

Upsurging the media for optimal growth and sporulation *Sphaerellopsis paraphysata*

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Abstract: Several biotic and abiotic factors distress the pearl millet and significantly influence the production. Leaf rust, caused by *Puccinia substriata*, is the most important disease among the biotic factors, which reduced the yield up to 76 percent. *Sphaerellopsis paraphysata* is a rust mycoparasite with a wide host range that inhibits rust spore germination which could be cultured and could be grown on potato dextrose agar media. The effect of different carbon and nitrogen sources on the growth of *S. paraphysata* TNAU Sp1 showed that the dextrose and sodium nitrate supported the maximum mean mycelial growth and recorded 60.00 and 58.67 mm, respectively. Similarly, maximum mycelial growth and pycnidial production of *S. paraphysata* were observed at 20°C (38.3 mm) temperature and 6.5 pH (55.0 mm).

Key words: Pearl millet rust, mycoparasite, *Sphaerellopsis paraphysata*, artificial media, growth and sporulation

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1. Introduction

Pearl millet (*Pennisetum glaucum*) is an important food grain crop and widely grown millet throughout the world. The major pearl millet producing states of India are Rajasthan, Gujarat and Tamil Nadu. Tamil Nadu occupies an area of 49,670 ha with the production of 1.02 lakh tonnes and productivity of 2059 Kg/ha. The pearl millet crop is affected by several biotic and abiotic factors causing significant reduction in the yield. The major diseases of pearl millet are downy mildew, ergot, rust and smut diseases [17]. Among these diseases, the pearl millet leaf rust caused by *Puccinia substriata* is the most destructive causing yield loss up to 76% [16]. The effect on grain yield reduction relies upon the severity, environmental conditions and age of the crop [15]. Though chemical fungicides were effective in rust disease management, the intensive use of fungicides which is hazardous to human and environment, hence reduction in the usage of chemicals must be practiced. *Sphaerellopsis* spp. is a hyperparasitic fungi which parasitizes the rust fungi and thus reduces the incidence of rust. Due to the wide host range of *Sphaerellopsis*

spp., it is a promising organism for biological control and integrated disease management. Hence, attempts were made to standardize the parameters required for optimum growth of *Sphaerellopsis paraphysata*.

2. Materials and Methods

2.1. Isolation of the biocontrol agent *S. paraphysata*

Two methods of isolation were followed for *S. paraphysata*. The first method was single pycnidial isolation, for this the pycnidia of *S. paraphysata* in the rust infected leaves were scrapped and these pycnidia were dipped in sodium hypochlorite for 30 sec and then mixed with water agar medium, after the solidification of the media the pycnidia dispersed randomly in the water agar were marked with a marker under stereo zoom microscope and incubated at 25° C for 48 hours and then the germinated spores were located and marked which are transferred to another Petri dish containing V8 juice agar. Streptomycin was added to prevent the bacterial contamination. Inoculated Petri

plates were incubated for 20°C for 25 days [6]. The biocontrol agent's pure culture was obtained by single hyphal tip technique [4]. Stock cultures were sub cultured at an interval of 30 days to keep the culture live. In another method of isolation, the conidia of *S. paraphysata* were collected and Shaked well. These conidia were suspended in sterile distilled water and 10 µl of this suspension was placed on the cavity slide and incubated on 20° C for 5 days. During this time the conidia germinated and formed a mat. This mat is taken and put on V8 juice agar and incubated at 25° C. It was sub cultured after 2 days.

2.2. Analyzing different carbon and nitrogen sources on mycelial growth of *S. paraphysata*

Potato dextrose agar medium was used as a basal medium by adding different carbon (sucrose, cellulose, starch, sodium, maltose) and nitrogen (Peptone, urea, potassium nitrate, beef extract, ammonium nitrate) sources to study their influence on the radial mycelial growth of *S. paraphysata*. The sources were added separately to the basal medium at 20 g per litre. The basal medium added with dextrose and sodium nitrate separately served as control. Every treatment was replicated thrice and the radial mycelial growth was measured 25 days after incubation [3]; [7].

2.3. Influence of different pH on the radial mycelial growth of *S. paraphysata*

Potato dextrose agar medium was used as a basal medium for studying the effect of different pH levels on the radial mycelial growth of *S. paraphysata*. The pH level of the basal medium was adjusted to different levels viz., 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 using a pH meter (model ELICO digital pH meter) by adding 0.1N sodium hydroxide or HCL. Twenty ml of autoclaved PDA medium was poured in a

petridish and then inoculated with the mycelial disc of the test fungus and incubated for 25 days. Each treatment was replicated thrice and 25 days after incubation the radial mycelial growth was measured [10].

