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Effects of different substrates on the morphological characteristics and proximate content of *Pleurotus pulminarius*

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Abstract
Morphological characteristics and proximate content of *Pleurotus pulminarius* cultivated on different substrates were investigated. The substrates used are cotton waste, sawdust, palm fruit waste, paper, mixture of sawdust and palm fruit waste and mixture of cotton waste and paper (1:1) were sterilized and inoculated with *Pleurotus pulminarius* spawn (sorghum-produced) aseptically, incubated under controlled temperature and humidity before exposure to sunlight. Length and, diameter of stalk and pileus were measured during stages of harvest. Protein, fat, carbohydrate, crude fibre, ash and moisture content were determined by standard methods. *Pleurotus pulminarius* appeared on saw dust and cotton waste; paper, combination of saw dust and palm fruit waste, mixture of paper and cotton waste; and palm fruit waste after 2 days, 3 days and 5 days respectively. For the first stage of harvest, Saw dust had the highest length of stalk (cm) of 5.40 ± 0.63 and the lowest value of 2.70 ± 0.14 was obtained from palm fruit waste only. Mixture of paper waste and cotton waste has the highest diameter of stalk (3.25 ± 0.61 cm) (second harvest), the lowest diameter of stalk (0.4 ± 0.1 cm) was recorded for palm fruit (third harvest). Cotton waste had the highest diameter of pileus (7.88 ± 1.29 cm) (first harvest) while the lowest value (1.78 ± 0.63 cm) was obtained from mushroom harvested on the mixture of paper and cotton waste (second harvest). *Pleurotus pulminarius* harvested on mixture of paper and cotton waste has the highest percentage protein of 40.54% while the least value 28.79% protein was obtained from mushroom harvested on saw dust. Mushroom harvested on saw dust has the highest percentage fat content of 0.99% and palm fruit waste gave the lowest percentage fat of 0.74%. Palm fruit waste displayed the highest percentage ash and crude fibre contents of 4.38% and 4.81% respectively while cotton and paper wastes had the least ash and crude fibre content of 1.81% and 0.47% respectively. Saw dust gave the highest percentage carbohydrate of 62.54%s. Moisture content of 0.51% was obtained for mushrooms harvested on Palm fruit waste, Sawdust and mixture of sawdust and palm fruit waste as the least value while the mixture of paper and cotton waste has the highest moisture content of 3.49%. The study concluded that mushroom can serve as cheap source of food supplements such as protein, crude fibre, ash (micro and macro nutrients), fat, carbohydrate and moisture with beneficial bioconversion of agricultural and other wastes materials in the environment.
Key words: *Pleurotus pulminarius,* cotton, sawdust, palm fruit, paper, waste

1. Introduction

Mushroom was defined as a macro-fungus with a unique fruiting body which can be epigeous or hypogenous in nature and conspicuous enough to be seen with the unaided eye and can be picked with hand by Chang and Miles (1992). Mushroom exhibit a saprophytic mode of nutrition which involves the production of a wide range of enzymes that can break down complex substances after which they are able to absorb the formed soluble substances (Chang and Miles, 1992).

Pleurotus pulminarius is an edible white rot fungi (WRF) commonly known as the Indian Oyster, Phoenix Mushroom, or the Lung Oyster (Jonathan *et al*., 2012). Mushrooms also appear to be a good source of vitamins, including thiamine, riboflavin, niacin, biotin and ascorbic acid, and of minerals (Isikhuemhen *et al*., 2009). Mushrooms also contain numerous medicinal compounds such as triterpenoids, glycoprotein, natural antibiotics, enzyme inhibitors that fortify health (Okhuoya, 2011). The bioconversion of agricultural and industrial wastes into food has attracted the world attention in recent years. The mushroom cultivation is a highly efficient method of getting rid of agricultural residues as well as producing nutritious and beneficial food (Ingale and Ramteke, 2010). Mushrooms can degrade virtually any lignocellulosic wastes which contain lignin, cellulose and hemicelluloses as their major components, this can be attributed to the possession of enzymes that can degrade these materials (Okhuoya, 2011).

Mushrooms are rich in protein, minerals, and vitamins, and they contain an abundance of essential amino acids (Sadler, 2003). Protein tends to be present in an easily digestable form, on a dry weight basis it ranges between 20 and 40% which is better than many legume sources like soybeans and peanuts, and protein-yielding vegetable foods (Chang and Buswell, 1996; Chang and Mshigeni, 2001). Moreover, mushroom proteins contain all the essential amino acids needed in the human diet and are especially rich in lysine and leucine which are absent in most staple cereal foods (Chang and Buswell, 1996; Sadler, 2003).

This study was carried out to investigate the use of various waste materials for the cultivation of edible mushrooms with the aim to determining their use in bio-waste management while it also serve as nutritional supplement to man.

