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Nutritional Requirements of *Pleurotus florida* (Mont.) Singer, A Nigerian Mushroom

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Abstract: The requirements for mycelial growth of *Pleurotus florida*, a Nigerian edible mushroom was carried out. The carbon nitrogen ratio of 5:1 was found to be the most suitable for the vegetative growth followed by 4:1 ratio and the least was 1:5. The most suitable vitamin for the mycelial growth was thiamine followed by pyridoxine while the least was cobalamine. With regard to phytohormones, gibberellic acid was found to be the best for the mycelial growth followed by indole acetic acid (IAA) while the poorest was 2, 4 dichlorophenoxy acetic acid. Calcium was found to be the best required macro nutrient while zinc was the best micronutrient. The implication of these results was discussed.

Key words: Macro nutrients, micro nutrients, mycelial growth, *Pleurotus florida*, vitamins, phytohormones

Introduction

Pleurotus florida is an excellent edible and highly nutritious mushroom which is a common species in tropical West Africa and Southern part of Asia. The edible fruit bodies develop in large numbers as a group on fallen trees, logs of wood and wooden poles. The cap measurement may range from 1.5 to 7.5cm diameter while the stipe is 0.5cm to 2.5cm long, annuls is absent and the spore print is cream – white in colour (Jonathan, 2002).

Wolter *et al*. (1997) proposed that *P. florida* is suitable for bioremediation of contaminated soils because of its ability to degrade highly condensed polycyclic aromatic hydrocarbons (PAH) and its high tolerance of these substrates. Many work have reported the requirements for the vegetative growth of many Nigerian Mushrooms; *Pleurotus tuber-regium* (Fasidi and Olorunmaiye, 1994), *Tricholoma lobayensis* (Jonathan and Fasidi, 2003a), *Lepiota procera* (Jonathan and Fasidi, 2000), *Psathyerella atroumbonata* (Jonathan and Fasidi, 2003b) while little work have been done on *P. florida*. It was therefore the objective of this investigation to examine the nutritional requirements of this fungus in order to improve mycelial growth.

Materials and Methods

Fungal cultivation and incubation: Pure mycelial culture of *Pleurotus florida* was obtained by tissue culture method (Jonathan and Fasidi, 2003a). The mycelial culture was maintained on Potato Dextrose Agar (PDA). Growth requirements of the fungus were determined by a mycelial dry weight method described by Fasidi and Olorunmaiye (1994). The basal medium used was that described by Chandra and Purkayastha (1977). The ingredients required to form the basal medium and streptomycin sulphate (0.5g) were dissolved in 1 litre of distilled water and pH regulated to 6.5. This liquid medium was dispensed into 150ml bottles (30ml per bottle) and the mouth of each bottle was sealed with aluminum foil, and autoclaved at 1.02 kg/cm^3 (10.0 pa) pressures at 121^{o}C for 10mins. After cooling, each bottle was inoculated with a 7mm - diameter disc of vigorously growing mycelium and incubated for 7 days at 30±2^{o}C. Each treatment was replicated thrice. The mycelium in each bottle was filtered through a pre- weighed 9cm diameter Whitman filter paper, oven- dried at 80^{o}C for 10 hours and weighed (Jonathan and Fasidi 2003a).

C: N Ratio: The basal medium consisted of KH_2 PO_4 (0.5g), $MgSO_4.7H_2O$ (0.5g) thiamine hydrochloride (500 µg) and 1000ml of distilled water (Chandra and Purkayastha, 1977). Varying ratios of glucose and tryptophan were used as carbon and nitrogen sources respectively (Jonathan and Fasidi, 2001). A concentration of 0.15g litre of glucose and tryptophan in the basal medium served as the 1:1 ratio, other ratios were prepared proportionately.

Vitamins: The basal medium was similar to that used for investigating the effect of C/N compounds except that thiamine hydrochloride was omitted. The vitamins used were ascorbic acid, biotin, cobalamine, folic acid, nicotinic acid, pyridoxine, riboflavin and thiamine. Two control experiments were set up. One contained all the vitamins and the other no vitamins. The media were then filter and sterilized. Vitamins were added to the basal medium separately to give a concentration of 500µg per 1000ml and each set – up was replicated thrice.

Phytohormones: The basal medium used was made up of Fructose (10.0g), Alanine (1.0g), $NaNO_3$ (2.0g), KH_2PO_4 (2.0g), $MgSO_4.7H_2O$ (0.2g), (aCl_2 CO.2g), thiamine hydrochloride (500µg) and 1000ml of deionized water (Jonathan and Fasidi, 2001). The phytohormones used included 2, 4 dichlorophenoxy acetic acid (2, 4 D) gibberellic acid (GA_3) and Indole acetic acid (IAA). These

Table 1: Effect of carbon/nitrogen ratios on mycelial growth of *P. florida*.

