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Evaluation of plant extracts on mycelial growth and viability of the sclerotia of *Rhizoctonia solani* Kuhn *In vitro* and in soil

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Abstract
In the present study, efforts were made to explore the efficacy of plant extracts against *R. solani.* Efficacy of six extracts *viz*., neem, garlic, pongamia, datura, lantana and calotropis were tested. The neem (*A. indica*) leaf extract and clove extract of *A. sativum* recorded maximum mycelia growth inhibition by 85.18 and 72.59 percent respectively at 20 percent concentration. The viability of sclerotia was also tested and among them only two *viz*., neem and garlic were found to be effective in inhibiting sclerotial germination even at lower incubation period of 30 min. both in soil application and *in vitro*. Lantana and datura inhibited sclerotial germination at higher incubation periods *i. e.*, 18 h and 24 h. rest of the plant extracts tested were ineffective in inhibiting sclerotial germination.

Keywords: Sheath blight, sclerotia, *Rhizoctonia solani*

1. Introduction
Rice Sheath blight is caused by *Rhizoctonia solani* (Kuhn). This disease was first recorded from Japan (Miyake, 1910) [8]. The fungus produces brown sclerotia depending upon the environmental conditions. Sclerotia are superficial, more or less globose but flattened, white when young and becomes brown. Individual sclerotium measures upto 5mm but may unite to form large mass in culture (Ou, 1985) [9]. The fungus survives in the soil for years as hard, resistant structures. The sclerotial bodies float on the surface of the water in rice fields and when come in contact with the plant, initiates infection. The sclerotia survives for long periods and tend to accumulate in the soil (Lee and Rush, 1983) [7]. Therefore, the sclerotia of *R. solani* play an important role in the pathogen survival in rice fields. Phytochemicals derived from various bio-active plant species offer a promising and natural source of safer agrochemicals (Isman, 2006) [4]. Antifungal activity of plant extracts may be more effective than some commercial fungicides due to presence of naturally occurring substances in plants with antimicrobial properties that have been recognized and tested against a wide range of pathogenic microbes (Tamuli *et al*., 2014) [12]. In recent years, plant extracts mainly, neem derivatives are gaining importance for the control of plant diseases due to their antifungal and antibacterial properties (Yin and Cheng, 1998; Anil Sahejpal *et al.*, 2009) [13, 1].
Hence, in the present study plant extracts were used to evaluate their effect in controlling the germination of sclerotia.

2. Material and Methods
The present experiments were carried out in the Department of Plant Pathology, S.V. Agricultural College, Tirupati.

2.1 Preparation of plant extracts
Fresh test material from the above individual plant species was collected and washed thoroughly in tap water followed by washing in distilled water. The test material was ground with sterile water at the rate of 1g plant material in 1ml of water using a pestle and mortar and the macerate was filtered through a muslin cloth to get the crude extract.

2.2 Effect of plant extracts on the growth of *Rhizoctonia solani*
The extracts of each plant products at four concentrations were tested for its efficacy by poisoned food technique (Nene and Thapliyal, 1986) by measuring the radial growth of the fungus. The list of plant products with their concentrations used in the study are presented in the Table 1.

The Petri plate containing PDA medium inoculated with *R. solani* alone served as control. The Petri plates were incubated at room temperature (27±1 ºC). Three replications were maintained for each treatment. The inhibition of growth of the fungi was calculated by using the formula given below by Vincent (1927):

$$I = \frac{C - T}{C} \times 100$$

Where, I = Per cent reduction in growth of test pathogen, C = Radial growth (cm) in control, T = Radial growth (cm) in treatments.

2.3 Effect of plant extracts on the sclerotial viability of *R. solani in vitro*

For each treatment, plant extract was prepared according to the concentration given in the Table 2 using distilled water. Ten sclerotia of the test pathogen were taken for each replication and dipped into the respective herbicidal solution for 30 min, 6 h, 18 h, 24 h. Control was maintained by dipping sclerotial bodies in distilled water. Then the sclerotia were retrieved and placed on the PDA medium for testing their viability.

