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Nutritional requirements of *Volvariella speciosa* (Fr. Ex. Fr.) Singer, a Nigerian edible mushroom

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Abstract

Nutritional requirement studies were carried out on synthetic and semi-synthetic media, as well as different agro-industrial wastes, to evaluate vegetative growth of *Volvariella speciosa* (Fr. Ex. Fr.) Singer, a Nigerian edible mushroom. The optimum temperature that supported the best growth of this fungus was 30 °C while the optimum pH was 6.0. The moisture contents were observed to vary with different substrates. The best vegetative growth was obtained at 40% moisture content, on sawdust, while it was 80% on *Andropogon gianus* straw. Among the different media used, the best mycelial extension (92.0 mm) was observed on semi-synthetic, potato dextrose agar while the least growth (74.0 mm) was recorded on laboratory formulated sorghum agar. Maize and *A. gianus* straw stimulated the best mycelial extension (92.0 mm) while fresh and fermented horse dung supported moderate growths of 70.0 and 67.0 mm, respectively. The least growth (36.0 mm) was observed on fresh cow dung. These findings are discussed in relation to the cultivation of *V. speciosa* in Nigeria.

Keywords: Nutritional requirements; *Volvariella speciosa*; Mycelial growth; Media; Agricultural substrates

1. Introduction

Volvariella speciosa (Fr. Ex. Fr.) Singer is an edible cosmopolitan mushroom that is found growing in many regions of the world, especially in the tropics (Zoberi, 1972). In Nigeria, this fungus is common during the rainy season and grows wildly on animal manure ground, lawns, gardens, fields and woods (Zoberi, 1972).

The pileus of *V. speciosa*, which may be white or pink ranges from 5 to 15 cm in diameter. The stipe, which lacks annulus, is firm, tough and may range between 4.5 and 14.0 cm in length. The spore print has a deep salmon colour and the spore size ranges from 9–18 cm to 6–10 cm along the major and minor axes, respectively (Jonathan, 2002).

Edible mushrooms are highly priced in Nigeria, because they are important sources of food and medicines (Fasidi & Kadiri, 1993; Jonathan & Fasidi, 2001a; Oso, 1977). Because commercial production of mushrooms is not common in Nigeria, people generally depend on mushrooms, which are collected from the wild. The sporophores of different Nigerian mushrooms are usually collected by mushroom hunters and sold in local markets or hawked along the major roads. *V. speciosa* has been reported to be a very good source of essential vitamins, amino acids, glycogen and mineral elements, such as potassium and phosphorus (Alofe, 1985).

Unfortunately, many Nigerian mushrooms (including *V. speciosa*) are gradually disappearing from our vegetation, which is increasingly being exploited for animal grazing, agriculture and urban development. A lot of information exist on the cultivation of *V. volvacea* and *V. esculenta* (Chang-Ho & Yee, 1977; Fasidi & Jonathan, 1994; Fasidi & Kadiri, 1993) but, very little is known about *V. speciosa*. Therefore, this study is aimed at providing

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useful information about the nutritional requirements of *V. speciosa*, which will enhance cultivation biotechnology of this edible fungus in Nigeria.

2. Materials and methods

2.1. The microorganism

The fruit bodies of *V. speciosa* were collected from the animal manure soil at the Teaching and Research farms, University of Ibadan, Nigeria. The mycelial culture was maintained on PDA (oxoid) plates (Jonathan & Fasidi, 2001a).

2.2. Effect of pH on mycelial growth of V. speciosa

The pH requirement of *V. speciosa* was determined using the mycelial dry weight method (Odebode & Che, 2001). The basal medium used was composed of dextrose (10.0 g), histidine monohydrochloride (0.1 g), methionine (0.02 g), biotin (0.4 mg), riboflavin (0.2 mg), $CaCl_2$ (0.4 mg), $FeCl_3$ (0.2 mg), $CuSO_4$ (0.4 mg), KI (0.10 mg), NaCl (0.1 g), KNO_3 (0.7 g), and KH_2PO_4 (0.1 g) dissolved in 1 l of de-mineralized water. The basal medium was homogenized in a water bath and dispensed in 30 ml lots into 250 ml conical flasks. The pH of the medium was adjusted (2.0–10.0) and autoclaved at 1.02 kg cm^{-2} pressure (121 °C) for 15 min. After cooling, the content of each triplicated conical flask was inoculated with 6.0 mm (diameter) mycelium of *V. speciosa* and incubated at 30 ± 2 °C for 7 days. Each treatment was triplicated. After the seventh day, the mycelial mat in each conical flask was filtered through a pre-weighed No. 1 Whatman filter paper in a Buckner funnel. These were dried in the oven at 80 °C for 24 h and weighed.

