CULTIVATION AND MOLECULAR CHARACTERISATION OF *Auricularia* SPECIES IN SOUTHWESTERN NIGERIA

BY

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A THESIS IN THE DEPARTMENT OF BOTANY SUBMITTED TO THE FACULTY OF SCIENCE IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF DOCTOR OF PHILOSOPHY OF UNIVERSITY OF IBADAN

JANUARY, 2017

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ABSTRACT

Mushrooms such as *Pleurotus*, *Vovariella* and *Auricularia* species are cultivated for food and medicinal purposes in the world. However, cultivation of *Auricularia* in Nigeria is limited due to inadequate information on its characteristics, nutritional contents and cultivation requirements. Hence, this study was designed to characterise *Auricularia* species in Southwestern Nigeria and determine suitable substrates for their cultivation.

Fifty-four samples of *Auricularia* species were randomly collected based on availability from secondary forests in Osun (11), Oyo (10), Ondo (9), Ekiti (8), Ogun (8) and Lagos States (8). Pieces of tissue from each sample were cultured on Potato Dextrose Agar (PDA). Samples with mycelial growth were later cultured on sterilised sorghum grains to produce spawns. Six substrates comprising *Mansonia altissima. (*A. Chev) A. Chev sawdust, cotton waste *Gossypium hirsutum* Linn, rice straw *Oryza sativa* Linn, each in polyethylene bags and drilled logs of *Mangifera indica* Linn, *Gliricidia sepium (*Jacq) Walp, and *Cedrela odorata* Linn, were purposively selected for spawn inoculation to produce mushrooms. Morphological identification (colour, shape and texture) of *Auricularia* species, the growth parameters (days of spawn run, days of pin head formation), and yield of the mushrooms were determined using standard procedures. Nutrient (nitrogen, phosphorus, and potassium) and proximate analysis (Protein, Fat, and Carbohydrate) were carried out using AOAC methods. Fifteen Random Amplified Polymorphic DNA (RAPD) primers were used for PCR amplification of the DNA of *Auricularia* samples, to determine the degree of genetic diversity. Phylogenetic relations were determined by cluster analysis, Polymorphic Information Content (PIC) and genetic diversity determined using standard procedures. Data were analysed using descriptive statistics, clustering and Principal Component Analyses (PCA).

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Morphologically, 31 samples of *A. auricula*, (yellow brown, auriform, leathery texture) and 12 samples of *A. polytricha* (dark brown, discoid, gelatinous) were identified, while 5 samples were unidentified and 6 samples did not grow. *Auricularia* species samples cultivated on *M. altissima* sawdust, cotton waste, rice straw in bags produced mycelial growth, but did not fructify while samples on drilled logs had mycelial growth and fructified. *Mangifera indic*a had highest days of spawn run (24.3±0.7), pin head formation (28.7± 0.6) and highest yield (10.0±0.4 g). The highest nitrogen content (13.6 ±0.7 mg/kg) was recorded in *A. polythrica*, phosphorus (39.3±7.6 mg/kg) in *A. auricula* and calcium (61.9 ± 3.6 mg/kg) in *A. auricula*. The highest protein (7.0±0.8%) and crude fibre (25.1±2.5%) were obtained in *A. polythrica.* Fat content (7.0 ± 0.1mg/kg) was highest in *A. auricula.* Cluster analysis and morphological traits produced 6 distinct groups while the PCA produced eigenvalues of 23.0 %, 16.0 %, 14.0 %, 11.0 %, 10.0 % and 9.0 % on six corresponding axes. The RAPD primers grouped the *Auricularia* species into 6 distinct clusters based on morphological traits. The PIC ranged from 0.5594 (OPH-15) to 0.7819 (OPB-12) and gene diversity from 0.5930 (OPH-15) to 0.7977 (OPB-12). Primer OPB-12 was the most informative for genetic diversity of *Auricularia* species.

Auricularia species exhibited genetic variations and *Mangifera indica* enhanced their growth. *Auricularia polytricha* was the most nutritious species recorded.

Keywords: Mushroom cultivation, *Mangifera indica* substrate, Polymorphic information content.
Word Count: 495

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ACKNOWLEDGEMENTS

The completion of this research has fulfilled yet another ambition that I have nursed for a number of years. It's success is due to the support and encouragement from a number of people who have assisted in various ways during the course of this study.

My most sincere gratitude goes to my supervisor, Dr Clementina O. Adenipekun for her constructive criticisms and kind advice. My sincere thanks go to members of staff of the Department of Botany, especially Prof A. C. Odebode, Prof A. E. Ayodele, Prof O. Osonubi, Prof S. G. Jonathan, Prof A. A Jayeola, Dr K. S. Chukwuka, Dr I. A. Adesoye, Dr A.M. Salaam, Dr O. J. Oyetunji, Dr A.A. Sobowale, Dr E. O. Shokefun, Dr O. J. Olawuyi, Dr Taiye Fasola, Dr Idayat Gbadamosi and Mr O. Popoola. Also from other departments are Dr Sherifah Wakil, Dr B. Ogunkanmi, Dr A. A. Ogunjobi, Dr I. A. Oladosu, Dr Helen Alabi, for making me to understand the various areas of this research work. I say thank you to Mr Rotimi Adebayo, Mr. Donatus. Esimekhuai, Mr. O. Oladiran, Mrs A. Adeshina, Mrs E. Allu, and Dr. O. Osowole of statistics Department University of Ibadan for their assistance. My appreciation also go to Dr Funmilayo Idowu, Mr D. Igwe, Dr B. Ojuederie, Dr Fisayo Kolade, Sir S. Korie and Mr A. Shittu, of National Institute of Horticulture (NIHORT) and International Institute of Tropical Agriculture (IITA) Ibadan respectively.

My appreciation will not be complete if I fail to thank the following organisations and Research centers, Zero Emission Research Initiative (ZERI) Foundation, Africa University Mutare Zimbabwe, USAID Cotonou, Biotech laboratory IITA and SONGHAI CENTER Port Novo, where the opportunity and exposure on mushroom cultivation and Biotechnology was first given to me. Others are Prof J. A Okhuoya and Dr Gbehou Pierre of University of Benin, Nigeria and University of Benin Calavi-Cotonou Benin Republic respectively, for their encouragement throughout the duration of this research work.

Finally, I thank my loving wife, Mrs Edith Ekun who has encouraged me in all ways leading to the completion of this project, especially for her prayers and sacrifices throughout this period. Sincere appreciation goes to my children, Emmanuel, Kelvin, Valentina and Yvonne are not left out in the appreciation for their love and understanding. Also to my wonderful parents-inlaw Late, (Sir) Lawrence Ehimen and Lady Hannah Ehimen.

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DEDICATION

Dedicated to the Almighty God for His special grace and mercy on me, to my loving parents, Hon. Jerome Ekun and late (Mrs) Theresa Yemisi Ekun for their love and care towards me.

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CERTIFICATION

I certify that this work was carried out by Victor Segun EKUN under my supervision and guidance in the Department of Botany, University of Ibadan.

______________________________	____________________
Supervisor **Dr. Clementina O. Adenipekun** **B.Sc, M.Sc, Ph.D (Ibadan)**	**Date**

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CHAPTER ONE

1.1 Introduction

Mushroom is a macro-fungus with a unique fruiting body, that can be easily seen with the naked eye and large enough to be picked by hand, which can be either epigeous or hypogenous (Chang and Miles, 2004) Macrofungi are lower plants that lack flowers and fruits of the higher orders (Adejumo and Awosanya, 2005; Ayodele and Akpaja, 2007). There are two major groups of macrofungi which are toadstools and mushrooms. Mushrooms locally known as 'olu' in the Southwestern Nigeria are a group of fungi belonging to the class Basidiomycetes and order Agaricales. Basidiomycetes or Ascomycetes are found in moist wood, soils rich in organic matter and humus, animal wastes in the form of vegetative mycelium (Zied *et al.,*2011).

The people of West Africa still depend on wild edible mushrooms for their livelihood especially as a low-cost alternative for plants and animal proteins and vitamins in diets. Mushroom gives a prominent source of subsistent income and raw material in traditional medicine (Guissou *et al.,* 2008).

There are nearly a hundred species of mushroom that can be cultivated (Food and Agriculture Organisation, 2004). Many of the common edible species have therapeutic effects, and several medicinal mushrooms are also eaten. Mushrooms are cultivated in controlled biological environment and it has been extensively used as food since ancient time, due to its nutritive and medicinal values (Manzi *et al.,* 2001). Mushroom cultivation can help reduce malnutrition, because mushrooms can serve as substitutes for other sources of protein. Mushrooms are an important protein source that also provide vitamins (B1, B2, C) and minerals as well as other nutrients (Ekpo and Aluko, 2002; Daodu, 2003). Mushrooms have been reported to be low in cholesterol and offer an especially promising opportunity to discover anti-cancer genes and pathways (Bechtel *et al*., 2002; Borchers *et al*., 2004).

Okhuoya, (2011) grouped mushrooms into four categories:

(i) Edible Mushrooms: Examples of this group are *Agaricus bisporous*- the most cultivated mushroom; *Lentinus* edodes (shiitake), *Volvariella volvacea*, *Auricularia auricula*, *Pleurotus squarrosullus*, *P. tuberregium*, *Termitomyces africanus,* e.t.c.

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(ii) Poisonous mushrooms: Examples are *Amanita phalloides*, *A. verna*, *A. virosa* (the destroying angel) *A. muscaria.*

(iii) Medicinal mushrooms: Examples are *Pleurotus tuberregium*, *Lentinus edodes*, *Schizophylum commune*

(iv) Miscellaneous mushrooms: *Stereum hirsutum*, *Phallus indusiatus*, *Earthstars* e.t.c. (These groups can have more than one character among the already mentioned groups. Therefore, that there are mushrooms that could be in more than one category. For example there are edible species that are equally medicinal).

