

EFFECT OF DIFFERENT NITROGEN SOURCES ON GROWTH OF *TRICHOPHYTON MENTAGROPHYTES* AND *SPERGILLUS FUMIGATUS*

BY

SALIL KUMAR BASAK* AND N. SAMAJPATI

Department of Botany, University of Calcutta
Calcutta-700 019

The present investigation has been carried out to provide some informations about the role of different sources of nitrogen as a nutritional factors on the growth of *Trichophyton mentagrophytes* (Robin) Blanchard and *Aspergillus fumigatus* (Fresenius) Thom and Church. They have been found to cause skin and nail diseases of human beings. Experimental procedures for this investigation have been discussed. Of the different sources of organic and inorganic nitrogen used, aspartic acid and ammonium chloride have been found to be the best sources in case of *T. mentagrophytes* and in case of *A. fumigatus*. Glycine and ammonium nitrate have been found to be the best sources among organic and inorganic nitrogen respectively. Of the different concentrations used 0.15% has the maximum stimulatory effect on the growth of both the test fungi.

INTRODUCTION

Nitrogen, one of the essential elements, is used by fungi for their structure and life processes. Fungi usually utilise nitrogen from simple inorganic compounds to complex organic forms yet they differ in their ability to utilise different forms of nitrogen.

The nutritional requirement of dermatophytes has been reviewed by Howard (1985). Mosher *et al.* (1936) reported the role of nitrogen on the growth of *Trichophyton mentagrophytes*. Robbins and Ma (1945) also studied the nitrogen requirement for growth of *T. mentagrophytes*. Rippon and Le Beau (1965) and Silva (1958) reported the utilisation of single amino acid as sole source of carbon and nitrogen of *Microsporon audouinii* and *T. rubrum* respectively. Hilger *et al.* (1979) reported the utilisation of single amino acid as sole source of carbon and nitrogen by *T. mentagrophytes*. Pine (1972) reported the growth of *Histoplasma capsulatum* and *H. duboisii* on amino acids.

Barash *et al.* (1967) and Hashimoto *et al.* (1972) reported the effect of nitrogen nutrition on morphogenetic changes in *Microsporum gypseum* and

*Present address : Department of Botany, Bankura Christian College,
P.O. & Dist—Bankura-722 101, West Bengal.

T. mentagrophytes respectively. Mc Vickar (1951) reported the toxicity of peptone on the growth of *Histoplasma capsulatum*. Georg (1952) reported the histidine requirement of *Trichophyton gallinae* and *T. magninii*.

In the present investigation attempts have been made to study the effect of different nitrogen sources on the growth of two pathogenic fungi causing human mycoses 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 the leg.

MATERIALS AND METHODS

Both the fungi were isolated aseptically from the two patients and maintained in *Sabourauds Glucose Agar* medium at 30°C.

Glucose-Asparagine basal medium (Lilly and Barnett, 1951) was selected for the present study.

The standard basal medium was first prepared but without any source of nitrogen. After preparation, only one source of nitrogen was added to each set of the medium in several flasks as 0.05%, 0.1% and 0.15% concentration of nitrogen. The source of nitrogen used in this experiment were cysteine, glycine, lysine, aspartic acid, glutamic acid, ammonium chloride, ammonium nitrate, potassium nitrate and potassium nitrite. The media was then sterilized, inoculated separately by the spore suspension (Basak, 1974) of both the test fungi and incubated at optimum temperature under diffused light. Several flasks with the medium but without any source of nitrogen were also kept as control. The pH of the medium was adjusted to their respective optimum values with citrate - phosphate buffer.

Sufficient culture vessels were 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 are given in Tables 1-3. From the tables it is evident that all the sources of nitrogen have a stimulatory effect on the growth of both the test-fungi. Among the organic sources aspartic acid has the maximum stimulatory effect on the growth of *Trichophyton mentagrophytes* followed by glutamic acid, glycine, lysine and cysteine. In case of inorganic sources, ammonium chloride comes first. Ammonium nitrate, potassium nitrite and potassium nitrate come in successive preferences. In case of lysine upto 4 days no noticeable growth has been found in *T. mentagrophytes*.

Table 1. Data (mean) showing the effect of different nitrogens on the growth of the two test-fungi at different incubation periods

Source of Nitrogen	Concentration of Nitrogen (%)	Fungus		Nitrogen (T) means
		<i>T. mentagrophytes</i>	<i>A. fumigatus</i>	
(NH ₄)Cl	0.5	99.8	143.2	121.5
	1.0	121.4	217.0	169.2
	1.5	134.0	238.6	186.3
(NH ₄)NO ₃	0.5	112.8	199.4	156.1
	1.0	124.8	223.0	173.9
	1.5	132.6	240.6	186.6
KNO ₃	0.5	99.2	126.5	112.8
	1.0	117.0	158.2	137.6
	1.5	124.0	180.8	152.4
KNO ₂	0.5	86.0	112.6	99.3
	1.0	116.8	140.2	128.5
	1.5	131.4	203.0	167.2
Cysteine	0.5	40.6	86.4	63.5
	1.0	58.4	127.8	93.1
	1.5	94.8	151.5	123.1
Glycine	0.5	74.6	138.0	106.3
	1.0	90.6	178.6	134.6
	1.5	107.0	209.0	158.0
Lysine	0.5	47.2	92.6	69.9
	1.0	69.0	137.7	103.3
	1.5	83.8	167.8	125.8
Aspartic acid	0.5	39.8	105.6	72.7
	1.0	59.4	135.8	97.6
	1.5	144.0	181.0	162.5
Glutamic Acid	0.5	61.0	106.0	83.5
	1.0	104.4	132.0	118.2
	1.5	140.0	158.0	149.0
Control		36.4	58.2	47.3
Fungus (F) means		94.6	155.3	—

