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To determine the susceptible age of stem rot disease caused by *Sclerotium rolfsii* Sacc. in groundnut

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Abstract
An experiment was carried out in net house of Department of Plant Pathology, Junagadh Agricultural University, Junagadh, during *Kharif* -2017 under pot culture. Seven stages *i.e*., 0, 6, 12, 18, 24, 30, 36 days after sowing (DAS) of groundnut plants were selected for their susceptible reaction against stem rot causal pathogen *S. rolfsii*. Groundnut plants were inoculated 16, 22, 28, 34, 40, 46 and 52 days after last pot sowing (days) by actively mycelium and sclerotia developed on sorghum grains places near the stem of groundnut plants. Observations recorded after 7 days developed stem rot symptoms. Result revealed that, stem rot developed in all inoculated plants but severity decreased with increasing plant age at inoculation. Highest disease severity (73.33%) was recorded at 45 DAS inoculated plants. Our findings suggest that plants are more susceptible to infection at early development stages. However, susceptibility to stem infection was reduced after 45 DAS of inoculation. Moreover, young stage of maturity was more susceptible to *S. rolfsii*.

Keywords: *Sclerotium rolfsii*; Stem rot, Disease severity, susceptible ages

Introduction
Groundnut is regarded as "King of oilseed crops". A large number of diseases attack groundnut in India (Mayee, 1987; Mayee and Datar, 1988; Ganesan and Sekar, 2004) [4] [5] [3]. Groundnut is susceptible to wide range of microorganisms which may include fungi, viruses, mycoplasma, nematodes and bacteria. Among them, groundnut stem rot caused by *S. rolfsii* is found throughout groundnut producing areas of the world and causes the severe damage during any stage of the crop growth. As such, warm and moist climatic conditions favor disease development. The younger plants were found more susceptible as the infection was more and rapid. The stem and pod rot caused by *S. rolfsii* Sacc. is major constraints and potential to reduces *Kharif* groundnut production in Saurashtra region of Gujarat state. The objective of this study was to determine how plants ages affect susceptibility of plants exposed to *Sclerotium rolfsii*.

Materials and Methods
Groundnut plants with typical showing stem rot symptoms collected from field of Main Oilseed Research Station, Junagadh Agricultural University, (J.A.U.) Junagadh during *Kharif* 2017. The pathogen *S. rolfsii* was isolated from the stems of infected groundnut plants by tissue segment method (Rangaswami and Mahadevan, 1999) [7] on potato dextrose agar (PDA) medium. Small pieces of tissue of about 0.2 to 0.5 cm from infected collar region with some healthy tissue were cut with sterile scalpel. The pieces were surface sterilized with 1% sodium hypochlorite solution for 30 second. The tissue pieces were subsequently washed in three changes of sterile distilled water to eliminate excess sodium hypochlorite. After that excess water was removed with sterilized blotting paper then the pieces were transferred on to PDA medium in Petri plates. Plates were incubated at 28 ± 1° C and observed periodically for growth of the fungus. The pathogen was obtained by single hyphal tip method, further purified using single sclerotial body and maintained on PDA throughout the present investigation. Purified culture was maintained on PDA slants by storing it under refrigeration (4° C).

Preparation of Inoculums

The pathogen *Sclerotium rolfsii* was multiplied on sorghum grains. Sorghum grains were washed thoroughly in tap water and half boiled. After removing the excess water, grains were allowed to air dry and cooled at room temperature. Polypropylene bags were filled up with about 200 grams of grains. Mouth of these bags were packed with 4 cm piece of PVC pipe and nonabsorbent cotton. Then it was autoclaved at 121° C and 15 psi for 20 min for sterilization. Than after inoculated with 2-3 discs of size 5 mm diameter from 4 days old culture of test pathogen put in BOD incubator after seven days of incubation.

