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Carbon Dioxide, Humidity, Oxygen and Light Effect on the Growth of *Pleurotus pulmonarius*

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ABSTRACT: Carbon dioxide, humidity, oxygen and light was evaluated on the mycelial growth of *Pleurotus pulmonarius*. Hundred grams (100 g) of paper wastes was weighed into screwed capped bottles and sterilized in an autoclave at 121 °C for 15 min thereafter innoculated with 10 g of *Pleurotus pulmonarius* spawn and incubated for 5 weeks. Mycelia of *P. pulmonarius* after 5 weeks of incubation were exposed to 10 cc of carbon dioxide and 20 cc of oxygen gas ran into the glass jar for a period of 7 days beneath the clamps of mushroom growing in the glass jar. The pressure of flow of each gas was 30 psi while the temperature at the time of introducing the gas was 24.7 °C. The glass jar containing the mushroom was weighed daily before introducing another dosage of gases. A glass jar containing mushroom was also exposed to light and humidity with their weight taken on a daily basis. Results of weekly mycelia growth of *P. pulmonarius* on paper wastes was observed to increase over the incubation period with full mycelia ramification obtained at week 5. Assessment of mineral content in *P. pulmonarius* revealed that control induced the highest contents of Na (0.308 g), K (0.114 g), S (0.258 g), P (0.189 g), Cu (0.860 g) and Zn (6.340 g) while humidity influenced the highest Pb (0.027 g) but the lowest Na (0.024 g), K (0.072 g), Ca (0.073 g), Mg (0.101 g) and S (0.202 g) respectively. The influence of light induced the highest Ca (0.097 g), Mg (0.121 g) and Mn (1.368 g). Proximate content revealed that control had the highest crude protein (3.077 g), crude fat (0.757 g), crude fibre (1.940 g) and ash (1.993 g) while humidity induced the lowest crude protein (1.940 g), crude fat (0.263 g), crude fibre (1.463 g) and ash (1.435 g) but influenced the highest moisture content (88.703 g) while the influence of light induced the least moisture (85.875 g) respectively. This study will help inform mushroom amateurs, consumers and growers the best parameters needed to increase yield in mushroom cultivation.

Keywords: Carbon dioxide; humidity; oxygen; light, *Pleurotus pulmonarius*

Introduction

In Nigeria, higher fungi including mushroom are important constituent of forest produce. These organisms grow on virtually all agro-industrial wastes including most abundant bio molecule of the biosphere, known as cellulose (Chang 2018; Gbolagade, 2005). Mushrooms have been utilized as food in different regions of the world and besides, they are gaining much attention in pharmaceutical industries, medicines and agro allied companies (Aina *et al*., 2012a; Jonathan *et al*., 2018). Mushrooms are eukaryotic organisms that have a cell containing the polysaccharide, chitin, along with lipids and proteins (Aina *et al*., 2012b; Jonathan *et al*., 2018). Some reproduce asexually in a variety of ways (Zoberi, 1972; Alofe *et al*., 1998). Edible mushroom are nutritionally endowed fungi (mostly Basidiomycetes) that grow naturally on the trunks, leaves and roots of trees as well as decaying woody materials (Chang and Miles, 1992; Gbolagade *et al*., 2006).

Mushrooms are not plants, and require different conditions for optimal growth. Plants develop through photosynthesis, a process that converts atmospheric carbon dioxide into carbohydrates, especially cellulose. While sunlight provides an energy source for plants, mushrooms derive all of their energy and growth materials from their growth medium, through biochemical decomposition processes. This does not mean that light is an

