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ANTIFUNGAL POTENTIAL OF WEED BIOMASS AGAINST SCLEROTIUM ROLFSII SACC. CAUSING STEM ROT OF GROUNDNUT

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ABSTRACT
Groundnut is attacked by over 55 pathogens including viruses. Among these, stem rot caused by *Sclerotium rolfsii* Sacc. is one of the most devastating diseases of Groundnut. Control of this fungus is difficult as it does not produce asexual spores and overwinters as sclerotia on plant debris and in soil. Various methods of control have been investigated but often cannot economically and consistently control stem rot. Considering these facts, the present investigation was undertaken to study efficacy of common, dominant and easily available plants i.e., weeds in the crop fields against *Sclerotium rolfsii* Sacc. in a cost-effective and eco-friendly manner. Among all the test weed plants, *Commelina benghalensis* L. leaf, stem and root extracts were found to be effective against *Sclerotium rolfsii.* Among all concentrations (1 %, 5 % and 10 %) of Aqueous and Ethanolic extracts of leaf, stem and root, 10 % Aqueous and Ethanolic extracts of leaf, stem and root of the test weed was found to be most effective. The 10 % Aqueous and Ethanolic extracts of leaf, stem and root inhibited 100 % mycelial growth of the test pathogen. However, among all other test plants, *Tridex procumbens* L. was found least inhibitory against test pathogen.

KEYWORD: Weeds, Groundnut, *Sclerotium rolfsii*, Stem rot.

INTRODUCTION
The peanut (*Arachis hypogaea* Linn.), better known as Groundnut is considered as most important crop in the World (13th) and is most important source of edible oil (4th). It is recognized as a palatable "poor man's nut" and is eaten and relished by all classes of people (Smith, 2002).

Groundnut is attacked by over 55 pathogens including viruses. The important one among them responsible for huge losses in yield are collar rot, charcoal rot, wilt and stem rot. Among these diseases, stem rot caused by *Sclerotium rolfsii* Sacc. is one of the most devastating diseases of Groundnut and is known to render Groundnut production highly unstable (Misra and Ghewande, 1989). Control of this fungus is difficult as it does not produce asexual spores and overwinters as sclerotia on plant debris and in soil.

Various methods of control have been investigated including genetic control, chemical control, cultural practices and biological control (Singh *et al.*, 2009). Crop rotation, sanitation, reduced irrigation, resistant varieties, protectant chemicals often cannot economically and consistently control stem rot caused by *Sclerotium rolfsii*. Disease control by fungicides is one of the promising strategies. However, use of fungicides poses certain problems of residues left over on crops, which has gained considerable importance in recent years, not only in India but also throughout the world.

Today there is a global search for alternatives to chemical fungicides, as part of this process various efforts have been carried out to use and apply natural products for disease control and crop production. Many plants and plant products have been known for their medicinal and antifungal properties since ancient times (Kuntal Das *et al.*, 2010).

Considering these facts the present investigation was undertaken to study efficacy of common, dominant and easily available plants i.e. weeds in the crop fields against the soil borne pathogen *Sclerotium rolfsii* Sacc. in a cost-effective and eco-friendly manner.

MATERIALS AND METHODS
1. Collection of infected plant parts
Diseased Groundnut plants (variety TAG-24) showing typical symptoms of stem rot i.e., wilting of total plants, white mycelial growth at collar region of plant were collected from the fields of study area.

2. Isolation and identification of pathogen causing stem rot
Diseased samples showing typical symptoms of stem rot were selected and used as sample source for the isolation

of causative agent. For the isolation of plant pathogen, method given by Appolinaire, (2004) was followed.

Identification and cultural characters of *S. rolfsii* Sacc. were confirmed with the help of ICRSAT Information Bulletin No. 36, (1992) and Mesquita, (2007).

3. Pathogenicity test of *S. rolfsii* Sacc.

i) Mass production of *Sclerotium rolfsii* Sacc.

