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Physico—Chemical studies on *Volvariella esculenta* (Mass) Singer, a Nigerian edible fungus

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Abstract

Studies were conducted on the effects of temperature, pH, vitamins and plant hormones on the vegetative growth of *Volvariella esculenta* (Mass) Singer, a Nigerian edible higher fungus. This mushroom had its optimum radial growth at 35 °C with mycelial extension of 85.0 mm. The pH that supported best growth was 6.0. Pyridoxine was the most utilizable vitamin with mycelial dry weight of 123 mg/30 cm^3. This was followed in order by thiamine and folic acid ($P = 0.01$) while the least growth (33.0 mg/30 cm^3) was sustained by pantothenic acid. Among the tested phytohormones, 2, 4-D (10.0 ppm) stimulated the best growth of 150 mg/30 cm^3 but, 0.1 ppm of GA_3 supported poor growth (53.0 mg/30 cm^3). The significance of these observations is discussed.

Keywords: Physicochemical; *Volvariella esculenta*; Vitamins; Phytohormones; Radial growth

1. Introduction

Volvariella esculenta (Mass) Singer, is a common tropical edible mushroom which belongs to the phylum basidiomycota, order agaricales family pluteaceae (Alexopolous, Mim, & Blackwell, 1996; Zoberi, 1972). It usually grows abundantly during the raining season and frequently appears in hollow trunks of rotten palm trees. It is known locally as 'Ogiri agbe' (farmers seasoning) because of its meaty taste, desired flavour and medicinal value (Fasidi & Jonathan, 1994; Jonathan, 2002).

Volvariella esculenta is an appetising delicacy among the Yoruba people of SouthWestern Nigeria. This fungus has been reported to be an excellent source of protein, glycogen, lipids and mineral elements (Fasidi & Kadiri, 1993). Fasidi and Jonathan (1994) investigated the effects of various carbon, nitrogen and mineral elements on myceial growth of *V. esculenta*. They reported that the growth of this mushroom was enhanced by glucose, peptone, potassium and zinc as carbon, nitrogen and mineral sources, respectively.

More research work needs to be carried out on this promising fungus to make its cultivation possible on a large scale. Therefore, this present study focuses on temperature, pH, vitamin and phytohormone requirements of *V. esculenta*. This will act as additional information that could be employed to improve the cultivation technology of this mushroom in Nigeria.

2. Materials and methods

2.1. Sample collection and preparation

The young fruit bodies of *V. esculenta* were collected from an oil palm wastes dump at Apoje Ogun State, Nigeria. The mycelia of this fungus were obtained by tissue culture (Jonathan & Fasidi, 2001) and maintained on potato dextrose agar (PDA) supplemented with 0.5% peptone.

2.2. Temperature and pH

The temperature requirement of this mushroom was investigated on potato dextrose agar (oxoid) plates. This medium was autoclaved at 1.02 kg cm^{-2} pressure at

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121 °C for 15 min. It was poured onto 10 cm Petri dishes sterilised by dry heat in an oven at 160 °C for 6 h. Streptomycin sulphate (0.05 g) was added to the medium after it had cooled to about 40 °C to inhibit bacterial contamination. On solidification, the dishes were inoculated with a 7.0 mm diameter disc of 5-day-old *V. esculenta* and incubated at 0, 10, 15, 20, 25, 30, 35, 40, 45 and 50 °C. The radial colony diameter was measured after every 2 days for 2 weeks. Each treatment was replicated thrice. The pH requirement of this fungus was determined by the mycelial dry weight method, using a liquid medium (Jonathan, 2002). The pH of the basal medium was adjusted (4.0–9.0) and it was dispensed in 30 cm^3 lots into 250 cm^3 milk bottles. The bottles were autoclaved at 1.02 kg cm^{-2} and a temperature of 121 °C for 15 min. After cooling, each bottle was inoculated with an actively-growing mycelial disc (7.0 mm) of a 5-day-old culture of *V. esculenta* and incubated at 30±2 °C for 7 days. Mycelia were harvested, dried and weighed (Fasidi & Jonathan, 1994).

