Utilization of agro-industrial wastes for the cultivation of *Pleurotus tuber-regium* (Fries) Singer, a Nigerian edible mushroom

Segun Gbolagade Jonathan and Busayo Babalola
Mycology & Biotechnology Unit, Department of Botany, University of Ibadan, Nigeria
E-mail: sg.jonathan@mail.ui.edu.ng

Abstract
In this study 16 agro-industrial wastes as well as simple organic and inorganic compounds were annexed for vegetative growth and fruitbody production of *Pleurotus tuber-regium*, a Nigerian edible mushroom. Utilization of these wastes for mushroom growing will be helpful in their conversion to edible protein in terms of mushroom fruit bodies. Different wastes (substrates) were used to cultivate this functional food. Each investigation was carried out in three replicates and the experimental set up was in a completely randomized block design using standard methods. The results obtained were subjected to analysis of variance (ANOVA) using general linear model options of SAS, while test of significance was determined by Duncan´s multiple range test at 0.5% level of probability. *Pleurotus tuber-regium* produced different degrees of mycelia biomass on varieties of agro-industrial wastes used. *Khaya ivorensis* sawdust produced the best mycelia extension (65.0mm), followed by *Terminalia ivorensis*, cotton wastes and rice straw ($p > 0.05$), while poultry manure stimulated mycelia extension by 3.0mm, which was the least. Among different growth media used, malt extract agar stimulated the best growth of 98.3 mm, while potato dextrose agar produced mycelia growth of 95.0 mm, closely followed by yeast extract, sorghum, millet, and corn meal agar with mycelia extension of 87.7, 86.0, 85.0, and 80.0mm, respectively. Sweet potato agar produced the least mycelia extension of 10.0 mm. Effect of organic carbon and nitrogen compounds on the growth of *Pleurotus tuber-regium* in submerged liquid culture showed that glucose and yeast extract were the best carbon and nitrogen compounds with 205.3 and 210, 0 mg per 30 cm3, respectively. Solid state fermentation of agro-industrial wastes showed that composted *Khaya ivorensis* sawdust produced an average of 15 fruitbodies after the first flush, while non composted sawdust of the same wood produced an average of 9 fruitbodies after the first flush.

Keywords: Agro-industrial wastes, cultivation, environment, *Pleurotus tuber-regium*, pollution

Introduction

Pleurotus species belong to the kingdom fungi, phylum basidiomycota and order agaricales (Alexopolus *et al.,* 1996) .They are generally referred to as 'oyster mushrooms because of their naturally occurring flavour. Edible oyster mushrooms are excellent delicacies in many regions of the world (Iwase *et al*.,2000; Jonathan *et al*.,2008).Among Pleurotus mushrooms, the edible species include *P. florida, P. pulmonarius, P. osteatus* and *P. tuber-regium* (Fasidi *et al*,2008) .In Nigeria, the most popular edible oyster mushroom is *P. tuber-regium* (Gbolagade,2006)**.** *P. tuber-regium* is one of the most common Nigerian edible mushrooms.

This fungus usually grows wildly on decomposing wood during the rainy season where it always produce sclerotium during unfavorable weather condition (Oso, 1977). It could be easily identified by the leathery texture of matured carpophores, velvety stipe and funnel-shaped whitish pileus (Zoberi 1972). The spore print of *P.tuber-regium* is white and spore size ranged between 3–7 and 2–5m along the major and minor axis respectively (Zoberi, 1972).The sporophores of *P. tuber-regium* are soft and flexible when young (12–36 h)but become very tough and leathery when fully matured(4–9 days old) (Jonathan *et al*.,2008). This fungus, locally called 'olu awo' (Oso, 1977), could be chewed for a long time and, because of its desired taste, it serves as a good alternative to meat among rural populace whom may not be able to afford the exorbitant price of meat, due to high cost of living.

