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In vitro evaluation of botanicals and organic amendments on mycelial growth and viability of the sclerotia of *Sclerotium rolfsii* Sacc.

VK Bagul, SN Hasabnis, AS Rothe and Satyam

Abstract
In the current investigation, the pathogen causing collar rot disease was isolated from chickpea-growing regions and identified as *S. rolfsii* by using morphological observations. *In vitro* efficacy of eight plant extracts and six organic amendments were tested against the mycelial inhibition and sclerotial viability. Efficacy of plant extracts *viz., L. inermis*, *O. tenuiflorum*, *A. indica*, *Vinca rosea*, *P. longifolia*, *M. longifolia*, *J. adhatoda* and *Z. officinale* and in oil cakes Mahua, neem, groundnut, Karanja, sunflower and castor were tested. Eight plant extracts and six organic amendments were evaluated for their *in vitro* efficacy against sclerotial viability and mycelial inhibition. All the tested concentrations, the 20% concentration was shown to be significantly better than the 5 and 10% concentrations. All the plant extract tested, *O. tenuiflorum* (tulsi) at (20% conc.) found that the highest mycelium inhibition (75.12%) of pathogen under *in vitro* condition. All the organic amendments tested, Mahua oil cake at 20% concentration was shown to be the most effective organic amendment in terms of highest mycelial inhibition (83.70%) of pathogen under *in vitro* conditions. Additionally, viability of sclerotia was evaluated at incubation period of 30 minute, 8 hours, and 24 hours of incubation. All the treatment tested at 20% conc. *O. tenuiflorum* (tulsi) revealed that the, total inhibition of sclerotial germination (100%) at 8 and 24 hours as well as 83.33% at 30 minute of incubation. In oil cakes, the sclerotial germination was totally inhibited by mahua oil cake extracts is 100% at 18 and 24 hours and at 30 minutes, it was 84.44%.

Keywords: Sclerotia, plant extracts, organic amendments, viability

Introduction
Sclerotium rolfsii (Sacc.) is a facultative parasite that is a polyphagous, ubiquitous and most destructive soil borne fungus. It mostly occurs in the tropics, sub-tropics and warm temperate regions of the world. It has wide host range, about 500 plant species including nearly all agricultural and horticultural crops affected by this fungus (Aycock, 1966) [1]. It causes a wide range of diseases, including foot rot, stem blight, root rot, collar rot, sclerotium wilt, seedling blight, damping off, and blight on valued crops. This fungus frequently attacks lower stems which are near to the soil surface or the collar region, but it can infect any part of a plant that is susceptible if the favorable environmental conditions were met. Wilt is the first symptom. Wilted plants usually weaken quickly and die. Every stage of a crop growth is severely damaged by this disease. Although yield loss is typically 25%, it can occasionally reach 80–90% in certain situations when there is moisture condition. (Grichar and Bosweel, 1987) [5]. The pathogen when host plant is not available, the pathogen thrives as a saprophyte on plant debris, including that of non-host crops and creates overwintering structures known as sclerotia. (Punja, 1985) [11]. As a result, sclerotia is important for survival. A natural and potential source of safer agrochemicals is phytochemicals, which are obtained from a variety of bioactive plant species (Isman, 2006) [6]. Plant extracts and oil cakes have antifungal properties that may make them more successful at controlling soil-borne pathogens than some commercial fungicides or pesticides. These products can have harmful side effects, such as residual toxicity, the development of pathogen resistance and pollution of the soil, water and air. In addition, these pesticides have the potential to destroy beneficial species and leave harmful residues in the soil, which can lead to an increase in pathogen resistance to synthetic chemicals. The inherent antimicrobial compounds found in plants give plant-based treatments their preventive, curative and antagonistic actions against various kinds of diseases. Therefore, the goal of the current study was to assess the antifungal effectiveness of oil cakes and plant extracts against *S. rolfsii*, caused chickpea collar rot disease.

Materials and Methodology

The current studies were conducted at the Mahatma Phule Krishi Vidyapeeth in Rahuri, Maharashtra, in the Department of Plant Pathology.

Efficacy of botanicals against the mycelial growth and viability of sclerotia of *S. rolfsii*

Preparation of plant extracts

Fresh test material from each species of plant was collected and properly washed in tap water before it was washed in distilled water. Using a mortar and pestle, the test material was crushed with distilled water. The ground material was passed through three layers of muslin cloth. For getting 1:2 (w/v) ratio in 100 g plant parts, 200 ml of distilled water was added. A detailed list of the botanicals utilized in the experiment, along with their respective concentrations presented in Table 1.

