PROPAGATION OF THREE MUSHROOMS GENERA IN SUBMERGED CULTURE OF MANGO STONE INFUSION

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ABSTRACT

Factors affecting the production of mushroom mycelium from *Agaricus bisporus, Pleurotus ostreatus* and *Pholiota aegerita* grown in mango stone infusion for 7 days were studied. The optimum conditions enhancing the economic coefficient (EC) of mushroom mycelium were 25, 30 and 20^0C for incubation temperature, 4.0, 4.5 and 5.5 for pH values, 300 r.p.m. for shaking rate and 25 : 1 for C/N ratio in case of *Agaricus bisporus, Pleurotus ostreatus* and *Pholiota aegerita* with high EC of 66.8, 51.9 and 63.9, respectively. In comparison under these optimum conditions, molasses media (as control) reflected lowered EC than that of mango stone infusion. The dry mycelium of *A. bisporus* showed a higher protein and fat contents than *Pleurotus ostreatus* and *Pholiota aegerita.* Inorganic elements, Mg, Ca, Na, K, Cu, Fe, Mn, Zn, Pb and Cd were also detected.

Keywords: fermentation, mushroom, food wastes.

INTRODUCTION

Food industries produce large amount of various types of wastes causing environmental pollution. Various methods for the utilization and recovery of by-products, were reviewed by many investegators (Joshi & Joshi, 1990, Ballero, 1991, Kuzmanova *et al.,* 1991, Ethiraj & Suresh, 1992, Zhu & Hi 1999). There is an increasing demand for natural ingredients and flavourings in food industry. However, submerged germentation is a fast and attractive technological process for production of highly flavoured mushroom biomass and is otherwise applied by either traditional or laboratory procedures (Hadar & Dosoretz, 1991 and Allan & Kennedy, 1997).

Mushroom mycelium was found to have approximately the same nutritive value as fodder yeast when grown in waste products of the citrus industry, molasses and waste sulfite (Kuzmanova *et al.,* 1991 and Zhu & Hi, 1999). Cirillo (1960) suggested that, mushroom mycelium produced in waste sulfite liquor can be used as a protein supplement for human food or for animal feed.

Litchfield *et al.* (1963) showed that, morel mushroom mycelium could be grown in submerged culture with glucose, maltose, lactose or wastes containing these sugars as substrate. Sugihara & Humfeld (1954) reported that, excellent yields of mushroom mycelia were produced when mycelia were grown on orange juice, citrus press water and chemically defined media.

Mushroom makes a very welcomed addition to the diet in certain areas of the world and is considered to be the most favorable fungus for its pleasant flavour, high nutritive value and medical effect (Chiang *et al.,* 1986, Anon, 1994, Johl *et al.,* 1997). Furthermore, Kuniori *et al.* (1976) reported that the quality of mushroom protein is valued, better than that of cereal grains and legumes because all the nine essential amino acids are present. In addition, mushroom is also a source of some nutrients such as phosphorous, iron, thiamine (Vit. B_1), riboflavin (Vit. B_2) and niacin (Kalac & Svoboda, 2000).

This investigation aimed to propagate three of some common edible mushroom mycelia in a submerged culture prepared from mango stone infusion which could be used in a future study as food supplements or flavouring materials.

MATERIALS AND METHODS

Materials :

Fungi: The mushroom strains used in this study were *Agaricus bisporus* MIRCEN 180 and *Pleurotus ostreatus* MIRCEN 201, obtained from Microbiological Resource Centre (MIRCEN), Ain Shams University, Egypt.

Pholiota aegerita was kindly offered in form of spawn from AZ. AGR. Paolo Costa, Produzione Micelio E Funghi, Italy.

The spawn of *Pholiota aegeritai* was cultured on wood dust as described by Subba (1989). Tissue culture technique was carried out to select fresh pure mature mushroom. Gentle washing under running water is necessary to remove surface dirt. The surface is also wiped gently with 70% alcohol. Using a sterile scalpel, a small slit from the bottom of the mushroom was removed. Mushroom was cut into two halves avoiding touching the inner surface. A few pieces of tissue from the center of the mushroom was transferred to malt extract agar plates. The cultures were incubated for few days. Some tiny bits of the mycelia were transferred to another agar plates.

Wastes and by-products: Mango stone wastes were obtained from El-Nasr for Food Preservation "Kaha", Abo-Kaber factory, Sharkia, Egypt.

