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Growth requirements of *Volvariella speciosa* (Fr. ex. Fr.) Sing., a Nigerian mushroom

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Growth requirements of *Volvariella speciosa* (Fr. ex. Fr.) Sing. were studied. All the tested carbohydrates except cellulose significantly enhanced mycelial growth. Mannitol was the most utilized, followed in order by fructose and maltose. All the organic and inorganic nitrogen sources investigated significantly improved growth, with tryptophan being the best. C/N ratios also affected growth and a ratio of 1:4 was the most stimulatory. Calcium, magnesium, sodium, potassium and zinc significantly enhanced growth whereas hormones and vitamins did not. The implication of these results is discussed in relation to cultivation of *V. speciosa* in Nigeria.

INTRODUCTION

Volvariella is cosmopolitan in distribution and its species have been reported from tropical, subtropical and temperate regions of both eastern and western hemispheres (Shaffer, 1957). Although more than 100 species, subspecies and varieties of the genus *Volvariella* have been described throughout the world, the most popular species is *V. volvacea* which is the third most-widely cultivated edible commercial mushroom in the world (Shaffer, 1957).

In Nigeria, *V. esculenta* and *V. speciosa* are found during the rainy season. *Volvariella speciosa* grows commonly on richly manured soil, lawns, gardens, fields and woods (Zoberi, 1972). Alofe (1985) reported that *V. speciosa* contains 26.8% protein, 4.89 mg g^{-1} potassium and 0.16 mg g^{-1} phosphorus. Edible mushrooms are eaten in Nigeria as alternatives to meat and also for medicinal purposes. They are collected from the wild and sold in local markets or along roadsides as mushroom farms are rare.

A lot of information exists on the cultivation of *V. volvacea* but little is known about *V. speciosa*. It was therefore the objective of this study to investigate the growth requirements of this fungus. This will provide additional information for cultivating this edible fungus in Nigeria.

MATERIALS AND METHODS

A pure mycelial culture of *V. speciosa* was obtained by tissue culture of the sporophore, and maintained regularly on potato dextrose agar (PDA). The growth of the fungus was determined by the mycelial dry weight method. The ingredients of the basal medium in addition to supplementary compounds and streptomycin sulphate (0.01 g) were dissolved in 1 litre of distilled water. Thirty millilitres of this medium were dispensed into a 350 ml bottle and adjusted to pH 5.5. The mouth of each bottle was scaled with aluminium foil and autoclaved at 1.02 kg cm^{-2} pressure at 121°C for 15 min. Each medium was inoculated with a 6 mm (diameter) mycelial disc and incubated at 30 ± 2°C for 7 days. The mycelia were then harvested, oven-dried at 85°C for 10 h and weighed.

Carbon nutrition

The basal medium comprising peptone (2.0 g), KH_2PO_4 (0.5 g), $MgSO_4 \cdot 7H_2O$ (0.5 g) and distilled water (1 litre) was supplemented separately with carbohydrates (Chandra & Purkayastha, 1977). The concentration of carbon in each carbohydrate was equivalent to that present in 1% glucose. For starch, dextrin and cellulose, 10 g $litre^{-1}$ was used. The basal medium served as the control. Each treatment was thrice replicated.

Nitrogen nutrition

The basal medium used consisted of KH_2PO_4 (0.5 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), thiamine hydrochloride (500 μg), fructose (10 g) and distilled water (1 litre) (Chandra & Purkayastha, 1977). The amount of nitrogen in each supplementary compound was equivalent to that in 2 g $NANO_3$. For peptone, urea, yeast extract and casein, supplementation was at the rate of 2 g $litre^{-1}$.

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Carbon:nitrogen ratio

A mixture of 0.15 g $litre^{-1}$ of mannitol and tryptophan (the best carbon and nitrogen compounds) served as a C:N ratio of 1:1; other ratios were prepared proportionately. The basal medium was similar to that used for investigating nitrogen nutrition except that fructose was omitted.

