Dynamic Biochemistry, Process Biotechnology and Molecular Biology ©2008 Global Science Books

The Effects of Some Physical and Nutritional Factors on the Vegetative Growth of *Pleurotus ostreatus* var. *florida* Eger. under Tropical Conditions

Ikechukwuka Cyriacus Okwulehie* • **Ikechukwu Adiele Okwujiako**

Department of Biological Sciences, Michael Okpara University of Agriculture Umudike, P.M.B. 7267, Umuahia, Abia State, Nigeria

Corresponding author: * **phylyke@yaoo.com**

ABSTRACT

Pleurotus ostreatus var. *florida* culture was grown on different media and agricultural wastes and also subjected to a range of temperatures and pH to investigate their influence on the performance of the mycelium. The mushroom mycelium grew best between 25 and 35°C and least at 15°C. Mycelial growth sharply decreased at ≥35°C. Similarly, a pH range of 6.0-8.0 produced maximum mycelial extension, 72.90–78.66% more than the control. Mycelial yield decreased at pH below 6 and above 8. Least mycelial extension was recorded at pH 3.0. However, pH values higher than 6.0–8.0 retarded the growth of the mycelium. Mushroom mycelium grew best on oat meal yeast agar (OMYA), followed by corn meal yeast agar (CMYA), then by potato dextrose yeast agar (PDYA). Poorest growth occurred on malt extract yeast agar (MEYA), although not significantly different from that on CMYA and PDYA. The density and growth (i.e. distinct mycelial masses) of the mushroom had the highest rating on OMYA followed by CMYA, PDYA and MEYA. CMYA produced the heaviest mycelial mass followed by OMYA. Similarly the mushroom mycelium grew well on *Andropogon gayanus*, *Pennisetum purpureum* and *Oryza sativa* straws, but very poorly on *Panicum maximum* straw. The fastest mycelial extension occurred on *A. gayanus* straw followed by *P. purpureum* and *O. sativa* straws, although not significantly so. Our results are discussed in relation to the importance of mycelia as soup ingredients, in the production of different flavours and cellulolutic enzymes in the food industry and as biological control agents to trap nematodes and other plant disease agents.

Keywords: agro-waste, growth, medium, pH, temperature, vegetative
Abbreviations: CMYA, corm meal yeast agar; **MEYA**, malt extract yeast agar; **OMYA**, oat meal yeast agar; **PDYA**, potato dextrose yeast agar

INTRODUCTION

In mushroom cultivation, spawning is done to produce increased mycelial mass to inoculate the substrate (Stamets 1993; Oei 1995). Dry mycelia of certain fungi are used to make soup ingredients, while aromatic compounds from mushrooms are used to produce different flavours in the food industry (Gallois *et al.* 1990). Cellulolytic enzymes have also been obtained from submerged liquid cultures (Cai *et al*. 1999; Velazquez-Cedeno *et al.* 2004). Fungal mycelia have also been employed as biological controls to trap nematodes or for other plant disease control. For instance, *Epicoccum nigrum* is considered as a potential biological control agent for certain plant diseases, such as white mould of bean caused by *Sclerotinia sclerotiorum* (Zhou *et al.* 1996).

