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Studies on Sclerotial Root-Rot Disease of Groundnut (*Arachis hypogaea* L.) Caused by Sclerotium rolfsii sacc.*

by

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Synopsis: The results of a detailed study of the sclerotial root-rot disease of groundnut (Arachis hypogaea. L.) caused by *sclerotium rolfsii* sacc are reported in this paper.

Introduction: The root-rot disease of groundnut caused by *Sclerotium rolfsii* is prevalent in some of the states like Bengal, Uttar Pradesh, Madhya Pradesh and Madras. In Madras, the disease has been noted to occur in a serious form in recent years in the taluks of Pollachi and Udumalpetai in Coimbatore district. Very little work has been carried out in India on this disease. The reaction of some groundnut varieties has been studied by Babu Singh and Mathur (1953). A detailed study of this disease has therefore been carried out by the present author and the results are presented here.

Materials and Methods: The fungus *sclerotium rolfsii* was isolated from wilted groundnut plants collected from Pollachi and maintained in oats agar plants. In addition, two more isolates one from chilli (*Capsicum* sp.) and another from *Amorphophallus* were obtained from the culture collections in the Mycology section and used in cross inoculation studies.

The studies were carried out in pots and sterilised soil was used for the experiments. In all the pot culture experiments, the plants were inoculated with the fungus multiplied in sand-maize medium for a fortnight at room-temperature. The surface soil from the pots was removed without disturbing the plants, the inoculum spread on the surface and the soil was replaced over the inoculum. In the studies of pre-emergence damping off, the inoculum was mixed with the top soil thoroughly prior to sowing. Plants grown in sand were fed with knop's solution diluted ten times.

Groundnut plants 30–45 days old were used in all the experiments. Five plants were maintained in each pot. In pathogenecity test, the short duration groundnut variety TMV. 2 was used.

Methods of Inoculation: On solid media, single sclerotium was placed in the centre of the plate after surface sterilising in 1 : 1000 mercuric chloride solution for one minute to kill the adhering mycelium and then washing in sterilised water. On liquid media, mycelial disks of 5 mm diameter from vigorously growing colonies

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in oats agar medium were cut with a sterilised cork borer and one such disk was inoculated in each flask. One hundred ml of liquid was taken in each flask for all the liquid culture experiments. Not less than three replications were maintained throughout.

Results: Symptoms of the Disease: The first symptom observed is the drooping and fading of leaves and branches, then the plant begins to wilt, the leaves, stem and branches become dry. Deep brown lesions are observed on the lower portion of the stem. Sometimes wilting of single branches is also noticed. The brown lesions become covered with white mycelium which is seen both on the roots and stem. Abundant brown sclerotia are seen on the mycelium. The roots are seen shredded and the bark peels off easily. All parts covered by the mycelium turn brown and decay rapidly.

Morphology of the Fungus: The mycelium is silky or cottony white, much branched and spreads out in strands producing a fan-like appearance, the hyphae being septate. Usually when the growth has completely covered the plates, small whitish mycelial masses begin to appear which enlarge and a corky covering is formed from the outer layers. Later a downy covering sloughs off leaving the surface dark brown, smooth and shiny. The sclerotia are usually spherical but sometimes irregular in shape. The side to which the mycelial strand is attached is slightly concave. Large drops of liquid are noted on the surface of the sclerotia before maturation. In pure cultures, the sclerotia are bigger in size, the range being 0.92 to 1.40 mm in oats agar. In general, the appearance of the fungus agrees with *S. rolfsii* described by Higgins (1927) and Taubenhaus (1919).

1. Pathogenicity: Infection of seedlings was obtained in sterilised and unsterilised sand and soil. Pre-emergence infection of the seeds was also secured both in sterilised and unsterilised soil. In soil, the mortality was 80–84% while in sand 100% mortality was obtained. Preemergence pathogenicity was 84–92%. The incubation period was 4–5 days.

2. Varietal Resistance Study: The following eight varieties of groundnut were used in this study:

1. TMV. 1 (Spreading type), 2. TMV. 2 (Erect or Bunch), 3. TMV. 3 (Spreading), 4. TMV. 4 (Spreading), 5. A. H. 6719 (Semi-spreading), 6. A. H. 6481 (Bunch), 7. A.H. 477 (Semi-spreading) and 8. A. H. 3490 (Erect).

