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STUDIES ON THE PHYSIOLOGY OF SOME FUNGI CAUSING HUMAN MYCOSIS : I. EFFECT OF TEMPERATURE AND HYDROGEN-ION-CONCENTRATION ON GROWTH OF *TRICHOPHYTON MENTAGROPHYTES* (ROBIN) BLANCHARD AND *ASPERGILLUS FUMIGATUS* (FRESENIUS) THOM AND CHURCH.

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Trichophyton mentagrophytes (Robin) Blanchard and *Aspergillus fumigatus* (Freserius) Thom and Church have been found to cause skin and nail diseases of human respectively. In order to investigate the relationship that exists between the fungus and the environment and the cause of infection to the human, the study of environmental factors *viz.*, temperature and hydrogen-ion-concentrations on the growth of both the fungus have been investigated. It has been found that 37°C and *p*H 6.5 has maximum stimulatory effect on growth. The possible explanations of the actions of these environmental factors have been given.

Introduction

It is customery to have comprehensive knowledge of physiology of the parasites for erradication of the diseases concerned. As such the study of physiology of these two fungi has been taken up in order to have a better in sight into the mode of infection, host parasite relationship and their very specialised nature of parasitism. In the present investigation attempts have been made to study the effect of temperature and *p*H on the growth of two pathogenic fungi causing human mycosis in Bankura district of West Bengal. Of these one is *Trichophyton mentagrophytes* causing skin disease and the other one *Aspergillus fumigatus* causing nail degeneration of the big toe of leg and both of the patient has been attended in the (skin) out door department of Bankura Sanmilani Medical College and Hospital.

The investigation on the effect of temperature on the growth of fungal mycelium has been reviewed by Wolf and Wolf (1947), Hawker (1950), Cochrane (1958), Dasgupta, Shome and Majumdar (1960), and Devarall (1965).

It is well known that both temperature and *p*H have a direct or indirect effect on cellular activities of microorganisms. There is a optimum temperature in which growth increases directly with the rise of temperature. The rate of growth of a fungus is greatly influenced by the different grades of hydrogen-ion-concentration of the substratum and optimum *p*H, however, remain towards acidic side in most cases.

Material and Methods

Both the fungi have been isolated aseptically from the two patient and maintain in *Sabouraud Glucose Agar* medium at 30°C. *Glucose-Asparagine* basal medium (Lilly and Barnett, 1951) has been selected for the present study.

Spores have been harvested from 7-days old culture by covering the culture with distilled water and brushing gently and superficially with an inoculation loop. The spore suspension thus obtained has been filtered through a sintered glass funnel to remove hyphæ and conidiophores. It is then washed by centrifuging for 10 minutes at 3000 g. The spores have then been suspended in sterile water and shaken on a SICO flask-shaker to break up clumps of spores. Then the spores have been counted in a hæmocytometer. The spore suspension has then been used as inoculum. During inoculation the spore suspension has then been constantly shaken to avoid variation in inoculum size due to sedimentation. Each culture vessel has been inoculated aseptically with 7×10^5 spores.

The effect of following temperature on the growth of fungi have been evaluated, 35°, 37° and 40°C, keeping in view the temperature of the human blood in mind. The effect of hydrogen-ion-concentrations, *i.e.* *p*H has also been investigated. The following grades of *p*H was prepared by using N/15 phosphate buffer, 6.0, 6 5, 7.0 and 7.5. The requisite amount of buffer solution has been used in each case to get the desired *p*H.

Sufficient culture vessels have been inoculated to provide five replicates for each experiments. Every fourth day harvesting was done and after drying the mycelia at 60°C for 24 hours the weight was noted.

Results

The results obtained during the experimental periods on the effect of different temperature and hydrogen-ion-concentrations are presented in Tables 1 to 6.

From the table 1—3, it is evident that the optimum temperature for growth of both the test-fungi is 37°C. It may be further noted that the growth of both the fungi is rather better at 40°C than at 35°C.

In case of different concentrations of hydrogen-ion, (Tables 4—6), it has been found that *p*H 6 5 has maximum stimulatory effect for both the test-fungi which is followed by *p*H 7.0, 6.0 and 7.5.