2.3. Effect of temperature on mycelial growth of V. speciosa

The effect of temperature on growth of this fungus was investigated on potato dextrose agar plates (Jonathan & Fasidi, 2004). The medium was autoclaved at 1.02 kg cm^{-2} (121 °C) for 15 min. Streptomycin sulphate (0.05 g) was aseptically added to the medium after it had cooled to 40 °C to prevent bacterial contamination. The agar medium was then dispensed into Petri-dishes, allowed to solidify, inoculated with a 6.0 mm (diameter) disc of vigorously growing (5-day old) culture of *V. speciosa* and incubated at 10, 15, 20, 25, 30, 35 and 40 °C. Each set was triplicated. Mycelial extension and density were then determined using the method described by Fasidi (1996).

2.4. Moisture requirements of V. speciosa

Agricultural wastes, such as sawdust of *Terminalia ivorensis,* straw of rice, maize and *Andropogon gianus*, were used. To prepare a substrate containing 30% water, 30 ml tap water was added to each of 100 g of the substrates and mixed thoroughly in different 250 ml plastic containers. In this way, substrates with 40%, 50%, 60%, 70%, 80%, 90% and 100% water contents were prepared. These were filled into 140×20 mm test tubes and covered with aluminium foil. Each treatment was triplicated. These tubes were autoclaved at 1.02 kg cm^{-2} pressure (121 °C) for 15 min. After cooling, each tube was inoculated with 0.7 cm diameter mycelia from a vigorously growing (6-day old) culture of *V. speciosa* and incubated at 30 ± 2 °C for 10 days. Growth was measured by increase in mycelial length down the boiling tube and mycelial densities were visually assessed (Fasidi, 1996).

2.5. Effect of different growth media on mycelial growth of V. speciosa

Food grains, such as sorghum, wheat, rice, beans, soybeans and corn (white and yellow), were milled using an electric grinding machine. Thirty grammes of each milled grain were weighed and suspended in 250 ml of distilled water and boiled. The suspension was strained through muslin cloth and the filtrate was made up to 1000 ml. Twenty grammes of agar-agar (oxoid) were added to each medium and homogenized in the water bath. The media were autoclaved at 1.02 kg cm^{-2} pressure (121 °C) for 15 min. Potato dextrose agar was also prepared and sterilized (Jonathan, 2002). Each medium was poured into 10 cm Petri dishes and 0.05 g of streptomycin sulphate was added, after sterilization, to inhibit bacteria contamination. On cooling, the plates were inoculated with a 0.7 cm diameter mycelial disc of actively growing mycelia (6-day old) culture of *V. speciosa* and incubated at 30 ± 2 °C. Radial colony diameters were recorded after the 10th day.

2.6. Effects of different growth substrates on vegetative growth of V. speciosa

Sawdust and coconut fibres were separately soaked in hot water for 1 h. These were squeezed between fingers in a muslin cloth to drain excess water and dispensed into 10.0 cm diameter Petri dishes. Agricultural wastes, such as cassava peels, rice straw, *Andropogon gianus* straw, were prepared according to the method of Fasidi and Ekuere (1993). Other substrates, such as fresh and fermented cow dung, poultry wastes and horse dung were soaked with 10% water before dispensing each into 10.0 cm diameter Petri dishes.

These plates were sterilized at 1.02 kg cm^{-2} pressure (121 °C) in the autoclave for 15 min. After cooling, the plates were inoculated with a 0.7 cm, mycelial disc of actively growing (5-day old) fungal isolate. Each treatment was triplicated. Incubation was carried out at 30 ± 2 °C for 10 days, after which the diameter of mycelial extension was observed and measured.

2.7. Analysis of data

The results obtained were subjected to analysis of variance (ANOVA), while the test of significance was carried out by Duncan's multiple range test.