All the groundnut varieties tested were susceptible to the fungus but in varying degrees. The variety A.H. 3490 (Control) recorded the maximum infection. The time taken for wilting in all the varieties ranged from 7–9 days and the earliest being in A.H. 3490 (Table I).

3. In the study to find out the optimum age at which the groundnut plants were most susceptible, the seeds of the variety A.H. 3490 were sown in pots at intervals of 15 days so that plants of ages 15, 30, 45, 60, 75, 90 and 105 days were obtained. These plants were simultaneously inoculated. It was

TABLE I.

Varietal resistance or susceptibility of the groundnut varieties to Sclerotium rolfsii.

S. No.	Variety	Habit of the variety	Total number of plants		% of infection	Time taken for wilting in days
			Inoculated	Wilted		
1.	TMV. 1	Spreading	25	11	44	8
2.	TMV. 2	Erect	25	17	68	9
3.	TMV. 3	Spreading	25	10	40	9
4.	TMV. 4	Spreading	25	10	40	8
5.	A. H. 6719	Semi-spreading	25	11	44	9
6.	A. H. 6481	Bunch	25	12	48	8
7.	A. H. 477	Semi-spreading	25	13	52	8
8.	A. H. 3490 (Control)	Erect	25	23	92	7

S. E. 0·52
Significant at 5% level. C. D. 1·5

8 2 7 6 5 1 3 4

observed that the plants were susceptible to the fungus at all the ages but the younger plants were more susceptible than the older ones. The percentage of infection decreased as the age of the plants increased. The time taken for wilting also varied from 10 to 14 days, the earliest being in younger plants up to 45 days old and the maximum in 90 to 105 days old plants. (Table II.)

TABLE II.

Susceptibility of groundnut plants at different ages

S. No.	Age of plants in days	Total No. of plants		% of infection	Time taken for wilting in days
		Inoculated	wilted		
1.	15	25	15	60	10
2.	30	25	14	56	10
3.	45	25	12	48	10
4.	60	25	11	44	11
5.	75	25	10	46	11
6.	90	25	8	32	14
7.	105	25	8	32	14

4. In the study to determine the inoculum dose for successful infection, the fungus was multiplied in 50, 100, 200, 300 and 400 gm sand-maize medium and each pot containing 1800 gm soil was inoculated with the respective dose of inoculum. Complete wilting was secured only in pots receiving 400 gm inoculum for 1800 gm soil. (Table III).

TABLE III.

Inoculum potential

Treatment No.	Quantity of the inoculum in 1800 g. soil	Total No. of plants		Percentage of infection in days from inoculation				
		Inoculated	Wilted at the end of 16 days	3rd day	5th day	8th day	11th day	16th day
1.	400 g.	25	25	8	72	100	...	...
2.	300 g.	25	22	4	40	80	88	88
3.	200 g.	25	15	4	20	44	48	60
4.	100 g.	25	...	...	...	...	...	...
5.	50 g.	25	...	...	...	...	...	...

5. Pathogenicity of the fungus at different moisture levels 20, 40, 60, 80 and 100% was studied, the moisture level being adjusted once in 24 hrs and it was observed that the fungus was highly pathogenic at 40% moisture level. At 20% level even the control plants wilted but the inoculated plants showed the symptoms of the disease which were absent in the former. In others, as the moisture level increased, the pathogenicity of the fungus decreased and at 100% level, no infection was observed (Table IV).

TABLE IV.

Pathogenicity of the fungus at different moisture levels

S. No.	Moisture level in %	Total No. of plants		% of infection
		Inoculated	Wilted	
1.	20	12	7	58.4
2.	40	12	11	91.7
3.	60	12	2	16.7
4.	80	12	1	8.3
5.	100	12	...	...

6. In the host range study, the following plants belonging to dicotyledons and monocotyledons were used:

Allium cepa, Sorghum durra, Abelmoschus esculentus, Capsicum annuum Cyamopsis tetragonaloba, Gossypium hirsutum, Lycopersicon esculentum, Ricinus communis, Solanum melongena, Vigna sinensis and Arachis hypogaea.

Sorghum durra and *Allium cepa* were not infected. Only 8% of *Gossypium hirsutum* plants were infected. In *A. esculentus*, *S. melongena*, *Vigna sinensis* and *A. hypogaea* 64, 88, 80 and 92% of the plants respectively died. In the rest, 100% pathogenicity was obtained.