Efficacy of plant extract against the mycelial growth of *S. rolfsii*

The effect of plant extracts evaluated on mycelial growth of *S. rolfsii*. Through the use of the poisoned food technique (Nene and Thapliyal, 1986) [10], the radial growth of the fungus was measured to assess the effectiveness of extracts of each treatment at three different concentrations *i.e.*, 5, 10, and 20%. The Petri plate with PDA medium inoculated with *S. rolfsii* alone served as control. The Petri plates were kept for incubated at room temperature (27±1°C). Three replications were maintained. The growth inhibition of the fungi was calculated by using the formula given below by Vincent (1927) [13]:

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

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

Effect of plant extracts on the viability of sclerotia of *S. rolfsii in vitro*

For each treatment, plant extract was prepared of 20% conc. given in the Table 1 using distilled water. For each replication, ten sclerotia of the test pathogen were retrieved and immersed in the appropriate solution for 30 minutes, 8 hours and 24 hours. Control was maintained, by immersing the sclerotial bodies in distilled water. After that, the sclerotia were removed and place on the PDA medium to see whether they would still survive. The formula used to calculate the percentage of sclerotia germination was as follows:

$$(\%)\ \text{Germination of sclerotia} = \frac{\text{No. of sclerotia germinated}}{\text{Total no. of sclerotia added in cup/plate}} \times 100$$

Table 1: Botanicals and their concentration tested

Tr. No	Treatments	Plant part	Conc. (%)
T1	*Lawsonia inermis* (Heena)	Leaf	5, 10, 20
T2	*Ocimum tenuiflorum* (Tulsi)	Leaf	5, 10, 20
T3	*Azadirachta indica* (Neem)	Leaf	5, 10, 20
T4	*Vinca rosea* (Periwinkle)	Leaf	5, 10, 20
T5	*Justicia adhatoda* (Adulsa)	Leaf	5, 10, 20
T6	*Madhuca longifolia* (Mahua)	Seed	5, 10, 20
T7	*Polyalthia longifolia* (Ashoka)	Leaf	5, 10, 20
T8	*Zingiber officinale* (Ginger)	Rhizome	5, 10, 20
T9	Control	-	-

Efficacy of organic amendments against the mycelial growth and viability of sclerotia of *S. rolfsii*

Preparation of oil cake extracts

For each treatment, 100 grams of oil cakes (cold water extract) were taken and ground into a powder. After being soaked at a ratio of 1 g to 1.25 ml of sterile distilled water, it was allowed to stand overnight. After that, the material was grounded with a pestle and mortar. It was then filtered through two layers of muslin cloth and the filtrate was centrifuged for fifteen minutes at 10,000 rpm. The supernatant served as the standard oil cakes extract solution (100%).

2.2.1

Table 2 contains a list of the specifics of the organic amendments utilized in the experiment, along with their concentrations.

Efficacy of oil cakes against the mycelial growth of *S. rolfsii in vitro*

2.2.2

The effect of organic amendments on mycelial growth of *S. rolfsii*. Through the use of the poisoned food technique (Nene and Thapliyal, 1986) [10], the radial growth of the fungus was measured to assess the effectiveness of extracts of each plant component at three different concentrations *i.e.*, 5, 10, and 20%. The Petri plate with PDA medium inoculated with *S. rolfsii* alone served as control. The Petri plates were kept for incubated at room temperature (27±1 °C). Three replications were maintained for each treatment. The formula is mentioned above was used to calculate inhibition of growth of the fungi.

Effect of oil cake extracts against the viability of sclerotia of *S. rolfsii in vitro*

The oil cake extracts of each treatment evaluated at the 20% concentration shown in Table 2. For each replication, ten sclerotia of the test pathogen were retrieved and immersed in the appropriate solution for 30 minutes, 8 hours, and 24 hours. Sclerotial bodies were kept in control plate by soaking them in distilled water. After that, the sclerotia were removed and placed on the PDA medium to see whether they would still survive. The percent germination of sclerotia was calculated.

Table 2: Organic amendments and their concentration tested

Tr. No	Treatments	Conc. (%)
T1	Mahua	5, 10, 20
T2	Neem	5, 10, 20
T3	Groundnut	5, 10, 20
T4	Karanja	5, 10, 20
T5	Sunflower	5, 10, 20
T6	Castor	5, 10, 20
T7	Control	-

Results and Discussion

Efficacy of plant extracts against the mycelial growth inhibition of *S. rolfsii*: The plant extracts, *viz., L. inermis, O. tenuiflorum, A. indica, Vinca rosea, P. longifolia, M. longifolia, J. adhatoda* and *Z. officinale* were tested against *S. rolfsii* for their bio efficacy by poisoned food technique. The results are shown in Table 3 and Plate 1 observed that, all the extracts tested at 5, 10 and 20 percent concentrations were significantly more effective than the control.

