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Growth, Yield and Nutritional Quality of *Pleurotus pulmonarius* and *Pleurotus ostreatus*, Grown on Different Substrates Amended with Wheat Bran

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Pleurotus species (oyster mushroom) cultivation is receiving global attention owing to their cheap labour requirements and nutritional/health benefits. In this study, 10 g of *Pleurotus pulmonarius* and *Pleurotus ostreatus* each were cultivated on 400 g of sugarcane bagasse and cotton waste individually. Each substrate was supplemented with wheat bran separately at varying compositions (0%, 5%, 10%, 15% and 20% w/w) in triplicate and incubated at 28 ± 2°C for 35 days. After incubation, the mushroom growth parameters and qualities (yields, minerals, and proximate

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composition) were evaluated. Overall, *P. pulmonarius* cultivated on sugarcane bagasse with wheat bran additives, irrespective of percentage concentration, had better mushroom quality/size, yields, biological efficiency, mineral content, and proximate composition than *P. ostreatus. P. ostreatus* cultivated on cotton waste performed best compared to P*. pulmonarius* at flush 1 ($P \leq 0.05$). Also, relative low calcium, magnesium, and phosphorus contents were observed on both substrates but were significantly rich in potassium (1.32 – 6.817%) and protein (26.60 – 30.46%) contents. Thus, this study will guide farmers in selecting mushroom strains, substrates and percentage additives for a healthy and vigorous mushroom.

Keywords: Additives; nutrient; oyster mushroom; substrates; yield.

1. INTRODUCTION

The cultivation of oyster mushrooms (*Pleurotus* spp.) has received a tremendous boost over the past decade due to its low-cost production technology, which relies on substrate materials that are considered wastes or by-products from industry, households, and agriculture at low charges or for free [1]. This crop is popularly grown for its pleasant taste, high-quality protein [2], and a major source of bioactive compounds that have been harnessed by the pharmaceutical industry as having antitumour, immunomodulatory, antigenotoxic, antioxidant, anti-inflammatory, hypocholesterolemic, antihypertensive, antiplatelet-aggregating, antihyperglycaemic, antimicrobial and antiviral activities. [3,4] (Chang, 2007).

The available statistics show that developing countries like Nigeria do not consume Mushroom on a larger percentage, the Food and Agriculture Organization (FAO) recommended 70g/capital/day protein requirement for individuals but consume 51g/capital/day [5,6]. Agricultural wastes such as chopped cocoa pods, cotton waste, dried chopped maize straw, oil palm (fibre and bunch) wastes, tobacco straw, tea leaves, rice straw, sugarcane bagasse, newsprint, old rags, and sawdust have been successfully used in cultivating mushrooms [7].

However, due to the recalcitrant nature and insufficient nutrient composition of substrates, the use of additives such as wheat bran, rice bran, poultry manure and peat moss has helped in increasing the production of fruiting bodies and high-quality mushrooms as well as shortening mushroom production periods, which indirectly lead to the rapid biodegradation of agro-wastes [8,9].

Therefore, this study investigated or evaluated the morphological response and proximate quality of two oyster mushrooms to cotton waste and sugarcane baggasse under varied additive levels. The influence of cotton waste and sugarcane baggasse on the growth, yield and nutritional quality of two oyster mushrooms (*P. ostreatus* and *P. Pulmonarius)* under different additive percentage composition levels.

2. MATERIALS AND METHODS

2.1 Collection of Samples

2.1.1 The spawn

pure culture of *P. pulmonarius* and *P. ostreatus* were collected from the Forest Research Institute of Nigeria (FRIN) Ibadan.

2.1.2 The substrates

Gossypium hirsutum, Sorghum grains, *Saccharum officinarum* were used as the substrates. *Gossypium hirsutum* was obtained from the Bode market, Molete, Ibadan, Oyo State while the *Saccharum officinarum* (sugarcane bagasse) was collected from the sugarcane section of Bodija market, Ibadan, Oyo State. These substrates were air-dried in a clean open space in the Department of Botany University of Ibadan for seven days to reduce moisture content and prevent decomposition.

2.1.3 The additive

Wheat bran was used as additives. Wheat bran was collected from the feed mill of the popular Bodija Market, Ibadan.

2.2 Spawn Multiplication

The pure spawn was multiplied from sorghum grains using the procedure described by Okhuoya et al. [10]. The sorghum grains were boiled in water for fifteen minutes to soften the grains and squeezed using a muslin cloth until no water oozed out. 100 g of the softened sorghum

grain was mixed thoroughly with 10g of wheat bran (additive) and loaded into 350 mL sterile bottles, covered with aluminum foil and autoclaved at 151bs pressure and 121°C temperature for 15mins. The bottles were later allowed to cool before inoculating with 5 g of the pure spawn and incubated at 28 ± 2°C for 21 days until the inoculated bottles were completely ramified to form a spawn. 150 bottles of pure spawn were prepared for this experiment. The spawn bottles were stored in the refrigerator at a temperature <10^{o}C.

2.3 Preparation of Substrates

The culture conditions were carried out following the method described by Lawal et al. [11] but modified as follows; 400 g of each dried substrate was weighed and moistened with 75 % distilled water (w/v). Calcium trioxocarbonate (iv) ($CaCO_3$) was mixed with the additive (wheat bran) separately then thoroughly mixed with each substrate respectively at varying percentages (0, 5, 10, 15 and 20%). The mixture was packed into well-labeled polythene bags, tied immediately and pasteurized in drums for 4hours. Each substrate mixed was prepared in three replicates.

2.4 Inoculation of Substrates Bags and Spawn Run

After pasteurization, the substrate bags were allowed to cool before inoculating with 10g of *P. pulmonarius* and *P. ostreatus* respectively into each sterilized substrate bag. The inoculated substrate bags were incubated at 28 ± 2 °C for 5 weeks.

Spawn run (mycelia extension) was observed regularly until a whitish mycelia growth (colonization) had spread to both the lower and upper sides of the bags from the inoculated zone The tied bags were opened. Three times watering per day was done on the substrate before the first harvest (first flush).

2.5 Harvesting and Determination of Fresh weight

Harvesting was done by hand holding the stipes at the base and twisting lightly. Stipe length, pileus diameter, and Mushroom height were measured in centimeters with a meter rule. Mushrooms were harvested, counted and weighed. At the end of the third flush, total yield and biological efficiency (BE) were determined. The fresh weight of the freshly harvested mushrooms from all the supplements was determined by weighing them with an electronic weighing balance [9].

