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Effect of physico-chemical factors and semi-synthetic media on vegetative growth of *Lentinus subnudus* (Berk.), an edible mushroom from Nigeria

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Abstract

Attempts were made to investigate the effect of different semi-synthetic growth media, pH, temperature, carbon and nitrogen compounds, as well as additives, on vegetative growth of *L. subnudus* (Berk.), an edible mushroom from Nigeria. The best mycelial extensions (92.7 and 92. mm) were obtained on potato dextrose agar (PDA) and yellow corn agar (YCA), respectively. Moderate growths (87.0, 85.0 and 80.0 mm) were observed on white corn agar (WCA), yeast extract agar (YEA) and malt extract agar (MEA), respectively, but least growth (38.6 mm) was recorded on 'Ife brown' beans agar (IBBA). This fungus grew within a temperature range of 15–40 °C (optimum 30 °C) and, pH range of 5.0–8.0 (optimum 5.5).

Among the 17 tested carbon compounds, the best growth (193.3 mg/30 cm^3) was supported by fructose, followed in order by maltose, glucose and myo-inositol ($P \leqslant 0.05$). Twenty-one nitrogen sources were used and yeast extract enhanced the greatest mycelial dry weight (200.0 mg/30 cm^3) while the least growth (33.0 mg/30 cm^3) was obtained with L-glutamic acid. The most utilizable carbon/nitrogen ratio was 4:3, followed, in order, by ratios 5:3, 3:2 and 5:2 ($P \leqslant 0.05$), while the least growth was observed with the ratio 1:1. The additive that supported the best vegetative growth on *Trema orientalis* sawdust was 30% rice bran (98.0 mm). Moderate growth was also obtained with 1% fermented cow dung but very poor growth was observed with poultry manure. The implication of these findings in relation to the cultivation of *L. subnudus* in Nigeria is considered.

Keywords: *Lentinus subnudus*; Growth; Media; pH; Temperature; Additives; Carbon and nitrogen compounds

1. Introduction

Lentinus subnudus (Synonym *L. squarrosulus*) is one of the most common Nigerian edible mushrooms. This highly priced fungus usually grow wildly on decaying wood during the rainy season (April–October). It could be easily identified by the tough texture of matured sporophores, velvety stipe and funnel-shaped whitish pileus (Jonathan, 2002). The spore print of *L. subnudus* is white and spore size ranged between 5–8 and 2–4 μm along the major and minor axis, respectively (Jonathan, 2002; Zoberi, 1972).

The fruit bodies of this highly desired Nigerian mushroom are soft and brittle if harvested young (12–36 h) but become very tough and leathery when fully matured (3–5 days old). This fungus, locally called 'erokiro', could be chewed for a long time and, because of its meaty taste; it serves as a good alternative to animal protein among rural dwellers and average Nigerians who cannot afford the high cost of meat, due to the daily rise in cost of living. It has been reported to be a very good source of amino acids, glycogen, sugar, lipid and ascorbic acid (Fasidi & Kadiri, 1990). In Nigeria, *L. subnudus* and other edible

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mushrooms are important dietary components. They can be made into a variety of delicious dishes because they add flavour to food and act as condiments (Alofe, 1985; Ohuoya & Etugo, 1993). Among Nigerian mushroom dishes are: mushroom with green vegetable, mushroom with melon soup, mushroom with vegetable and melon soup, mushroom in okro soup, mushroom in stew and mushroom alone.

Despite the high nutrient composition of this fungus, people in Nigeria still depend on its climatically controlled (seasonal) occurrence, which could not be ascertained. It is unfortunate that this mushroom is only available during the rainy season! Up to now, no edible Nigerian mushrooms (including *L. subnudus*) have been cultivated on a large scale. Fasidi and Kadiri (1993) investigated the use of rice straw and dry wood for the cultivation of *L. subnudus* and they attained fructification on logs of *Spondias mombin* and unfermented compost. This result is quite promising.

More research work needs to be carried out on this fungus in order to make its cultivation a profitable venture in Nigeria. In the present study, attempts were made to investigate the effects of environmental factors, semi-synthetic media and agricultural wastes on vegetative growth of this Nigerian mushroom. This information will be helpful for improving cultivation technology of *L. subnudus* in Nigeria.