Some researchers have examined the possibility of growing mushroom mycelia over a wide range of conditions. On a global scope, it has been reported that modifications of culture conditions, especially the choice of nitrogen and carbon source, influences the composition of the fungal odorous profile (Yong and Lim 1986). The addition of lipids to the culture media has been reported to increase the mycelial mass, the rate of various metabolites, antibiotics and lactones of mushrooms (Asther *et al.* 1988; Fasidi and Kadiri 1993). Fasidi and Olorunmaiye (1994) investigated the media requirements for the vegetative growth of *Pleurotus tuber-regium* mycelium. According to their study, glucose promoted high mycelial dry weight of the mushroom, followed by the sugar alcohol, mannitol and the disaccharide maltose. To provide information that could lead to the improved production of the mycelium of the mushroom *Epicoccum nigrum*, Zhou *et al.* (1996) investigated the effects of nutrients including specific carbohydrate sources and amino acids, on mycelial growth sporulation and germination of conidia of the fungus. Their investigation concluded that standard mycological media supported faster radial growth of the mycelia than media with single carbohydrate sources and individual amino acids. Kang *et al.* (2002) investigated the effects of various plant oils with their major components of fatty acids added to the growth media on the mycelial growth and pinhead formation of *Hericium erinaceum*, and reported that palmitic acid was most stimulatory for the mycelial growth by 18.3% resulting in 80.0 ± 3.9 mm of growth during 3 weeks of incubation as opposed to the contol (67.6 ± 2.7). Similarly Okwulehie (2004) investigated the effects of five media types, including a liquid medium, on mycelial extension of *Macrophomina phaseoli* and reported that the fungus grew in all media, although the highest growth was recorded in peanut leaflet oatmeal agar. Nwanze *et al.* (2005) cultured *Lentinus squarrosulus* and *Psathyrella atroumbonata*, two edible indigenous mushroom species in four different submerged liquid media supplemented with coconut, cotton, groundnut, butterfat, palm kernel and palm oil at five different rates. The purpose was to investigate the effect of the media and oil types (applied at 5% v/v) on the mycelial wet and dry weights of the two mushrooms. According to their findings the medium which contained twice the glucose level present in each of the other liquid media used, induced the heaviest mean wet and dry mycelial weights of both mushroom species used. Similarly, butter fat induced the heaviest weight.

Received: 11 June, 2008. Accepted: 8 July, 2008.

Original Research Paper

The effect of acidity of culture media on dry weight yield of fungal mycelium has also been investigated. Fasidi and Ekuere (1993) indicated that pH 6–7 was optimum for *P. tuber-regium* within a range of 4–9 and Fasidi (1996) reported pH 6.0 as the optimum for *Volvariella esculenta*

The primary objective of the present study was to determine the ideal media, temperature, pH and substrate that could be used to produce maximum yield of *Pleurotus ostreatus* var. *florida* mycelia in a local setting, namely in Nigeria.

MATERIALS AND METHODS

The original culture of *P. ostreatus* var. *florida* was obtained from stock culture maintained by Dr. I. A. Okwujiako, in the Department of Biological Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria. The mushroom culture was sub-cultured by aseptically introducing a 4 mm square mycelial mat on sterile medium of malt extract agar (MEA) and potato dextrose agar (PDA) in sterile Petri dishes. The dishes were wrapped in aluminum foil and stored at 28 ± 2°C and used for the investigation when required.

All chemicals and reagents were purchased from the commercial centre of Aba, Abia State, Nigeria and were of analytical grade.

Determination of the best medium for vegetative growth of *P. ostreatus*

To determine the best medium for the growth of *P. ostreatus*, malt extract yeast agar (MEYA), corn meal yeas agar (CMYA), oatmeal yeast agar (OMYA) and potato dextrose yeast agar (PDYA), were tested based on the radial extension of fungal mycelium on solid media following the methods of Fasidi (1996), Amadioha (2003) and Okwulehie (2004). Each of the media was sterilized in an autoclave at 0.1 MPa (121°C) for 15 min. Streptomycin sulphate (0.1 g) was added to each of the conical flasks containing 250 ml of the different sterilized media after cooling to about 45°C before dispensing into 9-cm Petri dishes. The methods for making these media have been described by Stamets (1993). Twenty ml of each medium were dispensed into each 9-cm Petri dish and allowed to gel. The dishes were inoculated each with a 4 mm (diameter) disc of 4-day old mycelium of *P. ostreatus* from the previously prepared cultures and incubated at 29 ± 2°C. Two diagonal lines were drawn on the underside of each of the Petri dishes containing the inoculated media, to intercept at the centre where the inoculum was placed (Okwulehie 2004). Mycelial diameter was measured at 12 h intervals for 4 days along these lines. Each treatment was replicated four times. The mycelial density was compared visually based on the "fluffiness" of the mycelium (Fasidi 1996). The data obtained were statistically analyzed using Analysis of Variance (ANOVA) in Microsoft Excel and the means were separated using the Least Significant Difference (LSD) at P = 0.05.

