 2: Sclerotial characters of different isolates of *S. rolfsii* on PDA media

S. No	Isolate	Time taken for sclerotial production (days)	Colour	Number per plate (90mm)	Site of production
1	S.r-1	8	Reddish brown	132	Spread
2	S.r-2	13	Reddish brown	95	Periphery
3	S.r-3	13	Reddish brown	75	Periphery
4	S.r-4	14	Dark brown	187	Periphery
5	S.r-5	12	Light brown	55	Spread
6	S.r-6	12	Light brown	47	Spread
7	S.r-7	13	Light brown	84	Spread
8	S.r-8	8	Light brown	92	Spread
9	S.r-9	14	Reddish brown	125	Periphery
10	S.r-10	14	Dark brown	176	Periphery

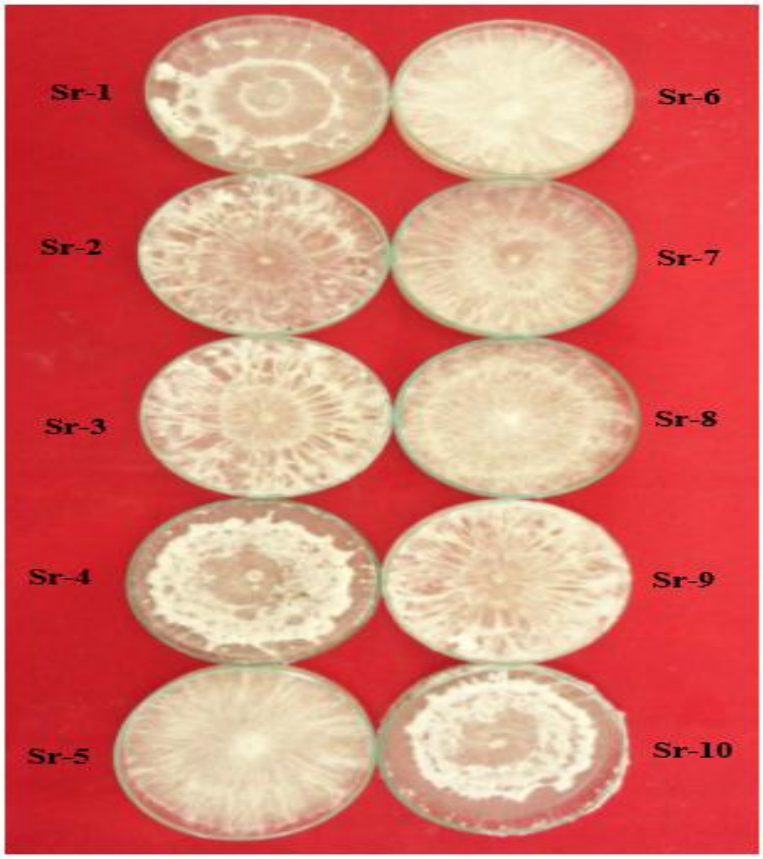

Fig. 2: Sclerotial production in different isolates of *S. rolfsii*

Mycelial compatability groups (mcg) of Sclerotium rolfsii

The Mycelial Compatibility Grouping studies were conducted among the 100 combinations of 10 isolates of *S. rolfsii* the results were showed in fig – 3 a. The observations were taken based on compatibility and incompatibility among the isolates.Based on compatibility and incompatibility in between *S. rolfsii* isolates, grouping was done and a dendrogram drawn using SPSS package (Fig-3 b).