irrelevant requirement, since fungi use light as a signal for fruiting (Chang *et al*., 2004). However, all the materials for growth must already be present in the growth medium. Mushroom grow well at relative humidity levels of around 95-100 % and substrate moisture levels of 50 to 75% (Adejoye *et al.*, 2006; Chang *et al*., 2004). Edible mushrooms provide high quality proteins that can be produced with greater biological efficiency than animal protein. Mushrooms are rich in fibres, minerals and vitamins and have low crude fat content and high proportion of poly unsaturated fatty acids (Harkonen *et al*., 1995). Furthermore, mushroom proteins contain all the essential amino acids required for man. About 50-75 % dry weight are carbohydrates, 15-50%dry weight are proteins and 1-15 % dry weight are fats (Harkonen *et al*., 2003; Jonathan *et al*., 2018) and containing vitamins and inorganic minerals (Miles and Chang, 1997; Chang, 2018; Adejoye *et al*., 2009).
Since growth of the fungus produces carbon dioxide as it decomposes the substrate, introduction of 'outside' air reduces carbon dioxide build up and increases oxygen levels. Fungal mycelium is extremely tolerant of carbon dioxide, thriving at 20% carbon dioxide levels. Oxygen is required for formation of fruit bodies. A significant decrease in ambient CO_2 level and increase in oxygen is critical for the initiation and development of primordial. Thus, sufficient air circulation within mushroom fruiting site is vital. Excessive influence of outside air, however, greatly affects both temperature and humidity of the environment (Stamets, 2000).
Mushroom grow best at relative humidity level of around 95-100%, and substrate moisture levels of 50-75%. Mushroom contain essential amino acids, white button mushrooms for example, contain more protein than kidney beans. Shiitake mushroom are less nutritious, but are still a good source of protein (Royce and Schister, 2000). Humidity affects the fruiting bodies' shape. Optimal cultivation conditions vary with strains. High carbon dioxide concentration inside mushroom houses is one of the major causes of abnormalities in fruiting bodies. Proper ventilation is needed in order to reduce co2 concentration. However, too much air caused by excessive ventilation also induces abnormalities in fruiting body shapes. An increase of carbon dioxide concentration can decrease cap sizes and increases length of stipes. However, even stipes are short at CO2 concentrations of more than 0.5%. This study intends to evaluate the sustainability of paper wastes obtained locally from the environment as fruiting substrate on cultivation of edible mushroom *Pleurotus pulmonarius* and to detect the efficiency of factors such as light, oxygen, carbon dioxide and humidity on the growth of cultivated mushroom. The aim of this study is to determine the effect of light, oxygen, carbon dioxide and humidity on the growth of *Pleurotus pulmonarius.*

Materials and methods

Sample collection: Pleurotus pulmonarius spawn was provided by Dr. O.D. Adejoye of the Department of Biological Sciences, Tai Solarin University of Education, Ijagun, Ijebu Ode, Ogun State. Substrate used in this study was paper wastes which were obtained locally within the university environment. The substrate (paper wastes) was chopped and soaked in warm water for one hour and then pressed to expel excess water. One hundred (100 g) of the wet substrate was weighed in replicates into clean screwed capped bottles. The substrates was covered immediately with foil paper and sterilized at 15 kg/m for 15 min using an autoclave. The substrates was allowed to cool to 30±20 °C and thereafter inoculated with 10 g of *P. pulmonarius* spawn and incubated for 5 weeks in a dark room at 30±20 °C. The samples were monitored weekly for mycelia growth. After 5 weeks of incubation, samples were exposed to carbon dioxide, oxygen, light and humidity to determine the effect on the fruit bodies' production. The fresh weight, proximate composition and mineral content were determined.
Evaluation of growth: The growth rate of substrate was observed and measurements of the growth were being taken using a meter rule.
Effect of carbon dioxide, oxygen, ightl and humidity: After five weeks of incubation, the gases were introduced beneath the clumps of mushroom growing in the glass jar. The gases were collected in 8.5 kg cylinder and fitted with pressure gauge and reducing valves.10 cc of carbon dioxide was slowly ran into the glass jar placed in a fume hood over a period of 7 days while 20 cc of oxygen gas was ran into the glass jar for a period of 7 days. The pressure of flow of each gas was 30 psi while the temperature at the time of introducing the gas was 24.7 °C. The glass jar containing the mushroom was weighed daily before introducing another dosage of gases. A glass jar containing mushroom was also exposed to light and humidity with their weight taken on a daily basis.
Proximate and mineral element content determination: Samples were analyzed chemically according to the official methods of analysis described by the Association of Official Analytical Chemist (A.O.A.C., 18th edition, 2005). All analysis was carried out in duplicate. Calcium, potassium, sodium and phosphorus determination were carried out using Spectrophotometric method.
Data analysis: All data obtained was analysed using analysis of variance (ANOVA) by Statistical Package for Social Science (SPSS), significant mean was done using Duncan Multiple Range Test at p(<0.05).