1.2 Cultivation of Mushrooms

Artificial cultivations of mushroom has been going on for many centuries. Scientists have been dedicating their time to domesticating wild mushrooms and breed them to get better quality mushrooms. Long before the development of mushroom production systems in the United States, the Chinese have been cultivating different species of edible mushrooms (Quimio *et al*., 1990).

The cultivation of Mushrooms in most rural areas remain an extra- agricultural activity that is practiced at different economic levels.It varies from subsistent to small scale business categories. In Cameroon, Bénin and Ghana, portable logs hitherto colonized by mushrooms are relocated from the wild to home gardens where they are nurtured and tendered until the appearance of the next flush. This practice equally sufficed as mushroom cultivation which according to Aguilar *et al*. (2002) can be described as "Flushes" in the cultivation of mushroom. It is characterized by increasing yield per flush until the log exhausts the nutrient requirement for mushroom development. Moreover, small scale mushroom cultivation are restricted to local bamboo or termite resistant wooden sheds lined with flat topped benches and covered in thatched roofs in shades.

Mushrooms of the genus *Auricularia,* commonly known as wood ear mushrooms are edible fungi which have been domesticated for cultivation in different parts of the world (Kirk *et al.,* 2001). Currently, the genus *Auricularia* is the fourth most important cultivated mushrooms after *Agaricus, Lentinula* and *Pleurotus* (Yan *et al.,* 2004). *Auricularia auricula* (St Amans, commonly called "jelly Ear) is a

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basidiomycetes of the family Auriculaceae. It is a fungus that has its fertile surface downward, it is usually gelatinous and tan to brown in color, but has sterile surface upward with veined and irregular, flesh, with gelatinous or rubber texture. (Zied *et al*., 2011) This may be attributed to variations in the ecosystems which may differ in climate, synecology, litter fall dynamics and composition, succession and geography (Adebiyi *et al.,* 2016) .

The rapid increase in domestication of this genus from the wild is attributed to its nutritional and medicinal properties (Chang and Miles, 2004). Musngi *et al*. (2005) used phenotypic differentiation to classify the strains of *Auricularia* in the Philipines. Knowledge of the varietal differences can be used as sources of cell lines for researchers and in breeding programs (Pei-Sheng and Chang, 2004). *Auricularia polytricha* is an edible mushroom, also known as black jelly. This mushroom is cultivated in tropical regions because its mycelium can grow at temperatures ranging from 10 to 40 ^{0}C (Jonathan *et al.,* 2009). Unlike other mushroom species, cultivation of *A. polytricha* is easy and fast to yield fruiting bodies. It does not require expensive facilities. In addition, various forestry and agricultural wastes have been used as substrates for cultivation of this mushroom (Irawati *et al.,* 2012).

In nature, mushrooms have not only been a source of food for man and animals, but also have played an important role in the cycling of carbon and other elements through the breakdown of lignocellulosic plant residues and animal dung which serve as the substrates for the saprophytic fungi. In this way, mushroom species as agents of decay, which help to keep the environment from being overwhelmed by the dead organic debris of plants and animals. Simultaneously, mushrooms can produce a wide range of enzymes that degrade complex substrates, following which they absorb the soluble substances. Strong consumer demands and threats of depletion of mushrooms have stimulated increased worldwide production in the past few decades (Chang and Miles, 2004).The increased demand of mushrooms is due to their unique culinary and medicinal properties (Yan *et al.,* 2003). However, Africa contributes a paltry 1% of the annual worldwide production of mushrooms (Adejumo and Awosanya, 2005).

West Africa is ranked among other parts of the world and recognized as one of the major growing areas of fungi. Currently world fungal diversity estimate is based on

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the ratio of 5:1 (higher plants to fungus) Hawksworth (2004). In addition, using documented plant species data (Hawksworth, 2001) reported a total of 140,000 global species of macrofungi for which only 10% were already documented and anesti-mate of 35,000 - 40,000 remaining "unknown" species as reported by Mueller *et al.* (2007*)*. The author suggested an estimated 25,000 known species of macrofungi to Africa.

Traill *et al*. (2013) reported that there is a relationship between rainfall and species availability, composition and spread, and as the most important denominator that separates West Africa from Central, East and South African sub-regions. Human' settlement provides easy access to food, fruits, water and security are typified by the overwhelming presence of forests, rivers in the region. The favorable climatic condition of this region accordingly, helped to enhance the mushroom development and diversity (Kauserud., 2008).

Mushroom cultivation has been reported as an alternative way of alleviating poverty in developing countries due to its possibility of low cost of production, high profit and quick returns (Masarirambi *et al.,* 2011). Farmers can utilize agricultural wastes, such as dried sugar cane leaves, saw dust, maize Stover and banana leaves as substrates for mushroom production (Beetz and Kustida, 2004; Lourdes *et al*., 2008).

Mushrooms were recorded to be obtained from forests, plantations, farmlands and grasslands along with other non-wood forest products (NWFP). The presence of mushroom has also been documented around and within human dwellings (Crous, 2006). This could have been made possible by the dynamics of prolonged mutually beneficial interactions down evolutionary lane that logically caused improved survival and adaptive mechanisms reflecting in their growth, nutritional diversity, reproductive capacity, habitat range and dispersal characteristics. The knowledge of edible mush-rooms is limited to their visible fruit bodies and their development transcends generations. (Kalu *et al*., 2013) .

According to Osemwegie *et al.* (2014) as shown in Table 1, Proper inventory of diverse wild edible and medicinal mushrooms sold in these local markets is required for the development of a mushroom genetic resource germplasm, cultivation of species yet uncultivated. It may also have accounted for the visible underdevelopment

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of mushroom cultivation practices and undermines their commercial scale production for priority export or foreign exchange earnings. The ignorance of their domestication knowledge and cultivation technology became the major cause of dependence on mushroom hunting practice as presented in Table 1.

Wild edible mushrooms are popular in some rural communities and their appearance during the planting season when food is scarce in these areas is seen by the locales as nature's food providence (Odebode, 2005). The practice of traditional mushroom hunting from the wild when in season is still prevalent (Adebiyi *et al* 2016) and the harvest is either used fresh by the locales for nutritional and medicinal purpose (Odebode, 2005) or retailed in local markets to augment family income (Osemwegie *et al.,* 2014).

Edible mushroom include many fungal species that are either harvested wild or cultivated. Easily cultivated mushroom and common wild mushroom are often available in market and those that are more difficult to obtain may be collected on a smaller scale by private gatherers. Some preparation may render certain poisonous mushroom fit for consumption. (Ndem and Oku 2016). Before assuming that any wild mushroom is edible, it should be identified. Proper identification of a species is the only safe way to ensure edibility. Some mushroom that are edible for most people can cause allergic reaction in some individuals and old or improperly stored specimens can cause food poisoning.

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Table 1: Some wide spread edible mushrooms that dominate ethnomycology literatures of West African origin.

S/n	Scientific name	Uses	Country
1	*Agaricus groossenniae*	Food	Bénin, Ivory Coast, Ghana, Cameroon
2	*Amanita* sp	Food and folk medicine	Bénin, Ivory Coast, Nigeria, Senegal
3	*Auricularia auricula*-Judae	Food and folk medicine	Bénin, Cote D'Ivoire, Ghana, Ivory Coast, Nigeria
4	*Auricularia. cornea*	Food	Bénin, Togo
5	*Calvatia*. Cyathiformis	Food and folk medicine	Bénin, Cameroon, Cote D'Ivoire, Ghana, Ivory Coast, Nigeria
6	*Cantharelluscongolensis*	Food	Bénin, Ivory Coast, Ghana, Cameroon, Nigeria, Senegal
7	*Cantharellus Platyphyllus*	Food	Bénin, Ivory Coast, Cameroon, Nigeria
8	*Cantharellus Floridulus*	Food	Bénin, Cameroon, Nigeria
9	*Chlorophyllum Molybdites*	Food	Ivory Coast, Cameroon and Nigeria
10	*Coprinus africanus*	Food	Burkina Faso, Ivory Coast, Nigeria
11	*Coprinuss* sp.	Food	Burkina Faso, Ivory Coast, Nigeria
12	*Daldinia concentric*	Folk medicine	Bénin, Cameroon, Cote D'Ivoire, Ghana, Ivory Coast, Nigeria
13	*Ganoderma lucidum*	Folk medicine	Bénin, Cameroon, Cote D'Ivoire, Ghana, Ivory Coast, Nigeria
14	*Ganoderma Applanatum*	Folk medicine	Cameroon, Nigeria
15	*Macrolepiota procera*	Food	Cameroon, Guinea, Nigeria
16	*Lactariuss* spp	Food	Bénin, Ghana, Nigeria, Senegal
17	*Lentinus squarrosulus*	Food and folk medicine	Cameroon, Ghana, Nigeria
18	*Lentinu Subnudus*	Food	Ivory Coast, Nigeria
19	*Lentinus tuber-regium*	Food and folk medicine	Bénin, Cameroon, Cote D'Ivoire, Ghana, Ivory Coast, Nigeria
20	*Pleurotus pulmonarius*	Food	Bénin, Cameroon, Nigeria
21	*Phlebopus sudanicus*	Food	Bénin, Burkina Faso

Extracted from Osemwegie *et al.* (2014).

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Table 1: Continued

S/n	Scientific name	Uses	Country
22	*Psathyrella atroumbonata*	Food	Cameroon, Nigeria
23	*Russula* sp	Food and folk medicine	Bénin, Cameroon, Nigeria
24	*Termitomyces microcarpus*	Food and folk medicine	Bénin, Cameroon, Cote D'Ivoire, Ghana, Senegal, Nigeria
25	*Termitomyces robustus*	Food and folk medicine	Bénin, Cameroon, Cote D'Ivoire, Ghana, Senegal Nigeria
26	*Termitomyces striatus*	Food and folk medicine	Bénin, Cameroon, Cote D'Ivoire, Ghana, Ivory Coast, Nigeria
27	*Schizophyllum commune*	Food and folk medicine	Bénin, Cameroon, Cote D'Ivoire, Ghana, Ivory Coast, Nigeria
28	*Volvariella esculenta*	Food	Bénin, Cameroon, Cote D'Ivoire, Ivory Coast, Senegal
29	*Volvariella. Volvacea*	Food and folk medicine	Bénin, Cameroo,Ghana, Ivory Coast, Nigeria, Senegal, Togo

Extracted from Osemwegie *et al.* (2014).

