*Plant Archives* Vol. 19, Supplement 1, 2019 pp. 1294-1296 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

# EFFECT OF LIGHT AND AERATION ON THE GROWTH OF *SCLEROTIUM ROLFSII* CAUSING STEM ROT OF CLUSTER BEAN

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## Abstract

*Sclerotium rolfsii* is one of the devastating soil-borne plant pathogens which cause severe loss at the time of seedling development. It also causes leaf spots in several crops and wild plants. In this experiment, exposure of pathogen to different light period and aeration in order to assess the mycelial growth, biomass production of *S. rolfsii* was done. Three-fourth area of three plates, 50% area of three plates and 100% area of three plates were sealed with cellophane tape. The other three plates were not sealed. Two sets of such plates were prepared. All the plates were incubated at 28±2 °C. One set was incubated in light whereas the other set was incubated in the dark. The results reveal that there was no significant difference in mycelial growth and number of sclerotia among them but significant difference was observed when compared with the control, that is, the plates which were not sealed. Sclerotial formations were directly influenced by air as completely sealed plates failed to produce sclerotia. 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.

***Key words*:** Cluster bean, Light, Air, Mycelial growth, Sclerotial production.

## Introduction

Cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.), commonly known as guar, is a member of Leguminosae (Fabaceae) family. It is a short duration, drought resistant, low investment and high return cash crop. It can be grown in mixture with bajra and jowar but pure crop of guar supplies highest tonnage of palatable nutritious green fodder for longer duration without much deterioration in quality with the age of the crop. It is rich in nutrients with crude protein content and total digestible nutrients on dry matter basis of 18.1 and 60.0 per cent respectively. Besides fodder, it can also be grown for grain, green manure, cover crop and for vegetable purposes. In India, cluster bean is mostly grown in Rajasthan, Haryana, Punjab, Uttar Pradesh and Madhya Pradesh. Rajasthan occupies first position in India both in area and production. It accounts for almost 82.1 per cent area and 70% production in India. Haryana and Gujarat have second and third position respectively. Rajasthan has an area of 46.30 lakh hectare, production of 27.47 M tones with a productivity of 593 kg/ha (Anonymous, 2015-16). Although, cluster bean being a hardy crop is very sensitive to the biotic and abiotic stresses. The crop has succumbed to number of diseases caused by fungi, bacteria and viruses. Of the all diseases, 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 (Ronakkumar and Sumanbhai, 2014). The main aim of the present study is to isolate and identify the pathogen and to expose the pathogen to different light period and air in order to assess the mycelia growth and biomass production of *S. rolfsii*.

## Materials and Methods

### Isolation and Maintenance of Pathogen

The stem rot symptoms were collected from major cluster bean growing tracts of Tamil Nadu pertaining to districts such as Cuddalore, Dindigul, Erode, Namakkal and Salem. The 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 (Ainsworth, 1961) impregnated with streptomycin, and incubated for seven days at 28±2°C.

A total of seven isolates ($I_1$ to $I_7$) causing stem rot was isolated from infected plant samples collected from different tracts of Tamil Nadu. The fungal growth on 5$^{th}$ 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 isolates were identified as *Sclerotium rolfsii* based on morphological and colony characteristics (Punja and Damini, 1996; Sarma *et al*., 2002; Watanabe, 2002b). Based on the pathogenicity studies the highly virulent isolate ($I_1$) was used for further studies

**Effect of air on sclerotial development of *S. rolfsii* ($I_1$) in potato dextrose agar medium**

Fifteen ml 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, 3/4$^{th}$ 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***

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. To carryout study on solid media, 15 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**

The data on the effect of the treatments on the growth of pathogen was analyzed by analysis of variance (ANOVA) and treatment means were compared by Duncan's multiple range test (DMRT). The package used for analysis was IRRISTAT version 92-1eveloped by the Biometrics Unit of the International Rice Research Institute, The Philippines (Gomez and Gomez, 1984).

## Results and Discussion

The results of the present study revealed that the number of sclerotia in 2/3 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. In all completely sealed plates, there was no sclerotium formation even after 12 days after inoculation. In 2/3 and 1/2 sealed plates, the number of sclerotia were less but they were bigger in size as compared to the control. In control plates, mature sclerotia became brownish at 12$^{th}$ day after inoculation but in 1/2 and 2/3 sealed plates, such sclerotia were seen after 10 days (Table 1). 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 (Sudarshan *et al*., 2010). Basamma *et al*. (2012) 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.* Muthukumar and Venkatesh (2013) 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.

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 2). 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). Light has a profound effect on the growth of fungi. The preliminary studies carried out in the present investigation with *S. rolfsii* indicated that maximum mycelial growth and dry weight was seen when pathogen is exposed to alternate cycles of light and darkness. This was followed by continuous light. This is in agreement with the findings of Punja (1985); Basamma (2008) and Muthukumar and Venkatesh (2013).

## Conclusion

From the above studies it could be concluded that the pathogen *S. rolfsii* require alternate cycles of 12 hrs light, 12hrs darkness and free air for their growth and activity of fungi.

**Table 1:** Effect of air on sclerotial development of *S. rolfsii* ($I_1$) in potato dextrose agar medium.

| 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; ++= Whitesclerotia; $+^F$ =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 light on the growth of *S. rolfsii* ($I_1$)

| 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] |

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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