Experimental design used was CRD and three replications were maintained per treatment.

Table 1: List of the plant extracts and their concentration tested

S. No.	Botanical name	Plant part used	Concentration (%)
1.	*Azadirachta indica*	Leaves	20
2.	*Calotropis indica*	Leaves	20
3.	*Datura stramonium*	Leaves	20
4.	*Lantana camara*	Leaves	20
5.	*Allium sativum*	Cloves	20
6.	*Pongamia glabra* Vent.	Leaves	20
7.	Untreated control	-	-

Table 2: *In vitro* efficacy of plant extracts on mycelia growth of *Rhizoctonia solani*

S. No.	Plant extracts	Radial growth (cm)*				Percent Inhibition			
		Concentration of plant extract				Concentration of plant extract			
		5%	10%	15%	20%	5%	10%	15%	20%
1.	*Azadirachta indica*	3.16	1.86	1.43	0.66	29.62 (32.95)**	58.51 (49.88)	68.14 (55.61)	85.18 (67.34)
2.	*Pongamia glabra*	3.96	3.53	3.26	2.96	11.85 (20.11)	22.22 (28.11)	31.40 (34.34)	42.96 (40.93)
3.	*Calotropis indica*	4.03	3.56	3.26	2.96	10.36 (18.75)	20.74 (27.07)	27.40 (31.53)	34.07 (35.69)
4.	*Lantana camara*	3.23	2.43	1.96	1.53	28.14 (32.02)	45.92 (42.64)	56.29 (48.59)	65.92 (54.26)
5.	*Datura stramonium*	3.52	2.83	2.53	1.76	21.62 (27.69)	37.03 (37.46)	43.70 (41.36)	60.92 (51.18)
6.	*Allium sativum*	2.76	1.90	1.70	1.23	38.51 (38.34)	57.77 (49.45)	62.22 (52.05)	72.59 (58.42)
7.	Control	4.50	4.50	4.50	4.50	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	CD					1.52	1.05	1.50	1.66
	SEm±					0.49	0.34	0.49	0.54
	CV					3.54	1.78	2.26	2.13

2.4 Effect of plant extracts on the viability of sclerotia of *R. Solani* mixed with the soil.

Dry soil of paddy field was used in this experiment. 10 g of soil was taken into plastic cups and ten sclerotia of the sheath blight pathogen were mixed with the soil. This is a unit representing a replication of a treatment. The plant extracts which were found effective in the previous experiments were chosen for evaluation. The respective plant extract was added to the plastic cup containing sclerotia and soil mixture upto saturation and incubated for 10 days. In control distilled water was added to the plastic cup containing sclerotia and soil mixture upto saturation. After 10 days the sclerotia were retrieved and placed on PDA medium for testing their viability. Per cent inhibition of sclerotial germination was calculated. (Harikrishnan and Yang, 2001) [3]

3. Results and Discussion

3.1 Effect of plant extracts on growth of *R. solani in vitro*.

The plant extracts, *viz.*, *Azadirachta indica*, *Calotropis indica*, *Datura stramonium*, *Lantana camara*, *Allium sativum* and *Pongamia glabra* were tested against *R. solani* for their bio efficacy using poisoned food technique. The data presented in Table 3 revealed that all the extracts tested at 5, 10, 15 and 20 per cent concentrations were significantly superior to control in checking the mycelial growth of the fungus.

Table 3: *In vitro* efficacy of plant extracts on the sclerotial viability of *Rhizoctonia solani*

S. No	Plant extracts	Conc.	Per cent inhibition of sclerotia			
			30 min	6 h	18 h	24 h
1	*Azadirachta indica*	20%	60.00 (50.74)	90.00 (71.53)	100.00 (90.00)	100.00 (90.00)
2	*Pongamia glabra* Vent.	20%	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	30.00 (33.19)
3	*Calotropis indica*	20%	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	10.00 (18.42)
4	*Lantana camara*	20%	36.66 (37.21)	56.66 (48.82)	60.00 (50.74)	70.00 (56.76)
5	*Datura stramonium*	20%	26.66 (30.98)	50.00 (44.98)	53.33 (46.90)	63.33 (52.75)
6	*Allium sativum*	20%	70.00 (56.76)	100.00 (90.00)	93.33 (77.69)	100.00 (90.00)
7	Control	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	CD		3.45	2.22	7.46	2.32
	SEm±		1.12	0.72	2.43	0.75
	CV%		7.79	3.44	11.13	2.69