Results

Weekly mycelia growth of Pleurotus pulmonarius on paper wastes: The mycelia growth on the substrate was measured and recorded weekly after inoculation as shown in Fig 1. The highest rate of growth was recorded in week 5 (12.00 cm) while the least mycelia length was observed in week 1 (3.50 cm). Table 1 revealed that control had the highest crude protein (3.077 g), crude fat (0.757 g), crude fibre (1.940 g) and ash (1.993 g) while humidity induced the lowest crude protein (1.940 g), crude fat (0.263 g), crude fibre (1.463 g) and ash (1.435 g) but had the highest moisture (88.703 g) while light influence had the least moisture (85.875 g). The result shows that there was significant difference in proximate content of *P. pulmonarius* under the different parameters.

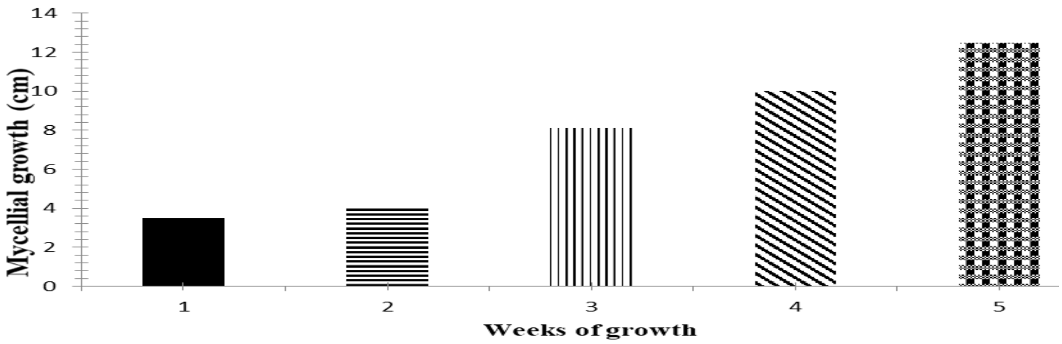

Fig. 1: Mycelia growth of mushroom on paper wastes

Table 1: Proximate content of *Pleurotus pulmonarius* on different parameters

Sample	C. Protein	C. Fat	C. Fibre	Ash	Moisture
Control	3.077 ± 0.018^{c}	0.757 ± 0.009^{c}	1.940 ± 0.006^{c}	1.993 ± 0.009^{c}	85.913 ± 0.015^{a}
CO_2	2.532 ± 0.177^{b}	0.472 ± 0.049^{b}	1.700 ± 0.084^{b}	1.685 ± 0.107^{b}	88.220 ± 0.105^{b}
O_2	2.472 ± 0.090^{ab}	0.423 ± 0.015^{b}	1.635 ± 0.038^{b}	1.623 ± 0.060^{b}	88.320 ± 0.048^{b}
Humidity	2.188 ± 0.043^{a}	0.263 ± 0.013^{a}	1.463 ± 0.009^{a}	1.435 ± 0.010^{a}	88.703 ± 0.015^{c}
Light	3.068 ± 0.041^{c}	0.748 ± 0.025^{c}	1.938 ± 0.020^{c}	1.963 ± 0.029^{c}	85.875 ± 0.019^{a}

Values followed by the same superscript along each vertical column are not significantly different by Duncan P (<0.05).
Keys: CP: Crudeprotein ; CF: Crude fat; CFIB: Crude fibre; MC: Moisture content; A: Ash

Mineral content of Pleurotus pulmonarius on different parameter: Table 2 shows that control had the highest Na (0.308 g), K (0.114 g), S (0.258 g), P (0.189 g), Cu (0.860 g) and Zn (6.340 g) while humidity had the highest Pb (0.027 g) but had the lowest Na (0.024 g), K (0.072 g), Ca (0.073 g), Mg (0.101 g) and S (0.202 g). Light was observed to induce the highest Ca (0.097g), Mg (0.121g) and Mn (1.368g) respectively.