2. Materials and methods

2.1. Preparation of inoculum

The fruit bodies of *L. subnudus* (Berk.) were collected from the decaying wood of *Spondias mombin* at the Nursery section of the Botany and Microbiology Department, University of Ibadan, Nigeria. The sporophores of this fungus were tissue-cultured to obtain pure mycelial starter culture, which was maintained on potato dextrose agar supplemented with 0.5% yeast extract (Jonathan & Fasidi, 2003).

2.2. Effect of semi-synthetic media on mycelial growth of *L. subnudus*

The growth agar used is shown in Table 1. Food materials, such as yellow and white corn, wheat, rice, 'Ife' brown beans, sorghum and Irish potato, were bought from Bodija market Ibadan, Nigeria. Soybeans, cassava and yam tubers were collected from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Synthetic media used were malt agar (Lab. M), corn meal agar (Oxoid) and yeast extract agar (Oxoid).

Fifty grammes (50.0 g) of each food source was weighed and suspended in 250 cm^3 of distilled water and boiled. This was ground with a pestle and mortar and strained through cheese-cloth and, the filtrate was made up to 1000 $cm^{3.}$ Twenty grammes of agar–agar (Oxoid) were added and the whole autoclaved at 1.02 kg/cm^2 for 15 min at 121 °C. Streptomycin sulphate (50 mg) was added to the medium after sterilization to suppress bacterial growth. The media were dispensed into 100 mm petri dishes and allowed to solidify. The plates were then inoculated with 5.0 mm diameter discs of five-day-old mycelium of *L. subnudus* and incubated at 30 ± 2 °C. The radial colony diameters were then measured after the 10th day of incubation.

Table 1
Growth of *L. subnudus* on different semi-synthetic media

Growth media	Radial mycelial growth (mm)	Mycelial density
Corn meal agar	70.7c	5+
Cassava agar	40.3fg	1+
'Ife Brown' beans agar	38.6f	2+
Malt extract agar	92.3a	8+
Potato dextrose agar	92.7a	8+
Rice agar	57.3de	5+
Sorghum agar	66.3cd	5+
Soybean agar	61.0cd	4+
White corn agar	80.0b	6+
Yam agar	50.3e	3+
Yeast extract agar	85.0ab	6+
Yellow corn agar	87.0ab	7+
Wheat agar	66.7cd	4+

Each value represents a mean of three replicates. Means followed by the same letter(s) are not significantly different by Duncan's multiple range test ($P \leqslant 0.05$).

2.3. Temperature and pH

The effects of temperature and pH on the mycelial growth of *L. subnudus* were determined by the mycelial dry weight method (Madunagu, 1988). The basal medium used contained $FeSO_4$ (0.01 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), KH_2PO_4 (0.05 g), fructose (10.0 g), yeast extract (2.5 g), KNO_3 (1.55 g) and 10 cm^3 of micronutrients made up to 1000 cm^3 with de-ionized water. The composition of the micronutrient solution in 1000 cm^3 of de-ionized water was: H_3BO_4 (1.4 g), $MnSO_4 \cdot H_2O$ (1.8 g), $ZnSO_4$ (0.22 g), $CuSO_4$ (0.08 g), $(NH_4)_6MO_7 \cdot 4H_2O$ (0.05 g) and 0.01 g of $FeCl_2$ (Alofe, 1985). The method employed for temperature and pH determination was that described by Jonathan and Fasidi (2003).