Media preparation

Various formulations have been developed for the cultivation of edible mushrooms on a semi-solid agar medium. The methods used in this study to determine the most suitable medium for *P. ostreatus* var. *florida* growth were prepared following slight modifications of Stamets' (1993) protocols.

Effect of temperature on mycelial growth of *P. ostreatus*

To establish the maximum temperature required for the best mycelial growth of this mushroom, a standard method of Fasidi (1996) and Okwulehie (2004) was followed. Pure mushroom culture was established on CMYA. 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 (Fasola *et al.* 2007). The medium was then aseptically dispensed into 9-cm Petri dishes at 20 ml per dish. After gelling, CMYA plates were inoculated at the centre, with a 6.0 mm (diameter) disc of vigorously growing (4-day old) culture of *P. ostreatus* and incubated at 10, 15, 20, 25, 30, 35, 40 and 45°C. Each treatment was replicated four times. Readings were taken at 12 h intervals by measuring the mycelial diameter along the lines on the underside of the dishes. Mycelial density was visually determined by observing the "fluffiness" of the mycelia and rating it on a 2–10 scale (Fasidi 1996).

Effect of pH on mycelial growth of *P. ostreatus*

The pH requirements for vegetative growth of *P. ostreatus* were determined gravimetrically using liquid medium. The basal medium used was composed of dextrose (10 g), $CaCl_2$ (0.4 mg), $FeCl_3$ (0.2 mg), $CuSO_4$ (0.4 mg), KI (0.10 mg), NaCl (0.10 g) KNO_3 (0.7 g) and KH_2PO_4 (0.1 g) dissolved in de-mineralized water (Fasola *et al*. 2007). The based medium was dispensed in 30 ml lots into 250 ml conical flasks. The pH of the medium was adjusted to 3, 4, 5, 6, 7 and 8 values using limestone and autoclaved at 1.02 kg cm^{-2} pressure (121°C) for 15 min. After cooling, the contents of each triplicated conical flask was inoculated with 6.0 mm (diameter) mycelium of *P. ostreatus* and incubated at 30 ± 2°C for 6 days. After the sixth day, the mycelial mat in each flask was filtered through a pre-weighted No. 1 Whatman filter paper in a Buchner funnel. The filtrates were dried in an oven at 70°C for 24 h and weighed.

Effect of different growth substrates on vegetative growth of *P. ostreatus*

Agricultural wastes such as *Andropogon gayanus*, *Panicum maximum*, *Pennisetum purpureum*, *Oryza sativa* and *Zea mays* straw were tested. To prepare the substrates, sun-dried portions of straws were chopped into pieces using a sharp cutlass, and then shredded into about 0.5-cm pieces using a Victoria Grain Mill hopper (ref 600009), following slightly modified methods of Fasidi and Kadiri (1993), Kadiri (1994) and Fasidi (1996). Fifty g each of the substrates were separately soaked overnight in sterile distilled water, in different 250-ml beakers. Excess water was squeezed out through four layers of muslin cloth. Three g of each of the substrates were put in different 9-cm Petri dishes. The dishes of substrates were autoclaved at 1.02 kg cm^{-2} pressure (121°C) for 15 min. After cooling, each dish was inoculated with 0.6 cm (diameter) mycelial disc from a vigorously growing 4-day old culture of *P. ostreatus*, and incubated at 28 ± 2°C for 6 days (Fasola *et al.* 2007). The linear growth (diameter) of the mycelium was observed and measured to the nearest cm while the density of the mycelia were visually evaluated and rated (Fasidi 1996). Each treatment was replicated four times.