Mass multiplication of *S. rolfsii* was carried out in Potato Dextrose broth at room temperature for 3 weeks (Ordentlich *et al.*, 1987) and then the numbers of sclerotia produced were used for the preparation sick pots.

ii) Preparation of sick pots

Sterilized soil sand (1:1) mixture was artificially infested with sclerotia of *S. rolfsii* at 1 sclerotia/ gm soil (Fouzia Yaqub and Saleem Shahzad, 2005). Six pots were disinfected with 5 % $CuSO_4$ solution and out of 6 pots, three pots were filled with inoculated soil at 150 gm soil per pot and three pots filled with sterilized uninoculated soil, were maintained as control. The pots containing inoculum were incubated for 15 days at room temperature, frequently watered for colonization of fungus in the soil. Surface disinfected seeds of Groundnut variety, TAG-24 were sown in plastic pots. All these pots were kept at room temperature and watered regularly. Observations were recorded on germination and mortality of the plants. This experiment was conducted in triplicates.

4. Studies on antifungal activity of plant extracts against *Sclerotium rolfsii* Sacc.

i) Preparation of plant extracts

For the preparation of plant extracts the methods described by Kuntal Das, (2010) was adopted. Accordingly, fresh samples were washed in tap water and finally washed thrice using sterilized distilled water. They were crushed in a pestle and mortar by adding a little quantity of sterile distilled water just enough to crush the sample easily. The extracts were collected by filtering through the two layers of muslin cloth. The filtrates thus obtained from were used as stock solution (Kuntal Das, 2010).

In order to study the antifungal activity of plant extract, poisoned food technique was followed as suggested by Grover and Moor, (1962).

The stock solution prepared were used at three concentrations (1 %, 5 % and 10 %) prepared by mixing aseptically 1 ml, 5 ml and 10 ml of stock solution in 100 ml semisolid sterilized potato dextrose agar medium (Tiwari *et al.*, 2005) and the PDA plates were prepared. PDA plates without supplementation of plant extracts served as control.

Each plate was seeded with 5 mm mycelial discs aseptically taken from the periphery of 7 days old culture and incubated at room temperature till the growth of the colony touched the periphery in control plate. Mean colony diameter in each case was recorded. Three replications were maintained for each treatment.

ii) Efficacy of weed extracts against *Sclerotium rolfsii* Sacc.

The efficacy of weed extracts was expressed as per cent inhibition of mycelial growth over control which was calculated by using the formula as given by (Verma and Kharwar, 2006).

$$\% \text{ inhibition} = \frac{\text{Mycelial growth (control)} - \text{Mycelial growth (treatment)}}{\text{Mycelial growth (control)}} \times 100$$

5. Statistical analysis

Statistical analysis was done as per the method described by Panse and Sukhatme, (1978).

RESULTS AND DISCUSSION

The entire research work is divided into two parts. The first part deals with collection, isolation, identification and pathogenicity of *Sclerotium rolfsii* Sacc. and the second part is devoted for evaluation of plant extracts against *Sclerotium rolfsii* Sacc.

Part: I

A. Studies on Isolation, Identification and Pathogenicity tests of *Sclerotium rolfsii* Sacc.

1. Collection of infected plant parts

Diseased samples were collected from different locations of Parbhani district of Marathwada region and brought to laboratory, Department of Botany, B. Raghunath Arts, Commerce and Science College, Parbhani in poly-ethylene bags (Photo plate 1).

2. Isolation and identification of pathogen causing stem rot or wilt

For this purpose, infected groundnut plants were used. Diseased samples showing typical symptoms of stem rot i.e. wilting of total plants, white mycelial growth at collar region of plant were selected and used as sample source for the isolation of causative agent (Photo plate: 1). Infected portion of stem was cut into small pieces with sterilized scalpel, cleaned with distilled water, then surface sterilized with 0.1% $HgCl_2$ solution for 30 second and again washed thrice with sterile distilled water. Small 1 to 2 pieces were transferred aseptically on Potato Dextrose Agar (PDA) plates containing Chloramphenicol (30 mg/100 ml) with the help of sterilized forceps under aseptic condition (Appolinaire,

2004). Inoculated Petri plates were incubated at 25±2°C for 5-7 days for growth of the pathogen.