7. Cross-inoculation experiment on groundnut plants with the isolates of *S. rolfsii* from *Capsicum* sp. and *Amorphophallus* showed that all the isolates were pathogenic to groundnut plants but the isolate from groundnut caused 80% mortality, the isolate from chilli 56% and that from *Amorphophallus* only 16% pathogenicity.

8. Evaluation of fungicides and fungicidal trial: The different fungicides taken up for evaluation were 10, 100 and 1000 ppm of Cerenox, Ceresan (wet), Cheshunt compound, Copper oxychloride, Dithane Z. 78, Flit 406 and penta chloronitrobenzene. The evaluation was done in two ways (1) Plate method and (2) Soil vial method [Tube method of Zentmyer (1955) and later modified by Kandrick (1959)]. Four fungicides, Bordeaux mixture one per cent, copper oxychloride, cheshunt compound and wet ceresan (0.1%) were used as drench to find out their efficacy and quarter pint of each was used per pot, 24 hours after inoculation of the fungus.

Evaluation of fungicides *in vitro* showed that ceresan was fungicidal in all the concentrations of 10, 100 and 1000 ppm and cheshunt compound was fungicidal in 100 and 1000 ppm. Flit at 1000 ppm and PCNB in 100 and 1000 ppm, had fungistatic effect. Copper oxychloride, Cerenox, Dithane, Cheshunt compound in 10 ppm, Flit in 10 and 100 ppm, PCNB in 10 ppm. had no effect at all. The growth was good in the control plates. The study in soil vials revealed that ceresan in 100 and 1000 ppm had good fungicidal effect. In the fungicidal trial in pots also, wet ceresan, gave good control of the disease and this agreed with that of the results obtained from plate and soil vial tests in the evaluation of fungicides.

Cultural Studies: The fungus was grown in various solid and liquid media to find out the suitable medium that favoured its growth and sclerotial production. The solid media used were oats agar, potato-dextrose agar, groundnut root extract agar, Richard's czapek's and Leonian's agar. The liquid media used were Richard's czapek's, Leonian's, groundnut root extract and Brown's solution. The dry mycelial weight after three week's incubation was recorded. The fungus grew well in all solid media except Richard's agar but was especially vigourous in oats agar. Sclerotial production was also good in oats agar, the size being the largest in this medium. In the liquid culture studies, Richard's medium was found to be the best for growth and sclerotial production. (Table V.)

In the study to find out the effect of various nitrogen sources on the growth and production of sclerotia of the fungus, both organic and inorganic nitrogen were used, the organic being peptone, asparagine and urea and the inorganic being ammonium sulphate, potassium nitrate and sodium nitrate. Asthana-Hawker

TABLE V.

Growth and production of sclerotia in solid and liquid media

Media	Solid		Liquid	
	No. of sclerotia	Size in mm	Dry wt. of mycelium in mg.	Sclerotial production
Oats	375	0.92—1.40	—	—
Potato dextrose	267	0.72—1.26	—	—
Groundnut root extract	15	0.74—1.33	621	+
Leonian's	132	0.90—1.33	—	—
Czapek's	45	0.68—1.08	1592	++
Richard's	nil	— —	2315	+++
Brown's			849	++

+++ Good, ++ Moderate, + Poor.

medium was used as the basal medium in which the potassium nitrate was replaced by the various compounds of nitrogen equivalent to the weight to supply equal quantity of nitrogen. Peptone was found to be superior to all other nitrogen sources for the growth and sclerotial production of the fungus both in solid and liquid media (Table VI).

TABLE VI.

Effect of various sources of nitrogen on the growth and production of Sclerotia

S. No.	Source of nitrogen	Dry weight of mycelium in mg.	Sclerotial production
1.	Urea	41.25	+
2.	Asparagine	18.50	—
3.	Peptone	163.50	++
4.	Ammonium sulphate	22.00	—
5.	Sodium nitrate	22.25	—
6.	Potassium nitrate	38.50	+
7.	Control	10.00	—

++ Good, + Poor, — Nil.

S. E. 14.9

C. D. 43.9

Significant at 1% level. 3 1 6 5 4 2 7

The various carbon sources included to find out the effect on the growth and production of sclerotia of the fungus were monosaccharides, disaccharides and polysaccharides. Asthana Hawker-medium was used with slight modification

in the study of the various carbon sources in solid medium. Potassium nitrate was replaced by peptone which was found by experiment to be the best nitrogen source. Five grams of carbon sources each was added to one litre of the medium. The fungus utilised all the carbon sources on solid media except lactose in which there was poor growth. In liquid medium, maltose and dextrose were superior than others and sclerotial production was good in maltose (Table VII).

