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Research article

Molecular characterization of most cultivated *Pleurotus* species in sub-western region Nigeria with development of cost effective cultivation protocol on palm oil waste

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ABSTRACT

Major problems associated with the study of mushrooms in Nigeria are inaccuracy in identification, low bio-efficiency of commonly used substrate and difficulty in composting of substrates. The current study attempts the identification and characterization of *Pleurotus* species commonly cultivated in Western region of Nigeria, and evaluates the effectiveness of agricultural wastes in mushrooms cultivation. Molecular characterization was carried out for species identification. Different substrates combinations at ratio 1:1 were used. Molecular characterization revealed that the *Pleurotus* species grown in most farms and research institutes in South-western Nigeria are predominantly *P. ostreatus* and *P. pulminarius*. Palm bunch + Rice bran (8.24 ± 0.16) ramified almost twice faster than sawdust alone (4.98 ± 0.31) or any of the other substrates containing it. Fermented bunch (7.36 ± 0.19), the only substrate not sterilized also ramified faster than sawdust alone. All the other substrates compounded with palm bunch and shaft ramified faster than sawdust as lone substrate. In terms of yield, Palm bunch + Rice bran gave the highest (1774.75 g), followed by shaft + rice bran (1483.70 g), while the least value of 326. 94 g was obtained from sawdust. The highest value of biological efficient (BE) (100.57 g) and productivity (PT) (17.46 g) were obtained from shaft + Wheat bran and Palm bunch + Rice bran respectively, while sawdust gave lowest values of 13.08% and 3.23% for BE and PT respectively. Results obtained have shown that bunch and shaft supplemented with wheat and rice bran gave better yields and can be recommended for commercial mushrooms cultivation.

1. Introduction

Mushrooms are higher fungi, with fruiting bodies or basidiocarps that represent the reproductive stage in the basidiomycetes life cycle. Edible mushrooms like *Pleurotus* are known to be among the largest of fungi (Musieba et al., 2012). The genus *Pleurotus* is in the order Agaricales and family Pleurotaceae. *Pleurotus* is currently known as one of the most commonly edible mushrooms in the world. It was Paul Kummer who defined the genus in 1871 having being classified in the genus *Agaricus* before then. Its numerous advantages and beneficial properties made it gained great popularity among the cultivated mushrooms (Ijeh et al., 2009). This nutritious edible mushroom is ranked the second among commercial cultivated mushrooms (Adebayo et al., 2014). The ecological diverse, nutritional, medicinal and health importance as generally regarded macrofungi as one of major bioresource (Odeyemi et al., 2014).

Mushrooms have wide geographical distribution, with *Pleurotus* species being the greatest commercial importance, both in temperate areas and tropical regions (Andrew et al., 2013). However, the description and morphological features of commercial *Pleurotus* species are determined by different climates in countries of cultivation, methods of cultivation and different substrate materials used in cultivation (Rashid et al., 2016). *Pleurotus* species have high phenotypic variability across wide geographical ranges.

In several countries mushrooms are seasonal, always present in wet season and different substrates are employed in cultivation (Khan et al., 2019). Morphologically, mushrooms are classified as bracket, puffballs, gilled, and stinkhorns and they may either be poisonous or edible.

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Traditionally, *Pleurotus* species are identified using some unique features such as margin of carpophore, texture of stipe, colour of basidiocarps, spore-bearing surface and any other fruiting body features (Odeyemi and Adeniyi 2015). This traditional characterization method may be inaccurate and misleading, because of close resemblance of different species of genus *Pleurotus* at fruit body stage.

Challenges of traditional method of nomenclature such as inconsistency of morphological classification, unnoticeable features and indiscrimination among fungal species are overcome by modern molecular techniques (Nilsson et al., 2009). Remarkable progress in the breeding of mushroom new strains necessitates a better and efficient method of identification and characterization (Staniaszek et al., 2002). To make biological assessment suitable for population identification, structure and evolutionary determination within and among species, the genotypic identity is important (Mahmood et al., 2009). Due to high influence of morphological and physiological characteristics by cultivation parameters, it is always necessary to employ advanced techniques such as DNA finger printing in species identification (Iqbal et al., 2010). The expression of a gene is a cumulative effect of both genetic and environmental interaction on a specie/strain of mushroom (Astarini et al., 2004). Biochemical markers can be used to elicit the genetic variability because they are direct product of genes. In genetic diversity assessment in plant biotechnology, one of the efficient tools used is DNA finger printing (Mehmood et al., 2008). For long, combinations of morphological traits and different DNA markers have been used for the determination of genetic variations at molecular level (Sajida et al., 2009). The genetic makeup of the cultivated edible mushrooms determined its productivity and quality (Kaur and Sodhi 2012). Increase in the quality, reduction in the costs of cultivation and farmers' revenue increase of cultivated mushrooms could be aided by genetically improved strains (Avin et al., 2012).

