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Impact of Environmental Conditions on The Growth and Sporulation of *Sclerotium Rolfsii* Sacc. Inciting Stem Rot of Clusterbean

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ABSTRACT
Sclerotium rolfsiii one of the devastating soil-borne plant pathogens which cause severe loss at the time of seedling development.Different levels of temperature and pH, light and aeration were tested for their effect on the growth of Sclerotium rolfsii Sacc. causing stem rot of cluster bean plant. Studies on effect of temperature on the growth of S. rolfsii revealed that temperature of 30°C was found to be more conducive for the mycelial growth of S. rolfsii (89.33 mm) under in vitro recording the highest mycelial dry weight of 240.00 mg, and more sclerotail production (+++) which was followed by 35°C. pH of 6.5 recorded maximum mycelial growth (89.33 mm), mycelial dry weight (250.66 mg) and sclerotial production was more which was followed by exposing the pathogen to pH 6.0 under in vitro. Studies on the effect of light revealed that the exposure of the pathogen to alternative cycles of 12 hours light and 12 hours darkness for ten days resulted in the maximum mycelial growth and dry weight of S. rolfsii (89.00 mm; 236.66 mg, respectively). In 3/4 and 1/2 sealed plates placed under light and darkness affected mycelial growth and number of sclerotia significantly whereas the number of sclerotia was more in unsealed plates kept under light and dark condition.Sclerotial formations were directly influenced by air as completely sealed plates failed to produce sclerotia.
Keywords-*Temperature, pH, Light, Aeration, Sclerotium rolfsii.*

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INTRODUCTION

Cluster bean (*Cyamopsiste tragonaloba* (L.)Taub) which is commonly known as guar means 'cow food' (in Hindi) belonging to the family leguminaceae. It is an annual arid and semi-arid legume crop [18] grown as green manure, as forage crop for cattle and as a vegetable crop for human consumption. The crop has got a special importance because of gum content in its seed. It is primarily grown for seed, animal feed, fodder, vegetable and green manuring purposes. The green pod contains 82.5% water, 9.9% carbohydrate, 3.7% protein, 0.2% fat, 2.3% fiber and 1.4% other minerals which is essential components of human consumption [6]. Cluster bean is a rich source of high quality galactomannan gum and protein rich (40-50%) guar meal as animal feed. Seed gum is used in various industries such as textiles, paper, cosmetics, explosives and food processing. Besides the gum preparation, cluster bean is emerging as a potential source of vegetable protein for human beings. The green and tender pods of guar are cooked as favourite vegetables in many parts of country including South India [5].

India is the largest producer of Guar with 80% among the world production, followed by Pakistan with 10-15%. In India, Rajasthan is the major producer of Guar followed by

Haryana, Gujarat and Punjab [2]. Due to high prices of Guar the production has now extended to other states like Madhya Pradesh, Chhattisgarh, Andhra Pradesh, Karnataka and Tamil Nadu. In Tamil Nadu, it is grown in Cuddalore, Dindigul, Erode and Salem districts. In 2014-2015, the production was 2415 tons with yield of 567 kg in an area of 4255 ha [3].

The fungal infection causes variations in protein contents of plant parts. Being a multipurpose crop, there is a great demand of organic cluster bean. Sustainable cluster bean cultivation is continuously challenged by diseases that cause quantitative and qualitative losses in yield. The cluster bean plants infected with several fungal diseases *viz.*, stem rot caused by *Sclerotium rolfsii* [14]; wilt, root rot and damping-off caused by*Fusariumsolani* [17]; *Alternaria* blight caused by *A. cyamopsidis* [9]; powdery mildew caused by *Leveillulataurica* [6] and root rot caused by *Rhizoctoniasolani* [5].Of these, stem rot caused by *Sclerotium rolfsii* is a soil-borne disease which causes considerable damage to the crop and yield loss was estimated up to 50-70 per cent under field condition [14].Though stem rot and sclerotial basal rot of cluster bean were reported way back in 1997 from Bangalore [7] followed by Ronakkkumar and Sumanbhai [14] from Gujarat. Thereafter, no further studies were undertaken on this disease. The pathogen produces sclerotia which over winter in soil and on plant debris and can survive for a long period causing disease in the following season [12].These pathogens exhibit variation in their morphological, biological and immunological characteristics and pathogenicity or resistance against harmful environment.In the present study, a detailed investigation was carried out on the variations with regard to temperature, pH, light and air on the growth and biomass production of *S. rolfsii*.

