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Effect of phytochemical composition of agricultural wastes on yield and proximate compositions of *Pleurotus pulmonarius* (fries.) que'let

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ABSTRACT

Four substrates, coir fibre (obtained from coconut husk), oil palm waste, sawdust of *Gmelina arborea* and rice straw were used for this study. The highest protein content (29.42%) was found in *P.pulmonarius* harvested from coir fibre, the lowest (29.03%) in those from sawdust with 20% rice bran level (RBL) producing *P.pulmonarius* with the highest protein content respectively. There was no significant difference in the values of protein obtained across the four substrates. The highest carbohydrate contents (82.44%) was obtained in *P.pulmonarius* harvested from 40% RBL coir fibre with the lowest (40.88%) from 0% RBL rice straw. The fat content of *P.pulmonarius* across the four substrates used were found to be relatively low (ranging from 0.79%) for coir fibre to 2.09% for sawdust). The highest ash content (7.99%) was found in coir fibre at 20% RBL. while the lowest (5.98%) was found in 40% RBL of sawdust with no significant difference across the RBL within the substrates used. The crude fibre obtained (ranging from 12.10-12.74%) also shows no significant difference across the substrates with the highest obtained from *P.pulmonarius* cultivated at 40% RBL across the four substrates. Phytochemical screening of the substrates shows the presence of tannin, alkaloid, saponin, flavonoid, phenolic compound, oxalate and cardiac glycoside. Phenol and flavonoid were highest in oil palm waste with values of 12.9mg/g and 33.01mg/g. The lowest values of phenol and flavonoid were found in rice straw with values ranging from 1.05mg/g and 11.75mg/g. The mean spawn running in percentage as exhibited by different substrates and RBL in percentage on weekly basis shows that all substrates were fully ramified at the fourth week. Biological efficiency (B.E) in percentage as exhibited by different substrates shows that there was significant difference between the substrates used. © 2013 Trade Science Inc. - INDIA

KEYWORDS

Pleurotus pulmonarius;
Phytochemical constituents;
Coir fibre;
Biological efficiency.

INTRODUCTION

Mushrooms are important constituents of forest produce^[7,14]. These non-photosynthetic organisms are

regarded as higher fungi with distinctive fruiting body. The carpophores could either be epigeous or hypogeous and they develop large sporophores which could be large enough to be seen with the naked eyes and

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picked by hands^[1,5,11].

Mushrooms are eukaryotic organisms that have cells containing the polysaccharide, chitin, along with other sugars, lipid and proteins. Some reproduce sexually while some reproduce asexually^[8]. They lack chlorophyll hence, they are unable to carry out photosynthesis^[10,38]. Geologically, mushrooms existed on earth before man, as evident from the fossil records of the lower cretaceous period. Anthropologically speaking, there is every possibility that man used the mushrooms as food when he was still a food gatherer and hunter on the chronology of cultural evolution^[6,7,15].

The edible mushrooms are highly nutritious and compare favourably with meat and other sources of proteins. Efforts to accurately measure protein nutritional quality started at the beginning of the 19th century. Two of the most commonly used techniques include net protein ratio (NPR) and protein efficiency ratio (PER), both of which are used to assess damage to proteins during processing and to rank foods according to their protein quality^[11,12,21]. Mushrooms are rich in protein, minerals and vitamins; they contain an abundance of essential amino acids^[22,33]. Thousands of years ago, fructifications of higher fungi have been used as a source of food^[16,23]. Due to high amount of proteins, they can be used to bridge the protein malnutrition gap. They are used as nutrient supplements to enhance immunity in the form of tablets^[19,30]. Yilmaz *et al.*^[37] and Pedneault *et al.*^[29] reported that fat fraction in mushrooms is mainly composed of unsaturated fatty acids. Mushrooms are one of the best sources of vitamins especially Vitamin B^[23]. Mushrooms are low in total fat content and have a high proportion of polyunsaturated fatty acids (72 to 85% relative to total fat content, mainly due to linoleic acid. The high content of linoleic acids is one of the reasons why mushrooms are considered as health food^[10,31,33]. Edible mushrooms provide high quality proteins that can be produced with greater biological efficiency than animal protein. Mushrooms are rich in fibers, minerals and vitamins and have low crude fat content and high proportion of polyunsaturated fatty acids^[17-19,27]. Furthermore, mushroom proteins contain all the essential amino acids required for man. About 50-70% dry weight are carbohydrates, 15-50% dry weight are proteins and 1-15% dry weight are fats^[18] and containing vitamins and inorganic minerals^[9,10]. Today,

P.pulmonarius is a potential protein source especially in developing countries where animal protein is scarce and expensive^[29]. Presently, sawdust is the major substrate used in commercial cultivation of *P.pulmonarius* of in Nigeria. Due to technology advancement, the so called waste (sawdust) is now been used greatly for the production of briquette, shelf, board, office table and furniture generally. The ongoing publicity of mushroom as a high source of protein with low cholesterol content which over ride meats and other fatty foods, may soon diminish due to the fore-scarcity of the sawdust^[10,20].