I – Incompatibility; C – Compatibility

Isolate	S.r-1	S.r-2	S.r-3	S.r-4	S.r-5	S.r-6	S.r-7	S.r-8	S.r-9	S.r-10
S.r-1										
S.r-2	I									
S.r-3	I	I								
S.r-4	I	I	I							
S.r-5	I	I	I	I						
S.r-6	I	I	C	I	I					
S.r-7	I	I	I	I	C	I				
S.r-8	I	I	I	I	C	I	C			
S.r-9	I	C	C	I	I	C	I	I		
S.r-10	I	I	I	I	I	I	C	C	I	

Fig. 3: a .Mycelial Compatibility Grouping map

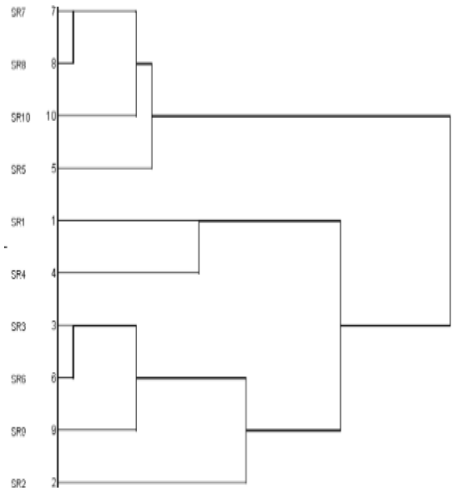

Fig. 3: b Dendrogram representing different *S. rolfsii* isolates

The prominent outcome of this analysis is that, all isolates were grouped into 4categories and designated as MCG-1 to 4. The MCG-1 included S.r-2 and S.r-9 isolates, MCG-2 includedS.r-3, S.r-6, and S.r-9, MCG-3 includedS.r-5, S.r-7 and S.r-8 and MCG-4 included S.r-7, S.r-8 and S.r-10 (fig 4a & 4b).Whereas, S.r-1and S.r-4 isolates were incompatible with all other isolates (fig 5a & 5b). Chenzhao Xie[6] studied mycelial compatibility and pathogenic diversity among S*clerotium rolfsii* isolates in South-eastern United States and reported that isolates collected from tomato were very diverse, with 31 isolates distributed into 12 MCG groups in this study, from which 29 of the isolates were collected from different tomato production areas of Florida. Nalim *et al*[11]., described 25 MCGs among 366 peanut isolates in Texas where the number of MCGs found in several surveyed fields ranged from one to five. Cilliers *et al*[7]., found that isolates from the same host plant appeared to group into different MCGs as well.

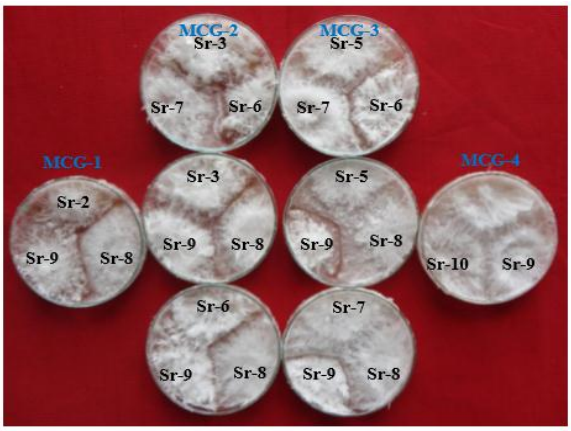

Fig. 4:a. Mycelial Compatibility between *S. rolfsii* isolates

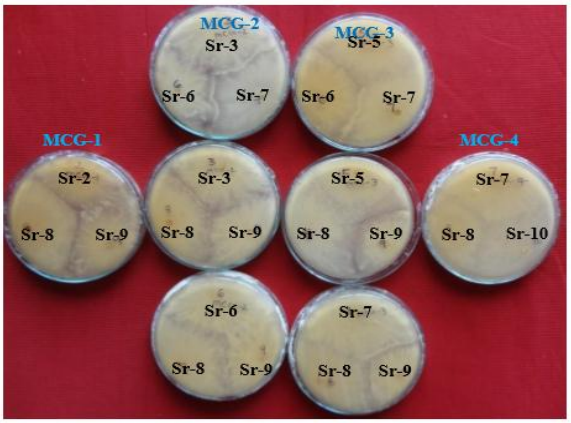

Fig. 4:b. Mycelial compatibility between *S. rolfsii* isolates (reverse)