MATERIALS AND METHODS

Isolation and maintenance of pathogen The stem rot symptoms were collected from farmer's field of Chidambaramtaluk, at Cuddalore District. The symptoms in the field were recognized by yellowing of lower leaves followed by loss of vigour and gradually wilting of lateral branches. Leaves of affected branches became chlorotic and then turn brown and gradually the whole plant dry. There was whitish mycelial growth creeping around the collar region of affected plants at or near the soil surface, imparting a white wash appearance to the base of the affected plants. Sometimes, mycelial growth radiated out over the soil surface when the soil surface become moist. Lesions on infected stems were initially light brown, which later became dark brown. Advanced symptoms developed when lesions coalesced to girdle the lower stem, become shredded and the sheathing mycelium quickly produced abundant spherical sclerotia on the surface of the affected plant parts or on the soil surface adjacent to affected plants. The sclerotia were initially white but soon turned dark brown as they maturedThe infected plant materials brought back from the field were washed, cut into 5 mm segments including the advancing margins of infection. The segments were surface sterilized in 0.5% sodium hypochlorite solution for 5 min. and rinsed in three changes of sterile distilled water. The segments were separately dried in between sheets of sterile filter paper and placed (3 pieces per plate) on fresh potato dextrose agar (PDA) medium [1] impregnated with streptomycin, and incubated for seven days at 28±2°C.

The pathogen causing stem rot symptoms were isolated and the fungal growth on 5th day, which arose through the sclerotial bodies was cut by inoculation loop and transferred aseptically to the PDA slants and allowed to grow at room (28±2°C) temperature to obtain the pure culture of the fungus. The culture thus obtained was stored in refrigerator at 5°C for further studies and was sub cultured periodically. The purified isolate was identified as *Sclerotiumrolfsii* based on morphological and colony characteristics [13, 16, 21].

Effect of different temperature levels on the mycelial growth and dry weight of *S. rolfsii* under*in vitro*

Solid medium: Aquantity of fifteen ml of the sterilized potato dextrose agar medium was poured into 90 mm sterile Petri plates. The plates were inoculated with six mm mycelial disc of the pathogen obtained from seven days old culture and incubated at different temperature *viz.,* 5, 10, 15, 20, 25, 30 and 35°C for seven days in an incubator. The mycelial growth of the pathogen was measured in mm at the end of incubation period.

Liquid medium: Erlenmeyer flasks (250 ml) containing 50 ml of potato dextrose broth were sterilized, inoculated and incubated at different temperature *viz.,* 5, 10, 15, 20, 25, 30 and

35°C for ten days in BOD incubator. At the end of the incubation period the mycelial mat was filtered through Whatman No. 41 filter paper of known weight. The filter paper with mycelial mat was dried in hot air oven at 105°C for 48 h. and the mycelial dry weight was calculated.

Three replications were maintained for each treatment and sclerotial production was noticed at 9 days after incubation.

Effect of certain pH levels on the mycelial growth and dry weight of *S. rolfsii in vitro*

Solid medium: Influence of different pH levels on the growth of *S. rolfsii* was studied. Eight different pH levels study from 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 were adjusted to the PDA medium. This was done before autoclaving with the help of HCL (0.1N) and NaOH (0.1N) by using digital pH meter. Fifteenml of molten PDA medium was dispensed into each of 90 mm sterile Petri plate. Mycelial discs taken from the advancing margins of seven days old culture of respective *S. rolfsii* isolate by the aid of cork borer were separately placed at the centre of the plate containing PDA medium. The plates were incubated at room temperature (28±2°C). The mycelial growth of the pathogen was measured in mm at the end of incubation period.

Liquid medium: Potato dextrose broth with different pH levels *viz.,* 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 were prepared and sterilized. They were inoculated with six mm mycelial disc of the pathogen obtained from seven days old grown on PDA. The flasks were incubated for ten days at 28±2°C in BOD incubator. After incubation, the fungal biomass was separated through filtration in a previously dried and weighed filter paper (Whatman No. 41). Then the mycelial dry weight was calculated.

Three replications were maintained for each treatment and sclerotial production was noticed at 9 days after incubation.

Effect of air on sclerotial development of *S. rolfsii*in potato dextrose agar medium

Fifteenml of molten PDA medium was dispensed into each of 90 mm sterile Petri plate. Mycelial discs taken from the advancing margins of seven days old culture of respective *S. rolfsii* isolate by the aid of cork borer were separately placed at the centre of the plate containing PDA medium. The inoculated plates were sealed with the help of lab seal in the following manner i.e., no sealing (control), half sealed, 3/4th and complete sealing. Each set contained three plates. After inoculation and sealing, Petri plates were incubated at 28±2°C (light and dark) and the other sealed plates were wrapped with black paper and incubated above. Visual observations were periodically made for sclerotial initiation, sclerotial development and number of sclerotia per plate. Three replications were maintained for each treatment.

