EFFECT OF CERTAIN EPIDEMIOLOGICAL FACTORS ON THE GROWTH AND SCLEROTIAL PRODUCTION OF *SCLEROTIUM ROLFSII*

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Abstract: Laboratory studies were conducted on the effect of temperature and pH levels on the growth and biomass production of *Sclerotium rofsii* Sacc. causing stem rot of cluster bean. The results reveal that the maximum mycelial growth and bio-mass production *S. rolfsii* was at 30°C (89.33 mm; 240 mm) which was reduced significantly below 20°C and above 35°C. Of the pH levels tested, acetic pH (6.5) produced maximum mycelia growth (89.33 mm) and mycelial dry weight (250.66 mg) which was followed by exposing the pathogen to pH 6.0. While increasing or decreasing the pH 7.5 and 5.5 detrimental to the growth *S. rolfsii*.

***Keywords*: Guar, Temperature, pH, Mycelial growth, Bio-mass production**

Introduction

Cluster bean (*Cyamopsis tetragonaloba* (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 (Singh *et al*., 2001) 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. Cluster bean is a rich source of high quality galactomannan gum and protein rich (40-50%) guar meal as animal feed. 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 (Anonymous, 2014). 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 havesecond 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). Cluster bean 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). These pathogens exhibit wide variation in their morphological and, biological characteristics. Morphogenic and pathogenic variations are known in many fungal pathogens and as such detailed investigation was carried out on the variations with regards to the effect of, temperature and pH on 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 different temperature levels on the mycelial growth and dry weight of *S. rolfsii* (I_1) under *in vitro*

Solid medium

A quantity 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, 35 and 40°C for seven days in an incubator. The mycelial growth of the pathogen was measured in mm at the end of incubation period. Further the plates were examined for sclerotial production.

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, 30,35 and 40°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* (I_1) *in vitro*

Solid medium

Influence of different pH levels on the growth of *S. rolfsii* was studied. Eight different pH levels study from 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 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.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 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. Further the plates were examined for sclerotial production

Liquid medium

Potato dextrose broth with different pH levels *viz.*, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 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.

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

Effect of different temperature levels on the mycelial growth and mycelial dry weigth of *S.rolfsii* (I_1)

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. 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 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 sclerotial production which was followed by 35°C. Similarly, Muthukumar and Venkatesh (2013) and Zape *et al.* (2013) reported that the growth of *S. rolfsii* was maximum at 30°C which was reduced significantly below 20°C and above 30°C. The pathogen was unable to grow and produce sclerotial

at and minimum and maximum temperature of 10 to 40°C. Temperature of 25°C was optimum for highest mycelial growth and dry weight of *S. rolfsii* followed by 30°C and 35°C (Prasad and Mahapatra, 2014).The above results lend support to the present findings.

Table 1. Effect of different temperature levels on the mycelial growth and dry weight of *S. rolfsii* (I_1).

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	0.0 f	0.0 f	-

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)

+	-	Sclerotial initial	++++	-	Dark brown sclerotia
++	-	White sclerotia	-	-	No sclerotial initials
+++	-	Fewer sclerotia initials			

Effect of different pH levels on the mycelial growth and dry weight of *S. rolfsii* (I_1)

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 sclerotail 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).

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.

Table 2. Effect of different pH levels on the mycelial growth and dry weight of *S. rolfsii* (I_1)

pH levels	Mycelial growth (mm)	Mycelial dry weight (mg)	Sclerotial production (after 9 days)
4	14.66h	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	+

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)

+	-	Sclerotial initial	++++	-	Dark brown sclerotia
++	-	White sclerotia	-	-	No sclerotial initials
+++	-	Fewer sclerotia initials			

In the present study, acetic pH (6.5) was found to be better and produced maximum mycelial growth, dry weight and sclerotial production was also more. This finding is in line with the earlier reports made by Sarker *et al.* (2013); Muthukumar and Venkatesh (2013) and Chaurasia *et al*. (2013).

Conclusion: It is concluded that the soil-borne pathogen *S. rolfsii* grow well and produced more number of sclerotia at a temperature of 30°C, pH of 6.5 (acetic). Whereas, a temperature of below 20°C, above 35°C, increasing or decreasing the pH of 7.5 and 5.5 detrimental to the growth *S. rolfsii*.

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