Pleurotus species have been successfully cultivated on agricultural wastes such as sawdust, rice straw, banana leaves, maize shaft, olive cake, date-palm leaves (Yang et al., 2013; Alananbeh et al., 2014; Adebayo et al., 2014; Marlina et al., 2015; Rezania et al., 2017; Yamauchi et al., 2019). However, its mycelium growth and yield are greatly affected by the methods of substrates preparation, type and level of supplementation of the substrate, and technicalities involve in composting. Strain degeneration and loss of genetic diversity are major problems encountered by cultivated mushrooms (Wang et al., 2012), forcing mushroom farmers to use new improved strains and new cultivation methods in order to achieve high performance (Adebayo et al., 2013). However, diverse growth conditions in mushroom cultivation cause inconsistency in the genetic components which set them apart from animal and plants. Environmental factor influences fruiting body morphology greatly, thus, problems frequently arise if the classification is based entirely on morphological characteristics.

Therefore, this study was undertaken to characterize the most cultivated *Pleurotus* species in the study area using molecular tools, and develop effective cultivation protocol using agricultural wastes for commercial oyster mushrooms cultivation.

2. Materials & methods

2.1. Samples collection and tissue culture preparation

Pleurotus species cultured were collected from two different States (Osun and Oyo) of South West Nigeria. Eight (8) different species of *Pleurotus* were purchased from four (4) renowned mushroom commercial centres from the two States. The details and particulars of the collected species were stated in Table 1. The tissue cultures of the organisms were prepared on potato dextrose agar (PDA) media and stored at 4 °C for further uses (Adebayo et al., 2013).

2.2. Grains spawn cultivation

Millets grains were weighed (100 g/bottle), washed (four times), boiled for 45 min and dried. Calcium carbonate ($CaCO_3$) (1% w/w) were mixed with the dried grains, dispensed in bottles and sterilized at 121 °C for 30 min. Six plugs (6 mm) of actively growing culture were used to inoculate sterile grains, incubated at 23 ± 2 °C and mycelium running was recorded three days interval (Adebayo et al., 2013).

2.3. Collection and preparation of oil palm substrates

Oil palm bunches and shafts were got from Onikoko idigba, Iresaapa, Ogbomoso (8.0758° N, 4.3481° E), Oyo State, washed, dried and shredded into smaller particle size by matchetting. Other agricultural wastes used are sawdust, rice bran and wheat bran.

2.4. Substrates formulation protocols

Different substrates combinations were used as stated in Table 2. Approximately 1.92 L of water was used to wet 10 kg of shredded substrate to attain 65% moisture content and 1 kg each was bagged in heat resistant polythene bag. A total of 10 bags of each treatment were produced. All the bags were packed into locally improvised autoclave (steamer) and sterilized for 4 h at over 100 °C. They were left overnight to cool down to prevent the high temperature from killing the spawn. Only the fermented bags (substrate soaked in water for 24 h, drained and covered with jute bag for 72 h) were exempted from sterilization.

2.5. Substrate inoculation

Spawn (5–10 %w/w) was used to inoculate each of the ten (1.0 kg) bags from both ends of the bags to ensure even distribution of spawn and ramification of the bags. Incubation of bags was done in the dark room at 25 ± 3 °C, until fully colonized. Fully colonized bags were transferred into the growth-room (dark, humid and between 22 °C to 28 °C) where

Table 1. Details of collected *Pleurotus* species with coordinates of the location.

Sample CODE	Name of State/Town	Sample Name	Mushroom cultivation Centre	Longitude & Latitude
PPLTC	Osun/Osogbo	*Pleurotus pulmonarius*	LTC mushroom farm	4° 30′ 0E and 7° 46′ 60N
PPNH	Oyo/Ibadan	*P. pulmonarius*	NIHORT	8° 23′ E and 11° 23′ N
POFR	Oyo/Ibadan	*P. ostreatus*	FRIN	3.8628° E and 7.3913° N
PSFR	Oyo/Ibadan	*P. sajor-caju*	FRIN	3.8628° E and 7.3913° N
POAM	Osun/Ede	*P. ostreatus*	AM mushroom farm	4°25′ 60E and 7°43′ 60N
+170119	Osun/Osogbo	*P. abalone*	LTC mushroom farm	4° 30′ 0E and 7° 46′ 60N
E2712	Osun/Osogbo	ELM oyster	LTC mushroom farm	4° 30′ 0E and 7° 46′ 60N
11GR190119	Osun/Ede	Gray oyster	AM mushroom farm	4°25′ 60E and 7°43′ 60N

*NIHORT: National horticultural research institute; *FRIN: Forestry research institute of Nigeria.
*AM: Agrikk Matas.

Table 2. Substrate formulation protocols.

Code	Substrate material	Percentage Composition (%)
SDT	Sawdust alone	100% sawdust
BCH	Bunch alone	100% bunch
SHT	Shaft alone	100% shaft
FBH	Fermented bunch (unsterilized)	100% bunch
BSD	Bunch + Sawdust	50% each of bunch & sawdust
BRB	Bunch + Rice bran	50% each of bunch & rice bran
BWB	Bunch + Wheat bran	50% each of bunch & wheat bran
SSD	Shaft + Sawdust	50% each of shaft & sawdust
SRB	Shaft + Rice bran	50% each of shaft & rice bran
SWB	Shaft + Wheat bran	50% each of shaft & wheat bran

they were opened and watered twice per day (morning and evening) for sporophore production. The ramification rates, weight of the fruit body, biological efficiency and productivity were determined according to Adebayo et al. (2014).