Effect of light on the growth of *S. rolfsii*

Liquid medium: Potato dextrose broth and agar were used in this experiment. Conical flasks of 250 ml capacity and each contained 100 ml of liquid broth were inoculated and exposed to different length of light hours*viz.*, alternate cycles of twelve hours light and twelve hours darkness, continuous light and continuous darkness in an environmental conditions. Flasks were inoculated with six mm mycelial disc obtained from the periphery of seven days old culture of *S. rolfsii* and incubated at different light intensities. Each treatment was replicated thrice and incubated for ten days. Mycelial dry weight was obtained as described earlier.

Solid media:Fifteen ml of potato dextrose agar was poured in 90 mm sterile Petri plate. Such plates were inoculated with six mm mycelial disc obtained from the periphery of seven days old culture of *S. rolfsii* and incubated at different light intensities. Each treatment was replicated thrice and incubated for seven days. The mycelial growth was recorded at the end of the incubation period.

Statistical analysis: All the experiments were of completely randomized design (CRD) and repeated twice. Data were subjected to analyses of variance and treatment means were compared by Duncan's multiple range test ($P<0.05$). The IRRISTAT package version 92-1, developed by the International Rice Research Institute Biometrics Unit, Philippines, was used for analysis [8].

RESULTS AND DISCUSSION

Effect of temperature on the growth of pathogen

Each pathogen has got its own cardinal temperature and understanding the temperature requirement of the pathogen will help to standardize the management practices. It plays an important role in the growth and reproduction of fungi. Among the temperature levels (5, 10, 15, 20, 25, 30, 35 and 40°C) tested, 30°C was found to be more conducive for the mycelial growth of *S. rolfsii* (89.33 mm) under *in vitro* recording the highest mycelial dry weight of 240.00 mg, and more sclerotail production (+++) which was followed by 35°C (Table 1). The exposure of *S. rolfsii* to high temperature i.e. 40°C was found to be highly detrimental to the growth of *S. rolfsii*.While the exposure of pathogen to lowest temperature of 5 and 10 °C recorded nil mycelial growth. Similarly, Muthukumar and Venkatesh [10] and Zape et al. [32] reported that the growth of *S. rolfsii* was maximum at 30°C which was reduced significantly at below 20°C and above 30°C. The pathogen was unable to grow and produce sclerotial at minimum and maximum temperature of 10 to 40°C. Contrary to the results obtained by Prasad and Mahapatra [11] recorded that temperature of 25°C was optimum for highest mycelial growth and dry weight of *S. rolfsii* followed by 30°C and 35°C.The above results lend support to the present findings.

The fungi generally utilize substrate in the form of solution only if the reaction of the solution is conducive to fungal growth and metabolism. This brings the importance of hydrogen ion concentration for the better fungal growth. In general, the pathogen showed preference for pH level towards acidic side. The results of the present study showed that among the pH levels (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0) tested, exposure of pathogen to acidic pH (6.5) recorded maximum mycelia growth (89.33 mm), mycelial dry weight (250.66 mg) and sclerotial production was more which was followed by exposing the pathogen to pH 6.0 under *in vitro*. Increase (or) decrease in pH beyond 7.0 and below 4.0 was not conducive for the growth of the pathogen (Table 2). This finding is in line with the earlier reports made by Sarker et al. (2013[15]); Muthukumar and Venkatesh (2013[10]) and Prasad and Mahapatra [11].

Effect of light on the growth of pathogen

The exposure of the pathogen to alternative cycles of 12 hours light and 12 hours darkness for ten days resulted in the maximum mycelial growth and dry weight of *S. rolfsii* (89.00 mm; 236.66 mg, respectively) which was significantly superior over other treatments tested (Table 3). The mycelial growth of pathogen exposed to continuous light resulted in moderate growth (67.00 mm; 202.33 mg) and continuous darkness resulted in minimum mycelial growth and dry weight of pathogen (48.33 mm; 90.00 mg).Muthukumar and Venkatesh [10] reported that generally, the light condition induces the production of more number of sclerotia than dark condition. In another study, the exposure of pathogen to different light periods revealed that alternative cycles of 12 h light and 12 h darkness for ten days resulted in the maximum mycelial growth and dry weight, more number of sclerotia and weight of sclerotia was also seen when compared with other treatments. The above results lend support to the present findings.

