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Effect of vitamins and minerals on the mycelial growth of *Lentinus squarrosulus, L. polychrous, Agrocybe broadwayi* and *Astraeus hygrometricus*

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Thiamine was the most required source for the mycelial growth of *Lentinus squarrosulus, L. polychrous, Agrocybe broadwayi* and pyridoxin and thiamine were the required ones for A. *hygrometricus.*

L. squarrosulus, L. polychrous showed compartively best growth in absence of iron. *l. squarrosulus* and *A. broadwayi* required copper and *A. hygrometricus* required zinc and manganese for its good mycelial growth.

Key words : Mycelial growth in submergred culture, fungi, nutritional factors - vitamins and trace elements

INTRODUCTION

Fungi, like other organisms, require vitamins, viz. thiamine, riboflavin (B_2) pyridoxin (b_6), niacin, folic acid, cyanocobalmin (B_{12}) etc. (Lilly and Barnet, 1951 ; Cochrane, 1958). Macronutrients like potassium, phosphorus etc. and micronutrients or trace elements like iron, zinc, copper, manganese etc. in minute or specific amount for good mycelial growth were also established (Cochrane, 1958).

In the present experiment effect of five different vitamins viz. ascorbuc acid, pyridoxine, riboflavin, thiamine, inositol and four different sources of trace elements on the growth by the mycelia of *Lentinus squarrosulus, L. polychrous, Agrocybe broadwayi* and *Astraeus hygrometricus* have been studied under submerged condition. The trace elements viz. 'Fe', 'Zn', 'Cu' 'Mn' were added to the basal medium as sulphate.

MATERIALS AND METHOD

A tissue culture was prepared from the fresh and young basidiocarp of the test fungi, collected from the different parts of Bankura district, W. Bengal during the period from July to September 1994. The cultures were isolated into 2% malt agar slants and maintained on the same medium.

To test the effect of different vitamins and trace elements sources, the experiments were carried out in 'Lutz' basal medium [NH_4 - NO_3 - 1.0 g., $(NH_4)_2$ HPO_4 -1.0 g., Mg SO_4. $7H_2O$ - 0.1 g., Fe $(SO_4)_3$ - 0.1 g, Mn SO_4 - 0.025 g, distilled water 1000 ml.] for *L. squarrosulus, L. polychrous, Agrocybe broadwayi* and 'Hagem' basal medium [KH_2 PO_4 - 0.5 g, $M_g SO_4$. 7 H_2O - 0.5 g., NH_4Cl - 0.5 g., 10 drops of 1% solution of ferric chloride, distilled water - 100 ml.] for *Astraeus hygrometricus.*
For removing any trace of vitamins the liquid synthetic basal medium was boiled with activated charcoal; (5g per litre for 15 minutes) and then filtered. Different vitamins such as ascorbic acid,

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pyridoxine, riboflavin, thiamine and inositol were added to the basal medium at the rate of 100 µg each per litre. The basal synthetic medium without any source of vitamins was kept as control. Subsequently for the effect of each vitamin, the said vitamin was omitted from the complete medium (basal medium with all vitamins)

The trace element contaminants were removed from the liquid synthetic basal medium properly. The main sources of trace elements contaminants lies in sugar, amino acid and the salts as medium ingredients. For the purification of other medium ingredients, the necessary amounts of medium's ingredient were dissolved separately in glass distilled water. Each solution was shaken with 2% 8-hydroxyquinoline solution in a separating funnel once at pH 7.2 and again at pH 5.2. After each extraction, the solution was washed several times with chloroform to make it free from traces of 8-hydroxyquinolone. Double glass distilled water used in this experiment.

The synthetic basal medium with all the four trace elements viz Fe, Zn, Cu and Mn, was prepared as complete medium. The basal synthetic media without any trace elements served as control. The each trace element was omitted from the complete medium to evalutate its individual role and the trace elements were added as 0.01 mg per 1 litre.

