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# STUDIES ON THE PHYSIOLOGY OF HIGHER FUNGI : XIV. EFFECT OF TEMPERATURE, LIGHT AND HYDROGEN-ION CONCENTRATIONS ON GROWTH OF *PYCNOPORUS SANGUINEUS* (L. EX FR.) MURR., *DÆDALEA FLAVIDA* LE'V. AND *TRAMETES CINGULATA* BERK.

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In this present study informations have been provided on the role of different environmental factors on growth of three heterothallic basidiomycetous fungi, *viz.*, *Pycnoporus sanguineus* (L. ex Fr.) Murr., *Dædalea flavida* Le´v. and *Frametes cingulata* Berk. Of the environmental factors, the optimum temperature for growth of *P. sanguineus* and *D. flavida* has been fomd to be 30°C. while it has been 25°C. for *T. cingulata*. The optimum source of light for growth of all the test-fungi has been found to be alternate light and darkness. The optimum *p*H for growth of *P. sanguineus* has been recorded as 4.5, of *T. cingulata* 6.5 while it has been 6.0 for *D. flavida*.

## Introduction

On reviewing the available literature it has been revealed that modern study on the physiology of wood-rot ing basidiomycetes has started only in the early decades of twentieth century. Most of the literature describing the effect of temperature on the growth of mycelium has been reviewed by Cartwright and Findlay (1946), Wolf and Wolf (1947), Lilly and Burnett (1951), Hawker (1950), and Cochrane (1958). The references on the effect of light on the growth of fungi have been given in the critical reviews by Banbury (1959), Carlile (1965) and Page (1965). Several researchers have found that fungi are able to tolerate a wide range within a *p*H grade. The works on the effect of hydrogen-ion concentration on growth of fungi have been extensively reviewed by Cartwright and Findlay (1946), Hawker (1950), Wolf and Wolf (1947), Lilly and Burnett (1951), and Cochrane (1958).

In this present investigation an attempt has been made to evaluate the roles of temperature, light and hydrogen-ion concentration on the growth of three common species of wood-rotting basidiomycetes *viz.*, *Pycnoporus sanguineus* (L. ex Fr.) Murr., *Dædalea flavida* Le'v, and *Trametes cingulata* Berk.

## Materials and Methods

The basidiocarps of *Pycnoporus sanguineus* (L. ex Fr.) Murr., *Dædalea flavida* Le'v. and *Trametes cingulata* Berk. have been collected from different logyards of Calcutta. These have been found to develop on felled timbers of Sal *(Shorea robusta* G.f.*)* during the rainy season every year throughout the period of (1969 – 1973) of this investigation. Freshly collected basidiocarps of all the fungi have been brought into the laboratory from the field and from these several monosporus cultures have been prepared (Smith, 1954). Subsequently, these cultures have been paired in various combinations and in some cases, the resulting secondary mycelia with typical clamp-connexions have developed at places of contact in each species. These have then been subcultured for further development. Both the primary and the secondary mycelia of all the three species have been subcultured at regular intervals, examined for their purity and maintained as stock cultures. For the present investigation, *Glucose-casein hydrolysate* basal liquid medium (Leonian and Lilly, 1945) has been selected. Skyrex Erlenmeyer flasks (250 ml) have been used as culture vessels. In each of the required number of flasks, 25 ml. of the medium has been taken. They have been plugged and sterilized at 15 lbs pressure for 15 minutes. Lindeberg's (1944) method of inoculation has been employed but with slight modification following Norkrans (1953) which has been described in the previous paper (Samajpati and Banerjee, 1969). Sufficient number of flasks has been incubated in order to provide five replicates for each treatment for 20 days. Every seventh day, five replicates of each treatment have been harvested three times and the contest have been filtered through tared filter paper, using a Buchner funnel. The residual mycelia have been washed separately with distilled water to remove any trace of the medium and then dried at 60°C in an oven for 24 hours. After drying these have been kept in a closed vacuum dessicator, cooled and weighed in a chemical balance.

Each flask has been inoculated separately with the primary and the secondary mycelia of each test-fungus and incubated (stationary) for 21 days at a fixed temperature. The different temperatures used have been 15°, 20°, 25°, 30°, 35°, and 40°C.

