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Bio-efficacy of organic amendments and plant oils against *Sclerotium rolfsii* Sacc.: The incitant of Southern blight of tomato

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Abstract
Tomato is one of the important vegetable crops cultivated worldwide. The diseases of tomato act as a major constrain for its cultivation globally. In the present study, the pathogen associated with collar rot of tomato plants was isolated from different tomato growing areas of Tamil Nadu and confirmed by morphological observation as *Sclerotium rolfsii. In vitro* efficacy of nine organic amendments and five plant oils were tested against the pathogen. Among the organic amendments, mahua oil cake (10% concentration) recorded the maximum inhibition (80.11%) on the mycelial growth (1.79 cm) of the pathogen under *in vitro* condition. Among the plant oils, neem oil (0.5% concentration) showed the maximum inhibition (82.67%) on the mycelial growth (1.56 cm) of the pathogen under *in vitro* condition. The inhibition of organic amendments and plant oils might be due to release of fungitoxic substance in the medium which suppressed the mycelial growth of *S. rolfsii*.

Keywords: Tomato, *Sclerotium rolfsii*, organic amendments, plant oils, collar rot

Introduction
Tomato is one among the three important horticultural crops cultivated worldwide. It is known as productive as well as protective food. It is a rich source of minerals like iron and phosphorus; vitamins like Vitamin A, C and E; pigments like lycopene and beta carotene; organic acid, plant amino acids and dietary fibers. The crop is infected by a large number of fungal and bacterial pathogens. In Florida, Rolfs (1893) [10] first noticed an unknown fungus that caused tomato blight. Saccardo (1911) [11] named the fungus as *Sclerotium rolfsii*. Southern blight or collar rot caused by *Sclerotium rolfsii* has been a very destructive and fast acting crown rot disease that rapidly kills the plant resulting in substantial yield losses. This pathogen causes yellowing and drooping of foliage, followed by browning and drying of leaves and plants (Mukhopadhyay, 1971) [6]. Initially white sclerotia are formed which darken to tan, brown or reddish brown as they mature resembling mustard seeds in size and colour (Xu *et al.*, 2008) [16]. The brown sclerotia survives in soil for many years tolerating chemical and biological degradation due to melanin present in the outer membrane (Chet, 1975) [2]. This pathogen has emerged as a major constrain in tomato cultivation due to extensive cultivation of tomato crops in nontraditional areas. Chemical fungicides are not very satisfactory as the sclerotia of the pathogen are able to survive for long time in the soil and use of chemical fungicides are harmful to environment. Therefore, an attempt was made to manage the disease through an alternate eco-friendly approach by combining organic amendments and plant oils.

Materials and methods
Collection and isolation of *Sclerotium rolfsii*
Tomato plants showing the characteristic symptoms of collar rot caused by *Sclerotium rolfsii* were collected from different tomato growing areas of Tamil Nadu. The collar rot disease incidence was assessed by counting the number of affected plants out of total number of plants observed in each plot (25 m^2). In each area, three fields were assessed and the mean disease incidence was calculated. The pathogen was isolated from the infected tomato plants by tissue segment method (Rangaswami and Mahadevan, 1999) [8] on sterilized potato dextrose agar (PDA) medium. The pathogen was identified based on the morphological characters as described by Punja (1988) [7].

Pathogenicity test
The pathogenicity experiment was conducted to test the virulent isolate of the pathogen and to prove the Koch's postulates.

The isolates of *S. rolfsii* were multiplied on sand-maize medium (Riker and Riker, 1936) [9]. The medium containing 1900 g of sand and 100 g of maize powder (19:1) was mixed, moistened with 400 ml of water per kg and then filled in saline (glucose) bottles. The bottles were sterilized at 1.4 kg cm^{-2} pressure for 2 hours for two alternate days and inoculated with actively growing two nine mm mycelial disc of *S. rolfsii* and incubated for 15 days at room temperature (28±2˚C), till the sclerotia turned brown indicating maturity.

