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Cultivation and Chemical Composition of The Paddy-Straw Mushroom (*Volvariella volvaceae*)

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ABSTRACT

The cultivation of the paddy-straw mushroom (Volvariella volvaceae) *was investigated. Four spawn media for growing* V. volvaceae *were tested. The presence or absence of urea and the use of barley or sorghum had no effect on the mycelial growth. The addition of yeast extract to the barley grain medium gave heavy growth of mycelium as compared with other media. Seven different media were prepared for the cultivation of paddy-straw mushroom, using local materials such as orange juice waste, rice straw, bagasse, pea-canning waste, horse manure and molasses. The media containing orange juice waste, bagasse, rice straw, horse manure, molasses and urea gave the highest yields of mushroom (5·029 and 4·3 kg/m^2). The chemical analysis of mushrooms harvested from orange juice waste–bagasse medium was as follows (percentage on dry weight basis): 4·92 total nitrogen, 1·64 non-protein nitrogen, 2·71 ether extract, 17·4 crude fibre, 6·07 reducing sugars, 4·23 non-reducing sugars, 19·2 starch, 5·29 glycogen, 9·86 mannitol and 12·6 ash. Of fourteen amino acids present, seven are essential, while methionine, arginine, histidine and serine are absent. Tryptophan concentration (14·7 g/100 g protein) was higher than in any other wild mushroom or legume seed.*

INTRODUCTION

The cultivation of edible fungi in the western world is confined almost exclusively to *Agaricus bisporus*, while in East Asia the oyster mushroom (*Pleurotus oestreatus*) is grown in increasing quantities. The paddy-straw mushroom (*Volvariella volvaceae*) is cultivated from West Africa to India and the Far East (Feinberg, 1966; Smith, 1972). Large-scale mushroom production of *V. volvaceae* is extending to Taiwan, Malaysia and South Korea (Hayes, 1976; Del Caire, 1978).

Several substrates rich in organic matter have been used for mushroom cultivation. Vegetable wastes and manure (Hayes, 1974) garbage, and other organic wastes are used for *A. bisporus* (Block, 1965). The paddy-straw mushroom (*V. volvaceae*) has been cultivated on rice straw and dried banana leaves (Gray, 1977). Hayes (1977) described the materials used in the formulation of media for mushroom cultivation. These materials are as follows: (a) Vegetable-based materials which provide a reservoir of cellulose, hemicellulose and lignin. (b) Supplements for activating growth; namely, manures which are used as a source of carbohydrates and nitrogen. (c) Available nutrients such as molasses, vegetable wastes for carbohydrates, urea, ammonium sulphate and nitrate for nitrogen. (d) Supplements designed to rectify mineral deficiencies such as gypsum, potash and superphosphate. Hayes & Haddad (1976) reported that the cultivated mushroom, *A. bisporus*, is a relatively good source of protein, phosphorus, iron, thiamin, riboflavin and niacin. Mushrooms are low in calories, calcium and ascorbic acid and are devoid of vitamin A activity. Mushroom protein contains all the essential amino acids required by man. The Food and Agriculture Organization (1972) reported the following proximate analysis for *V. volvaceae*: 12·2%, 10·1%, 58·6%, 11·1% and 10·1% for crude protein, fat, total carbohydrates, crude fiber and ash, respectively.

Wastes from food industries and agricultural residues are available in increasing quantities in Egypt. The cultivation of mushrooms on such wastes increases the food supply and improves the nutritional value of agricultural wastes for animal feeding.

Climatic conditions in Egypt are suitable for the cultivation of the paddy-straw mushroom (*V. volvaceae*) and this has been investigated using different local materials. The chemical composition of the harvested mushroom was determined.

MATERIALS AND METHODS

Materials

Cultivation of paddy-straw mushroom

Media preparation. Nine different raw materials were used in different combinations (Table 1) to prepare seven media for the cultivation of *V. volvaceae*. The first two media (Nos 1 and 2) were prepared as follows. The rice straw and bagasse were cut into short pieces (about 3–5 cm long). Gypsum (calcium sulphate) and ammonium nitrate were dissolved in water, boiled and poured over the chopped wastes. The ingredients were