2.4. Utilization of carbon compounds for growth by *L. subnudus*

To determine the effect of carbon compounds on vegetative growth of *L. subnudus*, the mycelial dry weight method in a liquid medium was employed. The basal medium consisted of KH_2PO_4 (0.05 g), $MgSO_4 \cdot 7H_2O$ (0.05 g), $FeSO_4$ (0.01 g), KNO_3 (1.55 g) and 1000 cm^3 of de-ionized water. For monosaccharides, disaccharides and sugar alcohol, 0.8% carbon of each carbon source was supplemented in the basal medium. Complex carbon compounds were supplemented at the rate of 10 g/1000 cm^3. The basal medium without any carbon source served as the control. The liquid

medium was dispensed into 250 cm^3 bottles (30 cm^3 per bottle) and pH adjusted to 5.5. The mouth of each bottle was sealed with aluminium foil and autoclaved at 1.02 kg cm^2 (121 °C) for 15 min. On cooling, 50.0 mg of streptomycin sulphate were added to suppress bacterial growth. The bottles were then inoculated with a 6.0 mm diameter disc of vigorously growing (five-day-old) mycelia of *L. subnudus* and incubated at 80 ± 2 °C for a week. The mycelia were then harvested, oven-dried at 80 °C for 12 h and weighed (Fasidi & Akwakwa, 1996).

2.5. Utilization of nitrogen compounds for growth by L. subnudus

Twenty-one nitrogen compounds, including both inorganic types, amino acids and complex organic sources, were separately added to 30 cm^3 of basal medium in each of 250 cm^3 jam bottles. The basal medium used was made up of thiamine hydrochloride (500 μg), KH_2PO_4 (0.05 g), $MgSO_4 \cdot 7H_2O$ (0.05 g), fructose (10.0 g) and 1000 cm^3 of de-ionized water. The amount of nitrogen supplemented was 0.1% for inorganic compounds and amino acids. For complex organic nitrogen sources (casein, yeast extract, malt extract, peptone and urea), supplementation was at the rate of 2.0 g/1000 cm^3 of basal medium only, without any nitrogen source. Mycelial dry weight was then determined (Jonathan & Fasidi, 2003).

2.6. Carbon/nitrogen ratios

The basal medium used was that described by Alofe (1985) but fructose was omitted. Equal quantities (0.15 g) of the best carbon source (fructose) and best nitrogen source (yeast extract) were supplemented in 1000 cm^3 of basal medium to form a ratio of 1:1. Likewise, the ratio 2:1 was prepared by mixing 0.30 g of fructose with 0.15 g of yeast extract in 1000 cm^3 of liquid medium. Other ratios were also prepared by mixing appropriate quantities of both organic compounds. These were autoclaved at 1.02 kg cm^{-2} (121 °C) for 15 min and treated as described in the previous experiments.

2.7. Effect of additives on growth of L. subnudus

To determine the effect of different additives on mycelial growth of *L. subnudus*, *Trema orientalis* saw dust was used as the main substrate. This was used since *L. subnudus* usually grown on wood. The additives investigated were fermented cow dung, fermented poultry manure and milled rice bran. Ninety grammes of *T. orientalis* sawdust were separately mixed with 10.0 g of additive (fermented cow dung, poultry manure and rice bran). This formed 10%. To prepare 20% additive, 80 g of the sawdust was mixed with 20.0 g of a particular additive. In this way, 30%, 40%, and 50% additives were prepared. For each preparation, the substrate was mixed thoroughly with additive and soaked with water before filling it into 100 mm Petri dishes. Each treatment was replicated three times. The control plates lacked any additive source. The plates were autoclaved at 1.02 kg cm^{-2} pressure (121 °C) for 15 min and allowed to cool. The plates were then inoculated with a 6.0 mm diameter disc of five-day-old mycelium of *L. subnudus* incubated at 30 ± 2 °C for 10 days. Growth was then measured by increase in mycelial diameter extension of the inoculated fungus while mycelial densities were assessed visually.

2.8. Analysis of data

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

3. Results and discussion

Out of the entire 13 tested semi-synthetic agars, potato dextrose agar and malt extract agar supported the best growth of *L subnudus* with mycelial extensions of 92.7 and 92.3 mm, respectively (Table 1). Yellow corn and yeast extract agar also enhanced very good growth with values, which were not statistically different from each other ($P \leqslant 0.05$).