Table 2: Mineral content of *Pleurotus pulmonarius* on different parameters

Sample	Na	K	Ca	Mg	S	P	Cu	Mn	Zn	Pb
Control	0.308 ± 0.231^{b}	0.114 ± 0.001^{c}	0.096 ± 0.001^{c}	0.119 ± 0.001^{c}	0.258 ± 0.001^{c}	0.189 ± 0.001^{c}	0.860 ± 0.006^{c}	1.350 ± 0.006^{c}	6.340 ± 0.006^{c}	0.017 ± 0.001^{ab}
CO_2	0.052 ± 0.009^{a}	0.089 ± 0.004^{b}	0.081 ± 0.003^{b}	0.109 ± 0.003^{b}	0.223 ± 0.008^{b}	0.171 ± 0.003^{b}	0.603 ± 0.040^{b}	1.203 ± 0.026^{b}	6.050 ± 0.087^{b}	0.021 ± 0.002^{b}
O_2	0.047 ± 0.005^{a}	0.084 ± 0.001^{b}	0.076 ± 0.001^{ab}	0.106 ± 0.001^{ab}	0.220 ± 0.003^{b}	0.168 ± 0.001^{b}	0.575 ± 0.013^{b}	1.190 ± 0.015^{b}	5.993 ± 0.035^{b}	0.019 ± 0.001^{b}
Humidity	0.024 ± 0.001^{a}	0.072 ± 0.001^{a}	0.073 ± 0.001^{a}	0.101 ± 0.002^{a}	0.202 ± 0.001^{a}	0.158 ± 0.002^{a}	0.495 ± 0.021^{a}	1.085 ± 0.010^{a}	5.805 ± 0.036^{a}	0.027 ± 0.001^{c}
Light	0.076 ± 0.002^{ab}	0.110 ± 0.004^{c}	0.097 ± 0.002^{c}	0.121 ± 0.002^{c}	0.256 ± 0.003^{c}	0.188 ± 0.003^{c}	0.855 ± 0.028^{c}	1.368 ± 0.019^{c}	6.335 ± 0.033^{c}	0.014 ± 0.001^{a}

Note: values followed by the same superscript along each vertical column are not significantly different according to Duncan multiple range test (P <0.05).

Discussion

The effect of carbon dioxide, humidity, oxygen and light on the yield of *Pleurotus pulmonarius* was evaluated in this study. Mycelia growth was observed for 5 weeks and record was taken from the 1st week of growth to the last week. The substrate used was paper wastes, mycelia length increases as incubation period increases. This reveals that waste materials contain nutrients that support the growth of mushroom (Akinmusire *et al.*, 2011). Dlaminiet *al*. (2013) reported that *Pleurotus* species have the ability to convert agricultural wastes to protein, the protein content of mushroom depend on substrate medium. Okhuoya and Okogbo (1991) attributed the ability of mushroom substrates to support the growth of mushrooms to the presence of lignin, cellulose and mineral elements as well as its residual oil.

The proximate and mineral content of mushroom at different parameters revealed that protein value ranged from 2.188-3.077 % with least value obtained from humidity and highest value obtained from control. Increase in CP contents may be due to secretion of extracellular enzymes which are proteinous in nature into the waste during break down and its subsequent metabolism (Kadiri, 1999). Loeffen (1995) reported on carbon dioxide production in terms of temperature and he noted other studies on CO_2 change as related to variation in yield. The result of mineral content revealed that light is the most suitable parameter that gave the highest result followed by CO_2, O_2 and humidity. This shows that light and other environmental factors are suitable for the cultivation of mushroom and this is similar to the work of Trukhonoverts (1991) who reported that appropriate growth of fruiting bodies is also affected by length of lighting period in the 24 h rhythm. Kadiri and Kehinde (1999) reported that mushroom growth is highly influenced by several factors such as spawn, media, pH, temperature, moisture content and light intensity.

Result from this study revealed that humidity induced low mineral elements and this is in agreement with the work of Stamets (2000) who reported that excessive influence of outside air, however, greatly affects both temperature and humidity of the environment. Sharma (2012) reported that light is not necessary for development of *Pleurotus* mushroom mycelia, but it is important for proper growth of mushroom fruiting bodies (Royse and Zaki, 1991; Trukhonovets, 1991). Jonathan *et al.* (2018) reported that variation in protein value of mushroom may be due to biological, chemical difference and C/N ratio of growth media. Nutritional compositions of substrates are crucial in determining how mycelia growth initiation seems and also due to the available proportion of carbon and nitrogen. Sharma (2012) reported that all outstanding growth of mycelium is a vital factor in mushroom cultivation.

One of the way by which wastes can be recycled is through the cultivation of mushroom which grows on agro industrial wastes. It has a lot of economic importance which include food for human because of its nutritional value, medicinal value which helps in the curing of many diseases, job opportunities and to alleviate poverty in the society. Therefore, the cultivation of mushroom requires a good substrate in which it grows upon and environmental factors such as light, humidity etc. for suitable development of fruiting bodies.

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