Effect of air on the growth of pathogen

The results of the present study revealed that the number of sclerotia in 3/4 and 1/2 sealed plates placed in light and darkness affected the mycelial growth and number of sclerotia significantly as compared to the control (unwrapped plates). In control plates, sclerotia initials were observed after 6 days of inoculation as tiny, pinhead-like structures and after 6-8 days exudation commenced. In completely sealed plates, the fungal growth was relatively very slow, compact and profusely growing mycelium was observed after 6 to 8 days as compared to the control (Table 4). In all completely sealed plates, there was no sclerotium formation even after 12 days after inoculation. In 3/4 and 1/2 sealed plates kept under dark condition (89; 100) the number of sclerotia were less but they were bigger in size as compared to the control (130). Whereas, in 3/4 and 1/2 sealed plates were kept under light condition, the number of sclerrotia were slightly more (170; 174) compared to dark condition, but more number of sclerotia were observed only unsealed plates (286).Dark and light conditions did not affect the fungal growth, size and number of sclerotia. In 3/4 and 1/2 sealed plates placed in light and darkness affected mycelial growth and number of sclerotia significantly as compared to the control (unwrapped plates). The number and sclerotial weight were affected drastically due to improper aeration as average number of sclerotia was more in unsealed plates [19]. Basamma *et al.* [4] reported that the exposure of *S. rolfsii* to alternative cycles of 12 hrs light and 12 hrs darkness for 10

days resulted in the maximum radial growth and dry matter production of *S. rolfsii.* Quadri and Fatima [20] reported that growth of *S. rolfsii* on potato dextrose agar medium were not affected by blue, yellow, green and sealed, but it affected by red and black. But in this investigation total number and weight of sclerotia found affected by sealed and black light after 15 days of incubation periods.

Table 1. Effect of different temperature levels on the mycelial growth, dry weight and sclerotial production of*S. rolfsii*

Temperature (°C)	Mycelial growth (mm)	Mycelial dry weight (mg)	Sclerotial production (after 9 days)
5	0.00 f	0.00 f	-
10	0.00 f	0.00 f	-
15	23.33 e	106.33 e	+
20	46.66 d	186.33 d	+
25	67.00 c	204.66 c	++
30	89.33 a	240.00 a	+++
35	72.66 b	222.33 b	+++
40	0.00 f	0.00 f	-

+=Sclerotial initial ++= White sclerotia; $+^F$ =Fewer sclerotia initials
+++ = Dark brown sclerotia - = No sclerotial initials
Mean of three replications
Values in each column followed by the same letter are not significantly different according to the DMRT method (P = 0.05)

Table 2. Effect of different pH levels on the mycelial growth, dry weight and sclerotialproduction *S. rolfsii*

Temperature (°C)	Mycelial growth (mm)	Mycelial dry weight (mg)	Sclerotial production (after 9 days)
4	14.66 h	138.33 h	+
4.5	46.00 g	165.00 f	+
5.0	57.33 f	176.66 e	+
5.5	68.00 d	200.33 c	++
6.0	87.00 b	228.00 b	+++
6.5	89.33 a	250.66 a	+++
7.0	78.00 c	200.00 c	++
7.5	66.33 e	185.00 d	+
8.0	22.00	160.66 g	+

+=Sclerotial initial ++= White sclerotia; $+^F$ =Fewer sclerotia initials
+++ = Dark brown sclerotia - = No sclerotial initials
Mean of three replications
Values in each column followed by the same letter are not significantly different according to the DMRT method (P = 0.05)

Table 3. Effect of light on the growth of *S. rolfsii*

Sl. No.	Treatments	Mycelial growth (mm)	Mycelial dry weight (mg)
1.	Continuous light	67.00 b	202.33 b
2.	Continuous dark	48.33 c	90.00 c
3.	Alternate cycle of 12 hour light and 12 hour darkness	89.00 a	236.66 a

+=Sclerotial initial ++= White sclerotia; $+^F$ =Fewer sclerotia initials
+++ = Dark brown sclerotia - = No sclerotial initials
Mean of three replications
Values in each column followed by the same letter are not significantly different according to the DMRT method (P = 0.05)

Table 4.Effect of air on sclerotial development of *S. rolfsii*

Sl. No.	Treatment	Observation								
		In dark visual observation after (days)				Average no of sclerotia / plate	In light visual observation after (days)				Average no. of sclerotia / plate
		6	8	10	12		6	8	10	12	
1.	No sealing (control)	+	++	++	+++	130 a	+	++	++	+++	286 a
2.	1/2 sealing	+F	++	+++	+++	100 b	+F	++	+++	+++	174 b
3.	2/3 sealing	+F	++	+++	+++	89 c	+F	++	+++	+++	170 c
4.	Complete sealing	-	-	-	-	0 d	-	-	-	-	0 d

+=Sclerotial initial; ++=White sclerotia; +F=Fewersclerotia initials; +++ = Dark brown sclerotia; - = No sclerotial initials

Mean of three replications

Values in each column followed by the same letter are not significantly different according to the DMRT method (P = 0.05)

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