2.4. Effect of different temperature on the radial mycelial growth of *S. paraphysata*

To study the effect of temperatures on the radial mycelial growth of *S. paraphysata* potato dextrose agar medium was used as basal medium. The autoclaved medium was dispensed in Petri plates, inoculated with non-sporulating growing tip culture of test fungus and incubated for ten days at different temperatures viz., 5, 10, 20, 30 and 35° C BOD incubators. Four replications were maintained for each treatment and radial mycelial growth was measured at twenty days after incubation at different temperatures [13]; [9].

3. Results and Discussion

3.1. Effect of different carbon sources on the growth of *S. paraphysata*

The effect of different carbon sources on the growth and dry mycelial weight of *S. paraphysata* TNAU Sp1 was evaluated. The results showed that the maximum mycelial growth (58.8 mm) and mycelial dry weight (9.85g) were observed when dextrose was used a carbon source followed by cellulose which recorded 54.5 mm and 9.28 g as mean mycelial growth and dry weight, respectively. Mannose had not supported the growth of *S. paraphysata* and recorded least mycelia growth and dry weight (Table.1).

Table 1. Effect of different carbon sources on the growth of *S. paraphysata*

S.No	Carbon sources	Mycelial growth (mm)	Dry Weight of Mycelia (g)	Pycnidial production
1.	Sucrose	46.0 ^c	8.48 ^c	+
2.	Cellulose	54.5 ^b	9.28 ^b	++
3.	Starch	54.0 ^b	8.43 ^c	+
4.	Mannose	28.0 ^d	2.25 ^e	+
5.	Maltose	45.0 ^c	5.08 ^d	+
6.	Dextrose	58.8 ^a	9.85 ^a	+

Values are mean of three replications; **Pycnidia (-) – zero; (+) – (0 to 25 Nos.); (++) – (25 to 50 Nos.); (+++) – (50 to 75 Nos.) and (+++++) – (75 to 100 Nos.)

Means in a column followed by the same alphabet are not significantly different according to DMRT at p<0.01

3.2. Effect of different nitrogen sources on the growth of *S. paraphysata*

The effect of different nitrogen sources on the growth and dry mycelial weight of *S. paraphysata* TNAU Sp1 in solid media was assessed (Table. 2). Among the tested nitrogen sources, sodium nitrate has obtained the highest mean mycelial growth (58.8 mm) and mycelia dry weight (3.70g). While, Potassium nitrate and urea recorded 56.3 mm and 56.5 mm mean mycelia growth respectively. The least mycelial growth was obtained in ammonium nitrate (38.8 mm).

Table 2. Effect of different nitrogen sources on the growth of *S. paraphysata*

S.No	Nitrogen sources	Mycelial growth (mm)	Dry Weight of mycelia (g)	Pycnidial Production
1.	Peptone	46.8 ^b	2.48 ^c	+
2.	Urea	56.5 ^a	2.95 ^{bc}	-
3.	Potassium nitrate	56.3 ^a	2.65 ^c	++
4.	Beef Extract	55.0 ^a	3.40 ^{ab}	++
5.	Ammonium nitrate	38.8 ^c	1.38 ^d	++
6.	Sodium nitrate	58.8 ^a	3.70 ^a	++

Values are mean of three replications; **Pycnidia (-) – zero; (+) – (0 to 25 Nos.); (++) – (25 to 50 Nos.); (+++) – (50 to 75 Nos.) and (+++++) – (75 to 100 Nos.)

Means in a column followed by the same alphabet are not significantly different according to DMRT at p<0.01

3.3. Effect of different temperature levels on the growth of *S. paraphysata*

This experiment was conducted to find out the optimum temperature requirement for maximum growth and pycnidial production of *S. paraphysata* and presented in Table 3. Among different temperature levels, maximum mycelial growth (38.3 mm) and pycnidial growth were observed at 20°C followed by 25°C (34.8 mm) and 15 °C (25.0 mm), respectively. Least mycelial growth was observed at 10°C (10.33 mm) while, there was no pycnidial production was observed at 10°C, 30°C and 35°C temperature levels tested.

