J. Mycopathol Res. 30(2) : 177-183 (1992) *Printed in India*

Effect of macro- and micro-elements on the growth and protein production of *Collybia diminuta, Tricholoma lobayense* and *Oudemansiella canarii* in stationary liquid medium

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Various sources of macro- and micro-elements were used in the culture medium to determine their requirements for the growth and protein production of *Collybia diminuta, Tricholoma lobayense* and *Qudemansiella canarii*. *C. diminuta* required Mg, K and Cu for the maximum mycelial growth. *T. lobayense* required Mg, Ca and Zn for the maximum mycelial growth while *O. canari* required Ca, K and Zn for its maximum growth. However, *C. diminuta, T. lobayense* and *O. canarii* required Mg, Zn and Zn respectively for the maximum production of protein.

Key words : Macro- and Micro-elements, Growth, Protein production, *Collybia diminuta, Tricholoma lobayense, Oudemansiella canarii*

INTRODUCTION

Stoller (1941) showed that supplementation of trivalent ions such as Al, Fe and Mn were necessary to enhance the growth of mushroom mycelium. Humfeld and Sugihara (1949) used P, K, S, Mg, Fe and Zn in the synthetic medium for the growth of *Agaricus campestris*. Iron, S, Zn, P and K had no effect on growth of *Tricholoma nudum* whereas Mg stimulated the growth by 17% and also increased the protein production by 11% (Falanghe *et al.*, 1964). Jandaik (1976) found that *Pleurotus sajor-caju* grew best with ferrous sulphate added in the medium. Chandra and Purkayastha (1977) reported that *Agaricus campestris* grew well in Mg depleted medium. Medium without Cu or Fe was most suitable for the growth of *Volvariella volvacea* and *Lentinus subnudus*. Kosaric and Nabuo (1981) reported that both K and Fe were essential for submerged growth of morel mushroom in cheese whey. Meischhans Ulrich (1981) showed Cd as a growth factor of *Agaricus abruptibulas* which enhanced

the growth upto 80%. Prikhod' Ko (1982) reported that $Al_2(SO_4)_2$, $FeSO_4$ and $MgSO_4$ at 0.5 g/litre in the medium enhanced the mycelial growth of *Agaricus bisporus*. Rao (1983) reported that *Agaricus trisulphuratus*, *Rhodocybe subgliba* and *Agrocybe praecox* required K and Mg for maximum growth.

The report describes the effect of macro-and micro-elements on the growth and protein yield of mycelia of *Collybia diminuta* (Berk. & Br.) Sacc., *Tricholoma lobayense* Heim. and *Oudemansiella canarii* Hohn. in submerged culture.

MATERIALS AND METHODS

Fungi

The tissue culture of *Collybia diminuta*, *Tricholoma lobayense* and *Oudemansiella canarii* were obtained from the stock culture collection, Mycology Laboratory, Department of Botany, Calcutta University.

An aliquot of 1.0 ml of the mycelial cell suspension was used as inoculum. The procedure for the preparation of inoculum was described previously (Saha and Samajpati, 1987).

Removal of element contamination

To remove the contaminating minerals from the growth medium, the following precautionary measures were taken. The water used in this experiment was deionized and double distilled. The main sources of trace element contaminants were sugar, amino acid and the salts used as medium components. For the purification of medium components, the necessary amounts were dissolved seperately in glass distilled and demineralized distilled water. Each solution was shaken twice with 2 per cent 8-hydroxyquinoline solution in chloroform 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 wash it free from traces of 8-hydroxyquinoline.

Media and growth conditions

In this experiment three macro-elements (which are required relatively large quantities as nutrients) namely Ca, K and Mg and five micro-element (which are required in much smaller amounts as nutrients) namely Zn, Cu, Mn and Fe were selected for the study Here, Ca, K, Mg, Zn, Cu, Mn, Mo and Fe were added as $CaSO_4$, $K SO_4$, $MgSO_4.7H_2O$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, $MnSO_4.H_2O$, MoO_3 and $FeSO_4.7H_2O$ in the amount of 500 mg, 250 mg, 500 mg, 0.1mg, 0.1 mg, 0.1 mg and 0.1 mg litre medium respectively. Following Litchfield *et al.* (1963) the synthetic liquid medium (Glucose 30g, Yeast extract 2g, Inositol 50 mg, Thiamine 100μg, Biotin 50 μg, Folic acid 100 μg and distilled