Macroelements

The basal medium utilized comprised mannitol (10 g), tryptophan (1.3 g), $NaNO_3$ (2 g), KH_2PO_4 (2 g), $MgSO_4{\cdot}7H_2O$ (0.2 g), $CaCl_2$ (0.3 g), thiamine hydrochloride (500 μg) and distilled water (1 litre) (Chandra & Purkayastha, 1977). To investigate the effects of macroelements on growth, Na, K, Mg and Ca were replaced by ammonium radicals in their respective compounds. Two sets of control were employed one containing Na, K, Mg and Ca and the other none.

Trace elements

The trace elements studied were Cu, Fe, Mn and Zn (in their sulphate forms). The basal medium utilized was that used for investigating the effects of macroelements on growth. The trace element to be tested was omitted from the medium. Complete medium (basal medium plus four trace elements) and basal medium were used as controls.

Vitamins

Eight vitamins were investigated and the concentration of each in the medium was 0.5 μg ml^{-1}. Complete medium (basal medium plus eight vitamins) and basal medium were used as controls. The basal medium used for investigating macroelements was employed.

Phytohormones

The basal medium used in this investigation was that employed for investigating macroelements.

Analysis of data

Data were analysed by ANOVA and Duncan's multiple range test.

RESULTS AND DISCUSSION

All the tested carbon compounds except cellulose significantly enhanced the mycelial growth of *V. speciosa* ($P < 0.01$) and mannitol was the most utilizable. Chandra & Purkayastha (1977) and Guha & Banerjee (1971) reported mannitol as the most suitable sugar for growth. The preference of mannitol may be related to the formation of fructose through oxidation and its subsequent incorporation in the respiratory pathway after phosphorylation (Cochrane, 1958). This view is supported by our finding that fructose was the second most utilized sugar (Table 1). Fructose has been widely reported to be suitable for the growth of many mushrooms (Voltz & Beneke, 1969; Voltz, 1972; Oso, 1977).

Table 1. Effect of carbon sources on the mycelial growth of *V. speciosa*

Carbon source	Mycelial dry weight (mg per 30 cm^3)	Final pH of medium
Control	70^{e}	7.7
Monosaccharides		
Glucose	160^{bc}	5.9
Frutcose	187^{a}	5.1
Galactose	153^{c}	6.5
Sorbose	127^{d}	5.7
Mannose	150^{c}	6.5
Oligosaccharides		
Sucrose	157^{bc}	5.9
Maltose	180^{ab}	5.6
Lactose	127^{d}	6.6
Sugar alcohols		
Mannitol	197^{a}	4.6
Inositol	140^{cd}	6.0
Complex carbohydrates		
Dextrin	143^{cd}	5.6
Starch	160^{bc}	5.8
Cellulose	73^{e}	7.5

Means followed by the same letter within each vertical column (in Tables 1–6) are not significantly different ($P > 0.01$) by Duncan's multiple-range test.

The nitrogen compounds significantly enhanced growth ($P < 0.01$) and tryptophan was the best, whereas methionine was the least-utilized nitrogen source (Table 2). Thimann (1935) has shown that *Rhizopus suinus* can convert tryptophan into 1AA and Audus (1959) reported that tryptophan is the most favoured precursor for 1AA biosynthesis. The stimulatory effect of tryptophan on growth may therefore be due to production of 1AA, a growth promoter. Casein supported the second best growth, this was followed in order by leucine, NH_4NO_3 and glutamic acid (Table 2). Casein hydrolysate supported the greatest mycelial growth of *V. volvacea* (Chandra & Purkayastha, 1977). The preference of casein may be due to its complex nature (Cochrane, 1958; Nolan, 1970). Glutamic acid and leucine have been reported as good nitrogen for *Pleurotus ostreatus*, *Agaricus campestris* and *V. volvacea* (Yusef & Allam, 1967; Chandra & Purkayastha, 1977). Ammonium nitrate was the most utilizable among the inorganic nitrogen sources tested. Garcha *et al.* (1979) obtained a substantial growth of *V. volvacea* in NH_4NO_3, whereas the other inorganic nitrates tested inhibited growth. It is obvious from our study that *V. speciosa* is able to utilize inorganic nitrogen, especially NH_4NO_3), $Ca(NO_3)_2$ and KNO_3 (Table 2). The ability to utilize nitrates suggests the presence of nitrate reductase in the mycelia of *V. speciosa* (Garraway & Evans, 1984).

