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Identifying the susceptibility stage of groundnut plant to stem rot disease

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Abstract
Groundnut stem rot is caused by *Sclerotium rolfsii*, a soil-dwelling, polyphagous, facultative parasite. Symptoms of stem rot caused by *S. rolfsii* on groundnut plants included the formation of a deep brown lesion on the plant's stem near the ground level, followed by yellowing of groundnut leaves, then loss of vigor and premature death. Current research was done under pot experiments to describe how the age of groundnut plants affected their susceptibility to *Sclerotium rolfsii*. Groundnut plants were inoculated with actively mycelium and sclerotia mass multiplied on sorghum grains at 0, 15, 30, 45, and 60 days after sowing (DAS). Results showed that *S. rolfsii* infects groundnut plants at every stage of their growth, from seed germination throughout the harvesting. Maximum susceptibility was recorded in 30 days old inoculated plants showing significantly highest stem rot disease incidence 91.67% which provide evidence that young stages of maturity are more susceptible to the *S. rolfsii* and the severity of infection was more and rapid. Susceptibility of groundnut seedlings to *S. rolfsii* decreases as the age of the groundnut plants increases.

Keywords: *Sclerotium rolfsii*, groundnut, stem rot, susceptibility

Introduction
Groundnut (*Arachis hypogaea* L.) is a major legume and an important oil seed crop in India, accounting for roughly half of all oilseed production. It is also known as peanut, goober (US), pindar (US), or monkey nut (UK). Groundnut is grown across the world; in India it is cultivated both in *Kharif* and *Rabi* seasons. India is the second largest producer of groundnut after China. It is widely grown in the tropics and subtropics, with both small and major commercial growers relying on it. Groundnut is a member of the Fabaceae (or Leguminosae) plant family, also known as the legume, bean, or pea family. Groundnut is grown for both commercial and subsistence purposes. In commercial cultivation, it is primarily grown as a single crop with heavy inputs, however in subsistence farming, it is planted as a mixed or intercropping crop. They grow best in light, sandy loam soil with a pH between 5.9 and 7.

Low production of groundnut is caused by a variety of reasons, including diseases such as leaf spot, collar rot, stem rot, bud necrosis, and others. *Sclerotium rolfsii* Sacc. causes stem rot, which is a severe problem and an economically significant soilborne disease. Stem rot reduces pod yields by 10% to 25%, but under severe disease conditions, yield losses might reach up to 80%. (Rodriguez Kabana *et al*., 1975) [12]. *Sclerotium rolfsii* is a soil-borne necrotrophic fungal plant disease that produces a lot of white mycelium on infected plants and in culture. Rolfs (1892) was the first to report *Sclerotium rolfsii*, and Saccardo (1911) later termed the disease *Sclerotium rolfsii.* Groundnut plants that are infected by *S. rolfsii* causes stem rot, sclerotial wilt and stem and pod rot on more than 500 plant species including practically all the agricultural and horticultural crops (Domsch *et al*., 1980; Farr *et al.*, 1989) [5, 6]. Stem rot has been found in areas where moisture and temperature levels are high enough for *S. rolfsii* to grow and survive. In India, stem rot, commonly known as white mold or southern blight, is a deadly soil-borne disease. *S. rolfsii* infected groundnut plants during all stages of growth, including the seed germination stage, causing pre-emergence rot and young plant stem rot. The time it took for the plants to wilt ranged from 8 to 15 days. Younger plants were found to be more susceptible to infection because it was more widespread and quicker (Patil MB and Rane MS., 1983) [11].

