IX. EFFECT OF DIFFERENT CONCENTRATIONS OF ASPARAGINE WITH GLUCOSE AS THE CARBON SOURCE ON THE MYCELIAL GROWTH OF Tricholoma erasum (Berk.) Sacc. IN SUBMERGED CULTURE

The concentration of a chemical in the medium seems to be a limiting factor in the physiological studies as it plays an important role in its utilization by any organism. It is needless to say that the combination of the nitrogen source with carbon source in the medium yielding a particular ratio is specifically very important for the mycelial growth and this varies with organism to organism and also with the environmental conditions.

Several investigators opined that different concentrations of nitrogen in a nutrient medium played a vital role in the utilization of sources, growth and production of mycelial biomass and protein by the test-organism. Atacador-Ramos et al (1967) found that Volvariella volvacea had its maximum mycelial yield when 0.4% urea as nitrogen source was used in the medium. Srivastava and Bano (1970) opined that for Pleurotus flabellatus as the concentration of nitrogen increased, the yield of dry matter also increased up to a certain stage, beyond which there was no increase in the yield but the crude protein of the mycelium increased. For Volvariella Voltz (1972) used 0.47g N/l while Chang-Ho and Yee (1977) used 0.25g N/l and the concentrations of
nitrogen used for *Volvariella* growth by Garcha *et al* (1979) and Chang-Ho (1980) were 0.416 g and 1.32 g N/l respectively. Tzeng (1974) studied six nitrogen compounds at three concentrations and he found that 0.2% nitrogen was optimum for four of the six compounds tested by him and only in two 0.4% nitrogen yielded better growth than the control. Sattler (1975) investigated on the temperature depending growth at various concentrations of different nitrogen sources of *Phycomyces blackeleanus*, *Botrytis* sp., *Agaricus bisporus*, *A. bisporus* var. *albidus*, *Clitocybe clavipes*, *Lentinus tigrinus*, *Polyporus melanopus* and *Sphaerobolus stellatus*. In nutritional study of the fruit-body formation of *Psilocybe panaeoliformis*, Kitamoto *et al* (1975) revealed that nutrients supplied at both supra-optimal concentrations caused abnormal fruiting but casamino acids or a mixture of 11 amino acids at 0.032% nitrogen concentration were found to be more beneficial. Watkinson (1975) opined that *Serpula lacrimans* grew well vegetatively in a medium with high concentrations of both sucrose and aspartate. The change in the concentrations of nitrogen source, had little effect on the total number of sclerotium initials but it had pronounced influence on sclerotial maturation in *Coprinus lagopus* (= *C. cinereus*) (Moore and Jirjis 1976). Nag Chowdhuri (1977) had indicated that the mycelial production and protein production were increased by increasing the concentration of both carbon and nitrogen, but both became limiting at supra-optimal concentrations in
Tricholoma gigantium. High amylolytic activity and mycelium yield of Lentinus edodes were obtained with 0.5% yeast extract (El-Zalaki et al 1980). Hong et al (1981) studied the mycelial growth of Agaricus bitorquis and Pleurotus ostreatus and opined that the growth depended on the carbon and nitrogen concentration of the medium. They also observed that the higher the concentration of glucose and peptone, the more was the increase in the mycelial yield of A. bitorquis and P. ostreatus. Kurtzman and Chang-Ho (1982) and Kurtzman and Zadrazil (1982) presented reviews on concentrations of nitrogen used for physiological studies of Volvariella and Pleurotus respectively by various authors.

The present investigation deals with the influence of different concentrations of nitrogen by keeping the concentration of carbon constant on the mycelial growth of T. crassum (Berk) Sacc.

MATERIALS AND METHODS

Test-organism:

The culture of the test-organism T. crassum (Berk) Sacc. was maintained by regular subculturing in PDA slants (peeled potato (decoction), 400g; glucose, 25g; agar, 20g; and distilled water to 1000 ml). The glucose-casein hydrolysate basal medium of Lilly and Barnett (1951) was modified
and used as the basal synthetic liquid medium.

Culture vessels:

Erlenmeyer flasks (250 ml) cleaned with sulphuric acid and potassium dichromate solution, carefully washed with tap water and distilled water, were used in the experiment.