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Okhouya (2011) reported that the various shapes mushrooms exhibit show that every shape is significant, from the top to the cap down to the base of the mushroom. These shapes contribute greatly to the identification of the available mushrooms. Umbrella shaped mushrooms, which are commonly referred to as Agaric mushrooms because of their unique cap/stipe configuration are most diverse, fleshy, gilled, and usually stalked, frail and easily broken at maturity.

Another prominent group is the Coma or bracket shaped mushrooms, which usually have undersurface pores with a wide range of textures and mode of substrate attachments. They may be leathery, papery, woody or corky, rubbery and so on. In addition, there are also tuberous and bulb shaped type of mushrooms. Mushrooms in this category exhibit similar habitat patterns. Their texture is hard or stony or puffy in the case of bulbous types with a visible sporocarp that lacks undersurface pores or gills but rather produce spores in a cloudy deposition in its surface. Cup and clubshaped mushrooms have rubbery to cartilaginous or papery texture, and may be stalked or sessile.They were observed to be growing gregariously or in a scattered pattern on logs, woods, fallen tree branches, coarse woods and rarely on twigs, and appearing in various sizes with cup depth that vary from one species to the other. (Okhouya, 2011)

Funnel shaped mushrooms are agaric mushrooms with very depressed cap and possessing pseudo- or gill-like ridges gills. Lastly, we have Star and coral shaped/Brush like mushrooms. Bounty harvests of edible and medicinal mushrooms are usually sold for little subsistent income to tourists, hoteliers as well as visitors from the cities. According to Osemwegie *et al* (2014) as shown in Table 1, the pattern of marketing wild indigenous edible mushrooms however varies from one West African country to another and even amongst tribes. In Nigeria, Ghana and Bénin mush-rooms are sold openly during village market days or hawked on trays along highways outside market days. They are displayed in different basket sizes in relation to their quantity and price. However, we can find little quantities in some supermarkets

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1.2 Justification for the Study

Mushrooms are currently faced by threats of depletion due to destruction of its forest habitat (Gateri *et al.*, 2004). It is important to cultivate wild mushrooms for domestic and commercial values. Information on the morphological and molecular characterization of *Auricularia* spp in the forests is not enough, so more Research work on the cultivation of *Auricularia* spp needs to be done.

The establishment of the nutritional contents and other chemical analysis of *Auricularia* spp are required in order to encourage its utilization. Morphological and molecular characterization of *Auricularia* spp in Southwestern Nigeria is therefore of utmost importance. The mode of cultivation and the chemical composition need to be known. Keeping in view the usefulness of morphological and molecular primers, the present study was aimed at investigating the genetic diversity of *Auricularia* spp.

Specific Objectives of the Research

Therefore, the objectives of this research were to:

1) survey and collect *Auricularia* species from Southwestern states in Nigeria.
2) culture them in the laboratory for spawn production.
3) determine the substrate that will favour the growth of the mushroom
4) determine the yield of *Auricularia* species when cultivated on some agricultural wastes
5) evaluate the proximate *and* nutrient composition of *Auricularia* species
6) carry out molecular characterisation of *Auricularia* species *in So*uthwestern Nigeria

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CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and Eco-geographical distribution of *Auricularia* spp

Auricularia polytricha belongs to the family Auriculariaceae, and also known as wood ear, Jew's ear, or red ear. *Auricularia* species. belong to the Fungi kingdom and is grouped in division Basidiomycota, subdivision Basidiomycotina in class Heterobasidiomycetes, order Auriculariales and in the family Auriculariaceae. The genus *Auricularia* is believed to be derived from the Greek word Auricula, meaning ear. Chang and Miles, (2004) reported that there are 15 to 20 species of the *Auricularia* worldwide with eight species namely *A. auricula, A. polytricha, A. mesenterica, A. delicate, A. fusfocucolnea, A. peltata, A. cornea* and *A. hispida* identified mostly in China (Chang and Miles, 2004). Among these various species, *A. auricula and A. polytricha* are the most popular and the most cultivated around the world.

Auricularia spp are very common and has a worldwide distribution from the temperate to the tropics growing mostly on living and dead broad-leaved trees, decayed stumps or logs (Reichard *et al.,* 2005). The mushrooms commonly known as Wood Ear or Cloud Ear mushrooms are actually two identical species of jelly fungi, *Auricularia polytricha* and *Auricularia auricula-judae*, respectively. The main difference between the two species is size. The Asian fungus is also called Tree Ear, Black Fungus, and Judas' ear. Wood Ear mushrooms received their common name from their odd shape, which is very similar to that of a human ear. A. *polytricha* is one of the most important medicinal fungi in China, and it has been reported to have several recognized medicinal functions. To mention a few, it has been used in promoting blood circulation, treating hemorrhoids, and having analgesic properties, antitumor agent, immuno-stimulating, effects (Yang *et al*., 2002 Dai *et al.,* 2009).

It is widely adopted in tropical, sub-tropical and temperate zone. *Auricularia auricula* commonly known as wood ear mushrooms is native to Kenya and occurs in Kakamega forest in Western Kenya.. Three main strains (brown, dark brown and yellow brown) occurring in this forest were previously identified through characterization using morphological markers (Onyango *et al.,* 2010). Wood Ear mushrooms are brown to dark brown and can be anywhere from two to 8 inches in size. The gill-less mushrooms are somewhat cup-shaped, with a thick, smooth, wavy

cap and almost no stem. The color of the skin often takes on the color of the tree that it grows on. With age, the mushroom darkens and the skin can turn black. The texture of the Wood Ear mushroom is crisp and crunchy. In other parts of Africa, the wood ear mushrooms have been reported in diverse places such as Nigeria where it is being conserved through cultivation on palm substrates (Osemwegie and Okhuoya, 2009).

In Kenya, the wood ears have not been previously cultivated. The mushrooms are currently faced by threats of depletion due to destruction of its forest habitat to clear land for settlement and agriculture (Gateri *et al.*, 2004). About 14 species of *Auricularia* were reported in China (Yan *et al*., 1998), among which, were common species while other six species are narrowly distributed in certain region such as *A. xishaensis* that only exists in Paracel Islands. Among the 14 species *A. auricula-judae* (Bull.) Quel, also known as wood ear was widely cultivated in China and also won favor around the world for its special nutrition and medicinal value in prevention of diabetes (Kim *et al*., 2007) and heart attacks.

It is the fourth important edible mushroom in the world, and the world total export value has reached 7.6 million dollars in China. The cultivation and production of *A. auricula-judae* plays an increasingly crucial role in China mushroom industry. China has abundant germplasm resources of *Auricularia* species, but the identification and classification of *Auricularia* strain are under confusing circumstance. It is imperative to rapidly and accurately distinguish *A. auricula-judae* from other *Auricularia* species which is very important for domestication and large-scale cultivation. The common way to classify different *Auricularia* species is based on morphological characters such as size, shape and color of the fruiting body. But these morphological characters are susceptible to environmental changes and fruiting body cultivation and are also time-consuming (Oyetayo, 2011).

The rapid increase in domestication of this genus from the wild is attributed to its nutritional and medicinal properties (Chang and Miles, 2004). Musngi *et al*., (2005) used phenotypic differentiations to classify the strains of *Auricularia* in the Philipines. Knowledge of the varietal differences can be used as sources of cell lines for researchers and in breeding programs (Pei-Sheng and Chang, 2004). *Auricularia auricula-judae* grows upon the wood of deciduous trees and shrubs. In up to 90% of cases, the mushroom is found on elder but it is often incorrectly assumed to grow

exclusively on elder. *Acer pseudoplatanus sycamore*), beech, ash, spindle, and in one particular case, the sycamore draining board of an old sink in Hatton Garden. In Australia, it is found in wood land and rainforests; in the rainforests, it can grow in very large colonies on fallen logs. It favors older branches, where it feeds as a saprophyte (on dead wood) or a weak parasite (on living wood). Commonly growing solitarily, it can also be gregarious (in a group). This dead and moist part of the bark of *Mangifera indica* supports germination of fungal spores and the growth of fungal hyphae of the saprophytic fungi like *Auricularia* sp. (Rajput and Rao 2004).

Spores are ejected from the underside of the fruit bodies with as many as several hundred thousand an hour, and the high rate continues when the bodies have been significantly dried. Even when they have lost some 90% of their weight through dehydration, the bodies continue to release a small number of spores. It is found all year, but is most common in autumn. It is widespread throughout temperate and sub-tropical zones worldwide, and can be found across Europe, North America, Asia, Australia, (Conte and Laessoe, 2008)

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2.2 Morphology of *Auricularia* species

The fruit body of *A. auricula-judae* species is normally 3 to 8 cm (1.2 to 3.1 in) across, but can be as much as 12 cm (4.7 in). It is distinctively shaped, typically being reminiscent of a floppy ear, though the fruit bodies can also be cup-shaped. It is normally attached to the substrate by the back surface of the cup, though there can also be a rudimentary stem. The species has a tough, gelatinous, elastic texture when fresh, but it dries hard and brittle (Sterry and Hughes 2009). The outer surface is a bright reddish-tan-brown with a purplish hint, often covered in tiny, downy hairs of a grey color. It can be smooth, as is typical of younger specimens or undulating with folds and wrinkles. The color becomes darker with age. The inner surface is a lighter grey-brown in color and smooth. It is sometimes wrinkled, again with folds and wrinkles, and may have "veins", making it appear even more ear-like.