2.6 Determination of Yield and Biological Efficiency

2.6.1 Yield performance

Total fresh yield (g)= Total fresh weight of the mushrooms harvest per unit production (bottle) for the two flushes (g)/ Subtracted dry weight (400g)

2.6.2 Biological efficiency

Total fresh yield (g)= Total fresh weight of the mushrooms harvest per unit production (bottle) for the two flushes (g)/ Subtracted dry weight (400g) ×100%

Where: BE = biological efficiency

3. PROXIMATE ANALYSIS/COMPOSITION

3.1 Moisture Content

The harvested mushrooms were weighed and dried in the oven at 60^{0}C for 48hours. The dried samples were weighed and the percentage difference in weight was taken as the moisture content according to Yuxi et al., [12].

3.2 Ash Content

Powdered mushroom samples (2g) were reduced to ash in a Gallenkamp furnace in crucibles that had been ignited, cooled in a $CaCl_2$ desiccator to prevent re-absorption of water, and weighed at 550^{0}C for 6hours [13].

3.3 Protein Content

Micro-Kjeldahl automated method was used. Aliquots (0.2g) of dried mushrooms were weighed with digestion tubes, 15 mL conc. H_2SO_4 and 7 Kjeldahl catalyst tablets were added with the digestion pre-set at 410 ^{0}C. Digestion was done for 45min. the tube was then placed in a distilling unit and 5 mL of 40% NaOH was dispersed into it. The distillate was digested into 35 mL of 4% boric acid for 5min.

3.4 Crude Fibre

The crude fibre of each mushroom sample was determined according to the standard method of the Association of Official Chemists [14].

3.5 Sugar Content

One gram of powdered sample was extracted overnight with 25 mL of 80% ethanol. The extract

was filtered out and the filtrate made up to 100 mL with distilled water. The quantity of sugar in the extract was determined using the phenol-sulphuric acid method [15].

3.6 Lipid Content

A powdered sample (2g) was extracted with 30 mL chloroform in a Soxhlet extractor for 4hours. The extract was evaporated to dryness in a pre-weighed flask and was then dried at 80^0C for 2 hours, cooled in a desiccator and reweighed. The difference in final and fresh weight was taken as the lipid content.

3.7 Statistical Analysis of Data an Experiment Layout

The experiment was set up using a complete randomized design (CRD).Data obtained were subjected to analysis of variance (ANOVA) while the means were separated by Duncan Multiple Range Test (DMRT) at ($P \leq 0.05$).

4. RESULTS

The mushroom growth performance was assessed quantitatively through measurements of stipe length (cm), stipe width (cm) and pileus width (cm) (Table 1). Overall at first flush, on sugarcane bagasse, *P. Pulmonarius* had the highest stipe length (4.47 cm), stipe width (3.37cm) and pileus width (6.23 cm) at 15% wheat bran concentration levels. The 5% wheat bran supplemented with cotton waste yielded the stipe length of *P. Pulmonarius to* 6.33 cm above other concentration levels. Conversely, 15% wheat bran concentration level produced the best stipe length, stipe width and pileus width of *P. Ostreatus* above other concentrations. At flush 2, a slight reduction in stipe length from flush 1 was recorded for both mushrooms in each substrate. Interestingly, *P. Pulmonarius* cultivated on 15% wheat bran on sugarcane bagasse, recorded the highest pileus width with 5.13cm compared with other levels of wheat bran concentration of *P. ostreatus.* On the other hand, *P. Pulmonarius* cultivated on 10% wheat bran supplemented with cotton waste recorded a superior stipe length of 5.17cm above other additive concentration levels compared with that of *P. ostreatus.* However, each percentage concentration levels of wheat bran have no significant difference on both mushrooms except for 20% concentration level at $p \leq 0.05$. 0 and 5% additive concentration levels had the highest Stipe, width and pileus width most on *P. ostreatus* with 4.40 and 8.77cm respectively.

At the third flush, a slight decrease in stipe length from flush 2 was recorded for both mushrooms in sugarcane bagasse. 5% wheat bran on sugarcane bagasse best enhanced the growth of *P. pulmonarius* with 4.50cm above other additive concentration levels and that of *P. ostreatus.* Also, *P. ostreatus* cultivated on 15% wheat bran additive recorded the best performance with 4.77cm and 6.43cm respectively for stipe width and pileus width. On the cotton waste with 10% wheat bran supplementation, *P. ostreatus* recorded the superior stipe length with 4.17cm above other additive concentration levels coupled with that of *P. pulmonarius.* However, 15% wheat bran concentration best enhanced the stipe width and pileus width most on *P. ostreatus* with 4.63 and 6.30cm respectively.

The result of the effect of substrates and additives on fresh weight, yield and biological efficiency (B.E) of the harvested *P. ostreatus and P. Pulmonarius*in Table 2 shows that *P.ostreatus* cultivated on sugarcane bagasse supplemented with wheat bran had higher fresh weight compared with *P. pulmonarius*. Also, the mushroom fresh weight decreases with an increase in flush number. At 15% wheat bran concentration, the highest fresh weight was observed in *P. ostreatus* cultivated from flush 1 to 3 with 4.63g, 4.55g, and 4.41g respectively compared with *P. pulmonarius.*

At 10% wheat bran concentration, the highest fresh weight was observed in *P. Pulmonarius* cultivated from flush 1 to 3 with 10.87g, 15.29g and 8.38g respectively compared with that of *P. ostreatus.*

At 15% wheat bran concentration, the total yield (13.59g) and biological efficiency (3.40g) of *P. Ostreatus* cultivated on sugarcane bagasse was superior to other additive percentage levels compared with *P. pulmonarius*. Contrarily, *P. pulmonarius* had the highest total yield and biological efficiency of 34.53g and 8.63g respectively for 10% wheat bran concentration in cotton waste.

In flush 3. *P. ostreatus* cultivated on sugarcane bagasse supplemented with wheat bran had a higher number of fruit bodies per flush compared with *P. pulmonarius*. At 15% wheat bran concentration, the highest number of fruit bodies per flush was observed in *P. ostreatus* cultivated from flush 1 to 3 with 14.33, 18.33 and 12.00 respectively compared with that of *P. pulmonarius.* No significant difference was

observed among the percentage concentrations of wheat bran for each flush (P ≤0.05).