The stimulation of better growth of *L. subnudus* on potato dextrose agar is not a surprise. This is because Fasidi and Akwakwa (1996), Lu (1997), and Oso (1997) obtained similar results for *Pleurotus tuber regium*, *Volvariella* species and *Coprinus comatus*, respectively. Both potato dextrose and malt extract agar have been found to support good mycelial growth of fungi, (Alofe, 1985; Huang, 1993; Jeffers & Martin, 1986; Kadiri, 1990). Likewise, Jonathan and Fasidi (2001) obtained luxuriant mycelial growth of *Psathyrella atroumbonata* when 0.05% yeast extract was supplemented in potato dextrose agar. The poor mycelial extension obtained on 'Ife brown' beans agar may be attributed to inability of this fungus to metabolize the ingredients. This highly proteineous medium may also be toxic to the fungal cell wall thereby preventing growth.

Table 2 shows that *L. subnudus* grew fairly well in acidic, neutral and alkaline environments (pH 5.0–8.0). It was observed that the best growth (120.3 mg/30 cm^3) was obtained in acidic medium of 5.5. The vegetative growth of 100.0 mg/30 cm^3, which was the second best, was also recorded in acidic medium (pH 5.0), followed by growth at pH 6.0. It could therefore be deduced that *L. subnudus* preferred an acid medium to neutral or alkaline pH.

There were no observable growths at pH 4.0, 8.5 and 9.0. This suggests that very strong acidic or alkaline environments were inhibitory to growth. This result agrees favourably with those of Chandra and Purkayastha (1977), Fasidi and Akwakwa (1996) and Oso (1997), who separately obtained very good mycelial growth *of A. campestris*, *P. tuber-regium* and *V. speciosa* at acidic pH values of 5.5, 6.0 and 6.5, respectively. Jonathan (2002) suggested that good growth of mushrooms could be obtained at moderately or slightly acidic pH.

Table 2
Effect of pH on mycelial growth of *L. subnudus*

PH	Mycelial dry weight (mg/30 cm^3)
4.0	–
5.0	100.0b
5.5	120.3a
6.0	65.0c
6.5	60.3c
7.0	47.3c
7.5	28.7d
8.0	15.0d
8.5	–
9.0	–

Each value represents a mean of three replicates. Means followed by the same letter(s) are not significantly different by Duncan's multiple range test ($P \leqslant 0.05$).

The results obtained when *L. subnudus* was grown in a different temperature range (Table 3) reveal that best mycelial yield (150.0 mg/30 cm^3) were attained at 30 °C. Therefore, this temperature was the optimum for the vegetative growth of this fungus. The minimum and maximum temperatures of growth were 15 and 40 °C (Table 3). The ability of this fungus to grow within this temperature range probably enables it to survive in a warm tropical climate. This observation is similar to that reported by Chang and Chu (1969) and Jonathan (2002) for *Volvariella volvacea* and *Lepiota procera*, respectively. Growth of *L. subnudus* was inhibited at extremely low and high temperatures (0, 10 and 40 °C). This observation agrees with the report of Garraway and Evans (1984) who suggested that metabolic activities of fungi are always reduced at extremely low temperature and denaturation of fungal enzymes occur at high temperature.

All 17 carbon compounds tested (except sorbose and arabinose) significantly enhanced mycelial growth of *L. subnudus* ($P \leqslant 0.05$; Table 4). The most stimulatory carbohydrate source was fructose with mycelial growth of 193.3 mg/30 cm^3, followed by maltose and glucose with mycelial yields of 168.3 and 150.0 mg/30 cm^3, respectively. Moderate growths were also observed with galactose, dextrin and myoinositol while poorest growths were observed with sorbose and galactose.

Table 3
Effect of temperature on mycelial growth of *L. subnudus*

Temperature (°C)	Mycelial dry weight (mg/30 cm^3)
0	–
10	–
15	18.3d
20	20.7d
25	116.3b
30	150.0a
35	63.5c
40	10.0d
45	–

Each value represents a mean of three replicates. Means followed by the same letter(s) are not significantly different by Duncan's multiple range test ($P \leqslant 0.05$).