The need for commercial production of *P.tuber-regium* and other edible mushrooms in Nigeria cannot be over emphasized in view of their potential contribution to agricultural, nutritional, environmental and health values (Jonathan *et al*, 2008; Oluranti *et al*, 2012). Mushrooms could be regarded as source of cheap protein especially for adults that required low cholesterol in their diet (Aina *et al*.,2012).They could be cultivated on various waste products of human, agricultural, forestry and industries, sources.(Jonathan

and Esho,2010). Thus, the growth of fungi on these substrates has helped to prevent environmental and health hazards posed by indiscriminate dumping of these materials (Jonathan *et al*., 2012). Edible fungi may also be utilized medically (Olawuyi *et al*., 2010; Oluranti *et al*., 2012).The objective of this investigation were to asses the agro-industrial wastes as well simple as organic and semi synthetic substrates for biomass and fruitbodies production of *P. tuber-regium*, a Nigerian edible mushroom

Materials and Methods

The wild fungus (*Pleurotus tuber-regium*) sporophores were collected from Ibadan University Botanical Gardens. Tissue culture procedure of Jonathan and Fasidi (2001) was employed to obtain mycelia starter culture of this fungus. The pure culture obtained was maintained on plates of PDA supplemented 0.5% malt extract. Sixteen agro-industrial wastes used for this investigation (Table 1) were sun dried for 7days.10.0g of each wastes was separately mixed with 20.0 ml of distilled water and dispensed into100mm (diameter) plates. These Petri-dishes were sealed with aluminum foil and sterilized at 1.02 kg/cm2 pressure at 121^0C for 15 min. After cooling, each plate (replicated 3 times) was inoculated with an actively growing mycelia disc (10.0 mm) of 5-day-old culture of *P. tuber-regium* and incubated at 30 ± 2^0C for 7 days. Growth rates were determined by measuring radial mycelia extension. Influence of 17 growth media (Table 3) on biomass production in *P.tuber-regium* was also determined using the method of Aina *et al* (2012).The effect of carbon, nitrogen and mineral elements on biomass yield of this fungus were also carried out (Jonathan and Fasidi 2001).The fruitbodies production on 10 agro -industrial wastes were assessed using the procedure Okhuoya and Okogbo (1991) as modified by Jonathan *et al (*2012).

Data Analysis

The data were subjected to analysis of variance using SPSS version 16 ,while treatment means were carried out by Duncan Multiple Range Test at $P<0.05$.

Results and Discussion

Table 1 shows that *P. tuber-regium* could thrive on all the 16 agro-industrial wastes used in this study. This fungus produced the best mycelia biomass on *K. ivorensis* sawdust with mycelia extension of 65.0mm, followed in order by *T. ivorensis*, cotton wastes and rice straw ($P\geq0.05$) while poultry manure stimulated mycelia extension of 3.0mm which was the least. This observation was agreement with reports of Okhuoya and Okogbo (1991); and that of Jonathan *et al (*2012).These authors suggested that oyster mushrooms have high saprophytic ability to grow on variety of agro industrial wastes. The poor growth of this fungus obtained on poultry manure may be due to the acidic nature of this substrate. Among different growth media used, malt extract agar stimulated the best growth of 98.3 mm, while potato dextrose agar produced mycelia growth of 95.0 mm, closely followed by yeast extract, sorghum, millet, and corn meal agar with mycelia extension of 87.7, 86.0, 85.0, and 80.0mm, respectively (Table2). Sweet potato agar produced the least mycelia extension of 10.0 mm. The high supportive nature of malt extract to the vegetative growth of this fungus may be related to its high nutrient composition (Jonathan and Fasidi, 2001).

The effect of organic carbon and nitrogen compounds on the growth of *P. tuber-regium* in submerged liquid culture (Table 3) showed that glucose and yeast extract were the best carbon and nitrogen compounds with 205.3 and 210,0mg/30cm^3 respectively. The preference for glucose to other organic carbon compounds used in this study could be linked to the ease with which this sugar is metabolized during cellular respiration Ability of glucose and yeast extract in supporting the biomass production in other mushrooms have been documented (Jonathan and Fasidi,2001;Aina *et al*,2012). Dextrin and urea were observed to stimulate least grow of this fungus. Inability of dextrin and urea to stimulate vegetative growth of *P. tuber-regium* in sub merged medium may be due to their toxicity to fungal cells .It may also be due to the fact that this mushroom may lack ability to produce hydrolyzing enzymes which could convert these substrates to utilizable cellular products.