Hence, other agricultural wastes such as pawpaw leaves, rice straw, coconut husk, corn cob, sugar cane bargass etc. which are not in high demand presently, can be a remedy for the above mentioned problem. With the upsurge in unemployment rate in developing countries, small-scale mushroom cultivation with these agriculture and forest fruit wastes could serve as a means of employment and for more income generation. Meanwhile, with new findings on the yield and nutritional compositions of *P.pulmonarius* grown on agricultural wastes, based on this we would be able to establish the yield performance and nutritional composition of *P.pulmonarius* as well as the phytochemical contents of agricultural wastes. The objectives of this work are therefore to determine the proximate and nutritional composition of *P.pulmonarius* and to determine the influence of phytochemical compositions of the substrates on the yield of *P.pulmonarius*.

MATERIALS AND METHODS

Collection of samples

Pleurous pulmonarius used for this experiment was collected from the Mushroom Unit of Forestry Research Institute of Nigeria. The substrates used for this research were: Coir fibre (Coconut husk fibre), Oil palm waste, Sawdust (*Gmelina arborea*) and Rice straw (*Oryza sativa* L.), Coconut husk was obtained from Araromi Badagry, Lagos Nigeria, Oil palm waste was obtained from Oje market, Ibadan Oyo State Nigeria, saw dust was collected from Bodija saw mill in Ibadan while Rice straw was obtained from African Rice Unit, of the International Institute of Tropical Agriculture, (IITA.) Ibadan. Oyo State, Nigeria.

The additives used were rice bran (*Oryza sativa* L.)

and Lime (CaCO₃). The lime was added to obtain PH ranging from 6.5 to 7.5 as suggested by Owen^[25]. The rice bran was obtained from African Rice Unit of the International Institute of Tropical Agriculture (IITA) Ibadan, Oyo State.

The wheat grain used for spawn production was purchased from Bodija market, Ibadan.

Experimental set up

The experiment was set up using Complete Randomised Design (CRD) with 3×4×5 arrangements.

Preparation of substrates

The coconut husks were pulverised at the Department of Geology, University of Ibadan using Highland Park Milling Machine after pounding with mortar. The rice straw were cut into 3-5cm^[35] at Botany Department of The University of Ibadan

Bagging of the substrates

2.8kg of each of the substrates was weighed on the weighing balance in five places. Since the substrates were non-composted the actual weights were taken as 70% of the total weight that is;

$2.8\text{kg} \times 70/100 = 1.96\text{kg}$ / (This is the Actual Weight) To the first 2.8kg of each substrate, 1% actual weight of lime was added that is;

$1.96\text{kg} \times 1/100 = 0.0196\text{kg}$ of lime added no rice bran (This represented the control 0% level of rice bran) To the second 2.8kg of each substrate, 1% actual weight of lime and 10% Actual weight of rice bran were added that is;

$1.96\text{kg} \times 10/100 = 0.196\text{kg}=196\text{g}$ was added, (This represented the 10% level of rice bran) To the third 2.8kg of each substrate, 1% actual weight of lime and 20% Actual weight of rice bran were added that is;

$1.96\text{kg} \times 20/100 = 0.392\text{kg}=392\text{g}$ was added, (This represented the 20% level of rice bran) To the fourth 2.8kg of each substrate 1% actual weight of lime and 30% Actual weight of rice bran were added that is;

$1.96\text{kg} \times 30/100 = 0.588\text{kg}=588\text{g}$ was added, (This represented the 30% level of rice bran)

To the fifth 2.8kg of each substrate, 1% actual weight of lime and 40% Actual weight of rice bran were added that is;

$1.96\text{kg} \times 40/100 = 0.784\text{kg}=784\text{g}$ was added, (This represented the 40% level of rice bran) Each of

these aforementioned measured substrates was then mixed with water enough to soak the substrate but which when squeezed with the hands dropped no water. After mixing thoroughly with rice bran, lime and water, 500g of each rice bran level substrates were packed into autoclavable polythene bags in three replicates. The mouth of each polythene bag was tied with rubber band and pasteurized in drums for 6hrs.

Inoculation of substrates bags

The substrate bags were allowed to cool down after pasteurization, holes were bored aseptically into the substrate bags and 25grams (5%) of *P. pulmonarius* spawn was used to inoculate the various bags of rice bran level (0-40% substrates). The bags were kept in a dark room for mycelia ramification of the various substrates, the mycelia growth was measured each week for five weeks and the various substrate bags when fully ramified were exposed and kept in the mushroom house of Pathology unit of Forestry Research Institutes. These were watered daily and when the mushroom started sprouting, they were harvested, weighed for fresh and dry weight, pileus diameter, stipe length and mushroom height were also measured.

