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MORPHOLOGICAL AND VIRULENCE VARIABILITY OF *Sclerotium rolfsii* Sacc. COLLECTED FROM GROUNDNUT GROWING AREAS OF NAGARKURNOOL DISTRICT OF TELANGANA STATE

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Groundnut [*Arachis hypogaea* L.], is an important edible oilseed crop used extensively for oil extraction, cooking, and domestic purposes. The groundnut seed is valued for its oil (35-56 %) and protein (25-30 %) which contributes significantly to the nutritional requirements of human and animals (Gulluoglu *et al*., 2016). It also provides carbohydrates (9.5-19 %), lipids, minerals (phosphorous, calcium, magnesium and potassium) and vitamin (E, K and B group) content and it (Gulluoglu *et al*., 2016). With a total production of 53.64 million metric tonnes and productivity of 1699.1 kg ha^{-1} groundnut crop is grown in an area of 31.57 million ha land globally (FAOSTAT, 2022). With an area of 6.02 million ha, a production of 10.24 million metric tonnes, and a productivity of 1703 kg ha^{-1}, India is the second-largest groundnut producer after China (INDIASTAT, 2022). In India, Gujarat (2.16 million ha) has the most area under groundnut followed by Andhra Pradesh, Rajasthan, Karnataka, Tamil Nadu, and Maharashtra. In Telangana state, groundnut is predominantly cultivated as a *Rabi* crop. The major groundnut growing regions in the state are Nagarkurnool, Wanaparthy, Mahabubnagar, Gadwal, Mahabubabad, Vikarabad, Suryapet, and Nalgonda. Despite being a crop that can be grown throughout the year, diseases including seedling rot, leaf spots, rust, stem rot, pod rot, and bud necrosis hamper groundnut production (Joshi *et al.*, 2020; Hawaldar *et al.,* 2022). Among the various diseases affecting groundnut, stem rot caused by *Sclerotium rolfsii* Sacc. is a major threat to successful groundnut cultivation and can cause heavy yield losses.

S. rolfsii is a ubiquitous, polyphagous, soil-borne pathogen and is reported on different species from various countries. Host range of *S. rolfsii* includes the majority of agricultural and horticultural crops with legumes, crucifers and cucurbits being the most common hosts (Punja, 1985; Wokocha *et al.,* 1986; Wydra, 1996; Okabe *et al*., 1998). In India, stem rot was first recorded by Butler and Bisby (1931). The disease is severe in Maharashtra, Gujarat, Madhya Pradesh, Andhra Pradesh, Telangana, Orissa and Tamil Nadu (Krishnakanth *et al.,* 1999). The pathogen often infects irrigated groundnut crops grown in India during the post-rainy and summer seasons (Kumar *et al.,* 2013). After the main crop, the fungus can persist for several days in the soil or infected plant residues as sclerotia or mycelium which can germinate and infect the the collar region producing typical silky white mycelium. Eventually, the pathogen will invade plant tissues and cause yellowing, wilting, and plant death instilling more economic loss to growers (Hawaldar *et al.,* 2022). During stem rot outbreaks, disease incidence can reach up to 80% especially when wet climatic conditions prevail at pod filling (Akgul *et al.,* 2011). Considerable variability in the cultural and sclerotial characteristics and variability in virulence have been reported in various studies (Punja, 1985; Le *et al.,* 2011; Thilagavathi *et al.,* 2013; Kumar *et al.,* 2014; Eslami *et al.,* 2015; Akram *et al.,* 2015). The present study attempted to study morphological and virulence variability of isolates of *Sclerotium rolfsii* Sacc. collected from groundnut growing areas of Nagarkurnool district of Telangana state.

A roving survey was conducted in groundnut growing areas of Nagarkurnool district of Telangana state during *Rabi* 2019-20 for the collection of stem rot infected plant samples. The external signs and symptoms such as the presence of white mycelial growth, sclerotia, lesion on the stem, wilting, drying, or dead plants were used to determine the incidence of the stem rot disease in the fields surveyed. Five plots were selected such that one plot was in the center of the field and the rest were randomly placed on the four corners leaving 1 m from the border. The total number of plants and number of stem rot infected plants were counted in each plot and per cent disease incidence was calculated by the following formula:

$$\text{Per cent disease incidence(PDI)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