2. Methodology

2.1 Collection of the substrates.

The sawdust and waste paper was obtained at Ile-Ife, cotton waste was obtained at International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, while the palm fruit waste was collected at the palm oil industry at the Obafemi Awolowo University Farm, Ile-Ife.

2.2 Cultivation of *Pleurotus pulminarius*

The methods involved in the cultivation of *Pleurotus pulminarius* on the above substrates include:

2.3 Preparation of tissue culture

Tissue culture was prepared by first selecting a strong and healthy mushroom which is not too mature, not too young, not too humid and has a stiff stalk. Sterilized needle was allowed to cool and used to cut a small piece after tearing the mushroom lengthwise. The sterile needle was inserted into the bottle and inoculated by placing small piece of cut mushroom in the middle of the PDA's surface, Petri dish was closed immediately near Bunsen burner flame and labelled. After 10 days mycelia were observed to be grown.

2.4 Preparation of *P. pulminarius* spawns.

Sorghum was washed, per-boiled and then allowed to drain and air dried. About 100 g of sorghum, thoroughly mixed with 2 g of calcium carbonates was then packed in bottles. This was autoclaved at 121^{o}C for 15 minutes and then allowed to cool. The sterile sorghum was inoculated with the tissue culture preparation and then incubated at room temperature for 2 weeks for mycelia running (ramification).

2.5 Cultivation of *P. pulminarius* on substrates.

Five hundred grammes (500 g) of cotton waste, sawdust, palm fruit waste, paper, mixture of sawdust and palm fruit waste (1:1) and mixture of cotton waste and paper (1:1) respectively were weighed, soaked and washed in water containing calcium carbonate, the water was drained out and the cotton was shredded. The cotton was then filled into polyethylene bags and sterilized at 121 ^{o}C for 15 minutes.

Each of the substrate was mixed thoroughly with 10 g of calcium carbonate. This was then packed in transparent polythene bags. For the sawdust, a hole was created at the center using small pipe, washed and per-boiled sorghum was poured into the hole, a plastic tube was used to hold the polythene bag together and corked with cotton wool). The substrates were in triplicates. The prepared substrates were autoclaved at 121^{o}C for 15 minutes and allowed to cool. It was inoculated with already prepared spawn and then incubated at room temperature in the dark for 2 weeks for mycelia running. After 2 weeks it was exposed to light and watered at intervals. Harvesting started after 2 days of exposure and watering for some of the substrates. This was done by twisting the mature mushroom and uprooting from the base to prevent breakage as decaying mushroom affects growth of the new ones. Mature mushroom was identified by curled margin of the pileus.

2.6 Preservation

Harvested mushrooms were sun dried and preserved for proximate analysis.

2.7 Determination of proximate analysis

Proximate analysis (moisture, crude fibre and ash) for the mushroom samples were determined by the AOAC procedures (AOAC, 1995). Protein content was determined following the method of Leco Manuel "Thermal conductivity" by the Kjeldahl (Dundar *et al*., 2009). Fat and carbohydrate contents were determined by the method of Watt and Merill (1975).

3.0 Results

3.1 Appearance of mushrooms on the various substrates

The result of this study revealed the appearance of mushroom on saw dust and cotton waste as 2 days, while paper, combination of saw dust and palm fruit waste, and mixture of paper and cotton waste occurred after 3 days while palm fruit waste had the longest appearance time of 5 days (Plate 1-6).

3.2 Morphological characteristics of the mushroom harvested from the various substrates during the first harvest

The length of stalk (cm) of mushrooms obtained during the first harvest from the saw dust had the highest value of 5.40 ± 0.63 followed by mixture of saw dust and palm fruit waste, and cotton waste with 4.88 ± 0.44 and 4.53 ± 0.46 respectively with the lowest value of 2.70 ± 0.14 obtained from palm fruit waste only. Similar result was also obtained with the diameter of the stalk as saw dust, mixture of saw dust and palm fruit waste and cotton waste had 1.55 ± 0.17, 1.45 ± 0.13 and 1.43 ± 0.13 cm respectively with the least being palm fruit waste only with a value of 1.15 ± 0.21 cm. The diameter of pileus revealed that cotton waste has the largest diameter of 7.88 ± 1.29 followed by saw dust and mixture of saw dust and palm fruit with 7.50 ± 1.78 and 7.18 ± 1.93 cm respectively while the least value of 3.00 ± 0.14cm was obtained from mixture of paper and cotton waste (Figure 3.1).