TABLE VII.

Effect of carbon sources on the growth and production of Sclerotia in liquid media

S. No.	Source of carbon	Dry weight of the fungus in mg.	Sclerotial production
1.	Glucose	70·3	++
2.	Sucrose	35·3	++
3.	Dextrose	89·6	++
4.	Lactose	25·3	+
5.	Maltose	153·6	+++
6.	Starch	71·3	++
7.	Control	...	—

+++ Good, ++ Fair, + Poor, — Nil.

S. E. 24.0

C. D. 73.9

Significant at 5% level. $\overline{5\ 3}\ 6\ 1\ 2\ 4$

The effect of 'H' ion concentration on the growth of the fungus was studied by growing the fungus in pH ranging from 1 to 9 (1, 2, 3, 4, 5, 6, 7, 8 and 9), Richard's solution being used as basal medium and the dry mycelial weight recorded after three weeks. The fungus tolerated a wide range of pH from 2 to 9 and the optimum was pH 6. Sclerotial production was good at pH 4, 5 and 6 (Table VIII).

Temperature Studies: The effect of temperature on the germination and production of sclerotia and on the mycelial growth of the fungus was studied at 5°, 10°, 15°, 20°, 25° and 30°C in oats agar medium. The fungus grew well at 30°C. As the temperature decreased, the sclerotia took longer time for germination. Sclerorial production was best at 25°C.

The thermal tolerance of sclerotia at 40°, 45°, 50° 55° and 60°C was also assessed and it was observed that Sclerotia retained their viability when exposed to 50°C for five minutes. Exposing the sclerotia at 60°C for the same period completely killed them.

TABLE VIII.

Effect of pH on the growth and production of Sclerotia

pH	Dry weight of the fungus in mg.	Sclerotial production
1	—	—
2	50·3	—
3	398·6	+++
4	400·6	++++
5	447·3	++++
6	470·3	++++
7	360·3	+++
8	157·0	++
9	135 0	+

++++ Good, +++ Fair, ++ Poor, + Very poor, — None

S. E. 49·7

C. D. 205·3

Significant at 1% level. 6, 5, 4, 3, 7, 8, 9, 2

TABLE IX.

Effect of Temperature on the germination, growth and production of sclerotia of the fungus. The daily increase in the average diameter of the colonies is given in mm.

S. No.	Temperature in contigrade	Time interval in Hours											Time taken for the Sclerotia to germinate in days	Mean number of sclerotia
		24	48	72	96	120	144	168	192	216	240	264		
1.	5°	—	—	—	—	—	—	—	—	—	—	—	Did not germinate even after 60 days	—
2.	10°	—	—	—	—	—	—	—	—	—	—	—	26	—
3.	15°	—	—	—	—	6·3	17·3	27·0	35·0	46·3	70·3	Plate full	5	8
4.	20°	—	—	3·0	16	32·7	49·3	68·3	Plate full	—	—	—	3	177
5.	25°	—	6·7	21·0	47·5	70·7	Plate full	—	—	—	—	—	2	507
6.	30°	—	3·0	17·0	51·3	Plate full		—	—	—	—	—	2	326

S. E. 3·8

C. D. 11·7

Significant at 1% level. 30°c, 25°c, 20°c, 15°c.

Discussion: In the pathogenicity study, successful infection was obtained both in soil and sand but the wilting was somewhat quicker and complete in the latter. This might probably be due to the fact that aeration was better in sand than in soil encouraging rapid development of the fungus. Taubenhaus (1919) Miller and Harvey (1932), Doolettle (1953) and Dubey (1958) also reported great loss due to *S. rolfsii* in sandy soils.

In the varietal resistance study, while all the varieties were found to be susceptible there were considerable differences in the percentage of wilt between varieties. This observation agrees with that of McClintock (1917) and Miller and Harvey (1932) who reported varietal differences in susceptibility.

In the determination of the inoculum dose for successful infection, it was found that optimum inoculum potential was about 22%. It was found that younger plants were more susceptible than older ones which is in conformity with the observations of Rayes (1937) and Clinton (1957).