3. Results and discussion

The mycelial growth of *V. speciosa* was obtained in the pH range 3.0–9.0 (Table 1). It was observed that this fungus had its minimum, optimum and maximum growths at the pH of 3.0, 6.0 and 9.0, respectively. Generally, vegetative growth of this mushroom was reduced at very strong acidic and alkaline pH. The least growth (36.7 mg/ml) was observed at pH 3.0 while there were no observable growths at pH 2.0 and 10.0. This result is similar to those obtained by Fasidi (1996) (for *V. esculenta*) and Jonathan (2002) (for *L. procera*). Hopkins (1995) reported that, at very strong acidic or alkaline pH, cell wall and tonoplast of plants may corrode and selective permeability function of the cell membrane may be impaired. This may be the reason why there were no growths at pH 2.0 and 10.0.

The best mycelial growth (160.0 mg/ml) was observed in the slightly acidic pH of 6.0 while good growths (123.3 and 110.0 mg/ml) were also obtained at pH 5.0 and 7.0 (Table 1). This result implies that *V. speciosa* prefers acidic pH values, tending toward neutrality. Likewise, Chandra and Purkayastha (1977) and Jonathan and Fasidi (2004) obtained very good growths of *Agaricus campestris* and *V. esculenta* at pH 6.0. The ability of *V. speciosa* to grow over a wide range of pH could explain why it grows on various agricultural wastes.

Table 2 shows the effect of temperature on mycelial growth of *V. speciosa*. The minimum, optimum and maximum cardinal temperatures of growth were 20, 30 and 35 °C, respectively. The best mycelial growth (90.0 mg/ml), obtained at 30 °C, was as a result of increased enzyme activity attained at this temperature for *V. speciosa* (Griffin, 1994). Garraway and Evans (1984) suggested that, at optimum temperature of growth of each fungus, enzyme and metabolic activities will increase, energy will be released and good mycelial growth will be enhanced. This result is different from that of Chang and Chu (1969) who reported 35 °C as the optimum temperature for mycelial growth of *V. volvacea*.

Table 1
Effect of pH on mycelial growth of *V. speciosa*

pH of basal medium	Mycelial dry weight (mg/30 ml)
2.0	–
3.0	36.7e
4.0	56.7d
5.0	123.3b
6.0	160.0a
7.0	110.0bc
8.0	103.3c
9.0	93.3c
10.0	–

Data were means of triplicates. Means followed by the same letter(s) are not significantly different by Duncan's multiple range test ($P \leqslant 0.05$).

Table 2
Effects of temperature on mycelial growth of *V. speciosa*

Temperature (°C)	Mycelial extension (mm)	Mycelial density
10	–	–
15	–	–
20	37.0d	2+
25	55.0c	3+
30	90.0a	6+
35	75.0b	4+
40	–	

Data were means of triplicates. Means followed by the same letter are not significantly different by Duncan's multiple range test ($P \leqslant 0.05$).

The water (moisture) requirement of *V. speciosa* varied with different substrates used. The best mycelial growth was observed at 40% moisture content on sawdust while it was 80% on *Andropogon gianus* straw (Table 3). For the maize straw, the most stimulatory water content was 50% but, for rice straw, the optimum growth was obtained at 70% moisture content. The variation observed for different agricultural wastes suggests that a specific amount of water is needed to wet these agricultural substrates for optimal production of *V. speciosa* mycelia. This result is similar to the earlier observation of Alofe (1985) who reported that *L. subnudus* and *P. tuber-regium* grew on agricultural wastes at different moisture contents. Jonathan (2002) also suggested that mushroom mycelia would grow very well, provided that there are good substrates with required nutrients and optimal physical factors (temperature, pH and relative humidity). Although the water content for different substrates varies, it is essential that substrates are freely permeable to air and are able to hold water without being water-logged.

All the media used supported mycelial growth of *V. speciosa* (Table 4). Although potato dextrose agar stimulated the best mycelial growth of this fungus, there were no statistical differences between the values obtained for PDA and Ife brown beans, wheat, white corn and yellow corn agar ($P \leqslant 0.05$). The enhancement of growth by PDA is not a surprise, because this medium has been widely reported to support growth of mushroom mycelia (Alofe, 1985; Fasidi, 1996; Huang, 1993; Oso, 1977). Jonathan and Fasidi (2001a, 2001b) also obtained better mycelial yields of *P. atroumbonata L. subnudus* and *S. commune* when PDA was supplemented with 0.5% yeast extract.