Symptomology
Under field conditions, the symptoms of stem rot caused *by S. rolfsii* on groundnut plants included the formation of a deep brown lesion on the plant's stem near the ground level,

followed by yellowing of groundnut leaves, then loss of vigour and premature death. The plant that was infected exhibited rotting of the stem region and poor root development. The lesion was soon covered by a cottony white mycelium with the rotting underneath it. The mycelial mat can extend several centimeters above the soil line, up to the stem. Numerous tan to brown about mustard seed size, spherical sclerotial bodies attached around the infected stem region in later stages of infection (Aken and Dashiell, 1991) [1]. The plant died more quickly under dry conditions, when necrosis showed instead of browning. Light brown lesions appeared on young pods, and mycelium and sclerotia formed even inside the pods. At advanced stages of plant growth, the kernels were also infected and become small and shriveled in size (Akram *et al*., 2008) [2]. The cortical deterioration of the stem base at ground level was noticed by Mehrotra and Aneja (1990) [9], as was the emergence of visible white mycelium that spread into the soil and on organic debris. Beatle (1954) [3] also observed similar kind of symptoms on groundnut infected plants.

Materials and Methods

Groundnut stems and pods with characteristic stem rot (*S. rolfsii*) disease symptoms were gathered in paper bags from the groundnut farms. The sick samples were taken to the lab and tissue isolation on Potato dextrose agar medium was performed aseptically. A total of 10 isolates of *Sclerotium rolfsii* were collected each from 10 different districts of southern Odisha and northern regions of Andhra Pradesh. These collected samples were isolated and purified.

Isolation and maintenance of the pathogen (*Sclerotium rolfsii*)

Groundnut plants showing typical symptoms of stem rot were used for isolation of *S. rolfsii*. The adhering dirt particles and other debris were removed from the infected stem region of each diseased sample by thorough washing under running tap water. Following that, infected stem sections were chopped into 1 cm pieces and surface sterilised by soaking them in 0.1 percent mercuric chloride for 30 seconds. To remove residues of mercuric chloride, the stem parts were washed three times in sterile distilled water and blotted dry on clean, sterile tissue papers. Later, these bits were aseptically transferred to the petriplates containing PDA medium inside laminar air flow chamber and incubated at 27 ± 2 °C for 3 to 4 days. With the use of a sterile needle, mycelium from sick stem pieces was transplanted directly onto the medium. Periodical transfers were used to keep pure cultures of the pathogen *S. rolfsii* on PDA.

Proving the pathogenicity

Mass multiplication of *S. rolfsii*

Sorghum grain medium was employed to achieve enormous multiplication of the inoculum. 200 g sorghum grains were steeped in a 2 percent sucrose solution for 16 hours before being poured into 1000 ml conical flasks and autoclaved for 45 minutes at 121 °C. Each flask was seeded with a 1cm mycelial plug from a 10-day old *S. rolfsii* culture cultured on PDA and incubated for 20 days at 27± 2 °C.

Pathogenicity of all ten isolates of *S. rolfsii* was confirmed by raising groundnut seedlings of the susceptible genotypes Dharani were sown in sterilised soil in polythene bag containers with a diameter of 20 cm and depth of 35 cm, four seeds were dispersed equidistantly in each pot. Each isolate was replicated thrice and were maintained. The pots were watered for two consecutive days. One month old seedlings were inoculated with 20 days old inoculum developed on sorghum grain medium by distributing the inoculum on surface of the soil. Plants in the untreated control group were not inoculated with inoculum. Observations on stem rot infection were made after 10 days of inoculation. Re-isolation was done using such symptoms produced on plant tissues to check for compliance with the original isolate.

Identification of susceptible stage of the crop to stem rot of groundnut

The sensitive reaction of groundnut plants at five stages (0, 15, 30, 45, and 60 DAS) against stem rot causal pathogen *S. rolfsii* was studied under pot culture. These plant stages along with control plants were maintained in eighteen 20 cm diameter polythene bag containers that were replicated three times and filled with sterilized soil. In each pot, 4 groundnut seeds (Dharani) were planted, and the recommended fertilizer dose was applied. After raising all the stages of plants, the sorghum grain inoculums were applied to each groundnut plant near the stem, up to 7-10 grain per plant. The inoculated pots were left out in the open for monitoring, and they were irrigated as needed. At 15, 30, 45, 60, and 75 days after inoculation, the severity of the stem rot disease was determined, and the number of plants that showed typical symptoms such as stem rot, stem lesion, leaf weathering, and dead plants due to *S. rolfsii* were recorded, and the percent disease incidence was calculated using formula.