The pathogenicity of the fungus was high in 40% moisture level and as the moisture level increased the percentage of infection decreased and in 100% level there was no infection at all. Flados (1958) reported that increased relative humidity favoured organisms that inhibit the growth of *S. rolfsii*. When the moisture content was increased an anaerobic condition was produced thereby making it unfavourable for the growth and activity of the fungus. The fungus did not appear to be killed at high moisture level as it was observed that growth of the fungus revived when the soil dried at the end of the experiment.

Monocotyledonous hosts like *Sorghum durra* and *Allium cepa* were immune to infection. Ciccarone and Platone (1949) reported that *sorghum* was resistant to this fungus. Reyes (1934) reported *Allium cepa* as susceptible to *S. rolfsii*, isolated from *Helichrysum bracteatum* and not from groundnut. The fact that *S. rolfsii* has several strains in nature might account for the apparently contradictory results. Taubenhaus (1919) reported the occurrence of S. *rolfsii* on *Lycopersicon esculentum, Gossypium hirsutum. Vigna sinensis, Solanum melongena* etc. Street (1948) reported the fungus on *Cyamopsis tetragonoloba.* In the cross inoculation study, it was observed that S. *rolfsii* isolated from chillies and *Amorphophallus* were pathogenic on groundnut but the isolate from groundnut was more pathogenic than the former two. Among the former two, the chilli isolate was more pathogenic. Work done in this laboratory showed that on chilli plants, the isolate from groundnut, *Amorphophallus,* and chilli were equally pathogenic (Nambiar, 1960). There seems to be some difference therefore between the isolate from chilli and groundnut in their behaviour indicating perhaps strainal difference. Edson and Shapovalov (1923) Nakata (1927), Curzi (1932), Fajardo and Mendoza (1935), Goto (1935, 1938) and Epps, Patterson and Freeman (1951) reported strainal difference in S. *rolfsii.*

In all the three methods of evaluation of fungicides, ceresan (wet 0·1%) proved to be the most effective fungicide which is in conformity with the report of Ramakrishnan and Sarojini Damodaran (1955) and Kandaswamy and Sundaram (1956).

Oats agar was found to be the best medium favouring the growth of the fungus which is in conformity with the observation of Ramakrishnan (1930) who studied an isolate of this fungus from *Zinnia*. Peptone was best utilised both in solid and liquid media for the growth and production of sclerotia. The fungus was able to utilise all the carbon sources except lactose, and this agrees with the observation of Higgins (1927) Ramakrishnan (1930) and Abeygunawardana and Wood (1957). The fungus tolerated a wide range of pH from 2 to 9 which is in close conformity with the observation of Chowdhury (1946) and Higging (1927). The optimum pH was round about pH 6 which corresponds to that given by Chowdhury (1946) and Rosen and Shaw (1929) who found the optimum to be pH 6.4 and pH 6 respectively eventhough they employed solid media. Sclerotial production was good in pH 4 to 6 which closely agreed with the report of Chowdhury (1946) and Clinton (1957).

The fungus grew well at 25°C and 30°C which closely agree with the report of Higgins (1922) and Fajardo and Mendoza (1935). The sclerotia germinated well at 25°C and 30°C and germination was delayed as the temperature decreased. This agrees with the observation of Fajardo and Mendoza 1935. Sclerotial production was best at 25°C. This does not agree with the observation made by Nakata (1928) and Tounsend (1957). Sclerotia exposed to 60°C (moist heat) for five minutes had lost their viability and this result was somewhat in accordance with the finding of Fajardo and Mendoza (1935) and Chowdhury (1945).

Summary: *Sclerotium rolfsii* isolated from groundnut caused wilt and pre-emergence death on the same host on artificial inoculation. All groundnut varieties tested were susceptible, but there were considerable differences in degree of susceptibility. Younger plants were more susceptible. The optimum inoculum dose was 400 gm inoculum in 1800 gm soil. Increasing soil moisture reduced pathogenicity. The fungus did not infect *sorghum* and onion while the dicotyledonous hosts tested were susceptible to varying degrees.

Ceresan (wet) gave the best control of the disease. The fungus utilised peptone best. It utilised glucose, sucrose, maltose, dextrose and starch well while lactose was utilised poorly. It tolerated a pH range of 3–9, but grew best at pH 6. A temperature range of 25–30°C was best for growth and sclerotial production. Sclerotia were killed when exposed to 60°C for five minutes.

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* Originals not seen.