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Research Article

Studies on different substrate effects on the growth, yield and nutritional contents of *Pleurotus ostreatus* (oyster mushroom) and *Calocybe indica* (milky mushroom)

Tolulope Ewekeye[1], Akinshola Omikunle[1], Promise Emenaha[1], Abdulrazak Adebayo[1], Adetayo Sanni[1,] Esther Okubena-Dipeolu[2] and **Oyedamola Oke[1]**

1. Department of Botany, Faculty of Science, Lagos State University, Ojo, Lagos, Nigeria.
2. Department of Agriculture, School of Agriculture, Lagos State University, Epe, Lagos, Nigeria.

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Corresponding Author
Tolulope Ewekeye
E-mail: tolulope.ewekeye@lasu.edu.ng

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Abstract

This study was conducted to compare the growth and nutritional contents of *Pleurotus ostreatus* and *Calocybe indica* grown on various substrates. Spawns of *P. ostreatus* and *Calocybe indica* were inoculated into sterilized cornhusk, sugarcane bagasse, sawdust and plantain stem. Calcium diphosphate and rice bran were mixed with them to optimize the growth of the mushrooms. The linear growth on the different substrates was determined by measuring the length from the top of each treatment bag to the point where the spawn was showing a whitish colour. Each substrate's initial flushes were collected and air-dried to a consistent weight. According to standard procedures, the nutritional contents were assessed; these include the amount of moisture, ash, protein, carbohydrate, and vitamins. After twelve weeks of inoculation, three of the substrates produced fruiting bodies with sawdust showing the highest mycelial growth of *P. ostreatus* while sugarcane bagasse did not produce fruiting bodies. Results from the nutritional analysis showed that carbohydrate, crude fat, and ash contents were significantly higher ($P<0.05$) in *P. ostreatus* grown on plantain stem, the protein was higher in sawdust substrate and crude fiber was higher in cornhusk. Also, Vitamins A and E recorded high values in *P. ostreatus* grown on sawdust followed by cornhusk and plantain stem which had the lowest value. The findings from this study showed that the utilization of different agricultural wastes as substrates for the production of mushrooms could be economically valuable to meet the health and nutritional needs of the world's population.

1. Introduction

The fruiting bodies of numerous fungi species are commonly referred to as mushrooms. The major structures of these fungal bodies are normally above ground; however, the structure above the ground only makes up a minor fraction of the overall fungal body. From the taxonomic point of view, mushroom-forming fungi are primarily basidiomycetes, while certain ascomycetes species also exist. There are an estimated 140,000 species of mushrooms on earth, of which about 10% have been identified [1]. From the approximately 14,000 species, less than 2% are suitable for human consumption; of these, 650 or so have therapeutic qualities [2]. Mushrooms have significant biological and economic effects on humans.

From ancient times, man has probably eaten wild mushrooms with nourishment because of their flavour and taste [3]. Mushrooms are an essential source of nutrition and energy in terrestrial food

chains as they are consumed by a variety of animals, including rodents and birds. Mushrooms are not considered to be true plant since they lack leaves, contain no chlorophyll, have no roots, or seeds, and essentially do not require light to thrive. They thrive in the dark and disperse by producing spores. They typically consist of a stem bearing a pileus known as the head.

There are some edible species of mushroom that can be found in the genus *Pleurotus*. These members are also known as tree or oyster mushrooms. They are saprotrophic and can be distinguished by their short, peculiar stalks, not centrally connected, and delicate fruiting bodies. One of the most common edible mushrooms found in this genus is *Pleurotus ostreatus*. It was first artificially cultured at the beginning of the 20th century by Richard Falck [4] and it is known to be a rich source of dietary fibers and carbohydrates. However, the sum of intrinsic non-digestible carbohydrates in mushrooms, primarily chitin, constitutes the total dietary fiber (TDF) in such foods [5]. *P. ostreatus* is a carnivorous fungus that feeds on roundworms by employing a calcium-dependent poison that paralyzes the prey within minutes of contact, causes necrosis, and produces slurry to allow consumption as a source of protein-rich food [6].