3.3 Morphological characteristics of the mushroom harvested from the various substrates during the second harvest

Figure 3.2 presents the morphological characteristics of mushrooms harvested during the second period. The mushroom obtained during the second harvest revealed that saw dust has the highest length of stalk of 3.75 ± 0.77 cm while the least value of 1.98 ± 0.51 cm was obtained with the mixture of saw dust and palm fruit waste. Mixture of paper waste and cotton waste has the highest diameter of stalk of 3.25 ± 0.61 cm followed by cotton waste (1.28 ± 0.15 cm) and saw dust (1.23 ± 0.08 cm) respectively while the lowest value of 0.65 ± 0.17 cm was obtained with paper waste. Mushroom from cotton waste displayed the largest diameter of pileus of 4.90 ± 1.05 cm followed by 4.63 ± 0.86 cm and 3.68 ± 0.84 cm for saw dust and mixture of saw dust and palm fruit waste respectively. The lowest value of 1.78 ± 0.63 cm was obtained from mushroom harvested from the mixture of paper and cotton waste.

Plate 3.1. *Pleurotus pulminarius* as observed growing on sawdust after 2 days of exposure and watering

Plate 3.2. *P. pulminarius* growing on palm fruit waste as observed after 5 days of exposure and watering

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Plate 3.3. *P. pulminarius* as seen growing on combination of sawdust and palm

Plate 3.4. *P. pulminarius* growing cotton wastes as observed after 2 days of exposure

fruit waste after 3 days of exposure and watering

and watering

Plate 3.5. *P. pulminarius* growing on paper as observed after 3 days of exposure and watering

Plate 3.6. *P. pulminarius* growing on mixture of cotton waste and paper as observed after 3 days of exposure and watering

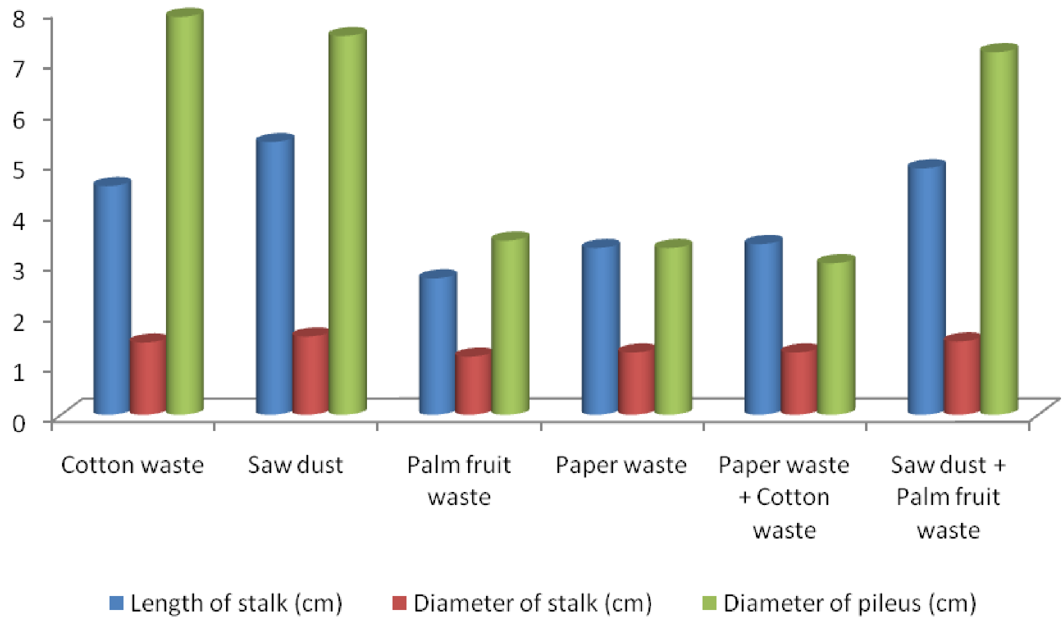

Figure 3.1. Length of stalk (cm), diameter of stalk (cm) and diameter of pileus (cm) of mushrooms harvested on the various substrates (First harvest)