The effect of sugar cane bagasse and wheat bran on the dry weight of *P. ostreatus and P. pulmonarius* is shown in Table 2. At flush 1, there was an increase in the dry weight of both fungi as the wheat bran increased. The highest dry weight of 0.850g was observed in *P. pulmonarius* cultivated with 10% wheat bran when compared with other additive concentrations and that of *P. ostreatus*. At flush 2, a slight increase in dry matter was observed in both fungi. *P. pulmonarius* cultivated with 10% wheat bran had the highest dry matter (0.867g) when compared with other additive concentrations and that of *P. ostreatus*. At flush 3, a significant increase in dry matter was observed in both fungi. The highest dry weight was observed in *P. pulmonarius* cultivated with 0% wheat bran (0.920g) compared with other additive concentrations and that of *P. ostreatus*. *P. pulmonarius* cultivated with 10% wheat bran showed a significant difference when compared with that of *P. ostreatus* (P ≤0.05).

The dry weight of the harvested *P. ostreatus* and *P. pulmonarius* on cotton waste is presented in Fig. 1. Irrespective of the wheat bran percentage concentration, *P. pulmonarius* had a superior dry matter compared to that of *P. ostreatus*.

The mineral composition of *P. ostreatus and P. pulmonarius* cultivated on different substrates with varying additive percentage composition is presented in Table 3.

Generally, both *P. ostreatus and P. pulmonarius* had low calcium, magnesium and phosphorus contents.

The Proximate analysis of *P. ostreatus and P. pulmonarius* cultivated on sugarcane bagasse and cotton waste with varying additive percentage composition are shown in Tables 4 and 5.

P. pulmonarius cultivated on 5% wheat bran additive had the highest protein content of 30.46% compared to other concentrations and that of *P. ostreatus*. *P. ostreatus* cultivated on 15% wheat bran additive had the highest nitrogen content with 4.87% compared to other concentrations and that of *P. ostreatus.* At 15% wheat bran additive, the highest ash and ether extract contents of 8.16% and 2.57% were respectively observed in the harvested *P. pulmonarius.* Also, *P. Pulmonarius* cultivated on 10% wheat bran additive had the highest carbohydrate content with 59.45% compared to other concentrations and that of *P. Ostreatus* (Table 4).

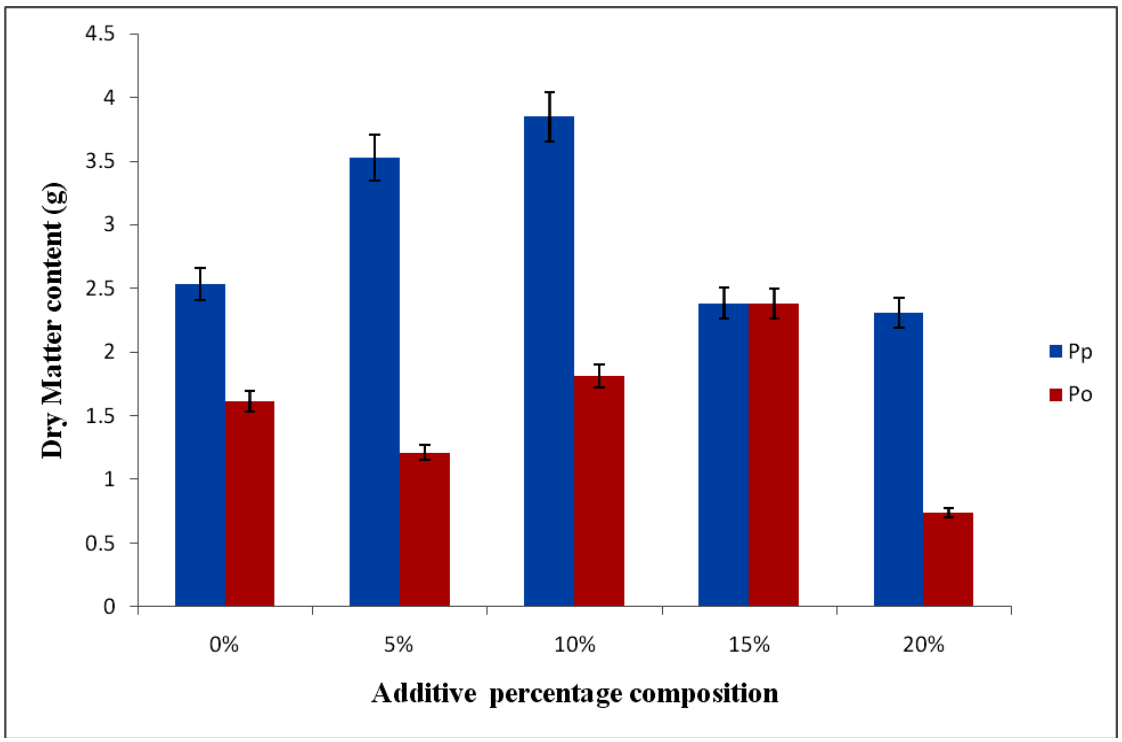


Fig. 1. The dry weight of the harvested *P. ostreatus* and *P. pulmonarius* on cotton waste

P. ostreatus cultivated on cotton waste with 20% wheat bran additive had the highest protein content with 28.73% compared to other concentrations and that of *P. pulmonarius* (Table 5). *P. ostreatus* cultivated on cotton waste with 0% wheat bran additive had the highest Ash and ether extract contents with 8.07% and 2.31% respectively compared to other concentrations and that of *P. ostreatus.* At 10% wheat bran additive in cotton waste, the highest crude fibre content of 10.77% was observed in the harvested *P. pulmonarius.* Also, *P. ostreatus* cultivated on 15% wheat bran additive had the highest moisture and carbohydrate contents with 89.77% and 58.50% respectively compared to other concentrations and that of *P. pulmonarius.*

The result in Fig. 2 shows that the stipe length of *P. ostreatus* is positive and significantly correlated with the stipe width, pileus width, Carbohydrate, Phosphorus and moisture content at $P<0.05$. There is a negative correlation between Calcium and Crude fibre.

Pileus width is positively related to Phosphorus, Moisture content and Carbohydrates but, negatively associated with Calcium, Ether and Crude fibre.