The phenolic and tannin components of *P. ostreatus* have been observed to have antibacterial activity, which is similar to that of many medicinal plants. The antibacterial effects are characterized by the lysis of cell membranes, the inhibition of protein synthesis, the release of proteolytic enzymes, and the production of microbial adhesins [7]. As a result, *P. ostreatus* serves as a useful source of antioxidant food additives [8]. Due to the nutritional properties of *P. ostreatus*, its production on a large or small scale is fast increasing over the years.

Calocybe indica, also referred to as a milky white mushroom, is a tropical mushroom that blooms in the summer and is valued for its nutrients. Both mushroom eaters and prospective farmers have become interested in it due to its sturdy size, sustained yield, appealing colour, delicacy, long shelf life, and lucrative market value. *C. indica* has a high protein, lipid, fiber, carbohydrate, and vitamin content as well as a large number of important amino acids and low-fat ingredients [9]. Mushrooms can be grown on a variety of substrates, depending on the type of mushroom being cultivated. In addition to the substrate, mushrooms require the right temperature, humidity, and air exchange to grow successfully. The specific requirements will vary depending on the species of mushroom being cultivated. The local availability of agricultural waste and production effectiveness determine the substrate used for mushroom production in a given area. This may offer a partial solution to the country's waste management issues as well as the pollution challenges surrounding the environment along with the nutritional benefits that would result from eating the cultivated mushroom. This study was aimed at cultivating and determining the growth rate of *Pleurotus ostreatus* and *Calocybe indica* and also assessing the proximate and nutritional compositions of fruit bodies obtained from four different substrates.

2. Materials and methods

The spawn of *Pleurotus ostreatus* and *Calocybe indica* were purchased at Bosol Company Ltd, Mokola, Ibadan, Oyo State, Nigeria.

2.1 Sterilization of substrates

The substrates used for the cultivation of *P. ostreatus* and *C. indica* were Corn husk (CH), Sugarcane bagasse (SB), Sawdust (SD), and Plantain stem (PS). These substrates were chosen because of their local availability within the research study site. While plantain stem was acquired at Lasu Parks and Gardens, the supplements used—calcium and rice bran—were both purchased at Lagos State farm settlement Agric, located along the Lagos-Badagry Expressway. Cornhusk was obtained from a corn farm next to the Lagos State University Post Service bus stop, while sawdust was collected from the Ipaye sawmill on the LASU-Isheri highway in Lagos State. At the Alaba Rago bus stop on the Lagos-Badagry expressway, sugarcane bagasse was bought. The substrates were soaked in water for a short while and allowed to air dry on clean, well-sanitized laboratory benches. The cornhusk was shredded into pieces about 1cm in length and dried for two weeks alongside the sawdust, plantain stem and sugar cane. After drying; 1.5kg of the dried substrates were distributed equally into a plastic container and irrigated with 2.5 liters of water, 600g of rice bran was added and 3g of calcium was applied to reduce the pH content of the substrate and then mixed thoroughly. Five hundred grams of the mixture was packed into

separate black polythene bags. All these treatments were carried out with six (6) replications. The substrates were bagged and sterilized in an autoclave at 121^{0}C and 1.1kg/cm^2 for 30 minutes. The bags (containing 500g) of replicates of all four substrates were left to cool on a cabinet shelf for three days; the shelf was initially cleaned with cotton wool and absolute ethanol.

2.2 Inoculation of spawns

The bottles of spawns were initially preserved in a refrigerator and were removed from the refrigerator 12 hours before inoculation into the substrate. The first step of spawning was the washing of hands and knives to be used. After the bags were untied, 10g of spawn material was mixed with the upper part of the substrate of each bag with the help of the knife such that the spawn covered almost all the whole substrates. Ten grams of spawn was used per 500g bag of the substrate [10]. The workbench was thoroughly cleaned and disinfected with ethanol.