2.6. Analysis of lignocellulose contents

The lignocelluloses such as hemicellulose, cellulose and lignin were analyzed using gravimetric method as described by Ayeni et al. (2015). Lignocellulosic biomass was extracted using Soxhlet extraction from 2.5 g of raw biomass substrates at 170 °C in 150 mL acetone. Hemicellulose was determined by using 1 g of dried extractive-free biomass. Lignin determination was done using 300 mg of dried extractive-free biomass, while cellulose content was calculated by difference, with the assumption that the materials (substrates) contain only cellulose, hemicellulose, lignin, ash and extractives (Ayeni et al., 2015).

2.7. Molecular characterization and DNA isolation protocols

The DNA Extraction and Purification was performed using a modified method of Vesty et al. (2017), with Zymo Research Kit. 75 mg (wet weight) fungal cells were centrifuged at 10,000 x g re-suspended in 200ul of isotonic buffered (PBS) ZR Bashing™ Lysis Tube. A lysis solution was added to help lyse cells during the mechanical lysis step. The supernatant of the lyses solution was filtered using a Zymo-Spin™ IV Spin Filter, then DNA was bound to a Zymo-Spin™ IIC Column in the presence of DNA Binding Buffer, containing 0.5% (v/v) beta-mercaptoethanol. DNA was washed and eluted with 100 µl of DNA Elution Buffer. DNA quantity and quality analysis of isolated DNA was done by Nanodrop Spectrophotometer.

2.8. DNA amplification and sequencing of amplified DNA

Internal transcribed spacer (ITS) region of the rRNA gene was amplified following a modified method of Gardes and Bruns (1993), with primers ITS4-F and ITS5-R. PCR reaction volume of 25 µl as final concentration consisting of 200 µM each of dATP, dCTP, dGTP and dTTP, 2.5 mM $MgCl_2$, 10X Taq, DNA polymerase and 20 pmol of each of the two primers (Banglore Genei). Initial denaturation step of 94 °C for 85 s followed by 25 amplification cycles of denaturation, annealing and extension were used as PCR profiles. Times and temperature adopted in this reactions were as follows; 95 °C for 35 s, 55 °C and 55 s and 72 °C for 2 min with further incubation at 72 °C for 10 min. Agarose gel of 1.2 % was used to resolve the amplified PCR products, and stained with ethidium bromide. DNA marker of 1 kb ladder (GeneRulerTM) was used as a size standard. The DNA obtained was sequenced using Sanger sequencing method (Sanger et al., 1977), with 3µl of the sample and 1µl of primer diluted with 3µl milliq water added together. The sequence was analysed using sequence analysis software v 6.2 from Thermos Fisher Inc.

2.9. Analysis of data, blasting and phylogenetic assay

Data were presented as Mean ± SEM. Statistical comparison were done by using one way ANOVA followed by Duncan Multiple Range Test (DMRT) using SPSS software (Version 20). The level of significance was set at $p < 0.05$. The sequenced data were analyzed using Geneious Prime and Biologics software (Version 9.0.5) (Biomatters Ltd, New Zealand). The blast was conducted with query sequences against a specific online repository sequence of organisms from GenBank database. Pairwise and multiple alignment was done and phylogenetic tree analysis was obtained by neighbor-joining and nucleotide alignment consensus tree (Kearse et al., 2012).

3. Results & discussion

The identities such as sample name at collection point, code, accession number and actual name of the organisms of identified commonly grown *Pleurotus* species in Southwestern part of Nigeria were shown in Table 3. Eight different samples were collected and characterized to the species level and comprises of six (6) species of *P. ostreatus* and two (2) species of *P. pulmonarius*. Adebayo et al. (2013) reported the isolation and characterization of *P. pulmonarius* (LAU09 - JF736658) and *P. ostreatus* (LAU 10- JF736659) from a wild basidiocarp growing on dead palm oil tree inside a Nigerian cocoa plantation in Ogbomoso area (347 m altitude) which also located in the Southwest Nigeria. *P. pulmonarius* (MF037415) and *P. ostreatus* (MF037419) were isolated on Dead *Mangifera indica* log and Dead *Elaeis guineensis* log respectively in Environmental Pollution Science and Technology (ENPOST) farm, Ilesa, Southwest Nigeria which located between (Longitude 4°42′30″E to 4°42′45″E and Latitude 7°36′55″N to 7°37′10″N) (Adeniyi et al., 2018). The characterization of *P. ostreatus* and *P. pulmonarius* in this study will resolve the problem of inaccuracy in identification in both species among mushroom farmers in Southwest Nigeria.

BLASTn results with DNA sequence revealed that *Pleurotus ostreatus* dominated (6 out of 8) samples collected, and also indicated the wrong names assigned to some species collected by mushroom growers overtime. Six (6) *Pleurotus ostreatus* and two (2) *Pleurotus pulmonarius*, were

Table 3. Wrong and actual name of the organism with summary of BLASTn similarity.