Sugar cane molasses was obtained from Egyptian Sugar and Distillery Company (Hawamdia Factory), Egypt.

Methods:

Preparation of standard inoculum: All inocula were prepared by the transference of mycelia bits from the agar slants to 250ml Erlenmeyer flasks containing 40ml of 5% malt extract broth and then were incubated at 25^0C for 10 days. The whole contents of the flasks were then blended for 30 sec., in a sterile waring blender. The resulted homogeneous suspensions were centrifuged at 2000 rpm for 5 min. and resuspended in 40ml sterile distilled water (Falanghe, 1962).

Fermentation process: For the preparation of the molasses medium (as control), the molasses was dissolved in distilled water to give 5% sugar concentration, then 4.0g ammonium sulfite, 0.1g potassium phosphate, 0.5g magnesium sulfate and 0.003g. ferrous sulfate were added to one litre of the diluted molasses.

From the other hand, mango stone infusion was prepared by dipping 5Kg of mango stones in 10 litres of hot water (70°C for 7 hrs. with 15 min. interval stirring), then filtered. Ammonium tartrate 1.1g/1000ml were added as nitrogen source. All the media were sterilized in 500ml flasks where each was containing 200ml of medium. Each flask was inoculated with 2ml of the prepared standard inoculum and incubated under different experimental conditions.

Chemical analysis: Moisture, total solids, fat, crude fiber and ash contents were determined according to A.O.A.C (1980). Atomic absorption Unicam 969 spectrometer was used for inorganic element determination. Reducing and non reducing sugars were estimated according to the method described by Somgyi (1945). Semi-Kjeldahl method was used for crude protein estimation. Yield Coefficient (YC) and Economic Coefficient (EC) were estimated according to the following equations as described by Falanghe (1962).

YC = (mg mushroom mycelium formed/mg sugar utilized) × 100

EC = (mg mushroom mycelium formed/mg total sugars) × 100

RESULTS AND DISCUSSION

Several chemical constituents of mango stone infusion were studied for their possible application for production of mushroom mycelia. Table (1) shows the chemical components of mango stone infusion prepared by dipping one part of mango stones in two parts of water.

Table 1: Main components of mango-stone infusion

Items	Mango stone infusion
Total solids (%)	6.24
Total sugars (%)	4.43
Reducing sugars (%)	4.1
Non-reducing sugars (%)	0.33
Total acidity (as citric acid %)	0.13
pH	5.0
Protein % (N x 6.25)	0.4
Ash (%)	0.52

Accordingly, from these values, there is a possibility to use this infusion in a fermentation process for mushroom mycelia production.

Fig. (1) shows the effect of incubation temperature on Economic Coefficient (EC) of the produced mushroom mycelium grown in mango stone infusion at 75:1 C/N ratio for 7 days. From this figure, it is clear that 25^0C incubation temperature was favorable for the

high production of *Agaricus bisporus* mycelium. While, 30^0C was the most favorable temperature for the production of mycelium from *Pleurotus ostreatus.* On the other hand, *Pholoita aegertia* mycelium was produced with the highest EC when grown at 20^0C. The differences between the optimum temperatures may be attributed to the individual physiological properties of studied fungi (Gewaily, 1977).

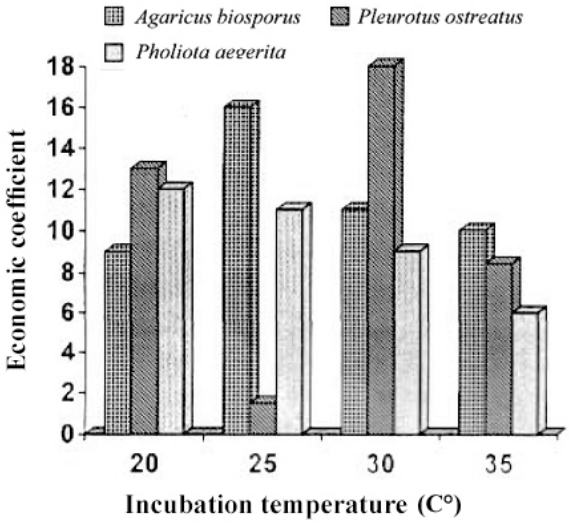

Fig. 1: Effect of incubation temperature on the economic coefficient of mushroom mycelia production