TABLE 1
The Constituents of Different Media

Ingredients (%)	*Medium number* 1	2	3	4	5	6	7
Orange juice factory waste	0·00	0·00	39·50	93·80	54·50	55·20	52·60
Rice straw	16·40	0·00	0·00	0·00	0·00	0·00	30·50
Bagasse	0·00	19·60	15·80	0·00	0·00	22·70	0·00
Horse manure	0·00	0·00	31·60	0·00	39·70	17·00	12·60
Molasses	0·00	0·00	5·20	4·90	5·80	4·20	3·70
Pea-canning waste	0·00	0·00	7·90	0·00	0·00	0·00	0·00
Urea	0·00	0·00	0·00	1·30	0·00	0·90	0·60
Gypsum	0·70	0·80	0·00	0·00	0·00	0·00	0·00
Ammonium nitrate	0·80	1·00	0·00	0·00	0·00	0·00	0·00
Water	82·10	78·60	0·00	0·00	0·00	0·00	0·00

mixed well and left for 3 h until water was completely absorbed. The ingredients of the other media* were chopped and mixed. Plastic jars (capacity, 2 kg) were completely filled with the mixtures, capped and airtight sealed with paraffin wax. The jars were stored for 3 months until the soluble carbohydrates disappeared (Nour *et al.*, 1980). Each compost was then cut into short pieces (about 3–5 cm long) using a cutter (Crypto model E B12). Water was added to bring the moisture content to about 75%.

* Thanks are due to Dr A. Nour, Department of Animal Production, College of Agriculture, University of Alexandria, for his assistance in the preparation of these composts.

Each medium was placed in 25 × 35 cm wooden trays, 5 cm deep, and autoclaved at 15 ppsi for 30 min.

Spawn preparation. A pure culture of *V. volvaceae* (CBS, 394-49, Baarn, The Netherlands) was maintained on a potato–glucose–agar–yeast extract (*Difco Manual of Dehydrated Cultures*, 1953). Four media (Table 2) were tried for growing spawn (Fritsche, 1978). Each medium was mixed in a 1-liter conical flask, and sterilized at 15 ppsi for 30 min. The cooled medium was inoculated with about 15 cm^2 agar mycelial block of *V. volvaceae* (San-Antonio, 1971) before incubation at 30 °C. When the mushroom mycelium appeared after 4 days of incubation, the flask was shaken to distribute mushroom mycelium throughout the grain medium. The grain mycelial block was obtained after 15–21 days of incubation. Addition of yeast extract (medium 'd') gave heavy growth of mycelium compared with the other media. It was therefore used for spawn preparation.

Cultivation and harvesting of mushroom

Two methods of inoculating the prepared media with the spawn material were tested—thumb-sized pieces of spawn were inserted into holes 2 cm deep and 10 cm apart, or spawn materials were spread on the surface of the medium. Since the latter method gave better results, it was used for inoculating the trays. After spawning, all the trays were placed in a chamber whose atmosphere was kept at high relative humidity (90 %) and temperature ranging from 28 to 30 °C. Each tray contained 3 kg of the medium, except medium No. 1 (where the tray contained 1·8 kg). Spawn inoculum was 100 g per tray. Watering was started on the fifth day from spawning. Water was sprayed, using an atomizer-type sprayer, for 2 days, but abundantly on the seventh day, and was repeated daily until

TABLE 2
The Composition of the Spawn Media

Ingredients (%)	*Medium*			
	a	*b*	*c*	*d*
Barley grain	49·5	0·0	49·3	49·4
Sorghum grain	0·0	49·5	0·0	0·0
Yeast extract	0·0	0·0	0·0	0·2
Urea	0·0	0·0	0·4	0·0
Calcium carbonate	1·0	1·0	1·0	1·0
Water	49·5	49·5	49·3	49·4

mushroom pin-heads developed. Watering was stopped during the first 2 days of the pin-head stage. A knife was used to harvest the buttons. The bed was rested for 2 days after the first harvest, and again another crop could be harvested every day.

Chemical analysis

The harvested mushroom was washed in running water and dried at 80 °C for 8 h. The samples were ground in an electric coffee mill to pass a 60 mesh sieve. The powder was placed in an airtight kilner jar and stored at 5 °C.

All determinations were carried out in triplicate. Moisture content, ether extract, phosphorus, reducing and non-reducing sugars (without interference of mannitol) and ash were determined according to the standard methods of the Association of Official Analytical Chemists (1975). Calcium, copper, manganese and iron were determined in the ash solution using a Shimadzu atomic absorption spectrophotometer, while sodium and potassium were measured according to the methods of Skoog & West (1963) by the EEL flame photometer. Total nitrogen (semi-micro-Kjeldahl method), crude fibre, vitamin C and starch (Lane and Eynon's method; Pearson, 1972) after being hydrolyzed with hydrochloric acid, were determined as described by Pearson (1972). Non-protein nitrogen was extracted according to the procedure of Kent-Jones & Amos (1967) and the nitrogen content of the extract was determined by the semi-micro-Kjeldahl method (Pearson, 1972). Glycogen content was assayed colorimetrically according to the method of Hassid & Abraham (1955). Mannitol content was determined colorimetrically by the periodate oxidation method (Abdel-Akhar *et al.*, 1965).