water 1000 ml) for eace test fungus was prepared containing the best carbon and nitrogen sources of respective fnngi in place of carbon and nitrogen of the synthetic liquid medium for growth (starch and yeast extract for *C. diminuta* ; maltose and yeast extract for *T. lobayense* ; glucose extract for *O. canarii*) at carbon : nitrogen ratio of 10 : 1 which was found to be optimum for all the test fungi (Saha and Samajpati,1987). The effect of macro-and micro-elements on the growth and protein production of the test fungi was studied by two methods. In the first method the synthetic liquid medium was supplemented with all the test elements as salts excepting the particular element under study. In this investigation, deficiency of a specific element was detected against the test fungi. In the second method the elements were added individually to the synthetic liquid medium to show the individual effect on growth and protein production of the test fungi. In both the methods described, the pH of the medium of *C. diminuta*, *T. lobayense* and *O. canarii* was adjusted to their respective optimum values of 6.5, 6.5 and 6.0 which was found to be optimum for all the test fungi (Saha and Samajpati, 1983) before sterilization. An aliquot of 50 ml of the medium was disposed in each 250 ml Erlenmeyer flask, plugged and sterilized at 10 p.s.i. for 20 minutes. The flasks containing medium without any element were kept as control. Each set of sterilized flasks with one type of treatment was inoculated separately with 1.0 ml of cell suspension of the mycelia of three test fungi and incubated (stationary) separately in complete darkness for 15 days and 30°C ; 20 days and 20 C; 20 days and 20 C for *C. diminuta*, *T. lobayense* and *O. canarii* respectively. The incubation days and temperature used in this experiment were found to be optimum for growth of respective fungi (Saha and Samajpati, 1983).

Measurement of Growth

After respective optimum incubation periods, three flasks from each treatment of the test fungi were harvested. The medium and the mycelium were separated by filtration through a tared sintered funnel (Jena IG-3). The filtered mycelia were washed repeatedly with distilled water to make it free from adhering medium and dried to a constant weight in a vacuum oven at 60°C for 24 hours, cooled in a desiccator and weighed.

Measurement of Protein

Total nitrogen content of the dried mycelium was determined colorimetrically (Folin and Wu, 1919 and Vogel, 1961) using a photo-electric colorimeter (Model AE-II, Tokyo, ERMA Optical Works Ltd., Japan). A conversion factor (N x 4.38) was used to estimate mushroom protein content (Crisan and Sands, 1978).

RESULTS AND DISCUSSION

The result indicated that *C. diminuta* grew best in the medium containing Cu (Table 1). It was also evident that all the other macro and micro elements when applied individually, have inhibitory role on the growth of *C. diminuta*. All the macro-and micro-element when applied at a time there was stimulation in growth of *C. diminuta* The complete macro-elements medium containing Mg and K but without Ca was found to have maximum stimulatory role on the growth of *C. diminuta*. The combined effect of Ca and Mg was also good for *C. diminuta*.

Table 1. Fungal mycelium (g DW/L) produced on different macro- and micro-elements

Elements tested	*Collybia diminuta*	*Tricholoma lobayense*	*Qudemansiella canarii*	Mean
Control	22.740a	5.858b	10.388c	12.996
Control + Ca	21.093	6.950	8.863	12.302
Control + Mg	19.710	7.120	10 117	12.316
Control + K	19.450	5.533	10.013	11.666
Control + Mn	20.150	7.750	10.697	12.866
Control + Zn	22.630	9.993	16.897	16.507
Control Fe	22.050	6.230	12.950	13.743
Control + Mo	22.350	8 810	12.553	14.571
Control + Cu	24.527	6.927	8.697	13.383
Control + Mn + Zn + Fe + Mn + Cu	24.097	7.930	14.883	15.637
Control + Ca + Mg + K	23.630	4.640	12 863	13.711
CM – Ca	26.230	5.763	12.697	14 897
CM—Mg	19.373	7.263	16.863	14.500
CM – K	24.873	8.663	10.472	14.669
CM—Mn	20.540	8.357	10.525	13.141
CM—Zn	19.540	7.063	12.700	13.101
CM - Fe	24.163	6.190	10.008	13.454
CM – Mo	24.217	7.317	10.772	14 102
CM—Cu	21.897	8.363	12.120	14.127
Mean	22.277	7.196	11.846	

L.S.D. at 5% of P level : Fungus (F) = 0.042 ; Treatment (T) = 0.106; CM = Complete Medium
Results are the mean of three replicas :
Composition of Control medium :

a. Starch 40g, Yeast extract 4g, Inositol 50 mg, Thiamine 100μg, Biotin 50μg, Folic acid 100μg and distilled water 1000 ml.
b. Maltose 40g, Yeast extract 4g, Inositol 50mg, Thiamine 100μg, Biotin 50μg, Folic acid 100μg and distilled water 1000 ml.
c. Glucose 40g, Yeast extract 4g, Inositol 50mg, Thiamine 100μg, Biotin 50μg, Folic acid 100μg and distilled water 1000ml.