After 7 days incubation on PDA plates, it was observed that the fungus produced abundant white septate mycelium, 1.5–3.0 µm diameter with clamp connections at each septation, aerial hyphae and also numerous spherical, or ellipsoidal, white sclerotia turning brown on maturation. Based on these morphological and cultural characteristics, the disease causing organism was identified as *Sclerotium rolfsii* Sacc. (Mesquita *et al.,* 2007). The results are presented in Photo plate 2.

3. Pathogenicity Tests of *Sclerotium rolfsii* Sacc.

During the present studies the sterile soil and sand mixture (1:1) was infested with sclerotia of the test pathogen filled in plastic pots. The surface sterilized seeds of the test variety of groundnut (TAG-24) were sown separately in the pots. Similarly, the pots filled with sterilized uninoculated soil and sand mixture (1:1) served as control. The pots were incubated at room temperature with regular watering for thirty days. The results are presented in Photo plate- 3.

It was observed that in the test variety, isolated strain of *Sclerotium* showed reduction in percent germination. Amongst the germinated seeds, initially yellowing of basal leaves was evident followed by drooping of leaves and wilting of plant. Some of the plants remained upright (Photo plate: 3 and 4). It was also observed that white mycelium and small sclerotia were present at the base of infected plants (Photo plate: 3 and 4). While uninoculated plants (control) remained healthy and free of signs and symptoms of disease. Among the infected plants mortality was calculated and was found to be 70.30 per cent.

To prove Koch's postulates, the diseased plants were removed and infected portion of stem was cut into small pieces and was used further for isolation of causative agent of the disease on PDA using standard microbiological method. The result obtained revealed that the isolated organism when compared with inoculated strain was found to be same. These results are indicative of the fact that *Sclerotium rolfsii* Sacc. used for artificial inoculation was a potent pathogen of Groundnut showing typical symptoms of stem rot or wilt.

Part: II

B. Evaluation of plant extracts against *Sclerotium rolfsii* Sacc.

This part of the work includes studies on antifungal activity of selected plant extracts against *Sclerotium rolfsii* Sacc.

1. Selection of plants to study antifungal activity against *Sclerotium rolfsii* Sacc.

During the studies on survey of incidence of *Sclerotium* wilt in the study area, it was observed fact that weeds produce a huge biomass. This fact promoted to select these weed plants to study their antifungal activity against *Sclerotium rolfsii* Sacc. Hence some common, dominant weed plants were selected to study antifungal activities which are mentioned in table 1.

2. Antifungal activity of plant extracts against *Sclerotium rolfsii* Sacc. **by Poisoned food technique.**

From the results presented in table 2 and Photo plate 5, it was observed that among all the test weed plants, *Commelina benghalensis* L. leaf, stem and root extracts were found to be effective against *Sclerotium rolfsii.* Among all concentrations (1 %, 5 % and 10 %) of Aqueous and Ethanolic extracts of leaf, stem and root, 10 % Aqueous and Ethanolic extracts of leaf, stem and root of the test weed was found to be most effective. The 10 % Aqueous and Ethanolic extracts of leaf, stem and root inhibited 100 % mycelial growth of the test pathogen.

However, among all other test plants, *Tridex procumbens* L. was found least inhibitory against test pathogen.

3. Efficacy of weed extracts against mycelial growth of *Sclerotium rolfsii* Sacc. **by poisoned food technique.**

The efficacy of weed extracts was expressed as per cent inhibition of mycelial growth over control which was calculated by using the formula as given by (Verma and Kharwar, 2006).

$$\% \text{ inhibition} = \frac{\text{Mycelial growth (control)} - \text{Mycelial growth (treatment)}}{\text{Mycelial growth (control)}} \times 100$$

The results are presented in table 3. From the results it is was observed that 10 % Aqueous and 10 % ethanolic extracts of leaf, stem and root of *Commelina benghalensis* L. were found to be most effective against *Sclerotium rolfsii.* Both the extracts showed maximum per cent mycelia inhibition (95 %) followed by 5 % Aqueous leaf extract of *Commelina benghalensis* L. (92 %).