Bio efficacy of organic amendments against the mycelial growth of *S. rolfsii in vitro*

Preparation of aqueous extracts of organic amendments

For the preparation of aqueous extracts of organic amendments (cold water extract) 100 g of organic amendment in each was taken and made into powder. It was soaked in sterile distilled water at 1 g in 1.25 ml of sterile distilled water and allowed to stand overnight. The material was again grounded using a pestle and mortar and filtered through two layer of muslin cloth and the filtrate was centrifuged at 10,000 rpm for 15 minutes. The supernatant served as the standard organic amendment extract solution (100%) (Dubey 2002) [4].The extracts of organic amendment were sterilized at 1.4 kg cm^{-2} pressure for 15 min before adding to the medium. The aqueous extracts at 2.5 ml and 5 ml were mixed with 50 ml of PDA medium to obtain 5 and 10 per cent concentration respectively and then again sterilized at 1.4 kg cm^{-2} for 15 min.

In vitro* efficacy of organic amendments against the mycelial growth of *S. rolfsii

The efficacy of extracts of organic amendments were tested against *S. rolfsii* using poisoned food technique (Schmitz, 1930) [12].The sterilized PDA medium containing 5% and 10% concentration of each aqueous extracts was poured onto sterilized Petri plates at 15 ml per Petri plate and then allowed to solidify. A nine mm mycelial disc of *S. rolfsii* was taken from actively growing culture and placed at the center of each Petri plate and incubated at room temperature for seven days (28±2˚C). The PDA medium without extracts of organic amendments served as control. The radial growth (cm) of *S. rolfsii* was recorded after seven days of incubation and the percentage of the mycelia growth inhibition was calculated using the formula:

$$PI = \frac{Dc - Dt}{Dc} \times 100$$

Dc = average diameter of fungal growth (cm) in control
Dt = average diameter of fungal growth (cm) in treatment
The experiment was conducted in a completely randomized design. For each treatment three replications were maintained.

Bio efficacy of plant oils against the mycelial growth of *S. rolfsii* under *in vitro*

The antifungal activity of different plant oils were tested against *S. rolfsii* by poisoned food technique. For preparation of the medium, 0.125 ml and 0.25 ml of each plant oil was mixed with 50 ml of PDA medium separately to obtain 0.25 and 0.5 per cent concentration respectively and again sterilized at 1.4 kg cm^{-2} pressure for 15 min. 15 ml of each plant oil medium was poured into sterilized Petri plates separately and allowed to solidify. A nine mm mycelial disc of *S. rolfsii* was taken from actively growing culture and placed at the center of each Petri plate and incubated at room temperature (28±2˚C). The PDA medium without any plant oils served as control. The radial growth (cm) of *S. rolfsii* was recorded after seven days of incubation and the percentage of the mycelia growth inhibition was calculated using the formula:

$$PI = \frac{Dc - Dt}{Dc} \times 100$$

Dc = average diameter of fungal growth (cm) in control
Dt = average diameter of fungal growth (cm) in treatment
The experiment was conducted in a completely randomized design. For each treatment three replications were maintained.

Statistical analysis

Experimental data were analyzed using analysis of variance (ANOVA) and the SPSS version 17.0. The treatment means were separated at 5% significance level using Duncan's Multiple Range Test (DMRT).

Results and Discussion

Collection and isolation of *Sclerotium rolfsii*

Tomato (*Solanum lycopersicum* L.) plants showing characteristic symptoms of collar rot disease caused by *Sclerotium rolfsii* were collected from six different tomato growing areas of Tamil Nadu *viz.,* Anaiyur, Chellampatti, Kinathukadavu, Madukkarai, Ottanchatram and Thondamuthur. The collar rot disease incidence was found to be maximum in Madukkarai area of Coimbatore district (60.75 per cent) and minimum in Chellampatti area of Madurai district (20.73 per cent) (Table 1). The pathogen was isolated from the infected collar region of tomato plants by tissue segment method (Rangaswami and Mahadevan, 1999) [8]. The isolates collected were identified as *Sclerotium rolfsii* based on the morphological characters.

Pathogenicity of the isolates of *S. rolfsii* on tomato plants (Pot culture)

All the isolates of the pathogen when artificially re-inoculated on the tomato plants, exhibited the typical collar rot symptoms and the Koch's postulates was proved. Among the six isolates, the isolate S-MK was found to be highly virulent by recording the maximum collar rot incidence of 92.56 per cent as compared with other isolates and was taken for further experiments. The isolates of S-KK, S-TM, S-AN, S-OC and S-CP recorded 84.73, 78.64, 71.59, 67.45 and 59.13 per cent collar rot disease incidence respectively (Table 2).

