Mycelia Biomass Yield of *Ganoderma lucidum* Mushroom by submerged culture.

Suberu, H. A, Lateef, A. A, Bello, I. M. and O. A. Y. Daudu. Department of Biological Sciences, School of Natural and Applied Sciences, Federal University of Technology, Minna, Niger State, Nigeria.

Abstract.

Biomass yield of G. lucidum in submerged culture was investigated on account of its requirements for sources of carbon, nitrogen and the carbon : nitrogen (C:N) ratio. The study was carried out with the culture medium of potato dextrose broth enriched with the different carbon (glucose, maltose, starch) and nitrogen (aspartic acid, nitrogen nitrate, peptone) sources over a period of 15 days following standard procedures. The highest biomass of 460±89mg was recorded in the broth medium incorporated with maltose which is significantly different ($p<0.05$) from that of glucose, 310±89mg, and lactose, 250±50mg. There was no growth on starch (0.00±0.000mg) while the control recorded 114±20mg. The highest biomass of 200±39mg among the nitrogen sources was recorded in the medium incorporated with aspartic acid which is also significantly different($p<0.05$) from that of peptone, nitrogen nitrate and the control. A carbon : nitrogen (C:N) ratio (i.e. maltose : aspartic acid) source of 2:2 supported the highest biomass when compared to other carbon : nitrogen ratios. It was therefore deduced, that the presence of maltose as a source of carbon and aspartic acid as a source of nitrogen respectively favoured a good growth of G. lucidum when supplied in a definite amount and ratio. The implications and reasons for these results were highlighted and discussed. It was concluded therefore, that various carbon and nitrogen sources and certain carbon : nitrogen (C:N) ratio have effect on the biomass yield of G. lucidum grown in Potato dextrose broth medium. The findings of this study will be an addition to the baseline information for the growth of the mushroom in submerged cultures for its pharmacological and medicinal potentials.

Keywords: Nitrogen source, Carbon source, Biomass, *Ganoderma lucidum*, Broth medium
Email: alateef123@yahoo.com, +2347039561316
Received: 2013/07/01.
Accepted: 2013/10/10

Introduction.

At present, the demand for natural products with pharmacological activity and medicinal uses is markedly increasing and this is the case with mushrooms from the genus *Ganoderma* (Gonzalez *et. al.*, 2002). In addition, attempts are being made to obtain useful mycelial products or to produce effective substances into a medium by means of liquid culture of the mycelia (Nasreen *et. al.*, 2005). Mycelia biomass powder can be used to formulate various types of health tablets and capsules. Many medicinal and edible mushrooms are capable of growing in the form of mycelia biomass in submerged cultures. The mycelia biomass obtained by submerged cultivation of many species also has higher nutritional value. *G. lucidum* is one of the oldest mushroom known to be used medicinally. *G. lucidum* (Fr.) Krast (Polyporaceae), a basidiomycete is a famous traditional Chinese medicine which has been used as tonic and invigorating medicine (Yan-Qun and Zhi-Cong 2012). *G. lucidum* has common socio-cultural uses across Nigeria as a traditional herb and as an ingredient in herbal preparations, spiritualism and mysticism (Wasser, 2005). *G. lucidum* has a worldwide distribution in both tropical and temperate geographical regions, including South and North America, Africa, Europe, and Asia, growing as a parasite or a saprotroph on a wide variety of trees. Nutritionally, *G. lucidum* contains mainly protein, fat, carbohydrate and fibre.

Medicinally, over 300 reports have been published concerning the constituents of *G. lucidum* and related species. The fruiting body, mycelia, and spores of *G. lucidum* contain approximately 400 different bioactive compounds, which mainly include triterpenoids, polysaccharides, nucleotides, steroids, fatty acids, protein/peptides and trace elements (Wasser, 2005). Recent pharmacological studies have proven that *G. lucidum* extract shows immune regulation (Zhang *et. al*., 2002) and anti-tumour (Li *et. al*., 2011; Liu and Zhong, 2011). Bioactive polysaccharides in mushroom can be extracted from the mycelia of the mushroom species for medicinal and pharmacological activity. Increasing cell biomass production and active ingredient concentrations can enhance the medicinal efficiency of *G. lucidum* (Yan-Qun and Zhi-Cong, 2012). The cultivation of *G. lucidum* mycelium in submerged culture solution was carried out to determine the effect of different carbon and nitrogen nutrient sources on biomass yield.

Materials and methods.

Sources of Materials: The fruiting body of *G. lucidum* used in this study was obtained from the base of a dead and decaying tree

in the Old Teaching and Research Farm, Federal University Of Technology, Minna, Bosso campus, Niger State.