Table No. 1. *Data (mean) showing the effect of different temperatures on the growth of the two test-fungi at different incubation period.*

Temperature (°C)	Fungi		Temperature (T) means
	T. mentagrophytes	*A. fumigatus*	
35	123.2	137.4	130.3
37	138.2	193.1	165.6
40	122.4	161.1	141.8
Fungi (F) means	127.9	163.8	

S.E. for F means = ±1.38 C.D. for F means at 5% of P = ±3.98
S.E. for T means = ±1.70 C.D. for T means at 5% of P = ±4.91
S.E. for F×T means = ±6.93 C.D. for F×T means at 5% of P = ±6.93

Table No. 2 *Data (mean) showing the role of different incubation periods on the effect of of temperatures on the growth of the two test-fungi.*

Incubation periods (days)	Fungi		Incubation days (I) means
	T. mentagrophytes	*A. fumigatus*	
4	99	155	127
8	133	171.3	152.1
12	150.7	203.5	177.1
16	139.3	157.3	148.3
20	117.6	132.2	124.9
Fungi (F) means	127.9	163.8	

S.E. for I means = ±2.19 C.D. for I means at 5% of P = 6.32
S.E. for F×I means = ±3.10 C.D. for F×I means at 5% of P = ±8.95

Table No. 3. *Data (mean) showing the role of different temperatures on the effect of incubation periods on the growth of the two test-fungi.*

Temperature (°C)	Incubation periods (Day)					Temperature (T) means
	4	8	12	16	20	
35	114	139.5	151.5	133	113.5	130.3
37	142	167	211.5	165.5	142.3	165.6
40	125	150	168.5	146.5	119	141.8
Incubation days (I) means	127	152.1	177.1	148.3	124.9	

S.E. for I×T means = ±3.80 C.D. for I×T means at 5% of P = ±10.98

Table No. 4. *Data (mean) showing the effect of different hydrogen-ion-concentrations on the growth of the two test-fungi at different incubation period.*

Different hyrogen-ion concentrations	Fungi		Hydrogen-ion concentration (T) means
	T. mentagrophytes	*A. fumigatus*	
6.0	103.4	126.5	115
6.5	132.06	175.5	153.8
7.0	109.5	154.4	131.9
7.5	92.3	124.6	108.5
Fungi (F) means	109.3	145.2	

S.E. for F means = ±1.93 C.D. for F means at 5% of P=5.57
S E. for I means = ±2.74 C.D. for I means at 5% of P=7.91
S.E. for F×I means = ±3.87 C.D for F×I means at 5% of P=11.18

Teble No. 5 *Data (mean) showing the role of different incubation periods on the effect of hydrogen-ion concentration on the growth of the two test fungi*

Incubation periods (days)	Fungi		Incubation days (I) means
	T. mentagrophytes	*A. fumigatus*	
4	91.1	154.2	122.7
8	115.4	153.7	134.5
12	136.6	163.5	150.0
16	112.5	140.1	126.3
20	91.0	114.7	102.8
Fungi (F) means	109.3	145.2	

S.E. for I means = ±3.06 C.D. for I means at 5% of P=8.84
S.E. for F×I means = ±4.33 C.D. for F×I means at 5% of P=12.51

Table No. 6. *Data (mean) showing the role of different hydrogen-ion-concentrations on the effect of incubation periods on the growth of the two test-fungi.*

Hydrogen-ion-concentration	Incubation periods (Days)					Hydrogen-ion-concentration (T) means
	4	8	12	16	20	
6.0	107.1	119.8	147 1	113.1	87.	115.0
6.5	145.2	159.5	167.8	159.8	136.5	153.8
7.0	132.6	137.1	150 1	132.1	107.6	131.9
7.5	105.6	121.8	135.1	100.1	79.6	108.5
Incubation days (I) means	122.7	134.5	150.0	126.3	102.8	

S.E. for I×T means = ±6.13 C.D. for I×T means at 5% of P=17.71

Discussion

A study on the responses exhibited by both the test-fungi under different and uniformly controlled environmental condition has made it possible to discuss regarding the relationship that exists between them and the environment.

Among the physical environmental factors 37°C has been found to be the optimum temperature for the growth of both the test-fungi. The influence of temperature on the metabolic activities of microorganisms are well known. Both the test-fungi are parasite on man and the temperature of the freshly shed blood is between 36° to 38°C. It can be assumed that 37°C being the optimum temperature for growth of both the test-fungi. is possibly the helpful temperature for the micro-organisms to parasitize the man.

The data on the effect of hydrogen-ion-concentrations on the growth of both the test-fungi have revealed that both of them are alkali loving with optimum temperature at *p*H 6.5. This characteristic *i e.* alkali loving nature, has possibly facilitated them to be parasite on human blood as both the arterial and venous human blood are alkaline in nature A venous blood appear to be less alkaline it can be assumed that both the parasites possibly will prefer the venous blood than the arterial blood during the initial establishment of the infection.

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