Regarding micro-elements the combined effect of Mn, Zn, Mo and Cu and also Mn, Zn, Fe and Cu had stimulatory role on the growth of *C. diminuta*. All the other combinations had inhibitory role on the growth of *C. diminuta*. It was also evident that Zn had the maximum stimulatory effect on growth which was followed by Mo, Mn, Mg, Ca, Cu, and Fe. However, K had inhibitory effect on

Table 2. Effect of d fferent macro- and micro-elements on protein content (%) of *C. diminuta*, *T. lobayense* and *O. canarii* in submerged culture

Element tested	*Collybia diminuta*	*Tricholoma lobayense*	*Oudemansiella canarii*
Control	8.190±0.06	17.526±0.08	15.575±0.04
Control±Ca	14.541±0.10	18.834±0.09	17.428±0.09
Control±Mg	18.702±0.08	24.090±0.07	25.280±0.05
Control+K	15.082±0.07	29.127±0.04	21.728±0.06
Control+Mn	9.380±0.05	25.185±0.10	22.120±0.03
Control+Zn	9.818±0.06	33.507±0.12	28.320±0.10
Control+Fe	9.958±0.11	22.770±0.11	23.870±0.11
Control+Mo	11.475±0.10	22.557+0.12	20.325±0.10
Control±Cu	11.256±0.07	20.367±0.09	18.080±0.09
Complete (Micro)	11.508±0.04	20.805±0.06	17 350±0.07
Complete (Macro)	12.720±0.10	21.024+0.07	18.098+0.05
Complete—Ca	14.789±0.12	21.900±0.04	19.920±0.08
Complete—Mg	12.570±0.09	22.580±0.10	21.165±0.09
Complete—K	14.780±0.04	21.027±0.03	20.264±0.12
Complete—Mn	16.293±0.08	28.320±0.04	22.621±0.11
Complete—Zn	13.446±0.11	27.180±0.06	21.730±0.10
Complete—Fe	12.132±0.10	21.580±0.08	20.180±0.05
Complete—Mo	12.572±0.09	18.420±0.07	17.980±0.06
Complete—Cu	13.082±0.14	18.980±0.10	16.580±0.07

the growth of *T. lobayense* (Table 1). It was found that Ca and Mg had stimulatory effect on growth but the effect of Mg+K and Ca+K had inhibitory role on the growth. All the combination of micro-elements had stimulatory effect on the growth of *T. lobayense* of which Mn, Zn, Fe and Mo was found to be the best and which was closely followed by Zn, Fe, Mo and Cu. It was found that Zn had the maximum stimulatory effect on growth of *O. canarii* which was followed by Fe, Mo and Mn. Whereas Ca, Mg and Cu had inhibitory role on the growth. Ca and K had stimulatory role on the growth. Mg+K and Ca+Mg had inhibitory role on the growth. Bul all the combinations of micro-elements had inhibitory role on the growth of *O. canarii*. The data on statistical analyses revealed that all the treatments are significant at 5% level. The results on

growth showed similarity with *A. campestris* (Humfeld and Sugihara, 1949) and requirement of Mg in *T. lobayense* for the growth also agree with the observation of Falanghe *et al.* (1964) on *T. nudum*. The role of Zn for the growth of *T. lobayense* and *O. canarii* showed similarity with the findings on *P. sajor-caju* by Jandaik (1976) and on *V. volvacea* and *L. subnudus* by Chandra and Purkayastha (1977). *C. diminuta* produced maximum amount of protein 18.702% in the medium containing Mg (Table 2) which was similar to *T. nudum* (Falanghe *et al*., 1964). Whereas *T. lobayense* and *O. canarii* produced maximum amount of protein 33.507% and 28.320% respectively in the medium containing Zn (Table 2) which was similar to *P. sajor-caju* (Jandaik, 1976).

ACKNOWLEDGEMENT

The authors are grateful to Prof. S. C. Datta, Head of the Department of Botany, University of Calcutta, for his keen interest and also to the Department of Planning and Development, Govt. of West Bengal for financial support.

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(Accepted for publication 3 May 1992)