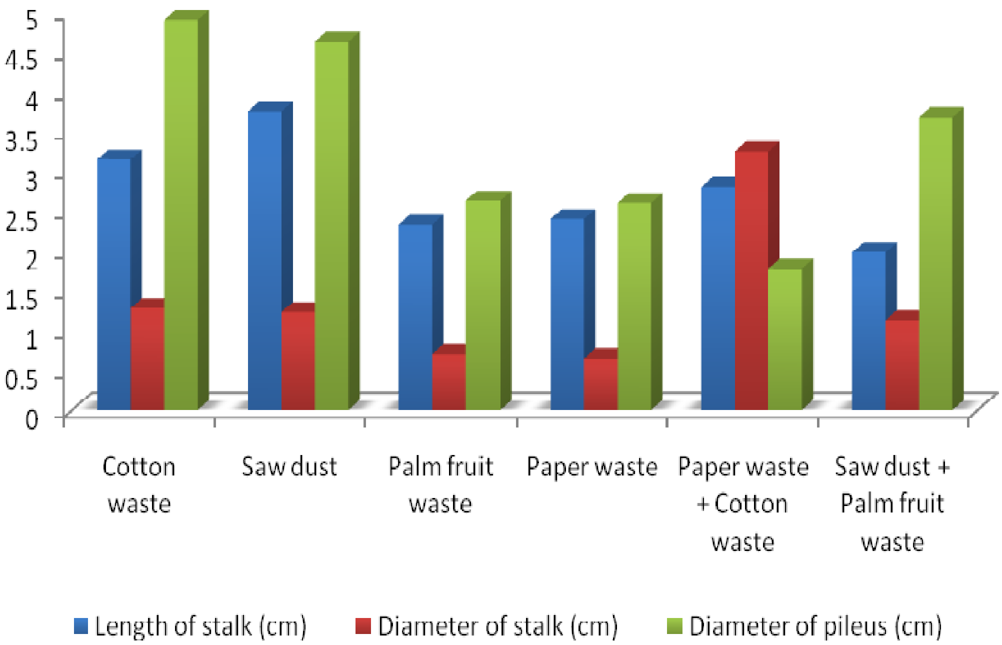

Figure 3.2. Length of stalk (cm), diameter of stalk (cm) and diameter of pileus (cm) of mushrooms harvested on the various substrates (Second harvest)

3.4 Morphological characteristics of the mushroom harvested from the various substrates during the third harvest

Saw dust mushroom displayed the highest length of stalk of 2.03 ± 0.32 cm while the least value of 1.53 ± 0.25 cm represent mushrooms harvested on mixture of paper and cotton wastes. Palm fruit and paper wastes had the same length of stalk of 1.90 ± 0.1 cm. The diameter of stalk of mushrooms harvested on the various substrates revealed that saw dust has the highest value of 0.9 ± 0.1 cm followed by cotton waste and, mixture of saw dust and palm fruit waste with 0.77 ± 0.25 cm and 0.57 ± 0.21 cm respectively, the lowest value of 0.4 ± 0.1 cm was obtained with mushrooms harvested on palm fruit waste. The highest and lowest diameter of pileus of 3.20 ± 0.20 cm and 1.80 ± 0.21 cm was obtained from saw dust and mixture of paper and cotton wastes respectively (Figure 3.3).

3.5 Proximate parameters of the mushroom harvested on the various substrates

Mushrooms harvested on mixture of paper and cotton waste has the highest percentage protein of 40.54% followed by paper and cotton wastes with 39.51% and 38.41% respectively. The least value of 28.79% was obtained from mushroom harvested on saw dust (Figure 3.4). Mushroom harvested on saw dust has the highest percentage fat content of 0.99% with 0.89% as that of mixture of paper and cotton wastes while the same value of 0.86% represents percentage fat from mushrooms harvested on paper, and mixture of saw dust and palm fruit waste. Palm fruit waste gave the lowest percentage fat of 0.74% (Figure 3.5). Palm fruit waste displayed the highest percentage ash (Figure 3.6) and crude fibre contents (Figure 3.7) of 4.38% and 4.81% respectively followed by mixture of saw dust and palm fruit wastes of 4.35% and 2.83% respectively. Saw dust gave the highest percentage carbohydrate of 62.54%, this is followed by mixture of sawdust and palm fruit waste, and palm fruit waste only with 59.95% and 56.36% respectively (Figure 3.8). Moisture content of 0.51% was observed for mushrooms harvested on Palm fruit waste, Sawdust and mixture of sawdust and palm fruit waste as the least value while the mixture of paper and cotton waste has the highest moisture content of 3.49% (Figure 3.9).

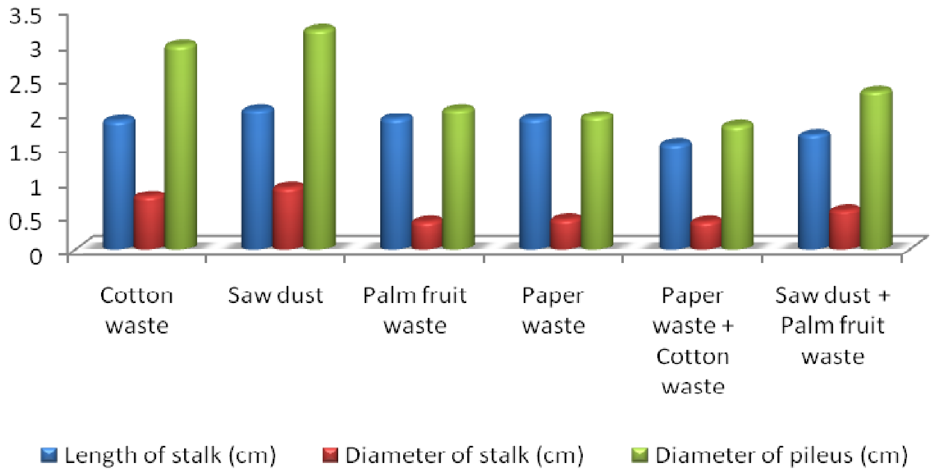

Figure 3.3. Length of stalk (cm), diameter of stalk (cm) and diameter of pileus (cm) of mushrooms harvested on the various substrates (Third harvest)