Yield and biological efficiency

The total weight of all fruiting bodies harvested for all the flushes were measured as Total Yield of the mushroom while the Biological Efficiency (B.E) was also calculated as given by Chang *et al.*^[10] using the formula:

$$\text{B.E} = \text{WM}/\text{WDS} \times 100\%$$

WM=Weight of fresh or dried mushroom harvested (g)

WDS=Weight of dried substrate

Proximate analysis

Analysis of moisture, protein, fat, crude fibre, ash, carbohydrate, protein and mineral elements (sodium, manganese, iron, copper, zinc, magnesium, calcium and potassium) contents were done by standard methods^[2] for *P.pulmonarius*.

Phytochemical analysis

Phytochemical screening of the substrates for tannin, alkaloid, saponin, flavonoid, phenols, oxalate and cardiac glycoside were done by standard methods. Tannin^[3,14], Alkaloids and flavonoid^[17], Saponin^[4,7] and

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Oxalate.

Statistical analysis

Data obtained were analyzed using Analysis of Variance (ANOVA). The means were separated with Duncan Multiple Range Test (DMRT) using Statistical Packages for Social Science (SPSS) Version 18.

RESULTS AND DISCUSSION



Plate 1: Freshly inoculated wheat grain for mother spawn



Plate 2: Ramifying wheat grain bottles



Plate 3: Fully ramified Spawn bottles



Plate 4 : *P. pulmonarius* cultivated on coir fibre



Plate 5 : *P. pulmonarius* cultivated on oil palm waste



Plate 6 : *P. pulmonarius* cultivated on sawdust



Plate 7 : *P. pulmonarius* cultivated on rice straw

Phytochemical analysis of the substrates

Phytochemical screening of the substrates shown in TABLE 1 reveals variation in the content of tannin, alkaloid, saponin, flavonoid, phenolic compound, oxalate and cardiac glycoside, while Steroids and an-

thraquinones were absent. Flavonoids and phenol were higher (12.93mg/g and 33.01mg/g) in oil palm waste followed by saw dust (6.79mg/g and 16.81mg/g). Rice straw had the least value of flavonoid and phenol (1.05mg/g and 11.75mg/g).

TABLE 1 : The Phytochemical analysis of different substrates used for mushroom cultivation

Substrates	Tannin	Alkaloid	Saponin	Flavonoid (mg/g)	Phenol	Oxalate	Cardiac glycoside
Coir fibre	1.51 ^c	3.7 ^{bc}	0.34 ^b	5.16 ^c	12.70 ^c	0.03 ^c	0.26 ^b
Oil palm waste	3.91 ^a	4.9 ^b	0.28 ^b	12.93 ^a	33.01 ^a	0.02 ^b	0.98 ^a
Sawdust	1.83 ^b	11.1 ^a	0.90 ^a	6.79 ^b	16.81 ^b	0.42 ^a	0.31 ^b
Rice straw	0.32 ^d	3.0 ^c	0.21 ^b	1.05 ^d	11.75 ^c	0.01 ^b	0.07 ^b

Each value is the mean for three replicates. Mean carrying the same alphabet are not significantly different (Pd^{**}0.05) using DMRT

Proximate composition analysis of *P.pulmonarius*

TABLE 2 shows the proximate composition of *P. pulmonarius*, from the four different substrates, added with different levels of rice bran in percentages. The protein content is higher in *P.pulmonarius* in all the four substrates used at 20% RBL. The highest protein content (29.4%) was obtained in *P.pulmonarius* grown on coir fibre followed by those from oil palm waste and rice straw (29.2%) then sawdust with protein value of 29.03%. There were no significant difference in the values of protein obtained for the four substrates. The carbohydrate contents was higher in *P.pulmonarius* harvested from coir fibre at 40% RBL followed by sawdust at 20% RBL, oil palm waste at 30% RBL and rice straw at 20% RBL with values of 82.4%, 47.1%, 43.93% and 40.9% respectively. The percentage moisture content was higher in the mushroom from rice straw at 20% RBL (8.4%) followed by saw dust at 30% RBL (7.9%), oil palm waste at 30% RBL (7.3%) and coir fibre at 40% RBL (6.0%). The ash content obtained in the mushroom from all the substrates shows no significant difference across the RBL within the substrates used. The highest ash content (8.0%) was found in those from coir fibre at 20% RBL. while the lowest (6.0%) was found in 40% RBL of sawdust. The crude fibre obtained shows no significant difference within the mushrooms. The highest crude fibre was found in 40% RBL across the four substrates with their values ranging from 12.1 to 12.7%.