The samples were collected from 12 fields in ten villages covering six mandals of Nagarkurnool district *viz.,* Bijinapalle, Tadoor, Kalwakurthy, Nagarkurnool,

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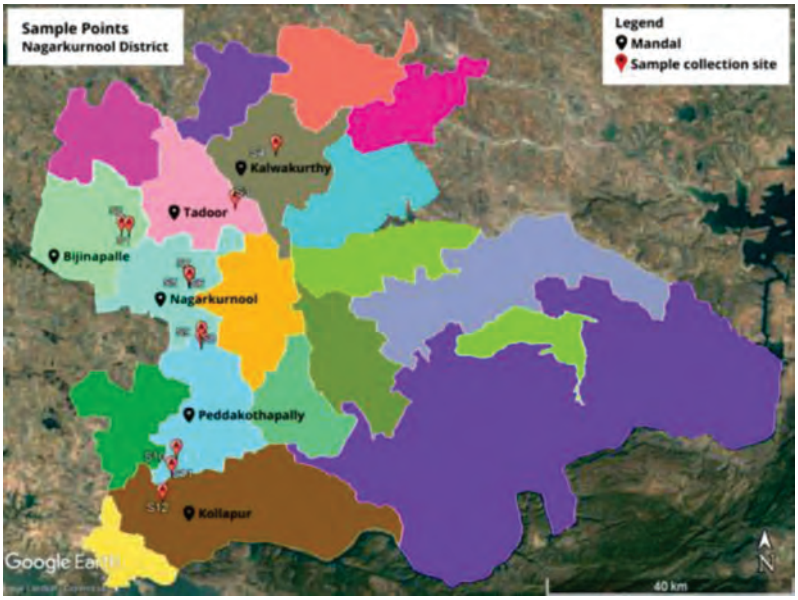

Fig. 1. Map showing sample collection sites in groundnut growing regions of Nagarkurnool district of Telangana during *Rabi* 2019-20

Fig. 2. Stem rot infected groundnut plants and sclerotial bodies observed during sample collection in groundnut growing fields of Nagarkurnool district of Telangana

Peddakothapally and Kollapur (Fig. 1).

Majority of farmers opted for only one season crop of groundnut and the fields remained fallow during the previous season after taking up groundnut. Solo cropping of groundnut variety Kadiri-6 was followed by majority of the farmers. Stage of groundnut crop in the fields surveyed wasd in flowering phase ranged between 30 to 50 days. The disease incidence of stem rot in the survey fields ranged between 7.84 and 20.63 per cent with a mean of 15.05 per cent (Table 1). The field in Deshitkyal village of Nagarkurnool mandal recorded the highest incidence of stem rot disease with 20.63 per cent followed by Adirala village (18.87 per cent) of Peddakothapally mandal and Choutabetla village (18.87 per cent) of Kollapur mandal while the lowest disease incidence of 7.84 per cent was observed in Vanapatla village of Nagarkurnool mandal (Table 1).

The pathogen *S. rolfsii* was isolated from the collar region of the infected groundnut plants with white mycelial growth at the base of the stem on potato dextrose agar (PDA) medium by tissue segment method (Rangaswami and Mahadevan, 1999). Briefly, using a sterile scalpel, small tissue bits around 0.5 - 1 cm from the infected collar region with some healthy tissue were cut. These tissue bits were surface sterilized with 1 per cent sodium hypochlorite solution for 30 seconds followed by three washes in sterile distilled water to remove excess sodium hypochlorite. They were placed in plates with solidified PDA and the plates were then incubated at 25 ± 2°C and observed periodically for growth of the fungus. Axenic culture of the pathogen was obtained by single hyphal tip method

Table 1. Data collected during sample collection from groundnut growing fields of Nagarkurnool district during *Rabi* 2019-20.