2.3 Incubation and cultivation

The cultivation of the two species of mushrooms on the four different substrates was done following the procedure of Anagho [11] with minor modifications**.** After all the bags have been inoculated with spawns and closed, they were kept in a dark cupboard for 21 days at room temperature (34 ± 2^{0}C) with a relative humidity of 84%. The bags were left to ramify entirely as the substrate begins to turn white and after the mycelia run has been completed with the colonization of the substrate bags for 21 days; the bags were carefully moved inside the screen house to start the fruiting process. To maintain proper temperature, moisture, and humidity, the room was watered daily and regular irrigation was done twice daily using a water sprayer in order to keep the bag moist for production. The substrate bags were slashed with a sterile razor blade at several points to allow the fruiting bodies to emerge well. The first flush from each substrate bag was harvested with clean hands and taken to the Department of Botany Laboratory to be weighed and air-dried. The mushrooms were air-dried on a sterile plain sheet of paper after they have been separated by cutting off the basal part of the stalk.

2.4 Morphological data collection

The measurement of length of the fruit bodies from the top of each treatment bag after sprouting was used to analyze the growth and yield of *P. ostreatus* and *Calocybe indica.* The linear growth was determined by measuring the length from the top of each treatment bag to the point where the spawn was showing a whitish colour (an indication of colonization). The assessment of the mycelia growth on the substrates was recorded every day from the second week up to the twelfth week. After this, *P. ostreatus* samples from each bag of substrates were collected gently, weighed, and air-dried to a constant weight. The samples were stored in polythene zip-lock bags.

2.5 Evaluation of nutritional composition and proximate analysis

The proximate analysis of the fruiting bodies collected from each substrate was estimated in accordance with the specified analytical techniques [12-14] at the University of Lagos, College of Medicine Laboratory, Idi-Araba, Lagos State. Moisture content was evaluated by drying fresh samples to constant weight at 105 °C in a hot air oven. The Kjeldahl technique was used to calculate the protein content and a conversion factor of 4.38 was used [15]. Ash content was determined by incineration using a muffle furnace for 24 hours at 550 ± 5 °C. Crude fat content was evaluated by extracting with petroleum ether using a Soxhlet apparatus. The crude fiber was obtained by subtracting the weight of ash from the rise in weight on the paper caused by the insoluble material after the acid hydrolysis of the fat-free samples and filtration into ashless filter paper. Total carbohydrate content was calculated by deducting the total percentages of protein, crude fiber, crude fat, moisture, and ash from 100 [16].

2.6 Vitamin A and E determination

The spectrophotometric approach published by Al-Sulimany and Townshend was used to determine the total vitamin A concentration [17]. In this approach, iodine was utilized as the chromogenic agent in the presence of 1, 2-dichloroethane, preventing interference from vitamin D2 and beta-carotene. Further verification and comparison of vitamin A concentration with Pearson's spectrophotometric approach were conducted [18]. The vitamin E content of the *P. ostreatus* mushrooms was determined using standard analytical methods [19].

2.7 Statistical analysis

The computation of data was done using SPSS

Table 1. Phases of spawn running (in weeks) on different substrates for *Pleurotus ostreatus*

Substrate	Week 2 (cm)	Week 4 (cm)	Week 6 (cm)	Week 8 (cm)	Week 10 (cm)	Week 12 (cm)
Corn husk	2.21	2.43	4.30	5.20	6.20	9.31
Plantain stem	6.55	9.82	11.21	13.40	10.20	7.20
Saw dust	2.72	4.12	8.40	12.50	15.20	19.00
Sugarcane bagasse	0.50	0.60	0.90	0.82	0.62	0.00

Table 2. Phases of spawn running (in weeks) on different substrates for *Calocybe indica*

Substrate	Week 2 (cm)	Week 4 (cm)	Week 6 (cm)	Week 8 (cm)	Week 10 (cm)	Week 12 (cm)
Corn husk	0.00	0.00	1.21	3.20	4.28	6.46
Plantain stem	0.00	0.00	2.20	3.42	4.42	5.30
Saw dust	0.00	0.00	3.50	5.40	6.20	7.60
Sugarcane bagasse	0.00	0.00	0.00	0.00	0.62	0.88

Version 20. Values are presented as Mean ± SD and were subjected to one-way analysis of variance (ANOVA). Where there is a considerable difference, Fisher's Least Significance Difference (LSD) was applied at $\alpha = 0.05$.

