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EFFECT OF NUTRITIONAL SOURCES ON THE GROWTH OF *SCLEROTIUM ROLFSII* CAUSING STEM ROT OF CLUSTER BEAN

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Abstract

Stem rot caused by *Sclerotium rolfsii* is one of the important soil-borne pathogen in cluster bean. The present study concerned with effect of different carbon, nitrogen and amino acid which are tested against *S. rolfsii*. The results revealed that the carbon sources tested sucrose as the best carbon source for the growth and bio-mass production of *S. rolfsii* (89.33mm; 650.66mg). With regard to the nitrogen sources tested, peptone supported good growth of *S. rolfsii* (89.66 mm;790.33 mg). In this context, different amino acids tested, among these, tryptophane and phenylalanine recorded the maximum growth of *S. rolfsii* (89.66 and 89.33mm; 785 .00 and 750.66 mg).

***Key words*:** Cluster bean, Carbon sources, Nitrogen sources, Amino acids.

Introduction

Cluster bean (*Cyamopsis tetragonoloba* (L.) Taub) popularly known as "Guar" is an important legume crop and mainly grown under rain fed conditions in arid and semi-arid regions of Rajasthan during Kharif season. The word "Guar" represents its derivation from sanskrit word "Gauaahar" which means cow fodder or otherwise fodder of the livestock. The crop is grown for different purposes such as vegetable, green manure and seed production. Among dryland crops, cluster bean occupies an important place in the national economy because of its industrial importance mainly due to the presence of gum in its endosperm (28 to 32%). Guar gum has its use in several industries viz., textiles, paper, petroleum, pharmaceuticals, food processing, cosmetics, mining explosives, oil drilling etc. Cluster bean is a leguminous crop and can fix 37-196 kg N/ha/year. 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). 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 utilization of nutrients. In the present study was aimed on the use of various nutritional 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 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 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 certain nutritional factors on the mycelial growth of *S. rolfsii* (I_1) *in vitro*

Carbon Source

Growth on Liquid Medium : The *in vitro* growth of the fungus was tested with nine different carbon sources *viz.,* cellulose, dextrose, fructose, glucose, lactose, maltose, mannitol, starch and sucrose. Czapek's broth was taken as the basal medium for the study. In the czapek's broth, sucrose was replaced with various carbon sources on equivalent weight basis and autoclaved at 15 lb pressure for 20 min. The final pH of the medium was adjusted to 7.0. After that, the medium was inoculated with six mm mycelial disc of pathogen obtained from seven days old culture grown on PDA. The inoculated media were incubated for ten days at room temperature (28±2°C). At the end of the incubation period the mycelial mats were filtered through previously dried and weighed filter paper (Whatman No. 41) and 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.

Linear Growth : Czapek's agar medium was amended with various carbon sources on equivalent weight basis and were dispensed in sterile Petri plate at fifteen ml quantities. After cooling they were inoculated with six mm mycelial disc of the pathogen obtained from seven days old culture grown on PDA in Petri plates and incubated for seven days. The linear growth of the pathogen was measured in mm at the end of the incubation period.

Nitrogen Source

Growth on Liquid Medium : The *in vitro* growth of the fungus was tested with nine different nitrogen sources *viz.,* ammonium chloride, ammonium nitrate, ammonium sulphate, calcium nitrate, peptone, potassium nitrate, sodium nitrite, sodium nitrate and urea. Czapek's broth was taken as the basal medium for the study. In the czapek's broth, sodium nitrate was replaced with various nitrogen sources on equivalent weight basis and autoclaved at 15 lb pressure for 20 min. The final pH of the medium was adjusted to 7.0. After that, the medium was inoculated with six mm mycelial disc of pathogen obtained from seven days old culture grown on PDA and the media were incubated for ten days at room temperature (28±2°C). At the end of the incubation period the mycelial mats were filtered through previously dried and weighed filter paper (Whatman No. 41) and 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.

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Amino Acids

Growth on Liquid Medium : The *in vitro* growth of the fungus was tested with eight different sources of amino acids *viz.,* alanine, asparagine, cysteine, glutamic acid, phenylalanine, tryptophan, tyrosine and valine. Czapek's broth was taken as the basal medium for the study. In the czapek's broth, the nitrogen source was replaced with various amino acids on equivalent weight basis and autoclaved at 15 lb pressure for 20 min. The final pH of the medium was adjusted to 7.0. Then the medium was inoculated with six mm mycelial disc of pathogen obtained from seven days old culture grown on PDA and the media were incubated for ten days at room temperature (28±2°C). At the end of the incubation period the mycelial mats were filtered through previously dried and weighed filter paper (Whatman No. 41) and 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.

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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 carbon sources on the mycelial growth and dry weight of *S. rolfsii* (I_1)

Among the nine carbon sources (cellulose, dextrose, fructose, glucose, lactose, maltose, mannitol, starch and sucrose) tested, sucrose recorded maximum mycelial growth of 89.00 mm and dry weight of 238.33 mg (Table 1). It was succeeded by dextrose and glucose. However, mycelial growth and dry weight was minimum with lactose as carbon source (44.00 mm; 169.00 mg, respectively). Fungi exhibit wide variation in the utilization of carbon source. Among the nine carbon sources tested, sucrose recorded the maximum mycelial growth and dry weight of *S. rolfsii.* which was similar to the findings of Xiao *et al.* (2012); Muthukumar and Venkatesh (2013) and Saha *et al*. (2014). Almost half of the dry mycelial weight of the fungal cell consists of carbon which is the main structural element (Lilly and Barnett, 1951). The utilization of various carbon compounds may depend on either of the activity of the fungus to utilize simpler forms or on its power to convert the complex carbon compounds into simpler forms, which may be easily utilized. Sucrose being the major component of photosynthesis in plants is generally utilized as a good source by most of the plant pathogenic fungi.