Bio-efficacy of organic amendments against the mycelial growth of *S. rolfsii in vitro*

In the present study, nine different organic amendments *viz.,* neem oil cake, groundnut oil cake, cotton oil cake, vermicompost, farm yard manure, castor oil cake, pungam oil cake, mahua oil cake and sesame oil cake were tested for antifungal activity against the virulent isolate (S-MK) of *S. rolfsii.* The results revealed that the minimum mycelial growth (1.79 cm) was obtained in mahua oil cake extract (10% concentration) and with the maximum growth inhibition of 80.11 per cent which was in accordance with the findings of Alice *et al.,* 1998[1]. Vermicompost extract was found to be the least effective with 30.22 per cent growth reduction over control (Table 3; Plate 1). The mahua oil cake was used to control fungal infections because of the high saponin content which reduced phytopathogenic fungi (Gupta, 2013) [5].

Bio-efficacy of plant oils against the mycelial growth of *S. rolfsii in vitro*

The effect of five different plant oils *viz.*, eucalyptus oil, coconut oil, neem oil, gingelly oil and groundnut oil were tested against the mycelial growth of the virulent isolate (S-MK) of *S.rolfsii in vitro.* Among these plant oils, neem oil (0.5%) recorded the maximum mycelial growth reduction of 82.67 per cent over control which was in accordance with the findings of Singh *et al.*, 1989 [14]. Coconut oil was found to be the least effective with 43.11 per cent growth reduction over control (Table 4, Plate 2). The most active principle present in neem oil was azadirachtin and sulphur (Siddiqui, 1992) [13]. Dubey and Kumar (2003) [3] reported the fungicidal effect of azadirachtin as good as the fungicides bavistin and mancozeb. The presence of sulphur along with other compounds in neem oil might be responsible for the maximum effect as sulphur compounds have been used as a practical control measure against a number of fungal plant diseases (Singh *et al.*, 1980) [15].

Table 1: Collection of isolates of *Sclerotium rolfsii* causing collar rot disease of tomato in different areas of Tamil Nadu

S. No.	Isolates	Places of collection	District	Per cent Disease Incidence (%)*
1	S-AN	Anaiyur	Madurai	41.25 (39.96)**
2	S-CP	Chellampatti	Madurai	20.73 (27.08) **
3	S-KK	Kinathukadavu	Coimbatore	45.52 (42.43) **
4	S-MK	Madukkarai	Coimbatore	60.75 (51.21) **
5	S-OC	Ottanchatram	Dindigul	25.16 (30.11) **
6	S-TM	Thondamuthur	Coimbatore	43.02 (40.99) **
CD (p=0.05)				2.13

*Mean of three replications

**Values in the parentheses are arc sine transformed values

Table 2: Pathogenicity and virulence of different isolates of *S. rolfsii* on tomato plants

S. No.	Isolates	Places of collection	Disease incidence (%)*
1	S-AN	Anaiyur	71.59 (57.80)**
2	S-CP	Chellampatti	59.13 (50.26) **
3	S-KK	Kinathukadavu	84.73 (67.00) **
4	S-MK	Madukkarai	92.56 (74.17) **
5	S-OC	Ottanchatram	67.45 (55.21) **
6	S-TM	Thondamuthur	78.64 (62.47) **
CD (p=0.05)			2.83

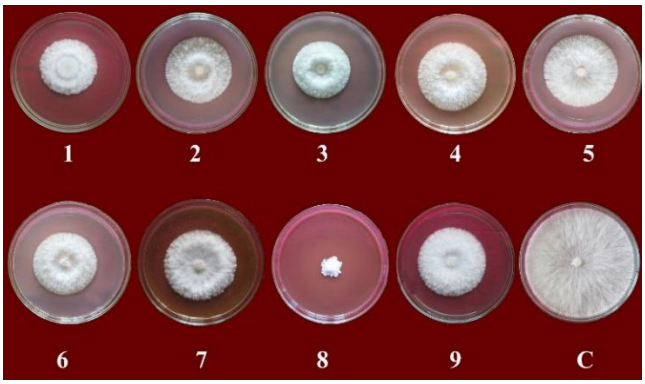
*Mean of three replications

**Values in the parentheses are arc sine transformed values

Table 3: Bio-efficacy of organic amendment against the mycelial growth of *S. rolfsii* (S-MK) *in vitro*