As described by Stamets (1993), the spores of *A. polytricha* usually have club-like structure and their spore print is mostly white in color. The mycelia of this species are longitudinally linear and as they grow older, the mycelia mat is thickening with age, to form a dense cottony white mat and becoming mottled with brown discoloration in cultures. *Auricularia polytricha* fruiting bodies have no stipe and are covered by

medulla of fine hairs. The surfaces are very smooth but wrinkled towards the center and upturned towards the outer edge of the mushroom. The fruiting bodies when fresh are usually brownish to reddish brown, yellowish brown or dark brown, ear-shaped and have a consistency of jelly – firmly gelatinous and rubbery texture. Upon drying, they usually look purplish brown to black; they shrink greatly to a minute portion of their original size. When contacted with water, they rehydrate and enlarge true to form again. Black Jelly mushroom has an atypical texture when eaten but it is not really flavorful when compared to other popular edible mushrooms (Stamets, 1993).

Species of *Auricularia* As of May 2015, Index Fungorum lists 28 species of *Auricularia*

- *Auricularia albida*
- *Auricularia americana*
- *Auricularia auricula-judae*
- *Auricularia cornea*
- *Auricularia delicata*
- *Auricularia discensa*
- *Auricularia eximia*
- *Auricularia fibrillifera*
- *Auricularia fuscosuccinea*
- *Auricularia goossensiae*
- *Auricularia hainanensis*
- *Auricularia hispida*
- *Auricularia hispidula*
- *Auricularia incrassata*
- *Auricularia indica*
- *Auricularia mesenterica*
- *Auricularia minor*
- *Auricularia nigricans*
- *Auricularia peltata*
- *Aurucularia. polythrica*
- *Auricularia rosea*
- *Auricularia scissa*
- *Auricularia semipellucida*
- *Auricularia sordescens*

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- *Auricularia stellata*
- *Auricularia subglabra*
- *Auricularia tenuis*
- *Auricularia wrightii*
- *Auricularia xishaensis*

Source: Adapted from Kirk (2015)

2.3 Agriculture and Agro-industrial Wastes or By-products used as Substrates in Nigeria

Africa generates huge quantities of organic wastes annually through activities in agriculture by farmers, forestry and food processing industries that uses agricultural products. The generated waste has adverse environmental effects related to their disposal (Gateri *et al.,* 2009). The situation in Nigeria is the same with our waste to wealth approach of growing mushroom as an aspect of agriculture. Yet, with the application of appropriate bioconversion technologies like biogas production, these wastes are also potentially useful substrates for the production of mushrooms (Chang and Buswell, 2003). Although various strategies have been developed to utilise part of the large quantities of waste lignocellulose generated annually, one of the most significant, in terms of producing a higher value product from the waste, is the cultivation of edible mushrooms by solid-state fermentation.

Large amounts of agriculture and agro-industrial residues or wastes are excessively produced by agriculture and agro-industrial activities. Generally, solid agro-industrial residues consist of cellulose, hemicelluloses and lignin and also pectin, starch and other polysaccharides and are insoluble in water. The wastes derived from agricultural activities can be used as a resource for sustainable production of food and value-added food products like mushroom. Expensive treatments or disposal is required if these wastes are not recycled or used to generate a value-added product. Adverse effect on the environment would also occur if these wastes are not managed effectively and left in the waste stream. It is also very expensive to dispose these agricultural wastes in incinerator, if not used for mushroom cultivation (Kalu *et al*., 2013).

Farmers can therefore utilize agricultural wastes, such as dried sugar cane leaves, saw dust, maize Stover and banana leaves which constitutes nuisance and health effects on

the population, and use them as substrates for mushroom production (Beetz and Kustida, 2004; Lourdes *et al*., 2008).

2.3.1 Starting a culture from spores

A mushroom culture can be started in one of two ways. Most growers start a culture from spores. The advantage of using spores is that they are viable for weeks to months after the mushroom has decomposed. The other way of obtaining a culture is to cut a piece of interior tissue from a live specimen, in effect a clone. Tissue cultures must be taken within a day or two from the time the mushroom has been picked, after which a healthy clone becomes increasingly difficult to establish (Stamets, 1993).

2.3.2 Taking a Spore Print

To collect spores, sever the cap from the stem of a fresh, well cleaned mushroom and place its gills down on a piece of clean white paper or a clean glass surface such as a microscope slide. If a specimen is partially dried, add a drop or two of water to the cap surface to aid in the release of spores. To lessen evaporation and disturbance from air currents, place a cup or glass over the mushroom cap. After a few hours, the spores will have fallen according to the radiating symmetry of the gills. If the spore print has been taken on paper, cut it out, fold it in half, seal in an airtight container and label the print with the date, species and collection number. When using microscope slides, the spores can be sandwiched between two pieces of glass and taped along the edges to prevent the entry of contaminant spores. A spore print carelessly taken or stored can easily become contaminated, decreasing the chance of acquiring a pure culture. (Chang and Miles, 2004).

2.4 Cultivation of *Auricularia*

Artificial cultivation of mushroom has been going on for many centuries. Scientists have been dedicating their time to domesticating wild mushrooms and breed them to get better quality mushrooms. Long before the development of mushroom production systems in the United States, the Chinese has been cultivating different species of edible mushroom (Quimio *et al.,* 1990). From the recent studies carried out on mushroom, It has been reported that more than 2000 species of mushrooms exist in nature, but unfortunately, approximately only 22 species are intensively cultivated from these large number (Manzi *et al*., 2001), while the most cultivated worldwide

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species are from *Agaricus, Pleurotus, Lentinula, Auricularia, Flammulina* and *Volvariella* genus to mention a few.

Although these edible mushroom species have the capability to degrade lignocellulosic materials both in their natural or composted form.They exhibit differences regarding the production of enzymes necessary to degrade lignocellulosic substrates and consequently different capabilities to grow and fruit on lignocellulosic-residue substrates (Baldrian and Valaskova, 2008).

2.5 Major steps in the cultivation of Mushroom

Techniques for cultivating mushrooms, whatever the species, follow the same basic pattern. Whereas two species may differ in temperature requirements, pH preferences or the substrate on which they grow, the steps leading to fruiting are essentially the same. They can be summarized as follows according to Stamets (2005):

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1. Preparation and pouring of agar media into Petri dishes.

2. Germination of spores and isolation of pure mushroom mycelium.

3. Expansion of mycelial mass on agar media.

4. Preparation of grain media.

5. Inoculation of grain media with pure mycelium grown on agar media.

6. Incubation of inoculated grain media (spawn).

7. Inoculation of grain spawn into bulk substrates.

8. Initiation—lowering temperature, increasing humidity to 95%, increasing air circulation, decreasing carbon dioxide and/or introducing light.

9. Cropping—maintaining temperature, lowering humidity to 85-92%, maintaining air circulation, carbon dioxide and/or light levels.

REFERENCES: Stamets (2005)

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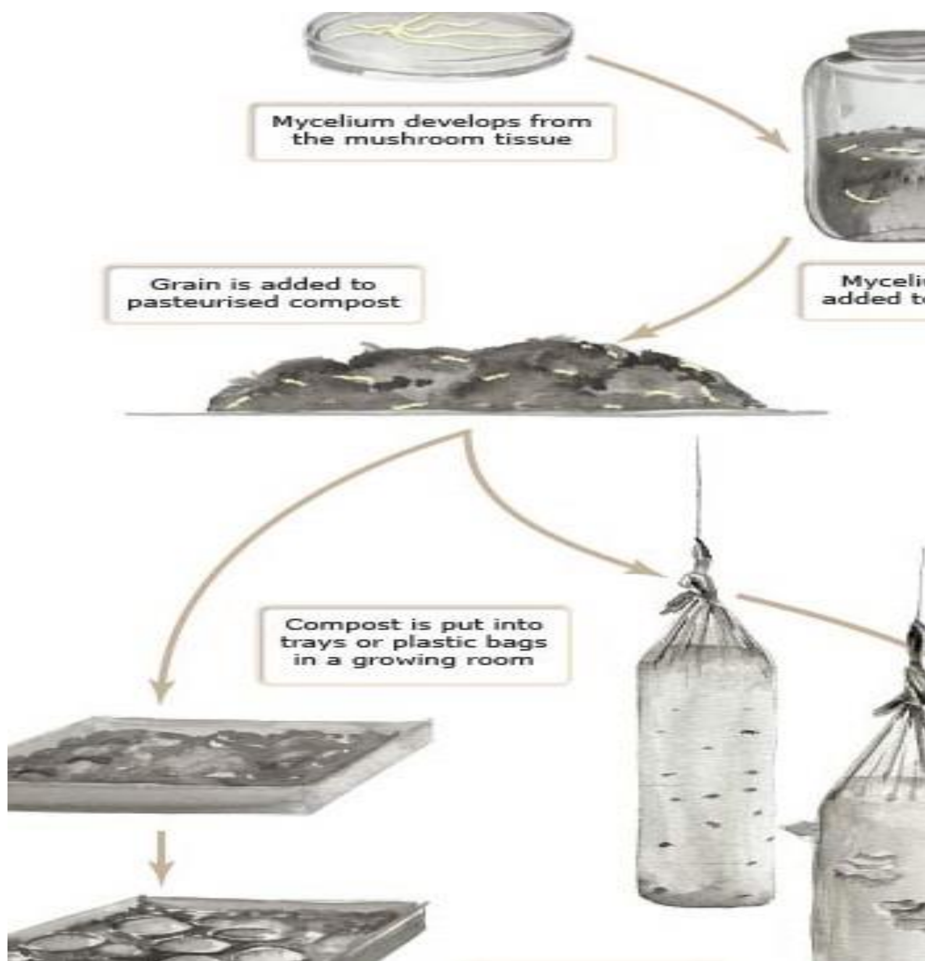

Fig.1.0

Adapted from: A practical guide to growing mushroom at home by Stamets (2005)

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Types of wastes

Agricultural wastes can be divided into crop-based wastes which are generated in the field and processing-based wastes, generated during the processing of crop. Crop based wastes are plant materials left behind in the field or farm after removal of the main crop produce, consisted of different sizes, shapes, forms and densities, such as straw, stalks, leaves, roots and branches which were originally burnt before the advent of using them for mushroom cultivation.