C/N Ratio	Mycelial dry weight (mg/30cm^3)	Final pH
Basal medium (Control)	30.0^{k}	6.2
1:1	80.0^{g}	5.7
1:2	51.7^{l}	6.2
1:3	50.0^{j}	6.7
1:4	33.3^{k}	6.8
1:5	21.7^{l}	6.6
2:1	110.0^{e}	6.7
2:3	65.0^{h}	5.8
2:5	45.0^{g}	5.9
3:1	133.3^{d}	6.5
3:2	96.7^{f}	5.9
3:4	50.0^{g}	5.5
3:5	41.7^{kgj}	6.8
4:1	176.7^{b}	6.2
4:3	93.3^{f}	5.9
4:5	63.3^{h}	7.1
5:1	220.0^{a}	6.7
5:2	151.6^{e}	6.9
5:3	63.3^{h}	6.2
5:4	46.7^{y}	5.7

Mean values followed by the same letters are not significantly different ($P \leq 0.01$) by Duncan's multiple range test.

plant hormones were added separately to the basal medium to give 0.1, 1.0, 10.0, 15.0 and 20.0ppm. The basal medium without phytohormone source was used as the control experiment. The phytohormones were filter sterilized and each treatment was replicated thrice.

Macro-elements: The basal medium was that described by Jonathan and Fasidi (2001). To investigate effects of Macro nutrients on growth, Na, K, Mg and Ca compounds in the liquid medium were replaced by their ammonium radicals (e.g. $NaNO_3$) was replaced by NH_4NO_3 and $MgSO_4$ replace by $(NH_4)_2\ SO_4$. Two sets of control were employed. Control I had all the macro-elements while control 2 had none.

Micro elements: The same basal medium described above for testing the effect of macro-elements was used. The trace elements (Cu, Mn, C, Fe and Zn) in their sulphate forms were supplemented separately in the basal medium to give 1ppm. Two sets of control were employed; one contained all the four trace elements, whereas the second did not contain any trace element.

Analysis of data: The data obtained were analyzed by ANOVA and tests of significance were determined by Duncan's multiple range test.

Results

In this study, the carbon: nitrogen ratios significantly affected growth of *P. florida* and growth at 5:1 ratio was the best (Table 1). This was followed by 4:1 which were significantly different from each other at $P \leq 0.01$. The least mycelial growth was 21.7mg/30cm^3 recorded in basal medium with C/N 1:5 which was not significantly different from control at $P \leq 0.01$.

Table 2: Effect of different vitamins on mycelial growth of *P. florida*

Vitamins	Mycelial dry weight (mg/30cm^3)	Final pH
Ascorbic acid	73.3^{e}	6.4
Biotin	100.0^{c}	6.6
Folic acid	86.7^{d}	6.3
Cobalamine	48.3^{f}	5.8
Nicotinic acid	53.3^{l}	7.2
Pyridoxine	133.3^{b}	6.8
Riboflavin	75.0^{e}	6.1
Thiamine	146.7^{a}	7.4
(All Vitamins) control 1	50. 0^{f}	6.6
Control 2 (basal medium only).	51.7^{f}	6.3

Mean values followed by the same letters are not significantly different ($P \leq 0.01$) by Duncan's multiple range test.

Table 3: Effect of phytohormones on mycelial growth of *P. florida*

Phytohormones (ppm)	Mycelial dry weight (mg/30cm^3)	Final pH
Gibberellic acid (GA3)		
0.1	130.0^{a}	6.4
1	83.3^{b}	5.9
10	56.7^{c}	6.8
15	46.7^{d}	6.3
20.0	-	6.4
Indole acetic acid (IAA)		
0.1	101.7^{a}	5.9
1	80.0^{b}	6.1
10	50.0^{c}	6.7
15	48.0^{d}	6.7
20	31.7^{e}	6.6
2, 4 Dichlorophenoxy acetic acid (2, 4D)		
0.1	71.7^{b}	7.1
1	100.0^{a}	6.8
10	43.3^{de}	6.3
15	33.3^{e}	6.1
20	18.3^{f}	6.4
Basal medium (control)	51.7^{c}	6.8

Mean values followed by the same letters are not significantly different according to Duncan's multiple range test ($P \leq 0.01$).