3. Results

3.1 Spawn running/mycelia growth

The mycelia growth of *Pleurotus ostreatus* throughout the spawning period in sawdust and corn husk showed increase in length from the second week as the week progresses while plantain stem and sugar cane bagasse mycelia growth decreased gradually. At the end of the twelfth week, sawdust had the highest mycelia growth of 19.00cm while sugarcane bagasse had a growth decline from 0.50cm to 0.00cm (Table 1). For *C. indica*, there was no spawn running on all the substrates until the sixth week when mycelia growth was recorded with the exception of sugarcane bagasse which only had 0.62cm and 0.88cm in weeks 10 and 12 respectively. A minimal increase in mycelia length occurred on the other substrates (Table 2).

3.2 Formation of fruiting body

For *P. ostreatus*, PS_1 substrate was the first to complete its mycelia run after 27 days of inoculation and the first to have its first flush of mushrooms after 33 days of inoculation. PS_2 and PS_3 produced their fruiting bodies after 32 days and 36 days of inoculation respectively. The sawdust treatment took 38 days to yield its first flush of mushrooms which were collected the following day. Its samples of mushrooms were weighed to be 22.0g, 22.5g and 22.3g respectively. Corn husk substrates were the last to finish their mycelia run, and the fruiting bodies were harvested after the second day. The mushrooms' total dry weights for CH_1, CH_2, and CH_3 were 39.6g, 39.9g, and 39.1g. Sugarcane bagasse treatment was unable to produce any fruiting bodies of mushroom, however, one of the bags that completed its mycelia run after 51 days but failed to produce any fruiting bodies till this research work was brought to an end. For *Calocybe indica,* although there was a mycelium run on all the substrates inoculated with the spawn of *C. indica,* there was no production of any fruiting bodies till the termination of this research.

3.3 Results of proximate analysis

The carbohydrate content among all the mushroom samples differs in their percentage composition with PS having the highest carbohydrate content of 50.38%, followed by corn husk with 47.10% and then sawdust (45.09%). Ash content in the mushroom sample from PS was greater than those from SD and CH, which showed values of 6.32%, 5.68%, and 4.81%, respectively. Also, values recorded from the crude fat contents showed (0.91%, 1.07%, and 1.05%) for CH, SD, and PS respectively (Table 3). SD demonstrated the highest protein concentration of the three (3) substrates examined, followed by cornhusk (16.90%) and plantain stems (15.82%). Table 3 shows that the moisture content varies from 8.27% for PS to 12.31% for the SD mushroom sample. Corn husk displayed the highest crude fiber content of 19.64%, sawdust at 18.14% and plantain stems recorded the least (18.06%). Table 4 shows the Vitamin A and E values for the mushroom samples; vitamin A was the most prevalent varying from 48.7 to 51.9 mg/100g while Vitamin E content in the samples ranged from 1.50 to1.86 mg/100g with a sample from sawdust treatment being the highest (1.86).

Table 3. Nutritional values of fruiting bodies from *P. ostreatus* on different substrates

Substrates	Carbohydrate (%)	Protein (%)	Crude Fat (%)	Moisture (%)	Ash (%)	Crude Fiber (%)
CH	47.12 ± 0.17^{b}	16.90 ±0.24^{b}	0.91 ± 0.06^{a}	10.93 ± 0.12^{b}	4.81 ± 0.05^{a}	19.64 ± 0.29^{b}
SD	45.09 ± 0.16^{a}	17.70 ± 0.07^{c}	1.07 ± 0.45^{b}	12.31 ± 0.32^{c}	5.68 ± 0.35^{b}	18.14 ± 0.44^{a}
PS	50.38 ± 0.46^{c}	15.82 ± 0.33^{a}	1.15 ± 0.15^{b}	8.27 ± 0.08^{a}	6.32 ± 0.31^{c}	18.06 ± 0.20^{a}

Means with the same superscript alphabets and in the same column are not significantly different (p>0.05). CH= Corn husk, SD= Sawdust, PS=Plantain stem.