For the light experiment, the inoculated flasks have been incubated at the optimum temperature in a big projection glass chamber illuminated internally by three day light flourescent (Osram, 80 watt) tubes. (Samajpati and Banerjee, 1969).

The intensity of light in the treatment boxes have been 2100 lux. The different types of light used are continuous light (500 – 650 mμ), alternate light (12 hrs), and darkness (12 hrs), red light (610 – 750 mμ), blue light (435 – 800 mμ), green light (500 – 560 mμ), orange light (595 – 610 mμ) and complete darkness.

The normal basal medium after preparation has been neutralized and then by the use of 0.02 M citrate phosphate buffer, the different grade of *p*H have been prepared. The range of *p*H used in their experiment has been 9.0, 8.5, 8.0, 7.5, 7.0, 6.5, 6.0, 5.5, 5.0, 4.5, and 4.0.

The other necessary experimental procedures, however, have been described in a previous paper (Samajpati and Banerjee 1969).

## Results

### Temperature

The results obtained during the experimental periods have been given in Tables 1 – 3. From the fore-going Tables 1 – 3, it will be evident that for both the primary and the secondary mycelia of *P. sanguineus* and *D. flavida*, the optimum temperature for growth is 30°C, while it is 25°C for *T. cingulata*. The minimum and maximum temperature for growth of all the test-fungi, however, are the same in all cases, which lie at 10°C and 40°C respectively.

Table No. 1. *Data (mean) showing the effect of different temperature on the vegetative growth (mg/25 ml) of the test-fungi.*

Temperature (T) × Fungi (F)

| Temperature (°C) | *P. sanguineus* | | *D. flavida* | | *T. cingulata* | | T-means |
|---|---|---|---|---|---|---|---|
| | Primary mycelium | Secondary mycelium | Primary mycelium | Secondary mycelium | Primary mycelium | Secondary mycelium | |
| 10 | 19.5 | 24.8 | 18.5 | 24.0 | 19.6 | 31.7 | 23.0 |
| 15 | 27.5 | 33.1 | 27.5 | 32.8 | 29.7 | 38.4 | 31.5 |
| 20 | 37.0 | 46.0 | 33.7 | 42.8 | 44.7 | 53.7 | 43.0 |
| 25 | 48.4 | 64.6 | 73.0 | 99.8 | 58.0 | 80.2 | 70.7 |
| 30 | 74.7 | 105.4 | 46.0 | 64.1 | 81.0 | 120.2 | 81.9 |
| 35 | 51.6 | 63.2 | 44.0 | 54.6 | 47.5 | 62.7 | 53.9 |
| 40 | 28.5 | 41.1 | 26.1 | 41.1 | 26.4 | 41.3 | 33.7 |
| F-means | 41.0 | 54.0 | 38.4 | 51.0 | 43.2 | 61.2 | |

S.E. for F = ±0.00046 C.D. for F at 5% of P=0.00126
S.E. for T = ±0.00048 C.D. for T at 5% of P=0.00137
S.E. for F × T = ±0.00167 C.D. for F × T at 5% of P=0.00316

Table No. 2. *Data (mean) showing the effect of incubation period on the growth (mg/25 ml) of the test-fungi.*

Incubation period (I) × Fungi (F)

| Incubation period (day) | *P. sanguineus* Primary mycelium | *P. sanguineus* Secondary mycelium | *D. flavida* Primary mycelium | *D. flavida* Secondary mycelium | *T. cingulata* Primary mycelium | *T. cingulata* Secondary mycelium | I-means |
|---|---|---|---|---|---|---|---|
| 7 | 26.8 | 39.0 | 25.4 | 36.9 | 29.6 | 43.4 | 33.5 |
| 14 | 38.6 | 50.6 | 34.9 | 47.7 | 41.4 | 57.9 | 45.2 |
| 21 | 57.7 | 72.5 | 54.9 | 68.4 | 60.4 | 82.2 | 66.0 |
| F-means | 41.0 | 54.0 | 38.4 | 51.0 | 43.2 | 61.2 | |

S.E. for I = ±0.00041 C.D. for I at 5% of P=0.000867
S.E. for F × I = ±0.000160 C D. for F × I at 5% of P = 0.00296

