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Studies on phytohormones, vitamins and mineral element requirements of *Lentinus subnudus* (Berk) and *schizophyllum* commune (Fr. Ex. Fr) from Nigeria

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Abstract

The effects of phytohormones, vitamins and mineral elements on growth of *Lentinus subnudus* (Berk) and *Schizophyllum commune* (Fr. ex Fr), two Nigerian edible mushrooms, were studied. Among the phytohormones tested, 1.0 ppm of 2,4 D stimulated the best growth in *L. subnudus*, followed by 0.1 ppm of GA3. As for *S. commune*, 10.0 ppm of 2, 4 D enhanced the best growth while 1.0 ppm of GA3 was the second best. The least stimulatory hormone for the two mushrooms was GA3 at 20.0 ppm concentration. Among the vitamins, biotin was the most utilizable for *L. subnudus*, followed in order by thiamine and folic acid. Pyridoxine promoted best growth for *S. commune* while the poorest growth was supported by pantothenic acid. In both higher fungi, macroelements (Mg, K and Ca) promoted good mycelial growth but Na was least utilised. Similarly, trace elements (Zn, Fe and Mn) also supported growth to a significant level ($P = 0.01$). The implication of the obtained results are discussed in relation to the cultivation of *L. subnudus* and *S. commune* in Nigeria.

1. Introduction

Lentinus subnudus (Berk) belongs to the family polyporaceae (Zoberi, 1972) which is the largest and most diverse group of phylum basidiomycetes (Pegler, 1983; Singer, 1986). This edible fungus grows naturally during the rainy season on dead pieces of wood, logs, trunks of buried or exposed roots of trees at different stages of decay (Alofe, 1985; Alofe, Odu, & Illoh, 1998; Kadiri & Fasidi, 1994). *Schizophyllum commune* (Fr. ex. Fr) is an edible mushroom which belongs to the family schizophyllaceae (Alexopolous, Mims, & Blackwell 1996; Singer, 1986). This fungus has been the object of numerous studies, such as sexuality, genetics and morphogenesis (Alexopolous, Mims, & Blackwell, 1996; Raper, 1988; Wessel, 1987). It is one of the most common gill-bearing bracket fungi of world wide distribution (Zoberi, 1972). It can be easily identified by the peculiar structure of its gills, which cover hymenium during unfavourable climatic conditions (Alexopolous et al.: Singer, 1986).

Both *S. commune* and *L. subnudus* are used to prepare delicious dishes among the Yoruba people of southwestern Nigeria. Kadiri and Fasidi (1994) reported that *L. subnudus* is rich in protein and minerals while Oso (1981) reported the medical importance of *S. commune*. Kadiri and Fasidi (1994) conducted an investigation on carbon and nitrogen requirements of *L. subnudus* and found fructose and peptone as the best carbohydrate and protein sources, respectively. The present studies were aimed at providing useful information on phytohormones, vitamins and minerals required for the vegetative growth of *L. subnudus* and *S. commune*. This will further improve their cultivation technology in Nigeria.

2. Material and methods

2.1. General

Fruit bodies of *L. subnudus* and *S. commune* were tissue-cultured to obtain mycelia, using the method described by Fasidi and Ekuere, (1993). The pure culture mycelia were maintained on plates of PDA supplemented with 0.5% of yeast extract. The requirements of these two fungi for phytohormones, vitamins and minerals were assessed by the mycelial dry weight method.

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Different compounds required to form the basal medium were dissolved in 1000 cm^3 of de-ionised water and 0.05 g of streptomycin sulphate was added to inhibit bacteria growth. The basal medium (30 cm^3 per bottle) was dispensed into 250 cm^3 milk bottles and pH adjusted to 6.0 for *L. subnudus* and 6.5 for *S. commune*. Phytohormones and vitamins were sterilised by millipore filtration while minerals were autoclaved at 121°C and pressure of 0.69 kg cm^{-3} for 10 min. The mouth of each bottle was covered with aluminium foil and each treatment was replicated three times. After sterilisation, each bottle was inoculated with a 0.7-cm disc of actively growing mycelia (6-day-old) and incubated at 30 + 2°C for 7 days. The mycelia from different treatments were harvested, oven dried at (80°C) for 24 h and weighed.