The stipe length of *P. pulmonarius* in Fig. 3 is positively correlated with pileus width, Potassium and crude fibre, Potassium and Phosphorus are also related to crude fibre while there is a positive relationship between moisture content and crude fibre. Phosphorus and crude fibre are negative and correlated with crude protein, ash and ether.

The prin 1 accounted for 58.0% of the total variation in Fig. 4. The principal component axes based on morphological characters, nutrient and proximate compositions shows that ash and magnesium as well as Phosphorus, moisture content, Carbohydrate, stipe length, stipe width and pileus width as well as ether, crude protein, calcium and crude fibre are closely related to one another (Fig. 4).

Crude fibre and calcium are closely associated compared to ether and crude protein. The SB and CW are not related to other parameters (Fig. 4).

The result in Fig. 5 reveals that prin 1 accounted for 43.6% of the total variation. In *Pleurotus pulmonarius.* All the parameters are related one way or the other except SB and CW. The (CP and ash), (PW, K and Mg) as well as CHO, SW and Mo are closely related to one another (Fig. 5).

5. DISCUSSION

The capability of sugarcane bagasse and cotton waste with varying percentage concentrations of wheat bran to support the growth of *P. pulmonarius* and *P. ostreatus* from complete mycelia ramification to fruit bodies formation lends credence to the findings of Adenipekun and Omolaso [9] who reported that *Pleurotus spp* is capable of growing on recalcitrant lignocellulosic biomass through their aggressive mycelia colonization of such waste.

The outstanding fruiting body quality size of *P. Pulmonarius* cultivated on sugarcane bagasse with varying percentage concentrations of wheat bran and *P. ostreatus* cultivated on cotton waste at flush 1 indicate the suitability of these substrates for mushroom cultivation since relatively large mushroom cap diameter and longer mushroom stipe length are desirable characteristics for marketable quality mushrooms [16]. Agreeing with the opinion of Tripathy et al., [17], the superiority of *P. ostreatus* cultivated on sugarcane bagasse with 15% wheat bran and *P. pulmonarius* on cotton waste with 10% wheat bran at flush 2 and 3 indicates the ultimate influences mushroom substrate structure for the growth of the mycelium, as it allows penetration of the mycelium, which aids in mushroom quality/size increments. Also, the production of extensive enzyme systems by these mushrooms which are capable of utilizing complex organic compounds during the decomposition of the substrate further leads to mushroom growth which occurs in substrates [18].

The results of stipe length are by findings made by Khattab (2000), who reported that rice straw and sugarcane bagasse gave fruit bodies the tallest stipe length as compared to the other substrates. In addition, Radwan (2005) reported that the highest cap diameter of mushroom fruit bodies was obtained from sugarcane bagasse, compared to other tested substrates. Noteworthy, the failure of *P. ostreatus* cultivated on sugarcane bagasse with 15% wheat bran and *P. pulmonarius* on cotton waste with 10% wheat bran to have an outstanding quality/size at flush 2 could probably be the presence of excess water in the substrates, high nutrient concentration and lack of proper aeration since *Pleurotus spp* mycelia cannot grow and spread adequately talk less of fructification in poor aerated environment [19].

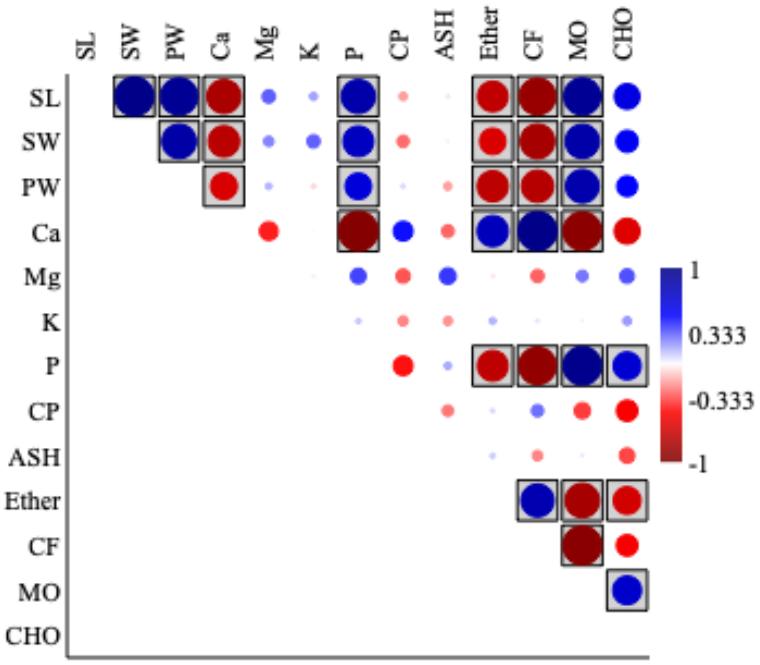
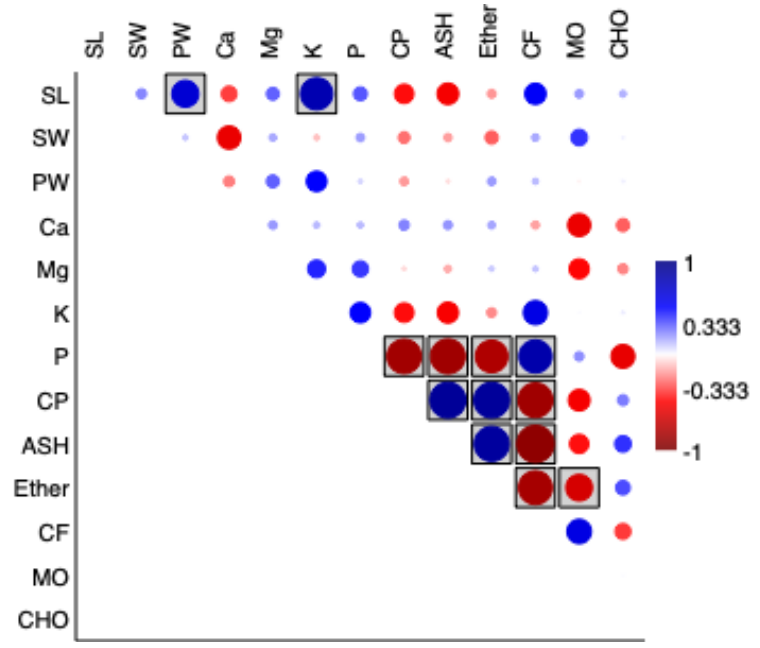