Table 3. Effect of different temperature levels on the growth of *S. paraphysata*

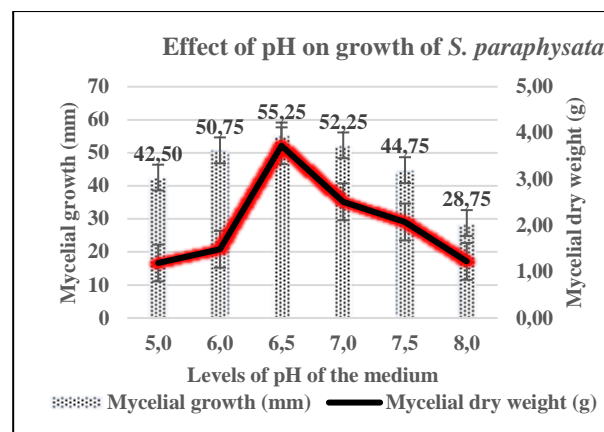
S. No	Temperature (°C)	Mycelial growth (mm)	Pycnidia Production
1.	10	10.3 ^e	-
2.	15	25.0 ^c	++
3.	20	38.3 ^a	+++
4.	25	34.8 ^b	++
5.	30	20.8 ^d	-
6.	35	10.0 ^e	-

Values are mean of three replications; **Pycnidia (-) – zero; (+) – (0 to 25 Nos.); (++) – (25 to 50 Nos.); (+++) – (50 to 75 Nos.) and (+++++) – (75 to 100 Nos.)

Means in a column followed by the same alphabet are not significantly different according to DMRT at $p < 0.01$

3.4. Effect of pH on the radial mycelial growth and mycelial dry weight of *S. paraphysata*

The growth of *S. paraphysata* was tested at six different pH levels viz., pH 5.0, 6.0, 6.5, 7.0, 7.5 and 8.0. Among pH levels, 6.5 supported the maximum mean mycelial growth (55.3 mm) followed by pH 7.0 (52.3 mm) and pH 6.0 (50.8 mm). The mycelial growth was very poor when the pH lowered than 6.0 and above 7.0. The lowest mycelial growth was obtained at pH 8.0 (28.8 mm). The highest dry mycelial weight was obtained in pH 6.5 (3.72g), followed by pH 7.0 (2.51g) and the lowest was obtained in pH 8.0 (1.22g). The pycnidial production was higher in pH 7.0 followed by pH 6.5 (Fig. 1).



The carbon sources such as sucrose, cellulose, starch, sodium, maltose and dextrose were tested for optimizing the efficient carbon source for the growth of *S. paraphysata*. The maximum mycelial growth (58.8 mm) and dry mycelial weight of *S. paraphysata* was obtained when dextrose used as carbon source. The results obtained was slightly deviating and does not agree with [12], and they have reported that the fructose followed by sucrose were responsible for the growth and development of *S. paraphysata*.

Among the nitrogen sources, sodium nitrate obtained the maximum mean mycelial growth (58.8 mm) while the ammonium nitrate (38.8 mm) recorded the least. The results obtained was similar to [11], who reported that peptone and yeast extract has yielded maximum mycelial growth for *A. quisqualis*. In liquid media the highest dry mycelial weight was obtained at sodium nitrate (3.70g) followed by beef extract (3.40 g) followed by urea (2.96 g) the least growth was obtained in ammonium nitrate (1.37 g). This was in accordance with [11], who stated that maximum mycelial growth of *A. quisqualis* was obtained in peptone.

Among different pH levels tested, pH 6.5 has obtained the highest mean mycelial growth (55.25 mm) and the

minimum growth was obtained in pH 8.0 (28.75 mm). The results obtained in liquid media also same as that of solid media, the highest dry mycelial weight was obtained in pH 6.5 (3.72g) and the lowest was obtained in pH 8.0 (1.22g). This suggests that *S. paraphysata* grows well in slightly acidic and neutral pH. [8] stated similar results of growth of *A. quisqualis*, a mycoparasite which has evolutionary relationship with *S. paraphysata* and found that it grows between the pH 5.0 to 7.0 [13] has also said that the maximum mycelial growth of *A. quisqualis* was obtained at pH 6.5. This was supported by [18], who obtained maximum mycelial growth in pH 7.0.

In all the previous reports in *S. paraphysata* the growth temperature was maintained at 20 - 25°C (Swendsrud and Calpouzos, 1970; [2]; [6]. Among the temperature, the maximum mycelial growth was obtained in 20°C (38.3 mm). Similarly [13] grown *Ampelomyces quisqualis* (reported to have evolutionary relationship with *Sphaerellopsis* spp. under various treatment temperatures and reported that that it could grow under temperature ranges from 10° C to 35°C and [1] obtained maximum mycelial growth of *A. quisqualis* at 20° C (89.0 mm) followed by 25° C (81.0 mm).

4. Conclusion

With the above results it is obvious that the *Sphaerellopsis paraphysata* – a mycoparasite on pearl millet rust pathogen *Puccinia substriata* could be potentially exploited as a biocontrol agent.

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