Many wood decomposing fungi utilize lignocelluloses efficiently and this characteristic can be attributed to their ability to metabolize lignin (Baysal *et al.*, 2003). Huge lignocellulosic materials can be used in edible and medicinal mushroom cultivation and at the same time also protecting and regenerating the environment against land Pollution. Chiu and Moore (2001) reported that commercial mushroom production is a relatively short but efficient biological process of food protein recovery from negative-value lignocellulosic materials by utilizing the degrading capabilities of mushroom fungi. Currently, the mushrooms are considered and universally accepted as the most profitable and environment friendly method for recycling of the vast lingo cellulosic wastes in agricultural and forestry based industries

Farmers can utilize agricultural wastes, such as dried sugar cane leaves, saw dust, maize Stover and banana leaves as substrates for mushroom production (Beetz and Kustida, 2004; Lourdes *et al.,* 2008). Cotton waste material has been used as substrate for growth of mushrooms. Lin *et al*. (1993) reported that dead branches, fallen leaves and pruning wastes from tea plants were suitable for cultivation of *A. polytricha*. A nature-imitated cultivation method was developed in China by cultivating *Auricularia* mushrooms in corn fields, and thereafter, using the spent compost as organic fertilizer and soil conditioner (Yan *et al.,* 2004). Sharma and Puttoo, (2004) revealed that wheat straw when supplemented with 10% wheat bran was the most suitable as it provided 93% bio-efficiency in mushroom production while Thiribhuvanamala *et al.,* (2005) reported that paddy straw, mixed saw dust and wheat bran substrates resulted in early spawn running with uniform mycelial growth of *A. polytricha* with bio efficiency of 46.4%.

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Wood ear mushrooms (*Auricularia spp*.) are commonly cultivated in Asia. Plastic bag cultivation is gaining popularity due to the scarcity of suitable logs and the ease with which different species of *Auricularia* can be cultivated on sawdust (Veeralakshmi *et al.,* 2014). *Auricularia auricula-judae* is also in cultivation elsewhere in the world, for instance, in Ghana. In the Bron Ahafo and Ashanti regions, it is grown with what is referred to as the "plastic bag method". Sawdust is packed into polypropylene bags and then sterilized by steam for several hours. Once the sawdust has cooled, Sorghum grain spawn is added, and the bags are kept in moderately dark conditions. Once the sawdust is exposed to a humid environment, *A. auricula-judae* fruit bodies begin to grow.

Onyango *et al.* (2011), reported that organic substrates and supplements tested were significantly different ($p<0.05$) in suitability for wood ear mushroom cultivation. Generally, maize cob substrate consistently gave the best results followed by wheat straw, sugar bagasse and grass straw in that order. On the other hand, wheat bran supplement proved to be better than rice bran. Composted and non-composted substrates and supplements were nutritionally analyzed to determine their lignin, ash, cellulose, crude protein and moisture content using *Auricularia auricula* (Onyango *et al.,* 2011). The substrates and supplements were significantly ($p<0.05$) different in their nutritional content with maize cobs and wheat bran containing higher cellulose, crude protein and moisture content. To make mushroom cultivation sustainable and highly productive, novel improved strains with improved characteristics are greatly needed. However, mushroom strains are very difficult to discriminate due to lack of clearly distinguishable characters.

Lawal *et al.* (2011) carried out a work on the positive effect of additives on the cultivation of *Auricularia auricula.* Mycelial growth was observed in each of the substrate-supplement combinations.It was noted that saw dust supplemented with 10% brewer's grain gave the best yield of mushrooms while the least was observed on and the least yield (1.50gram) was observed in saw dust supplemented with 10% Oil palm fibre.

Adenipekun *et al*. (2015) cultivated *Auricularia auricula (St. Aman's*) Berk on *Mansonia altissima* sawdust with various additives (Brewer's grain (BG), Corn chaff (CC), Oil palm fibre (OPF), Sorghum chaff (SC) and Wheat bran (WB) at different

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percentages (0%, 5%, 10% and 20%). The treated and untreated substrates with different percentages of additives were analyzed for lignocelluloses composition, macro element, C-N ratio and proximate composition. *A. auricula* was able to reduce the lignocelluloses composition of *M. altissima* sawdust exhibited an increase in performance with increase in additives.About 20% inclusion level being the most efficient for all the additives used.

Wood meals of 3 tropical hardwood species (*Falcataria moluccana, Shorea sp*., and *Tectona grandis*) from Indonesia were used as basal cultivation substrates for *A. polytricha* by Irawati *et al*. (2012). The fastest mycelia growth was found in the substrate made of *Shorea sp*., and the highest glucosamine content was found in the substrates made of *Shorea sp.* and *F. moluccana.* No significant difference in the period of time to the first harvest was found between *F. moluccana* (23 days) and *Shorea sp*. (25 days), whereas a significant difference was found in the interval between the following harvesting periods (7 and 10 days for substrates made of *F. moluccana* and *Shorea sp*., respectively). Over the entire cultivation period, the substrates made of *F. moluccana* produced the highest fruiting body yield, greatest biological conversion, and greatest weight loss from the substrate. These results indicated that *F. moluccana* wood meal is the appropriate basal substrate for *A. polytricha* cultivation.

According to Veeralakshmi *et al*. (2014), the studies conducted at Mushroom Research and Training Centre, TNAU, Coimbatore revealed that the paddy straw+wheat bran (3:1) ratio recorded minimum days for spawn run (21.3 days), pin head formation (31.3) and first harvest (35.6 days). The same combination also recorded the highest yield of 147.6 g/bed bioefficiency of 59.04%. The total cropping period was also the minimum in the same treatment. In the trials conducted at Vijaya Mushrooms, Coimbatore (North), paddy straw+wheat bran (3:1) ratio again recorded a significantly higher yield of 132.0 g/bed and bio efficiency of 58.20% with minimum cropping period of 47.3 days. The yield performance trials conducted at Maha Mushroom, Kovaipudur, and Coimbatore (South) also revealed the same trend as paddy straw+wheat bran (3:1) again recorded significantly higher yield of 130 g/bed and bioefficiency of 52.00%.

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The wood waste, however, comes from many different tree species. Mycelial growth and fruiting body formation are greatly affected by tree species and quality (Ohga, 2000).

2.6 Basic Substrates for Mushroom Cultivation

Materials that can be used as substrate for mushroom cultivation are very well diverse and abundant in the environment. In Nigeria, as in other parts of Africa, substrates for mushroom cultivation have not been fully exploited. A close look at the habitat of wild mushrooms indicates that they are normally found on such sites of natural wastes like leaf litter, fallen logs or on wastes accumulated on sites of farm processing of agricultural products. This common observation indicates the potentials of such wastes for the cultivation of mushrooms (Okhuoya, 2000, Okhuoya *et al*., 2010).

A vast variety of wood and wide range of wastes or by-products from agriculture and forestry industries can be explored to be used as substrate materials because the majority of cultivated mushroom are saprophytic-typed, which exist on dead organic matter. These organic materials contain lignin and cellulose, besides other compounds that can be easily broken down by the extensive enzyme system in mushroom. The substrates chosen and used in mushroom cultivation are numerous and can include both field-based residues such as oil palm frond, corn husk, rice straw and wheat straw and also processing based-residues such as sugarcane bagasse, brewers/spent grains, rice bran and palm pressed fiber. (Adenipekun *et al*., 2015)

However, supplements containing sugars and starch as well as fats can be added to the basic ingredient because these supplements are time-lasting nutrient sources and are more slowly degraded. This is because the composition of nutrients of the substrate are one of the many important factors limiting fungi colonization on substrates and also affecting the yield quantity and quality of cultivated mushrooms.

Carbon, nitrogen, minerals and vitamins are the four basic chemical compounds needed by mushroom for growth. Therefore, to ensure success in mushroom cultivation, all four compounds should be sufficiently present in the basic substrate with emphasis on a balance content of carbon and nitrogen ratio. This is because carbon and nitrogen play bigger roles on overall growing process of mushroom. Varied form of carbon source such as monosaccharide, oligosaccharide and

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polysaccharide are essential for the growth of mycelium on growing medium, especially polysaccharide such as cellulose and hemicelluloses. Most polysaccharides are hydrolyzed to produce sugar. Nitrogen sources such as acid amino, urea, ammonium and nitrate are needed by the fungus to synthesize proteins, purines, pyrimidines and also help to produce chitin compared to other plant materials, wood has low level of nitrogen content but high in lignin. High concentrations of carbon and nitrogen sources are generally needed for high mycelium biomass, but in some types of mushrooms, it is observed that high glucose concentration inhibits mycelium growth. (Wang *et al*., 2010)

Different levels of carbon and nitrogen content are needed by different types of mushroom and thus require different optimum C:N ratio for both mycelium growth and fruiting body formation. For example, *V. volvacea* can be grown on plant materials with low nitrogen content but evidently some mushroom types require high nitrogen content. All Basidiomycetes mushroom like *A. polytricha* also require several element of mineral to stimulate their growth. (Chang *et al*. 1981).

According to Chang (1982), most fungi are able to synthesize their own vitamin and low concentration of vitamin resulted in optimum growth of fungi, reported in some cases. Thiamin and B1 vitamin are needed for mycelium growth, primordia formation and also fruiting bodies. In general, higher level of vitamin is required by mushroom during primordia and fruiting body formation phase compare to vegetative phase or mycelia growth stages of the mushroom.

2.7 Supplement for mushroom growth

Supplements are used to enhance nutritional content, accelerate growth as well as to increase mushroom yield during cultivation (Royse, 1997). There are a wide variety of protein-rich materials used in mushroom cultivation, such as rice bran, wheat bran, spent grain, spent yeast, molasses, cotton and coffee wastes and many more. According to Stamets (1993), supplementing a substrate risks competition from contaminants and insects because supplementation changes the number and the type of organisms that can be supported. This means that contamination can be easily occur if supplementation of the substrate is not done appropriately for the benefit of the mushroom.

