

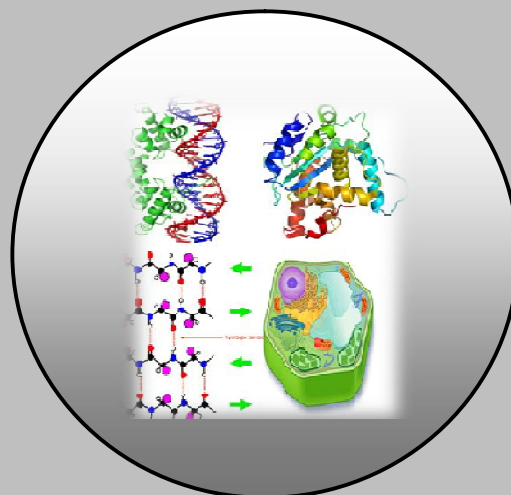
# Growth, Yield and Nutritional Quality of *Pleurotus tuber-regium* (Fries) Singh grown on Sawdust of Different Wood Types from South-Western Nigeria

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## **Growth, Yield and Nutritional Quality of *Pleurotus tuber-regium* (Fries) Singh grown on Sawdust of Different Wood Types from South-Western Nigeria**

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### **ABSTRACT**

Studies were conducted on the growth, yield and nutritional quality of *Pleurotustuberregium* grown on sawdust of different wood origin (*Afzeliaafricana*, *Cordiamillenii*, *Tectonagrandis*, *Triplochytonscleroxylon*, *Chlorophoraexcelsa*, *Gmelinaaborea*). This was aimed at evaluating the influence of these sawdust sources on the performance of the mushroom using complete randomized design with three replications. Significantly highest mushroom yield and biological efficiency (93.10g and 88.63%) were obtained on *G. aborea* and the lowest (50.87g and 48.44%) on *C. excelsa*. Longest daily mycelium growth (0.63cm) was recorded on *C. millenii* and the least (0.38cm) was on mixed bed. However, with regards to protein (13.96 g/100g), phosphorus (0.38 g/100g), fat (0.86 g/100g) and ash (3.94 g/100g) contents, the mushroom grown on *C. excelsa* had the highest. Conversely, the mushroomcultivated on *Afzelia Africana* were the least. In terms of yield, *G. aborea* is adjudged as the best wood type for the cultivation of *P. tuberregium*.

**Keywords:** Mycelium growth, Sclerotium, *Gmelinaaborea*, Biological efficiency andPhytochemical content.

### **INTRODUCTION**

Mushroom cultivation is an effective way of extracting biological resources left behind in agricultural and industrial wastes and at the same time protecting the environment from pollution either by burning or careless dumping of such wastes.

The recycling of these wastes through mushroom growing may be a solution to the conversion of inedible biomass wastes into nutritious protein rich food in the form of mushrooms (Mshandete and Cuff 2007, 2008).

Food rich in essential nutrients like protein, vitamins and minerals obtainable from meat, fish, eggs, beans, fruits and vegetables are not within the reach of the rural poor and the levels of the intake of these food values determine their health status. In Nigeria, carbohydrate is culturally the major source of food intake, one of the reasons why malnutrition is rampant in the country (Iyangbe and Orewa 2009). Majority of Nigerians do not consume the Food and Agriculture Organization (FAO) recommended 60g per person daily protein requirement for individuals (FAO 1992) Nigeria's per capita daily protein intake is estimated to be 45.4g as against the FAO's minimum protein recommendation.