Carbon:nitrogen ratios significantly affected the growth of *V. speciosa* and growth at 1:4 was the best (Table 3). This was followed in order by 1:5, 3:1 and 5:1. The growths obtained at 1:5, 3:1 and 5:1 were not different statistically ($P > 0.01$). This result is similar to that obtained by Chandra & Purkayastha (1977) who found 1:3 as the most suitable C:N ratio for *V. volvacea*. However, most workers recommend high C:N ratios for the cultivation of mushrooms (Cochrane, 1958; Chang-Ho & Yee, 1977). For mushrooms that prefer low C:N ratios, growers can look for cheap nitrogen sources.

Of the eight vitamins tested, only pyridoxine increased growth whereas pantothenic acid supported the same amount of growth as the vitamin-free medium. Thiamine, riboflavin, ascorbic acid, biotin, folic acid, nicotinic acid and complete medium (containing eight vitamins) supported slightly less growth than vitamin free medium (Table 4). These results suggest that the supply of vitamins is not an absolute requirement for the growth of *V. speciosa*. Lilly & Barnett (1957) observed that some fungi synthesize their vitamins. Perhaps *V. speciosa* is in this category.

The basal medium, supplemented with Ca, Mg, K and Na, significantly stimulated mycelial growth, suggesting that the macroelements are essential for *V. speciosa* growth. This result is similar to those reported by Treschow (1944), Humfeld & Sugihara (1952) and Chandra & Purkayastha (1977). Calcium-free medium induced the least growth among the macroelements whereas K-free medium stimulated the greatest growth (Table 5). This implies that Ca was the most utilized and K the least utilized. Fasidi & Olorunmaiye (1994) reported that Ca supported the highest mycelial growth of *Pleurotus tuber-regium* among the macroelements tested. Fasidi & Kadiri (1993) had earlier reported that Ca is required to strengthen the stipe of *V. esculenta*.

Basal medium containing Cu, Fe, Mn and Zn (complete medium) inhibited growth (Table 5) showing that the four microelements are not required for mycelial growth. However, Cu-free medium significantly improved growth, showing that Fe, Mn and Zn are stimulatory while Cu is inhibitory to growth. Chandra & Purkayastha (1977) and Humfeld & Sugihara (1952) reported that Cu inhibited the growth of *A. campestris*, *V. volvaces* and *Lentinus subnudus*. Zinc-free medium produced the least growth (Table 5),

Table 2. Effect of organic and inorganic nitrogen compounds on the mycelial growth of *V. speciosa*

Nitrogen source	Mean mycelial dry weight (mg per 30 cm^3)	Final pH of medium
Control	37^{e}	4.0
Inorganic		
$NaNO_3$	80^{cd}	8.4
KNO_3	97^{cd}	8.3
NH_4NO_3	113^{bc}	6.7
$Ca(NO_3)_2$	107^{cd}	8.0
$(NH_4)_2SO_4$	73^{d}	2.4
Organic: amino acids		
Glycline	93^{cd}	7.8
DL-Leucine	123^{bc}	4.0
L-Glutamic acid	113^{bc}	8.5
L-Aspartic acid	100^{cd}	8.3
L-Asparagine	77^{cd}	7.3
L-Tryptophan	177^{a}	7.9
DL-Phenylalanine	100^{cd}	3.6
Nethionine	70^{de}	3.0
Citrulline	103^{cd}	7.4
Complex		
Peptone	80^{cd}	5.0
Urea	73^{de}	8.6
Yeast extract	90^{cd}	6.5
Casein	130^{b}	5.5