For both vitamin and trace element experiments the pH of the medium was adjusted to 6.0 by 0.2 M phosphate buffer before sterilization. All these media were then dispensed in 250 ml Erlenmeyer flaks, as 50 ml, plugged and sterilized at 10 psi. for 20 minutes. All these flasks were then inoculated separately with an agor block (5 mm. diameter) containing 7 days old mycelia of the test organisms mentioned before and incubated for 14 and 21 days under static condition in complete darkness. The inoculated flasks were incubated at 30°C (±0.5°C).

The medium and mycelia were separated by filtration through a tared sintered funnel (Jena IG-3). The filtered mycelium was washed repeatedly with distilled water to make them free from adherent medium and dried to constant weight at 60°C for 96 hours. The dry weight of the mycelium thus obtained was taken as an index of growth. The reported data are the average of three replicates only.

RESULTS AND DISCUSSION

The growth responses during the experimental period of test organisms to different vitamins and trace elements are recorded in Tables 1 and 2 respectively.

It is evident from the Table 1, that of all the vitamins, thiamine is the most required one for growth by both the species of *Lentinus* and *Agrocybe broadwayi*. However, in case of *A. hygrometricus*, it is pyridoxine. Next to thiamine and pyridoxine, riboflavin is required by both the species of *Lentinus* and inositol by the species of *Agrocybe* and *Astraeus*. Besides these, all the other vitamins have some stimulatory effect on the growth of all the test fungi. However, complete medium with all the vitamins does not show very good result so far growth is concerned which might be due to the interaction of the vitamins in the medium.

Jennison *et al.* (1955) reported that thiamine was essential for the growth of the most white and brown wood rotting fungi. Banerjee and Nandi (1965) reported that when thiamine was added in a medium, considerable increase takes place in mycelial growth of *Lentinus praerigidus* and similar observation was noticed in case of *L. subnudus* by Chandra and Purkayastha (1977).

Inositol heterotrophy is also noticed from the present data and it may be assumed that all the four test fungi will be good sources of ascorbic acid, pyridoxine and riboflavin as they have shown autotropic responses to these vitamins.
It is evident from the Table 2, that the test organisms exhibited rapid mycelial growth in a complete medium in comparision to control sets. *Lentinus squarrosulus* and *L. polychrous* show relatively

Table 1. Data (mean)* showing the effect of different vitamin sources on the mycelial growth of *Lentinus squarrosulus, Lentinus polychrous. Agrocybe broadwayi* and *Astraeus hygrometricus* at different incubation periods

Vitamin sources	Dry weight of mycelial yield (mg./50 ml.)* Incubation period (days)							
	14	21	14	21	14	21	14	21
	L squarrosulus.		*L. polychrous*		*A. broadwayi*		*A. hygrometricus*	
**								
'C M' (-) Ascorbic acid	80.6 ± 3.2	73.3 ± 2.0	130.6 ± 5.6	132.3 ± 3.0	53.0 ± 6.0	42.0 ± 3.0	50.3 ± 6.5	77.0 ± 5.0
'C M' (-) Pyridoxine	85.3 ± 3.0	80.6 ± 2.0	144.6 ± 6.4	134.6 ± 3.7	49.0 ± 2.6	34.3 ± 4.5	39.6 ± 2.5	68.6 ± 5.6
'C M' (-) Riboflavin	62.2 ± 6.1	62.0 ± 2.6	131.0 ± 1.0	129.0 ± 2.0	45.3 ± 8.3	39.3 ± 2.0	56.6 ± 4.9	95.0 ± 4.3
'C M' (-) Thiamine	58.6 ± 3.5	52.0 ± 4.0	122.3 ± 5.8	113.6 ± 5.1	38.0 ± 1.0	37.0 ± 4.3	47.0 ± 5.5	75.6 ± 3.5
'C M' (-) Inositol	68.0 ± 8.0	63.0 ± 5.1	158.0 ± 6.0	141.6 ± 4.5	43.0 ± 4.5	41.6 ± 6.6	43.0 ± 5.5	80.6 ± 6.1
'C M'.	88.0 ± 5.5	75.0 ± 5.36	160.00 ± 3.6-	147.3 ± 4.0	61.6 ± 6.1	48.3 ± 2.5	56.6 ± 2.0	104.3 ± 5.6
Basal Medium'(-) all vitamins (control)	51.0 ± 6.0	49.6 ± 4.0	129.3 ± 4.1	123.3 ± 7.5	35.0 ± 2.0	35.0 ± 2.0	41.0 ± 5.5	62.6 ± 8.5