Figures in parentheses are angular transformed values

All the plant extracts significantly inhibited the growth of *R. solani* compared to the control. Over all the concentrations, leaf extract of *A. indica* was found to be the most effective in inhibiting the growth of *R. solani* (85.18%) followed by clove extract of *A. sativum* (72.59%), leaf extracts of *L. camera* (65.92%) and *D. stramonium* (60.92%). The leaf extracts of *P. glabra* (42.96%) and *C. indica* (34.07%) recorded the least mean inhibition of *R. solani* growth at 20 per cent concentration.

The plant extracts at 20 per cent concentration brought about the highest inhibition of growth followed by the extracts at 15 per cent concentration. The extracts at 5 per cent concentration recorded the lowest inhibition of growth. Increase in the concentration of the extract increased inhibitory effect on the growth of *R. solani*.

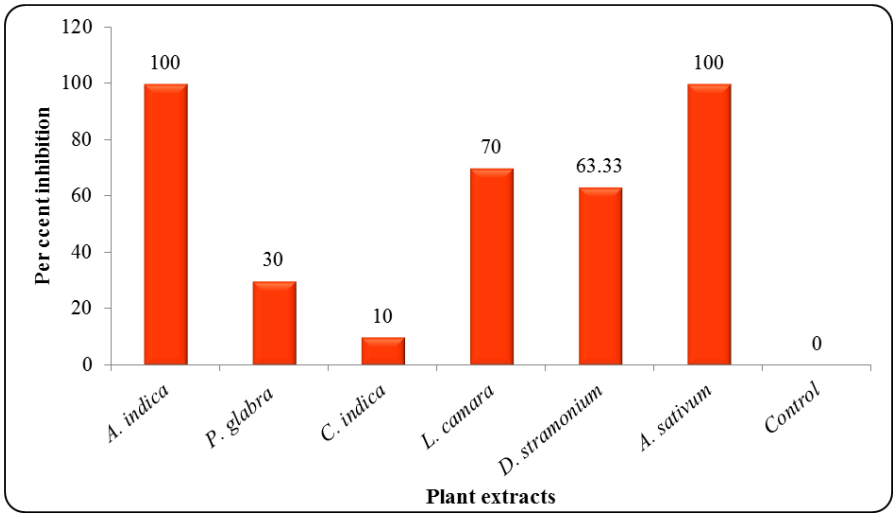
Many reports are available on the efficacy of plant extracts inhibiting the mycelial growth and sclerotial germination of *R. solani* and other soil borne pathogens. Generally it was found that higher the concentrations of extracts, greater will be the inhibition of mycelial growth and sclerotial germination (Patole and Narture, 2011, Anil Sehajpal *et al.*, 2009, Kane *et al*., 2002, Prasad *et al*., 1998, Shivapuri *et al*., 1997) [10, 1, 5, 11].

3.2 Effect of plant extracts on the sclerotial viability of *R. solani in vitro.*

Among all the treatments, *A. indica* showed the (100%) inhibition of sclerotial germination at 18 h and 24 h of incubation followed by (90%) at 6 h and (60%) at 30 min of incubation. *A. sativum* showed the (100%) inhibition of sclerotial germination at 6 h and 24 h of incubation followed by (93%) at 18 h and (70%) at 30 min of incubation.