Fig. 2. The Correlation matrix between morphological growth, nutrient contents and proximate composition of *P. ostreatus* under varied additive supplements

Key: SB- Sugar Bagasse, CW- Cotton Waste, SL- Stipe Length, SW- Stipe Width, PW- Pileus Width, CP- Crude Protein, Mo- Moisture content, CHO- carbohydrate The red and blue circles represent negative and positive correlation respectively at (P<0.05). The extent of correlation is indicated by pie fill area, i.e., larger to smaller pie fill area indicates high to low correlation



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Key: SB- Sugar Bagasse, CW- Cotton Waste, SL- Stipe Length, SW- Stipe Width, PW- Pileus Width, CP- Crude Protein, Mo- Moisture content, CHO- carbohydrate. The red and blue circles represent negative and positive correlation respectively at (P<0.05). The extent of correlation is indicated by pie fill area, i.e., larger to smaller pie fill area indicates high to low correlation

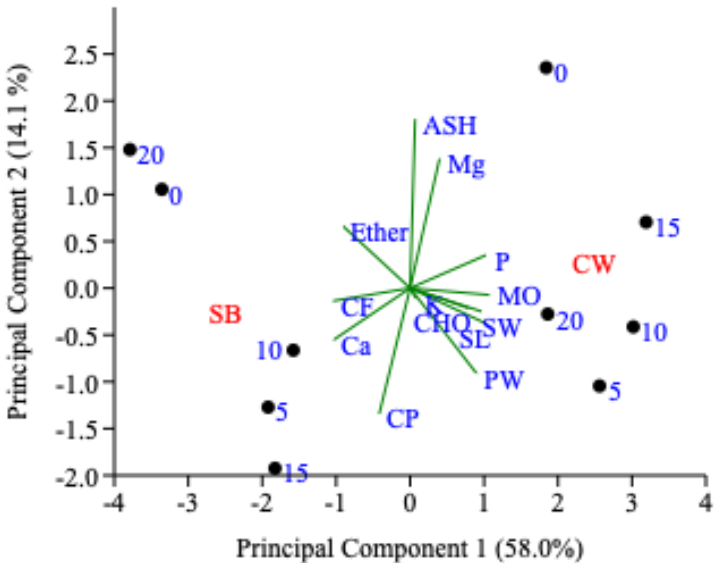

Fig. 4. The principal component analysis based on morphological growth, nutrient contents and proximate composition of *P. ostreatus* under varied additive supplements

Key: SB- Sugar Bagasse, CW- Cotton Waste, SL- Stipe Length, SW- Stipe Width, PW- Pileus Width, CP- Crude Protein, Mo- Moisture content, CHO- Carbohydrate

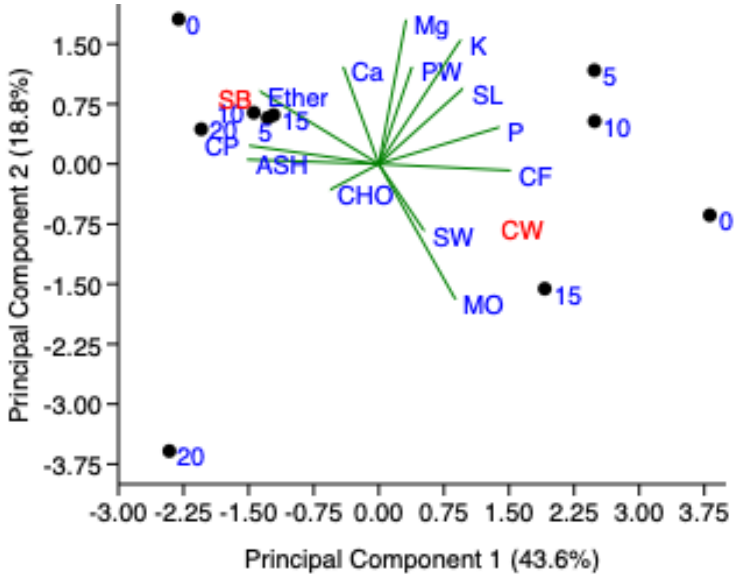

Fig. 5. The principal component analysis based on morphological growth, nutrient contents and proximate composition of *P. pulmonarius* under varied additive supplements

Key: SB- Sugar Bagasse, CW- Cotton Waste, SL- Stipe Length, SW- Stipe Width, PW- Pileus Width, CP- Crude Protein, Mo- Moisture content, CHO- Carbohydrate

Shen and Royse [20] observed that accumulation of CO_2 during spawn running causes reduction in mushroom productivity. The total yields and biological efficiency of *P. ostreatus* cultivated on sugarcane bagasse and *P. pulmonarius* cultivated on cotton waste the mushroom was significantly influenced by wheat bran additives in conformity with the report of Adenipekun and Omolaso [9]. Also, agreeing with the opinion of Earnshaw [21], the superiority of wheat bran additive at the lowest concentration in both substrates further explained that lowering quantity of wheat bran additives in substrates improves mushroom yields, sizes, and durability. Similarly, Chandra et al. [22] reported that high yield and biological efficiency of *Pleurotus sajor-caju* was achieved at a low concentration of rice bran additive on the substrate.

Table 1. Growth parameters per flush of *P. ostreatus and P. pulmonarius* on sugarcane bagasse