Proximate analysis of mineral and energy content of edible mushrooms indicate their potentials for use as sources of good quality food and dietary supplements. The amount of crude protein, ash, and crude fibre found in most edible mushroom species compare favourably with, and in some cases surpasses those of legumes except groundnut and soya beans (De-Veries 1979; Ologbogbo 1981 and Oso 1977). In view of the increased popularity of health foods, the dietary and medicinal importance of mushrooms cannot be over emphasized; it is therefore desirable to encourage the production and consumption of edible mushroom species. *Pleurotustuberregiumis* a tropical mushroom that forms both fruit body and a resting structure called sclerotium either in the soil or in any substrate on which it is growing, the sclerotium is globose to ellipsoid in shape. It is popularly consumed in Nigeria for its exotic, nutritive and medicinal effects (Aletor and Aladetimi 1989). The fruit body is cooked alongside with other vegetable mixes while the sclerotium is added with ground egusi melon, seasoned with traditional spices, wrapped in banana leaves, and steamed and this is served along with palm wine during important traditional rites such as weddings and is a common practice among the Igbo ethnic group in Nigeria and they generally refer to this mushroom as "osun" (personal communication).

The biological conversion of agricultural and agro-industrial wastes into protein-rich edible mushroom is a developing enterprise with a lot of advantages (Ayodele *et al.* 2007). In Nigeria, studies on the cultivation of mushrooms include those of (Kadiri and Fasidi 1990; Isikhuemhen 1999, Idowu, 2003 and Idowu *et al.* 2009)

Wood debris and byproduct of wood processing (sawing and sawdust), pollutes the environment. In many parts of the world they are simply dumped or burned. Even though is a material suited for biodegradation (Williams 2001). In Nigeria wood waste are not properly disposed, they are usually burned or dumped in water ways especially along the coastal region of the country, (Adewolue *et al.* 2009) reported that one of the sources of organic waste into the Lagos lagoon is the waste material from Okobaba sawmill industry located along the coast of the Lagos lagoon system which houses about 2,150 sawmills at the bank of the Lagos lagoon (Dosumu and Ajayi 2009) and which is highly destructive to the inhabitants of these coastal areas who are majorly fisher men.

Through the production of edible fungi, value can be added to these wood processing by-products (sawdust) by using them to produce protein rich food in form of edible and medicinal mushrooms which is an economically viable biological technology for the

Conversion of this waste into high quality protein rich food and this will naturally open up new job opportunities especially in the rural areas (Hussein *et al.* 2003). The aim of this present investigation is to evaluate the use of sawdust from different wood sources on the growth, yield and nutritional quality of *P. tuberregium*.

## MATERIALS AND METHODS

This study was carried out in the mushroom production unit of the Vegetable Programme of National horticultural research Institute (NIHORT), Ibadan, Nigeria.

Pure culture of *Pleurotustuberregium* was obtained from the tissue culture of the mushroom fruit body generated from the sclerotium of the mushroom and maintained on potato dextrose agar throughout the period of investigation.

**Table 1. Growth and yield of *P. tuberregium* grown on sawdust of different wood sources.**

Sawdust sources	Initial substrate weight (g)	Final substrate weight (g)	Total sclerotia yield (g)	Production efficiency (%)	Mycelia extension (cm)
<i>Afzeliaafricana</i>	300	188.06	59.57	31.68	12.24
<i>Cordiamillanii</i>	300	200.2	58.16	29.05	13.2
<i>Gmelinaaborea</i>	300	256.88	93.10	36.24	11.84
<i>Triplochitonscleroxylon</i>	300	247.45	63.38	25.61	10
<i>Chlorophoraexcelsa</i>	300	281.68	50.87	18.06	10.96
<i>Tectonagrandis</i>	300	263.45	52.35	19.87	12.16
Mixed/bed	300	183.22	66.02	36.03	8.08
LSD	ns	1.42	0.97	0.43	0.92

## Spawn preparation

Spawn was prepared using *sorghum bicolor* (guinea corn) seeds which were washed in running tap water to remove empty kernels and soaked overnight to allow for moisture absorption. The seeds were drained the following day and parboiled for 15 minutes and thereafter drained until water was no longer dripping, the seeds were added with 10% (v/v) rice bran and the pH was adjusted with 1% calcium carbonate. The mixture was then filled into 200ml spawn bottles, covered with cotton wool wrapped in aluminum foil and sterilized at 121°C for 30minutes. After cooling down to room temperature, the substrate bottles were then inoculated with actively growing mycelium block from agar slant and incubated at ambient temperature for twenty one days when the mycelium had fully grown throughout the entire substrate. This is the mother spawn and it was used to prepare the planting spawn which was used to inoculate another set of sorghum seeds prepared as above which was then used to inoculate the sawdust substrates for both mycelium growth and fruit body production.

