Indian J. mycol. Res., 9 (1 & 2), 26—32 (1971)

EFFECT OF DIFFERENT TEMPERATURE, LIGHT AND HYDROGEN-ION CONCENTRATIONS ON GROWTH OF SOME SPECIES OF *POLYPORUS* AND *FOMES*

BY

MADHU JYOTSNA BHATNAGAR

Agra College, Agra, U. P.

The role of different temperature, hydrogen-ion concentration and light on the vegetative growth of all the test-fungi has been described. The optimum temperature for the vegetative growth of *Fomes lividus* and *Polyporus zonalis* is 30°C while for that of *Polyporus cinnabarinus* it is 35°C. Optimum *p*H for the growth of *Polyporus cinnabarinus* and *Fomes lividus* is on the acidic side of neutrality while that of *Polyporus zonalis* is on the alkaline side. Complete darkness has the best effect on the growth of all the fungi used. Of the different spectral range of continuous light, blue and green have the maximum stimulatory effect on growth in all cases and orange has the minimum.

INTRODUCTION

The role of different environmental factors on the growth of the wood-rotting fungi have been studied by different workers. The effect of different environmental factors include temperature, hydrogen-ion concentration and light. Temperature is one of the most important environmental factor affecting the metabolic activities of fungi. In appreciation of this fact many workers from time to time have concerned themselves with problems involving the influence of temperature upon selected species of fungi. Extensive studies have been carried out to correlate temperatures that one favourable or inhibitory to infection and subsequent development of disease or decay with these that are favourable or inhibitory to the growth of the pathogens. However, it is not possible to completely isolate temperature as an environmental factor in studies with fungi. Non-temperative-factors, such as relative humidity, character of the substratum, *p*H and æration of the medium also play an important role in such studies. Most of the literature describing the effect of temperature on growth of the fungal mycelium has been reviewed by Hawker (1950), Cockrane (1958) and Devarall (1965). Studies on temperature requirements for growth and fruiting of various basidiomycetes show that the temperature requirements of species within the famalies Thelephoraceæ, Polyporaceæ and Agricaceæ vary between species, even within the same genus (Cartwright and Findlay, 1934). The optimum temperature for majority of basidiomycetes, however, lies between 20°C and 30°C, (Madelin, 1956).

Variations in the hydrogen-ion concentration of the substratum of a fungus influences its growth characteristics. Fungi have an inherent capacity of exerting an effort to adjust the *p*H of the substratum on which they grow, as a result of which they have been found to tolerate wide range of *p*H. The optimum *p*H, showing maximum matabolic activity of different wood-rotting fungi however, remains on the acidic side of the grade in most cases (Cartwright and Findlay, 1943), though a few show their preference for the grades of *p*H on the alkaline side. Meacham (1918) reported *p*H 3.0 to be optimum for *Lenzities sapiaria, Fomes r. seus, Merulius lacrymans* and *Coniophora cerebella*, while 6.0 to 7.0 was found to be the optimum *p*H for the growth of *Fomes fraxinus* (Montgomery, 1936). Certain fleshy basiodiomycetes, such as species of *Coprinus*, actually required alkaline conditions for best growth (Grainger, 1946; Treschow, 1944). The *p*H requirements for the optimum growth of *Fomes lignosus* was 7.75 (Robbins, 1950).

Light is known to influence greatly the growth and sporulation of numerous fungi. Certain fungi do not sporulate if grown in complete darkness, others require short exposure to light before they can produce normal fertile spore-fruits. Most of the literature dealing with the effect of light on fungi have been reviewed by March *et. al.* (1959). Long and Harsh (1918) found that basidiocarps of *Polyporus cinnabarinus P. forlowii*, and *Trametes seriales* developed in culture in complete darkness. Basing upon his studies on the effect of light on 12 species of Polyporaceæ, Bose (1930) concluded that the diffused light of the laboratory induced basidiocarp formations, but vegetative growth was rather poor. From their studies on the effect of light on six species of Polyporaceæ, Banerjee and Bakshi (1945) concluded that in the presence of light vegetative hyphæ became more compact due to early condensation, while darkness intensified the colour of the culture. References on the effect of light on the growth of fungi were given in critical reviews by Banbury (1959), Carlile (1965) and Page (1965).

In the present study, the effect of temperature, light and *p*H on the growth of the test-fungi, viz., *Polyporus cinnabarinus*, *Polyporus zonalis* and *Fomes lividus* have been investigated.

Materials and Methods

For the present investigation three species of basidiomycetes, viz., *Fomes lividus Kalchbr, Polyporus zonalis* Berk, and *Polyporus cinnabarinus* Jacq. ex Fries have been collected from Calcutta and suburbs. Primary and secondary mycelial cultures, prepared from basidiospores of each of these test-fungi, were used in the experiment.