Name of the mandal and village		Field location		Variety grown	Stage of the crop (DAS)	PDI of *Sclerotium* stem rot (%)
Mandal	Village	Latitude	Longitude			
Bijinapalle	Palem	16.51448	78.25046	Kadiri-6	50	16.95
	Palem	16.51645	78.23946	Kadiri-6	50	17.46
Tadoor	Polmur	16.55449	78.41018	Kadiri-6	45	12.31
Kalwakurthy	Tharnikal	16.63257	78.47112	Kadiri-6	50	16.67
Nagarkurnool	Vanapatla	16.44346	78.34063	Kadiri-6	30	7.84
	Vanapatla	16.44224	78.34194	Kadiri-6	45	11.48
	Deshitkyal	16.44037	78.34143	Kadiri-6	45	20.63
Peddakothapally	Adirala	16.36383	78.35995	Kadiri-6	45	18.87
	Chandrakal	16.35254	78.35992	Kadiri-6	45	15.09
	Chennapuraopally	16.19221	78.32291	Kadiri-6	30	9.26
Kollapur	Ankiraopally	16.16729	78.31594	Kadiri-6	45	15.25
	Choutabetla	16.12870	78.30240	Kadiri-6	45	18.87

PDI-Per cent disease incidence DAS- Days after sowing

and maintained on PDA throughout the investigation. The pathogen was identified as *S. rolfsii* based on its mycelial and sclerotial characters (Barnett and Hunter, 1972). A total of 7 pure cultures of *S. rolfsii* were obtained from the collected infected plant samples and these were considered for studying morphological and virulence variability (Table 2; Fig. 3).

Table 2. List of isolates of *S. rolfsii* obtained.

Isolate ID	Location	
	Mandal	Village
GNS1	Bijinapalle	Palem
GNS2		Palem
GNS3	Nagarkurnool	Vanapatla
GNS4	Peddakothapally	Adirala
GNS5		Chandrakal
GNS6		Chennapuraopally
GNS7	Kollapur	Ankiraopally

For morphological characterization, pathogen isolates were inoculated in sterile PDA plates and incubated at 25 ± 2°C. The time taken by mycelial growth to cover the entire plate was recorded for each isolate of *S. rolfsii*. Further, colony characters such as colony colour, colony type and growth type along with sclerotial characters such as the time required for production and maturation of sclerotia, number of sclerotia produced per plate, arrangement of sclerotia produced in plate, sclerotial colour, sclerotial weight per 100 sclerotia and sclerotial size were recorded for each isolate of *S. rolfsii*.

All the isolates of *S. rolfsii* produced white cottony mycelial growth on PDA medium except isolate GNS7, which produced a dull white colony (Table 3). Characteristic profuse to highly profuse colony growth was observed in all isolates of *S. rolfsii*. The colonies of the isolates GNS1, GNS4 and GNS6 showed highly profuse growth whereas the isolates GNS2, GNS3, GNS5 and GNS7 showed profuse growth. It was also observed that the isolates which produced profuse growth appeared flat except for isolate GNS5 which appeared raised at ends. The colony of the isolates GNS1, GNS4 and GNS6 which produced highly profuse growth were raised at the ends. The average time for the mycelial growth to cover the entire plate differed significantly and ranged from 3.33 (isolate GNS7) to 6.67 days (isolate GNS3). The mycelial growth of the isolate GNS7 of *S. rolfsii* took only 3.33 days to cover the entire plate and was on par with isolate GNS2 which took 3.67 days while the isolate GNS3 took a maximum of 6.67 days to cover the entire plate followed by isolate GNS4 which took 5.67 days. The isolates GNS5 and GNS6 took a time of 5.33 days to cover the entire plate (Table 3).

Kumar *et al.* (2014) reported two *S. rolfsii* isolates ISR-1 and ISR-2, which covered entire petriplate within 96 hours of incubation as fast growing isolates. In the current study, isolate GNS7 covered the entire plate in a lesser time of 80 hours (3.33 days) and can be considered as fast growing isolate.

The isolates showed significant variability in sclerotial characteristics on PDA medium. The average time required to form sclerotia by seven isolates of *S. rolfsii* differed significantly and ranged from 6.33 days (isolate GNS7) to 16.67 days (isolate GNS1). Similarly,