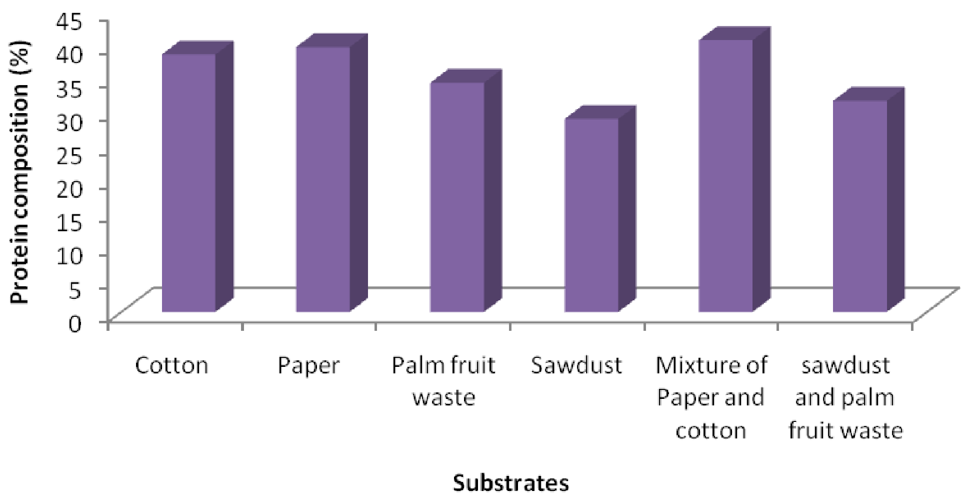

Figure 3.4. Percentage protein composition of mushrooms harvested on various substrates

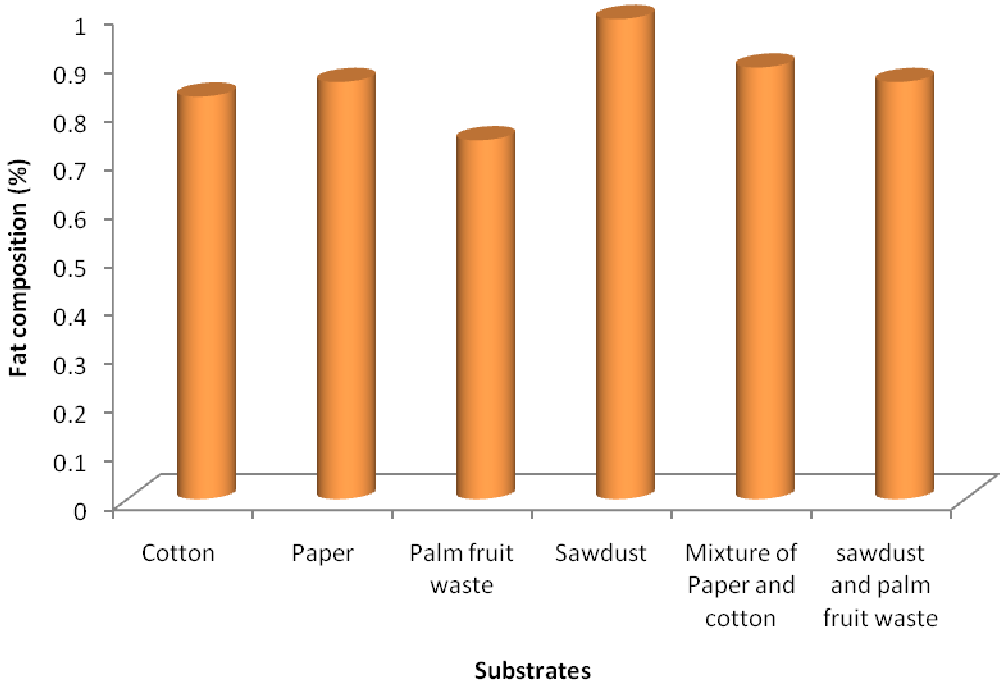

Figure 3.5. Percentage fat composition of mushrooms harvested on various substrates