Sample CODE	Sample name at collection point	Accession number	BLASTn identity of sample	Percentage (%) identity	Actual name of the organism
LAU1901	*Pleurotus pulmonarius* (OS)	MK751847	*P. ostreatus* (MN244437)	99.07	*Pleurotus ostreatus*
LAU1902	*P. pulmonarius* (OY)	MK751118	*P. ostreatus* (MN244437)	88.62	*P. ostreatus*
LAU1903	*P. ostreatus* (OY)	MK751119	*P. ostreatus* (MN244437)	95.30	*P. ostreatus*
LAU1904	*P. sajor-caju* (OS)	MK751848	*P. ostreatus* (MN244435)	98.31	*P. ostreatus*
LAU1905	*P. ostreatus* (OY)	MK751120	*P. pulmonarius* (MN622735)	99.21	*P. pulmonarius*
LAU1906	*P. abalone* (OS)	MK751849	*P. ostreatus* (MN893871)	88.62	*P. ostreatus*
LAU1907	ELM oyster (OS)	MK751850	*P. ostreatus* (KT273368)	99.69	*P. ostreatus*
LAU1908	Gray oyster (OS)	MK751851	*P. pulmonarius* (MN622735)	98.32	*P. pulmonarius*

OS: Osun; OY: Oyo.

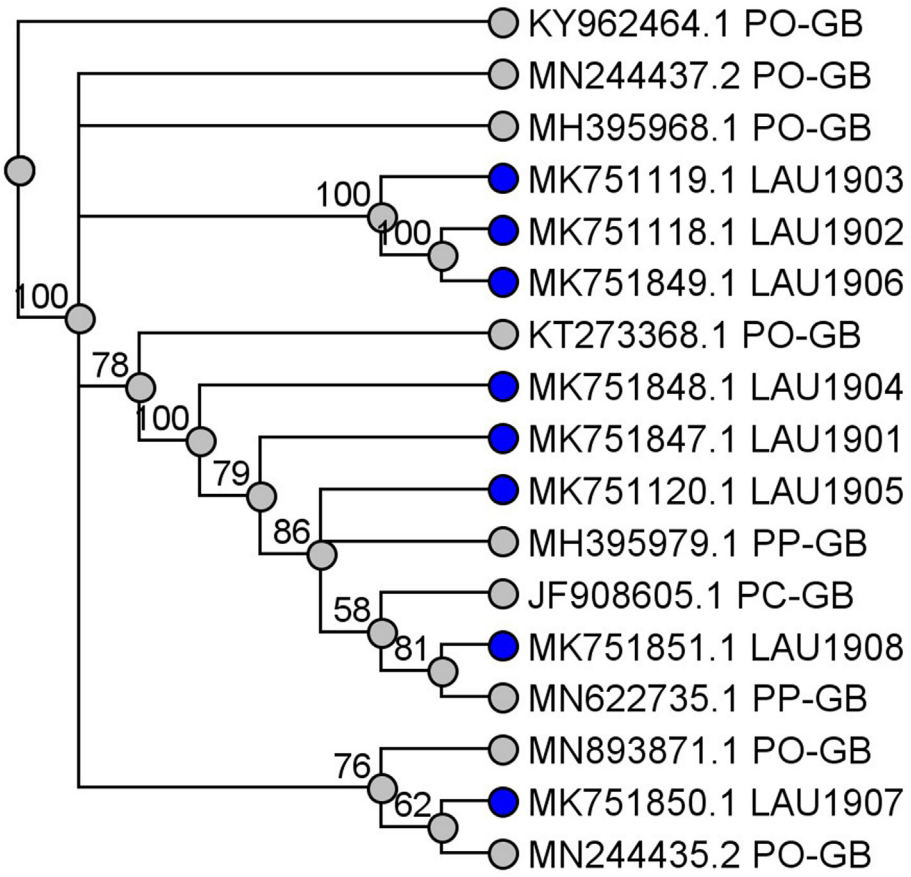

Figure 1. Phylogenetic tree of characterized cultivated *Pleurotus* species in Oyo and Osun State compared with other species in the database.

identified against the pieces of information given by the mushroom farmers as follow: two (2) *P. pulmonarius,* two (2) *P. ostreatus,* one (1) *P. sajor-caju,* one (1) *P. abalone,* one (1) ELM oyster and one (1) Gray oyster. Three species *P. ostreatus* (MK751847, MK751118 and MK751119) identified were closest to *P. ostreatus* (MN244437) with percentage similarity ranging between 88.62 and 99.07%. Other *P. ostreatus* species identified were closest as follow: *P. ostreatus* (MK751848) to *P. ostreatus* (MN244435), *P. ostreatus* (MK751849) to *P. ostreatus* (MN893871) and *P. ostreatus* (MK751850) to *P. ostreatus* (KT273368) with their percentage similarity 99.21, 88.62 and 99.69% respectively. The two *P. pulmonarius* (MK751120 and MK751851) were closest to *P. pulmonarius* (MN622735) with percentage similarity of 99.21 and 98.32% for species 1 and 2 respectively (Table 3).