Statistics

The data obtained were statistically analyzed using Analysis of Variance (ANOVA) and the means were separated using the Least Significant Difference (LSD) at P = 0.05. Each treatment was replicated four times.

RESULTS AND DISCUSSION

Effect of temperature on mycelial growth

The results of the growth response of the *P. ostreatus* var. *florida* mycelium are presented in **Fig. 1**. The result showed that *P. ostreatus* mycelium grew best between 25 and 30°C within the incubation period. The least growth of the mushroom sharply decreased at ≥35°C. Least growth occurred at 15°C. Similar results of optimal growth temperatures of 25-30°C for mycelia of many mushroom species that were adapted for tropical growth were noted by Okwulehie (2004), Akinyele and Adetuyi (2005) and Ko *et al.* (2005). According to Stamets (1993), the incubation temperature for most *Pleurotus* spp. is 24-30°C but each has different temperature requirements. For instance *P. citrinopitatus* and *P. euosmus* require 24-29°C and 21-27°C, respectively for mycelial growth, while *P. ostreatus florida* has a 24°C optimum temperature (Stamets 1993), a slight deviation from the 25°C was recorded in the present study, possibly as an

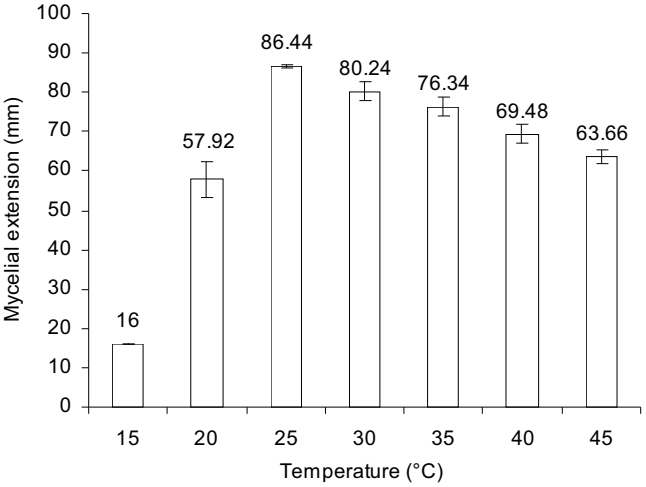

Fig. 1 The effects of temperature on the mycelial extension (mm) of *P. ostreatus* var. *florida*.

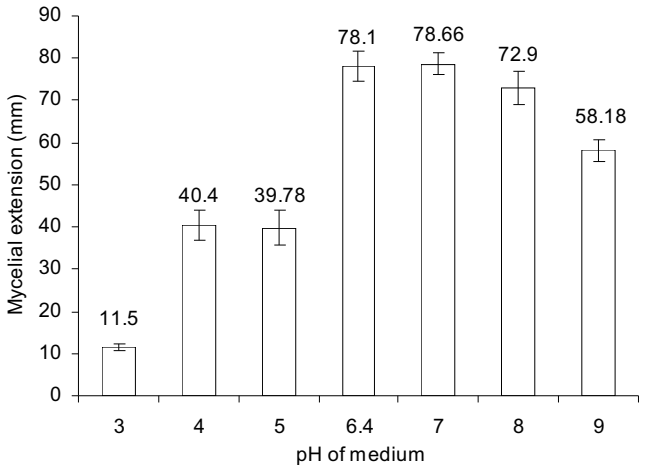

Fig. 2 The effects of pH of the medium on mycelial extension (mm) of *P. ostreatus* var. *florida*.

adaptation to tropical conditions. The best temperature range for maximum production of *P. ostreatus* mycelium in Nigeria is 25-30°C.