Medium for Tissue culture of *G. lucidum*

Potato Dextrose Agar was used for the cultivation of *G. lucidum*in accordance with Ayodele *et. al*. (2007). 300g of Irish potato (*Solanum tuberosum*) was peeled and boiled with 20g glucose, 20g agar powder, 0.5g chloramphenicol and 250ml distilled water . The filtrate was made up to 1 litre with distilled water. The medium was autoclaved at 121^{o}C for 15min and poured in aliquot of 20ml inside sterile Petri dishes. The Petri dishes with uncontaminated medium were employed for further experiments.

Tissue Culture of *G. lucidum*

The harvested fruiting body was washed under running tap water and then rinsed with sterile distilled water. After drip drying, it was cut with a sterile razor blade into small pieces. One each was placed on the agar medium in the Petri dishes. The dishes were then incubated at normal room temperature of 27±3^{o}C for (6) six days to allow the mycelia to fully colonize the medium.

Preparation of Potato Dextrose Broth (PDB)

Potato dextrose broth (PDB) was prepared in the laboratory using the same procedure as for PDA except for the absence of agar powder. The broth was then distributed into (30) thirty conical flasks of 100ml; each containing 30ml of the broth.

Carbon Nutrition

0.3g of each type of carbon source (glucose, maltose, starch) was added to five (5) conical flasks, each containing 30ml of the growth medium according to the method of Gbolagade (2006). The control medium was constituted without any carbon nutrition.

Nitrogen Nutrition

0.06g of each type of nitrogen source (aspartic acid, nitrogen nitrate, peptone) was added aseptically to five (5) conical flasks each containing 30ml of the growth medium according to the method of Gbolagade (2006). The control medium was constituted without any nitrogen nutrition.

Carbon + Nitrogen Nutrition

A mixture of maltose and aspartic acid served as carbon : nitrogen (C:N) ratio incorporated in the ratios of 1:1, 1:2, 2:1, 2:2 in the 30ml of the growth medium.

Innoculation

Actively growing culture plates of *G. lucidum* mycelium was punched at the advancing edge (Song *et al.*, 2007) with a 2mm cork borer. A disc of the mycelium was then transferred into the broth media in all the conical flasks. The inoculated broth media was then incubated on bench top in the laboratory at room temperature of 27±3^{o}C for (15) fifteen days according to Nasreen *et. al.* (2005).

Harvesting

After the incubation period, the culture media were decanted and each medium was separately filtered using Whatman filter paper No. 1 to get the fresh mycelia after washing with distilled water. The mycelia were oven dried at 70^{0}C (Gbolagade *et. al.*, 2006) until a constant dry weight was obtained. The dry mycelia were weighed in milligrams using Mettler balance and recorded.

Statistical analysis.

The data obtained were analysed using Analysis of Variance (ANOVA) and the group means compared by Duncan Multiple Range Test (DMRT) using the SPSS version 16 computer package.

Results.

The highest biomass 460± 89mg of *G. lucidum* mycelial yield was obtained using maltose as source of carbon, followed by 310mg± 89 with glucose and 250± 50 mg using lactose, while no growth was obtained using starch (0.00mg) as compared with the control 114±20mg (Table 1).

Table 1: Biomass of *G. lucidum* spawn grown on Potato dextrose broth using different carbon sources.

Carbon source	Biomass (mg/30g) at 15 days
Glucose	310± 89^{c}
Maltose	460± 89^{d}
Lactose	250± 50^{c}
Starch	0.00± 0.0^{a}
Control	114±20^{b}

Means with the different superscript are significantly different from each other at 95% confidence level tested by DMRT (Duncan Multiple Range Test).

Different nitrogen sources also influenced mycelial yield in *G. lucidum*. The highest biomass of 200± 39mg was obtained using aspartic acid. It was, however, not significantly different from that obtained using

$NaN0_3$ (130± 92mg) and peptone (172± 45mg), but different from the control (114±20mg) which gave the lowest biomass as shown in Table 2.

Table 2: Biomass of *G. lucidum* spawn grown on Potato dextrose broth using different nitrogen sources

Nitrogen source	Biomass at 15 days (mg/30g)
$NaN0_3$	130± 92[c]
Aspartic acid	200± 39[d]
Peptone	172± 45[c]
Control	114±20 [b]

Means with the different superscript are significantly different from each other at 95% confidence level tested by DMRT (Duncan Multiple Range Test

With respect to carbon: nitrogen ratio, 2:2 was most supportive of mycelial yield, giving 270±83mg, and was significantly different from all other ratio combinations. Ratio 1:1 was next most supportive with 144±27mg, followed by ratio 1:2 producing 130±38mg. The control experiment recorded 114±20mg, supporting better development of mycelium than ratio 2:1 which recorded 84±19mg (Table 3).