The closest similarity obtained with the genus *Pleurotus* confirmed the collected samples are of the same ancestral origin. The information from the GenBank database further clarified the species at which each collected sample was belonging. *P. ostreatus* (MK751847-OS, MK751118-OY) were wrongly named *Pleurotus pulmonarius, P. ostreatus* (MK751848-OS) was given *P. sajor-caju, P. pulmonarius* (MK751120-OY) erroneously called *P. ostreatus* and *P. ostreatus* (MK751849-OS) was given *P. abalone*. While *P. ostreatus* (MK751850-OS) and *P. pulmonarius* (MK751851-OS) were given arbitrary names ELM oyster and Gray oyster respectively (Table 3).

The earliest characterization by mushroom growers was based solely on morphological features which have resulted into inaccuracy and misinformation in the particulars of the commonly growing *Pleurotus* species in the study area. It is clearly shown that there are overlapping in characterizing *Pleurotus* species using morphological features, which needed to be corrected by using molecular features. The current work corroborates Adeniyi et al. (2018) who ascertained the identity of *P. ostreatus* and *P. pulmonarius* from Ilesa, Southwest region Nigeria with 93 and 100% similarity. Appiah et al. (2017) also ascertained the identity of *P. ostreatus* from central region of Ghana with 98 similarities, while ITS 1 and 2 had previously been used to identify *P. pulmonarius* in Malaysia (Avin et al., 2014). The current study will clear the doubt of any mushroom growers on the particulars of the identified species especially in the area where their most cultivated.

In relationship with out-group, the phylogenetic tree has five main clusters. The analysis revealed that samples LAU1902 (*P. ostreatus* – OY), LAU1903 (*P. ostreatus* - OY) and LAU1906 (*P. ostreatus* – OS) clustered together under main cluster three (3) with 100 boostraps values (Figure 1). The 100 boostraps value on the same cluster indicated the close similarity of the organisms and is evidence that they are species of the same ancestral origin not minding the place of collection or cultivation. In the same vein, a close similarity was found in LAU1901 (*P. ostreatus* – OS) and LAU1904 (*P. ostreatus* - OS) also has 100 boostraps value at sub-cluster two under main cluster four which shared a distant relationship with another *P. ostreatus* (KT273368) from GenBank database at boostraps value of 78% at sub-cluster one (Figure 1).

The close relationship with may be referred to as identical with 100% boostraps value found in both LAU1901 (*P. ostreatus* – OS) and LAU1902 (*P. ostreatus* – OY) that were erroneously named *P. pulmonarius* by mushroom growers, with other *P. ostreatus* from GenBank database has clearly shown that both LAU1901 and LAU1902 belong to species *ostreatus* but not *pulmonarius.* The phylogenetic analysis further confirmed that both LAU1905 and LAU1908 belong to species *pulmonarius* but not *ostreatus* and Gray oyster as previously called respectively by the mushroom growers. This was established by both LAU1905 and LAU1908 clustered with *P. pulmonarius* (MH395979 and MN622735) GenBank database at boostraps values of 86 and 81 respectively. LAU1907 which was previously called ELM oyster (OS) was revealed to be *P. ostreatus* with clear evidence of clustering in both sides with

Table 4. Lignocellulose contents (wt%) of the substrate materials used in the study.

Samples	Hemicelullose	Cellulose	lignin
Palm oil bunch	25.73 ± 0.31[b]	37.88 ± 0.33[b]	27.39 ± 0.21[b]
Wheat bran	23.87 ± 0.38[c]	11.12 ± 0.41[e]	5.30 ± 0.29[e]
Palm oil shaft	26.01 ± 0.16[b]	28.57 ± 0.28[d]	28.84 ± 0.21[a]
Sawdust	29.38 ± 0.37[a]	48.33 ± 0.31[a]	21.33 ± 0.44[d]
Rice bran	22.33 ± 0.35[d]	34.38 ± 0.41[c]	22.40 ± 0.31[c]

P. ostreatus (MN893871 and MN244435) at cluster five with boostraps values of 76 and 62 respectively (Figure 1).

The result from the Phylogenetic analysis clearly shown that six of the collected samples falls under the species *P. ostreatus* while only two are *P. pulmonarius* species. Phylogenetic analysis of the samples showed high genetic relatedness with reference organisms, this is indication that mushroom samples are similar from the reference sequences from the Genbank (Adeniyi et al., 2018). Non cluster or low genetic relatedness of the samples with the reference taxa from the GenBank database revealed distinct nucleotide signature different from existing organisms in the database (Togashi et al., 2001; Aremu and Babalola 2015). The close relatedness obtained in the samples collected in this study in relation with the reference taxa from the GenBank database revealed that organisms have close or related ancestral origin. Furthermore, it also confirmed that all the collected samples belong to genus *Pleurotus,* while further validation or correction as to which each of the species belong was established.