Effect of pH on mycelial extension

pH is generally considered to be one of the most important environmental factors that seriously affects the growth and extension of fungal mycelia (Kang *et al.* 2002; Akinyele and Adetuyi 2005; Okwulehie *et al.* 2006). The result of the effect of pH on mycelial extension of *P. ostreatus* is presented in **Fig. 2**. Maximum mycelial extension of 72.90-78.66% (relative percentage) was recorded at pH 6.0-8.0 but decreased at pH below 6 and above 8. Least mycelial extension was recorded at pH 3.0. This result differs from that of Okwulehie *et al*. (2006) for *P. pulmonarius* where pH 4.5 favoured the extension of the mycelium of the mushroom at pH 5.0 and 6.0 with least extension occurring at pH 6.5. That the least acidic media of pH 6.0-8.0 recorded significantly greater mycelial extension is surprising, since it has been reported by Zadrazil (1978), Bilgrama and Verma (1992) and Okwulehie *et al*. (2006) that fungal mycelia grew best in more acidic conditions. However, pH values higher than 6.0-8.0 retarded the growth of *P. ostreatus* mycelia, indicating that basic media still are not favourable for mycelial growth.

Effect of different growth media on mycelial extension

Mushroom mycelium grew best on OMYA (**Fig. 3**), significantly better than on CMYA and PDYA. Growth was poorest on MEYA although the value was not significantly different from those on CMYA and PDYA. This data confirms the conclusions made by Zoberi (1973) that *Pleurotus* species can efficiently utilize different carbon sources for growth. *P. ostreatus* mycelium (this study) favoured OMYA similar to *P. pulmonarius* (Okwulehie *et al*. 2006). Optimal production of fungal biomass may be obtained for *Pleurotus* spp. using oatmeal as a carbon source.

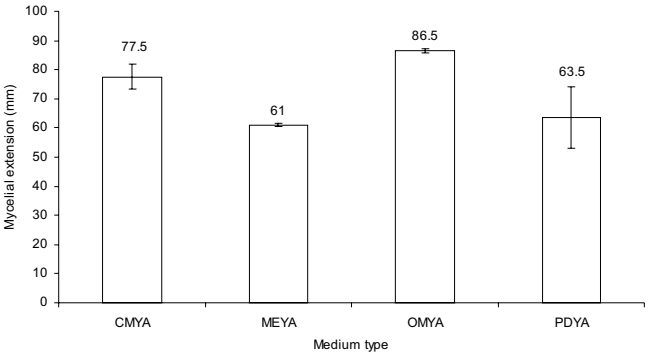

Fig. 3 The effects of different media on mycelial extension (mm) of *P. ostreatus* var. *florida*. CMYA: corn meal yeast agar; MEYA: malt extract yeast agar; OMYA: oat meal yeast agar; PDYA: potato dextrose yeast agar.

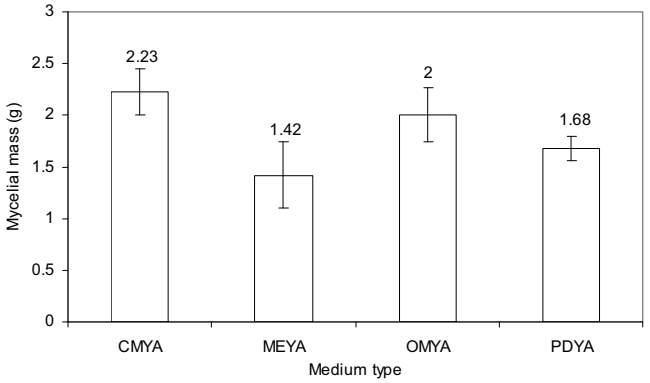

Fig. 4 The effect of different medium on mycelial wet weight (g) of *P. ostreatus* var. *florida*. CMYA: corn meal yeast agar; MEYA: malt extract yeast agar; OMYA: oat meal yeast agar; PDYA: potato dextrose yeast agar.

Table 1 The effects of different growth media on the density of *P. ostreatus* var. *florida* mycelium.

Media type	Mycelial extension (mm)
CMYA	7.50 ± 1.29 b
MEYA	4.75 ± 0.50.c
OMYA	8.75 ± 0.50 a
PDYA	7.00 ± 0.82 b

Means ± SD with the same letter(s) are not significantly different (P>0.05, 0.01) by LSD.