Acid and alkaline protein hydrolysates were prepared (Block *et al.*, 1958) for the determination of amino acids. The acid protein hydrolysate was subjected to a one-dimensional descending technique of paper chromatography using butanol: acetic acid: water (144:13:34 v/v/v) as solvent (Mikes, 1966). The chromatograms were dipped in ninhydrin reagent prepared as described by Roland & Gross (1954). The spots were identified by comparison with standards and eluted using 75 % ethanol (Majunders *et al.*, 1956). The colour intensities were measured at 515 nm for all amino acids except proline whose eluate was measured at 450 nm. Tryptophan was estimated colorimetrically in the alkaline protein hydrolysates according to the method of Miller (1967).

The 80% ethanol soluble sugars were extracted and the sugars were chromatographically separated and identified according to the method of Partridge (1946).

RESULTS AND DISCUSSION

Cultivation of *Volvariella volvaceae*

The results presented in Table 3 showed that media Nos 3, 6 and 7 gave good yields. The medium containing orange juice factory waste, bagasse, horse manure, molasses and urea (medium No. 6) gave the highest yield (5·029 kg/m^2). Medium No. 7, which is similar to No. 6 except that rice straw was substituted for bagasse, also gave a high yield of mushroom (4.32 kg/m^2). Medium No. 3, which contained more horse manure than the other two media, bagasse and pea canning waste, but no urea, gave a lower mushroom yield than media Nos 6 and 7. These three media (i.e. 3, 6 and 7) were more or less similar in time of first harvest, number of flushes

TABLE 3
Production of *Volvariella volvaceae* Mushroom on Different Media

	Medium number						
	1	*2*	*3*	*4*	*5*	*6*	*7*
Cultivation period (days)	33	42	36	42	42	37	42
Harvesting from time of spawning to first flush (days)	32	32	14	—	—	12	12
Number of flushes	4	—	10	—	—	10	10
Number of mushrooms per each flush	3	—	10	—	—	16	13
Average weight of each mushroom (g)	2·2	—	2·75	—	—	3·0	3·0
Total yield (g)	27	—	275	—	—	440	378
Time of cropping (days)	10	—	21	—	—	24	29
Yield/unit mass (g/kg)	15·0	—	91·6	—	—	146·6	126
Yield/unit urea (kg/m^2)	0·309	—	3·143	—	—	5·029	4·320

and time of cropping. Media Nos 4 and 5, which did not contain rice straw or bagasse, gave no fruit bodies despite the fact that horse manure was added to medium No. 5, and urea was added to medium No. 4, and that both media did contain orange juice factory waste.

Horse manure alone as a source of cellulose (medium No. 5) gave no yield. It became clear that bagasse and rice straw are good sources of cellulose materials when they are fortified by horse manure. Manipulation of the ratio of these three main components would maximize the yield.

A small yield (0·309 kg/m^2) was obtained from medium No. 1 which consisted mainly of rice straw, fortified gypsum (calcium sulphate) and ammonium nitrate. A very small amount of small fruit bodies (cap 1 cm wide and stem 5 cm long) was obtained from medium No. 2 which consisted mainly of bagasse fortified with gypsum and ammonium nitrate. Chang (1978) reported that yields of the straw mushroom differ widely, depending on cultural method and quality of the substratum. In Vietnam 1 m^2 of straw bed produced 1 kg of mushrooms in 2 weeks. In the Philippines average yields were 2·93 kg/m^2 on rice straw. The yields under indoor conditions are much higher than those obtained under outdoor conditions. For example, in Hong Kong, the average yield from outdoor conditions was 7 kg of mushroom per 100 kg of dry paddy straw, reaching 28·3 kg in indoor experiments. These results show that *V. volvaceae* can be grown under conditions similar to those pertaining in Egypt, using local agricultural wastes. Moreover, the significance of the source of cellulose-containing substances and the nature of the nitrogen source on the cultivation of *V. volvaceae* were clearly demonstrated.

Chemical composition of cultivated mushroom

The cultivated mushroom (*V. volvaceae*), which was collected from culture No. 6, was analyzed and the results are shown in Table 4 as compared with those reported by the FAO (1972).

Carbohydrates, ether extract, ash and minerals

The chromatographic identification of carbohydrates indicated that *V. volvaceae* had relatively higher contents of glucose and galactose than of ribose, arabinose and rhamnose.