An experiment was conducted in net house of Department of Plant Pathology, Junagadh Agricultural University, Junagadh, during *Kharif* -2017 under pot culture. All pots used for experiment were filled with sterilized soil. Completely randomized design with three repetition was performed. Ten seeds of groundnut variety GG -20 were sown at 5 cm depth in the twenty four plastic pots of 15 cm width x 15 cm depth. Seven stages *i.e.*, 0, 6, 12, 18, 24, 30, 36 days after sowing (DAS) of groundnut plants were taken for their susceptible reaction against stem rot causal pathogen *S. rolfsii*. These stages of plants were maintained with fertilizer does applied as per recommendation. Irrigation and insecticide control measures were also carried out as and when required. After raising all the respective stage, the sorghum grain inoculums (5 g pot^{-1}) were applied near the stem on each plants of groundnut. The test fungus inoculums added sixteen days after sowing of last stage and pots without inoculums was considered as control. Simultaneously, three pots were also inoculated with test pathogen at the time of sowing to know the effect of *S. rolfsii* on germinating seeds and seedling.

Stem rot and disease incidence and severity was recorded at 67, 61, 55, 49, 43, 37 and 31 days after sowing at respective stage, number of plants showed typical symptoms *i.e.*, stem rot lesion of stem, weathering leaf and mortal plants due to *S. rolfsii* was observed and per cent disease incidence and disease severity was calculated. Periodical observation after 7 days intervals (75, 68, 62, 56, 50, 44 and 38 period-II and 81, 75, 69, 63, 57, 51 and 45 period-III) at respective stage were carried out to precise the susceptible age of stem rot in groundnut.

$$\text{Per cent disease incidence} = \frac{\text{Number of infected plants per pot}}{\text{Total number of plants per pot}} \text{ x } 100$$

Symptoms on groundnut plants were observed as 1-5 rating scale in Table 1 (Shokes *et al*., 1996) [8].

Table 1: Symptoms on groundnut plants and rating scale

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

Disease severity (Ds) was carried out by using following formula (Filion *et al*., 2003) [2].

$$\text{Disease severity} = \frac{\Sigma\,(ab)}{AK} \text{x} 100$$

Where: a = No. of diseased plants having the same degree of infection, b = Degree of infection, A = Total no. of examined plants, K = Highest degree of infection

Different stages, inoculated of groundnut plant and observation days after sowing were given in Table 2.

Table 2: Different stages, inoculation of groundnut plant and observation days after sowing

S. No.	Treatments/ stages (days)	Inoculation after last pot sowing (days)	Observation DAS		
			Period-I	Period-II	Period-III
1	0	52	67	75	81
2	6	46	61	68	75
3	12	40	55	62	69
4	18	34	49	56	63
5	24	28	43	50	57
6	30	22	37	44	51
7	36	16	31	38	45

DAS – Days after sowing

Results and Discussion

The isolated pathogen was identified as *S. rolfsii* Sacc. based on morphological characters, the fungal mycelium was first silky white in color, fluffy mycelium growth later turned to dull white with radial spreading given fan like appearance and coarse hyphal stands have ropy appearance. The fungal culture was the aerial hyaline, thin walled, septate hyphae with profusely branched mycelium which measured 7.45 µm. The fungus was characterized by white mycelia and spherical brown sclerotia, which ranged from 0.5 to 1.2 mm in diameter.

Structural and morphological characters of sclerotial bodies at different stage of growth revealed that they were variable with respect to size, shape, colour, texture. The sclerotial colour changed white to off white (in immature bodies), later it became light brown (in partial mature bodies) and dark brown to black (in mature bodies). The immature bodies exhibited irregular round shape with smooth wall. The partial mature bodies were medium round and slightly rough walled, mature bodies appeared round with rough wall. Young sclerotia often exude droplets of clear to pale yellowish fluids.

Similar, cultural and morphological characters were given by Bekriwala *et al*. (2016) [1] while working with *S. rolfsii* in peanut crops.

To find out the susceptible stage of the groundnut to stem rot disease development, an experiment was conducted in pot conditions. The results are presented in the Table 3. The results revealed that there was no difference in disease incidence percentage among the different stage of plant. Forty three day old plant had maximum 63.33% disease severity which was statistically at par with forty nine day old plant. Least disease severity was recorded in 67 days old plants with 33.33% which was followed by 61 day old plant disease severity was recorded 43.33%. Whereas; 37, 31 and 55 day