S.E. for F means = ± 1.13 S.E. for T means = ± 4.2 S.E. for F x T means = ± 6.02 C.D. for F means at 5% of P = ± 3.26 C.D. for T means at 5% of P = ± 12.28 C.D. for F x T means at 5% of P = ± 17.39

In case of *Aspergillus fumigatus* is the best source of organic nitrogen followed by lysine, cysteine, aspartic acid and glutamic acid. Among the organic nitrogen sources ammonium nitrate has the maximum stimulatory effect followed by

Table 2. Data (mean) showing the role of different incubation periods on the effect of nitrogens on the growth of the two test-fungi

Incubation periods (Days)	Fungus		Incubation days (I) means
	<i>T. mentagrophytes</i>	<i>A. fumigatus</i>	
4	62.0	119.0	90.5
8	91.8	155.1	123.5
12	126.1	193.8	160.0
16	103.7	162.0	132.9
20	89.5	146.5	118.0
Fungus (F) means	94.6	155.3	—
S.E. for I means = ± 1.80		C.D. for I means at 5% of $P = \pm 5.20$	
S.E. for F x I means = ± 2.54		C.D. for F x I means at 5% of $P = \pm 7.34$	

ammonium chloride, potassium nitrite and potassium nitrate of the different concentrations used 0.15% has the maximum stimulatory effect on the growth of both the test-fungi. It has also been noted that in case of *T. mentagrophytes* organic source i.e., aspartic acid has the maximum stimulatory effect among all the inorganic and organic sources used, while in case of *A. fumigatus*, ammonium nitrate (inorganic source) is the best source of nitrogen among the different inorganic and organic forms used.

DISCUSSION

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

After detecting the role of different sources of nitrogen on the growth of *T. mentagrophytes* it has been noted that aspartic acid and ammonium chloride are the best sources among the organic and inorganic sources respectively. Aspartic acid is being followed by glutamic acid while ammonium chloride is followed by ammonium nitrate and potassium nitrite. The data of the present investigation have revealed that *T. mentagrophytes* is able to utilise glutamic acid next to aspartic acid, the optimum source. This obviously does not support the views of Hilger *et al.* (1979) and Georg (1952).

In case of *A. fumigatus* glycine and ammonium nitrate are the best sources of nitrogen among the organic and inorganic sources respectively. It has been further noted that *A. fumigatus* can utilise more efficiently ammonium nitrate than glycine. Of the different concentrations used (0.05, 0.10, 0.15%), 0.15% of all the sources of nitrogen used has the maximum stimulatory effect. However, the present findings do not provide any firm basis for differentiation of *T. mentagrophytes* and *A. fumigatus*.

Table 3. *Data (mean) showing the role of different nitrogens on the effect of incubation periods on the growth of the two test-fungi*

Source of Nitrogen	Concentration of Nitrogen(%)	Incubation periods (days)					Nitrogen (T) means
		4	8	12	16	20	
(NH ₄) Cl	0.5	97.0	128.5	158.5	124.5	99.0	121.5
	1.0	145.1	170.5	194.0	178.0	158.5	169.2
	1.5	164.5	189.0	214.0	189.5	174.5	186.3
(NH ₄)NO ₃	0.5	136.0	165.5	187.5	162.5	129.0	156.1
	1.0	153.0	170.5	206.5	175.5	164.0	173.9
	1.5	162.0	187.5	224.5	191.5	167.0	186.6
KNO ₃	0.5	102.0	109.0	125.3	108.0	120.0	112.8
	1.0	116.0	140.0	158.5	138.0	135.5	137.6
	1.5	130.5	157.0	170.5	154.5	149.5	152.4
KNO ₂	0.5	83.5	100.0	126.0	101.0	86.0	99.3
	1.0	107.5	126.0	150.5	134.0	124.5	128.5
	1.5	128.5	169.0	204.5	173.0	161.0	167.2
Cysteine	0.5	25.5	61.0	97.0	73.0	61.0	63.5
	1.0	38.5	89.5	132.6	107.5	97.5	93.1
	1.5	53.0	119.5	178.0	143.0	122.3	123.1
Glycine	0.5	69.5	101.5	142.5	116.5	101.5	106.3
	1.0	88.0	128.5	192.0	142.0	124.5	134.6
	1.5	106.5	137.5	210.5	178.0	157.5	158.0
Lysine	0.5	17.5	66.5	112.0	80.1	63.5	69.9
	1.0	19.0	97.0	160.5	134.8	105.5	103.3
	1.5	20.5	118.5	197.5	157.5	135.0	125.8
Aspartic Acid	0.5	49.5	66.0	103.5	75.0	69.5	72.7
	1.0	75.5	92.0	121.0	103.5	96.0	97.6
	1.5	129.0	153.5	202.5	169.0	158.5	162.5
Glutamic Acid	0.5	67.0	85.0	111.0	83.0	71.5	83.5
	1.0	89.5	120.5	148.5	127.5	105.0	118.2
	1.5	125.0	152.0	185.5	154.5	128.0	149.0
Control		35.0	57.0	67.5	47.0	30.1	47.3
Incubation days (I) means		90.5	123.5	160.0	132.9	118.0	—

S.E. for I x T means = ± 9.52 C.D. for I x T means at 5% of P = ± 27.51

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