Effects of different growth media on density (fluffiness) of mycelium

Growth of the macrofungus on OMYA was rated 8.75 (**Table 1**), significantly higher than the values recorded for CMYA, PDYA and MEYA: 7.50, 700 and 4.75, respectively. A higher rating indicates more "luxuriant" growth or fluffiness, with individual hyphae appearing more distinct.

Effects of media on mycelial wet weight

CMYA produced the heaviest mycelial mass followed by OMYA (**Fig. 4**), a result that appeared to conflict with the result of mycelial density but was similar to that reported by Okwulehie *et al*. (2006) where it was noticed that CMYA induced the highest mycelium extension of *P. pulmonarious*,

Table 2 The effects of different agricultural wastes on the mycelial extension (cm) of *P. ostreatus* var. *florida.*

Substrate type	Mycelial extension (mm)
Andropogon straw	13.48 ± 0.78 a
Panicum straw	3.90 ± 2.90 b
Pennisetum straw	12.15 ± 1.26 a
Oryza straw	11.63 ± 1.53 a

Means ± SD with the same letter(s) are not significantly different (P>0.05, 0.01) by LSD.

more than MEYA although the mycelium of the latter weighed more. Based on wet weight, CMYA is preferred to OMYA but based on radial extension and density OMYA is preferred to CMYA.

Effects of agricultural wastes on mycelial extension

Mushroom mycelium grew very well (speed-wise) on *A. gayanus*, *P. purpureum* and *O. sativa* straws (although not significantly different at P = 0.01 and 0.05), but very poorly on *P. maximum* straw (**Table 2**). This result confirmed that *Pleurotus* mushrooms have great saprophytic ability and can colonize and exploit the nutrients in various lignocellulose materials (Jandaik 1974; Zadrazil 1978). Rice straw could not support the fastest mycelial extension even though it is among the most common substrates for the cultivation of edible mushrooms world-wide (Chang and Fernandez 1980; Chang 1982). It is however, the natural substrate on which *Volvariella esculenta* grows hence its name, delicious straw mushroom (Hashioka 1962).

REFERENCES

Akinyele RJ, Adetuyi FC (2005) Effect of agrowastes, pH and temperature variation on the growth of *Volvariella*. *African Journal of Biotechnology* **4**, 1390-1395

Amadioha AC, Uchendu PN (2003) Postharvest control of tomato fruit rot, caused by *Fusarium solani* with with extracts of *Azadiracta indica. Discovery and Innovation* **15 (2)**, 83-85

Asther M, Lesage L, Drapon R, Corrieu G, Odier E (1988) Phospholipids and fatty acid enrichment of *Phanerochaete chrysosporium* INA-12 in relation to ligninase production. *Applied Microbiology and Biotechnology* **27**, 393-398

Bilgrami KS, Verma RN (1992) *Physiology of Fungi*, Villkas Publishing House Hvt. Ltd., London, pp 70-71

Ca, YJ, Chapman SJ, Buswell JA, Chang ST (1999) Production and distribution of endogluconase, cellobiohjydrlase and glycosidase components of the cellulolytic system of *Volvariella vovalceae*, the edible straw mushroom. *Appied Environmental Microbiology* **65 (2)**, 553-559

Chang ST (1982) The need for more varieties of cultivated mushrooms. *Mushrooms Newsletter of the Tropics* **3 (2)**, 2

Chang ST, Fernandez F (1980) Mushrooms, biogas and fertilizer from organic wastes. *Mushrooms Newsletter of the Tropics* **1 (2)**, 1

Fasidi IO (1996) Studies on *Volvariella esculenta* (Muss) Singer. cultivation on agricultural wastes. Proximate composition of stored mushrooms. *Food Chemistry* **55 (2)**, 161-163

