



RESEARCH ARTICLE

Effect of crop residues and root exudates on mycelial growth, sclerotial formation, and *Sclerotium rolfsii*-induced stem rot disease of groundnut

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ABSTRACT: The effect of aqueous leaf extracts, dried crop residues and root exudates of rotational crops commonly grown in groundnut production system *viz.*, sorghum, pearl millet, maize, wheat, cotton, castor, sesame, sunflower, safflower, mustard, soybean, pigeon pea, green gram, black gram, chick pea, cow pea, pea, garlic and onion on mycelial growth, sclerotial germination, number and size of sclerotia of *Sclerotium rolfsii* Sacc. and the incidence of stem rot in groundnut (*Arachis hypogaea* L.) caused by this pathogen were studied under *in vitro* conditions. Aqueous leaf extracts (ALE) (100 g/100 ml water) of garlic, onion, pearl millet, sunflower and sorghum at 5% concentration reduced mycelial growth and completely inhibited sclerotia formation. ALE of pigeon pea, green gram, and black gram moderately reduced mycelial growth but there was sclerotial formation. ALE of groundnut and soybean enhanced mycelial growth, size and number of sclerotia. The lowest germination of sclerotia was on crop residue of sunflower and garlic whereas the highest was on the crop residue of soybean and groundnut. In the pot experiment, the lowest incidence of stem rot was observed in sunflower and garlic crop residue, and the highest was in soybean followed by groundnut crop residue. Significant but varied level of inhibitory effect of root exudates of twelve crops *viz.*, sorghum, pearl millet, maize, wheat, garlic, onion, sesame, sunflower, safflower, mustard, cotton and castor on mycelial growth of *S. rolfsii* and reduction in germination of sclerotia was observed. The results of this study will be useful in deciding the rotational crops in the integrated disease management of groundnut.

Key words: Groundnut, *Sclerotium rolfsii*, stem rot, crop residues, root exudates

Stem rot caused by *Sclerotium rolfsii* Sacc. occurs in all the groundnut (*Arachis hypogaea* L.) growing states of India and is more severe in the states of Gujarat, Maharashtra, Madhya Pradesh, Orissa and Tamil Nadu, where approximately over 50,000 ha of groundnut fields are infected. Latur, Raichur, Dharwad, Junagadh and Hanumangarh have been identified as 'hot spots' for this disease. About 27% or more yield loss due to this disease has been reported from India (Chohan 1974). Mayee and Datar (1988) have reported yield losses of over 25% in Maharashtra. The indirect losses such as reduction in dry weight and oil content are also reported.

The management of stem rot of groundnut is particularly complex because this pathogen forms sclerotia that can survive in soil for long periods, frequently tolerating biological and chemical degradation due to the presence of melanin in the outer membrane (Chet, 1975). Methods employed to manage *S. rolfsii* are fungicides application, solarization, use of antagonistic microorganisms, deep ploughing, crop rotation, and incorporation of organic and inorganic residues (Punja, 1985). Despite the ability of *S. rolfsii* to survive for long periods in the soil as saprophyte on plant debris or in the form of sclerotia in the absence of host plants (McClintock, 1917; Garren, 1957), there are reports of the beneficial use of crop rotation in combating the menace of the pathogen. Some organic residues may suppress while others may stimulate sclerotial germination of *S. rolfsii* in soil (Beute and

Rodriguez-Kabana, 1979). Population of soil borne pathogens are known to diminish, to relatively harmless levels if various plant species are carefully arranged in a cropping sequence for reasonable periods of time (Baker and Cook, 1974).

Organic residues can be added to soil as the result of the natural cycling of plants. In some cases the residue products stimulate germination of the pathogen propagules which die in the absence of host, thereby reducing the pathogen population (Patrick and Toussoun, 1965). Other reports indicate that organic amendments stimulate microbial activity, which depletes the nitrogen level or changes its form so that the infection process by the pathogen is impaired (Huber *et al.*, 1965). Some research has been done on the effects of temperature, moisture, pH, salinity, fungicides, amendments, and volatile compounds on the germination of sclerotia of *S. rolfsii* (Beute and Rodriguez-Kabana, 1981; Maiti and Sen, 1988; Canullo *et al.*, 1992). However, there is limited information available on the effect of different crop residues and root exudates of these crops on the sclerotial germination, mycelial growth and incidence of stem rot of groundnut. The objective of this study was to evaluate the effect of leaf extracts, crop residues and root exudates of rotational crops commonly grown in groundnut production system. such as groundnut, sorghum, pearl millet, maize, wheat, cotton, castor, sesame, sunflower, safflower, mustard, soybean, pigeon pea, green gram, black gram, chick pea, cow pea, pea, garlic and onion.