2.1.1. Phytohormones

The basal medium consisted of fructose (10.0 g), peptone (2.0 g), KH_2PO_4 (0.5 g), $MgSO_4$ $7H_2O$ (0.5 g), thiamine hydrochloride (0.5 mg), and 1000 cm^3 of de-ionised water (Fasidi & Olorunmaiye, 1994). Phytohormones (2,4 dichlorophenoxyacetic acid (2,4,D), gibberellic acid (GA3) and naphthalene acetic acid (NAA) were added to the basal medium, separately, to give concentrations of 0.1, 1.0, 10.0, 15.0 and 20.0 ppm, respectively. The basal medium, without any phytohormone, served as the control.

2.2. Vitamins

The basal medium used was similar to that of phytohormones except that thiamine hydrochloride was omitted. Each vitamin (ascorbic acid, biotin, folic acid, cobalamine, nicotinic acid, pantothenic acid, pyridoxine, riboflavin and thiamine) was added to the basal medium, separately, to give a concentration of 0.5 mg 1000 cm^{-3}. Two sets of control were prepared; one contained all vitamins and a second lacked all.

2.3. Macroelements

The basal medium contained fructose (10.0 g), asparagine (10.0 g), $NaNO_3$ (2.0 g), KH_2PO_4 (2.0 g), $MgSO_4$. $7H_20$ (0.5 g), $CaCl_2$ (0.3 g), thiamine hydrochloride (0.5 mg), and 1000 cm^3 of de-ionised water. To investigate effect of sodium, $NaNO_3$ was replaced by NH_4NO_3. Likewise, KH_2P0_4, $Mg.S0_4$ and $CaCl_2$ were replaced by equal amounts of their ammonium conjugates ($NH_4H_2P0_4$, $(NH_4)_2$ SO_4 and NH_4Cl). Two sets of control were prepared, as for vitamins.

2.4. Microelements

The basal medium used was the same as for macroelements. Microelements (Cu, Zn Fe, Mn and Co) in their sulphate form were dissolved separately in the basal medium to give a concentration of 0.1 g 1000 cm^{-3}. Two controls were employed; in control 1 all the micronutrients were added to the medium while control 2 had none.

Table 1
Utilization of phytohormones for growth by *Lentinus subnudus*[a]

Phytohormones (ppm)	Dry weight of mycelia (mg cm^{-3})	Final pH
Gibberellic acid (GA_3)		
0.1	110.0±3.8	5.9
1.0	90.0±5.4	5.8
10.0	73.3±3.3	6.5
15.0	50.0±2.8	6.1
20.0	35.7±3.5	6.2
Naphthalene acetic acid (NAA)		
0.1	53.3±3.2	6.4
1.0	93.3±4.3	6.5
10.0	100.0±5.8	6.0
15.0	70.0±4.5	5.8
200	41.7±1.7	5.6
2,4-Dichlorophenoxyacetic acid. (2,4D)		
0.1	93.3±3.7	6.3
1.0	125.0±3.2	6.4
10.0	70.0±4.7	5.7
15.0	58.3±4.2	5.9
20.0	40.0±2.9	6.9
Control (basal medium)	53.3±1.7	6.4

[a] Data represented above are means of three treatments±S.E. at 1% level of probability.

2.5. Analysis of data

The data obtained were subjected to analysis of variance (ANOVA) and Duncan's multiple range test.

3. Results and discussion

The three phytohormones used in this study were found to enhance mycelial growth of *L. subnudus* and *S. commune* significantly ($P = 0.01$). The most stimulatory hormone for the two fungi was 2,4D at concentrations of 1.0 and 10.0 ppm, respectively (Tables 1 and 2). This result is similar to that obtained by Fasidi and Olorunmaiye, (1994) (on *Pleurotus tuberregium*) and Voltz, (1972) (on species of *Cantharellus* and *Volvariella*. Kurancowa (1963) also reported that this hormone was important for spore germination and mycelial growth of *Pleurotus ostreatus* and *Marasmius rotula*. Poor growths of these fungi were obtained at high concentration (15.0 and 20.0 ppm). This implies that high concentrations of these hormones are inhibitory to the growth of *L. subnudus* and *S. commune*. Hayes (1981) observed that neither low nor high concentrations of phytohormone had effects on vegetative growth and sporocarp production of *Agaricus bisporus*.