Mineral elements composition of *P. pulmonarius* cultivated on different substrates

TABLE 3 shows the mineral element composition of *P.pulmonarius* from different substrates at different RBL. The mineral content of *P. pulmonarius* harvested varied with substrates and RBL. *P. pulmonarius* was found to be richer in potassium followed by calcium and Magnesium while Manganese was the least. The values of Potassium ranges from 2000mg/100g for *P.pulmonarius* harvested at 0% RBL of rice straw to 3020mg/100g for those harvested at 10% RBL of rice straw. Calcium values obtained ranges from 230 mg/100g for *P.pulmonarius* harvested at 30% RBL for rice straw to 390mg/100g for those harvested at 10% RBL of saw dust. The calcium content ranges from 230-390mg/100g with the least value (230mg/g) from mushroom cultivated at 30% RBL rice straw and the highest value (390mg/100g) from those at 10% RBL sawdust. The Mg content of the mushroom obtained ranges from 97 to 267mg/100g in which the lowest was found at 0% RBL rice straw and the highest value was obtained for *P. pulmonarius* at 20% RBL of oil palm waste.

The highest Zn value of 76.0mg/100g was recorded for *P. pulmonarius* cultivated at 20% RBL of coir fibre. Highest Na content (115mg/100g) in *P. pulmonarius* cultivated on 20% RBL of rice straw and the least (37.5mg/100g) in 40% RBL of oil palm waste. The iron content varied from 8.1 to 12.4mg/100g with the highest value recorded for the mushroom cultivated with 10% RBL added to rice straw.

However, copper and Manganese had highest val-

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ues for *P. pulmonarius* grown with 10% rice bran added to rice straw (12.9mg/100g) and 40% of rice bran added to coir fibre (9.3mg/100g) respectively.

Growth analysis of *P. pulmonarius*

TABLE 5 shows that *P. pulmonarius* was harvested up to four times in oil palm waste and sawdust and five

times in coir fibre and rice straw. The mean Stipe length (cm) per flush measured, ranges from 4.3-7.3cm with *P. pulmonarius* harvested from rice straw having the highest mean stipe length at the second flush and those from oil palm waste having the least mean stipe at the second flush. The total mean value ranges from 4.08±2.16 to 6.68±0.39cm.

TABLE 2: Proximate composition of *P. pulmonarius* cultivated on different substrates at different rice bran level (%)

Substrates	Ricebran Level (%)	Proximate composition (%)					
		Fat	Ash	Moisture	Crude fibre	Protein	Carbohydrate
Coir fibre	0	1.06 ^a	7.02 ^a	4.64 ^b	11.27 ^b	28.86 ^a	36.19 ^a
	10	1.14 ^c	7.59 ^a	5.21 ^c	11.74 ^b	28.74 ^a	59.81 ^a
	20	1.35 ^c	7.29 ^b	5.47 ^c	12.66 ^a	29.42 ^a	63.34 ^a
	30	0.84 ^c	7.99 ^a	5.59 ^b	12.52 ^b	23.87 ^a	80.63 ^a
	40	0.79 ^b	6.08 ^a	6.04 ^d	12.74 ^a	22.11 ^a	82.44 ^a
Oil palm Waste	0	1.03 ^a	7.19 ^a	5.19 ^b	11.30 ^b	26.61 ^b	38.84 ^b
	10	1.30 ^b	7.36 ^b	5.86 ^b	11.78 ^b	27.75 ^b	41.02 ^b
	20	1.83 ^b	7.23 ^b	6.87 ^b	12.30 ^b	29.22 ^b	43.12 ^c
	30	1.06 ^b	7.46 ^b	7.26 ^{ba}	12.26 ^c	23.79 ^b	43.93 ^b
	40	0.90 ^{ba}	6.03 ^{ba}	6.42 ^b	12.50 ^b	21.61 ^b	42.81 ^b
Sawdust	0	1.00 ^a	7.20 ^a	5.30 ^a	11.02 ^c	26.12 ^c	39.44 ^b
	10	1.32 ^b	7.10 ^c	6.00 ^b	11.66 ^b	27.87 ^b	40.94 ^b
	20	2.09 ^a	6.99 ^c	6.70 ^b	12.20 ^b	29.03 ^c	47.09 ^c
	30	1.12 ^b	6.79 ^c	7.90 ^a	12.38 ^a	25.22 ^c	22.28 ^d
	40	1.00 ^a	5.98 ^b	6.98 ^a	12.67 ^{ba}	21.29 ^d	25.32 ^c
Rice straw	0	1.03 ^a	7.35 ^a	5.63 ^a	11.62 ^a	24.84 ^a	18.94 ^c
	10	1.44 ^a	7.39 ^b	6.37 ^a	11.94 ^a	26.64 ^c	22.32 ^c
	20	2.04 ^a	7.42 ^a	8.43 ^a	12.03 ^c	29.22 ^b	40.88 ^d
	30	1.22 ^a	7.59 ^b	8.28 ^a	11.89 ^d	22.78 ^d	28.59 ^c
	40	0.91 ^{ba}	6.02 ^{ba}	6.24 ^c	12.10 ^c	21.42 ^c	20.79 ^d

Each value is the mean for three replicates. Mean carrying the same alphabet are not significantly different (Pd^{0.05}) using DMRT

Mineral elements composition of *P. pulmonarius* cultivated on different substrates

TABLE 3 shows the mineral element composition of *P. pulmonarius* from different substrates at different RBL. The mineral content of *P. pulmonarius* harvested varied with substrates and RBL. *P. pulmonarius* was found to be richer in Potassium followed by Calcium and Magnesium while Manganese was the least.