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MATERIALS AND METHODS

Isolation of *Sclerotium rolfsii*

Fifty nine isolates of *S. rolfsii* were isolated from infected groundnut plants collected from groundnut growing areas of Maharashtra and Gujarat states of India. The pathogen from the infected portion was isolated on potato dextrose agar (PDA) medium at a temperature of $28\pm 1^\circ\text{C}$. The culture was incubated for 20 days for formation of sclerotia. Pure culture was obtained by growing single sclerotium. Isolates were screened for their virulence and the most virulent isolate NRCG-SR-07 was used in the study. Virulence was studied by growing *in vitro* in culture tubes at ambient temperature on PDA medium, and in the pots in net-house by artificially inoculating the soil with inoculums of *S. rolfsii*.

Effect of aqueous leaf extracts on mycelial growth and sclerotial formation

Aqueous leaf extract (ALE) of each crop were made by crushing the leaves in sterile distilled water in a ratio of 1:1 w/v and passing through two layers of muslin cloth. The effect of ALE on mycelial growth and sclerotial formation of *S. rolfsii* was tested by poisoned food technique. For this 15 ml of PDA medium amended with 5% ALE was poured in 90 mm Petri dish. The Petri dishes were inoculated with 5 mm disc excised from periphery of the actively growing 5-days old culture of the isolate NRCG-SR-07. Three replications were maintained for each, along with a control (without ALE). The Petri dishes were incubated at $28\pm 1^\circ\text{C}$ in a BOD incubator. The colony diameter was measured after 5 days by taking two measurements at right angles. The number of sclerotia per colony was counted after 20 days of incubation. Diameter of 10 sclerotia was measured. The data from the three replicated plates were averaged.

Effect of crop residues on sclerotial germination, number and size of sclerotia

Crop residues of each crop collected after harvesting were chopped up and soaked in sterile distilled water for one hour. Hundred gram of residue of different crops were filled in the Petri dishes (150 x 25 mm diameter) in three replicates. For the control treatment 100 g black soil was used. After sterilization in an autoclave, it was cooled down and 100 sclerotia were placed superficially onto residues and incubated in a BOD incubator at $28\pm 1^\circ\text{C}$. Observations on germination of sclerotia was recorded at an interval of 24 hours (up to 10 days).

To study the number and size of sclerotia, another set of Petri dishes having finely crushed crop residue were inoculated with 5 mm disc excised from 5-days old culture of *S. rolfsii* and incubated at $28\pm 1^\circ\text{C}$. The number of sclerotia per plate was counted after 20 days of incubation. Diameter of 10 sclerotia was measured. The data from the three replicated plates were averaged.

Effect of crop residues on incidence of stem rot

The experiment was done in plantation pots (30 x 30 cm) in the net-house during rainy season of 2008. The soil used for experiment was black calcareous, collected from groundnut cultivated fields of experimental farm. Inoculums of *S. rolfsii* were mass multiplied on sorghum grains. For this, the sorghum seeds were half boiled and air dried. To soak moisture and avoid bacterial contamination, gypsum (50 g for each 10 kg grains) was added. After air drying it was filled in autoclavable polyethylene bags of 250 g capacity and autoclaved. These bags were inoculated with 5 mm disc excised from 5-days old culture of *S. rolfsii* and incubated at 28°C for 20 days before use.

Each plantation pots were filled in by 10 kg sieved and autoclaved soil mixed with 250 g of finely chopped crop residue of different crops and 50 g inoculums of *S. rolfsii*. The treatments along with control were replicated thrice. Thirty days period was given for decomposition of crop residues followed by sowing of groundnut cultivar GG-20. One week after germination, the plants were thinned to 10 plants/ pot and allowed to grow up to 60 days. Enough moisture was maintained in the pots by watering at regular intervals. The day temperature was $30\text{-}35^\circ\text{C}$ and RH was 70-80%, respectively in the net house. Observations on incidence of stem rot were recorded at an interval of 10 days.