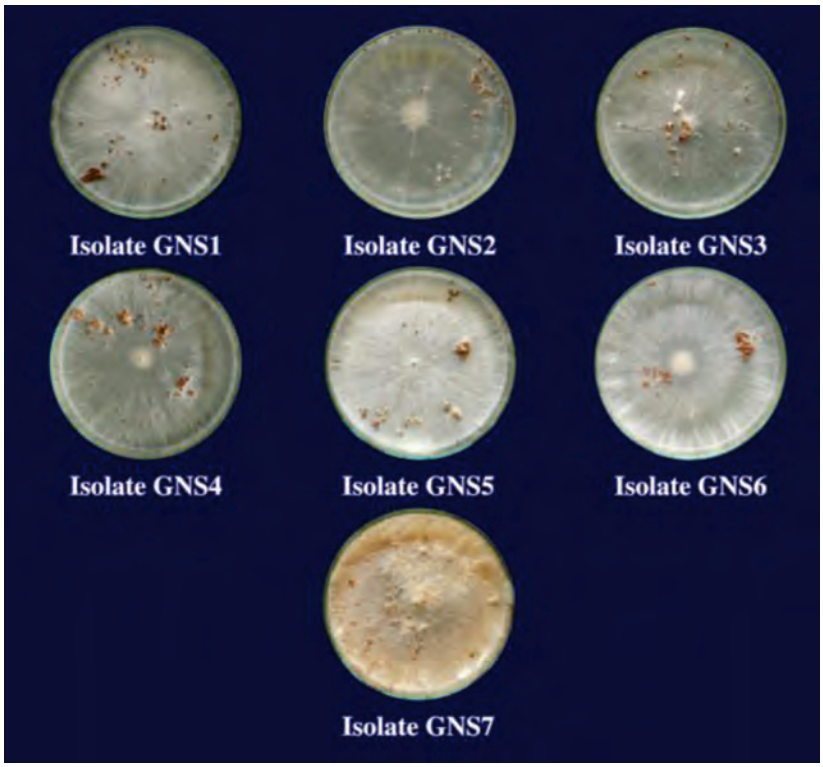

Fig. 3. Pure cultures of the isolates of *S. rolfsii* isolated from stem rot infected groundnut plant samples

Table 3. Cultural characteristics of different isolates of *S. rolfsii* on potato dextrose agar medium

Isolate ID	Colour	Colony type	Growth type	Time to cover entire plate (Days)
GNS1	White	Raised at ends	Highly profuse	4.67[b]
GNS2	White	Flat	Profuse	3.67[a]
GNS3	White	Flat	Profuse	6.67[d]
GNS4	White	Flat	Highly profuse	5.67[c]
GNS5	White	Raised at ends	Profuse	5.33[c]
GNS6	White	Raised at ends	Highly profuse	5.33[c]
GNS7	Dull White	Flat	Profuse	3.33[a]

Values expressed are mean of three replications; Same letters indicates insignificant difference at 5 per cent signifcance level.

the average time required for sclerotial maturation by the isolates ranged from 11.67 days (isolate GNS7) to 20.33 days (isolate GNS1). Initially, white colored sclerotia were formed which later changed to light brown to dark brown colour on maturity. The sclerotia were scattered in the entire plate in all the isolates of *S. rolfsii* except in isolate GNS2 which was characterized by a peripheral arrangement of sclerotia. The sclerotia were spherical to subspherical in shape. The average number of sclerotia produced by isolates of *S. rolfsii* ranged from 39.00 (isolate GNS5) to 138.67 (isolate GNS6). The average size of sclerotia produced by the isolates ranged from 1.14 mm (isolate GNS2) to 1.73 mm (isolate GNS1). The 100 sclerotial weight of the isolates of *S. rolfsii* ranged from 0.027 g (isolate GNS5) to 0.187 g (isolate GNS3) (Table 4; Fig. 3).

Similar observations in colony and sclerotia characteristics of isolates of *S. rolfsii* were reported by Le *et al.* (2011), Thilagavathi *et al.* (2013), Kumar *et al.* (2014), Akram *et al.* (2015) among others. Le *et al.* (2011) reported significant morphological variations among 103 isolates of *S. rolfsii* studied for days to produce sclerotia, days for maturation, sclerotia number and size. Likewise, Thilagavathi *et al.* (2013) reported the considerable variability among the isolates of *S. rolfsii* collected from Tamil Nadu concerning sclerotia number,

Table 4. Sclerotial characteristics of different isolates of *S. rolfsii* on potato dextrose agar medium