Table 4
Utilization of carbons sources for growth by *L. subnudus*

Carbon source	Mycelial dry weight (mg/30 cm^3)	Final pH
Simple sugars		
Arabinose	54.3f	7.3
Fructose	193.3a	6.7
Galactose	105.0e	6.6
Glucose	150.0bc	6.9
Mannose	78.3fgh	7.1
Sorbose	48.31	7.4
Rhamnose	58.3hi	6.8
Oligosaccharides		
Cellobiose	56.3hi	6.7
Lactose	60.0hi	6.8
Maltose	168.3b	7.2
Raffinose	90.0ef	6.2
Sucrose	85.3fg	7.0
Polyols		
Mannitol	93.3ef	7.6
Myo-inositol	133.3cd	7.4
Polysaccharides		
Cellulose	93.3ef	6.8
Dextrin	110.0de	6.9
Starch	70.0gh	7.1
Control (basal medium only)		

Means followed by the same letter(s) are not significantly different by Duncan's multiple range test ($P \leqslant 0.05$). Each value represents a mean of three replicates.

The preference for fructose over any other carbon compounds may be attributed to the ease with which this sugar is metabolized during cellular respiration (Griffin, 1994). Glucose, which also supported very good mycelial growth, is an isomer of fructose. These two compounds could undergo molecular rearrangement (isomerism) to one another in cells during metabolism (Morrison & Boiyd, 1992). Alofe (1985) and Kadiri (1990) reported that glucose and fructose were the most readily utilized carbohydrate sources for the growth of *P. tuber-regium* and *P. squarrosulus*, respectively.

Arabinose was the least utilizable carbon compound with a mycelial yield of 43.3 mg/30 cm^3(Table 4). This result is contrary to those obtained by Madunagu (1988) (for *P. squarrosolus*) and Jonathan and Fasidi (2001) (for *P. atroumbonata*) where arabinose stimulated better growth of these fungi. The result obtained for *L. subnudus* suggests that carbohydrate requirements of mushrooms may differ. The poor growth of *L. subnudus* with sorbose and arabinose may be attributed to its inability to produce enzymes, which could catalyze the breakdown of these sugars in cells.

In the series of tested nitrogen compounds (Table 5), the most stimulatory compound was yeast extract, followed, in order, by peptone, sodium nitrate and malt extract ($P \leqslant 0.05$). Among amino acids tested, asparagine, alanine, leucine and glutamine supported moderate growth (Table 5). All other amino acids had values that were not statistically different from that of the control ($P \leqslant 0.05$). It was also observed that complex organic nitrogen sources (except

Table 5
Influence of nitrogen compounds on mycelial growth of *L. subnudus*

Nitrogen sources	Mycelial dry weight (mg/30 cm^3)	Final pH
Amino acids		
L-aspartic acid	40.0fg	6.3
L-asparagine	113.3bc	5.4
D-alanine	100.0bcd	5.6
L-glutamic acid	33.3g	6.8
L-glutamic acid	40.0fg	7.0
D-methionine	50.0efg	6.2
L-tryptophan	40.0fg	6.4
Complex organic sources		
Casein	53.3fg	7.2
Malt extract	120.0bc	6.7
Peptone	140.0b	6.0
Urea	40.0fg	6.4
Yeast extract	200.0a	6.2
Inorganic sources		
Ammonium nitrate	73.3def	6.2
Ammonium sulphate	36.7g	6.7
Calcium nitrate	70.0def	6.3
Potassium nitrate	96.7cd	6.1
Sodium nitrate	130.0b	6.5
Control (basal medium only)	48.3fg	6.3

Means followed by the same letter(s) are not significantly different by Duncan's multiple range test ($P \leqslant 0.05$). Each value represents a mean of three replicates.

urea) generally supported better mycelial growth than did amino acids. This may be due to the fact that complex organic nitrogen compounds contained combined amino acids and carbohydrate, which were supportive of fungal growth (Bolton & Blair, 1982). This result is similar to that obtained for *Volvariella speciosa* by Fasidi and Akwakwa (1996). The inhibition of mycelial growth of *L. subnudus* by urea may be due to its toxicity to the fungal cells (Griffin, 1994).

The carbon/nitrogen ratios used in this study promoted growth significantly (Table 6). *L. subnudus* grew best in media with the ratio 4:3, followed, in order, by 5:3, 3:2 and 5:2, while the least growth was observed with the ratio 1:1. The results obtained for this fungus are different from those obtained by Kadiri (1990) for *P. tuber-regium*, Madunagu (1988) (for *P. squarrosulus*) and Jonathan (2002) (for *T. lobayensis*). The different variations obtained in these mushrooms suggest that each fungus utilizes specific C:N ratios, which may be entirely different from others.