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Therefore, extra caution is required to prevent contamination and ensure success. One of the methods to achieve this is by prolonging the sterilization cycle of the substrates. Many studies have reported an increase in mushroom yield by adding supplements to the basic spawn and fruiting substrates, depending on the type of mushroom cultivated, supplement types and concentration of supplements added. Nevertheless, excessive use of supplements gave a reduced effect of the substrates on mushroom production. Hadwan *et al*. (1997) recommended supplementation of the substrate with various materials such as rice bran, spent grain and wheat grain, prior to spawning for enhancement of the yield of mushroom.

As a way to provide optimum growth medium for mushroom cultivation, Royse (2001) suggested the addition of different starch –based supplements such as wheat and rice bran, rye, millet and maize powder to sawdust which will serve as major nutrients. Research conducted by Chang *et al.* (1981) exhibited substantial increase in the yield of fruiting bodies per unit weight by addition of supplements to wheat straw substrate in oyster mushroom cultivation. Wang (2010) also reported that supplementation of fruiting substrate resulted in a significant increase of oyster mushroom yield. This was originally mentioned by reports of Mau *et al*. (2002), Chang (1996) and Okhuoya *et al*. (2005) which has proved that supplementation of substrate was indeed improve the production, quality, flavor and also shelf life of cultivated mushrooms. The positive effect of supplementation can be correlated with the nutrients present in those supplements. According to Fasidi and Kadiri (1993), carbohydrates, amino acids and mineral elements present in rice bran can be the triggering factor for the increase of the productivity of Shiitake mushroom.

2.8 Mushroom growth and development

Mushroom is a macro-fungus with a unique fruiting body, can be easily seen with naked eye and large enough to be picked by hand, which can be either epigeous or hypogenous (Chang and Miles, 2004). This definition can be accepted as a working term in cultivation of edible mushroom, although it is not a perfect one. Mushroom, like all fungi, cannot undergo photosynthesis because of the lack of chlorophyll and thus get the necessary nutrients from organic materials. Mushrooms require carbon, nitrogen and inorganic compounds as their nutritional sources (Sharma *et al.,* 2013).

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2.9 Economic importance of *Auricularia*

Mushroom cultivation and its derived products can help reduce malnutrition, because mushrooms can serve as substitutes for other sources of protein like egg and meat. Mushrooms are an important protein source that also provide vitamins (B1, B2, C) and minerals as well as other nutrient (Ekpo and Aluko, 2002; Daodu, 2003). Mushrooms have been reported to be low in cholesterol and offer an especially promising opportunity to discover anti-cancer genes and pathways (Bechtel *et al*., 2002; Borchers *et al.,* 2004). Most Nigerians in the rural areas (65% of population) eat mushrooms. About 90% of these mushrooms are collected from the wild while the rest 10% is imported in form of pickled or canned materials usually from Britain, U S A, China and other countries from the far East (Isikhuemhen and Okhuoya, 1995).

Fresh mushrooms contain relatively large amounts of carbohydrate and fibre ranging from 51 to 88% and 4 to 20% (dry weight), respectively, for the major cultivated species. Okhuoya and Ayodele,(2007).Strong consumer demands and threats of depletion of mushrooms have stimulated increased worldwide production in the past few decades(Chang and Miles, 2004).The increased demand of mushrooms is due to their unique culinary and medicinal properties (Yan *et al*, 2003) However. Africa contributes a paltry 1% of the annual worldwide production of mushrooms (Adejumo and Awosanya, 2005).

In nature, mushroom have not only been a source of food for man and animals, but also have played an important role in the cycling of carbon and other elements through the breakdown of lignocellulosic plant residues and animal dung which serve as the substrates for the saprophytic fungi. In this way, mushroom species as agents of decay, help keep the environment from being overwhelmed by the dead organic debris of plants and animals. Simultaneously, mushrooms can produce a wide range of enzymes that degrade complex substrates, following which they absorb the soluble substances (Stamets 2005).

Auricularia auricula-judae has been the subject of research into possible medicinal applications. Experiments in the 1980s concluded that two glucans isolated from the species showed potent antitumour properties when used on mice artificially implanted with Sarcoma 180 tumours. This was despite the conclusion of earlier research indicating that, while aqueous extracts from several other fungal species had anti-

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tumour effects, extracts from *A. auricula-judae* did not. Further, research on genetically diabetic mice showed that a polysaccharide extracted from *A. auricula-judae* had a hypoglycemic effect; mice fed with food including the polysaccharide showed reduced plasma glucose, insulin, urinary glucose and food intake. Another chemical extracted from the species was an acidic polysaccharide (made up of mostly mannose, glucose, glucuronic acid which showed anticoagulant properties. The article concluded that "the polysaccharides from these mushrooms may constitute a new source of compounds with action on coagulation, platelet aggregation and, perhaps, on thrombosis"(Yoona *et al*., 2003).

Another study reported that the species may be effective in stopping platelet binding *in vitro,* with possible uses regarding hypercholesterolemia. Research has shown that *A. auricula-judae* can be used to lower cholesterol levels generally, and in particular, is one of two fungi shown to reduce the level of bad cholesterol (Yoona *et al*., 2003). Jonathan and Fasidi (2005), worked on the antimicrobial activities of some selected Nigerian Mushrooms. *A. polytricha* exhibited antagonistic effects of 18mm value. This was evidenced by the clear zone of inhibition produced by the bacteria and fungi around the tested mushroom extracts. He also observed that the antimicrobial activities of purified mushrooms extracts were generally higher than the crude extracts. The jelly-like fruit bodies have been shown to contain various bio-compounds that have anti-tumor, antiviral, antibacterial and anti-parasitic effects making it a choice food (Yan *et al*., 2003: Chang and Miles, 2004).

In addition, *Auricularia* mushrooms have been known to have marked ability to assist the body in healing complex ailments such as cancer, AIDS, diabetes and heart disease. Tambekar *et al*. (2006) indicated that mushrooms have been used extensively in traditional medicine for curing various types of bacterial infections. Palapala *et al.*, (2006) reported that Kenyan native wood ear mushrooms have the potential to be grown on locally available substrates such as wheat straws, sugar bagasse, sawdust, maize cobs and maize stalks. In order to achieve maximum yields, supplementation of substrates with other nutrient bases such as soybean meal, rice and wheat brans is necessary as they can reportedly increase mushroom yield two-fold.

Onyango *et al.* (2011) reported that maize cob substrate consistently gave the best results followed by wheat straw, sugar bagasse and grass straw in that order. On the

other hand, wheat bran supplement proved to be better than rice bran. Jonathan *et al.* (2009) reported that *A. polytricha* produced best biomass (310mg/100cm cube) at pH 6.5 after six days of incubation. For the effects of carbon compounds for mycelia biomass production, glucose a monosaccharide at the concentration of 1.6% produced better than sugar alcohol and complex sugar (cellulose) for biomass production. Moreover, he also observed that the best biomass yield was obtained between temperature range of 25°C - 30°C.

Okhuoya *et al.* (2010) carried out an investigation on *Auricularia auricula judae* (bull) as one of the edible mushrooms and discovered, it was one of the under-utilized none forest resources. In his report, *Auricularia auricula* mushroom is generally accepted by the three major tribes in Nigeria as food and for medicinal purposes. Despite the high level of progress made through global network and the advancement of mushroom cultivation industries in many developed nations, growing mushrooms in homes or even on a commercial scale is still uncommon in Nigeria. Researchers need to reduce the dependence on the naturally occurring mushrooms.

Edosomwan *et al.* (2013) did a study on the presence of heavy metals, microbiological and parasitological concentration of *Auricularia auricula*. Edosomwan *et al.* (2013) worked on the prevalence of identified helminth parasite eggs in *Auricularia auricula* from Ikpoba Hill market and the result showed 53.30% (*Toxocara canis*), 13.33% (*Trichuris ovis*), and 6.67% (*Moniezia benedeni*). Only iron (Fe) 860mg/kg, Zinc (ZN) 58mg/kg and Nickel (Ni) 1.60mg/kg concentrations were heavy metals identified in the study.

These values were higher than the World Health Organization Standard especially for Fe and Zn. While the average bacterial count was 3x103cfu/ml and the parasitological result showed 53.30% for *Toxocara canis* and 6.67% for *Moniezia benedeni*. After the characterization and identification of the Isolates, three genera of bacteria were isolated, *Citrobacter sp*, *Staphylococcus aureus. Bacillus sp* and *Mucor sp* were isolated. Mushrooms have the ability to easily absorb heavy metals from the soils. The consumption of mushrooms with high heavy metals concentrations poses a great risk of heavy metal toxicity. Mushrooms grow very close to the ground and can easily be contaminated by bacteria and helminth eggs which pose the risk of intestinal parasitic infection (Okechukwu *et al* 2011).

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2.9.1 Insects and Pests.

Pygmephorid mites were previously considered to be of doubtful pest status (Clift and Toffolon, 1981). However, some species e.g. *Microdispus lambi* (Krczol), feed on the mycelium of crop mushrooms, andcommonly cause 10%-20% yield losses, occasionallyeven a total crop loss on some farms in China (Gao and Zou,2001; Wu and Zhang, 1993), and up to 30% yield losses in Australia (Clift and Toffolon, 1981; Ferragut *et al*., 1997). Others species, such as *Pediculaster* spp., feed and develop on *Trichoderma viride* Pers, *Cladobotryum dendroides* (Bull), *Chrysonilia sitophila* (Montagne) and *Mycogoneperniciosa* (Magnus), common fungal parasites of commercial mushrooms (De Lillo, 1997) the cultivation of the button mushroom *Agaricus bisporus* (Lange). Large reddish brown clumps of mites accumulate on mushroom caps and lumps of peat, before the occurrence of third flash. Earwigs are elongate, flattened insects, ranging from light red-brown to black and are easily recognized by their forcep-like appendages (pincers) on the end of the abdomen. Young earwigs (nymphs) are similar to adults. They are white to olive-green and lack wings. Jonathan *et al*. (2012) reported that, Insect pests such as ants, beetles and true flies were encountered on the mushrooms, they werefound at the larval and adult stages. Insect orders such as Coleoptera, Hymenoptera, Collembola and Diptera were present.