2.3. Vitamins

The compositions of the basal medium used were fructose (10.0 g), peptone (1.0 g), $MgSO_4.7H_2O$ (0.5 g), KH_2PO_4 (0.5 g) and 1000 cm^3 of de-ionised water. The vitamins used were: ascorbic acid, biotin, folic acid, cobalamine, nicotinic acid, pantothenic add, pyridoxine, riboflavin and thiamine. These vitamins were supplemented in the basal medium at a concentration of 500 μg/1000 cm^3. Care was taken to handle riboflavin in dim light because strong light destroys this compound (Madunagu, 1988). A triplicate set of 250 cm^3 milk bottles containing 30 cm^3 of the medium with pH adjusted to 6.0 was prepared for each vitamin. The basal medium that contained all the vitamin sources served as control 1 while the basal medium without any vitamin source served as control 2. The vitamins were sterilised by millipore filtration (Jonathan & Fasidi, 2001).

2.4. Phytohormones

The phytohormones used were gibberellic acid (GA_3), 2,4-dichlorophenoxy acetic acid (2,4-D) and indole acetic acid (IAA). The same basal medium as employed for investigating vitamin sources was employed. Each phytohormone, which was filter-sterilised, was added to the autoclaved basal medium to give concentrations of 10.0, 1.0 and 0.1 ppm. Inoculation, incubation and harvesting were also carried out as described (Jonathan and Fasidi, 2001).

2.5. Analysis of data

The results generated from these studies were subjected to analysis of variance and test of ANOVA significance was carried out by Duncan's multiple range tests (DMRT).

3. Results and discussion

Of all the temperatures tested, the best radial mycelial extension (85.0 mm) was observed at 35 °C on the 12th day (Table 1). Thus, the optimum temperature for the growth of *V. esculenta* was 35 °C. There was considerable growth at 30 °C followed by 25 and 20 °C but growth was minimal at 40 and 15 °C, respectively. This observation is similar to that obtained by Chandra and Purkayastha (1977) who observed 32 °C as the optimum temperature for the vegetative growth of *V. volvacea*. Likewise, Jonathan and Fasidi (2003) reported that *P. atroumbonata* grew fairly well within the temperature range 25–35 °C.

At extremely low temperatures (0 and 10 °C), there was no observable mycelial growth of *V. esculenta* (Table 1). This may be due to the fact that, at these temperatures, the metabolic activities of this fungus were reduced considerably to allow the absorption of essential nutrients needed for growth (Garraway & Evans, 1984). Similarly, there were no growths at 45 and 50 °C. This could be due to the denaturation of important enzymes which catalyse fungal metabolic processes. Jonathan (2002) also reported that the growth of *S. commune* was inhibited at 45 and 50 °C.

The result obtained when *V. esculenta* was grown under different pH conditions (Table 2) shows that this fungus could thrive in acidic, neutral and alkaline environments (pH 4.0–9.0). It was observed that the optimum vegetative growth (155 mg/30 cm^3) was obtained in acidic medium of 6.0. The growth of 110 mg/30 cm^3, which was the second best, was recorded in

Table 1
Effect of temperature on radial mycelial extension of *V. esculenta on* PDA[a]

Temperature (°C)	Days						
	Radial Mycelial Extension (mm)						
	2	4	6	8	10	12	14
0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0
15	0	4.0c	5.0c	6.0d	6.0d	7.0d	7.0d
20	5.0a	7.0bc	7.0c	9.0d	10.0d	12.0d	22.0d
25	5.0a	11.0ab	18.0b	24.0c	27.0c	35.0c	38.0c
30	7.0a	12.0a	24.0a	35.0b	40.0b	50.0b	50.0b
35	7.0a	15.0a	29.0a	48.0a	53.0a	85.0a	85.0a
40	3.0a	5.0c	8.0c	8.0d	12.0d	19.0d	19.0d
45	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0

[a] Each value represents the mean of three replicates. Means followed by the same letter(s) are not significantly different by Duncan's multiple range test ($P = 0.01$).

the slightly acidic medium of 6.5 while the least growth (10.0 mg/30 cm^3) was observed in the alkaline medium of 9.0 (Table 2). These observations agree with those of Madunagu (1988) and Jonathan (2002) who separately reported that *P. squarrosulus* and *L. procera* grew best at pH of 6.0 and 6.5, respectively.

Among the vitamins investigated, pyridoxine stimulated the best growth of 123 mg/30 cm^3. This was followed in order by thiamine and folic acid with growths of 107 and 97.0 mg/30 cm^3, respectively (Table 3). Pantothenic acid sustained the poorest growth (33.0 mg/30 cm^3) among the vitamin sources. Similar utilization of pyridoxine was reported by Jonathan and Fasidi (2001, for S. *commune*) and by Fasidi and Olorunmaiye (1994, for *P. tuber-regium*). Conversely, Madunagu (1988) reported very poor growth of *P. squarrosulus* with the inclusion of thiamine in the growing medium. The support of growth by pyridoxine could be attributed to its conversion to functional phosphate, which is important in the synthesis of tryptophan (an amino acid) needed for growth (Hilgenberg & Hofmann, 1977).