[* Mean data of three replicates,] ** 'CM' - [=Complete medium i.e. Basal medium (+) all vitamins]

Table 2. Data (mean)* showing the effect of different trace elements on the mycelial growth of *Lentinus squarrosulus, Lentinus polychrous. Agrocybe broadwayi* and *Astraeus hygrometricus* at different incubation periods

Sources of Trace elements	Dry weight of mycelial yield (mg./50 ml.)* Incubation period (days)							
	14	21	14	21	14	21	14	21
	L squarrosulus.		*L. polychrous*		*A. broadwayi*		*A. hygrometricus*	
**								
'C M' (-) 'Fe'	112.6 ± 3.5	97.3 ± 7.2	153.3 ± 3.7	138.3 ± 6.8	66.0 ± 3.6	47.6 ± 7.0	46.6 ± 4.5	80.0 ± 2.0
'C M' (-) 'Zn'	78.6 ± 8.3	62.3 ± 5.8	145.3 ± 2.5	133.6 ± 3.7	60.0 ± 5.5	43.6 ± 2.0	37.3 ± 5.5	67.0 ± 2.0
'C M' (-) 'Mn'	90.6 ± 4.9	75.0 ± 6.2	142.6 ± 6.0	106.0 ± 5.2	58.6 ± 4.1	44.0 ± 4.5	47.3 ± 4.5	75.0 ± 4.5
'C M' (-) 'Cu'	99.3 ± 3.0	71.6 ± 4.7	116.0 ± 6.0	100.0 ± 1.0	54.6 ± 4.5	49.6 ± 2.5	50.3 ± 5.5	82.0 ± 5.5
'C M'	121.0 ± 4.0	93.6 ± 7.3	151.0 ± 3.0	120.3 ± 8.3	81.6 ± 7.5	54.0 ± 7.2	46.6 ± 7.0	91.3 ± 7.0
'Basal Medium' all trace elements (Control)	77.6 ± 6.8	68.6 ± 4.7	100.0 ± 1.0	78.6 ± 4.5	46.3 ± 6.5	40.0 ± 5.5	25.6 ± 5.1	51.0 ± 7.2

[* Mean data of three replicates only,] ** 'CM' - [=Complete medium i.e. Basal medium (+) all trace elements]

best growth in absence of iron which prove that both the fungi possibly do not require external source of iron for their growth. *L. squarrosulus* shows minium growth in absence of zinc and poorer growth in absence of manganese. These indicate that the fungus requires zinc and manganese as external supply for growth. Similarly *L. polychrous* and *A. broadwayi* require copper for their growth and also to some extent manganese and zinc. *Astraeus hygrometricus* requires manganese and zinc for its growth.

Fungi have definite requirement for micronutrient like iron, zinc, copper, manganese, molybdenum etc. in a very small amount. Humfeld and Sugihara (1952) observed that iron and zinc responsible for the best mycelial yield of *Agaricus campestris*. Chandra and Purkayastha (1977) showed very rapid mycelial growth of *A. campestris, Calocybe indica, Termitomyces eurhizus, L. subnudus* and *V. volvacea* in medium containing iron, zinc, manganese, copper and potasssium.

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