S. No.	Organic amendments	Concentration (%)			
		5%		10%	
		Mycelial growth (cm)*	Per cent growth reduction over control	Mycelial growth (cm)*	Per cent growth reduction over control
1	Neem oil cake	6.93^{d}	23.00	4.69^{e}	47.89
2	Groundnut oil cake	7.86^{bc}	12.67	5.69^{c}	36.78
3	Cotton oil cake	7.01^{d}	22.11	4.77^{e}	47.00
4	Farm Yard Manure	8.02^{bc}	10.89	5.84^{c}	35.11
5	Vermicompost	8.20^{b}	08.89	6.28^{b}	30.22
6	Castor oil cake	6.01^{e}	33.22	5.33^{d}	40.78
7	Pungam oil cake	7.76^{c}	13.78	5.92^{c}	34.22
8	Mahua oil cake	2.18^{f}	75.78	1.79^{f}	80.11
9	Sesame oil cake	5.72^{e}	36.44	5.83^{c}	35.22
C	Control	9.00^{a}	-	9.00^{a}	-
CD (p=0.05)		0.34	-	0.28	-

*Mean of three replications. Means with the same letter do not have significant difference according to Duncan's multiple range test at $p<0.05$.



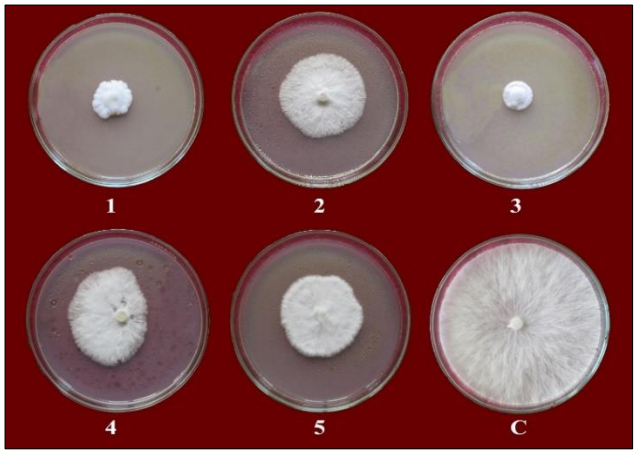
1. Neem oil cake
2. Groundnut oil cake
3. Cotton oil cake
4. Farm Yard Manure
5. Vermicompost
6. Castor oil cake
7. Pungam oil cake
8. Mahua oil cake
9. Sesame oil cake
C. Control

Plate 1: Bio-efficacy of organic amendment (10%) against the mycelial growth of *S. rolfsii* (S-MK) *in vitro*

Table 4: Bio-efficacy of plant oils against the mycelial growth of *S. rolfsii* (S-MK) *in vitro*

S. No.	Plant oils	Concentration (%)			
		0.25%		0.5%	
		Mycelial growth (cm)*	Per cent growth reduction over control	Mycelial growth (cm)*	Per cent growth reduction over control
1	Eucalyptus oil	6.84[c]	24.00	2.26[d]	74.89
2	Coconut oil	8.35[b]	07.22	5.12[b]	43.11
3	Neem oil	5.24[d]	41.78	1.56[e]	82.67
4	Gingelly oil	6.97[c]	22.56	4.43[c]	50.78
5	Groundnut oil	8.29[b]	07.89	4.59[c]	49.00
6	Control	9.00[a]	-	9.00[a]	-
CD (p=0.05)		0.27		0.29	

*Mean of three replications. Means with the same letter do not have significant difference according to Duncan's multiple range test at $p<0.05$.



1. Eucalyptus oil 2. Coconut oil
3. Neem oil 4. Gingelly oil
5. Groundnut oil C. Control

Plate 2: Bio-efficacy of plant oils (0.5%) against the mycelial growth of *S. rolfsii* (S-MK) *in vitro*

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