Table 3 : Biomass of the *G. lucidum* spawn grown on Potato dextrose broth using different Carbon (Maltose):Nitrogen(Aspartic acid) (C:N) ratio.

Carbon : nitrogen (C:N)ratio	Biomass at 15 days (mg/30g)
1:1	144±27 [a]
1:2	130±38 [a]
2:1	84±19 [a]
2:2	270±83 [b]
Control	114±20[a]

Means with the same superscript are not significantly different from each other at 95% confidence level tested by DMRT (Duncan Multiple Range Test).

Discussion.

Glucose, as a carbon source, had been reported to produce high yield of mycelium of *G. lucidum* at 60g/l concentration (Yuan *et. al*., 2012; Hsieh *et al*., 2006 and Lee *et. al*., 2007). In the present study, maltose was found to produce higher yield of mycelium than all the other carbon sources used, as had been reported by Tang and Zhong (2002). Kadiri and Fasidi (1994) reported that the best utilizable carbon sources for *Lentinus subnudus* were fructose, maltose, dextrin and glucose.The enhancement of biomass yield by maltose, an oligosaccharide, may be explained for reason that maltose has a higher percentage of carbon than the monosaccharides (Rahman *et al.,* 2012) and that the mushroom possesses hydrolytic enzymes which catalyse the hydrolysis of maltose to an assailable monosaccharide (Carlie and Watkinson, 1996).

Aspartic acid produced the highest biomass in *G. lucidum* among the nitrogen sources investigated. This is similar to that of Kuforiji *et al.* (2010) who also reported that peptone significantly increased the growth of *Pleurotus ostreatus* (0.2g/100ml) while inorganic nitrogen sources had no appreciable effect. This implies that the fungus had greater preference for peptone than the inorganic compounds such as sodium nitrate (NaNO3). The higher utilization of aspartic acid compared to sodium nitrate($NaN0_3$) and peptone may be because aspartic acid is an essential amino acid for the mycelia growth of *G. lucidum.* Also, aspartic acid is one of the predominant amino acids found in mycelia extract of *G. lucidum* depending on the composition of the growth medium (Tsung-che and Li-Lin, 1988).

Generally, it has been reported that organic nitrogen sources are more effective than inorganic sources for mycelia growth (Chandana *et. al.*, 2008). However, it was observed in this study that inorganic nitrogen source ($NaN0_3$) also comparatively enhanced mycelia growth of *G. lucidum* which is the same as reported by Kaur (2011) on the growth and sporulation of *Termitomyces triatus*. Also, Osman *et. al.* (2009) reported that Sodium nitrate ($NaN0_3$) at concentration of 2g/L was suitable for producing the highest weight of 12g/L of biomass and crude extract in the growth of two different strains of *Lentinus edodes*.

The ratio of carbon to nitrogen (C: N) balance in mushroom substrate is very important (Stamets, 2000). A well balanced carbon to nitrogen ratio enhances the growth and development of mushrooms while an imbalance of C:N impedes their growth (Stamets and Chilton, 1983; Okhuoya *et. al*., 2000). Since the use of concentrated materials in growing mushroom is intended to correct the Carbon : nitrogen (C:N) ratio and each mushroom has an optimum of C/N ratio for growth, allowing the highest yield in a short period of time, this study revealed that the growth of *G. lucidum* is favored by an equal quantities of carbon and nitrogen elements in the ratio of 2:2. This shows that *G. lucidum* has specific growth requirements, though this differs from the carbon/ nitrogen ratio of 2:3

obtained by Jonathan and Fasisdi (2001) on the growth of *Psathyerella atroumbonata.* This ratio is also different from that obtained by Neelam *et al.* (2013) on the growth of *Pleurotus ostreatus* and *Pleurotus florida* in which they obtained the best growth of 1.1g/L and 1.09g/L respectively.

This study has shown that various carbon, nitrogen and C:N sources have effects on the biomass yield of *G. lucidum* mycelium grown in PD Broth. Therefore, the medium used to grow *G. lucidum* mycelium should be enriched with maltose in order to produce a high biomass.
Further studies is required to determine the mineral elements and other growth factors for yield of the highest biomass of the mycelium and fruit body of *G. lucidum* in the shortest possible time.

References.

Ayodele, S.M., Akpaja, E.O. and F. Anyiador (2007). Evaluation of the yield of *Lentinus squarrosulus. Pakistan Journal of Biological Science*. 10: 4283-4286.

Carlie, M.J., and S. C. Watkinson (1996). Optimization of sub-merged culture conditions for a Nigerian edible fungus biomass production in *Pleurotus florida* (mont.) Singer, *The Fungi*. Academic Press, London. pp.82.

Chandana, J., Ahmed, I., Hyun, H., Geon, W.L., Tae, S.L. and U. L. Youn (2008). Favourable culture conditions for mycelia growth of Korean wild strains in *Ganoderma lucidum. Mycobiology*, 36: 28-33.