Lignocellulose composition containing hemicellulose, cellulose and lignin of the substrate materials used in the study are report in Table 4. Highest value (29.38 ± 0.37 wt%) and lowest value (22.33 ± 0.35 wt%) of hemicellulose were obtained in sawdust and rice bran respectively. Sawdust gave the highest value (48.33 ± 0.31 wt%) of cellulose, while lowest value of 11.12 ± 0.41 wt% was obtained from wheat bran. Highest lignin value (28.84 ± 0.21 wt%) and lowest lignin value (5.30 ± 0.29 wt%) were obtained in Palm oil shaft and wheat bran respectively. Highest lignocelluloses especially hemicellulose and cellulose obtained in sawdust might be responsible for the delay in incubation period (IP) reported during spawn running or substrate ramification, while the lowest lignocelluloses values obtained in other substrate materials may have aided quick incubation period. Delayed in the ramification in the substrates with higher value of lignocelluloses (hemicellulose and cellulose) may be resulted due to difficulty or delayed in metabolizing of these organic materials by the extracellular enzymes. The values of lignocellulose composition obtained in this current study falls within the range reported previously by Saini et al. (2015).

Schematic diagram of *Pleurotus* species cultivation protocol on supplemented and unsupplemented palm oil waste, which consists of grains spawn production and substrate processing (Figure 2). The pictorial representation revealed step by step procedure involves in oyster mushroom cultvation using palm oil waste as substrate, which was adopted in this study. Table 6 showed the ramification rate with significant increase at day 7 in SHT (5.46 ± 0.49) and SSD (5.44 ± 0.65) when compared with the others. They were closely followed by BUH (4.30 ± 0.23) and BSD (4.30 ± 0.15) while ramification rate was slow in SDT, FBH, BWB and SWB with SRB being the slowest. At Day 10, SDT, FBH, BWB, SWB and SRB growth were insignificantly different from each other. BUH, BSD, BRB, SHT and SSD were of the same ramification rates but are significantly higher than the rest.

At day 13, SWB ramification was significantly higher than SDT. Also, SRB, BWB and FBH ramification rates were significantly higher than SWB and SDT. However, SHT ramification rate was significantly higher than SDT, SWB, SRB, BWB and FBH while BUH, BSD, BRB and SSD were observed to be more significantly higher than SHT, SRB, BWB, FBH, SWB and SDT. FBH (6.36 ± 0.51) which started slowly had caught up with SHT (6.50 ± 0.51). It is also worthy to note that BRB (8.24 ± 0.16) which had one of the lowest values at Day 7 had overtaken all other substrates. At Day 16, FBH, BUH, BSD, BRB, SHT and SSD growth rates were

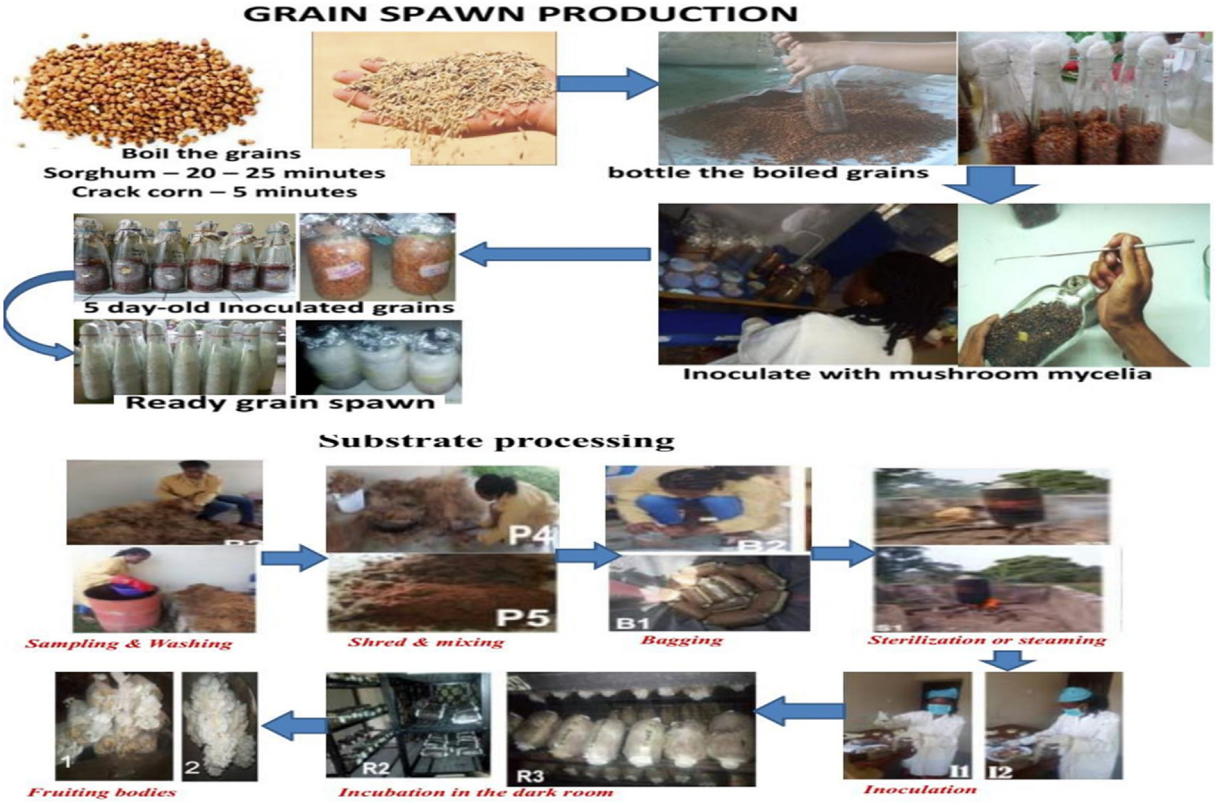

Figure 2. Schematic diagram of *Pleurotus* species cultivation protocol on supplemented and unsupplemented Palm oil waste.