### Growth of the Mycelium

Sawdust from the following trees, *Afzelia africana*, *Cordia millenii*, *Tectona grandis* (Teak), *Triplochyton scleroxylon* (obeche), *Gmelina aborea* and *Chlorophora excelsa* were collected from Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. They were separately sundried to prevent any form of degradation, bagged and stored until needed. Each sawdust of the various wood sources and a mix bed containing a mixture of equal volumes of all the sawdust employed were added with 1% calcium carbonate, these were separately moistened with water to a level of about 65% moisture content, covered with polyethylene sheet to prevent the substrates from drying out and these were left to compost for seven days after which test tubes of 25 x 150mm were filled with the composted sawdust the mouth of which were plugged with cotton wool wrapped in aluminum foil with each treatment replicated six times. These test tubes containing the various sawdust from the above tree sources were sterilized at 121°C for 15 minutes, after cooling down to room temperature, the test tubes were then inoculated with fresh mycelium disc (5mm diameter) of *Pleurotuberregium* according to the method of Fasidi and Ekuere, (1993) and were incubated at 30°C for 22 days. Vertical mycelium extension was taken four days after inoculation and thereafter taken every other day.

**Table 2** Proximate composition (g/100g dry matter) of *Pleurotuberregium* grown on sawdust of different wood types.

Wood type	N	protein	Phosphorus	Fat	moisture	ash	phenol
<i>A. africana</i>	1.68	10.53	0.31	0.84	7.40	2.72	0.21
<i>C. millenii</i>	1.61	10.03	0.36	0.71	5.27	3.23	0.23
<i>G. aborea</i>	1.58	9.87	0.30	0.77	8.31	3.52	0.32
<i>T. scleroxylon</i>	1.78	11.1	0.30	0.75	9.54	3.64	0.27
<i>C. excelsa</i>	2.23	13.96	0.38	0.86	7.48	3.94	0.13
<i>T. grandis</i>	1.99	12.42	0.36	0.76	8.46	3.14	0.17
Mixed bed	1.66	10.39	0.30	0.48	7.00	2.56	0.27
LSD	0.12	0.81	0.02	0.11	0.48	0.33	0.05

LSD: Least significant

### Cultivation of the Sclerotium

Substrate samples consisting of sawdust from the named trees above were treated as above 300g each of the various sawdust types were packed in polyethylene bags held in place with polyvinyl chloride (PVC) pipe plugged with cotton wool, covered with aluminum foil with each treatment replicated three times. These bags were then packed in a steamer and were steamed for 6 hours after which they were allowed to cool down to ambient temperature and thereafter inoculated with the freshly prepared spawn of *P. tuberregium*. The spawned substrates bags were incubated for 100 days in the dark at 30±2°C with each treatment replicated six times.

Sclerotia were harvested twice with the first harvest done 2 months after spawning and the second was done 40 days after the first harvest. The harvested sclerotia were weighed for the determination of biological efficiency (BE) which is the percentage ratio of fresh sclerotia harvested to substrate dry weight and production efficiency (PE) which is also the percentage ratio of fresh sclerotia harvested to substrate weight before sclerotia cropping.

### Proximate and mineral compositions

Proximate analysis was done using freshly harvested sclerotia from the different treatments. The samples were oven dried at 80°C for two days, weighed, powdered in a Moulinex blender and sieved through a 450µm sieve. The final powdered samples were stored in desiccators until needed for all the analysis to be done.

Nitrogen was determined by the micro-Kjeldhal method. Moisture content was done according to the method of AOAC (1998). Dry mushroom sample (1g) was placed in a pre-weighed crucible in a muffle furnace heated gradually 550°C and the resulting residue was weighed to determine the ash content (AOAC 1984). The residues from the incinerated samples above were used for the determination of macro and micro elements contents of all the samples.