Table 2
Effect of pH on vegetative growth of *Volvariella esculenta*[a]

PH	Mycelial dry weight (mg/30 cm^3) (Mean of three replicates)	Final pH of culture filtrate
4.0	15.3c	4.9
4.5	25.7c	5.4
5.0	45.0c	5.9
5.5	90.7b	6.3
6.0	155a	6.6
6.5	110b	6.7
7.0	87.3b	6.7
7.5	47.3c	7.2
8.0	30.3c	7.7
8.5	27.7c	7.9
9.0	10.0c	8.3

[a] Values followed by the same letters are not significantly different by Duncan's multiple range test ($P = 0.01$).

Table 3
Effect of vitamins on vegetative growth of *Volvariella esculenta*[a]

Vitamins	Mycelial dry weight (mg/30 cm^3) (Mean of three replicates)	pH of culture filtrate
Ascorbic acid	53.0cd	7.0
Biotin	70.0bc	5.6
Folic acid	97.0ab	6.8
Cobalamine	50.0cd	6.2
Nicotinic acid	60.0cd	5.9
Pantothenic acid	33.0d	5.5
Pyridoxine	123a	6.9
Riboflavin	63.0cd	6.5
Thiamine	107ab	6.1
Control 1 (all Vitamins)	77.0bc	6.4
Control 2 (basal medium)	80.0bc	5.8

[a] Values followed by the same letters are not significantly different by Duncan's multiple range test ($P = 0.01$).

The growths in the two control media were observed to be greater than those obtained for ascorbic acid, biotin, cobalamine, nicotinic acid, panthothenic acid and riboflavin (Table 3). This indicates that *V. esculenta* can grow on synthetic media without the external supply of these vitamins. Possibly this fungus is capable of synthesizing the listed vitamins. Therefore, exogenous incorporation of these organic compounds into a growing medium may not be necessary.

Table 4 shows that, among the phytohromones, 10.0 and 1.0 ppm of 2,4-D promoted the best and second best vegetative growth of 150 and 110 mg/30 cm^3, respectively. This was followed in order by 10.0 ppm of GA_3 and 0.1 ppm of IAA while the least mycelial growth was sustained by 0.1 ppm of GA_3. The utilization of these hormones (2,4-D and GA_3) correlates with the observation of Voltz (1972) who studied the requirements of phytohormones in some agaricales. The effectiveness of 2,4-D is not a surprise because Kurancowa (1963) reported that this hormone was important for spore germination and mycelial growth of *P. ostreatus* and *M. rotula*.

It was also observed that the mycelial growth of this fungus increased with increased concentration of 2,4-D and GA_3 (Table 4). This implies that better growth of this fungus could be achieved at high concentrations of these hormones. This result is, however, contrary to that reported by Jonathan and Fasidi (2001) that the growth of S. *commune* and *L. subnudus* decreased significantly with increased concentration of phytohromones. Hayes (1981), however, observed that neither low nor high concentrations of 2,4-D, GA3 and NAA had effects on vegetative growth and sporocarp production of *A. bisporus*.

From these observations, it can be concluded that *V. esculenta* mycelia grew best at 30 °C and pH of 6.0. This

Table 4
Effect of phytohormones on vegetative growth of *Volvariella esculenta*[a]

Phytohormones (ppm)	Mycelial dry weight (mg/30 cm^3) (means of three replicates)	pH of culture filtrate
Gibberellic acid (GA_3)		
10.0	100.bc	6.5
1.0	70.0bc	5.5
0.1	53.0c	6.2
Indole acetic acid (IAA)		
10.0	60.0c	6.0
1.0	75.0bc	6.4
0.1	90.0bc	5.8
2,4-Dichlorophenoxy acetic acid (2,4-D)		
10.0	150a	6.8
1.0	110ab	7.0
0.1	70.0bc	6.6
Control (basal medium)	63.0c	5.3

[a] Means followed by the same letter(s) are not significantly different by Duncan's multiple range test ($P = 0.01$).

fungus also requires an exogenous supply of pyridoxine and thiamine as vitamin sources. Similarly, phytohormones (2,4-D and GA_3) are required at 10.0 ppm. These various compounds could be incorporated into the growing media to promote mycelial production of *V. esculenta*. This will in turn enhance spawn production and consequently boost cultivation of *V. esculenta* in Nigeria.

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