Concentrations of reducing and non-reducing sugars are comparable to those reported in wild mushroom collected at Alexandria, Egypt

TABLE 4
The Chemical Composition of Cultivated Mushroom (*Volvariella volvaceae*) as Compared with that Reported by the FAO (1972)

Constituent	*Per cent*[a]	
	Present work	*FAO (1972)*
Moisture	92·1	90·1
Total nitrogen	4·92	4·84
Crude protein	30·8[b]	21·2[c]
Non-protein nitrogen	1·64	
True protein	20·5	
Ether extract	2·74	10·1
Crude fibre	17·4	11·1
Reducing sugars	6·07	
Non-reducing sugars	4·23	
Starch	19·2	
Glycogen	5·29	
Mannitol	9·86	
Vitamin C (mg/100 g fresh)	2·08	1·99
Ash	12·6	10·1
Sodium	1·16	0·374
Potassium	6·73	3·46
Calcium	0·37	0·071
Phosphorus	0·94	0·677
Iron (mg/100 g)	37·5	17·1
Copper (mg/100 g)	0·63	
Manganese (mg/100 g)	0·14	

[a] All the data presented on a dry weight basis except the moisture content.
[b] N × 6·25.
[c] N × 4·38.

(Zakhary *et al.*, 1983) (*Agaricus rodmani* and *Collybia* spp.), but higher than that in cultivated *Agaricus campestris* (McConnel & Esselen, 1947). However, the crude fibre and crude fat contents of *V. volvaceae* obtained in this study are in agreement with those obtained by McConnel & Esselen (1947) and Bano *et al.* (1971). On the other hand, the results reported by the FAO (1972) show a higher value for the ether extract and a lower value for crude fibre than the present work.

V. volvaceae had a low content of vitamin C (2·08 mg per 100 g fresh mushroom). The ash content of *V. volvaceae* was higher than that found in wild Egyptian mushroom (*Agaricus* sp. and *Collybia* sp.) (Zakhary *et al.*, 1983) and cultivated *A. campestris* (McConnel & Esselen, 1947). On

TABLE 5
The Amino Acid Contents of Cultivated *Volvariella volvaceae* as Compared with the Essential Amino Acids of Wild Mushrooms and Egg

Amino acid	*Concentration of amino acids as g/100 g protein*			
	V. Volvaceae	Agaricus[a] *sp.*	Collybia[a] *sp.*	*Egg*[b]
Iso-leucine +				8·0
Leucine	7·55	8·24	7·27	9·2
Lysine	5·20	4·32	5·66	7·2
Methionine	0·00	2·89	2·02	4·1
Cystine	0·95			
Phenylalanine	6·22	3·58	4·04	6·3
Tyrosine	4·79			
Threonine	4·88	4·59	4·44	4·9
Tryptophan	14·7	11·5	11·4	1·5
Valine	3·77	4·42	4·04	7·3
Arginine	0·00	4·42	5·06	6·4
Histidine	0·00	1·54	2·02	2·1
Alanine	7·14			
Aspartic acid	12·4			
Glutamic acid	27·9			
Proline	6·60			
Serine	0·00			

[a] Open cap stage of maturity as found by Zakhary *et al.* (1983).
[b] Bano *et al.* (1962).

the other hand, the results reported by the FAO (1972) for *V. volvaceae* gave a lower value for ash content.

The cultivated *V. volvaceae* had relatively high contents of potassium, sodium, phosphorus, copper and iron but no appreciable quantities of calcium as compared with wild Egyptian mushroom, i.e. *Agaricus* sp. and *Collybia* sp. (Zakhary *et al.*, 1983).

Protein and amino acids

The cultivated mushroom had a total nitrogen content of 4·92 % of which 33·3 % was of non-protein origin. Cultivated *V. volvaceae* had a lower amount of crude protein than wild Egyptian *Agricus rodmani* but the same amount of crude protein as *Collybia* sp. (Zakhary *et al.*, 1983).

However, these results were similar to those obtained by Bano *et al.* (1971).

The results of quantitative determination of the various amino acids in acid and alkaline hydrolyzates of *V. volvaceae* are given in Table 5. The data show the presence of the following fourteen amino acids: cystine, lysine, aspartic acid, glycine, glutamic acid, threonine, alanine, tyrosine, proline, valine, phenylalanine, leucine, *iso*-leucine and tryptophan.

V. volvaceae only contained seven essential amino acids since arginine, histidine and methionine were absent. Consequently, the quality of the protein of cultivated mushroom (*V. volvaceae*) was poor when compared either with egg (Bano *et al.*, 1962), or wild Egyptian mushroom, i.e. *Agaricus sp.* and *Collybia sp.* (Zakhary *et al.*, 1983). A noteworthy observation is that the cultivated mushroom, *V. volvaceae*, had higher amounts of tryptophan than any other known plant source. This finding makes it possible to use the cultivated mushroom as a supplement for legumes to overcome their deficiency in tryptophan.

Our amino acid results underline the importance of medium composition in regard to the nutritional quality of mushroom and previous workers have noted similar effects.

Maggioni *et al.* (1968), for example, has found that mushroom (*A. bisporous*) cultivated on composts supplemented with urea plus ammonium sulphate exhibited a lower total amino acid content with limited production of proline and arginine but an increased production of methionine, aspartic acid, valine and alanine when compared with mushroom grown on composts supplemented with ammonium sulphate alone.

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