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Cultural and Taxonomical Studies on *Pestalotiopsis funerea* Causing Leaf Spot of *Eucalyptus globulus**

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The effect of different media, temperature and *p*H on the growth and sporulation of *Pestalotiopsis funerea* Desm., causing leaf spot of *Eucalyptus globulus* Labill., was studied. The pathogen showed best growth on malt-extract (Blakeslee formula) and Czapek-Dox+0.05% yeast extract media. It exhibited excellent sporulation on corn meal. host extract, potato-dextrose and malt extract media; and grew and sporulated on a wide range of temperature (10 to 30°C) and *p*H (4 to 11) with an optimum at 25°C and *p*H 6 respectively.

Key Words: *Pestalotiopsis funerea*, **Media, *p*H, Temperature, Growth, Sporulation**

Introduction

Fungal growth and sporulation are greatly influenced by the medium, its *p*H and temperature. Variation in *p*H and temperature, leads to differences in growth and reproduction and therefore, for each fungus there is minimum, optimum and maximum of these two factors (Lilly & Barnett 1951, Cochrane 1958, Chauhan & Suryanarayana 1970, Tandon & Mitra 1963, Bilgrami & Verma 1978). The present investigation was undertaken to determine the effect of different media, temperature and *p*H on growth and sporulation of *Pestalotiopsis funerea* Desm. (Leaf spot pathogen of *Eucalyptus globulus* Labill.)

Material and Methods

Monosporic culture of *P. funerea* was maintained on Czapek-Dox+0.05% yeast extract agar at 24±1°C for study.

Seventeen different types of nutrient media-rice meal, oat meal, gram meal, corn meal,

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host extract, potato dextrose, Czapek-Dox, Czapek-Dox+0.05% yeast extract, (Asthana & Hawker's, Richard's, Martin's Thind & Mandhar's), malt extract (Leonian version), malt extract (Blakeslee formula), glucose-peptone, and glucose-aspargine were used to study their effect on growth and sporulation of the pathogen.

For temperature studies, the pathogen was grown on Czapek-Dox+0.05% yeast extract medium and incubated at 2°, 6°, 10°, 20°, 30°, 35°, and 40°C. To avoid lag effect, the flasks were kept at particular temperature at least 24 hr before inoculation. For *p*H experiment, the pathogen was grown on Czapek-Dox+0.05% yeast extract medium at *p*H 2.0, 4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 9.0 and 11.0. Desired *p*H of the media was adjusted using 0.1 N/NaOH or HCl with Elico *p*H meter model Li-12. The alteration in *p*H after growth was also recorded. Medium was not buffered owing to its demerits while studying physiology of fungi, particularly dealing with the effect of *p*H as reviewed by Lilly and Barnett (1951) and more over, we were interested with the effect of initial *p*H on mycelial yield and their drift after nutrient up-take (Upadhyay & Dwivedi 1978).

The above studies were performed by two methods:

Hyphal dry weight method: Fifty ml of the liquid medium was apportioned in each of the 250 ml Erlenmeyer flasks and autoclaved at 1.05 kg/cm^2 for 15 min. Each flask was inoculated with two 6 mm diam. agar discs of a vigorously growing culture of *P. funerea* and incubated at 25±1°C or at particular temperature (in case of temperature effect) for 15 days. The cultures were filtered through, dried and weighed on Whatman filter paper no. 42 and *p*H of the filtrate determined simultaneously. The mycelial mats were thoroughly washed with distilled water, dried to a constant weight at 60° for 48 hr and reweighed after cooling in a desiccator. Triplicate sets were used in each case and average dry weight was recorded. The growth in terms of hyphal dry weight (mg) was graded as: best (<634), next best (555–635), good (455–554), poor (255–454) and absent (0).

Sporulation was classified as excellent, good, fair, poor or absent on the basis of visual observations. The criteria were that of surface area of hyphal mats covered by black spores and their colour intensity as suggested by (Upadhyay et al. 1977).

Colony diameter method: Fifteen ml molten nutrient agar medium was poured in each of sterilized Petri plates which solidified after a short time. Agar discs of 6 mm diam. from a vigorously growing colony of the pathogen were placed over the medium in centre of the plate and incubated at 25±1°C or at particular temperature (in case of temperature effect). Colony diameter was measured after 120 hr of incubation and results presented in tables after calculating mean value of five replicate plates.