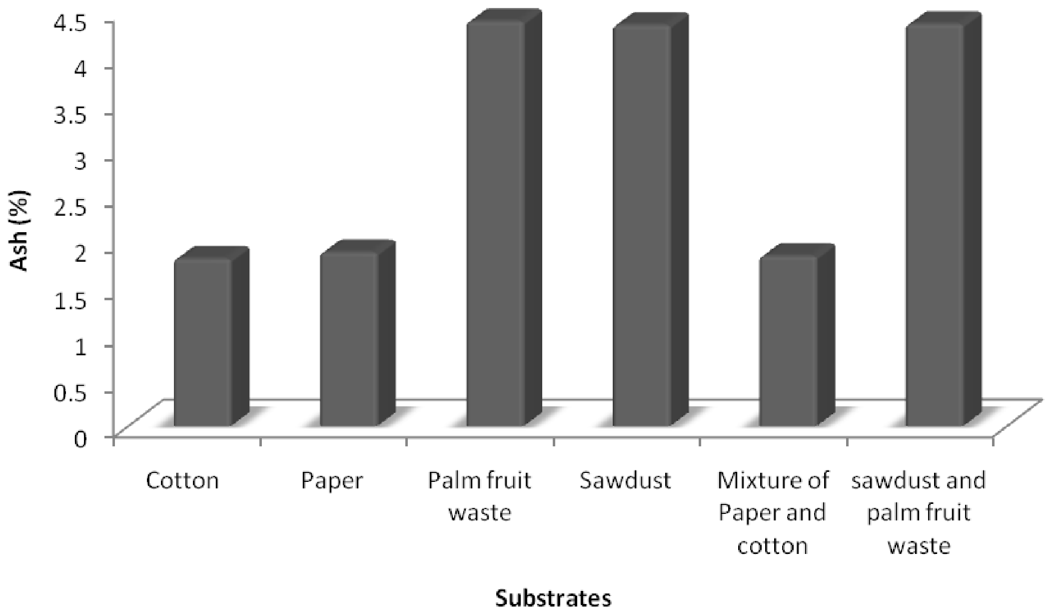

Figure 3.6. Percentage ash composition of mushrooms harvested on various substrates