The effect of initial pH values on the economic coefficient of the produced mushroom mycelium grown in mango stone infusion at 75:1 C/N ratio for 7 days are illustrated in Fig. (2). From this figure, it could be observed that, the initial pH of the mango stone infusion medium had a pronounced effect on the production of mushroom mycelia. The favorable pH values for mushroom mycelia production with the highest EC by *A. bisporus, Pl. ostreatus* and *Ph. aegerita* were 4.0, 4.5 and 5.5, respec-tively. These results disagree with those obtained by Block *et al.* (1953) who mentioned that the original pH of the medium had very little effect on the total yield of mycelium produced by *A. blozei* grown in synthetic medium. They detected that the mycelium buffered the medium at approxi-mately pH 5.5, in orange juice medium. Generally, changing the pH values towards acidity or alkalinity led to decrease the mycelium dry weight and EC. This could be attributed to (i) the production of small

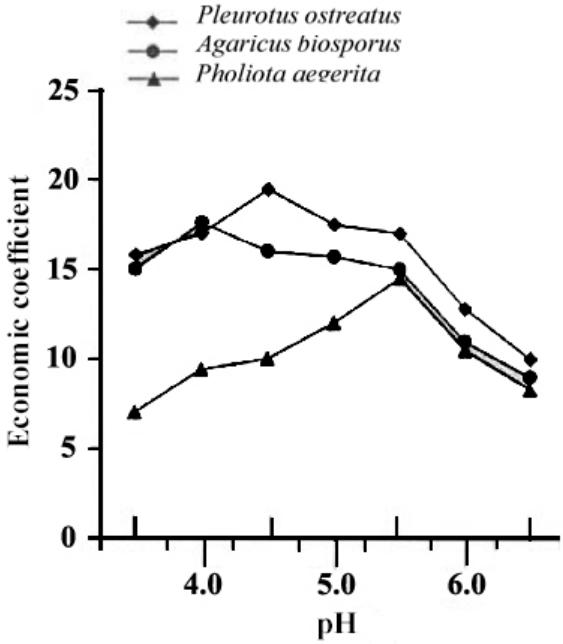

Fig. 2: Effect of different pH values on the economic coefficient of mushroom mycelia production

amount of organic acids like succinic acid produced during the growth, (ii) carbon dioxide produced by the cells and dissolved in the medium and/or (iii) use of the basic compounds such as ammonium tartrate by the cells (Yoshida *et al.,* 1990).

The effect of shaking rate on the EC of produced mushroom mycelium grown in mango-stone infusion at 75 : 1 C/N ratio for 7 days at the optimum temperature and pH for each fungus are shown in Fig. (3). The results indicated that the EC increased with increasing the shaking rate for the three studied mushrooms.

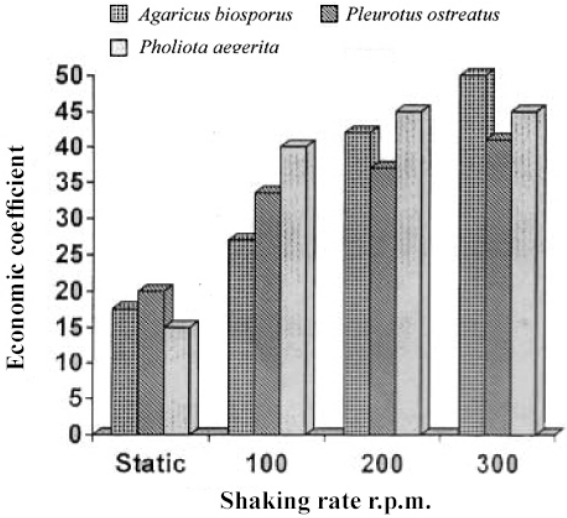

Fig. 3: Effect of shaking rate on the economic coefficient of mushroom mycelia production

Economic coefficient were 49.2, 41.9 and 46.5 or for *A. bisporus, Pl. ostreatus* and *Ph. aegerita* at 300 r.p.m., respectively. On the other hand, EC were 17.4, 19.4 and 14.8 in static culture for studied fungi, respectively. There are no residual sugars in mango stone infusion media with increasing shaking rate and mycelia dry weight increased. Generally, these findings sounded to be true since, fungi are strictly aerobic. Nevertheless, the quantitative relation of growth and oxygen supply were very considerable among different forms (Wix & Woodbine, 1959, Subba, 1989, Zhu & Hi, 1999).