Table 2 shows that thiamine was the best vitamin source (146.7mg/30cm^3) followed by pyridoxine (133.3mg/30cm^3), the results being significantly different from each other at $P \leq 0.01$. Generally, it was observed that high concentration of phytohormones reduced the vegetative growth of *P. florida* (Table 3). No growth was observed at 20.0ppm of Gibberelic acid while Gibberellic acid (0.1ppm), indole-acetic acid (0.1ppm) and 2, 4, D (1.0ppm) promoted the greatest growth, and the values were not significantly different at $P \leq 0.01$. Gibberellic acid (15ppm) and IAA (10.00ppm) were not significantly different from the basal medium control at $P \leq 0.01$ according to Duncan's multiple range tests.

In the series of macroelements, supplementation of basal medium significantly improved growth at

Table 4: Effect of different macro elements on the mycelial growth of *P. florida*

Macro elements	Mycelial dry weight (mg/ 30cm^3)	Final pH
Control 1 (basal medium with all macroelements)	126.7^b	7.3
Control 2 (basal medium minus macro elements)	38.0^{ef}	6.3
Complete medium Minus Na	163.3^a	7.5
Complete medium minus Ca	46.7^{de}	6.5
Complete medium minus K	75.0^c	6.4
Complete medium minus Mg	60.0^d	6.8

Mean values followed by the same letters are not significantly different ($P \leq 0.01$) according to Duncan's multiple range test

Table 5: Effect of micro element on mycelial growth of *P. florida*

Micro element	Mycelial dry weight (mg/ 30cm^3)	Final pH
Complete medium (Control)	136.7^a	7.0
Complete medium without Cu	90.0^b	6.5
Complete medium without Fe	53.3^d	5.5
Complete medium without Mn	70.0^c	5.7
Complete medium without Co	130.0^{ab}	6.8
Complete medium without Zn	50.0^{de}	6.6
Basal medium (Control 2).	40.0^e	6.1

Mean values followed by the same letters are not significantly different. ($P \leq 0.01$) by Duncan's multiple range test.

($P \leq 0.01$). The complete medium minus Na produced the greatest growth of 63.3mg/30cm^3. While the least growth was observed in basal medium without macroelements (Table 4). The complete medium without K significantly reduced the growth values (75.0mg/30cm^3) followed by Mg (60.0mg/30cm^3) and Ca-free media (46.7mg/30cm^3). Table 5 shows that certain trace elements are required for the growth of *P. florida*, the complete medium without Fe gave poor growth followed closely by a Zn-free medium. For Co-free and Co-containing media, identical growth values were observed (130.0mg/30cm^3 and 136.7mg/30cm^3). The basal medium supplemented with Cu, Zn, Fe, Mn and Co produced greater growth than the basal medium at ($P \leq 0.01$). The basal medium without the trace elements resulted in a slightly higher growth than that of the basal medium (Table 5).

Discussion

Pleurotus florida inoculated on basal medium with carbon / nitrogen ratio of 5:1 gave the best mycelial growth. This result agrees with the findings of Fasidi and Jonathan (1994) who mentioned the importance of carbon nitrogen ratio and that ratio 5:1 can be incorporated into the medium to produce better yield of mycelial growth of fungus and hence spawn production. Also basal medium supplemented with pyridoxine and thiamine gave the best growth compared to the rest vitamin source. This is similar to the findings of Jonathan and Fasidi (2001) in *L. subnudus* and *S. commune*. This is supported by the hypothesis of Fasidi and Olorunmaiye (1994) who stated that pyridoxine is converted by some higher fungi into functional phosphate form which is important in the synthesis of tryptophan.

The highest mycelial growth recorded in basal medium supplemented with gibberellic acid correlates with the report of Fasidi and Jonathan (1994) who studied the requirement of phytohormones for growth by some Agaricales as Gibberellins are known to promote extensive growth as a complementation of the action of natural auxins.

P. florida grew best on the medium containing all the essential Macro nutrients. This shows that each of the tested macro-nutrients is important but the degree of utilization varied. While the complete medium without Ca and Mg gave the lowest mycelial dry weight, this indicates that those two metals are important for the growth of *P. florida*. The importance of calcium to the growth of fungi was also reported by Fasidi and Kadiri (1990), Fasidi and Olorunmaiye 1994) reported that the ability of Calcium to support better growth of *P. florida* may be attributed to its role in the fungus metabolic process such as glycolysis and respiration.

The complete medium without Zn and Fe resulted in good mycelial growth compared to others. The highest mycelial was found in complete medium without Cu (Copper). This explains the report of Garraway and Evans (1984) that Zn is a component of variety of fungal enzymes ranging from those involved in the intermediary metabolism to those involved in the synthesis of DNA and RNA.

The present studies thus reveal that good yield of *P. florida* can be obtained from medium with 5:1 Carbon / nitrogen ratio thiamine, Gibberellin, Calcium, and Zinc. This is thus recommended as an excellent combination for the growth of *P. florida* for prospective mushroom farmers.

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