The name earwig is derived from a European superstition that these insects enter the ears of a sleeping person and bore into the brain. Earwigs develop from egg to adult through gradual metamorphosis with four to five nymphal instars or stages. They are rapid runners and feed on mosses, lichens, algae, fungi, insects, spiders and mites, both dead and alive. Some earwigs are predators, feeding on aphids and others feed on living plants, becoming pests in greenhouses and on certain crops such as vegetables, fruits, ornamentals, forages and field plants. Earwigs require moist, cool places and are found in damp crawl spaces, flower gardens near the home, in mulches, compost piles, trash, under boards and in wood piles. Since they are attracted to lights, reduce lighting around doors, windows and other potential entry sites. Earwigs need and are very attracted to moisture. High populations, practically invisible during the day, may be present around foundations (Stamets and Chilton 2013)

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2.9.2 Other uses of Mushrooms

Mushrooms can be used for dyeing wool and other natural fibers. The chromophores of mushroom dyes are organic compounds and produce strong and vivid colors, and all colors of the spectrum can be achieved with mushroom dyes. According to Mussak and Bechtold (2009) before the invention of synthetic dyes, mushrooms were the source of many textile dyes.

In Egypt, the total yield of bread grains does not satisfy the needs of the country. The total production of wheat grains cover only about 55% of the total needs. The way forward was to search for the native cereal sources or others which could be used with wheat flour bread making. Biscuits are convenient food products and the most popular bakery items consumed nearly by all levels of society in Egypt.(Hesham *et al.,* 2007) The studies carried by Hesham have shown the potential for developing protein-rich balady bread and biscuits with the partial replacement of wheat flour using fresh oyster mushroom (*Pleurotus.sajor-caju*, strain 290) The results obtained indicated that raw and germinated legumes (chick peas and kidney peas) flour and mushroom flour may be blended with wheat flour at levels as high as15% without adversely affecting baking performance of balady bread, but with some adverse effects on biscuits (Hesham *et al* 2007).

Chang and Miles (2004) came up with the new mushroom vocabulary "mushroom nutriceuticals" as extractable dietry food supplements from either the fungal mycelium or the fruiting body of the mushroom. Many have been associated with treatment of ailments. There are so many mushroom supplements in the herbal markets especially in Europe, Asia and America Due to nutritional and medicinal contents of some mushrooms, it is also now recommended to fortify foods with mushrooms especially when used in different available recipes.

Adenipekun *et al.* (2015) carried out a research on the biodegradation of polycyclic aromatic hydrocarbon (PAHS) in spent and fresh cuttings fluids contaminated soils using *Pleurotus pulmonarius* (Fries).Quelet and *Pleurotus ostreatus* (Jacq) Fr.Kumm. He discovered that the *P. spp* is useful in bioremediation of contaminated soils. Mushroom is also an agent of bioremediation especially in the recycling of lignocellulosic wastes of agricultural origin and in healing the soil (Stamets, 2005).

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Mushroom had been used from ancient times and is connected with mysticism (Griensven, 2009). Ironically, the first record of mushroom used as hallucinogenic agent was credited to the Yoruba tribe of Nigeria in Africa (Griensven, 2009). The record dates back to the Paleolithic period (7000 – 9000 years ago) (Samorini, 1992). In Eastern Countries like China and Japan the knowledge on the use of edible and medicinal mushrooms had been passed on from one generation to the other in documented form. For example, over 2,500 years ago, many medicinal mushrooms had been recorded and depicted in the earliest Chinese material medica book, *Shennong Bencao Jing*, and other succeeding Chinese medical book (Zhu, 2009). It was not so in Nigeria. Information on the indigenous use of mushrooms had been passed orally from one generation to another (Akpaja *et al.*, 2003). It is possible that some of this undocumented information had been lost. Women who sells vegetables and mushrooms and elderly people are usually most helpful in supplying information about ethnomycological uses of mushrooms in South west Nigeria (Oso, 1977). The same observations were also made in other parts of the country where survey were carried out (Akpaja *et al.*, 2003). In essence, the younger generation in Nigeria has little or no knowledge about ethnomycological uses of mushrooms. Some edible/medicinal mushrooms in Nigeria had also been extinct as a result of human activities during farming and annual wild fire out break (Ayodele *et al.*, 2009).

2.9.3. Processing and preservation for short and long-time utilization

In this study different methods were found used by different communities for both short and long term preservation to ensure all year supply of mushrooms. In the local open markets, both fresh and dry mushroom were sold Donatha, (2013). The dry mushrooms were sold at relatively higher prices compared to fresh mushrooms. Interrogating the collectors and traders, different ways deployed in improving the shelf life of the collected mushrooms were revealed as follows:

2.9.4. Fresh preservation

This involved soaking them in water where they remained fresh for 2–3 days the mushroom remains fresh simply because waters in these cooler areas are really cold, thus soaking mushroom in these water reduces the biological activity of the mushrooms as the same principle used in storing them in the fridges. In warmer areas they spread the mushroom on the tray from buckets and sacs and leave them outside the house over night before transporting them to the market (Okhuoya, 2011).

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2.9.5 Long preservation

Sun drying

This involved direct sun drying whereby mushroom were spread on the ground/wire meshed shelves and left them to dry by direct sunshine. This method is the best and has been recently found to be very good means of preservation. Sun drying and keeping them in airtight container can stay for a long period in good condition (Stamets and Chilton, 2013)

Smoking

According to Donatha (2013) preserving the collected mushroom on shelves involves using firewood which gives out heat and smoke that goes straight to the mushroom preserved on the shelves constructed above the cooking points. The heat help in drying the mushroom,.Smoked mushroom can stay up to three years in good conditions and consumer testified those smoked mushrooms are very delicious and tasty.

Salt drenching

This involves preserving mushroom in a supersaturated sodium chloride solution. This goes without a question that the saline condition kills most of the microbes that would have caused mushroom deteriorations thus remain in good condition. The method was mostly observed in Kigoma - Uvinza where they have salt panels in the area. Discussing the applicability of this method with other interviewee in other parts, they were doubtful on the method as it could be expensive since it will involve spending money for buying salt. (Romain *et al.,* 2006)

2.9.6 Morphological characterisation

Li *et al.,* (2011) worked on the conventional way to classify different *Auricularia species* which relied on morphological characters such as size, shape and color of the fruiting body. Onyango *et al.*, (2010) reported on the morphological characterization of Kenyan native wood ear mushroom. In his work, 9 basidiocarps were selected for characterization. Strain identification was based on basidiocarp morphology and structure of mycelia colonies. Three main basidiocarp colours were observed. These included yellow brown, brown and dark brown. With regards to basidiocarp shape, most of the yellow brown strains were ear shaped, majority of brown strains were

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discoid and campanulate while a good number of dark brown strains were flattened. In the case of colony formation, the findings showed that mycelia colonies were white and cottony, with abundant aerial hyphae, off white, velvety and low density mycelia with scarce aerial hyphae were also observed.

Mushrooms native to Kenya were successfully characterized using morphological characters by Onyango *et al.*, (2011). It was evident that wheat bran supplementation of millet and sorghum grains had a high potential for utilization for spawn production of Kenyan native wood ear mushrooms (Onyango *et al.*, 2011).

Members of the genus *Auricularia* are known to have a wide range of morphological plasticity due to the absence of clearly distinguishing characters (Wong and Wells, 1987). Previous work done on characterization has combined Basidiocarp features with characteristics of individual and colonial hyphae (Lowy, 1952; Duncan, 1972). Wong and Wells, (1987) emphasized the need for compatibility and inter-fertility studies in delimiting the members of this genus. More recently, Musngi *et al.,* (2005) used phenotypic differentiations to classify the strains of *Auricularia* in the Philipines.

2.9.7 Molecular characterisation of Mushroom

To make mushroom cultivation sustainable and highly productive, novel improved strains with improved characteristics are greatly required. However, mushroom strains are very difficult to discriminate due to lack of clearly distinguishable characters. This makes strain protection problematic, and impedes strain improvement (Chandra *et al.*, 2010). Molecular markers of rDNA sequencing, RFLP (Restriction Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), microsatellite and mitochondrial genotypes have all been used to discriminate mushroom species and/or strains of *Agaricus* (Castle *et al.*, 1987; Sonnenberg *et al.*, 1991; Khush *et al.*, 1992; Barroso *et al.*, 2000; Calvo-Bado *et al.*, 2000; Moore *et al.*, 2001; Ramirez *et al.*, 2001), *Auricularia* (Yan *et al.*, 1999), *Ganoderma* (Hseu *et al.*, 1996), *Lentinula* (Chiu *et al.,* 1996), *Stropharia rugoso-annulata* (Yan *et al.*, 2003), and *Volvariella* species (Chiu *et al.*, 1995).

The genetic diversity of mushrooms has been worked out using molecular markers especially random amplified polymorphic DNA (RAPD) (Staniaszek *et al.*, 2002; Stajic *et al.*, 2005; Ravash *et al.,* 2009). Khan *et al.*, (2011) reported the use of RAPD

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markers to determine the genetic diversity among *Pleurotus* species of mushroom. Seven different species were collected. Five species, naming *Pleurotus platypus* (P-6), *Pleurotus flabelatus* (P-7), *Pleurotus florida* (P-17), *Pleurotus ostreatus* (P-19) and *Pleurotus sajor-caju* (P-56) were from Canada and two *Pleurotus warm-stram* (P-9) and *Pleurotus eryngii* (P-16) from Phillipines. Out of 14 random primers used by Khan *et al*., (2011), the maximum polymorphism was observed by primers OPL3 (72.70 %) and OPL11 (70%). Two species P-56 and P-17 were observed to be most similar having value 86% and constituting a cluster 'A'.