Results and Discussion

Effect of media (table 1)

The pathogen showed the best growth in terms of hyphal dry wt. on malt extract (Blakeslee formula) and Czapek-Dox+0.05% yeast extract media; good growth on Czapek-Dox, oat-meal, potato-dextrose, host leaf extract, Richard's, gram-meal and glucose-asparagine; fair on malt extract (Leonian version), malt extract, Thind and Mandhar's and Martin's; and the least growth on Asthana and Hawker's and rice meal media. Sporulation was excellent on corn meal, host leaf extract, potato-dextrose and malt extract; good on Czapek-Dox +0.05% yeast extract; while it was absent on several other media.

Maximum radial growth of the pathogen was recorded on Czapek-Dox+0.05%

Table 1 *Effect of different media on the growth and sporulation of* P. funerea

Media	Radial colony growth (mm)	Hyphal dry weight (mg)	Sporulation
Rice meal	55	75	—
Oat meal	56	560	—
Gram meal	61	510	++
Corn meal	52	253	++++
Host leaf extract	49	540	++++
Potato-Dextrose (PDA)	55	560	++++
Czapek-Dox	60	565	++
Czapek-Dox+0.05% yeast extract	66	660	+++
Asthana and Hawker's	39	207	—
Richard's	46	521	—
Martin's	49	423	—
Thind and Mandhar's	48	433	+
Malt extract	21	336	++++
Malt extract (Leonian version)	56	450	—
Malt extract (Blakeslee)	56	719	+
Glucose-Peptone	45	348	—
Glucose-Asparagine	—	510	—

Growth on different media was highly significant indicated by the exceeding of the calculated value beyond the tabulated value 34.077>2.70 (at 1%with 16 and 34 degree of freedom
—Absent; +Poor; ++ Fair; +++ Good; ++++Excellent

yeast extract and Czapek-Dox, while it was minimum on Richard's and glucose-peptone media. Rice meal medium supported least hyphal yield in liquid medium. However, it exhibited good growth on nutrient agar medium. This difference in growth on solid and liquid media was due to very light and superficial growth of the pathogen, thereby covering more area; however, total hyphal yield was quite meagre.

The fungus thus exhibited good growth on some of the natural and semisynthetic media viz., oat meal, gram meal, potato-dextrose and Czapek-Dox. This finding is similar to the observations of Wahi (1967), Misra and Chatterji (1963) and Singh (1975). Czapek-Dox+0.05% yeast extract proved to be the best synthetic medium which is in accord with the findings of Wahi (1967) and Singh (1975).

Effect of temperature (table 2)

The pathogen was able to grow on temperatures ranging from 10 to 30°C. Growth was best at 25° and poor at 10°. The optimum temperature range for growth and sporulation of the pathogen was between 25 to 30°. The hyphal dry weight increased with the rise in temperature reaching maximum at 25°C which fell little at 30°C and declined suddenly thereon. Similarly, Tandon and Bhargava (1960) and Tandon and Mitra (1963) found 25°C temperature to be most suitable for the growth of different species of *Pestalotia* and *Pestalotiopsis*.

Table 2 *Effect of different temperatures on the growth and sporulattion of* P. funerea

Temperature (°C)	Radial colony growth (mm)	Hyphal dry weight (mg)	Final *p*H	Sporulation
5	0.0	0	5.8	—
10	14.0	331	6.7	—
20	41.0	550	6.7	+
25	66.0	660	6.7	++
30	45.5	510	6.8	++
35	0.0	0	5.8	—

Treatment with different temperature was highly significant by exceeding of the calculated value beyond the tabulated value 21896.5>5.04 (at 1%) with 5 and 12 degree of freedom

Table 3 *Effect of different* pH *on the growth and sporulation of* P. funerea

Initial *p*H	Radial colony growth (mm)	Hyphal dry weight (mg)	Final *p*H	Sporulation
2.4	0.0	0.0	2.0	—
4.0	62.9	555	6.4	+
5.0	66.5	611	6.4	++
5.5	66.5	653	6.3	++
6.0	68.1	660	6.9	++
6.5	67.0	658.7	7.0	++
7.0	67.0	620	7.2	++
9.0	66.8	600	7.3	++
11.0	47.2	430	7.3	+×

Different *p*H were highly significant indicated by exceeding of the calculated value beyond the tabulated value 27980.7>3.71 (at %) with 8 and 18 degree of freedom

*Effects of p*H (table 3)

The pathogen could grow on *p*H ranging from 4 to 11. Maximum growth and sporulation was at *p*H 5 followed in order by *p*H 6.5 and *p*H 5.5. Sporulation was good on a wide range of *p*H 5 to 11. Cochrane (1958), Munjal (1960), Martinez and Hanson (1963), and Chauhan and Suryanarayana (1970) also reported *p*H 6 to be optimum for the growth and sporulation of fungi studied by them.