The effect of C/N ratio on the economic coefficient of the produced mushroom mycelia from *A. bisporus, Pl. ostreatus and Ph oegerita* grown under the optimum conditions stated before in the present results are illustrated in Fig. (4). From this figure, it could be noticed that decreasing C/N ratio by adding ammonium tartrate increased economic coefficient for all the three mushrooms studied. Moreover, the fungi consumed the total sugars in the media, while the mycelium increased, this reflects the ability of these fungi to utilize citric acid as a carbon source. Generally, high economic coefficient was 66.82 for *A. bisporus* followed by 63.88 and 51.92% in case of *Ph. aegerita* & *P. ostreatus,* respectively. These results are in line with those obtained by Yoshida *et al.* (1990), who found that the optimal carbon/ nitrogen ratio in the medium was 20:1 for production

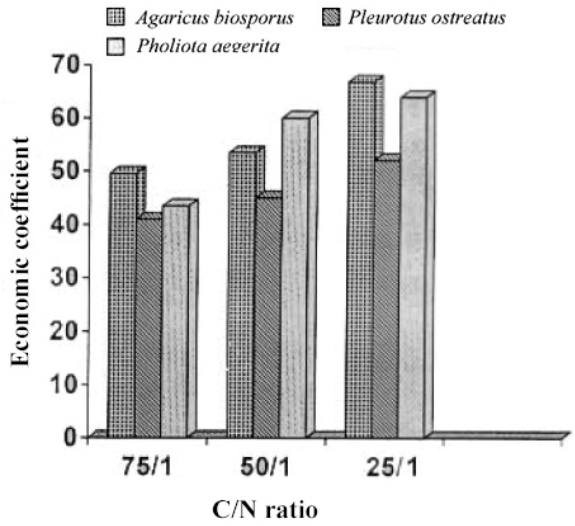

Fig. 4: Effect of C/N ratio on the economic coefficient of mushroom mycelia production

of *A. compeotris, Morchela hybride* mycelia when grown in liquid culture media.

Comparison between molasses and mango-stone infusion media on the production of mushroom mycelia from *A. bisporus, Pl. ostreatus and Ph. aegerita* under the optimum conditions for each fungi are tabulated in Table (2). The EC obtained from molasses medium was lower than that from mango stone infusion medium for the three studied mushrooms. The EC *for A. bisporus, Pl. ostreatus and Ph. aegrerita* were 33.6, 39.0 and 43.4 in case of molasses media and 66.82, 51.92 and 63.88 in case of mango stone infusion, respectively. This may be attributed to the difference in nutrients between the molasses media and/or fermentable sugars abounding in each medium. On the contrary Reusser *et al.* (1958) cultured four strains of *A. campeestris* on a synthetic medium and on media containing molasses or waste sulfite liquor. They found that, high yields of dry matter and protein were obtained on molasses medium, while yields on waste sulfite liquor were slightly lowered. On the other hand, Falanghe (1962) observed that, *A. compestris, and Tricholoma nudum* were capable of growing in submerged culture in medium of vinasse with added salts. Higher fermentative efficiencies were found under these conditions than in medium containing molasses or waste sulfite liquour.

Table (3) shows the proximate analysis of the dried mycelium of three mushroom strains studied grown in mango stone infusion media. It was pointed that, the dry mycelium of *A. bisporus* showed a higher protein and fiber contents than either *Pl. ostreatus* or *Ph. aegerita,* while *Pl. ostreatus* contained a higher fat and ash contents than either *A. bisporus* or *Ph. aegerita*. Carbohydrates content was higher in *Ph. aegerita* than the other two strains studied. This may be due to the physiological properties during growth of these strains. These results are in accordance with findings of Cavazzoni & Adami (1992) and Yoshida *et al* (1996).