Trema orientalis sawdust supplemented with rice bran enhanced better growth of *L. subnudus* than other tested additives (Table 7). The ability of this fungus to grow well on rice bran may be due to its carbohydrate, amino acids and mineral element composition. Hayes (1972) reported that rice bran was rich in linoleic acid which is a stimulator of better growth of *Agaricus bisporus*. Han, Ueng, Chen, and Chen (1981) and Quimio (1981) suggested that rice bran was a good nutrient supplement to sawdust for vegetative growth of *Auricularia polytrica* and *L. edodes*, respectively. Sawdust, supplemented with 30% rice bran, stimulated better mycelial growth of *L. subnudus* than 10%, 20%, 40% and 50%. This implies that additives could enhance mycelial growth of mushrooms to a tolerable limit. It was also observed that fermented cow dung supported moderate growth *of L. subnudus* mycelia at 10% concentration but, growth was poor between 30% and 50% (Table 7). Similarly, very poor growth was recorded for poultry manure. This result is similar to that obtained by Ohuoya and Etugo (1993) (for *P. tuber-regium*).

Table 6
Effect of different carbon/nitrogen ratios on mycelial growth of *L. subnudus*

Carbon/nitrogen ratios	Mycelial dry weight (mg/30 cm^3)	Final pH
1:1	45.0I	6.1
1:2	60.0hi	6.6
1;3	60.0hi	6.8
1:4	66.7hi	6.3
1.5	70.0 gh	5.9
2:1	100.0efg	5.7
2.3	96.7efg	6.2
2:5	90.0fgh	6.4
3:1	123.3cde	6.7
3:2	150.0bc	7.1
3:4	136.7cd	6.8
3:5	140.0c	6.7
4:1	110.0def	7.2
4.3	186.7a	6.9
4:5	133.3cd	6.2
5:1	100.0efg	5.9
5:2	136.7cd	6.4
5:3	166.7abc	6.6
5:4	150.0bc	6.2
Control 0:0 (basal medium only)	41.6I	6:3

Each value represents a mean of three replicates. Means followed by the same letter(s) are not significantly different by Duncan's multiple range test ($P \leqslant 0.05$).

Table 7
Effect of additives on vegetative growth of *L. subnudus* on *T. orientalis* sawdust

Additives	Radial mycelial growth (mm) (mean of three replicates)	Mycelial density
TOS + 10%FCD	75.0bc	3+
TOS + 20%FCD	60.0de	7+
TOS + 30%FCD	50.0ef	7+
TOS + 40%FCD	45.0f	6+
TOS + 50%FCD	35.0g	5+
TOS + 10%FPM	27.0h	3+
TOS + 20%FPM	45.0f	6+
TOS + 30%FPM	26.0hi	2+
TOS + 10%FPM	15.0i	2+
TOS + 10%RB	82.0b	9+
TOS + 20%RB	85.0b	9+
TOS + 30%RB	95.0a	10+
TOS + 40%RB	70.0cd	8+
TOS + 50%RB	50.0ef	6+
Control (TOS only)	30.0gh	2+

Means(s) followed by the same letters are not significantly different ($P \leqslant 0.05$).
Key: TOS, *Trema orietalis* sawdust; FCD, fermented cow dung; FPM, fermented poultry manure; RB, rice bran.

From our results, it was clear that luxuriant growth of *L. subnudus* was obtained on potato dextrose and malt extract agar. Also, mycelial growth was greatly enhanced by fructose and yeast extract as carbon and nitrogen sources, respectively. The best mycelial growth was obtained at 30 °C and pH 5.5. Likewise, high mycelial yield was obtained at a C:N ratio of 4:3 while 30% rice bran stimulated best mycelial extension on *T. orientalis* sawdust. This information may be useful for producing high yield mycelial starter cultures of *L. subnudus*. This could be utilized in the inoculation of agricultural substrates or spawns for the production *L. subnudus* fruit bodies in Nigeria.

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