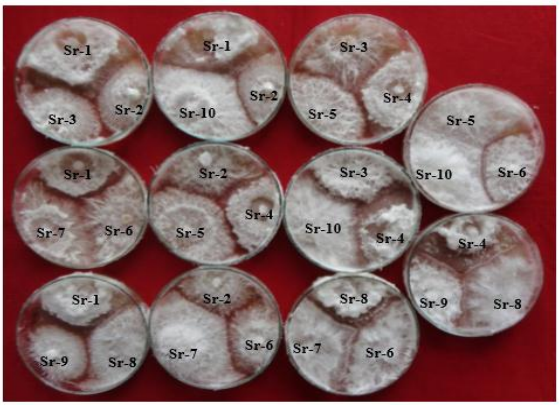

Fig. 5:a. Mycelial incompatibility between *S. rolfsii*

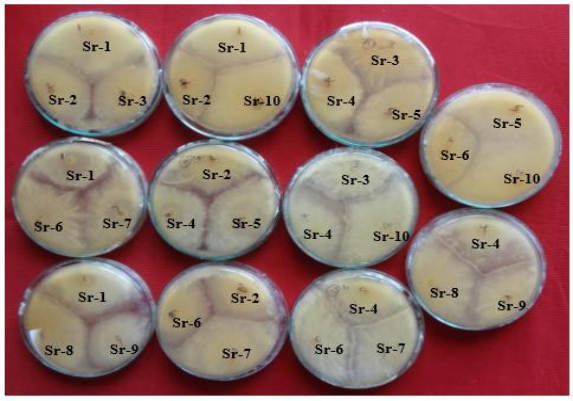

Fig. 5:b. Mycelial incompatibility between *S. rolfsii* isolates (reverse)

Pathogenic variability

The pot culture experiment was conducted to test the pathogenic potential of each isolate on groundnut variety TMV-2. Pathogen was inoculated in sorghum grain for mass multiplication and mixed with soil in pots. The seedling mortality was recorded at 15 days after pathogen inoculation and calculated the Per cent Disease Incidence (PDI).

Pathogenicity reactions were observed for all the 10 isolates tested. The pathogenicity reactions ranged from 46.33 to 100 per cent among the isolates (Table-3). The isolate S.r-9 exhibited maximum disease incidence (100%) followed by S.r-7 (90.67%). The lowest disease incidence (46.33%) was recorded in S.r-6 isolate. The isolate S.r-9 has taken only 6 days for seedling mortality whereas the isolate S.r-4 has taken 15 days (fig-6a & b)

Durga Prasad *et al*[8]., reported pathogenic variability among the twenty *Sclerotium rolfsii* isolates tested on groundnut variety TGCS888. Deva-cueva and Natural studied the pathological variations among the isolates of *S. rolfsii* from different crops. Sivaramakrishna *et al.*, assessed pathogenic variability among 15 isolates of *Sclerospora graminicola* by their disease causing potential on 12 pearl millet differential lines. Shukla and Pandey[14] showed the pathogenic variability of 10 *S. rolfsii* isolates recovered from diseased parts of *Parthenium*. The isolate Par#02 showed maximum disease incidence (80%) and the isolate Par#10 showed lowest 30% disease incidence.

Table 3: Pathogenicity of different isolates of *S. rolfsii* on groundnut (TMV-2)

S. No	Isolate	Time taken for disease expression (DAI)	Per cent disease incidence (PDI)
1	S.r-1	12	46.67(43.07)*
2	S.r-2	14	74.33(59.53)
3	S.r-3	11	64.67(53.50)
4	S.r-4	15	52.33(46.31)
5	S.r-5	9	61.33(51.53)
6	S.r-6	10	46.33(42.88)
7	S.r-7	7	90.67(72.18)
8	S.r-8	9	81.00(64.13)
9	S.r-9	6	100(90.00)
10	S.r-10	14	72.33(58.24)
11	Control		00.00(00.00)
	CD		0.669
	SE(m)		0.227
	SE(d)		0.321
	CV		0.743