Among the different agricultural substrates used, growth was enhanced mostly on maize and *Andropogon gianus* straw but greatest mycelial density was obtained with cassava peels (Table 5). This result is similar to that of Fasidi and Kadiri (1993) who obtained highest mycelial extension of *L. subnudus* with *A. tectorum* . The growth observed for this fungus on maize straw, on various substrates used, supports the suggestion of Joshua and Agiria (2002) that *Pleurotus* and *Volvariella* sp. could grow on various farm

Table 3
Moisture requirements of *V. speciosa* for ME (mm)

Moisture in %	Sawdust of *Terminalia ivorensis*		Straw of *Andropogon gianus*		Maize (Zea mays) straws		Rice (*Oryza sativa*)	
	ME	MD	ME	MD	ME	MD	ME	MD
30	6.9c	1+	9.3c	1+	10.5bc	5+	6.7e	1+
40	8.4a	8+	10.5b	2+	10.8ab	5+	8.3d	2+
50	7.4b	4+	10.9ab	5+	11.1a	8+	9.5c	3+
60	7.2b	4+	10.9ab	5+	10.0c	5+	10.3b	3+
70	7.2b	4+	11.1a	5+	8.5e	1+	11.4a	6+
80	7.4b	4+	11.2a	5+	8.8de	2+	11.1a	6+
90	6.9c	1+	10.8ab	2+	8.5e	1+	10.5b	3+
100	6.6c	++	10.8ab	2+	9.1d	3+	11.3a	6+
Control 0%	0	0	0	0	0	0	0	0

ME = mycelial extension, MD = mycelial density.
Means followed by the same letters along each vertical column are not significantly different by Duncan's multiple range test ($P \leqslant 0.05$).

Table 4
Effects of different growth media on mycelial growth of *V. speciosa*

Growth media	Mycelial extension (mm)	Mycelial density
Sorghum agar	7.4c	2+
Rice agar	8.0bc	2+
Soy beans agar	85.0ab	5+
Ife brown beans agar	90.0a	6+
Wheat agar	90.0a	7+
White corn agar	90.0a	4+
Potato dextrose agar	92.0a	8+
Yellow corn agar	90.0a	4+

Data were means of triplicates. Means followed by the same letter(s) are not significantly different by Duncan's multiple range test ($P \leqslant 0.05$).

Table 5
Vegetative growth of *V. speciosa* on different substrates

Growth media	Mycelial extension (mm)	Mycelial density
Fresh cow dung	36.0g	12+
Fermented cow dung	38.0fg	12+
Fresh poultry waste	38.0fg	5+
Fermented poultry waste	60.0d	5+
Fresh horse dung	7.0c	9+
Fermented horse dung	67.0ld	10+
Coconut fibre	55.0e	3+
Cassava peels	67.0ld	13+
Saw dust	46.0lf	1+
Rice straw	82.0b	7+
Rice husk	82.0b	3+
Maize straw	92.0a	8+
Andropogon gianus straw	92.0a	7+

Data were means of triplicates. Means followed by the same letter(s) are not significantly different by Duncan's multiple range test ($P \leqslant 0.05$).

wastes. Also, this fungus may be said to possess cellulase (an enzyme), which has the ability to catalyse the break down of cellulose in these wastes, to release simple sugars needed for cellular respiration and growth.

Growth of *V. speciosa* was poor on fresh cow dung and poultry droppings. The low growth on cow dung may be due to inability of this fungus to secrete hydrolyzing enzyme, which will convert the wastes to utilizable amino acids and carbon compounds necessary for growth. Likewise, growth was poor on poultry manure that has been reported to contain high amounts of ammonia, which are toxic to mycelial growth of fungi (Singer, 1961).

From this study, it is clearly shown that the optimum growth of *V. speciosa* mycelia was obtained at 30 °C and pH 6.0. The best medium of growth was PDA, while the moisture requirement varied, depending on the agricultural substances used. It was also observed that maize and *Andropogon gianus* straw enhanced the best vegetative growth of this fungus.

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