old plant disease severity were recorded 56.67%, 50.00% and 46.67%, respectively. However, few plants were emerged from inoculums incorporated with seeds at the time of sowing (the pots in which test pathogen fungus inoculated at the time of sowing). It may be due to poor germination or plant emergence due to production of organic acid by *S. rolfsii*, which are toxic to living cell. Therefore, this result was used to identify most susceptible stage for evaluation of genotypes under artificial condition. Periodical observation at 7 days interval was also recorded (first period, second period and third period) to know the stem rot severity and susceptible age of groundnut. During the second period observation, 44 day old plant had maximum 70.00% disease severity. Least disease severity was recorded in 74 days old plants with 36.67% which was followed by 68 and 62 day old plant disease severity was recorded 43.33% and 50.00%, respectively. Whereas; 56, 50 and 38 day old plant disease severity were recorded 63.33%, 63.33% and 56.67%, respectively.

At third period, the results revealed that 45 day old plant had maximum 73.33% disease severity. Least disease severity was recorded in 81 days old plants with 36.67% which was followed by 69 and 75 day old plant disease severity was recorded 50.00% and 43.33%, respectively. Whereas; 63, 57 and 51 day old plant disease severity were recorded 63.33%, 66.67% and 70.00%, respectively. The present findings revealed that groundnut plants were infected by *S. rolfsii* at all stage of plant growth from seed germinating to harvesting. Germinating stage of the seed causing pre-emergence rot and the susceptibility of groundnut plants against *S. rolfsii* was decreased with the increase in the age of groundnut plants. Highest stem rot severity was recorded in period-I at 43 (63.33%) and 49 (63.33%) DAS, period-II at 44 (70.00%) and period-III at 45 (73.33%) DAS. From this study, it can be decided that stem rot susceptible age was 45 days after sowing. The results obtained confirmed the findings of Patil and Rane (1983) [6] and Bekriwala *et al*. (2016) [1] who reported that, maximum 79.04 PDI recorded and as the age of groundnut plant increased, the infection of *S. rolfsii* decreased.

Conclusion

S. rolfsii appeared as white fluffy mycelium growth on PDA as well as around the basal portion of stem than it produced light brown and dark brown round to oval, globes or irregular mustard seed like sclerotia were produced. Groundnut plants were infected by *S. rolfsii* at all growth stages of plant from seed germinating to maturity. But the younger plants were found more susceptible to infection by *S. rolfsii* caused highest plant mortality results to reduced pod yield. The groundnut plants were infected by *S. rolfsii* at all stage of plant growth from seedling to harvesting. However the highest severity of 73.33% recorded at the age 45 days after sowing. Germinating stage of the seed causing pre-emergence rot and the susceptibility of groundnut plants against *S. rolfsii* was decreased with the increase in the age of groundnut plants.

Table 3: Determination of susceptible stage of groundnut crop to stem rot sown at different date in pot culture

S. No.	Treatment Days (Date)	Inoculat-ion DAS	Obs. DAS	Disease incidence	Disease Severity %# (P – I)	Obs. DAS	Disease Severity %# (P – II)	Obs. DAS	Disease Severity %# (P – III)
1.	0 (12/ 06/17)	52	67	100	5.80 (33.33)*	74	6.08 (36.67)	81	6.08 (36.67)
2.	6 (18/ 06/ 17)	46	61	100	6.55 (43.33)	68	6.55 (43.33)	75	6.55 (43.33)
3.	12 (24/ 06/17)	40	55	100	6.76 (46.67)	62	7.04 (50.00)	69	7.04 (50.00)
4.	18 (01/ 07/17)	34	49	100	7.99 (63.33)	56	7.99 (63.33)	63	7.99 (63.33)
5.	24 (07/ 07/17)	28	43	100	7.99 (63.33)	50	7.99 (63.33)	57	8.19 (66.67)
6.	30 (15/ 07/17)	22	37	100	7.54 (56.67)	44	8.38 (70.00)	51	8.38 (70.00)
7.	36 (21/ 07/17)	16	31	100	7.11 (50.00)	38	7.56 (56.67)	45	8.59 (73.33)
			S. Em. ±		0.47		0.42		0.42
			C.D at 5%		1.41		1.27		1.26
			CV%		11.34		9.85		9.56

It is average of three replication

* Data within parenthesis are original value (square root transformed values + 0.5 added)

P – I First period observation

P – II Second period observation

P – III Third period observation

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