Among the three concentrations, the 20% conc. of all plant extracts was shown to be significantly more effective than the 10% and 5% concentration. The least growth inhibition was observed in the extracts at a concentration of 5%.

An increase in extract concentration, increased inhibitory effect on the growth of *S. rolfsii*. A maximum of 75.12% inhibition of mycelial growth was seen in *O. tenuiflorum* at a concentration of 20% plant extracts. This was followed by *Z. officinale* (63.78%), *A. indica* (42.12%), *L. inermis* (36.56%), *J. adhatoda* (36.34%), *M. longifolia* (35.45%), and *P. longifolia* (34.78%). *Vinca rosea* showed the least inhibition (17.78%). This is in agreement with the studies conducted by, Begum *et al.* (2014) [2], who discovered that (74.81%) exhibited the significantly highest average inhibition (74.81%) among botanicals evaluated at 5 and 10% doses, followed by tulsi (67.10%) and nirgudi (65.81%). Similar results of aqueous extracts of plants on fungal mycelial growth on *S. rolfsii* Sacc reported by several researchers, Madhavi and Bhalliprolu (2011) [9], Kumar *et al.*, (2011) [8] and Kuldhar and Suryawanshi (2017) [7].

Effect of plant extracts on the viability of sclerotia of *S. rolfsii in vitro*

All the plant extract tested, *O. tenuiflorum* (tulsi) exhibited the highest percentage of inhibition of sclerotial germination (100%) at 18 and 24 hours of incubation and then (83.33%) at 30 minutes. *Z. officinale* (ginger) exhibited (100%) inhibition of sclerotial germination at 24 hours, followed by (80.00%) and (72.22%) reduction at 8 hours and 30 minutes. *A. indica* (neem) leaf extract inhibited sclerotial germination by 82.22% at 24 hours, 75.55% at 8 hours and 60.00% at 30 minutes. *M. logifolia* (mahua) demonstrated a 60.00% inhibition of sclerotial germination after 24 hours, followed by (36.66%) at 8 hours incubation period, (23.33%) at 30 min was ineffective in inhibiting the sclerotial germination. *J. adhatoda* (Ashoka) found that (52.44%) inhibition of sclerotial germination at 24 hours incubation followed by (22.22%) inhibition at 8 hours incubation, (15.55%) at 30 minutes was ineffective in inhibiting the sclerotial germination. Leaf extracts of *L. inermis* (Heena), *V. rosea* (Periwinkle) and *P. longifolia* (Adulsa) were the least effective in inhibiting sclerotial germination. The results are presented in the Table 3. The findings are in line with the observations of Rajeshwari *et al.* (2020), tested garlic and neem extracts were shown to be effective in inhibiting sclerotial germination, even at a shorter incubation time of 30 minutes. Sclerotial germination was suppressed by lantana and datura over longer incubation times, such as 18 and 24 hours. The remaining plant extracts that were examined did not effectively inhibit sclerotial germination. Dubey *et al.* 2009 [4] evaluated the neem cake and leaves extracts against *M. phaseolina* where highest inhibition of sclerotial germination was found after 96 hrs. of incubation.