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

The effect of various nitrogen sources on the mycelial growth and dry weight of *S. rolfsii* are summarized in Table 2 revealed that all the nitrogen sources (ammonium chloride, ammonium nitrate, ammonium sulphate, calcium nitrate, peptone, potassium nitrate, sodium nitrite, sodium nitrate and urea) favoured the growth of *S. rolfsii.* Among the nine nitrogen sources tested, peptone recorded maximum mycelial growth (89.00 mm) and dry weight of (240.00 mg) of *S. rolfsii*. This was followed by potassium nitrate and urea. Culturing of *S. rolfsii* in ammonium sulphate and calcium nitrate was found to be recording minimum mycelial growth (68.00 mm; 66.33 mm, respectively) and dry weight (164.33 mg; 151.66 mg, respcetively) of *S. rolfsii*. Nitrogen being a component of protein is an essential element and like carbon, it is used by fungi for functional as well as structural purposes. But all the sources of nitrogen are not equally good for the growth of fungi.

The fungus also showed variation when growth pattern was studied on media containing different nitrogen sources. The fungus produced maximum mycelial growth and dry weight on peptone and potassium nitrate containing medium. Similarly, Basamma (2008) and Muthukumar and Venkatesh (2013) reported that potassium nitrate and peptone recorded the maximum mycelial growth of *S. rolfsii*.

Effect of various amino acids on the mycelia growth of *S. rolfsii* (I_1)

The effect of various amino acids on the mycelial growth and dry weight of *S. rolfsii* are summarized in Table 3. Among the eight amino acids (alanine, asparagine, cysteine, glutamic acid, phenylalanine, tryptophan, tyrosine and valine) tested, tryptophane and phenylalanine supported maximum mycelial growth (89.33 mm, 89.00 mm, respectively) and dry weight (235.66 mg; 236.00 mg, respectively) of *S. rolfsii*. It was succeeded by valine (80.66mm, 210.00 mg, respectively). The minimum mycelial growth and dry weight was registered in Glutamic acid. A large number of amino acids are recorded as good nutritional sources for many fungi. However, the nutritive capacity of individual amino acids varies highly with organisms. Among the eight amino acids tested, phenylalanine and tryptophane amended medium supported the maximum mycelial growth and dry weight of *S. rolfsii*. Similarly, Muthukumar and Venkatesh (2013) who explained that maximum mycelial growth and dry weight of *S. rolfsii* was recorded in phenylalanine and tryptophan amended medium.

Conclusion

Fungi exhibit wide variation in their utilization of nutritional sources. Hence, it could be concluded that the pathogen *S. rolfsii* prefer sucrose as a carbon source, peptone as a nitrogen source, tryptophane and phenylalanine as a amino acid source for their growth and activity of fungi.

Table 1: Effect of different carbon sources on the mycelial growth and dry weight of *S. rolfsii* (I_1)

Sl. No.	Carbon sources	Mycelial growth (mm)	Mycelial dry weight (mg)
1.	Cellulose	50.66 e	210.00 f
2.	Dextrose	80.66 b	610.00 b
3.	Fructose	63.66 d	250.00 e
4.	Glucose	75.00 c	533.66 c
5.	Lactose	45.66 g	100.66 h
6.	Maltose	53.00 e	230.33 f
7.	Mannitol	47.00 f	170.33 g
8.	Starch	74.33 c	495.66 d
9.	Sucrose	89.33 a	650.66 a
10.	Control	52.00 e	70.33 i

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 nitrogen sources on the mycelial growth and dry weight of *S. rolfsii* (I_1)

Sl. No.	Nitrogen sources	Mycelial growth (mm)	Mycelial dry weight (mg)
1.	Ammonium chloride	67.33 e	410.00 g
2.	Ammonium nitrate	73.66 d	620.33 e
3.	Ammonium sulphate	70.00 d	590.66 f
4.	Calcium nitrate	65.33 e	350.00 h
5.	Peptone	89.66 a	790.33 a
6.	Potassium nitrate	85.00 b	750.00 b
7.	Sodium nitrite	84.33 b	730.66 c
8.	Sodium nitrate	80.00 c	690.66 d
9.	Urea	82.00 c	700.00 d
10.	Control	52.66 f	71.66 i

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 different amino acids on the mycelial growth and dry weight of *S. rolfsii* (I_1)

Sl. No.	Amino acids	Mycelial growth (mm)	Mycelial dry weight (mg)
1.	Alanine	70.00 c	600.00 d
2.	Asparagine	67.33 d	520.33 e
3.	Cysteine	72.66 c	670.00 c
4.	Glutamic acid	65.00 d	405.66 f
5.	Phenylalanine	89.33 a	785.00 a
6.	Tryptophan	89.66 a	780.66 a
7.	Tyrosine	60.00 e	320.00 g
8.	Valine	77.33 b	698.33 b
9.	Control	53.00 f	72.00 h

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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