Table 3. Effect of C:N ratios on mycelial growth of *V. speciosa*

C:N ratio	Mean mycelial dry weight (mg per 30 cm^3)	Final pH of medium
0:0 (basal medium)	3^{d}	6.0
1:1	30^{c}	7.9
2:1	23^{c}	7.2
3:1	60^{b}	7.2
4:1	47^{bc}	7.3
5:1	53^{b}	6.8
1:2	40^{bc}	8.2
1:3	43^{bc}	8.2
1:4	80^{a}	8.4
1:5	77^{ab}	8.5

Table 4. Effect of vitamins on mycelial growth of *V. speciosa*

Vitamin	Mean mycelial dry weight (mg per 30 cm^3)	Final pH of medium
Complete medium	50^{a}	6.0
Thiamine hydrochloride	50^{a}	5.2
Riboflavin	30^{a}	6.2
Pyridoxine	60^{a}	6.1
Ascorbic acid	47^{a}	6.3
Biotin	43^{a}	6.1
Folic acid	47^{a}	6.0
Nicotinic acid	33^{a}	6.4
Pantothenic acid	53^{a}	6.3
Pasal medium	53^{a}	6.0

Table 5. Mineral nutrition of *V. speciosa*

Macroelement	Mycelial dry weight (mg per 30 cm^3)	Final pH of medium
Macroelement		
Basal medium	43^{b}	6.3
— Mg	63^{a}	6.1
— Na	63^{a}	6.2
— K	67^{a}	6.0
— Ca	60^{a}	6.5
Complete medium	67a	5.9
Micronutrient		
Basal medium	103^{b}	5.7
— Cu	130^{a}	5.5
— Fe	97^{b}	6.0
— Mn	97^{b}	5.7
— Zn	87^{b}	6.1
Complete medium	100^{b}	5.7

Table 6. Effect of phytohormones on the mycelial growth of *V. speciosa*

Phytohormone concentration (ppm)	Mycelial dry weight (mg per 30 cm^3)		
	2,4-D	NAA	GA_3
0.1	91[a]	103[a]	88[b]
1	104[a]	88[a]	138[a]
10	82[a]	68[b]	80[b]
Basal medium	83[a]	83[a]	83[a]

showing that zinc is the most essential of the trace elements. Pratt (1944), and Fasidi and Olorunmaiye (1994) showed that zinc improved mycelial growth. Tsui (1948) and Nason (1950) reported that zinc was implicated in 1AA synthesis. The stimulatory effect of zinc on growth may be due to 1AA synthesis.

Supplementation of the basal medium with GA_3, NAA and 2,4-D (0.1–10.0 ppm) did not improve growth significantly (Table 6). This result is in contrast to that reported by Kurancowa (1963), Voltz (1972) and Fasidi and Olorunmaiye (1994). Hayes (1981) obtained increased mycelial growth and basidiocarp production in *A. campestris* by application of 10^{-5}–10^{-4} M 1AA and GA_3.

From our study, different carbohydrates, nitrogen sources and C:N ratios significantly enhanced the mycelial growth of *V. speciosa*. By preparing spawn from mycelia grown on medium enriched with mannitol and casein, high mycelial yield and vigour will be obtained. Similarly, to produce good yield, *V. speciosa* can be cultivated on compost having a C:N ratio of 1:4 and adequate amounts of Ca, Mg, Na, K and Zn.

REFERENCES

Alofe, F. V. (1985). The general characteristics and cultivation of some Nigerian mushrooms. Ph.D. thesis, Obafemi Awolowo University, Ile-Ife, Nigeria.

Audus, L. J. (1959). *Plant Growth Substances*, 2nd ed. Oxford and IBH Pub. Co., New Delhi, pp. 128.