Mushroom	Wheat	First flush			Second flush			Third flush		
	Bran level	Stipe length	Stipe width	Pileus width	Stipe length	Stipe width	Pileus width	Stipe length	Stipe width	Pileus width
	(%)	(cm)	(cm)	(cm)	(cm)	(cm)	(cm)	(cm)	(cm)	(cm)
P . pulmonarius	0	4.03±0.12^{ab}	1.90±0.21^{c}	3.77±0.30^{cd}	2.60±0.31^{b}	2.07±0.03^{cd}	3.93±0.27^{bc}	3.17±0.09^{a}	2.33±0.09^{c}	4.53±0.41^{a}
	5	3.70±0.21^{bc}	2.73±0.24^{abc}	4.70±0.50^{cd}	3.17±0.24^{ab}	2.23±0.19^{cd}	4.13±0.18^{b}	4.50±1.04^{a}	4.07±0.35^{ab}	5.93±0.54^{a}
	10	4.17±0.20^{ab}	2.07±0.07^{ab}	4.97±0.03^{bc}	2.97±0.27^{ab}	2.93±0.17^{ab}	4.77±0.12^{a}	4.30±0.84^{a}	3.77±0.28^{ab}	5.43±1.53^{a}
	15	4.47±0.26^{a}	3.37±0.76^{a}	6.23±0.49^{a}	3.80±0.38^{ab}	2.77±0.12^{ab}	5.13±0.13^{a}	4.23±1.16^{a}	3.80±0.00^{ab}	6.37±0.74^{a}
	20	4.07±0.22^{ab}	2.73±0.12^{abc}	4.50±0.50^{cd}	3.10±0.32^{ab}	1.93±0.07^{d}	2.93±0.23^{d}	3.47±0.29^{a}	2.93±0.37^{bc}	5.27±0.43^{a}
P. ostreatus	0	2.93±0.12^{d}	2.00±0.21^{c}	3.57±0.35^{d}	2.93±0.35^{ab}	2.47±0.13^{bc}	3.87±0.07^{bc}	2.67±0.18^{a}	3.07±0.07^{bc}	4.17±0.44^{a}
	5	3.30±0.21^{cd}	2.77±0.13^{abc}	4.40±0.31^{cd}	3.13±0.03^{ab}	2.53±0.12^{bc}	4.13±0.09^{b}	4.00±0.29^{a}	3.63±0.13^{ab}	5.37±0.52^{a}
	10	3.43±0.07^{cd}	3.20±0.25^{ab}	5.03±0.32^{bc}	3.73±0.54^{ab}	3.17±0.32^{a}	4.90±0.32^{a}	4.00±0.90^{a}	3.70±0.17^{ab}	5.00±0.53^{a}
	15	3.23±0.09^{cd}	2.50±0.25^{abc}	6.10±0.25^{ab}	3.93±0.69^{a}	3.23±0.15^{a}	5.03±0.23^{a}	4.07±1.31^{a}	4.77±0.90^{a}	6.43±0.97^{a}
	20	3.03±0.09^{d}	2.33±0.22^{bc}	3.87±0.49^{cd}	3.10±0.06^{ab}	1.90±0.15^{d}	3.47±0.15^{cd}	3.23±0.90^{a}	3.63±0.35^{ab}	4.27±0.15^{a}

Each value is a mean± standard error of three replicates. Values in the same column with different letters as superscripts are significantly different by Duncan multiple range test ($p \leq 0.05$)

Table 2. Fresh and dry weights of the harvested *P. ostreatus and P. pulmonarius* on sugarcane bagasse

	Wheat bran Level	First flush		Second flush		Third flush		Moisture content
	(%)	Fresh weight(g)	Dry weight (g)	Fresh weight(g)	Dry weight (g)	Fresh weight(g)	Dry weight (g)	(%)
P. pulmonarius	0	1.55±0.39	0.203±0.02	2.10±0.19	0.277±0.015	1.90±0.18	0.920±0.660	66.02±0.04
	5	2.79±0.23	0.337±0.05	2.55±0.12	0.400±0.006	3.34±0.67	0.417±0.041	71.25±0.22
	10	3.43±0.31	0.850±0.12	4.35±0.46	0.867±0.065	3.89±0.89	0.803±0.046	70.54±0.48
	15	4.29±0.56	0.453±0.02	4.21±0.34	0.550±0.029	4.15±0.80	0.570±0.031	73.94±0.03
	20	1.79±0.47	0.203±0.01	1.32±0.01	0.307±0.012	2.54±0.27	0.307±0.007	65.17±0.44
P. ostreatus	0	1.44±0.35	0.247±0.02	2.28±0.28	0.377±0.087	2.55±0.61	0.357±0.079	59.51±0.51
	5	2.72±0.36	0.437±0.02	2.76±0.18	0.513±0.019	3.07±0.84	0.483±0.017	69.57±0.45
	10	3.17±0.26	0.507±0.05	4.12±0.61	0.650±0.029	2.88±0.26	0.583±0.018	70.95±0.48
	15	4.63±0.33	0.493±0.10	4.55±0.49	0.593±0.092	4.41±1.40	0.557±0.084	70.73±0.41
	20	2.24±0.36	0.177±0.01	1.76±0.07	0.253±0.024	2.43±0.50	0.250±0.021	60.70±0.65

Each value is a mean± standard error of three replicates. Values in the same column with different letters as superscripts are significantly different by Duncan multiple range test ($p \leq 0.05$)

Table 3. Mineral composition of *P. ostreatus and P. pulmonarius* on sugarcane bagasse and cotton waste

Mushroom	Wheat bran Level (%)	Ca		Mg		K		P	
		SB	CW	SB	CW	SB	CW	SB	CW
P. pulmonarius	0	0.11±0.00^{a}	0.027±0.001^{a}	0.24±0.01^{c}	0.286±0.002^{f}	4.22±0.06^{b}	3.357±0.004^{g}	1.06±0.00^{a}	2.639±0.008^{a}
	5	0.02±0.00^{b}	0.010±0.002^{d}	0.35±0.01^{a}	0.260±0.001^{g}	2.24±0.19^{e}	6.817±0.036^{a}	0.75±0.00^{b}	1.471±0.022^{b}
	10	0.02±0.00^{b}	0.015±0.001^{c}	0.32±0.00^{b}	0.210±0.004^{j}	4.32±0.04^{b}	6.083±0.018^{b}	0.60±0.00^{e}	1.394±0.013^{c}
	15	0.02±0.00^{b}	0.021±0.001^{b}	0.24±0.01^{c}	0.223±0.004^{i}	3.71±0.14^{b}	4.263±0.005^{f}	0.63±0.00^{d}	1.450±0.033^{b}
	20	0.02±0.00^{b}	0.002±0.000^{e}	0.26±0.01^{c}	0.250±0.000^{h}	2.43±0.07^{e}	2.703±0.002^{h}	0.66±0.00^{c}	1.189±0.002^{fg}
P. ostreatus	0	0.02±0.00^{b}	0.003±0.001^{e}	0.32±0.01^{b}	0.341±0.001^{c}	1.32±0.16^{f}	4.828±0.014^{e}	0.50±0.00^{f}	1.254±0.006^{e}
	5	0.02±0.00^{b}	0.006±0.002^{e}	0.31±0.01^{b}	0.327±0.007^{d}	4.43±0.24^{b}	1.832±0.017^{j}	0.63±0.00^{d}	1.159±0.001^{gh}
	10	0.02±0.00^{b}	0.004±0.001^{e}	0.31±0.01^{b}	0.361±0.001^{b}	8.15±0.05^{a}	2.390±0.003^{i}	0.75±0.00^{b}	1.225±0.006^{ef}
	15	0.02±0.00^{b}	0.003±0.001^{e}	0.21±0.01^{d}	0.390±0.001^{a}	3.79±0.10^{c}	5.703±0.003^{c}	0.61±0.00^{e}	1.130±0.005^{h}
	20	0.02±0.00^{b}	0.003±0.000^{e}	0.36±0.01^{a}	0.295±0.001^{e}	3.08±0.03^{d}	4.907±0.004^{d}	0.61±0.00^{e}	1.312±0.004^{d}