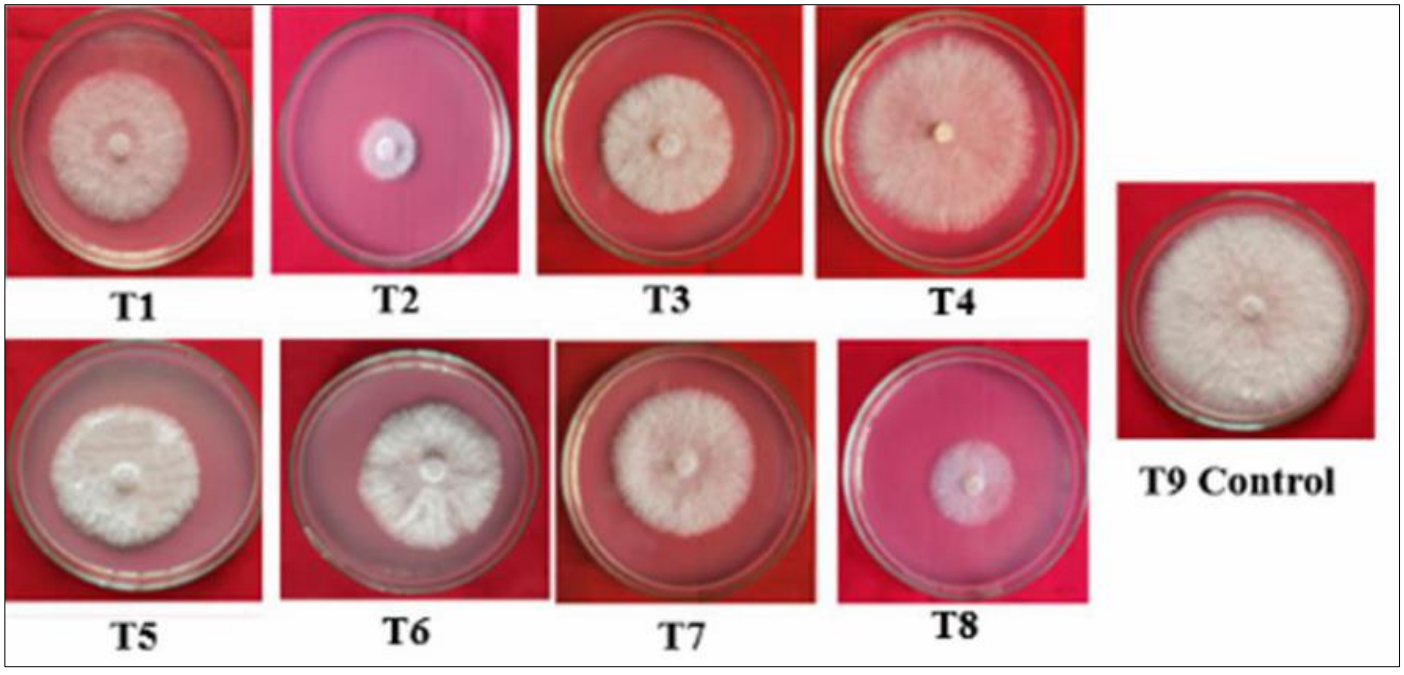

Plate 1: Efficacy of plant extracts against the mycelial growth of *S. rolfsii* at 20% concentration

Table 3: Efficacy of plant extracts against the mycelial growth and viability of sclerotia of *S. rolfsii*

Tr. No.	Treatments	Percent mycelial growth inhibition at various concentration			Average mean	Percent inhibition of sclerotia		
						20% concentration		
		5%	10%	20%		30 min	8 h	24 h
T1	*Lawsonia inermis* (Heena)	12.56 (20.75) *	17.89 (25.02)	36.56 (36.00)	22.33 (28.88)	0.00 (0.00) *	0.00 (0.00)	10.00 (18.43)
T2	*Ocimum tenuiflorum* (Tulsi)	46.23 (42.75)	68.00 (55.55)	75.12 (60.07)	63.11 (52.60)	83.33 (65.90)	100.00 (90.00)	100.00 (90.00)
T3	*Azadirachta indica* (Neem)	19.12 (25.92)	35.23 (36.40)	42.12 (40.46)	35.15 (36.36)	60.00 (50.77)	75.55 (60.37)	82.22 (65.07)
T4	*Vinca rosea* (Periwinkle)	4.45 (12.16)	17.34 (24.60)	17.78 (24.93)	13.19 (21.29)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T5	*Polyalthia longifolia* (Adulsa)	5.23 (13.19)	12.56 (20.75)	34.78 (36.13)	17.52 (24.74)	0.00 (0.00)	0.00 (0.00)	10.00 (18.43)
T6	*Madhuca longifolia* (Mahua)	23.45 (28.96)	33.34 (35.26)	35.45 (34.11)	30.74 (33.67)	23.33 (28.88)	36.66 (37.26)	60.00 (50.77)
T7	*Justicia adhatoda* (Ashoka)	18.66 (25.59)	25.12 (30.07)	36.34 (37.07)	26.70 (31.11)	15.55 (24.19)	22.22 (28.11)	52.44 (46.40)
T8	*Zingiber officinale* (Ginger)	38.78 (38.51)	60.69 (51.17)	63.78 (52.99)	54.41 (47.53)	72.22 (58.20)	80.00 (63.43)	100.00 (90.00)
T9	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	SE (m±)	0.39	0.60	0.53	-	0.23	0.35	0.36
	C.D. at 1%	1.17	1.78	1.59	-	0.70	1.05	1.07

Efficacy of organic amendments of against the mycelial growth of *S. rolfsii*

The bio efficacy of the organic amendments *i.e.,* Mahua, Neem, Groundnut, Karanj, Sunflower, and Castor were evaluated against *S. rolfsii*. The results are shown in Table 4 and Plate 2 found that all of the extracts tested at 5, 10, and 20% concentrations were significantly were significantly more effective than the control.