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total no. of observed plants}} \text{ X } 100$$

Table 1: Symptoms on groundnut plants were observed as per 1-5 rating scale

Disease rating	Description
1	Healthy
2	Lesions on stem only
3	Up to 25% of the plant symptomic (wilt, dead or dying)
4	26% to 50% of the plant symptomic
5	>50% of the plant symptomic

$$\text{Disease severity} = \frac{\Sigma ab}{AK} \text{ X } 100$$

Where, a=No. of disease plants having the same degree of infection, b=Degree of infection, A=Total no. of examine plant, K=Highest degree of infection

Results

S. rolfsii showed cottony white to white colour, compact to fluffy mycelium on PDA medium. Sclerotia development began 5- 12 days after incubation, numerous round to oval, globose or irregular light brown to dark brown or black colour mustard seed like sclerotial bodies were observed on the media. Symptoms on plants were observed 8- 11 days after inoculation, initially symptoms are observed on the basal part of stem as water soaked to dark brown lesions later the lesion were covered by a cottony white mycelium with the rotting underneath it which spreads on to the soil surface and produces numerous mustard seed like sclerotial bodies. The leaves of infected plants gradually become yellowing and dry

up leads to wilting and death of the plants. The pathogen was re-isolated from the infected groundnut plants which are inoculated and pathogenic nature of fungus was proved. Uninoculated control seedlings did not develop any symptoms.

Pathogenicity test

The first symptom appeared at 8 DAI on the groundnut plants inoculated with GSR 7 isolate. Among the 10 ten isolates tested for pathogenicity, three isolates (GSR 5, GSR 7 and GSR 9) showed highest disease incidence (100%) compared to others followed by GSR4 (91.7%), GSR 8 (91.8%), GSR 1(83.3%) and GSR 2(83.3%), whereas lowest disease incidence (75%) was observed on plants inoculated with GSR 3 and GSR 10 isolates. (Table 2 plate 2)

Table 2: Pathogenicity of *S. rolfsii* isolates on groundnut

Treatments	Isolates	Time taken for disease expression (DAI)	Disease incidence
T1	GSR1	9 DAI	83.33 (69.98)
T2	GSR2	9 DAI	83.33 (69.98)
T3	GSR3	10 DAI	75.00 (59.98)
T4	GSR4	8 DAI	91.67 (79.99)
T5	GSR 5	8 DAI	100.00 (90.00)
T6	GSR 6	12 DAI	75.00 (59.98)
T7	GSR 7	7 DAI	100.00 (90.00)
T8	GSR 8	10 DAI	91.67 (79.99)
T9	GSR 9	8 DAI	100.00 (90.00)
T10	GSR 10	11 DAI	75.00 (59.98)
T11	Control		0.00 (0.00)
	CD at 1%		14.83
	SE m±		5.03

Table 3: Impact of age on susceptibility of groundnut plant to stem rot disease development

Treatments	Days of inoculation (DOI)	Time taken for disease expression (DAI)	Disease incidence (%)	Disease severity (%)
T1	0	14 days	27.77 (26.74)	6.67 (14.75)
T2	15	11 days	66.67 (54.98)	31.67 (33.96)
T3	30	7 days	91.67 (79.99)	85.00 (71.13)
T4	45	10 days	75.00 (64.99)	73.33 (63.83)
T5	60	11 days	33.33 (34.99)	16.67 (23.85)
T6	Control		0.00 (0.00)	0.00 (0.00)
	CD at 1%		27.77 (26.74)	22.13
	SE (m)		66.67 (54.98)	7.10

Identification of susceptible stages of the crop

An experiment was conducted in green house to determine the most susceptible stage of the groundnut to stem rot disease development, as indicated in materials and methods, and the findings are shown in table 3 plate 2.