The basal liquid medium used during these studies is *Glucose-casein-hydrolysate* medium (Leonian and Lilly, 1945) which has been recommended as a well balanced medium by Lilly and Barnett (1949). The chemical composition of the medium is as follows : Glucose, 25gm. ; Casein hydrolysate, 2gm. ; $MgSO_4$, $7H_2O$, O.5gm. ; KH_2PO_4, 1.0gm. ; Fumaric acid, 1.32gm. ; Na_2CO_3, 1.12gm. ; Fe^{+++}as SO_4, 0.2mg. ; Zn^{+++}as SO_4, 0.2mg. ; Mn^{++}as SO_4, 0.1mg. ; and distilled water to make the volume 1000ml.

25ml. aliquots of the medium was taken in each of the required number of Erlenmeyer flasks (250ml). They were plugged and sterilised at 15 lbs. pressure for 15 minutes. In all cases inocula of uniform size (5mm diametre) were obtained by punching out agar discs from advancing zones of 10 days-old vigourously growing mycelia on *malt-agar* in Petridishes. These were then lifted aseptically and floated with mycelial side upwards on the surface of liquid medium, one in each, in the flasks. After inoculation the flasks were incubated (stationery) for 10 days. Sufficient number of flasks were inoculated in order to get 3 replicates. Harvesting was done on the tenth day. The mycelia were washed, dried at 60°C for 24 hours, and finally weighed. The *p*H values of the medium before and after sterilization and at the time of harvesting were recorded.

The different temperatures used in the experiment are 15°, 20°, 25°, 30°, 35° and 40°C.

In order to study the role of H-ion concentration on the vegetative growth of the test-fungi, nine grades of *p*H prepared were : 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0.

To acertain the effect of light on vegetative growth of the fungi under consideration a big projection glass chamber was used. The different types of light used were continuous light (500-600mμ), alternate light (12 hrs.) and darkness (12 hrs.), red light (610-750mμ), blue iight (435-800mμ), green light (500-560mμ) and orange light (595-610mμ). A set was also kept in complete darkness.

RESULTS

The results obtained during the experimental period are given in Tables 1-3.

From the foregoing Table 1, it is evident that the optimum temperature for the growth of both types of mycelia of *P. cinnabarinus* is 35°C., while for those of *F. lividus* and *P. zonalis* it is 30°C.. The minimum and maximum temperatures for the growth of two types of mycelia of *P. cinnabarinus* and *F. lividus* are 15°C and 40°C respectively. Although both the monosporous and polsporous mycelia of *P. zonalis* show maximum vegetative growth at 30°C., they are found to tolerate temperatures as low as 15°C and as high as 40°C..

Table 1. *Data (mean) showing dry weight (mg.) of mycelia of* P. cinnabarinus, F. lividus *and* P. zonalis *at different temperature.*

Fungi	P. cinnabarinus		F. lividus		P. zonalis	
Temperature (°C.)	Primary mycelium	Secondary mycelium	Primary mycelium	Secondary mycelium	Monosporous mycelium	Polysporous mycelium
15	2.20	2.83	3.66	14.06	48.83	33.90
20	3.56	5.60	19.63	44.30	61.50	47.10
25	17.70	20.23	58.13	79.33	80.40	59.53
30	32.93	35.33	88.56	122.76	109.13	87.93
35	38.00	44.70	72.43	106.96	59.90	46.83
40	21.43	28.26	20.30	32.53	34.53	8.96

From Table 2, it is evident that the optimum *p*H for both the primary and the secondary mycelia of *P. cinnabarinus* is 5.5. In case of *F. lividus* the optimum *p*H for both the types of mycelia is 6.5, while in case of the two types of mycelia of *P. zonalis* it is 8.5.

Table 2. *Data (mean) showing dry weight of mycelia (mg.) of* P. cinnabarinus, F. lividus *and* P. zonalis *at different hydrogen-ion-concentration.*

Fungi	P. cinnabarinus		F. lividus		P. zonalis	
Range of *p*H	Primary mycelium	Secondary mycelium	Primary mycelium	Secondary mycelium	Monosporous mycelium	Polysporous mycelium
5.0	20.90	28.83	28.13	65.03	24.33	20.06
5.5	34.80	49.40	41.26	78.16	34.00	27.26
6.0	20.43	24.00	72.90	92.46	38.30	31.96
6.5	15.20	19.76	92.96	117.40	43.93	35.70
7.0	12.03	14.96	67.96	79.33	53.96	43.43
7.5	9.33	12.43	48.56	68.33	74.16	68.30
8.0	8.16	9.40	26.43	43.16	92.40	80.76
8.5	7.63	8.70	14.16	17.80	140.23	126.90
9.0	7.03	7.93	8.60	12.53	109.66	102.26

It is evident from the foregoing Table 3 that in *P. cinnabarinus* and *F. lividus* the optimum vegetative growth takes place in complete darkness. In the case of *P. zonalis*, on the other hand, the optimum light for vegetative growth is alternate light (500-650mμ) and darkness. Orange light (595-610mμ) has an inhibitory effect on the growth of all the fungi under consideration. Of the different spectral ranges of continuous light, green light (500-560mμ) has the maximum stimulatory effect on the test-fungi.