Tables 3 and 4, show that *L. subnudus* and *S. commune* required an external supply of vitamins since least

Table 2
Utilization of phytohormones for growth by *S. commune*[a]

Phytohormones (ppm)	Dry weight of mycelia (mg cm^{-3})	Final pH
Gibberellic acid (GA_3)		
0.1	93.3±2.3	5.9
1.0	95.0±5.8	6.2
10.0	73.3±6.4	5.8
15.0	50.0±0	5.7
20.0	40.0±.7	5.6
Naphthalene acetic acid (NAA)		
0.1	58.3±4.4	5.7
1.0	63.3±3.7	5.7
10.0	70.0±3.8	6.5
15.0	73.3±6.7	6.4
20.0	45.0±5.0	5.9
2,4-dichlorophenoxy acetic acid (2,4D)		
0.1	70.0±3.7	5.6
1.0	76.7±3.5	6.2
10.0	128.3±5.0	6.1
15.0	78.3± 4.4	5.8
20.0	50.0±1.8	6.5
Control (basal medium)	63.3±3.3	6.4

[a] Data represented above are means of three treatments±S.E. at 1% level of probability.

growth was obtained in the negative control. Biotin was the best vitamin source for *L. subnudus,* followed, in order, by thiamine and folic acid ($P=0.01$). The importance of biotin in cellular processes has been attributed to its role as a co-factor for acetyl co A carboxylase, an enzyme which is important in lipid metabolism (Rao & Modi, 1968). Thiamine serves as a co-enzyme for several enzymes of intermediary metabolism (Gounaris, Turkenkopf, Averchia, & Greenlie 1975). The ability of *S. commune* to utilise pyridoxine supports the suggestion of Hilgenberg and Hofmann (1977), that pyridoxine is converted by some fungi into functional phosphate which is important in the synthesis of tryptophan (an amino acid needed for growth).

Among the macroelements employed, the best growth was observed on Na free medium (Tables 5 and 6). This indicates that Na is not required by the two mushrooms. Sodium ion has been shown to inhibit several biochemical processes, including respiration and fermentation in basidiomycetes (Garraway & Evans, 1984). Least growth observed on Mg-free medium (Tables 5 and 6) suggests that Mg is the most essential macronutrient for the growth of these two fungi. This result agrees favourably with that of Fasidi and Olorunmaiye (1994), Fasidi and Jonathan (1994) and Okorokov et al. (1975). Magnesium is needed for the transfer of phosphate groups during ATP formation (Mahier & Cordes, 1971). It is also required by iso-citrate dehydrogenase as a co-factor during the Krebs cycle (Faller, Baroudy, Johnson, & Ewall 1977). It was shown (Tables 5 and 6) that K is the second important macrometal. This result

Table 3
Effect of vitamins on growth of *L. subnudus*[a]

Vitamins	Dry weight of mycelia (mg cm^{-3})	Final pH
Ascorbic acid	70.0±5.8	6.1
Biotin	106.7±2.8	6.2
Cobalaniine	60.0±3.6	5.8
Folic acid	81.7±4.4	6.5
Nicotinic acid	60.0±3.5	6.2
Pantothenic acid	51.7±4.2	5.7
Pyridoxine	75.7±4.4	6.3
Riboflavin	76.7±6.7	6.8
Thiamine	90.0±5.8	5.9
Basal medium plus all vitamins	56.7±3.3	5.3
Basal medium only	48.3±1.7	5.6

[a] Data represented above are means of three treatments±S.E. at 1.0% level of probability.