Results revealed that time taken for first expression of disease ranges from 7 to 14 days after inoculation in groundnut plants. Earlier expression of disease was observed at 7 days after inoculation (DAI) in 30 days old inoculated groundnut plants followed by 10 DAI in 45 days old inoculated groundnut plants, 13 DAI in 45- and 15-day old inoculated groundnut plants. However, compared to earlier treatments 0-day old inoculated plants exhibit disease expression lately at 14 DAI.

Maximum susceptibility was recorded in thirty days old inoculated plants showing significantly highest stem rot disease incidence 91.67% followed by 45 and 15 days old inoculated plants with 75% and 66.67% disease incidence respectively. Significantly lowest disease incidence was recorded in 0-day old inoculated plant with 27.77% followed by 60 days old inoculated plant with 33.3% disease incidence. Similarly, the highest disease severity was recorded in 30 days old inoculated plants with 85% disease severity followed by 45 and 15 days old inoculated plants with 73.3% and 31.1% disease severity respectively and least disease severity was observed in 0 days old plants with 6.7% followed by 60 days old inoculated plant with 16.7% disease severity. Time taken for wilting of the infected plant ranges from 7 to 15 days. Maximum plant mortality was recorded in 30 days old inoculated groundnut plants whereas, minimum plant mortality was recorded in 60 days old plants.

The current findings demonstrated that groundnut plants are infected by *S. rolfsii* at every step of their growth, from seed germination to harvesting. However, inoculums mixed with seeds at the time of sowing produced only a few seedlings. It could be owing to limited germination or plant emergence, or it could be due to *S. rolfsii* producing organic acid, which is poisonous to living cells. As a result, this data was used to identify the most susceptible stages for genotype evaluation under artificial conditions.

Present findings revealed that young stages of maturity in groundnut plants are more susceptible to the *S. rolfsii* causing stem rot disease and the severity of infection was more and rapid. Disease incidence and severity were decreased as the age of the plant increases. Similar findings were reported by Bekriwala (2016) [4] who found that, groundnut plants were shown to be most sensitive to *S. rolfsii* attack during the first 45 days of growth and also reported that as the plant's age progressed, the severity of the disease reduced. Similar findings were made by several workers (Vinod, 2006; Muthukumar and Venkatesh, 2013) [13, 10]. The highest susceptibility was found when the plants were about 45 days old, with a disease incidence of up to 79.86%, followed by s 30-day old plants (75.45%) were reported by Kumar *et al.*, (2021) [8]. Kulakarni *et al*. (1994) [7] found that 105-day-old groundnut plants recorded the lowest mortality, and stated that susceptibility of groundnut seedlings to *S. rolfsii* decreases as the age of the groundnut plants increases.

Plate 1: Pathogenicity of *S. rolfsii* isolates on groundnut

Plate 2: Impact of age on susceptibility of groundnut plant to stem rot disease development

Conclusions

This research documented that groundnut plants that are infected by *S. rolfsii* causes stem rot, reduces pod yields by 10% to 25%, but under severe disease conditions, yield losses might reach up to 80%. Symptoms on plants were observed 6-10 days after inoculation, initially symptoms are observed on the basal part of stem later the lesion were covered by a cottony white mycelium which spreads on to the soil surface and produces numerous mustard seed like sclerotial bodies. The recent findings show that *S. rolfsii* infects groundnut plants at every stage of their growth, from seed germination through harvesting. Inoculums combined with seeds at the time of sowing, on the other hand, only produced a few seedlings. It could be due to *S. rolfsii* producing organic acid, which is toxic to living cells, or it could be due to limited germination or plant emergence. Young stages of maturity in groundnut plants are more susceptible to the *S. rolfsii* causing stem rot disease and the severity of infection was more and rapid. Disease incidence and severity were decreased as the age of the plant increases. As a result, this evidence was used to determine the most susceptible stages for genotype evaluation under controlled conditions.

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