Isolate ID	Time required to (Days)		Colour	Arrangement	Number/ Plate	Size (mm)	Weight (g/100 sclerotia)
	Produce	Mature					
GNS1	16.67^{f}	20.33^{e}	Dark brown	Scattered	107.33^{c}	1.73^{c}	0.035^{b}
GNS2	8.33^{b}	13.00^{b}	Dark brown	Peripheral	117.67^{d}	1.14^{a}	0.047^{c}
GNS3	11.33^{c}	14.67^{c}	Dark brown	Scattered	49.00^{b}	1.46^{b}	0.187^{g}
GNS4	12.67^{d}	16.33^{d}	Light brown	Scattered	104.33^{c}	1.45^{b}	0.131^{f}
GNS5	9.00^{b}	12.00^{a}	Dark brown	Scattered	39.00^{a}	1.23^{a}	0.094^{e}
GNS6	15.33^{e}	17.00^{d}	Dark brown	Scattered	138.67^{e}	1.50^{b}	0.069^{d}
GNS7	6.33^{a}	11.67^{a}	Brown	Scattered	110.33^{cd}	1.18^{a}	0.027^{a}

Values expressed are mean of three replications; Same letters indicates insignificant difference at 5 per cent signifance level.

100 sclerotia weight and sclerotial colour ranging from dark brown to light brown. Similarly, Kumar *et al.* (2014) reported morphological variations among isolates of *S. rolfsii* collected from groundnut growing areas of Andhra Pradesh concerning to presence or absence of sclerotia, sclerotial colour, number, sclerotial weight and arrangement of sclerotia produced. Akram *et al.* (2015) studied variability among isolates of *S. rolfsii* collected from various parts of India and reported that the isolates varied in colony morphology, mycelial growth rate, sclerotium formation, sclerotial size and colour.

Further, pathogenicity tests for each of the seven S. rolfsii isolates were carried out in the pot culture experiment using the susceptible groundnut variety Kadiri-6 to establish the virulence variability among them (Fig. 4). Pot covers of size 20 x 25 cm were filled with sterilized pot mixture (soil, sand and vermicompost in 2:1:1 ratio). Seeds were surface sterilized with 0.1 per cent sodium hypochlorite and fives seeds were sown per pot. Finally, three seedlings were maintained per pot. *S. rolfsii* was mass multiplied on sterilized sorghum grains pre-soaked overnight in 2 per cent sucrose solution (Upadhyay and Mukhopadhyay, 1986). Briefly, sorghum seeds were pre-soaked in 2 per cent sucrose solution overnight and autoclaved in conical flasks twice at 15 *psi* pressure (121.6°C) for 20 minutes. Each