**Table 3** Mineral composition of *Pleurotustuberregium* grown on sawdust of different wood types.

Wood type	Ca (%)	Mg (%)	K (%)	Na (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)
<i>A. africana</i>	0.09	0.09	0.26	62.14	6.90	39.98	1.83	12.69
<i>C millenii</i>	0.13	0.10	0.30	89.22	11.10	86.52	1.53	12.31
<i>G aborea</i>	0.19	0.10	0.26	334.97	16.97	92.30	1.15	13.60
<i>T. scleroxylon</i>	0.22	0.14	0.30	149.98	24.64	87.32	1.50	21.04
<i>C excelsa</i>	0.29	0.15	0.29	195.02	30.60	85.65	1.82	18.01
<i>T grandis</i>	0.16	0.12	0.30	110.48	12.94	58.22	2.19	15.32
Mixed bed	0.12	0.10	0.28	82.56	9.59	53.50	2.43	13.74
LSD	0.07	ns	ns	20.49	1.56	7.00	0.63	1.98

### Phytochemical content of the sclerotium

Oxalate content of the powdered sample of the mushroom sclerotia was estimated as oxalic acid equivalent using the method of (Lambert and Muir 1976) reported by (Hassan *et al.*, 2011). The presence of saponins in the mushroom sclerotia samples was done by the method described by (Odebiyi and Sofowora 1978). To 1g of powdered sample 30ml of tap water was added. The mixture obtained was vigorously shaken and warmed; the frothing that persisted for 30min. was taken as an indication of the presence of saponins. Tannin content was determined according to the method of Allen *et al.* (1974). The method of Ola and Oboh (2001) was adapted for phytate quantification.

## RESULTS AND DISCUSSION

All the sawdust sources employed in this study supported sclerotia production of *P.tuberregium* although the sclerotia yield varied with the sawdust source. *G. aborea* recorded the highest biological efficiency while *Tectonagrandis* and *Chlorophora excelsa* recorded the lowest (Fig 1). The mushroom flushed twice on all the sawdust of the wood types tested and the biggest flush sizes were at the first flush on all the wood types with the exception of *G. arborea* which flushed best at the second flush and recorded the highest sclerotia yield (Fig 2). Initial wet substrate weight for the different sawdust tested was the same but reduced at the time the sclerotia were ready for harvesting. The highest reduction in weight was observed on sawdust of *Cordiamillennii* and the lowest on that of *Chlorophora excelsa* and *Tectonagrandis*.

Production efficiency was highest on *G. aborea* and the lowest was on *Chlorophora excelsa*. The growth of the mycelium was best on *Cordiamillennii* and least on mixed bed (Table 1). These results are indications that *P. tuberregium* will grow on a large range of wood types and couple with the fact that sawdust is in abundance, cheap and almost free in Nigeria there is need to be guided as to which wood types support the mushroom growth and yield most. A report similar to the one obtained above was given by (Abott *et al.* 2009) in which the yield of *Lentinus squarrosulus* was observed to be influenced by sawdust sources. Final substrate weight was lower than initial substrate dry weight this could be as a result of production of hydrolyzing and oxidizing extracellular enzymes by the mycelium of *P. tuberregium* which brought about the degradation of the lignin content of the sawdust substrate as the mushroom is popularly referred to as "wood rot fungi" (Oei 2003). In a similar work by (Morais *et al.* 2000), they reported that during the cultivation of four strains of *Lentinus edodes* on sawdust substrate, cellulose and lignin content of the substrate were degraded resulting in weight loss of the final substrate when compared to the initial weight of the substrate at inoculation. *G. aborea* produced the highest sclerotia yield while *Chlorophora excelsa* produced the least, this result suggests that sawdust source influences both growth and yield of *P. tuberregium* hence the need for guided use of sawdust in the cultivation of this mushroom (Abott, *et al.*, 2009).