Effect of root exudates on sclerotial germination and mycelial growth

The seedlings of 20 crops *viz.* black gram, castor, chick pea, cotton, cow pea, garlic, green gram, groundnut, maize, mustard, onion, pea, pearl millet, pigeon pea, safflower, sesame, sorghum, soybean, sunflower, wheat were raised in earthen pots having 10 kg autoclaved soil + sand (1:1 ratio) for 20 days. Ten seeds were sown in each pot and kept in net house for growth with proper irrigation. After two weeks, the seedlings were removed along with root system and washed thoroughly in running water. The roots of seedlings were dipped in 20 ml sterile water in test tubes and kept for 72 hr after which it was filtered through Whatman filter paper No. 41. The exudates thus collected were assumed 100% stock and kept at 4°C for further study. The effect of root exudates on mycelial growth of *S. rolfsii* were evaluated using poisoned food technique with four different concentrations *viz.* 5, 10, 15, and 20% along with distilled water as control. For evaluating effect on sclerotial germination, in each concentration ten sclerotia were first soaked for 24 hr and then transferred on to PDA plates. The sclerotia soaked in sterile water served as control. The plates were incubated at $27\pm 1^\circ\text{C}$. The radial mycelial growth was measured on fourth day of incubation while the number of sclerotia germinated was counted after six days. The percent inhibition of mycelial growth and sclerotial germination over control was derived.

Statistical analyses

Data were subjected to analysis of variance and least significant differences between means were calculated at 5% probability level.

RESULTS AND DISCUSSION

Effect of leaf extracts on mycelial growth, number and size of sclerotia

ALE of five crops viz., garlic, onion, pearl millet, sunflower and sorghum had the maximum inhibitory effects on mycelial growth of *S. rolfsii* and no sclerotia were formed even after 20 days of inoculation. The colony diameter was minimum (17.1 mm) in garlic leaf extract and the maximum (90.0 mm) in soybean and groundnut leaf extracts compared to the control (83.3 mm) (Table 1). ALE of wheat, pigeon pea, maize, green gram and black gram moderately inhibited mycelial growth, and there was sclerotial formation but the size of sclerotia was smaller and was fewer in number as compared to the control. ALE of groundnut and soybean enhanced mycelial growth and number of sclerotia formed compared to the control. The number of sclerotia were highest in ALE of groundnut (427) followed by soybean (390) as compared to control (316). The size of sclerotia formed in culture were also maximum in ALE of groundnut (2.1 mm) followed by soybean (1.9 mm) as compared to control (1.7 mm). ALE of green gram, pigeon pea, black gram, wheat and maize reduced the number and size of sclerotia produced by *S. rolfsii* as compared to control. The differential effect of ALE indicated the presence of inhibitory or stimulatory compound in the leaves of these crops. The active compounds in extracts of *Allium* spp. was reported to be n-propyl and allylsulphides (Coley-Smith and Cooke, 1971).

Effect of crop residues on sclerotial germination, number and size of sclerotia

In the crop residues of sunflower, garlic, onion, pearl millet, sorghum, maize, wheat and pigeon pea, sclerotial germination was reduced (Table 2). The lowest germination

Table 1. Effect of leaf extracts of different crops on mycelial growth, number and size of sclerotia of *Sclerotium rolfsii*

Aqueous leaf extracts	Colony diameter (mm)*	Number of sclerotia*	Size of sclerotia (mm)**
Groundnut	90.0	427	2.1
Soybean	90.0	390	1.9
Pigeon pea	32.1	79	0.9
Green gram	39.1	272	0.7
Black gram	43.6	215	1.0
Sunflower	27.6	0	0.0
Sorghum	29.1	0	0.0
Pearl millet	22.1	0	0.0
Maize	33.3	126	1.1
Wheat	31.5	196	1.3
Onion	19.3	0	0.0
Garlic	17.1	0	0.0
Control	83.3	316	1.7
CD (p=0.05)	0.46	0.26	0.07

*Average of three replications **Average of 10 sclerotia

Table 2. Effect of crop residues on sclerotial germination, number and size of sclerotia of *Sclerotium rolfsii*

Crop residues	Sclerotial germination (%)*	Number of sclerotia*	Size of sclerotia (mm)**
Groundnut	81.7	978	1.3
Soybean	88.3	1105	1.0
Pigeon pea	57.8	669	0.6
Green gram	73.3	965	0.7
Black gram	62.3	871	0.5
Sunflower	15.2	18	0.5
Sorghum	27.3	51	0.8
Pearl millet	21.5	21	0.5
Maize	29.2	54	1.0
Wheat	47.5	60	1.3
Onion	19.2	18	0.6
Garlic	17.4	17	0.6
Control	61.0	578	0.9
CD (p=0.05)	0.62	1.46	0.13