Among the all test weeds, leaf and stem extracts of *Tridex procumbens* L. and *Euphorbia hirta* L. were found to be least effective in all concentrations and in both aqueous and ethanolic extracts.

Table 1: List of selected weed plants to study antifungal activity against *Sclerotium rolfsii* Sacc.

Sr. No.	Name of Plant
1	*Argemone maxicana* L.
2	*Commelina benghalensis* L.
3	*Cynodon dactylon* (L.) Pers.
4	*Cyperus rotundus* L.
5	*Euphorbia hirta* L.
6	*Parthenium hysterophorus* L.
7	*Phyllanthus amerus* Schumach.& Thonn
8	*Portulaca oleracea* L
9	*Solanum nigrum* auct.
10	*Tridex procumbens* L.

Table 2: Antifungal activity of plant extracts against *Sclerotium rolfsii* Sacc. by poisoned food technique.

Sr. No.	Name of Plant	Part Used	Mean colony diameter (in mm)					
			Aqueous Extracts			Ethanolic Extracts		
			1%	5%	10%	1%	5%	10%
1	*Argemone maxicana* L.	Leaves	34	32	32	32	32	30
		Stem	35	34	35	35	35	32
2	*Commelina benghalensis* L.	Leaves	10	08	05	11	10	05
		Stem	11	09	05	12	09	05
3	*Cynodon dactylon* (L.) Pers.	Leaves	38	38	37	39	38	38
		Stem	39	38	38	39	39	38
4	*Cyperus rotundus* L.	Leaves	40	40	39	39	39	39
		Rhizome	39	38	38	39	39	38
5	*Euphorbia hirta* L.	Leaves	42	40	40	42	40	40
		Stem	44	42	42	45	44	44
6	*Parthenium hysterophorus* L.	Leaves	36	38	36	39	39	38
		Stem	40	38	38	40	40	40
7	*Phyllanthus amerus* Schumach.& Thonn	Leaves	45	45	44	42	41	40
		Stem	43	45	42	40	40	39
8	*Portulaca oleracea* L	Leaves	36	32	32	36	30	30
		Stem	39	39	35	38	35	32
9	*Solanum nigrum* auct.	Leaves	39	39	35	38	35	32
		Stem	39	39	35	38	35	32
10	*Tridex procumbens* L.	Leaves	49	48	50	50	50	48
		Stem	50	52	50	50	49	48
11	**Control**		**100**			**100**		
	Leaves	S.E. ±	1.78	1.50	1.91	1.23	2.03	1.37
		C.D. at 0.05%	5.24	4.42	5.63	3.64	5.98	4.04
	Stem	S.E. ±	2.14	1.69	2.09	2.09	1.91	1.54
		C.D. at 0.05%	6.31	4.98	6.17	6.16	5.63	4.53
	Commelina benghalensis L.	Root	10	09	05	11	10	05
	Control		**100**			**100**		

Table 3: Efficacy of plant extracts against mycelial growth of *Sclerotium rolfsii* Sacc. by poisoned food technique.

Sr. No.	Name of Plant	Part Used	Per cent mycelial inhibition					
			Aqueous Extracts			Ethanolic Extracts		
			1%	5%	10%	1%	5%	10%
1	*Argemone maxicana* L.	Leaves	66	68	68	68	68	70
		Stem	65	66	65	65	65	68
2	*Commelina benghalensis* L.	Leaves	90	92	95	89	90	95
		Stem	89	91	95	88	91	95
3	*Cynodon dactylon* (L.) Pers.	Leaves	62	62	63	61	62	62
		Stem	61	62	62	61	61	62