The values of Potassium ranges from 2000mg/100g for *P. pulmonarius* harvested at 0% RBL of rice straw to 3020mg/100g for those harvested at 10% RBL of rice straw. Calcium values obtained ranges from 230 mg/100g for *P. pulmonarius* harvested at 30% RBL

for rice straw to 390mg/100g for those harvested at 10% RBL of saw dust. The calcium content ranges from 230-390mg/100g with the least value (230mg/g) from mushroom cultivated at 30% RBL rice straw and the highest value (390mg/100g) from those at 10% RBL sawdust. The Mg content of the mushroom obtained ranges from 97 to 267mg/100g in which the lowest was found at 0% RBL rice straw and the highest value was obtained for *P. pulmonarius* at 20% RBL of oil palm waste.

The highest Zn value of 76.0mg/100g was recorded for *P. pulmonarius* cultivated at 20% RBL of coir fibre. Highest Na content (115mg/100g) in *P.*

pulmonarius cultivated on 20% RBL of rice straw and the least (37.5mg/100g) in 40% RBL of oil palm waste. The iron content varied from 8.1 to 12.4mg/100g with the highest value recorded for the mushroom cultivated with 10% RBL added to rice straw.

However, copper and Manganese had highest values for *P.pulmonarius* grown with 10% rice bran added to rice straw (12.9mg/100g) and 40% of rice bran added to coir fibre (9.3mg/100g) respectively.

Growth analysis of *P.pulmonarius*

TABLE 3 shows that *P.pulmonarius* was harvested up to four times in oil palm waste and sawdust and five times in coir fibre and rice straw. The mean Stipe length (cm) per flush measured, ranges from 4.3-7.3cm with *P.pulmonarius* harvested from rice straw having the highest mean stipe length at the second flush and those from oil palm waste having the least mean stipe at the second flush. The total mean value ranges from 4.08±2.16 to 6.68±0.39cm

TABLE 3 : Effect of substrates, on stipe length per flush of *P.pulmonarius*

Substrate	The mean stipe length (cm)					Total Mean±SD
	flush1	flush2	flush3	flush4	flush5	
Coirfibre	6.4	7.1	5.7	6.2	6.3	6.34±0.46ab
Oilpalmwaste	5.8	4.3	5.1	5.2	-	4.08±2.16c
Sawdust	6.0	5.3	6.8	7.1	-	5.04±2.68b
Ricestraw	6.2	7.3	6.4	7.2	6.3	6.68±0.49a

Each value is the mean for three replicates. Mean carrying the same alphabet are not significantly different (Pd<0.05) using DMRT, SD=Standard Deviation

TABLE 4 shows that mushroom produced from rice straw had the highest mean values of pileus diameter (7.3cm) for second flush while oil palm waste (with four flushes only) had the lowest mean pileus diameter value of 5.1cm for third flush. The total mean pileus diameter ranges from 5.21±2.72 to 7.08±0.17cm. There is also significant (Pd<0.05) difference between the total mean pileus diameters per flush across the four substrates.

TABLE 5 shows that *P.pulmonarius* cultivated on rice straw and coir fibre has the highest mean mushroom height of 9.3cm at second flushes while those cultivated on oil palm waste has the least mushroom height of 6.0cm at the fourth flush. Total mean mush-

room height ranges from 5.18±2.72 to 8.86±0.39cm. There is significant (Pd<0.05) difference in the total mean height of *P.pulmonarius* produced from the four substrates.

TABLE 4 : Effect of substrates, on pileus diameter per flush of *P.pulmonarius*

Substrate	Themeanpileusdiameter(cm)					Total Mean±SD
	flush1	flush2	flush3	flush4	flush5	
Coirfibre	7.2	6.9	6.7	7.1	6.8	6.94±0.19b
Oilpalmwaste	6.1	6.0	5.7	5.2	-	4.60±2.40d
Sawdust	6.1	7.0	6.8	6.1	-	5.21±2.72c
Ricestraw	7.0	7.3	6.8	7.1	7.2	7.08±0.17a

Each value is the mean for three replicates. Mean carrying the same alphabet are not significantly different (Pd<0.05) using DMRT, SD=Standard Deviation

TABLE 5 : Effect of substrates on mushroom height per flush of *P.pulmonarius*

Substrate	Themeanmushroomheight(cm)					TotalMean
	flush1	flush2	flush3	flush4	flush5	
Coirfibre	8.2	9.3	7.9	8.6	8.2	8.46±0.49ab
Oilpalmwaste	7.4	6.4	6.1	6.0	-	5.18±2.72c
Sawdust	8.1	8.2	8.4	9.2	-	6.78±3.53b
Ricestraw	8.2	9.3	9.0	8.7	9.1	8.86±0.39a

Each value is the mean for three replicates. Mean carrying the same alphabet are not significantly different (Pd<0.05) using DMRT.