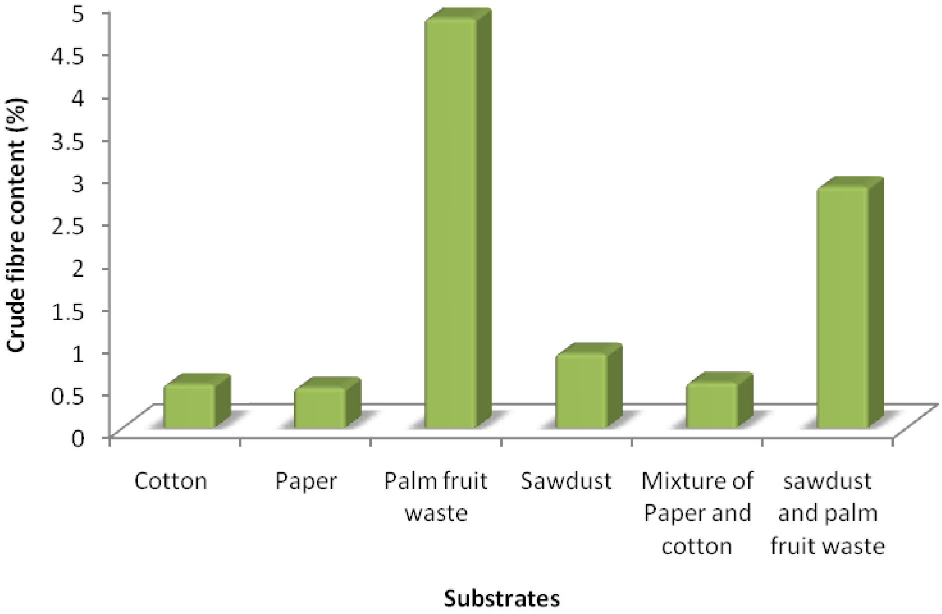

Figure 3.7. Percentage crude fibre of mushrooms harvested on various substrates

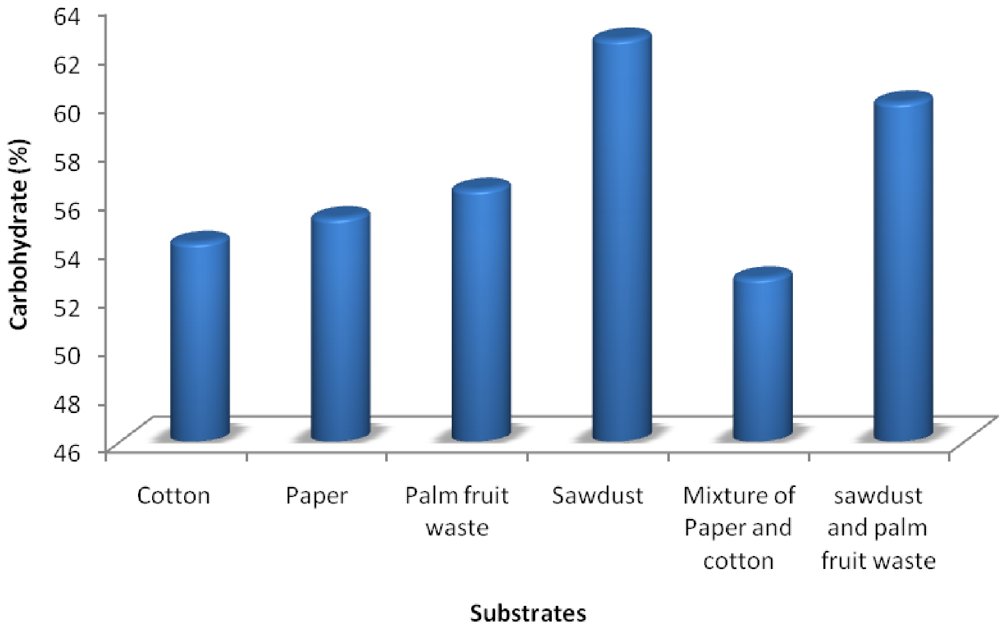

Figure 3.8. Percentage carbohydrate content of mushrooms harvested on various substrates

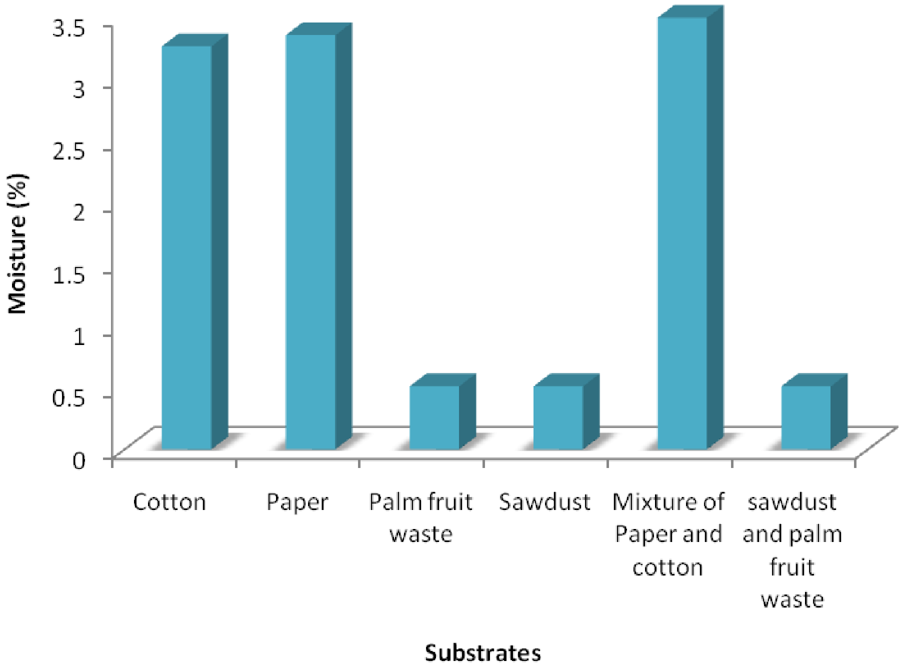

Figure 3.9. Percentage moisture content of mushrooms harvested on various substrates

4. Discussion

This study revealed that *Pleurotus pulminarius* possess the capability to grow on different ligno-cellulosic wastes such as cotton, paper, palm fruit waste, saw dust, mixture of paper and saw dust, and mixture of saw dust and palm fruit waste. This is a proof of the fact that *Pleurotus pulminarius* produces diverse enzymes such as cellulase, lipase and so on, for the breakdown of the complex organic compounds like cellulose, lipids, lignin to their absorbable forms, which is therefore used for their growth and development. This buttressed the statement that agricultural ligno-cellulosic waste represents the ideal and most promising substrates for cultivation by Ingale and Ramteke (2010). This has also been reporeted by Ugioro *et al*. (2012).

From the results obtained, it was shown that the shortest time of appearance was in saw dust and cotton waste (2 days), followed by paper, combination of saw dust and palm fruit waste, and mixture of paper and cotton waste (3 days) while palm fruit waste revealed the longest appearance time of 5 days (Plate 1-6). Islam *et al.* (2009) reported 2.45 and 4.75 appearance days on the average for sawdust and palm fruit waste respectively.