Table No. 3. *Data (mean) showing the effect of different temperature on the incubation periods of vegetative growth (mg/25 ml) of the test fungi.*

Temperature (T) × Incubation period (I)

| Temperature (°C) | Incubation period (day) | | | T-means |
|---|---|---|---|---|
| | 7 | 14 | 21 | |
| 10 | 11.0 | 19.2 | 39.0 | 23.0 |
| 15 | 16.4 | 28.2 | 49.8 | 31.5 |
| 20 | 28.5 | 39.1 | 61.4 | 43.0 |
| 25 | 53.2 | 67.5 | 91.3 | 70.7 |
| 30 | 63.7 | 77.3 | 104.6 | 81.9 |
| 35 | 40.1 | 51.3 | 70.4 | 53.9 |
| 40 | 21.9 | 33.6 | 45.6 | 33.7 |
| I-means | 33.5 | 45.2 | 66.0 | |

S.E. for I × T = ±0.00078 C.D. for I × T at 5% P=0.00249

**Light**

The results obtained during the experimental period have been given in Tables 4 – 6. From the fore-going tables it is evident that for both the primary and the secondary mycelia of all the test-fungi, the optimum source of light for their vegetative growth is alternate light and darkness, followed by complete darkness and continuous light in succession. Of all the different spectral ranges of light, blue (435 – 800 mμ) has minimum and orange (595 – 610 mμ) has maximum inhibitory effect on vegetative growth of the test-fungi.

Table No. 4. *Data (mean) showing the effect of various sources of light on the vegetative growth (mg/25 ml) of the test-fungi.*

Light (T) × Fungi (F)

| Surces of Light | *P. sanguineus* Primary mycelium | *P. sanguineus* Secondary mycelium | *D. flavida* Primary mycelium | *D. flavida* Secondary mycelium | *T. cingulata* Primary mycelium | *T. cingulata* Secondary mycelium | T-means |
|---|---|---|---|---|---|---|---|
| Light | 93.3 | 114.3 | 89.0 | 109.0 | 95.0 | 119.6 | 102.5 |
| Alternate light & Darkness | 104.0 | 123 6 | 95.3 | 119.0 | 106.0 | 131.0 | 113.7 |
| Darkness | 116.6 | 139.0 | 112.0 | 135.3 | 123.6 | 151 2 | 129.7 |
| Blue | 73 6 | 96.0 | 73.1 | 96.3 | 74.6 | 101.3 | 85.9 |
| Green | 58.0 | 81.3 | 57.3 | 82.0 | 62.3 | 89.0 | 71.6 |
| Red | 63.3 | 89.3 | 60.6 | 92.0 | 63.6 | 95.3 | 77.4 |
| Orange | 53.3 | 74.3 | 41.0 | 64.0 | 49.3 | 76.3 | 59.8 |
| F-means | 80.3 | 100.2 | 74.7 | 99.6 | 82.0 | 109.2 | |

S.E. for F = ±0.00050 C.D. for F at 5% of P=0.001116
S.E. for T = ±0.00051 C.D. for T at 5% of P=0.001436
S.E. for F × T = ±0.00013 C.D. for F × T at 5% P=0.003461

Table No. 5. *Data (mean) showing the effect of incubation period on the vegetative growth (mg/25 ml) of the test fungi*

Incubation period (I) × Fungi (F)

| Incubation period (day) | *P. sanguineus* Primary mycelium | *P. sanguineus* Secondary mycelium | *D. flavida* Primary mycelium | *D. flavida* Sec ndary mycelium | *T. cingulata* Primary mycelium | *T. cingulata* Secondary mycelium | I-means |
|---|---|---|---|---|---|---|---|
| 7 | 57.1 | 76.5 | 50.0 | 79 1 | 53.5 | 84.3 | 66.8 |
| 14 | 82.5 | 104.8 | 76 7 | 99.1 | 87.7 | 111.0 | 93.6 |
| 21 | 101.2 | 126.2 | 97.6 | 120.7 | 105.0 | 132.0 | 113.9 |
| F-means | 80.3 | 100.2 | 74.7 | 99.6 | 82.0 | 109.2 | |

S.E. for I = ±0.00051 C.D. for I at 5% of P=0.00 121
S.E. for F × I = ±0.00016 C D. for F × I at 5% of P=0.002712