4. Discussion

The mycelia growth and yield of *P. ostreatus* varied widely, depending on the nature of substrate used. Different substrates have been reported to have various effects on the growth and yield of mushrooms [20]. The mean mycelia length of the fruiting bodies of *P. ostreatus* cultivated on sawdust was highly significant than *P. ostreatus* cultivated on other agricultural wastes. The maximum growth of *P. ostreatus* on the substrate probably indicates effective bio-conversion of this waste (sawdust). Also, Akinyele et al. [21] reported that the capacity of *P. ostreatus* to break down sawdust makes them a valuable waste control strategy. The selection of substrate is therefore essential in the mushroom-growing process because it greatly affects how prolific oyster mushrooms are for better growth.

Table 4. Mean Vitamin Composition of Samples of the Fruiting Bodies from *P. ostreatus* on different substrates

Substrates	Vitamin A (mg/100g)	Vitamin E (mg/100g)
Corn husk	51.59 ± 1.11^{b}	1.53 ± 0.13^{a}
Sawdust	54.8 ± 1.12^{c}	1.86 ± 0.11^{b}
Plantain stem	48.7 ± 1.40^{a}	1.50 ± 0.26^{a}

Means with the same superscript alphabets and in the same column are not significantly different (p>0.05).

The proximate analysis of the various *P. ostreatus* samples from three of the four substrates that were analyzed showed different percentages of nutritional composition and vitamin contents. From this study, samples obtained from PS and SD recorded the highest carbohydrate and protein contents of 50.38% and 17.70% respectively. This shows that mushrooms can be recognized as alternative sources of good quality protein and carbohydrates from agro-wastes; and when compared to green vegetables, edible mushrooms have been reported to be a more protein-rich option [22]. *P. ostreatus* raw fiber content of those grown on cornhusk, sawdust, and plantain stem recorded 19.64%, 18.14% and 18.06% respectively. Crude fiber is known as being effective in the lowering of blood cholesterol [23].

The moisture level of food material has an impact on the freshness, consumption, and stability of the food [24]. The moisture content of the mushroom samples varies as a result of the different substrates used in treating them as it was found that the fruiting body harvested from sawdust contained a higher percentage of moisture compared to those from cornhusk and plantain stem. This complies with previous work that harvesting time, maturity time, and environmental factors all have an impact on the moisture content of mushrooms during the growing season [25]. Although oyster mushrooms have little fat, they do contain certain important fatty acids for humans. The crude fat content recorded a low percentage range of 0.91 to 1.15%; however, this is a significant supply of necessary fatty acids to meet the demands of the human body [26]. The presence of nutritionally significant mineral food components has been linked to ash content [27], the ash content obtained in this study was 6.32, 5.28, and 4.81% for plantain stem, sawdust, and cornhusk respectively. Ash serves as a marker for the presence of minerals.

Vitamins are necessary and must be obtained on a regular and periodic basis through nutrition in order for people to grow and function properly [28]. Vitamin A was significantly higher in all three (3) substrates when compared to Vitamin E. Similar research by Ewekeye *et al* [29] has also shown the occurrence of Vitamins A and E in SD and CH substrate treatments. The lack of production of fruiting bodies from *C. indica* could be an indication of contamination of its spawn material or the inability of the substrates to support their growth.

5. Conclusions

This study showed the different types of substrates which can be used to cultivate oyster mushrooms and the nutritional composition of the mushroom. The cultivated mushrooms can be regarded as superfoods because of their high nutritional content, particularly

in terms of protein, carbohydrates, dietary fiber, and vitamins. The findings from this study showed promising results which can enhance the development of functional foods by food and pharmaceutical industries using mushrooms or bioactive compounds from mushrooms. Thus, these mushrooms can be promoted as a potential source of nutrients to combat malnutrition.

Authors' contributions

Conceptualization, T.E., E.O., and O.O.; Methodology, A.O., P.E., and A.S.; Investigation, A.A..; Writing—original draft preparation, T.E., E.O., and O.O.; Writing—review and editing, Supervision, T.E., and O.O.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare no conflict of interest.

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