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# STUDIES ON THE PHYSIOLOGY OF SOME FUNGI CAUSING HUMAN MYCOSIS : III. EFFECT OF DIFFERENT NITROGEN SOURCES ON GROWTH OF *TRICHOPHYTON MENTAGROPHYTES* (ROBIN) BLANCHARD AND *ASPERGILLUS FUMIGATUS* (FRESENIUS) THOM AND CHURCH

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The present investigation has been carried out to provide some informations about the role of different sources of nitrogen as nutritional factor on the growth of *Trichophyton mentagrophytes* (Robin) Elanchard and *Aspergilius fumigatus* (Fresenius) Thom and Church. They have been found to cause skin and nail disease of human bcings. 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 forms yet they differ in their ability to utilise different forms of nitrogen.

The nutritional requirement of nitrogen by fungi has been reviewd by Lilly and Barnett (1951), Wolf and Wolf (1947) and Tove *et al.* (1949).

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 sources of nitrogen used in this experimeut were cysteine, glycine lysine, aspartic acid, glutamic acid, ammonium chloride, ammonium nitrate, potassium nitrate and potassium nitrite. The media was then sterilized, inoculated seperately by the spore suspension (Basak, 1974) of both the test-fungi and incubated at optimum temperarure 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 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 stimvlatory effect on the growth of *Trichophyton mentagrophytes* followed by glutamic acid, glycine, lysine and cysteine. In case of inorganie sources, ammonium chloride comes first. Ammonium nitrate, patassium nitrate and patassium nitrite 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 (%) | Fungi | | Nitrogen (T) means |
|---|---|---|---|---|
| | | T. mentagrophytes | A. fumigatus | |
| $(NH_4)CI$. | 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 | 116.0 | 140.2 | 128.5 |
| | 1.5 | 131.4 | 203.4 | 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 | 0.5 | 39.8 | 105.6 | 72.7 |
| Acid | 1.0 | 59.4 | 135.8 | 97.6 |
| | 1.5 | 144.0 | 181.0 | 162.5 |
| Glutamic | 0.5 | 61.0 | 106.0 | 83.5 |
| Acid | 1.0 | 104.4 | 132.0 | 118.2 |
| Control | 1.5 | 140.0 | 158.0 | 149.0 |
| Fungus (F) means. | | 94.6 | 55.3 | — |

S.E. for F means $= \pm 1.13$ C.D. for F means at 5% of P $= \pm 3.26$

S.E. for T means $= \pm 4.25$ C.D. for T means at 5% of P $= \pm 12.28$

S.E. for F $\times$ T means $= \pm 6.02$ C.D. for F $\times$ T means at 5% of P $= \pm 17.39$

Table 2. *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 | 156.0 |
| | 1.0 | 153.0 | 170.5 | 206.5 | 175.5 | 164 0 | 173.9 |
| | 1.5 | 162.0 | 187.5 | 224.5 | 191.5 | 167.5 | 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 | 135.0 | 137.6 |
| | 1.5 | 130.5 | 157.0 | 170.5 | 115.5 | 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 | 190.0 | 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 | 73.5 | 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 | 35.0 | 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 | 85.5 | 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 | 57.5 | 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 × T means = + 9 52 C D for I × means at 5% of P = + 27 51

Table 3. *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) | Fungi | | Incubation days (I) means |
|---|---|---|---|
| | T. mentagrophytes | A. fumigatus | |
| 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 = ± 5.20
S.E. for F × I means = ± 2 54 C.D. for F × I means at 5% of P = ± 7.34

In case of *Aspergillus fumigatus* glycine is the best source of organic nitrogen followed by lysine, cysteine, aspartic acid and glutamic acid. Among the inorganic nitrogen sources ammonium nitrate has the maximum stimulatory effect followed by ammonium chloride, potassium nitrate and potassium nitrite. Of the different concentrations used 0 15% has the maximum stimulatory effect on the growth of both the test-fungi. It has als 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 aspartie 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 nitrate. Dasgupta *et al.* (1959) have reported that *Trichophyton rubrum*, *T. mentagrophytes* and *Epidermophyton floccosum* have been able to utilise any form of inorganic nitrogen source but failed to utilise glutamic acid, tryptophane and histidine. 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 supportthe views of Dasgupta and Shome (1959). In case of *A. fumigatus* glyceine 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 concentratious used (0.05, 0.10, 0.15%), 0.15% of all the sources of nitrogen used has the maximam stimulatory effect.

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