Table No. 6. *Data (mean) showing the effect of light sources on the incubation periods of the vegetative growth (mg/25 ml) of the test-fungi.*

Light (T) × Incubation period (I)

| Sources of Light | Incubation period (day) 7 | 14 | 21 | T-means |
|---|---|---|---|---|
| Light | 78.7 | 102.1 | 126.6 | 102.5 |
| Alternate light & darkness | 86.6 | 116.0 | 136.8 | 113.7 |
| Darkness | 98.3 | 134.3 | 156.2 | 129.7 |
| Blue | 62.6 | 88.5 | 106.3 | 85.9 |
| Green | 46.8 | 73.8 | 94.3 | 71.6 |
| Red | 53.6 | 80.8 | 97.6 | 77.4 |
| Orange | 40.5 | 60.0 | 78.6 | 59.8 |
| I-means | 66.8 | 93.6 | 113.9 | |

S.E. for I × T = ±0.0026 C.D. for I × T at 5% of P=0.003127

Table No. 7. *Data (mean) showing the effect of pH on the vegetative growth (mg/25 ml) of the test-fungi.*

pH (T) × Fungi (F)

| pH | *P. sanguineus* | | *D. flavida* | | *T. cingulata* | | T-means |
|---|---|---|---|---|---|---|---|
| | Primary mycelium | Secondary mycelium | Primary mycelium | Secondary mycelium | Primary mycelium | Secondary mycellum | |
| 4.0 | 82.3 | 105.0 | 29.3 | 41.6 | 61.3 | 85.3 | 67.5 |
| 4.5 | 117.3 | 148.3 | 38.6 | 52.6 | 69.3 | 94.0 | 86.8 |
| 5.0 | 81.7 | 98.3 | 54.0 | 71.3 | 78.6 | 97.8 | 82.2 |
| 5.5 | 72.0 | 93.0 | 66.0 | 82.0 | 87.6 | 121.6 | 87.0 |
| 6.0 | 67.6 | 84.0 | 77.3 | 101.3 | 108.0 | 138.6 | 95.2 |
| 6.5 | 61.3 | 75.3 | 102.3 | 138.6 | 76.0 | 100.6 | 92.4 |
| 7.0 | 63.3 | 67.0 | 75.3 | 99.6 | 65.6 | 90.0 | 76.9 |
| 7.5 | 38.6 | 50.3 | 69.0 | 89.3 | 60.0 | 77.6 | 63.2 |
| 8.0 | 27.3 | 38.0 | 58.0 | 75.6 | 42.0 | 57.3 | 49.8 |
| 8.5 | 20.0 | 28.0 | 25.3 | 33.6 | 28.0 | 41.2 | 29.0 |
| 9.0 | No growth | No growth | 15.3 | 25.6 | 19.0 | 28.5 | 22.5 |
| F-means | 62.6 | 78.8 | 55.6 | 73.3 | 63.3 | 85.2 | |

S.E. for F = ±0.0008 C.D. for F at 5% of P=0 001867
S.E. for T = ±0.00086 C.D. for T at 5% of P=0.002612
S.E. for F×T = ±0.00636 C.D. for F×T at 5% of P=0.003633

Table No. 8. *Data (mean) showing the effect of incubation period on the vegetative growth (mg/ml) of the test-fungi.*

Incubation period (I) × Fungi (F)

| Incubation period (day) | *P. sanguineus* | | *D. flavida* | | *T. cingulata* | | I-means |
|---|---|---|---|---|---|---|---|
| | Primary mycelium | Secondary mycelium | Primary mycelium | Secondary mycelium | Primary mycelium | Secondary mycelium | |
| 7 | 40.9 | 52.0 | 35.1 | 57.0 | 42.0 | 63.0 | 47.4 |
| 14 | 54.6 | 70.3 | 54.9 | 72.9 | 62.4 | 85 9 | 66.9 |
| 21 | 75.0 | 92.2 | 76.4 | 97.3 | 85.2 | 106.6 | 88.9 |
| F-means | 62.6 | 78.8 | 55.6 | 73.8 | 63.3 | 85.2 | |

S.E. for I = ±0.00471 C.D. for I at 5% of P=0.001916
S.E for F×I = ±0.00336 C.D for F×I at 5% of P=0.003612