Table 4 showed that *K. ivorensis* sawdust produced average of 15 fruitbodies after the first flush while non composted sawdust of the same wood has average of 9 fruitbodies after the first flush. It was observed from the results that composted agro-industrial wastes significantly enhanced fruitbody production in *P.tuber-regium* than non fermented substrates. This result was similar to earlier observation

of Jonathan *et al* (2012), on *P. pulmonarius* .These workers reported that composting is a solid-state fermentation process, which exploits the principles of elimination of competing microorganisms. After the succession of different microorganisms in the composting substrates, the fermented wastes will now be utilized for the growth of mushroom mycelia with little or no competitors.

References

1. Aina, DA. Jonathan SG. Olawuyi, OJ. Ojelabi, DO and Durowoju, BM (2012).Antioxidant, antimicrobial and phytochemical properties of alcoholic extracts of *Cantharellus cibarius* – Nigerian mushroom. *New York Science Journal* 5(10):114-120
2. Alexopoulous, C.J., Mims, and Blackwell M. 1996. *Introductory Mycology*. 3rd Edition. John Wiley and Sons, New York
3. Fasidi I.O, Kadiri M, Jonathan SG, Adenipekun CO, Kutororiji CO (2008). Cultivation of edible tropical mushroom. Ibadan university press 81 pp.
4. Gbolagade J.S (2006). Bacteria associated with compost used for cultivation of Nigerian edible mushrooms: *Pleurotus tuber-regium* (Fr.) Singer, and *Lentinus squarrosulus* (Berk.) *African Journal of Biotechnology*.5 (4) :338-342.
5. Iwase K,Umezawa Y , Masuda K(2000).Cultivation of *Pleurotus ostreatus* with beer spent grains and utilization.Mush Sci 15(2):819-826.
6. Jonathan S.G. and Fasidi,I.O (2001). Effect of carbon, nitrogen and mineral sources on growth of *Psathyrella atroumbonata* ,Pegler. A Nigerian edible mushroom. *Food Chemistry*.72:479 – 483.
7. Jonathan,S.G;Fasidi,I.O:Ajayi,A.O and Adegeye,A(2008).Biodegradation of Nigerian wood wastes by *Pleurotus tuber-regium* (Fries) Singer. *Bioresource Technology* 99:807-811.
8. Jonathan SG,Adegboyega AA and Oyelakin AO.(2012). Cultivation of *Pleurotus pulmonarius* Fr. Singer on fermented and non fermented sawdust *New York Science Journal* 5(11):36-40.
9. Okhuoya, J.A. and Okogbo, F.O. (1991). Cultivation of *Pleurotus tuber-regium* (Fr.) Sing. on various farm wastes. *Proceedings of Oklahoma Academy of Science* 71:1-3.
10. Olawuyi OJ, Jonathan S.G**,** Aina DA and Baysah GI(2010).Studies on antibacterial activities of *Fomes lignosus*-A Nigerian mushroom. *Nigerian Journal of Microbiology* 24(1):2153-2159.
11. Oluranti OO,Olawuyi O.J and Jonathan S.G(2012). Therapeutic properties of some Nigerian higher fungi. *Nature and Science* 10(10):135-143.
12. Oso, BA. 1977. *Pleurotus tuber-regium* from Nigeria. *Mycologia.* 67(2): 271-277.
13. Zoberi, M. H. 1972. Tropical Macrofungi. Macmillan Press. London

Table1: Biomass yield (mycelial extension) of *P. tuber* regium on agro-industrial wastes