Chandra, A. & Purkayastha, R. P. (1977). Physiological studies on Indian mushrooms. *Trans. Br. Mycol. Soc.*, **69**(1), 63–70.

Chang-Ho, Y. & Yee, N. T. (1977). Comparative study of the physiology of *Volvariella volvacea* and *Coprinus cinereus. Trans. Br. Mycol. Soc.*, **68**(2), 167–72.

Cochrane, V. W. (1958). *Physiology of Fungi.* John Wiley and Sons, New York.

Fasidi, I. O. & Kadiri, M. (1993). Effect of sporophore maturity on chemical composition of *Volvariella esculenta* (Mass) Singer, a Nigerian mushroom. *Nahrung*, **37**(3), 269–73.

Fasidi, I. O. & Olorunmaiye, K. S. (1994). Studies on the requirements for vegetative growth of *Pleurotus tuber-regium* (Fr.) Singer, a Nigerian mushroom. *Food Chem.*, **50**, 397–401.

Garcha, H. S. Kalra, K. L. & Beg, G. M. (1979). Physiology of weed mushrooms. *Mushroom Sci.*, **10**(2), 645–52.

Garraway, O. M. & Evans, C. R. (1984). *Fungal Nutrition and Physiology.* John Wiley and Sons, New York, pp. 71–163.

Guha, A. K. & Banerjee, A. B. (1971). Effect of different carbon compounds on the submerged production of *Agaricus campestris. J. Food Sci. Technol.*, **8**, 82–3.

Hayes, W. A. (1981). Interrelated studies of physical, chemical, and biological factors in casing soils and relationships with productivity in commercial culture of *A. bisporus* Lange. *Mushroom Sci.*, **XI**, 103–29.

Humfeld, H. & Sugihara, T. F. (1952). The nutrient requirements of *Agaricus campestria* grown in submerged culture. *Mycologia*, **44**, 605–20.

Kurancowa, Z. (1963). Germination of spores of some species of the Agaricales and its dependence on the duration of storage of the spores, the season of the year, and the action of 2, 4-D and thiamine. *Am. Univ. Mariel Curie-Sklodowaka Sect. C. Biol.*, **17**, 433–51.

Lilly, V. G. & Barnett, H. L. (1957). *Physiology of Fungi.* McGraw-Hill, New York.

Nason, A. (1950). Effect of zinc deficiency on the synthesis of tryptophan by *Neurospora* extracts. *Science*, **112**, 111.

Nolan, R. A. (1970). The phycomycete *Catenaria anguillulae*: growth requirements. *J. Gen. Microbiol.*, **60**, 167–80.

Oso, B. A. (1977). *Pleurotus tuber-regium* from Nigeria. *Mycologia*, **69**(2), 271–9.

Pratt, H. K. (1944). Ph.D. thesis, University of California.

Shaffer, R. L. (1957). *Volvariella* in North America. *Mycologia*, **49**, 545–79.

Thimann, K. V. (1935). On the plant growth hormone produced by Rhizopus suinus. *J. Biol. Chem.*, **109**, 279–91.

Treschow, C. (1944). Nutrition of the cultivated mushroom. *Dan. Bot. Ark.*, **61**, 1.

Tsui, C. (1948). The role of zinc in auxin synthesis in the tomato plant. *Am. J. Bot.*, **35**, 172–9.

Voltz, P. A. (1972). Nutritional studies on species and mutants Lepista, Cantharellus, Pleurotus and Volvariella. *Mycopath. Mycol. Appl.*, **48**, 175–85.

Voltz, P. A. & Beneke, E. S. (1969). Nutritional regulation of basidiocarp formation and mycelial growth of Agaricales. *Mycopath. Mycol. Appl.*, **37**, 225–53.

Yusef, H. M. & Allam, M. E. (1967). The carbon and nitrogen nutrition of certain fungi. *Can. J. Microbiol.*, **13**, 1097–107.

Zoberi, M. H. (1972). *Tropical Macrofungi.* Macmillan Press, London, p. 158.