Each value is a mean± standard error of three replicates. Values in the same column with different letters as superscripts are significantly different by Duncan multiple range test ($p \leq 0.05$)
SB : Sugarcane Bagasse CW: Cotton Waste

Table 4. Proximate composition of *P. ostreatus and P. pulmonarius* cultivated on sugarcane bagasse with varying wheat bran composition

Mushroom	Wheat bran level	Crude Protein	Nitrogen	Ash content	Ether	Crude Fibre	Dry matter	Moisture	Carbohydrate
	(%)	(%)	(%)	(%)	Extract (%)	(%)	(%)	(%)	(%)
	0	29.70±0.20^{ab}	4.72±0.04^{ab}	7.77±0.13^{ab}	2.40±0.01^{bc}	4.84±0.10^{bc}	39.36±0.24^{a}	59.51±0.51^{e}	55.30±0.35^{cd}
	5	30.46±0.11^{a}	4.82±0.05^{a}	7.21±0.19^{cd}	2.45±0.04^{abc}	4.99±0.06^{abc}	30.00±0.12^{c}	69.57±0.45^{c}	54.89±0.19^{d}
P.p	10	26.60±0.22^{e}	4.17±0.03^{f}	8.06±0.19^{a}	2.46±0.06^{abc}	5.00±0.01^{abc}	28.49±0.29^{cd}	70.95±0.48^{b}	59.45±1.83^{a}
	15	28.37±0.23^{cd}	4.42±0.03^{de}	8.16±0.08^{a}	2.57±0.06^{ab}	4.55±0.13^{d}	30.00±0.06^{c}	70.73±0.41^{bc}	56.35±0.40^{bcd}
	20	29.46±0.32^{b}	4.70±0.01^{abc}	7.18±0.09^{cd}	2.54±0.06^{ab}	4.93±0.09^{abc}	35.90±3.56^{b}	60.70±0.65^{e}	55.89±0.22^{bcd}
	0	28.48±0.26^{c}	4.60±0.01^{bcd}	8.15±0.04^{a}	2.32±0.07^{c}	5.13±0.09^{a}	34.40±0.38^{b}	66.02±0.04^{d}	55.91±0.22^{bcd}
	5	27.90±0.21^{cd}	4.52±0.14^{cde}	6.74±0.22^{d}	2.34±0.08^{c}	5.10±0.03^{abc}	28.52±0.29^{cd}	71.25±0.22^{b}	57.91±0.38^{ab}
P.o	10	27.70±0.30^{d}	4.35±0.07^{e}	7.25±0.16^{bcd}	2.39±0.08^{bc}	5.14±0.04^{a}	29.46±0.25^{cd}	70.54±0.48^{bc}	57.30±0.29^{bc}
	15	30.34±0.30^{a}	4.87±0.04^{a}	7.43±0.32^{bc}	2.33±0.04^{c}	4.79±0.12^{cd}	26.05±0.03^{d}	73.94±0.03^{a}	55.11±0.54^{d}
	20	29.73±0.18^{ab}	4.75±0.03^{ab}	8.03±0.09^{a}	2.59±0.01^{a}	5.10±0.12^{ab}	35.10±0.21^{b}	65.17±0.44^{d}	54.55±0.21^{d}

Each value is a mean± standard error of three replicates. Values in the same column with different letters as superscripts are significantly different by Duncan multiple range test ($p \leq 0.05$)
P. o: P. ostreatus; P. p: P. pulmonarius

Table 5. Proximate analysis of *P. ostreatus and P. pulmonarius* cultivated on cotton waste with varying wheat bran composition

Mushroom	Wheat bran level	Crude Protein	Ash Content	Ether Extract	Crude Fibre	Moisture Content	Carbohydrate
	(%)	(%)	(%)	(%)	(%)	(%)	(%)
P.pulmonarius	0	13.20±0.21^{c}	4.24±0.10^{f}	1.26±0.15^{f}	10.19±0.03^{b}	79.72±0.28^{f}	53.58±0.33^{e}
	5	18.81±0.26^{bc}	4.72±0.13^{e}	1.89±0.02^{d}	8.62±0.01^{d}	73.35±0.29^{g}	57.97±0.26^{ab}
	10	20.33±0.51^{abc}	5.33±0.04^{d}	1.97±0.01^{cd}	10.77±0.14^{a}	86.57±0.19^{cd}	55.08±0.19^{d}
	15	22.97±0.21^{ab}	5.31±0.01^{d}	1.61±0.02^{e}	9.84±0.05^{c}	87.03±0.11^{c}	56.09±0.13^{c}
	20	27.79±0.12^{ab}	7.93±0.12^{ab}	2.13±0.03^{abc}	4.20±0.06^{ef}	86.17±0.18^{de}	57.94±0.21^{ab}
P. ostreatus	0	20.16±8.63^{abc}	8.07±0.07^{a}	2.31±0.05^{a}	4.38±0.09^{e}	86.17±0.18^{de}	57.94±0.21^{ab}
	5	28.50±0.29^{a}	7.40±0.06^{c}	2.12±0.04^{bc}	4.27±0.15^{ef}	88.97±0.09^{b}	57.72±0.36^{ab}
	10	27.72±0.15^{ab}	7.47±0.07^{c}	2.11±0.05^{bc}	4.23±0.04^{ef}	89.77±0.05^{a}	58.50±0.25^{a}
	15	27.36±0.42^{ab}	8.05±0.03^{a}	2.20±0.05^{ab}	4.08±0.04^{f}	85.87±0.41^{de}	57.25±0.36^{b}
	20	28.73±0.23^{a}	7.74±0.13^{b}	2.09±0.01^{bc}	4.20±0.06^{ef}	85.57±0.26^{e}	57.25±0.36^{b}