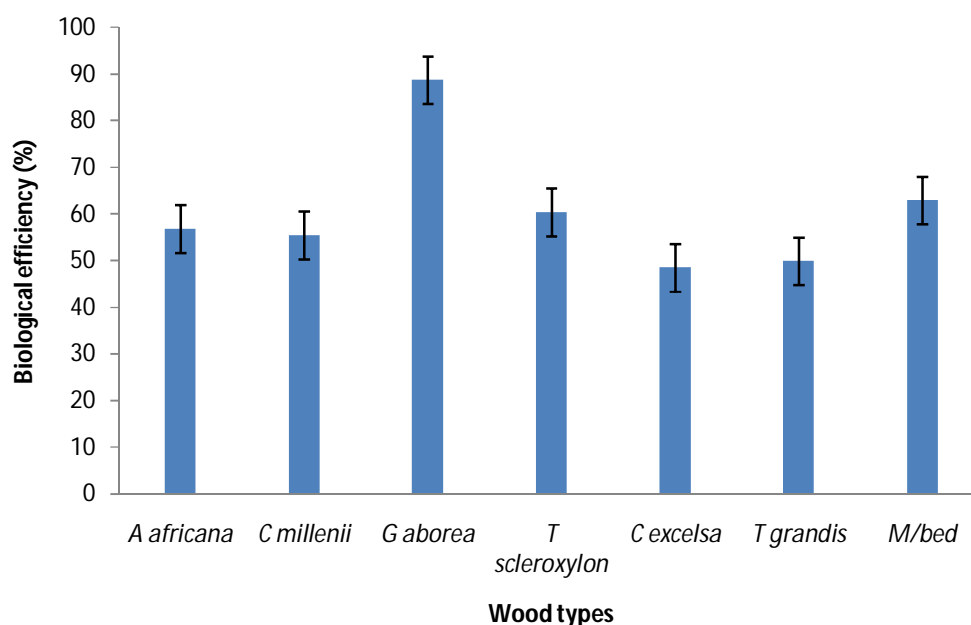
**Table 4. Phytochemical composition of *P. tuberregium* grown on sawdust of different wood types (mg/100g dry weight).**

Wood types	TANIN	SAPONIN	PHYTATE	OXALATE	PHENOL
<i>Gmelina aborea</i>	0.21	11.24	0.15	0.11	0.32
Mixed bed	0.17	10.24	0.22	0.16	0.27
<i>Azela africana</i>	0.31	9.75	0.12	0.13	0.21
<i>Chlorophora excelsa</i>	0.02	8.67	0.11	0.11	0.13
<i>Tectonagrandis</i>	0.12	10.48	0.13	0.12	0.17
<i>Triplochiton scleroxylon</i>	0.21	10.75	0.12	0.12	0.27
<i>Cordiamillennii</i>	0.15	9.78	0.11	0.12	0.23
LSD	0.10	0.93	0.03	ns	0.04