Table No. 9. *Data (mean) showing the effect of pH on the incubation period of the vegetative growth (mg/25 ml) of the test-fungi.*

pH (T) × Incubation period (I)

| pH | Incubation period (day) | | | T-means |
|---|---|---|---|---|
| | 7 | 14 | 21 | |
| 4 0 | 20.8 | 66.6 | 88.5 | 67.5 |
| 4.5 | 57.1 | 86.3 | 116.8 | 86.8 |
| 5.0 | 65.2 | 80.5 | 100.8 | 82.2 |
| 5.5 | 66.5 | 84.6 | 107.8 | 87.0 |
| 6.0 | 71.0 | 96.0 | 118.5 | 95.0 |
| 6.5 | 66.0 | 92.1 | 118.3 | 92.4 |
| 7.0 | 59.1 | 75.5 | 95.8 | 76.9 |
| 7.5 | 44.0 | 63.0 | 82.5 | 63.2 |
| 8.0 | 31.0 | 48.3 | 69.8 | 49.8 |
| 8.5 | 9.3 | 27.0 | 50.6 | 29.0 |
| 9.0 | 5.7 | 20.0 | 41.5 | 22.5 |
| I-means | 47.4 | 66.9 | 88.9 | |

S.E. for I×T = ±0.0036 C.D. for I×T at 5% of P=0.00378

**Hydrogen-ion concentration**

The results obtained during the experimental period have been given in Tables 7 – 9. From the fore-going Tables (7 – 9) it is evident that all the test-fungi have responded differently against different grades of hydrogen-ion concentrations. Both for the primary and the secondary mycelia of *P. sanguineus*, the optimum *p*H for growth is 4.5 In case of *T. cingulata*, it is 6.5, and that for *D. flavida* 6.0. Moreover, pigmentation in *P. sanguineus* becomes more intense in the lower range of *p*H (4 – 6) than that in the higher one (8 – 9).

## Discussion

A study on the responses exhibited by the primary and the secondary mycelia of *P. sanguineus*, *T. cingulata* and *D. flavida* under different environmental conditions has made it possible to discuss in a general way some of the salient features.

It has been found that 30°C is the optimum temperature for the growth of both the types of mycelia of *P sanguineus* and *D. flavida* while it is 25°C in case of *T. cingulata*. The influence of temperature, however, has become more invident as has been evident from the widely different responses in growth exhibited by the two test-fungi at the same optimum temperature. After studying the effects of different sources of light on the growth of both the types of mycelia of all the three test-fungi, it has been noticed that the optimum light source for growth has been found to be alternate light (500 – 650 m$\mu$) and darkness. The effect of continuous light and that of different spectral ranges have revealed that all of them have inhibitory effect on the growth of these fungi. The maximum stimulatory effect of alternate light and darkness can be explained on the basis of two metabolic reactions which are influenced by light. In the first reaction the growth has been stimulated in presence of light and inhibited in darkness, while in second reaction the growth has been inhibited in presence of light and stimulated in darkness. The available data on the action of spectrum has indicated that the effective wave length has appeared to be in the blue region which is close to the peak of action-spectrum of phototropism, and carotenoid being found in fungi, is absorbed maximally in the same region of the spectrum. From this it can be speculated that the photoreceptor in these three test-fungi may be a carotenoid compound. Mediator which has induced wall-synthesis during light stimulation.

The data on the effect of hydrogen-ion-concentrations on the growth of all the three test-fungi have revealed that all of them are acids loving though they differ widely among themselves with regard to their individual optimum *p*H requirement for growth. The optimum *p*H for *P. sanguineus* has been found to be 4.0, for *T. cingulata* is 5.5, and that for *D. flavida* is 6.0. It has been further found that all the test-fungi have grown well within the range of *p*H 4.0 and 7.0.

This deversity in the nature of optimum *p*H requirement for growth of different test-fungi has also been recorded in different species of fungi. The evidences so far obtained show convincingly that *p*H is an environmental factor of enormous importance in modifying their metabolic activity. These effects are always intricately correleted. As a secondary feature it is apparent that each species may differ in the limits of their *p*H range for the isoelectric point of the constituent proteins of the different species.

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