TABLE 6 : Effect of different substrates and different concentration of rice bran on yield (weight/g)of *P.pulmonarius*

Substrate	RiceBranLevel					Total MeanWeight±SD
	0%	10%	20%	30%	40%	
Coirfibre	8.22	16.16	19.20	13.21	9.17	66.36±10.01b
Oilpalmwaste	4.72	6.00	7.03	5.20	4.00	26.70±8.53d
Sawdust	6.28	15.09	18.80	10.48	7.30	58.94±3.58c
Ricestraw	13.61	23.74	25.72	15.63	14.65	93.33±32.03a

Each value is the mean for three replicates. Mean carrying the same alphabet are not significantly different (Pd<0.05) using DMRT, SD= Standard Deviation

Effect of substrates and rice bran level on *P.pulmonarius* yield

TABLE 6 shows that rice straw had the highest total mean yield followed by coir fibre, sawdust and oil palm waste with mean yield values of 93.33±32.02g, 66.36±10.1g, 58.94±3.58g and 26.70±8.53g respectively. There was significant difference between the yields

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obtained from the four substrates. At rice bran levels 20% RBL has the highest yield followed by 10% RBL across the different substrates.

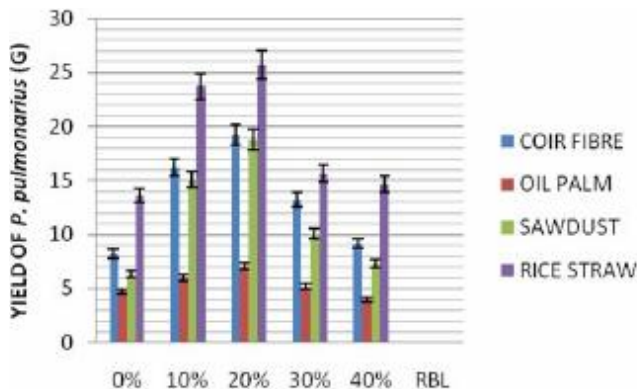


Figure 1: *P. pulmonarius* yield (g) as exhibited by different substrates at different rice bran level (RBL)

DISCUSSION

This study shows a negative correlation between the flavonoids and phenol content with the yield observed, the higher the flavonoids and phenols content the lower the yield. Observation made by Chang^[6], have shown that flavonoids protect plants from different biotic and abiotic stresses. Flavonoids have anti-inflammatory property^[21]. Oil palm waste with highest phenols and flavonoids content had the least yield probably due to high concentration of the flavonoid and phenol which might be inhibitory to *P. pulmonarius* at such concentrations.

Highest tannin value (3.91mg/g) was obtained in oil palm waste followed by sawdust (1.83mg/g) with the least value 0.32mg/g recorded in rice straw. There is also a negative correlation, the higher the tannin value, the lower the yield obtained. Tannin has been reported as antagonistic to gibberellin (a growth promoter) in plants by Kim.^[22] If *P. pulmonarius* is compared with other plants, the bioaccumulation of tannins might be responsible for the lower yield in oil palm waste and the saw dust.

The highest values of alkaloids and saponins (11.1mg/g and 0.90mg/g were obtained in saw dust while the least alkaloid value 3.0mg/g was recorded for rice straw. The least value of saponins 0.21mg/g was also recorded in rice straw. Saponins play important role in plant defence against phytopathogenic fungi, al-

lelopathic activity and defence against insect and pathogens^[15].

Highest oxalate value 0.42mg/g was obtained in saw dust. Rahman *et al.*^[31] reported that forage plants can sometimes accumulate oxalate to a potentially toxic concentration; the high concentration of oxalate in saw dust might be toxic to *P. pulmonarius* hence the low yield. Rice straw with the highest yield had the least value for all phytochemical components investigated.

The values of protein obtained are higher than those reported by Jonathan *et al.*^[20] for some protein rich foods such as green vegetables, cowpea seeds (22.5%) and lima beans (23.3%). Therefore, the observation from this study is in agreement with the findings of Mattila *et al.*^[24] and Degreef *et al.*^[7] who reported that mushroom can be ranked as protein rich food for both humans and livestock and thus, can support the protein need of the poor peasants and solve the problem of malnutrition. The carbohydrate values obtained in the mushroom from coir fibre might be as a result of the richness in nutritional composition of the substrate.

The percentage moisture content agrees with Bonatti *et al.*^[5] who reported that *P. sajor-caju* showed higher moisture when cultivated on rice straw, this also conforms to the findings of other authors^[34-37] that the pileus contains more moisture than other parts of the mushroom. The moisture content present in mushroom could be as a result of having larger pileus, It also might be as a result of water retaining ability of the substrate. The fat content for the mushroom from the four substrates was found to be relatively low (ranging from 0.8% for coir fibre to 2.1% for sawdust). This agrees with Chang and Mshigeni,^[10] and Sadler,^[33] who reported that Mushrooms are low in total fat content and have a high proportion of polyunsaturated fatty acids. The low fat content is an indication that mushrooms are low in cholesterol.