*Average of three replications **Average of 10 sclerotia

of sclerotia was on the crop residue of sunflower (15.2%) followed by garlic (17.4%), onion (19.2%), pearl millet (21.5%) sorghum (27.3%) and maize (29.2%) compared to the control (61.0%). The rate of mycelial growth and sclerotial formation on these crop residues was also very slow. The highest percentage germination of sclerotia was on crop residue of soybean (88.3%) followed by groundnut (81.7%), green gram (73.3%), black gram (62.3), and pigeon pea (57.8%). The rate of mycelial growth was also faster in these crop residues covering the entire surface of crop residue with higher number of sclerotia produced on them. The number of sclerotia produced by of *S. rolfsii* was significantly higher in crop residues of black gram, green gram, groundnut and soybean. After 20 days of incubation, the number of sclerotia produced on these varied from 669 to 1105 as compared to control (578). The size of sclerotia produced varied from 0.5 to 1.3 mm. Significantly bigger sclerotia than control (0.9 mm) were in crop residues of groundnut (1.3 mm), and wheat (1.3 mm). The population of sclerotia determines the inoculum load in soil and if this is reduced stem rot incidence can be reduced. During the decomposition of crop residues various volatile compounds are released which may have differential effects on the sclerotia. Linderman and Gilbert (1969) had reported that volatile compound emanating from alfalfa hay residues stimulated germination of sclerotia of *S. rolfsii* in soil followed by colonization of the residues.

Effect of crop residues on incidence of stem rot

The results revealed that the percentage of seedling was higher in soil amended with crop residues of sunflower, garlic, onion, pearl millet, sorghum and maize after 30 days of sowing (DAS) having zero incidence of stem rot compared to 10% incidence in the control (Table 3). Surface soil completely colonized with white cottony mycelium of *S. rolfsii* was evident after 15 DAS. The percent incidence of stem rot

Table 3. Effect of crop residues on incidence of stem rot

Crop residues	Incidence of stem rot (%)*		
	30 DAS	45 DAS	60 DAS
Groundnut	10.0	46.7	96.7
Soybean	16.7	83.3	100.0
Pigeon pea	3.3	60.0	86.7
Green gram	6.7	60.0	80.0
Black gram	6.7	63.3	83.3
Sunflower	0.0	3.3	3.3
Sorghum	0.0	6.7	13.3
Pearl millet	0.0	3.3	6.7
Maize	0.0	13.3	23.3
Wheat	3.3	50.0	63.3
Onion	0.0	3.3	6.7
Garlic	0.0	3.3	3.3
Control	10.0	70.0	93.3
CD (p=0.05)	0.25	0.50	0.53

*Average of three replications; DAS: Days after sowing

at 45 DAS and 60 DAS were also the lowest in pots which were amended with crop residues of sunflower, garlic, pearl millet, onion and sorghum. The incidence of stem rot ranged from 3.3 to 13.3% in these treatments compared to 70.0 to 93.3% in control at 45 and 60 DAS. The highest incidence of stem rot was recorded in soybean and groundnut amended pots (83.3 to 100% and 46.7 to 96.7% respectively) at 45 and 60 DAS. Soil amended with crop residues of sunflower, garlic, onion, pearl millet, sorghum and maize not only reduced the percentage incidence of stem rot but also affected plant height, number of flowers and pegs positively.

The present investigation clearly indicated that crop residues of sunflower, garlic, onion, pearl millet, sorghum had inhibitory effect on the various parameters studied while soybean and groundnut had stimulatory effect favouring the disease. Lack of stimulation in germination of sclerotia and formation of new sclerotia in treatments with sunflower, garlic, onion, pearl millet, sorghum and maize might be associated with the presence of antagonists or liberation of toxic or fungistatic compounds on decomposition. Beute and Rodriguez-Kabana (1979) had reported stimulation of mycelial growth induced by kudzu (*Pueraria lobata*) residues. These authors concluded that stimulation was due to the liberation of volatile compounds released by decomposition of amendments. In the present studies, germination of sclerotia was similarly stimulated by some amendments. Kokalis-Burelle and Rodriguez-Kabana (1994) and Soler *et al.* (1996) had reported a positive correlation between organic amendments and microbial activities.