Table 5. Ramification rates of the spawn running on formulated substrates.

Substrate	Day 7	Day 10	Day 13	Day 16
SDT	2.94 ± 0.15^{cd}	4.38 ± 0.29^{b}	4.98 ± 0.31^{e}	4.98 ± 0.31^{e}
FBH	2.64 ± 0.15^{cd}	4.42 ± 0.30^{b}	6.36 ± 0.38^{c}	7.36 ± 0.19^{ab}
BUH	4.30 ± 0.23^{b}	6.60 ± 0.09^{a}	7.60 ± 0.15^{a}	7.60 ± 0.15^{a}
BSD	4.30 ± 0.15^{b}	6.92 ± 0.19^{a}	7.44 ± 0.23^{a}	7.44 ± 0.23^{ab}
BWB	2.90 ± 0.43^{cd}	4.74 ± 0.40^{b}	6.02 ± 0.27^{cd}	6.92 ± 0.44^{b}
BRB	3.56 ± 0.07^{bc}	6.10 ± 0.36^{a}	8.24 ± 0.16^{a}	8.24 ± 0.16^{a}
SHT	5.46 ± 0.49^{a}	6.36 ± 0.52^{a}	6.50 ± 0.51^{bc}	7.26 ± 0.39^{ab}
SSD	5.44 ± 0.65^{a}	5.98 ± 0.47^{a}	7.36 ± 0.38^{ab}	7.36 ± 0.38^{ab}
SWB	2.40 ± 0.21^{d}	3.76 ± 0.41^{b}	5.18 ± 0.29^{de}	7.16 ± 0.47^{b}
SRB	2.24 ± 0.19^{d}	3.88 ± 0.11^{b}	5.86 ± 0.23^{cde}	5.86 ± 0.23^{c}

The values are expressed as mean ± SEM, each diet follow by the same alphabet across the column are not significantly different at $p < 0.05$.
SDT – Sawdust alone; FBH - Fermented Bunch; BUH – Bunch alone; BSD - Bunch + Sawdust; BWB - Bunch + Wheat Brain; BRB - Bunch + Rice Brain; SHT – Shaft alone; SSD - Shaft + Sawdust; SWB - Shaft + Wheat Bran; SRB - Shaft + Rice Bran.

Table 6. Distributions of the average mean of total fresh and total dry weight (g) over three harvests by the *Pleurotus ostreatus* (MK751847) in different substrates.

Substrate	IP	PP	1st FLUSH	2nd FLUSH	3rd FLUSH	TWW	TDW
SDT	30	38	15.18 ± 0.12^{c}	232.46 ± 0.12^{a}	79.3 ± 0.21^{b}	326.94	57.16
FBH	21	47	NF	339.16 ± 0.12^{a}	115.54 ± 0.72^{b}	454.7	79.8
BUH	14	54	784.43 ± 9.61^{a}	277.98 ± 0.68^{b}	241.00 ± 0.12^{c}	1313.41	230.18
BSD	14	54	753.44 ± 0.36^{a}	379.57 ± 0.55^{b}	235.99 ± 1.50^{c}	1369	240.73
BWB	15	53	138.11 ± 0.78^{c}	381.03 ± 0.73^{a}	318.66 ± 0.35^{b}	837.8	147.56
BRB	14	54	1360.21 ± 0.48^{a}	301.31 ± 0.57^{b}	113.23 ± 0.17^{c}	1774.75	311.60
SHT	21	47	208.69 ± 0.43^{b}	282.82 ± 1.16^{a}	136.39 ± 0.65^{c}	627.9	110.20
SSD	21	47	515.83 ± 1.11^{a}	263.39 ± 0.58^{b}	109.97 ± 0.12^{c}	889.19	156.40
SWB	30	38	NF	1025.33 ± 10.20^{a}	382.69 ± 1.03^{b}	1408.02	247.00
SRB	21	47	871.64 ± 0.87^{a}	369.44 ± 0.62^{b}	242.62 ± 1.09^{c}	1483.7	260.39

IP incubation period (required days for formation of primordia), PP production period, starting with the formation of primordia till third harvest. Weight of mushrooms obtained in 3 replicates.
The values are expressed as mean ± SEM, each diet follow by the same alphabet down the column are not significantly different at $p < 0.05$.
TDW- total dry weight; TWW-total wet weight.

insignificantly different from one another but significantly higher than SDT, SRB, BWB and SWB. At the end of ramification of the bags, the ramification rates could be ranged thus; BRB, BUH, BSD, FBH, SSD, SHT, SWB, BWB, SRB, SDT. Approximately the incubation period - IP (required days for formation of primordia) was less than 16 days in BRB, BUH, BSD and BWB (Table 5), which is as result of high ramification rates. This current study is in agreement with Adebayo et al. (2013), who reported incubation period (IP) of less than 16 days for *P. pulmonarius* (LAU 09-JF736658).