4	*Cyperus rotundus* L.	Leaves	60	60	61	61	61	61
		Rhizome	61	62	62	61	61	62
5	*Euphorbia hirta* L.	Leaves	58	60	60	58	60	60
		Stem	56	58	58	55	56	56
6	*Parthenium hysterophorus* L.	Leaves	64	62	64	61	61	62
		Stem	60	62	62	60	60	60
7	*Phyllanthus amerus* Schumach.& Thonn	Leaves	55	55	56	58	59	60
		Stem	59	58	58	60	60	61
8	*Portulaca oleracea* L	Leaves	64	68	68	64	70	70
		Stem	61	61	65	62	65	68
9	*Solanum nigrum* auct.	Leaves	61	61	65	62	65	68
		Stem	61	61	65	62	65	68
10	*Tridex procumbens* L.	Leaves	51	52	50	50	50	52
		Stem	50	48	50	50	51	52
11	**Control**			**00**			**00**	
	Leaves	S.E. ±	2.25	1.70	2.61	1.57	1.96	1.58
		C.D. at 0.05%	6.62	5.02	7.68	4.64	5.79	4.65
	Stem	S.E. ±	2.02	1.23	2.09	2.13	1.95	1.72
		C.D. at 0.05%	5.97	3.61	6.17	6.27	5.74	5.05
	Commelina benghalensis L.	Root	90	91	95	89	90	95
	Control			**00**			**00**	

Photo plate 1: Groundnut plant showing symptoms of stem rot.

Photo plate 2: Isolated *Sclerotium rolfsii* from infected Groundnut.

Photo plate 3: Pot assay for Groundnut var. TAG-24 showing symptoms of stem rot.

Photo plate 4: Groundnut var. TAG-24 showing wilting.

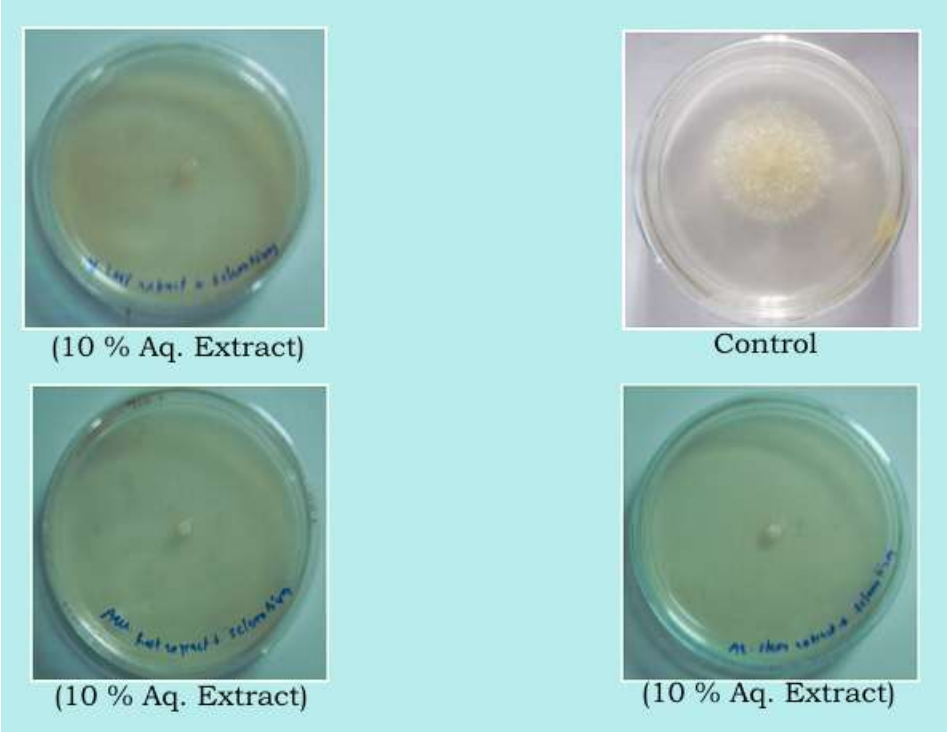

Photo plate 5: Antifungal Activity of *Commelina benghalensis* L. against *Sclerotium rolfsii* Sacc.

CONCLUSION

Survey to assess the incidence of stem rot or wilt need to be continued which may throw a light on hot spots for stem rot. Integrated management of the disease by employing components of management practices need to be done under field conditions. More extension work is needed for transfer of technology to farming community of the state. Developing resistant varieties against *S. rolfsii* either by following conventional breeding or transgenic is the need of the hour.

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