The proximate composition of the sclerotium of *P. tuberregium* grown on sawdust of different wood sources is summarized in table 2 below. Nitrogen content was significantly highest (2.23%) at  $p < 0.05$  in mushroom sclerotia harvested from *C. excelsa* followed by *T. grandis* (1.99%) and least in *G. aborea*. Protein was also highest significantly (13.96%) in sclerotia from *C. excelsa* and least (9.87%) in *G. aborea*. Highest fat content (0.86%) was obtained on mushroom grown on *C. excelsa* and the lowest was obtained on mixed bed. *T. scleroxylon* sawdust produced mushroom sclerotia with the highest moisture content (9.54%) with the lowest occurring on those from the mixed bed. Ash content was significantly highest (3.94%) in sclerotia harvested from *C. excelsa* and least on those obtained from the mixed bed. Phenol was significantly highest (0.32%) at  $p < 0.05$  in sclerotia obtained on *G. aborea* and least on those from *C. excelsa*. Table 3 presents the mineral element profile (mg/g) of *P. tuberregium* sclerotium grown sawdust of various wood sources. The most abundant nutritive element in the cultivated sclerotia on all the different sawdust was potassium which was similar to that obtained by earlier workers (Khanna *et al.* 1992 and Akindahunsi and Oyetayo 2005) who found the highest mineral to be potassium in various species of edible mushrooms analyzed. The abundance of potassium in the sclerotium tissue may be due to absorption and accumulation of this element from the substrate and this is essential as potassium plays an important role in the synthesis of amino acids and proteins (Malik and Stravastava 1982). Significantly highest percentage of potassium (0.3%) was recorded on sclerotia obtained on *C. millenii*, *T. scleroxylon* and *T. grandis* sawdust and the least obtained on the others. Copper was found to be the least abundant element of all the elements evaluated, it is required in trace quantity and useful in combination with manganese, play important roles in enzymatic catalyses and are crucial in all biological and physiological processes in living organisms (Saiqa, *et al.* 2008). The results of the phytochemical screening of all the mushroom sclerotia harvested from sawdust of different wood sources employed in this study are shown in table 4. The chemical analysis revealed the presence of tannin, saponin, phytate, oxalate and phenols which varied quantitatively in all the sclerotia harvested. Tannin concentration were generally low (0.02 to 0.31 mg/100g in sclerotia harvested on *C. excelsa* and *Cordia millenii*), saponin ranged between 8.67 in *C. excelsa* and 11.24 mg/100g in *G. aborea*, phytate content ranged from 0.11 to 0.22 mg/100g in, oxalate content, 0.11 to 0.16 mg/100g, 0.13 to 0.32 mg/100g. Content of phenols in all the mushroom sclerotia ranged between 0.13 to 0.32 mg/100g. According to (Enujiugha and Agbede 2000) tannin usually forms insoluble complex with proteins, thereby interfering with their bioavailability. Poor palatability is usually attributed to high tannins in diets (Mehansho *et al.* 1987). From the results of the analysis of the phytochemical contents of the sclerotia of *P. tuberregium* grown on sawdust of different wood types, tannin concentration (mg/100g) was generally low (0.02 to 0.31 mg/100g). These levels might not affect the nutritional potential of the mushroom sclerotia since they are all less than 10% of the total dry weight of the mushroom sclerotia sample (Osagie 1996; Akindahunsi and Oyetayo 2005). Saponins were the most abundant of all the phytochemicals in the mushroom sclerotium ranging from 8.67 mg/100g in sclerotium harvested on *C. excelsa* to 11.24 mg/100g on *G. aborea* and are known for their ability to lower the blood cholesterol levels and also inhibit the growth of cancer cells by interfering with their DNA (Alyssa, 2007).