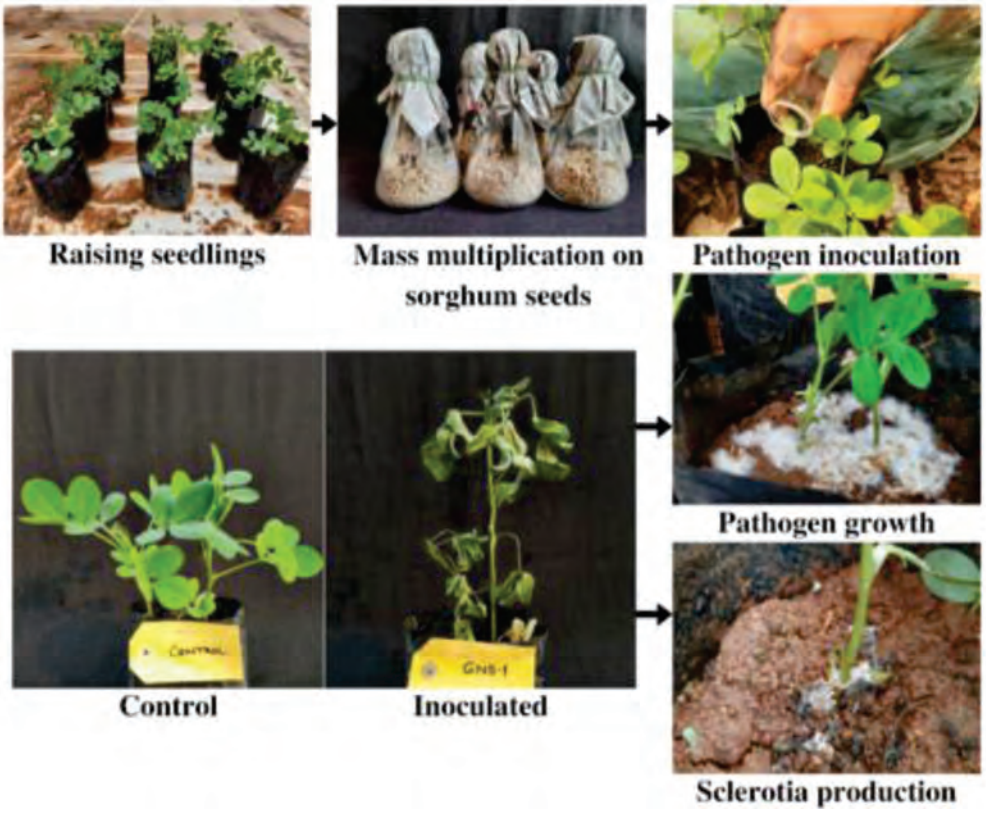

Fig. 4. Pathogenicity test of the isolates of *S. rolfsii* on groundnut variety Kadiri-6

Table 5. Incubation period and days to permanent wilting of the isolates of *S. rolfsii* on groundnut variety Kadiri-6 (K6)

Isolate ID	Incubation period (dpi)*	Days to permanent wilting (dpi)
GNS1	4.25[a]	12.25[a]
GNS2	5.25[c]	15.00[b]
GNS3	4.75[b]	14.75[b]
GNS4	5.25[c]	14.50[b]
GNS5	6.75[d]	18.25[d]
GNS6	5.25[c]	16.75[c]
GNS7	4.75[b]	21.25[e]

Values expressed are mean of four replications; Same letters indicates insignificant difference at 5 per cent signifance level; *dpi-days post inoculation.

flask was then seeded with a 1 cm mycelial disc from 5 days old actively growing culture of individual isolates of *S. rolfsii* and incubated at 25 ± 1°C for 20 days. Thirty days old seedlings were inoculated with 25 g inoculum per pot by spreading the inoculum on the surface of the soil. Simultaneously, an untreated control without the addition of inoculum was also maintained. Observations on incubation period (IP) and days to permanent wilting (DPW) were made from the next day after inoculation, observations on. The pathogen was re-isolated from symptomatic plant tissues and compared to the original isolate for conformity.

All the seven isolates showed a cent per cent disease incidence however they differed with respect to IP and DPW (Table 5). The average IP ranged from 4.25 (isolate GNS1) to 6.75 days (isolate GNS5) and differed significantly among the isolates tested. Similarly, the average period to permanent wilting ranged from 12.25 (isolate GNS1) to 21.25 days (isolate GNS7). The lowest time taken for incubation and permanent wilting by isolate GNS1 in pathogenicity test suggested that it was the most virulent isolate among all the seven isolates tested.

The results are in line with the findings of Le *et al.* (2011) who proved the pathogenicity of *S. rolfsii* isolates obtained from groundnut, tomato, and taro on groundnut and reported variation in their virulence levels. Similarly, Eslami *et al.* (2015) tested the virulence of 78 isolates of *S. rolfsii* on groundnut genotypes. They observed a positive correlation between virulence and per cent disease incidence and per cent stem area affected among the tested isolates. Based on the reaction with the groundnut genotypes, the 78 isolates of *S. rolfsii* were categorised as most virulent and less virulent.

Data obtained from the experiments were statistically analysed by one-way analysis of variance (ANOVA), and means were separated ($p \leq 0.05$) by Fisher's least significant difference (LSD) using statistical analysis software GRAPES 1.0.0, Kerala Agricultural University, Kerala, India (Gopinath *et al.,* 2021).

To conclude, stem rot infected groundnut plant samples were collected from 12 different fields in ten villages covering six mandals of Nagarkurnool district of Telangana state during *Rabi* 2019-20. The mean stem rot incidence in the groundnut fields surveyed was 15.05 per cent and it ranged between 7.84 and 20.63 percent. Seven isolates of *S. rolfsii* were obtained which were studied for their cultural and scleriotial characteristics. Considerable variability in colony morphology and sclerotial morphology was shown by the tested isolates of *S. rolfsii*. Further, pathogenicity test on susceptible groundnut variety Kadiri-6 identified *S. rolfsii* isolate GNS1 as the most virulent isolate with the lowest mean values for IP (4.25 days) and DPW (12.25 days). Further studies on molecular aspects of these isolates of *S. rolfsii* might offer more insight into the variability and diversity among them.

Authors' contribution

Conceptualization of research work and designing of experiments (AUA, VR, GU, ST, SNCVLP); Execution of field/lab experiments and data collection (AUA); Analysis of data and interpretation (AUA); Preparation of manuscript (AUA, VR).

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