* Values in parenthesis are angular transformed values

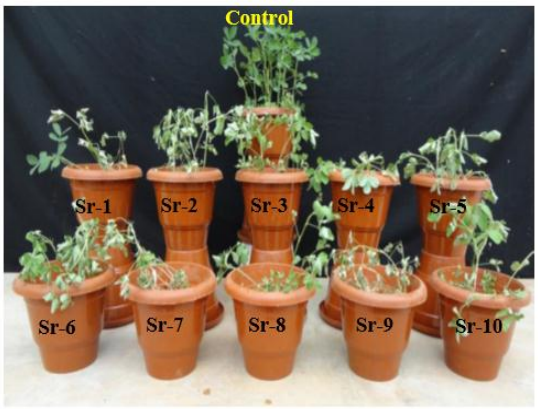

Fig. 6a. Pathogenicity of *S. rolfsii* isolates on groundnut (TMV2) at 15 days after inoculation

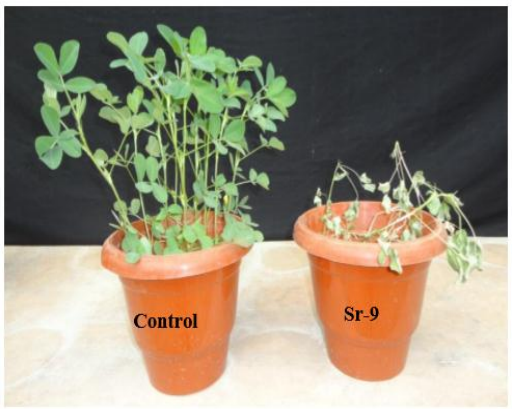

Fig. 6b. Virulent isolate S.r-9 showing wilting symptoms of groundnut plants compared with control

CONCLUSIONS

A total of ten *S. rolfsii* isolates were isolated from stem rot infected groundnut plants. The maximum radial growth and growth rate were recorded in the isolates S.r-1, S.r-2, S.r-3, S.r-5, S.r-6, S.r-7, S.r-8 and S.r-9 as 90.00 and 18.00mm respectively and least radial growth and growth rate was observed in S.r-4 (83.33 and 16.66mm). S.r-1, S.r-2, S.r-3 and S.r-9 isolates showed cottony white colour mycelium with pluffy growth at center and upright in position. S.r-4 and S.r-10 isolates shown extra white colour with upright growth habit and pluffy aggregated. S.r-5 and S.r-6 isolates observed as white in colour, cottony with sparse appearance. S.r-7 isolate was dirty white in colour with cottony dense at canter aggregated and S.r-8 isolate was light white in colour with suppressed thin strands.

Isolates of S.r-4, S.r-9 and S.r-10 have taken maximum time of 14 days, whereas the isolates S.r-1and S.r-8 have taken only 8 days for sclerotia production. Remaining isolates took 12-13 days for sclerotial production. Most of the isolates have produced reddish brown coloured sclerotia (S.r-1, S.r-2, S.r-3 and S.r-9), where as light brown colour sclerotia (S.r-5, S.r-6, S.r-7 and S.r-8). However, only 2 isolates (S.r-4 and S.r-10) produced dark brown sclerotial bodies. Among the isolates, S.r-4 produced 187 sclerotia/plate followed by S.r-10 (176/plate). S. r-2, S.r-3, S.r-4, S.r-9 and S.r-10 produced sclerotial bodies on periphery and in case of S.r-1, S.r-5, S.r-6, S.r-7 and S.r-8 spread over the plate.

Based on compatibility and incompatibility grouping, *S. rolfsii* isolates were grouped into 4 categories and designated as MCG -1 to 4. The MCG-1 included S.r-2 and S.r-9 isolates, MCG-2 included S.r-3, S.r-6, and S.r-9, MCG-3 included S.r-5, S.r-7 and S.r-8 and MCG-4 included S.r-7, S.r-8 and S.r-10. Whereas, S.r-1and S.r-4 isolates were incompatible with all other isolates.

Pathogenicity test was conducted for all the 10 isolates and isolate S.r-9 exhibited maximum disease incidence within 6 days and proved to be more virulent.

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