Based on the morphology of the cultivated mushrooms, mushrooms grown on saw dust had better length and diameter of stalks compared to other substrates during the three stages of harvest, cotton waste produces mushrooms with better diameter of pileus at the first and second stages of harvest. The better mushrooms morphology observed with saw dust can be attributed to its higher surface area which naturally will enhance digestibility by the fungal enzymes. Okhuoya (2011) stated that *Pleurotus pulminarius* produces enzymes that degrade sawdust at a faster rate than palm fruit waste leading to a faster and high yield of mushroom having greater stalk length with increased stalk diameter coupled with high pileus diameter relative to palm fruit waste. On the combination of the substrates, mixture of saw dust and palm fruit waste produced morphologically better mushrooms at the three stages of harvest except for length and diameter of stalk at the second stage of harvest, this may be due to the fact that both palm fruit and saw dust are natural waste materials unlike paper which had been chemically treated. Progressive decrease in the length and diameter of stalk, and diameter of pileus were observed after each stage of harvest, this can be attributed to depletion in the nutrient composition of the substrates over time in the course of the study.

The proximate analysis of the mushroom revealed that mixture of paper and cotton waste has the highest percentage protein content (40.54%) compared to other substrates, this is simply an evidence that this combination result in a better synergy as the protein content of the combination was higher than that of paper (39.51%) and cotton waste (38.41%) alone which were also high; unlike mixture of saw dust and palm fruit waste (31.49%) with lower protein content compared to saw dust (28.79%) and palm fruit waste (34.20%) singly. The mushrooms obtained in this study contain higher percentage protein (28.79% - 40.54%) than those reported by Dundar *et al*. (2009) for *Pleurotus ostreatus* cultivated on millet, wheat, cotton and soy bean stalks (14.06% - 22.15%). The result of this study is also better than the range of percentage protein (19 - 35%) stated by

Okhuoya (2011).
All the substrates produced mushrooms that are very low in fat with the highest been 0.99% on saw dust. Thus, they supply cholesterol free nutrients. It was reported by Bobek *et al*. (1991) that the consumption of mushroom-containing diet prevented serum cholesterol increase at the end of four week period and lowered by almost 40% as compared with control groups which have not had mushroom in their diet. This study reported lower percentage fat (0.74% - 0.99%) compared to the report of Dundar *et al*. (2009) for *Pleurotus ostreatus* cultivated on millet, wheat, cotton and soy bean stalks (2.45% - 3.15%); Bonatti *et al.* (2004) who reported 5.97% and 6.32% for mushrooms cultivated on banana and rice straw respectively; and 1.8% obtained by Shah *et al.* (1997).

The high percentage ash content of 4.34% for mushroom harvested on sawdust, 4.35% for mushroom harvested on combination of sawdust and palm waste, and 4.38% for mushroom harvested from palm fruit waste are proofs of mushroom serving as appropriate source of macro and micro-nutrients essential for the effective functioning of the body system. This observation is lower than 5.58% and 6.13% of ash content of *P. ostreatus* cultivated on banana straw and rice straw respectively reported by Bonatti *et al*. (2004). The mushrooms cultivated in this study possess appreciable amount of crude fibres which are essential in keeping the digestive system healthy, the highest value of 4.81% was observed for mushrooms harvested on palm fruit with 2.83% for mushrooms harvested on combination of sawdust and palm fruit waste. This study revealed low crude fibres compared to 34.8% for *P. ostreatus* cultivated on wheat straw by Justo *et al*. (1999). The high ash and crude fibre content of mushrooms harvested on palm fruit waste and palm fruit waste-combination can be attributed to readily absorbable nutrients in palm fruit waste.

All the substrates used produced mushrooms with high carbohydrate content which ranged from 52.72% to 62.54%, saw dust and, mixture of saw dust and palm fruit waste had the highest percentage carbohydrate of 62.54% and 59.95% respectively. This may be due to high carbon content of these substrates. The mushrooms obtained in this study contain higher percentage carbohydrate (52.72% - 62.54%) than those reported by Dundar *et al*. (2009) for *Pleurotus ostreatus* cultivated on millet, wheat, cotton and soy bean stalks (36.07% – 39.94%).

The highest moisture content (3.49%) was obtained for mushrooms harvested on the combination of paper and cotton wastes, this is again a synergistic effect in that this was higher than values reported for paper (3.35%) and cotton waste (3.26%) respectively. This result was very low compared to the report of Dundar *et al*. (2009) for *Pleurotus ostreatus* harvested on millet, wheat, cotton and soy bean stalks (7.37% - 7.40%).

Conclusion, the result of this study revealed that *Pleurotus pulminarius* can be used for agricultural waste management food supplements to serve as cheap source of protein to solve protein deficiency in humans most especially among the very poor who can neither afford the price of fish nor meat. The study also revealed that morphological characteristics and nutritional composition of mushroom depend on the substrate of cultivation. Conssidering that the wastes used in this study are ubiquitous in our environment and are being disposed off in ways that are detrimental to man’s existence, coupled with the nutritional composition of mushroom which present a cheap and highly economical alternative to the now scarce and expensive essential nutrients, it is high time we unearth this gold.

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Appendix

Plate 3.7. Fully ramified spawn