Table 3. *Data (mean) showing dry weight of mycelia (mg.) of* P. cinnabarinus, F. lividus *and* P. zonalis *at different hydrogen-ion concentration.*

Fungus	P. cinnabarinus		F. lividus		P. zonalis	
Source and nature of light	Primary mycelium	Secondary mycelium	Primary mycelium	Secondary mycelium	Monosporous mycelium	Polysporous mycelium
Light	22.00	44.66	66.56	99.63	122.26	109.63
Light and dark	31.96	50.00	71.93	104.10	140.70	126.90
Dark	35.46	55.66	75.00	105.40	135.80	121.60
Blue	19.26	42.36	61.10	82.53	111.93	85.43
Red	17.26	39.16	58.83	77.20	107.90	82.83
Green	19 43	43.26	61.30	83.43	114.70	85.00
Orange	15.23	32 70	43.26	70.73	99.23	71.70

DISCUSSION

The study of the growth responses of the aforesaid fungi under different and uniformly controlled environmental conditions make it possible to discuss in a general way the role of different environments controlling their vegetative activities.

The role of temperature on the vegetative growth of the test-fungi is in confirmity with the views of Cartwright and Findlay (1934) that the temperature requirements within Polyporaceæ vary between species, even within the same genus. *P. cinnabarinus* exhibits its best vegetative growth at 35°C. The temperature-growth-curves in all the cases are typically of skewed type. As noted by previous workers, the temperature growth-curves are always steeper in the supra-optimal region than those in the sub-optimal one. The relation between the rate of growth and temperature may not be linear but the curves become steeper as they approach the optimum which has also been noted by Hoffman (1910). Following Humphrey and Siggers (1933), *F. lividus* and *P. zonalis* are to be included in the 'intermediate-temperature group' (above 32°C.). Temperature is considered to influence to a number of metabolic processes, any one of which may be the limiting factor under particular circumstance. This effect of temperature may be direct or indirect.

Further critical investigations with these fungi are, therefore, necessary before any definite interpretation can be made on this point.

A study of the role of H-ion concentration on the growth of these fungi reveals great diversity in the nature of their optimum *p*H requirements. *P. cinnabarinus* and *F lividus* exhibit maximum vegetative growth in the acidic side of neutrality, their optimum being 5.5 and 6.5 respectively. *F. zonalis* grows well within *p*H 6.6 to 9.0, the maximum being 8.5. It has generally been recognised that the majority of the Basidiomycetes grow well only in acidic media. The alkaline range of *p*H for higher fungi, however, have also been reported (Grainger, 1946 ; Treschow, 1944 ; and Robbins, 1950). Different species may differ in the limits of their *p*H range. According to Robbins (1942), the underlying reasons lie on the isoelectric points of the constituent proteins of the different species. This mechanism of action of *p*H on growth has, however, not yet been ascertained.

After evaluating the role of different sources of light on the growth of all the fungi, it has been found, that the optimum light source for the vegetative growth of *P. zonalis* is alternate light (500 – 650mμ ; 12 hours) and darkness (12 hours). *P. cinnabarinus* and *F. lividus* grow best in complete darkness. The present findings in the case of *F. lividus* are in confirmity with the observations made by Fritz (1923) who reported that in complete darkness the growth and diagonistic characters of some species of *Fomes* are well menifested. These also support Boriss (1934) in that continuous light has definite inhibitory effect on the growth of this test-fungus. The different spectral range of light have shown inhibitory effects on growth of all of them. Of these, blue (435 – 800mμ) and green light (500 – 560mμ) have minimum and orange light (595 – 610mμ) has the maximum inhibitory effects when compared to those in alternate light and darkness and continuous darkness. The available data on the action spectrum indicate fairly consistently that the effective wavelength is in the blue region of the spectrum (Borriss, 1934 ; Medelin, 1956), which is close to the peak of action spectrum of phototropism. Most of the data, so far available on photoreceptors in fungi, have been found to be of carotenoid nature. It has been found that action spectrum of phototropism has a maximum at about 430 – 500mμ, and carotenoid being found in fungi is absorbed maximally in the same region of the spectrum (Walds, 1943). However, the nature of the photoreceptors in fungi which may be a flavin or flavoprotein (Galston and Baker, 1949) or a carotenoid (β-carotene) compound (Walds, 1943) needs further detailed investigation to reveal its chemical nature and role in the photoreception mechanism At present all the responses of light that have been studied in detail involve probably the synthesis of wall materials. How light induces these changes is still unknown. It is can be shown by further extensive researches that ATP is responsible for providing energy for wall-synthesis, a link between reception of light energy and a visible response in growth will be possible to establish in future. Further investigations in this line are in progress in order to establish this relationship.