Table 4
Effect of vitamins on growth of *S. commune*[a]

Vitamins	Dry weight of mycelia (mg cm^{-3})	Final pH
Ascorbic acid	60.0±5.8	6.6
Biotin	86.7±6.0	5.9
Cobalamine	81.7±5.2	5.7
Folic acid	93.3±3.5	6.3
Nicotinic acid	61.7±2.7	5.1
Pantothenic acid	48.3±3.3	5.0
Pyridoxine	120.0±5.8	6.2
Riboflavin	50.0±3.8	4.9
Thiamine	78.3±4.8	5.8
Basal medium plus all vitamins	50.0±3.8	6.4
Basal medium only	43.3±4.4	5.5

[a] Data represented above are means of three treatments ± S.E. at 1% level of probability.

favours the observation of Slayman and Tatum (1964), that all fungi require K for growth. Potassium regulates cellular osmotic potential and brings about turgur pressure necessary for growth (Garraway & Evans, 1984).

Poor mycelial yield obtained on the medium that contained no micronutrient (control 2) suggests that the two mushrooms need trace elements for better growth (Tables 5 and 6). The complete medium without Zn gave least growth. This implies that Zn is the most essential microelement for the growth of *L. subnudus* (Table 5). Similar observations were made by Chandra and Purkayastha (1977) (on *A. campestris*), Fasidi and Olorunmaiye (1994) (on *P. tuber regium*) and Jonathan and Fasidi (2001) (on *Psathyerella atroumbonata*). Faillar, Benedict, and Weinberg (1976) suggested that Zn is the functional component of enzymes needed for the synthesis of DNA and RNA. As for *S. commune,* Fe was the most utilisable trace element (Table 6). Iron is an enzyme-activator and component of hemes—like porphyrin—which is important for electron transfer needed for growth (Neiland, 1974).

Table 5
Mineral Nutrition of *L. subnudus*[a]

	Dry weight of mycelia (mg cm^{-3})	Final pH
Effect of macroelements on growth		
Complete medium (control 1)	106.7±6.7	6.3
Complete medium minus Mg	63.3±3.5	5.9
Complete medium minus Ca	80.0±5.8	6.7
Complete medium minus K	70.0 0	6.4
Complete medium minus Na	146.7±4.8	6.3
Basal medium (control 2)	43.3±3.3	6.0
Effect of microelements on growth		
Complete medium (control 1)	90.0±5.8	6.4
Complete medium minus Cu	123.3±12.0	5.7
Complete medium minus Fe	83.3±3.8	5.9
Complete medium minus Mn	123.3±3.4	6.4
Complete medium minus Co	143.3±4.4	6.7
Complete medium minus Zn	63.3±2.5	6.3
Basal medium (control 2)	46.7±3.3	6.2

[a] Data represented above are means of three treatments ± S.E. at 1% level of probability.

Table 6
Mineral nutrition of *Schizophyllum commune*[a]

	Dry weight of mycelia (mg cm^{-3})	Final pH
Effect of macroelements on growth		
Complete medium (control 1)	110.0±5.8	5.7
Complete medium minus Mg	63.3±3.3	6.3
Complet medium minus Ca	85.0±2.9	6.4
Complete medium minus K	70.0±2.8	6.2
Complete medium minus Na	100.0±10.0	5.9
Basal medium (control 2)	50±5.0	6.6
Effect of microelements		
Complete medium control 1)	133.3±3.3	6.0
Complete medium minus Cu	136.7±4.8	5.8
Complete medium minus Fe	70.0±2.9	5.9
Complete medium minus Mn	93.3±4.5	6.2
Complete medium minus Co	130.0±5.8	6.3
Complete medium minus Zn	96.7±4.3	6.7
Basal medium (control 2)	40.0±0	5.7

[a] Data represented above are means of three treatments ± S.E. at 1% level of probability.

In conclusion, it is clear from this study that synthetic growth medium for optimum yield of *L. subnudus* mycelia should comprise hormone (2,4D at 1.0 ppm concentration), biotin, Mg and Zn, while that of *S. commune* should have a concentration of 10.0 ppm of 2,4,D, pyridoxine, Mg and Fe. It is hoped that these findindgs will aid vegetative propagation as well as production of fruit bodies of S. commune and *L. subnudus* in Nigeria.

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