Gbolagade, S.J., Sobowale, A. and D. Adejoye (2006). Optimization of sub-merged culture conditions for biomass production in *Pleurotus florida* (mont.) Singer, a Nigerian edible fungus. *African Journal of Biotechnology*, 5 (16):1464-1469.

Gbolagade, S.J. (2006). The effect of different nutrient sources on biomass production of *Lepiota procera* in submerged liquid cultures. *African Journal of Biotechnology,* 5 (12): 1246-1249.

Gonzalez, M.R., Figlas, D., Devalis, R., Delmastro, S. and N. Curvetto (2002). Sunflower seed hulls as a main nutrient source for cultivating *Ganoderma lucidum. Micologia Aplicada International,* 14: 19-24.

Hsieh, C., Tseng, M.H. and C. J. Liu (2006). Production of polysaccharides from *Ganoderma lucidum* (CCRC. 36041) under limitations of nutrients. *Enzyme Microb. Technol.*, 38, 109–117.

Kadiri, M., and I. O. Fasidi (1994). Growth requirements of Lentinus subnudus Berk. A Nigerian edible mushroom. Chemical Microbiology Technology. 16: 80 – 84.

Lee, W.Y., Park, Y., Ahn, J.K., Ka, K.H., and S.Y. Park, (2007). Factors influencing the production of endopolysaccharide and exopolysaccharide from *Ganoderma applanatum*. *Enzyme Microb. Technol.* 40(2): 249-254.

Li, W., Nie, S., Chen, Y., Wang, Y., Li, C., and M. Xie, (2011). Enhancement of cyclophosphamide- induced antitumor effect by a novel polysaccharide from ganoderma atrum in sarcoma 180-bearing mice. *J. Agric. Food Chem*. 59: 3707-3716.

Liu, R.M. and J. J. Zhong (2011). Ganoderic acid Mf and S induce mitochondria mediated apoptosis in human cervical carcinoma HeLa cells. *Phytomedic*. 18: 349-355.

Nasreen, Z., Kausar, T., Nadeem, M. and R. Bajwa (2005). Study of different growth parameters in Ganoderma *lucidum*. *MicologiaAplicada International*, 17: 5-8.

Okhuoya, J.A, Isikhuemhen, O.S, and H. A. Tomo (2000). Effect of soil factors on growth and yield during sporophore induction from sclerotia of Pleurotus tuberregium (Fr.) Sing. *The Int. J. Mushroom Sci.*. 3: 3 – 7.

Rahman, N.A., Daud, F., Kalil, M.S., and S. Ahmad (2012). Tiger Milk Mushroom Cultivation By Using Submerged Culture Technique. *WSEAS TRANSACTIONS on BIOLOGY and BIOMEDICINE*, Issue 3, Volume 9, July 2012 . E-ISSN: 2224-2902.

Song, M., Kim, N., Lee, S. and S. Hwang (2007). Use of Whey Permeate for Cultivating *Ganoderma lucidum* Mycelia. *American Diary Science Association*.

Stamets, P. (2000). Growing gourmet and medicinal mushrooms. Ten Speed Press, California. 574p.

Stamets, P., and J. S. Chilton (1983). The mushroom cultivator. A practical guide to growing mushrooms at home. Agarikon Press Olympia Washinton. p. 415.

Tang, Y.J. and Zhong, J.J. (2002). Exopolysaccharide biosynthesis and related enzymes activities of the medicinal fungus, *Ganoderma lucidum*, grown on lactose in a bioreactor. *Biotechnology Letter*, 24: 1023-1026.

Tsung-Che, T. and L. Li-Lin (1988). Identification of strains by chemical compositions in mycelia extracts. *Botany Bulletin Academia Sinica*, 29: 189-199.

Wasser, S.P. (2005). Reishi or Ling Zhi (Ganoderma *lucidum*). Institute of Evolution, University of Haifa, Mount Carmel, Haifa, Isreal.

Yan-Qun, L. and Z. Zhi-Cong (2012). Promotion and inhibition to biomass and polysaccharides production of *Ganoderma lucidum* by *Sophora flavescens* extract. *African Journal of Microbiology Research* 6(3), pp. 568-574.

Yuan, B., Chi, X., and R. Zhang (2012). Optimization of Exopolysaccharides Production from a Novel Strain of *Ganoderma lucidum* CAU5501 in Submerged Culture. *Brazilian Journal of Microbiology* (2012): 490-497.

Zhang, J., Tang, Q., Zimmerman-Kordmann, M., Reutter, W., and H. Fan (2002). Activation of B lymphocytes by GLIS, a bioactive proteoglycan from *Ganoderma lucidum. Life Science*. 71: 623-638.