Leaf extract of *L. camara* showed (70%) inhibition of sclerotial germination at 24 h and followed by (60%) inhibition at 18 h incubation, (57%) at 6 h and however was ineffective in inhibiting the sclerotial germination at 30 min (37%). *D. stramonium* showed (63%) inhibition of sclerotial germination at 24 h incubation followed by (53%) inhibition at 18 h incubation, (50%) at 6 h and however was ineffective in inhibiting the sclerotial germination at 30 min (27%). Increase in incubation period increased inhibition in leaf extracts of *D. stramonium* and *L. camara*. Leaf extracts of *C. indica* and *D. stramonium* are the least effective in inhibiting both mycelial growth and sclerotial germination. The results are presented in the Table 3, Graph 1 and Fig 2.

Fig 1: Mature sclerotia of *R. solani in vitro*



Graph 1: *In vitro* efficacy of plant extracts on the sclerotial viability of *Rhizoctonia solani* at 24 h incubation

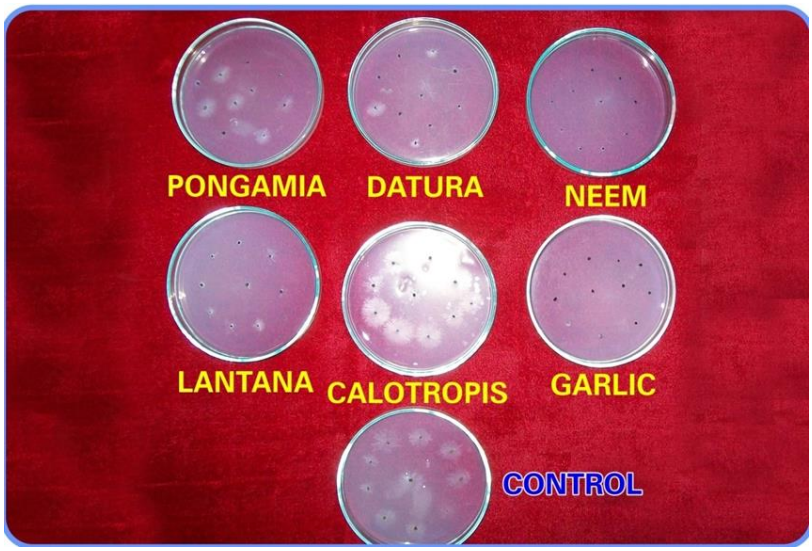


Fig 2: *In vitro* efficacy of plant extracts on sclerotial viability at 24 h incubation period

Karthika *et al.* (2017) [6] reported that the mycelilal regeneration from sclerotia was completely inhibited by soaking of sclerotia at 72 hours in 10 per cent of *A. sativum* extract. The findings pertaining to clove extract of *A. sativum* on inhibition of sclerotial germination was in agreement with the present results.

In the present study plant extracts at 20 per cent concentration exhibited different inhibitory effects on *R. solani*. However, soaking of sclerotial bodies for longer period had certainly resulted in increased inhibitory effect and thus exposure of sclerotia to the extracts for longer periods will reduce sclerotial viability. This is in agreement with the studies conducted by Dubey *et al.*, 2009 on the extracts of neem cake, leaves and bark against *M. phaseolina* where maximum inhibition of sclerotial germination was noticed after 96 hours of incubation.

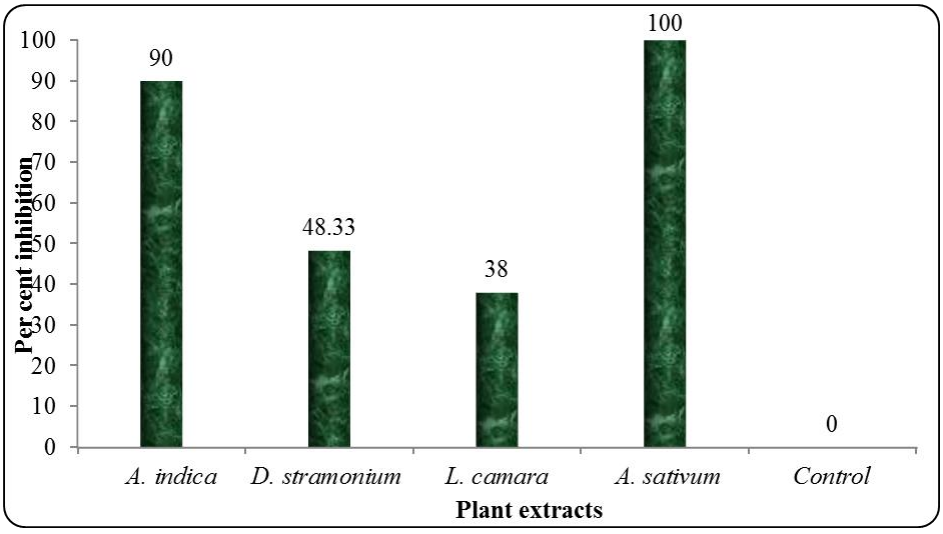
3.3 Effect of different plant extracts on the sclerotial viability of *R. solani* in soil.