ACKNOWLEDGEMENT

The author expresses her deep gratitude to Dr. S. N. Banerjee, M. Sc., Ph. D., for his kind guidance and valuable suggestions during the present investigation.

REFERENCES

Banbury, G. H. (1959). In *Handbuch der Pflanzenphysiologie* (W. Ruland, Ed.) Springer, Berlin, 530-578.
Banerjee, S. N. and Bakshi, B. K. (1945). Studies in the biology of wood-rotting fungi of Bengal, *J. Ind. Bot. Soc.*, **20** : 73-92.
Borriss, H. (1934). Über den Einfluss ausserer Faktoren auf Wachsturn Und Entwicklung der Fruchtkorper von *Coprinus lagopus*, *Planta*, **22** : 644-648.
Carlile, M. J. (1965). The photobiology of fungi. *Ann. Rev. Plant Physiology*, **16** : 100-148.
Cartwright K. St. G., and Findlay W. P. K. (1934). Studies in the physiology of wood-rotting fungi. II. Temperature and rate of growth, *Ann. Bot.*, **48** : 481-496.
Cochrane, V. W. (1958). *Physiology of Fungi*. Wiley, New York.
Devaralt, B. J. (1965). In *The Fungi*, Vol. I. (G. C. Ainsworth and A. S. Sussman ed.) p. 543-549.
Fritz, C. W. (1923). Cultural criteria for the distinction of wood-destroying fungi. *Trans. Royal Soc. Can.*, **17** : 191-288.
Galston, A. W. and Baker, R. S. (1949). Studies on the physiology of light action. II. The photodynamic action of riboflavin *Amer. J. Botany*, **36** : 773-784.
Grainger, J. (1964). *Trans. Bot. Mycol. Soc.*, **29** : 52-64, cital by Cochrane, V. W. (1958).
Hawker, L. E. (1950). *Physiology of Fungi*, University of London Press, London, pp. 360.
Humphrey, C. J. and Siggers, P. V. (1933). Temperature relations of wood-destroying fungi. *J. agric. Res.*, **47** : 997-1008.
Leonian, L. H. and Lilly, V. G. (1945). The comparative value of different test organisms in the microbiological assays of Bvitamins, West Va. Agric. Expt. Sta. Bull., 319.
Lilly, V. G. and Barnett, H. L. (1949). Growth rates of vitamin deficiencies of various fungi. *Proc. W. Va. Acad. Sci.*, **19** : 27-33.
Long, W. H. and Harsh, R. M. (1918). Pure cultures of wood-rotting fungi on artificial media. *J. Agri. Res.*, **12** : 53-82.
Marsh, P. B., E. E. Taylor and L. M. Bassler (1959). *Plant Disease Reptr. Suppl.*, **26** : 251-312.
Meacham, M. R. (1918). Note upon the hydrogen-ion-concentration necessary to inhibit the growth of four wood-destroying fungi. *Science*, **48** : 499-500.
Medelin, M. P. (1956). The influence of light and temperature on fruiting of *Coprinus lagopus* Fr. in culture. *Ann. Bot. N. S.* **20** : 467-480.
Montgomery, H. B. S. (1936). An investigation of the temperature lethal to some wood-decaying fungi. *Trans., Brit. mycol. Soc.*, **20** : 3-4, 293-298.
Page, R. M. (1965). The Physical Environment for Fungal Growth. 2, Light., In *The Fungi*, An Advance Treatise, Vol. I (Ed. by G. C. Ainsworth and A. S. Sussman.) Academic Press, New York, 559-574.
Plunkett, B. E. (1953). Nutritional and other aspects of fruit-body production in pure cultures of *Collybia velutipes* (Curt.) Fr. *Ann. Bot.*, N. S., **17** : 193-217.
Robbins, W. J. (1950). A survey of the growth requirement of some Basidiomycetes, *Mycologia*, **42** : 470-476.
Treschow, C. (1944). Nutrition of the cultivated mushroom. *Dansk. Botan. Arkiv.*, **11** : 180.
Urayama, T. (1960). *Mem Fac. lib. Arts & Ed. Miyazaki University*, **9** : 393-462 ; (Re-print).
Walds, G. (1943). *Vitamins and Hormones*, **1** : 195-227, cited by Cochrane, V. W. (1958).

(Accepted for publication 5th October 1970)