Wastes	Mycelia extension (mm)	Mycelia density
Rice straw	45.7± 0.3c	
Cotton waste	50.0± 1.4bc	+5
Khaya ivorensis	65.0± 3.7a	+5
Terminalia ivorensis	53.7± 2.3bc	+4
Androgon sp	35.0± 6.5dc	+3
Groundnut shell	6.3± 1.7 h	+1
Melon pericarp	7.7± 0.5 gh	+1
Cassava peels	10.0± 3.3gh	+1
Banana leaves	30.3± 1.6de	+1
Palm wastes	12.0± 3.4g	+1
Con cob	9.7±0.6gh	+1
Cocoa leaves	10.5± 3.0fg	+1
Coffee leaves	15.0 ± 3.0 fg	+1
Paper wastes	28.0± 0.3e	+2
Soybean wastes	8.3 ± 1.7gh	+1
Poultry manure	3.0 ± 0.7h	+0.5

Each value is the mean of 3 readings ± SE . Values followed by the same letter(s) are not significantly different by Duncan's multiple test ($P \leq 0.05$)

Table 2: Effects of different growth media on biomass production (mycelia extension (mm)) in *P. tuber-regium* (mean of 3 replicates)

Growth media	Mycelia extension	Mycelia density
Brown beans agar	55.0±1.6	+
Carrot agar	60.7±3.3	+7
Cassava peels agar	25.0±1.3	+1
Cellulose powder agar	45.3±2.3	+3
Corn meal agar	80.0±3.7	+9
Groundnut agar	40.7±0.9	+2
Millet agar	85.0±5.8	+10
Malt extract agar	98.3±3.3	+14
Peptone dextrose agar	50.0±2.9	+5
Potato dextrose agar	95.0±1.7	+13
Saboraud dextrose agar	70.3±2.3	+8
Sorghum agar	86.0±3.3	+10
Soy bean agar	67.3±2.7	+8
Sweat potato agar	10.0±0.9	+1
Yam peels agar	25.7±1.3	+1
Yeast extract agar	87.7±5.8	+12
Yellow maize agar	90.3±4.3	+11

Values followed by the same letter(s) are not significantly by Duncan's multiple range test (P≤0.05).

Table 3: Effect of organic carbon and nitrogen compounds biomass Production in *P. tuber* – regium

Organic compounds	Biomass yield (mg/30ml)	Final pH
Control (basal medium only)	20.3±1.3	7.1
(a) Carbohydrate sources		
Fructose	180.0±5.8b	6.0
Glucose	205.3±3.3a	6.7
Maltose	120.7±43c	6.6
Raffinose	85.0±1.6d	5.9
Mannitol	90.3±0.9d	6.3
Sorbitol	47.0±0.7f	6.2
Dextrin	40.3±2.3f	6.4
Soluble starch	75.0±0.7e	6.6
(b) Protein sources		
Alanine	140±2.3c	6.7
Glutamine	120±3.1d	5.9
Methionine	100±5.8d	6.2
Tryptophan	150±4.1c	6.3
Casein	70±0.9e	6.8
Peptone	170±4.3b	5.9
Urea	55±1.7f	5.8
Yeast extract	210±3.3a	6.8

Each value is the mean of 3 replicates ± SE. Means followed by the same letter(s) are not significantly different by Duncan's multiple range test (P≤ 0.05).

Table 4: Effect of fermented and non fermented agro-industrial wastes on fruitbody yield of *P. tuber* – regium

Substrates	(g) ANF(1-3F)	fresh weight(g)	dry weight(g)
Rice straw (F)	11a	5.7d	2.8c
Rice straw (NF)	7de	3.4ef	1.1f
Cotton wastes (F)	10c	8.3c	2.7c
Colton wastes (NF)	5f	2.5fg	0.8fg
Paper wastes (F)	6ef	2.8fg	0.9fg
Paper wastes (NF)	3g	1.8g	0.6g
T. ivorensis SD (F)	13ab	10.1b	3.2b
T. ivorensis SD (NF)	9cd	5.1d	1.8de
K. ivorensis SD(F)	15a	12.0a	3.9a
K. ivorensis SD(NF)	9cd	5.8d	2.0d

Values followed by the same letter(s) along column are not significantly different by multiple range test (P ≤ 0.05). **Key:** F = fermented; NF = Non fermented ;ANF =Average no of fruit bodies SD=saw dusts