The ash content conforms to the observation made by Patil *et al.*^[26] who reported that the ash content obtained from *P. ostreatus* cultivated on different lignocellulosic agro-wastes ranged between 5.9-6.7%. The crude fibre obtained is higher than the value obtained by Oso^[32] who reported that the value of crude fibre obtained from *P. tuber-regium* grown on different substrates ranged between 0.38 and 6.4%. This is in accordance with Stanley^[35] who reported that *Pleurotus*

sp. has high protein content and fibre content which facilitates digestion in man. The result obtained from the total mean yield is an indication that all the substrates used proved suitable for mushroom production though the oil palm waste yield was low with rice straw having the best performance. This is similar to the study of Obodai *et al.*^[26] in which it was observed that rice straw performed the best for *P. ostreatus* mushroom cultivation when compared with banana leaves, maize stover, corn husks, rice husks and elephant grass. The yield performance by these substrates can be attributed to the presence of supplements such as lime and rice bran which also play a vital role in mushroom cultivation by aiding the yield of fruiting body. Lime is known to neutralize the pH of the soil thereby making it possible for lime to neutralize the pH of different substrates thus aiding the microorganism activities on the substrate and as a result contributing to yield performance of the fruiting bodies. Similar findings was recorded by Jonathan *et al.*^[17] who addressed the effect of different supplement on the yield of *P. florida* in which it was discovered that supplement such as lime and wheat bran contributed to the high yield of mushroom and also aid sporophore emergence. The yields recorded in the mushroom produced were relatively high and this is an indication that all the substrates used supported the yield of *P. pulmonarius*. The yield obtained in saw dust was lower than those of rice straw and coir fibre also due to the fact that *P. pulmonarius* has to break down the lignin content of the wood. It was also observed that 20% RBL had the best mean yield for the four substrates followed by 10% RBL.

Result shows that 20% and 10% RBL were the most suitable for the production of *P. pulmonarius* with mean fresh weight of 74.03 and 73.38g respectively. This indicates that 20% and 10% are the most appropriate levels of additives to be added as supplement along with the substrate. This agrees with Fasidi and Kadiri^[8] who reported that rice bran supported the best mycelia growth in mushroom cultivation. The low yield recorded by oil palm waste despite different RBL might be due to complex lipid present in it. This also is in consonance with the findings of Lim^[23] who reported that oil palm waste may contain complex lipid which may hinder easy access of the fungus to simpler carbon sources, thus reducing the mycelia growth and yield.

Rice straw produced the highest dry mean which is in accordance with Obodai *et al.*^[25,26] who reported that rice straw appears to be best for *P. ostreatus* cultivation when compared with banana leaves, maize stover, corn husk, rice husk and elephant grass. There was significant difference between the four substrates but there was no significant difference between 10% and 20% RBL for dry *P. pulmonarius*. The results are similar to those obtained for fresh *P. pulmonarius*, indicating a close relationship between the fresh and dry yield. The result of B.E obtained shows that the yield is a function of B.E. thus meaning that B.E. is dependent on yield. The highest values of B.E. obtained in rice straw and coir fibre could be attributed to yield as both were found to give the best yield. At RBL, it could be deduced that the *pleurotus* made best use of the substrates at lower levels of rice bran (0-20%). The highest B.E. could have been due to efficient and effective utilization of substrates by *P. pulmonarius*. Shah *et al.*^[34] evaluated 64.69% of B.E. for mushroom grown on fermented sawdust. Obodai and Vowotor^[25] also obtained 50.93% of B.E. for *P. ostreatus*. The regression equation showing the relationship between B.E. and spent substrate in Figure 1 is an indication that *P. pulmonarius* made good use of the substrate since the R^2 was found to be high $R \sim 0.60$. Similar findings was observed by Jonathan *et al.*^[16] on the efficacy of different spawn types on sawdust media, in which the B.E. was strongly and positively correlated with yield.