The amounts of inorganic elements of the mushrooms mycelia grown at the optimum conditions for each in mango stone infusion are presented in Table (4). It was noticed that potassium and magnesium predominated over all the other elements. These elements followed by sodium and calcium. Trace elements (Zinc, Copper, Iron and Manganese) ranged between 0.43 and 19.6 mg/100g dry wt. Cadmium as a heavy metal was not found in all the mush-

Table 2: Comparison between molasses and mango stone infusion media on the production of mushroom mycelium under the optimum conditions for each

Media	Sugars (g/100ml)			Mycelium dry wt (g/100ml)	Yield co-efficient	Economic coefficient
	Total	Residual	Consumed			
Agaricus bisporus						
Molasses	5.00	0.29	4.71	1.78	37.79	35.60
Mango stone infusion	4.43	0.00	4.43	2.96	66.82	66.82
Pleurotus ostreatus						
Molasses	5.00	0.18	4.82	1.95	40.46	39.00
Mango stone infusion	4.43	0.00	4.43	2.30	51.92	51.92
Pholiota aegerita						
Molasses	5.00	0.62	4.38	2.17	49.54	43.40
Mango stone infusion	4.43	0.00	4.43	2.83	63.88	63.88

Table 3: Proximate analysis of dried mushroom mycelia grown in mango stone infusion media

Fungi	Composition (g/100g dry weight)				
	Carbohydrates	Protein	Fat	Crud fi-ber	Ash
Agaricus bisporus	40.92	31.60	7.60	10.7	4.18
Pleurotus ostreatus	48.28	24.50	8.00	7.0	6.22
Pholiota aegerita	50.19	26.90	7.30	9.3	3.39

rooms under study, while lead content ranged between 0.03 and 0.07mg/ 100g dry wt. Differences between mushrooms for their ability to accumulate inorganic elements in their mycelium may be due to physiological properties itself and/or mineral content of the substrate before cultivation (Patrabnsh & Madan, 1999). Low concentration of sodium and relatively high concentration of potassium suggest that mushroom mycelia may be useful as a potential anti-hypertensive food (Manzi *et al.,* 1999).

Table 4: Inorganic elements in mushroom mycelia grown under its optimum conditions, in mango-stone infusion.

Elements	Element content (mg/100g dry wt.) of mycelia		
	Agaricus bisporus	***Pleurotus ostreatus***	***Pholiota aegerita***
Calcium	27.0	34.0	30.0
Magnesium	140.0	160.0	110.0
Potassium	368.0	460.0	250.0
Sodium	70.0	65.0	73.0
Copper	16.5	6.0	7.3
Iron	5.3	5.9	4.2
Manganese	0.51	6.59	0.43
Zinc	19.6	11.0	7.0
Cadmium	0.00	0.00	0.00
Lead	0.03	0.07	0.04

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تنمية ثلاثة أجناس من عيش الغراب بواسطة المزرعة المغمورة في بيئة نقيع نوى المانجو

عطية محمد عطية المخزنجي

قسم التصنيع الزراعي معهد الكفاية الإنتاجية – جامعة الزقازيق

تم دراسة العوامل المؤثرة على إنتاج هيفات عيش الغراب من الفطريات التالية : *Agaricus bisporus*، *Pleurotus ostreatus* و*Pholiota aegerita* عند تنميتها في منقوع نوى المانجو لمدة ٧ أيام. وكانت الظروف المثلى والتي أعطت أعلى مكافئ اقتصادي (EC) للإنتاج هي : ٢٥ ، ٣٠ ، ٣٠°م كأحسن درجة حرارة تحضين، ٤، ٤,٥ و ٥,٥ كأفضل قيم pH و٣٠٠ لفة في الدقيقة كأحسن معدل للارج و١:٢٥ كأحسن نسبة للكربون إلي النتروجين لكل من السلالات المدروسة على الترتيب. تحت الظروف السابقة كان أعلى (مكافئ اقتصادي) هو ٦٦,٨، ٥١,٩ و٦٣,٩ على الترتيب. وبإجراء مقارنة تحت الظروف المثلى على المكافئ الاقتصادي في بيئة المولاس (بيئة مقارنة) لوحظ أن بيئة المقارنة أدت إلي انخفاض المكافئ الاقتصادي مقارنة بمستخلص منقوع نوى المانجو. ولوحظ من إجراء التحليل الكيماوي للهيفات الناتجة أن أعلى نسبة بروتين ودهون وجدت في هيفات *A. bisporus* مقارنة بالسلالات الأخرى. كما شملت الدراسة تقدير لعناصر المغناسيوم، كالسيوم، الصوديوم، البوتاسيوم، النحاس، الحديد، المنجنيز، الزنك، الرصاص والكوبلت في الميسليوم الجاف للفطريات الثلاثة موضع الدراسة.