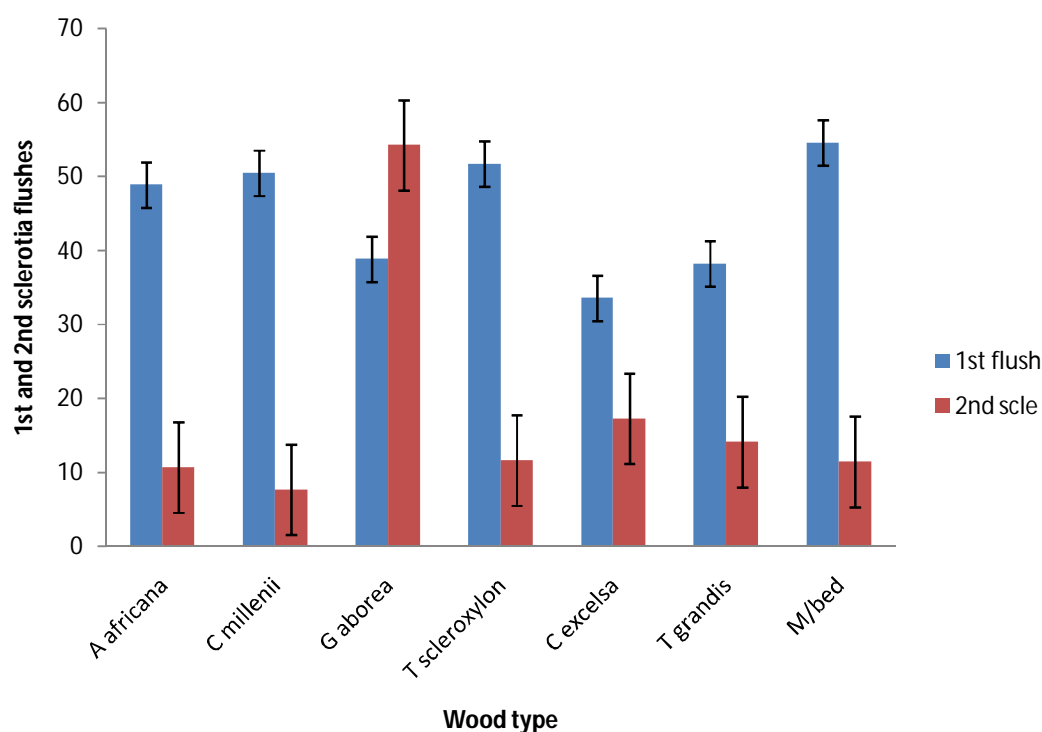
The phytate content in the mushroom sclerotia was low and ranged between 0.11mg/100g in *C. excelsa* and *C. millenii* and 0.22mg/100g in *G. aborea*. This concentration is lower than the amount reported by (Akindahunsi and Oyetayo, 2005) who reported  $385 \pm 0.2$  mg/100g in the sclerotium of wild *P. tuberregium* the difference could be as a result analytical procedure used and varietal differences (wild and cultivated). Generally, the phytate content of the mushroom sclerotium analyzed is low compared with green leafy vegetables whose phytate content is found to be exceptionally high (Odebiyi and Sofowora 1978). Phytic acid forms very stable complexes with mineral ions rendering them unavailable for intestinal uptake because the first step in mineral absorption requires that the mineral remain in the ionic state (Lopez, *et al.* 2002). thus indicating mineral deficiencies. Therefore the low phytate content is of nutritional significance here because it may allow for the bioavailability of many essential minerals and therapeutic effects of this sample towards disease is guaranteed.



**Fig 1. Effect of sawdust of different wood types on biological efficiency of *P. tuberregium*.**

Oxalate concentration in all the sclerotia ranged between 0.11 to 0.16mg/100g and it was the lowest occurring phytochemical in the mushroom sclerotia analyzed. (Saiqa *et al.* 2008) reported 0.667 and 0.539mg/100g of oxalate concentration in the fruit bodies of *Agaricus bisporus* and *A. bitorquis* respectively, this amount is higher than the one obtained in this study, the oxalate content of food can vary considerably between plants of the same species, due to differences in climate, soil quality, state of ripeness, or even which part of the plant is analyzed (Savage *et al.* 2002).

Content of phenol in the mushroom sclerotia harvested from the different sawdust sources ranged between 0.13 to 0.32mg/100g. it is the second most abundant of the phytochemicals analysed for. Phenols are known to be part of phytonutrients known to prevent cancer by blocking specific enzymes that cause autoimmune diseases, protecting against heart attacks and strokes. It also prevents the platelets in the blood from clumping, reversing nerve cells ageing and destroying hepatotoxins which damage the liver (Alyssa 2007). From the results obtained in this study, it can be inferred that *G. aborea* sawdust is suitable for the cultivation of *P. tuberregium* and that the mushroom has the potential to convert the substrate into nutritious food. The phytochemicals they contain is so low in concentration and are unlikely to produce any undesirable effect on the consumer.



**Figure 2. Effect of sawdust of different wood types on sclerotia flush of *P. tuberregium* 14 weeks after inoculation.** (A) First sclerotia harvest (1<sup>st</sup> flush) (B) Second sclerotia harvest (2<sup>nd</sup> flush).

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