Crop rotation is an important factor affecting incidence of stem rot. Deep burial of surface organic matter and crop debris by ploughing it to a depth of 8 to 10 inches during land preparation improved yield of groundnuts largely due to

elimination of food base of the pathogen and subsequent reduction of disease incidence (Garren, 1961; Young, 1967). Bicici *et al.* (1994) reported that in groundnut + maize or wheat rotation, incidence of stem rot was 50% less than in groundnut monoculture. Asghari and Mayee (1991) also reported that onion and garlic crop rotation with groundnut reduced stem rot incidence.

Effect of crop root exudates on germination of sclerotia

Out of twenty crop root exudates, significant reduction in germination of sclerotia was observed by root exudates of twelve crops *viz.*, sorghum, pearl millet, maize, wheat, garlic, onion, sesame, sunflower, safflower, mustard, cotton and castor at concentration 10, 15 and 20%. There was progressive reduction in sclerotial germination with increase in concentration from 10 to 20%. Hundred percent sclerotial germination was observed at 5% concentration (Table 4). The inhibition observed at 20% concentration of crop residue ranged between 21.3-72.7%, highest being in pearl millet followed by sunflower (72.0%) and maize (69.0%). The root exudates of soybean, pigeon pea, green gram, black gram, chick pea, pea, cow pea and groundnut had no effect on sclerotial germination.

Effect of crop root exudates on mycelial growth

The root exudates of twelve crops *viz.*, sorghum, pearl millet, maize, wheat, garlic, onion, sesame, sunflower, safflower, mustard, cotton and castor had significant but varied level of inhibitory effect on mycelial growth of *S. rolfsii* (Table 4). Inhibition of mycelial growth was observed even at 5% concentration. The percent inhibition of mycelial growth at 20% concentration varied from 10.0 to 72.2, highest being in maize followed by sunflower (70.0) and pearl millet (66.7) and the lowest in mustard.

The rotational crops which inhibited germination of sclerotia also inhibited mycelial growth. Zeidan (1986) had reported that onion bulb extract or root exudates inhibited both sclerotial germination and mycelial growth of *S. rolfsii*. Rao *et al.* (2002) had also reported that sorghum root exudates were the most effective in inhibiting mycelial growth and sclerotial germination. Abd-El-Moneem *et al.* (2003) reported that the types of sugar and amino acids in the root exudates, particularly the content of arabinose, glutamic acid, lysine, serine and tryptophan in cultivars of groundnut were responsible for resistance to *Fusarium oxysporum* and *S. rolfsii*. Inhibition of sclerotial germination decreased the inoculum potential of the fungus and disease incidence. Results of this study clearly indicated that the rotational crops *viz.*, sorghum, pearl millet, maize, wheat, cotton, castor, sesame, sunflower, safflower, mustard, garlic and onion adversely affected growth of *S. rolfsii* and reduced its inoculums by way of inhibiting sclerotial germination. If such crops are taken in the groundnut production system will certainly be helpful in managing stem rot disease of groundnut caused by *S. rolfsii*. It is plausible that root exudates of cereals may lead to the death of sclerotia of *S. rolfsii* in soil either directly or through the activities of antagonistic microorganisms which they support.

Table 4. Effect of root exudates of crop on sclerotial germination and mycelial growth of *Sclerotium rolfsii* at different concentrations

Root exudates	Germination of sclerotia (%)*				Colony diameter (mm)*			
	5%	10%	15%	20%	5%	10%	15%	20%
Sorghum	100	84	82	41	89	86	82	48
Pearl millet	100	68	45	27	87	81	55	30
Maize	100	57	34	31	86	80	53	25
Wheat	100	67	73	79	89	89	86	79
Garlic	100	55	56	57	83	83	82	59
Onion	100	75	63	51	89	84	80	49
Sesame	100	77	67	75	90	90	89	80
Sunflower	100	52	37	28	87	81	55	27
Safflower	100	49	61	47	88	83	69	47
Mustard	100	76	67	77	90	90	81	81
Cotton	100	79	69	69	90	89	84	73
Castor	100	88	81	68	90	89	80	69
Groundnut	100	100	100	100	90	90	90	90
Soybean	100	100	100	100	90	90	90	90
Pigeon pea	100	100	100	100	90	90	90	90
Green gram	100	100	100	100	90	90	90	90
Black gram	100	100	100	100	90	90	90	90
Chick pea	100	100	100	100	90	90	90	90
Pea	100	100	100	100	90	90	90	90
Cow pea	100	100	100	100	90	90	90	90
Control	100	100	100	100	90	90	90	90
CD (p=0.05)	-	0.19	0.48	0.50	0.19	0.14	0.22	0.43

*Mean of four replications

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