The effective plant extracts found in the previous experiment *i. e., A. stivum*, *D. stramonium*, *A. indica*, *L. camara* were selected for this experiment. Dried paddy soil was taken in plastic cups and ten sclerotia of *R. solani* were mixed in the soil, plant extract was added to the soil up to saturation. After ten days the sclerotia were retrieved and subjected to germination test on PDA. The results are presented in the Table 4, Graph 2, and Fig 3.

Table 4: Efficacy of plant extracts on the sclerotial viability of *R. solani* in soil

S. No.	Plant extracts	Concentration	Percent germination of sclerotia	Per cent inhibition of sclerotia
1	*Azadirachtaindica*	20%	10.00 (18.42)	90.00 (71.53)
2	*Daturastramonium*	20%	50.16 (45.07)	48.33 (44.02)
3	*Lantanacamara*	20%	60.20 (50.86)	38.00 (38.02)
4	*Alliumsativum*	20%	0.00 (0.00)	100.00 (90.00)
5	Control	-	100.00 (90.00)	0.00 (0.00)
	CD		0.21	2.17
	SEm±		0.06	0.68
	CV%		0.28	2.42

Figures in parentheses are angular transformed values



Graph 2: Efficacy of soil application of plant extracts on the sclerotial viability of *Rhizoctonia solani*

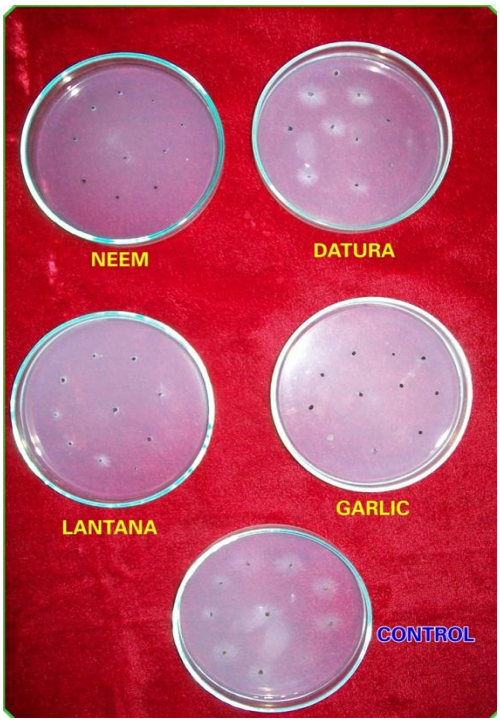


Fig 3: Efficacy of soil application of plant extracts on sclerotial viability at 10 days incubation period

All the treatments were significantly superior over control in recording low germination percentage of sclerotia of *R. solani* in soil. *A. stivum* was significantly superior to all other treatments by recording the lowest sclerotial germination (0.00%) followed by *A. indica* (10%), *D. stramonium* (50.16%) and *L. camara* (62%) at 15 per cent concentration Percent inhibition of sclerotial germination was recorded in the following order.

$T_5 < T_3 < T_2 < T_1 < T_4$

Dath (1981) [2] reported that soil amended with green manures the scleortia of *Corticum sasakii* lost viability in diancha amended soil followed by sunhemp. Whereas neem was ineffective in reducing the viability of sclerotia but, on increase in incubation period the viability of sclerotia reduced.

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