Values of the total fresh mushroom harvested containing three flushes, total wet weight, total dry weight, incubation period and production period are shown in Table 6. The shortest primodia formation day (14 days) was obtained with substrates BUH, BSD and BRB, while the longest, which is 30 day, occurred at SDT and SWB. Highest total mushroom yield (1774.75 g) was obtained with BRB, followed by (1483.7 g) from SRB, while the longest primodia formation day substrate SDT (30 days) gave lowest yield of total mushroom harvest (326.94 g). BRB has the highest total dry weight of 311.60 g and SDT has the lowest TDW of 57.16 g. Clearly it is shown here that both bunch and shaft supplemented with rice bran gave higher yield with considerably lower of incubation period. Sawdust alone has longest incubation period and consequently lower yield compared to all other substrates formulations.

Lignocellulose content of the substrates used as presented in Table 4 shows significant difference in the hemicellulose, cellulose and lignin contents of the five substrates. While sawdust has the highest hemicellulose, rice bran has the lowest. Also, sawdust has the highest cellulose content while wheat bran has the lowest. Palm shaft has the highest lignin while wheat bran has the lowest. The substrate made from bunch and rice Bran (BRB) gave the highest production and this may not be unconnected to the high lignocellulose contents of the two agricultural wastes used in making it. On the other hand, sawdust gave the lowest production and may be due to the low lignin content compared to rice bran and bunch which gave the highest. It must also be borne in mind that the best two substrates in terms of production were the ones containing Rice bran. This is a further proof that rice bran gives better yield than wheat bran. Moonmoon et al. (2011) reported significant increased yield of *Lentinus edodes* when maize powder was supplemented with wheat bran and rice bran. The average yield (biological efficiency and productivity) of *Pleurotus eryngii* (ATCC36047) was increased significantly with addition of rice bran with sawdust substrate from *Trema*

Table 7. Biological efficiency and productivity.

Substrates formulation	Biological efficiency (%)	Productivity (%)
SDT	13.08	3.23
FBH	22.74	4.55
BUH	77.25	13.13
BSD	59.52	13.69
BWB	33.51	8.39
BRB	73.95	17.46
SHT	20.25	6.28
SSD	28.68	8.89
SWB	100.57	14.08
SRB	38.04	14.84

orientalis (Peng et al., 2000). Kinge et al. (2016) also reported an improved yield production in *P. ostreatus* when different local substrates in Northwest Region of Cameroon were supplemented with rice bran. Yield increased of *Pleurotus ostreatus* was also reported with cotton seed cake supplemented with wheat straw and rice bran (Khan et al., 2019).

Table 7 shows the biological efficiency of the substrates with respect to their utilization and the quantity of the mushroom produced. In terms of substrates biological efficiency, they can be rated as follows in descending order; SWB, BUH, BRB, BSD, SRB, BWB, SSD, FBH, SHT and SDT. While the biological efficiency (productivity) in terms of the quantity of mushroom produced can be rated as follows: BRB, SRB, SWB, BSD, BUH, SSD, BWB, SHT, FBH and SDT, which also indicated mushroom productivity. Substrate prepared using shaft and wheat bran had the best biological efficiency, while bunch with rice bran had the best productivity and sawdust as a lone substrate had the lowest biological efficiency and productivity. This has clearly shown that in commercial mushroom cultivation, both shaft with wheat bran and bunch with rice bran could be easily recommended as suitable substrates. Both shaft and bunch might be more desirable in commercial mushroom cultivation as both materials are always got free of charge as a waste from palm oil products. In addition, the fermented bunch (FBH) which required virtually no labour in term of sterilization also may be more desirable in commercial mushroom cultivation compared to sawdust (SDT) when the yield output is considered.

This work has established the potentials of palm tree bunch and shaft as single substrates or in combination with wheat bran or rice bran as better substrates. In fact, the biological efficiency of both of them (bunch and shaft) as single substrates was found to be better than sawdust which is commonly used by most mushroom farmers.

4. Conclusion

In this study, it has been shown that both *Pleurotus ostreatus* and *P. pulmonarius* are predominantly cultivated in most mushroom farming in Oyo and Osun States of Southwestern region, Nigeria. The study has clarified the ambiguity and inaccuracy in the identification of some *Pleurotus* species mostly cultivated by mushroom growers in the study area. It is also established that amount of lignocelluloses component present in the substrate may determine how effective the substrate will be in cultivation of the mushroom. Cultivation protocols using palm oil bunch and shaft supplemented with either wheat and rice bran at the ratio of 1:1 has shown greater potentials in cultivation of *Pleurotus* species over all other evaluated substrates. The better yields in terms of total weight, biological efficiency and productivity has made the formulated substrates stand out and can be recommended for commercial mushrooms cultivation.

Declarations

Author contribution statement

Adebayo EA: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Elkanah FA, Afolabi FJ: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ogundun OS, Alabi TF: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Oduoye OT: Analyzed and interpreted the data.

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Data availability statement

Data associated with this study has been deposited at National Center for Biotechnology Information - GenBank Database under the accession numbers MK751847, MK751118, MK751119, MK751848, MK751120, MK751849, MK751850, MK751851.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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