Fasidi IO, Ekuere UU (1993) Studies on *Pleurotus tuber-reguim* (Fries) Singer. Cultivation, proximate composition and mineral contents of sclerotia. *Food Chemistry* **48**, 255-258

Fasidi IO, Kadiri M (1993) Use of agricultural wastes for the cultivation of *Lentinus subnudus* in Nigeria. *Revista de Biologia Tropical* **4 (3)**, 411-415

Fasidi IO, Olorunmaiye KS (1994 Studies on *Pleurotus tuber-regium* (Fries) Singer, a Nigerian edible mushroom. *Food Chemistry* **50**, 379-401

Fasola TR, Gbolagade JS, Fasidi IO (2007) Nutritional requirements of *Volvariella spaciosa* Fr. Ex.) Singer, a Nigerian edible mushroom. *Food Chemistry* **100**, 904-908

Galois A, Gross B, Lang LD, Puinler HE, Brunerie P (1990) Influence of culture condition or production of flavour compounds by 29 lignolytic basidiomycetes. *Mycological Research* **94 (4)**, 494-504

Hashioka V (1962) Culture of mushrooms in Thailand. *Tottori Mycological Institute* **2**, 31-35

Kadiri M (1994) Effect of additive on mycelial growth and fructification of *Pleurotus squarrosulus*. Polyporales. *Review of Biology of the Tropics* **42 (6)**, 45-52

Kang H, Hwang S, Lee H, Park W (2002) Efects of high concentrations of plant oils and fatty acids for mycelial growth and pinhead formation of *Hericium erinaceum*. *Transactions of the American Society of Agricultural Engineers* **45 (1)**, 257-260

Ko HG, Park HG, Park SH, Choi CW, Kim SH, Park WM (2005) Comparative study of mycelial growth in seven different species of edible mushroom genus *Hericium*. *Bioresource Technology* **96**, 1439-1444

Nwanze PI, Khan AU, Ameh JR, Umoh VJ (2005) The effect of media oil type and rate on the mycelial wet and dry weight of *Lentinus squarrosulus* (Mont. Singer) and *Psathyrella atrombonata* Pegler in submerged liquid culture. *African Journal of Biotechnology* **4 (3)**, 326-331

Oei P (1991) *Mushroom Cultivation with Special Emphasis on Appropriate Techniques for Developing Countries*, Tool Publications, Amsterdam, The Netherlands, pp 52-60

Okwulehie IC (2004) Studies on *Macrophomina phaseli* (Maub) Ashby growth and some physiological aspects of groundnuts (*Arachis hypogeal* L.) plants infected with the fungus. *Global Journal of Pure and Applied Sciences* **10 (1)**, 23-29

Okwulehie IC, Okwujiako IA, Igbojionu V (2006) Studies on nutritional requirements and growth of *Pleurotus pulmonanus* (Fries.) Quelet, an exotic mushroom. *Nigerian Journal of Botany* **19 (2)**, 308-316

Stamets PS (1993) *Growing Gourmet and Medicinal Mushrooms*, Ten Speed Press, Berkeley, USA, 554 pp

Valazquez-Cedeno MA, Farmet AM, Ferre E, Savoie JM (2004) Variation of lignocellulose activities in dual cultures of *Pleurotus ostreatus* and *Trichoderma longibranchiatum* on unsterilized wheat straw. *Mycologia* **9**, 712-719

Yong FM, Lim G (1986) Effect of carbon source on aroma production by *Trichoderma viride*. *MIRICEW Journal* **2**, 483-488

Zadrazil F (1978) Cultivation of *Pleurotus*. In: Chang ST, Hayes WA (Eds) *The Biology and Cultivation of Edible Mushrooms*, Academic Press, New York, pp 521-558

Zhou T, Reeleder RD, Sparace SA (1996) Influence of nutrient on growth of *Epicocum nigrum*. *Canadian Journal of Microbiology* **42**, 647-654

Zoberi MH (1973) Some edible mushrooms from Nigeria. *Nigerian Field* **38**, 81-90