The growth of the pathogen led to a change in the *p*H of the medium at the end of the growth period. It was noticed that whenever the initial *p*H was low the final one reached the neutral point or slightly on the alkaline side whereas in high alkaline media it decreased towards neutral side. According to Lilly and Barnett (1951) these changes in *p*H were due to variation in the relative amounts of acids and bases formed or withdrawn and to the ionization constant

of these compounds in the growth medium during formation of secondary and tertiary metabolites in idiophase of a growing fungus. Moreover, the utilization of nitrate ions or other anions such as phosphate or sulphate present in a growth medium for the formation of non-ionized constitutory compounds as primary metabolites during trophophase of a growing fungus had the effect of increasing hydroxyl ion concentration in the medium (drift towards alkaline side). On the contrary pathogen growing on alkaline medium, the more CO_2 evolved may lower the *p*H. Change in *p*H may alter the permeability of protoplasmic membrane, ionization of compounds, enzymic activity etc. which indirectly affected the growth and sporulation. These results are in general agreement with the observations of Tandon and Mitra (1963) on *Pestalotiopsis versicolor*.

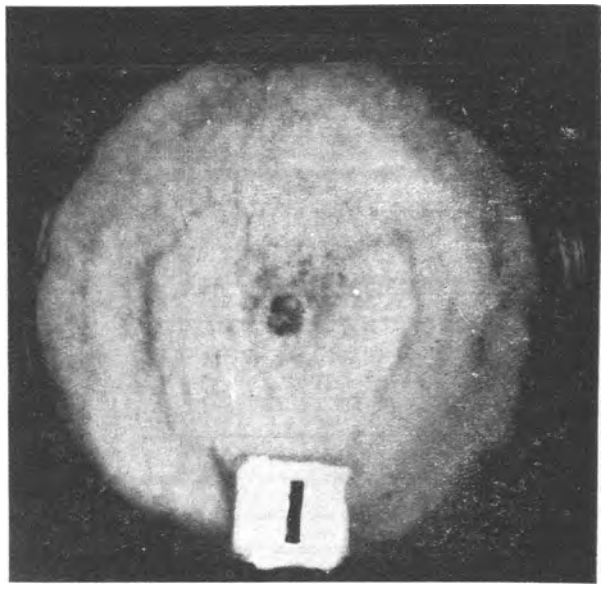

Figure 1 White colony of *Pestalotiopsis funera* on nutrient agar

Colony growth of P. funerea

The culture of the pathogen was white in colour with thick felty mass of mycelium spreading on the agar (figure 1). The speed of radial growth was found to be 13.2 mm/24 hr (average mean of 0 to 120 hr growth) on nutrient Czapek-Dox+0.05% yeast extract agar. The colony was divided into three regions viz., active zone of very light band of mycelium (outermost), inactive zone of thick band of mycelium (middle) and sporulating zone of very thick band of mycelium (central) after 96 hr of growth. The terms very light, thick and very thick band of mycelium were coined for the relative strength of the mycelium of growing colony. However, terms active, inactive and sporulating zone were physiological. The hyphal tips of the active zone of mycelium grew continuously, the middle one became inactive with reference to its extension and the central one which built up a level of staling growth products that inhibited extension of growth and stimulated sporodochia production. The reverse of the colony was yellowish in sporulating central zone, comparatively lighter in non-sporulating middle zone and colourless in outermost region. The actively growing region of the colony continuously added inactive mass of hyphae in middle zone which ultimately converted into sporulating central zone later on. Thus they formed rings of the sporulating zone after a long period of the colony growth by continuous gradual shifting of different zones centrifugally.

A detailed observation of the hyphae of active zone may be cited as follows (the distance was measured in the direction from outermost to the inner side of the colony):

1. 0–400μm = hyphae 7 μm in diameter, straight, oriented towards periphery and least branched;

2. 400–800μm =comparativey more branched, 4μm in diameter, turning in the direction of open area and then growing straight;

3. 800–1200μm =repeated branching resulting in close areas of agar, branches facing centrifugally in direction, 3 μm in diameter and formed reticulate structure;

4. 1200–1500μm =complicated branching. fusion of hyphae, developmemt of hyphal loops and coils, and hyphae 3 μm in diameter;

5. 1500 μm to central part =complicated branching, 3 μm in diameter and not oriented towards outside. Thick and felty mass of mycelium did not permit further direct observations by binocular. Complicated branching was denoted for the repeated branching followed by anastomosis among hyphae.