REFERENCES

- [1] F.R.Alofe, E.A.Odu, H.C.Iloho; *The Nigerian Fields*, **63**, 3-18 (1998).
- [2] AOAC, Official methods of analysis, 18th Edition, Association of Official Analytical Chemists, Washington D.C, (2005).
- [3] S.Amalesh, D.Gonranga, K.D.Sanjoy; *Int.J.Pharm.Sci.Tech.*, **6**, 1 (2011).
- [4] J.H.Brunner; Direct spectrophotometer determination of saponin. *Analytical Chemistry*, 1314-1326 (1984).
- [5] M.Bonatti, P.Karnopp, H.M.Soares, S.A.Furlan; *Food Chemistry*, **88**, 425-428 (2004).
- [6] S.T.Chang; *International Journal of Medicinal Mushrooms*, **1**, 291-300 (1999).
- [7] J.Degreef, F.Malaisse, J.Rammeloo, E.Baudart;

Full Paper

- Biotechnology, Agronomy, Society and Environment, **1**, 221-231, (1997).
- [8] I.O.Fasidi, M.Kadiri; Acta Botanica Hungarica, **36**, 167-172 (1991).
- [9] M.Figen; Journal of Cell and Molecular Biology, **5**, 13-17 (2006).
- [10] J.S.Gbolagade; Acta Phytopathologica. Et Entomologica Hungarica, **40(2-3)**, 333-34017 (2005).
- [11] J.S.Gbolagade; African Journal of Biotechnology, **5(4)**, 338-342.
- [12] J.S.Gbolagade, I.O.Fasidi, E.J.Ajayi, A.A.Sobowale; Food Chemistry, **99**, 742-747 (2006).
- [13] J.S.Gbolagade, A.I.Ajayi, I.Oku, D.O.Wankasi; Global Journal of Biotechnology and Biochemistry, **1(1)**, 16-21 (2006).
- [14] J.B.Harborne; Phytochemical methods: A guide to modern techniques of plant analysis. 1st Edition Chapman and Hall, London, (1973).
- [15] S.G.Jonathan, O.R.Adeoyo; Natural products, **7(3)**, 128-136 (2011).
- [16] S.G.Jonathan, B.M.W.Amos Tautua, O.J.Olawuyi; African Journal of Agricultural Research, **6(13)**, 3007-3012 (2011).
- [17] S.G.Jonathan, C.B.Okon, A.O.Oyelakin, O.O.Oluranti; Nature and Science, **10(9)**, 186-191 (2012).
- [18] S.G.Jonathan, O.J.Oyetunji, M.A.Asemoloye; Nature and Science, **10(10)**, 149-15622 (2012).
- [19] S.G.Jonathan, A.A.Adegboyega, A.O.Oyelakin; New York Science Journal, **5(11)**, 36-40 (2012).
- [20] S.G.Jonathan, O.J.Olawuyi and, O.O.Oluranti; Academia Arena, **4(9)**, 39-45 (2012).
- [21] S.G.Jonathan, O.J.Oyetunji, O.J.Olawuyi, M.D.Asemoloye; Academia Arena, **4(9)**, 49-56 (2012).
- [22] J.Y.Kim, S.J.Park, K.J.Yun, Y.W.Cho, H.J.Park, K.T.Lee, K.T.European; Journal of Pharmacology; **584**, 175-184 (2008).
- [23] W.C.Lim, Mushroom Science, **11**, 595-602 (1981).
- [24] P.Mattila, K.Kanko, M.Earola, J.M.Pihlava, J.Astola, L.Vahterist; Journal of Agriculture and Food Chemistry, **49**, 2343-2348 (2001).
- [25] M.Obodai, K.A.Vowotor, Journal of Food Technology in Africa, **7(3)**, 98-100, (2002).
- [26] M.Obodai, J.Cleland-Okine, K.A.Vowotor; Journal of Industrial Microbiology and Biotechnology, **30**, 146-149 (2003).
- [27] S.S.Patil, S.A.Ahmed, S.M.Telang, M.W.Baig; Innovative Romanian Food biotechnology, **7**, 66-76 (2010);
- [28] L.Pathmashini, V.Arulnandhyal, P.S.Wijeratman; Journal of tropical agricultural research and extention, **11**, 55-59 (2008).
- [29] K.P.Pedneault, A.Gosselia, R.J.Tweddell; Mycology Resources, **110**, 1179-1183 (2006).
- [30] T.H.Quimio, S.T.Chang, D.J.Royse; Technical guidelines for mushroom growing in the tropics. FAO plant production and protection, paper 106, Rome, Italy. *citrinopileatus* strain, (1990).
- [31] M.M.Rahman, M.Niimi, Y.Ishii, O.Kawamura; Grassl.Science., **52**, 161-166 (2006).
- [32] B.A.Oso; Mycologia, **69**, 271-279 (1977).
- [33] M.Sadler, Nutrition Bulletin, **28**, 305-308 (2003).
- [34] Z.A.Shah, M.Ashraf, M.Ishtiaq, Pakistan Journal of Nutrition, **3**, 158-160 (2004).
- [35] H.O.Stanley, C.N.Stanley; Agriculture and biology Journal of America, **7**, 2151-7517 (2003).
- [36] B.A.Wani, R.H.Bodha, A.H.Wani; Journal of Medicinal Plant resources, **4(24)**, 2598-2604 (2010).
- [37] N.Yilmaz, M.Solmaz, I.Turkekul, M.Elmastas; Food Chemistry, **99**, 168 (2006).
- [38] M.H.Zoberi; Tropical Macrofungi. Macmillan Press.London (1972).