Each value is a mean± standard error of three replicates. Values in the same column with different letters as superscripts are significantly different by Duncan multiple range test ($p \leq 0.05$)

The performance of 10 -15% wheat bran level above other supplement levels agrees with the report of Wei et al. [23] that the inclusion of 10-40 % wheat bran supplement is the optimum growing condition in substrates for mushroom production. Wang et al. [24] also reported that significantly high biological efficiency (19.1%) was obtained with the addition of wheat bran to 45% supplement level. Similarly, Khlood-Ananbeh and Ahmad-Almomany (2005) reported that 30% supplement level of olive cakes gave the highest yield and 80% biological efficiency. Peng-JinTorng et al. [25] also observed a significant increase in biological efficiency on substrate supplemented with wheat bran. Supplement with different levels of carbonates and nitrogen-based additives had been shown to enhance mushroom production (Fasidi and Kadiri, 1993). The differences in the fresh weight, total yield per flush and biological efficiency of mushrooms of both *P. ostreatus* and *P. pulmonarius* on these two substrates could be linked to differences in the mineral composition of the substrates [26].

The slight increase in the fresh weight of both mushrooms at flush 2 on the tested substrates is due to the easier way of getting sugars from the cellulosic substances [27]. Kumari and Achal (2008) cultivated *P. ostreatus*on different substrates and reported the highest yield on wheat straw, followed by the combination of paddy and wheat straw. The result from this study results is not similar to the findings of Das and Mukherjee [28] where a mixture of two substrates (weed plants and rice straw) in a ratio 1:1, recorded a significant increase in mushroom fresh weight, yield and biological efficiency than when used individually. Taurachand [29] reported that sugarcane bagasse contains cellulose and sucrose which is easily degraded by the oyster mushroom. It is also rich in nitrogen content. Even though it is rich in cellulose, sucrose, and nitrogen, its yield was found low in our experiment in comparison to other substrates. Furthermore, the low yield produced on sugarcane bagasse compared with cotton waste could be linked to its low nitrogen and high amount of lignin contents. The low degradation of lignocellulosic substances of sugarcane bagasse by *P. pulmonarius* might be another factor responsible for the overall low yield values.

The three flushes obtained on each substrate coupled with a decrease in the fresh and dry weight corroborate the findings of Idowu and kadiri [30] where *P. tuberregium*flushed twice on all the sawdust of *Chlorophoraexcelsa, Cordia millenii* and *G. aborea*. The biggest flush sizes were recorded at the first flush with the exception of *G arborea* which flushed best at the second flush. Similarly, Singh and Singh [19] also observed that the first flush of *Pleurotus citrinopileatus* fruiting bodies gave maximum yield in comparison to second and subsequent flushes.

The reduction in a number of fruitbodies per flush of the examined mushrooms could be due to a decrease in the supplement nutrient compositions after the first flush together with the accumulation of CO_2 and temperature fluctuations in the growth room during the spawn running phase since environmental conditions were not efficiently controlled. Shen and Royse [20] reported that the accumulation of CO_2 during spawn running causes a reduction in mushroom productivity. While temperature fluctuation also could cause the death of surface mycelia and sclerotia, which tends to reduce the number of fruiting bodies formed [31]. Similarly, Ambi et al. [32] reported that variations in the number of mushroom fruit bodies after the first flush be associated with the depletion of the nutrient content of the substrates. Furthermore, poor mycelia running due to depletion of supplements in the substrate which further leads to carbon and nitrogen imbalance in the substrates could be attributed to a reduction in a number of fruitbodies Okhuoya et al. [10]. Also, Lee Hoon et al. [13] reported that supplementation of the substrate is a way of increasing the productivity of mushrooms, especially in *Pleurotus spp*. Thus, the reduction and variation in the number of fruitbodies could be due to the difference in nutritional and physical composition as well as microclimates of the substrates [33]. Ayodele and Okhunya (2007) further explained that the level of supplement changes the sequence of decomposition of the substrate components, thereby reducing mushroom fruitbodies and yield of mushrooms for quality production.

The mineral elements in these substrates suggest that they can support the growth of mushroom since essential element as potassium plays an important role in the synthesis of amino acids and proteins while copper in combination with manganese, also play important roles in enzymatic catalyses which are crucial in all biological and physiological processes in living organisms [30,34].

Despite differences in substrates types, the proximate compositions of *P. ostreatus* and *P.*

pulmanarius harvested conforms with the findings of Ali et al., [35] where 17.40 to 30.31% protein; 3.90 to 6.16% lipid and 7.05 to 9.15% ash in *P. ostreatus* grown on wheat bran supplemented sugarcane bagasse was reported. Nuruddin et al. [36] also found 18.43 to 30.90% protein, 3.34 to 5.13% lipid, 33.50 to 49.58% carbohydrates and 6.33 to 8.40% ash in *P. ostreatus* grown on cow dung supplemented rice straw. Moni et al., [37], found 88.15 to 91.64% moisture, 18.46 to 27.78% crude protein; 1.49 to 1.90% crude fats, in oyster mushrooms. The fiber content obtained in the study agrees with 5.97-6.42% as reported by Wang et al. [24]. The high protein contents observed agree with the theoretical prediction, stating that an increase in the availability of nitrogen might enable increase protein contents of plants, animals, and fungi Chandel et al., (2010). Also, the nature of the protein depends on the used substrate [24]. Similarly, Lee Hoon et al. [13] reported that the mushroom protein level is generally higher than that of green vegetables. The moisture content of the cultivated mushroom is in line with the reports of Manzi et al., (1999) that the moisture content of fresh *Pleurotus spp* ranged between 70 -95%. Lawal et al. [11] reported that the high moisture content of *A. auricula* cannot permit it to be kept for long. This is because high water activities encourage microbial growth. The total carbohydrate content found in this study conforms with the finding of Patil et al. [38] where 50.50% - above was reported.

6. CONCLUSIONS

From the results, it was obvious that *P. ostreatus* cultivated on sugarcane bagasse with 15% wheat bran additives had higher quality and size, yields, biological efficiency, mineral composition and proximate composition than *P. pulmonarius* while *P. pulmonarius* had the best performance on cotton waste with 10% wheat bran. This observation accentuates the significance of the selection of appropriate substrate and additive percentage composition in profitable and healthy mushroom cultivation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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