All the tested concentrations, 20% concentration of all oil cake extracts was shown to be significantly better than the 5% and 10% concentrations. The least amount of growth inhibition was observed in the extracts at a 5% conc. An increase in extract concentration, increased inhibitory effect on the growth of *S. rolfsii*. Mahua cake exhibited the highest percentage of 83.70 percent inhibition of mycelial growth at a 20% conc. of plant extracts. This was followed by Castor cake (52.19%), Neem cake (42.14%), Sunflower cake (39.58%), and Groundnut cake (37.47%). Least growth inhibition was recorded in Karanja cake is (32.85%). The findings are in line with the findings according to Bhoraniya *et al*. (2003) [3], the incidence of *S. rolfsii* chili-induced stem rot disease was 78.57 percent reduced when castor oil cakes were evaluated against pathogen. These findings are consistent with those of Senjaliya (2015) [12], who examined six distinct organic extracts (mustard, groundnut, neem, castor, cotton cakes, and FYM) and found that all of them strongly suppressed the development of *S. rolfsii in vitro*, with the exception of FYM.

Effect of organic amendments on the viability of sclerotia of *S. rolfsii in vitro*

All the tested oil cakes, Mahua exhibited the highest percentage of inhibition of sclerotial germination (100%) at 8 and 24 hours of incubation and then (84.44%) at 30 minutes. After 24 hours of incubation, castor revealed a 100% inhibition of sclerotial germination, followed by 85.55% and 80.00% at 8 hours and 30 minutes, respectively. Neem oil cake extract (84.55%) inhibits sclerotial germination at 24 hours, with further inhibition occurring at 8 hours is 77.77% and at 30 minutes is 71.11%. Following a 24 hours incubation period, sunflower exhibited an 81.11% inhibition of sclerotial germination, followed by at 8 hours (70.00%) and 30 minute (64.44%) inhibition. Groundnut showed (71.11%) inhibition at 24 hours incubation followed by (60.00%) inhibition at 8 hours incubation, (55.55%) at 30 minutes was observed. Out of all the oil cake extracts, karanja has the least effectiveness in inhibiting germination of sclerotia. The results of the experiment are given in Table 4. This is in accordance with research by Dubey *et al.* (2009) [4], which examined, neem cake and bark extracts against *M. phaseolina* and found that after 96 hours of incubation, there was a maximum inhibition of sclerotia.

Plate 2: Efficacy of organic amendment against the mycelial growth of *S. rolfsii at* 20% concentration

Table 4: Efficacy of organic amendments against the mycelial growth and viability of sclerotia of *S. rolfsii.*

Tr. No.	Treatments	Percent mycelial growth inhibition at various concentration			Average mean	Percent inhibition of Sclerotia		
						20% concentration		
		5%	10%	20%		30 min	8 h	24 h
T1	Mahua	62.56 (52.27) *	71.30 (57.60)	83.70 (66.19)	72.52 (58.38)	84.44 (66.79) *	100.00 (90.00)	100.00 (90.00)
T2	Neem	24.70 (29.80)	34.85 (36.18)	42.14 (40.48)	33.89 (35.60)	71.11(57.50)	77.77 (61.89)	84.55 (66.88)
T3	Groundnut	12.29 (20.51)	35.58 (36.62)	37.47 (37.74)	28.44 (32.23)	64.44 (53.41)	70.00 (56.79)	81.11 (64.26)
T4	Karanj	13.78 (21.78)	21.14 (27.38)	32.85 (34.96)	22.59 (28.38)	52.22 (46.27)	58.89 (50.12)	66.66 (54.75)
T5	Sunflower	15.67 (23.31)	24.03 (29.35)	39.58 (38.99)	26.42 (30.93)	55.55 (48.19)	60.00 (50.77)	71.11 (57.50)
T6	Castor	41.64 (40.18)	50.34 (45.19)	52.19 (46.26)	48.05 (43.88)	80.00 (63.43)	85.55 (67.69)	100.00 (90.00)
T7	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	SE (m±)	0.34	0.30	0.44	-	0.74	0.51	0.68
	C.D. at 1%	1.03	0.91	1.33	-	2.25	1.54	2.07

Conclusion

The use of plant-based solutions, it reduces cost of cultivation eliminates health risks while offering an alternative to synthetic pesticides. From the *in vitro* findings, there are alternatives approach to replace the synthetic pesticides for management of the soil borne fungi (*S. rolfsii*). It is conceivable to create an economically sound crop production system alternative by combining these strategies (using plant extracts and organic additives).

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