Structure of conidia

Conidia consisted of three, thick-walled median cells capped by hyaline end cells (figure 2). Conidia fusiform, 9.5–10.5×2.5 μm, slightly constricted; intermediate cells deep olive buff, concolorous, 5–6×2.5 μm long; apical hyaline cells long, acute, bearing 2–3, usually 3, widely divergent setulae, 6–7×25 μm long, with visible lumen; basal hyaline cells tapering into filiform pedicels measuring 1–1.5×2.5 μm in length.

Taxonomic position

The classification of fungi within *Pestalo-*

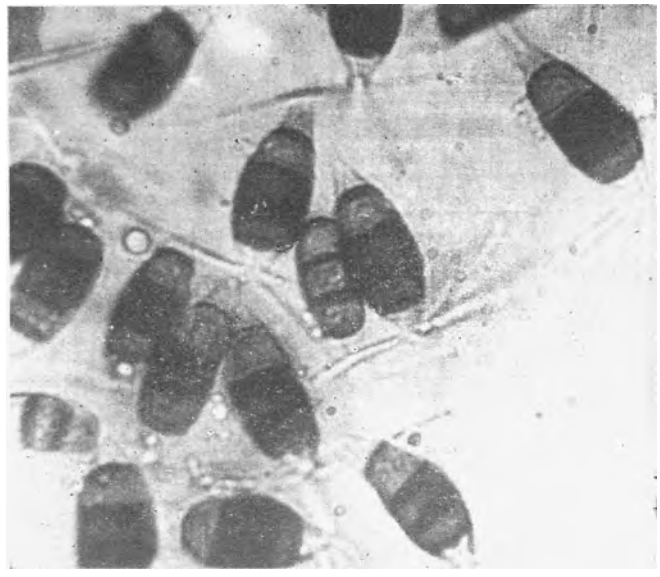

Figure 2 Photomicrograph of spores of *P. funerea*

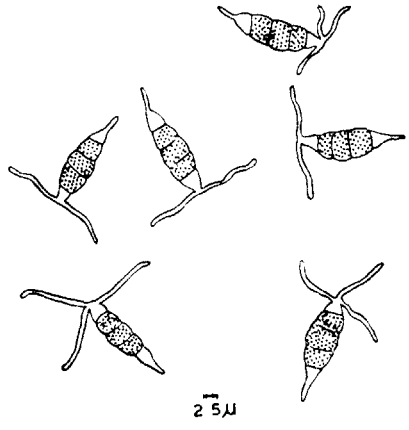

Figure 3 Camera lucida of drawing of spores of *P. funerea*

tia/ Monochaetia/ Seimatosporium complex is still hampered by differences of opinion regarding the basic criteria to be used in delimiting genera (Griffiths & Swart 1974). Steyaert (1949) introduced the idea of number of cells per conidium as a primary criterion. He created genus *Doliomyces* with *D. senegalensis* as the type species to accommodate such forms of *Pestalotia* or *Pestalotiopsis* which possessed only 4 celled conidia with pseudosepta. The present isolate (*P. funerea*), however, has typically 5-celled conidia, the cells being separated by true septa. Steyaert (1961) treated *Pestalozzia senegalensis* Speg., as synonym of *D. senegalensis* (Speg.) Stey., while Guba (1961) in his monograph described it as *Pestalotia senegalensis* Speg., and he did not recognize the genus *Doliomyces* Stey. Sutton (1969) supported Steyaert's ideas (number of cells per conidium) to a large extent by introducing an additional criterion, the wall structure of the conidium. *P. funerea* differed from *P. senegalensis* in the following characters:

Characters	*P. senegalensis*	*P. funerea*
1. Conidial size	14–18 × 5μm	9.5–10.5 × 2.5μm
2. Setulae	Chlorine colored 2–4, 15–20 μm long	hyaline 2–3, 6–7 × 2.5 μm long
3. Pedicel	short up to 5 μm	short 1–1.5 × 2.5μm
4. Middle coloured cells	The lower intermediate cell is bigger in size than the two upper cells	The lower intermediate cell is either of the same size or some what smaller than the next-upper cells

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