Growth medium:

The modified glucose-casein hydrolysate medium of Lilly and Barnett (1951) was first prepared without any nitrogen source. The basal medium was separated into five equal portions. To each set nitrogen in the form of asparagine were added. The amount of asparagine added per litre was 1g, 2g, 3g, 4g and 5g respectively. The percentage of nitrogen added were 0.021%, 0.042%, 0.063%, 0.084% and 0.106% respectively. Different sets were separately adjusted to pH 6.0 with 0.2M phosphate buffer, dispensed as 50 ml per flask (250 ml) used as culture vessels, plugged with non-absorbent cotton wool and sterilized at 15 p.s.i for 15 minutes.

Preparation of inoculum:

A small portion of the actively growing mycelium from agar slant of the test-fungus was aseptically transferred to a sterile 250 ml Erlenmeyer flask containing 50 ml of the basal medium and was incubated at room temperature (30°C ±0.5°C) on a shaking incubator (120 r.p.m) for 7 days in complete darkness. After the incubation period, the mycelial
mass was aseptically fragmented with the help of a Waring blender. The fragmented mycelium was washed several times with distilled water to remove any trace of the medium and then suspended in a phosphate buffer medium (pH 5.5) for 24 hours to overcome the shock encountered during blending. One ml of the mycelial suspension was used as the inoculum.

**Inoculation and incubation:**

The flasks of different sets were inoculated separately with 1.0 ml of the cell suspension of the mycelium of the test-fungus and incubated in a shaking incubator (at 120 r.p.m) in complete darkness at 30°C (optimal temperature) for 14 days. Sufficient number of flasks were incubated to have three replicates of each treatment.

**Harvesting and measurement of growth:**

After the incubation period, the mycelia were harvested by filtration through a tarred filter paper (Whatman No. 1) using a Buchner funnel. The filtered mycelia were washed repeatedly with distilled water to remove any trace of the medium and dried at 60°C in an oven for 24 hours. After drying, the mycelia were kept in a vacuum desiccator and weighed in a chemical balance (Sartorius). The dry weight of the mycelia thus obtained was taken as an index of growth.
Table 8: Data (Mean)* showing the effect of different concentrations (%) of nitrogen (asparagine) on the growth (g/l) of the mycelia of *T. crassum* (Berk.) Sacc. under submerged conditions at optimal temperature and pH with glucose concentration at constant

<table>
<thead>
<tr>
<th>Varying concentration of nitrogen (%) (asparagine)</th>
<th>Dry weight of the mycelium (g/l)</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.021</td>
<td>0.960 ± 0.025</td>
<td>± 0.016</td>
</tr>
<tr>
<td>0.042</td>
<td>1.206 ± 0.023</td>
<td>± 0.015</td>
</tr>
<tr>
<td>0.063</td>
<td>1.140 ± 0.023</td>
<td>± 0.015</td>
</tr>
<tr>
<td>0.084</td>
<td>0.920 ± 0.030</td>
<td>± 0.020</td>
</tr>
<tr>
<td>0.106</td>
<td>0.540 ± 0.023</td>
<td>± 0.015</td>
</tr>
</tbody>
</table>

* Values are averages of 3 replicates.
Text-fig. 11: Graph showing the effect of different concentrations (%) of nitrogen (asparagine) on the growth (g/l) of the mycelia of *T. crassum* (Berk.) Sacc. under submerged conditions at optimal temperature and pH with glucose concentration at constant.
Dry weight of mycelium (g/l) vs. concentration of nitrogen (V%) (Asparagine)

Text fig 11
RESULTS AND DISCUSSION

The experimental data are given in Table 8 and Text-fig. 11.

The results indicate that the best mycelial growth is influenced at 0.042% nitrogen concentration per litre which gradually decreases at supra-optimal concentration i.e. at 0.106%. However, on the other hand, the decrease in concentration does not have much of an influence. The usage of different concentrations of nitrogen are as follows: 0.042%, 0.063%, 0.021%, 0.084% and 0.106%.

The results coincide with the findings of Nag Chowdhuri (1977) that the mycelial yield increases with increase in the concentration of nitrogen source but becomes a limiting factor at supra-optimal concentration. The results, however, contrast with that of Hong et al (1981) that for Agaricus bitorquis and Pleurotus ostreatus, the higher concentration of glucose and peptone result in better mycelial yield. The results are further substantiated by the findings of Tzeng (1974) that in higher concentrations most of the amino acids tested by him yielded lesser growth of the mycelium.
REFERENCES