Du *et al*., (2011) did a research on the genetic diversity of wild *Auricularia polytricha*. Ten Sequence related amplified polymorphic (SRAP) primer combinations chosen for analysis produced a total of 426 and 91 SRAP loci in the wild and cultivated strains of *A. polytricha*, of which 425 and 37 loci were polymorphic, an average of 43 and 3.7 loci were amplified per primer pairs. The average percentage polymorphism in wild strains was 99.8%, which was over two times higher than that in the cultivated strains (40.7%). Hence, the genetic diversity of wild *A. polytricha* was higher than that in the cultivated strains. The size of DNA fragments ranged from 100 to 2000 bp, and few bands were more than 2000 bp and lower than 100 the number of loci amplified by different SRAP primer pairs varied from 38-46 in wild strains and 5-13 in cultivated ones.).

Phenotypic traits (physiological characteristics and somatic incompatibility) and genotypic traits (Target Region Amplification Polymorphism TRAP) were used to study the diversity of 32 main cultivars of *Auricularia auricula-judae* in China by Li *et al*., (2011). Twenty-seven important and stable physiological indexes were evaluated; Somatic Incompatibility Test (SIT) reaction was described from three aspects: type, pigment, and intensity; 16 pairs of TRAP primer combinations produced 535 unambiguous and reproducible DNA fragments, of these 524 (97.9%) were polymorphic. Dendrograms were constructed by Unweighted Pair-group Method with Arithmetic Averages (UPGMA) method, and the principal coordinate analysis (PCO) of the three methods (physiological characteristics, SIT intensity and TRAP) exhibited similar clustered patterns, revealing that all the tested strains could be divided into six distinct groups, each of which was correlated with different geographical regions.

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Most strains originated from the same area with a narrow genetic basis and could possibly be domesticated from the local wild-type strains, some strains were suspected to be synonymous. Molecular characterization studies on *A. polytricha* with *Pleurotus. platypus, P. florida* and *P. eous,* was carried out using ITS primers by Veeralakshmi *et al.*, (2014). The polymerase chain reaction primers, ITS-1 and ITS-4 were used to amplify the ITS of ribosomal DNA, which encompassed both ITS-1 and ITS-4 regions and the results indicated that all mushrooms exhibited similarity in ITS lengths among the mushrooms. It was difficult to isolate DNA from *Auricularia* strains because their mycelia contained high amounts of polysaccharides. Also, he compared several methods and modified the CTAB method slightly to obtain good quality DNA from liquid cultures of mycelia. According to Veeralakshmi *et al.* (2014), molecular characterization of *A. polytricha* was studied using ITS primers by comparing with P. platypus, *P. florida, and P. eous*. The polymerase chain reaction primers, ITS-1 and ITS-4 were used to amplify the Inter-Transcribed Sequence (ITS) of ribosomal DNA, which encompasses both ITS- 1 and ITS-4 regions. The results of the study indicated that all mushrooms exhibited similarity in ITS lengths. On gelelectrophoresis, the amplified region of *A. polytricha* showed fragment of 600-700 bp. The amplified product was eluted and sequenced

Sequence Related Amplified Polymorphism (SRAP) is a novel molecular marker, being firstly introduced by Li *et al*. (2003), it had been applied extensively in genetic linkage map construction, germ plasm identification, gene tagging and mapping, genetic diversity analysis, and comparative genetics of different fungal species and other fields (Li *et al.,* 2003). The method has been used for the identification of cultivars of several medicinal and edible fungi (Yu *et al*., 2008), but only few reports estimated the genetic diversity of wild strains (Chen *et al*., 2009).

Further, the forest habitats are rapidly being destroyed alongside the germplasm of this fungus to create land for settlement and agriculture (Onyango *et al*., 2010). Therefore, there is need to develop cultivation methods that will encourage propagation and conservation of this resource protecting it from extinction. The most crucial factor in domesticating wild mushrooms is development of appropriate protocols for spawn production (Zervakis *et al*., 2001, Oei, 2005) reported that understanding the nutritional and physiological preferences of mushroom mycelia is essential to its domestication. Grain spawns derived from sorghum and millet has

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successfully been used in the mushroom industry as the ‘seed’ for bulk inoculation of substrates (Royse *et al*., 1997, Oei, 2005).

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CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of samples

Three species of Auricularia (*A. polythrica, A. auricula* and *A.*sp) were collected from various locations across Oyo, Osun, Ondo, Ogun, Ekiti and Lagos states in Southwestern Nigeria. A total of fifty-four fresh mushroom samples were collected between September 2011 and July 2012 from forests, farms, botanical gardens and wood markets in some major towns.

The samples were randomly collected based on availability in Osun (11) Oyo (10) Ondo (9) Ekiti (8), Ogun (8) and Lagos states (8) from the 6 States. The *Auricularia* samples were identified at the department of Botany based on the morphological characters (colour, shape, texture and fruit body) as presented on Table 3.1

Fully matured mushroom samples were transported to the laboratory in slants of Potato dextrose agar to preserve freshness and their locations were noted. Three dimensional characteristics (color, shape and texture) were observed.

This study was conducted at the University of Ibadan, Oyo state. Ibadan is located in the Southwestern Nigeria approximately between Latitude N 7o 261 Longitude E 3o 531 and an Altitude of 190m. The city ranges in elevation from 150m in the valley area to 275m above sea level. Ibadan has a tropical wet and dry climate with mean monthly temperatures fluctuating between 23° C to 30° C and humidity is usually from 55% to 75%.

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Table 3.1: Areas of sample collection in Southwestern Nigeria

S/N	Sample Code	Local Government	Town	State
1	OG1	Abeokuta North	Abeokuta	Ogun State
2	OG2	Ewekoro	Itori	Ogun State
3	OG3	Ifo	Ifo	Ogun State
4	OG4	Ijebu Ode	Ijebu Ode	Ogun State
5	OG5	Ikenne	Ikenne	Ogun State
6	OG6	Shagamu	Shagamu	Ogun State
7	OG7	Odeda	Odeda	Ogun State
8	OG8	Odogbolu	Odogbolu	Ogun State
9	LA1	Agege	Ikeja	Lagos State
10	LA2	Ojo	Ojo	Lagos State
11	LA3	Apapa	Ikeja	Lagos State
12	LA4	Badagry	Badagry	Lagos State
13	LA5	Epe	Epe	Lagos State
14	LA6	Shomolu	Shomolu	Lagos State
15	LA7	Ikorodu	Ikorodu	Lagos State
16	LA8	Mushin	Ikeja	Lagos State
17	OY1	Akinyele	Moniya	Oyo State
18	OY2	Egbeda	Egbeda	Oyo State
19	OY3	Ido	Ido	Oyo State
20	OY4	Iseyin	Iseyin	Oyo State
21	OY5	Ogbomosho North	Ogbomosho	Oyo State
22	OY6	Oluyole	Idi Ayunre	Oyo State
23	OY7	Oyo	Oyo	Oyo State
24	OY8	Olorunsogo	Igbeti	Oyo State
25	EK1	Ado Ekiti	Ado Ekiti	Ekiti State
26	EK2	Ilejemeje	Iye	Ekiti State
27	EK3	Ikole	Ikole	Ekiti State
28	EK4	Oye	Oye	Ekiti State
29	EK5	Irepodun	Igede	Ekiti State
30	EK6	Ikere	Ikere	Ekiti State
31	EK7	Ijero	Ijero Ekiti	Ekiti State
32	EK8	Emure	Emure Ekiti	Ekiti State

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Table 3.1: Continued

S/N	Sample Code	Local Government	Town	State
33	OD1	Idanre	Idanre	Ondo State
34	OD2	Ilaje	Igbokoda	Ondo State
35	OD3	Ile Oluji	Ile Oluji	Ondo State
36	OD4	Odigbo	Ore	Ondo State
37	OD5	Okitipupa	Okitipupa	Ondo State
38	OD6	Ose	Ifon	Ondo State
39	OD7	Owo	Owo	Ondo State
40	OD8	Ifedore	Igbara-Oke	Ondo State
41	OS1	Bolunduro	Ota Aiyebaju	Osun State
42	OS2	Ejibo	Ejigbo	Osun State
43	OS3	Ifedayo	Oke-Ila Orangun	Osun State
44	OS4	Ifelodun	Ikirun	Osun State
45	OS5	Ila	Ila Orangun	Osun State
46	OS6	Irepodun	Ilobu	Osun State
47	OS7	Iwo	Iwo	Osun State
48	OS8	Obokun	Ibokun	Osun State
49	OS9	Irewole	Ikire	Osun state
50	OS10	Oriade	Ilesha	Osun state
51	OS11	Oriade	Ipetu Ijesha	Osun state
52	OY9	Akinyele	Ojo	Oyo state
53	OY10	Ibadan North	Bodija	Oyo state
54	OD9	Akure South	Akure	Ondo State

KEY: EK1-EK8 = Ekiti State, OD1 –OD9 = Ondo state, OS1-OS11= Osun state, LA1-LA8 = Lagos State, OY1-OY10 = Oyo state, OG1-OG8= Ogun state

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3.2 Spawn And Sample Preparation

The preparation of spawn and substrates were carried out at the Mushroom growing Unit, of the National Institute of Horticulture (NIHORT) Ibadan and at the Department of Botany, University of Ibadan.

The molecular study was carried out at the Bioscience laboratory of the International Institute of Tropical Agriculture Ibadan (IITA).

The substrates for the cultivation were:

1. Saw dust (*Mansonia altissima*) from Bodija Market Ibadan.
2. Cotton waste (*Gossypium* spp) from Bodija Market Ibadan.
3. Rice straw (*Oryza* spp). From WARDA-IITA Ibadan.
4. Wood of *Mangifera indica* from Botanical garden, University of Ibadan
5. Wood of *Cedrela odorata* from Botanical garden, University of Ibadan
6. Wood of *Gliricidia sepium* from Botany department, University of Ibadan

Figure 3.1 shows the locations of *Auricularia spp.* collected in Southwestern Nigeria. The areas of sample collection in Southwestern Nigeria are presented in Table 3.1. Plate 3.1 shows the collection of *Auricularia* spp growing on wood in Ibadan location. Plates 3.2- 3.4 shows woods of *Gliricidia sepium, Cedrela odorata and Mangifera indica,* respectively.

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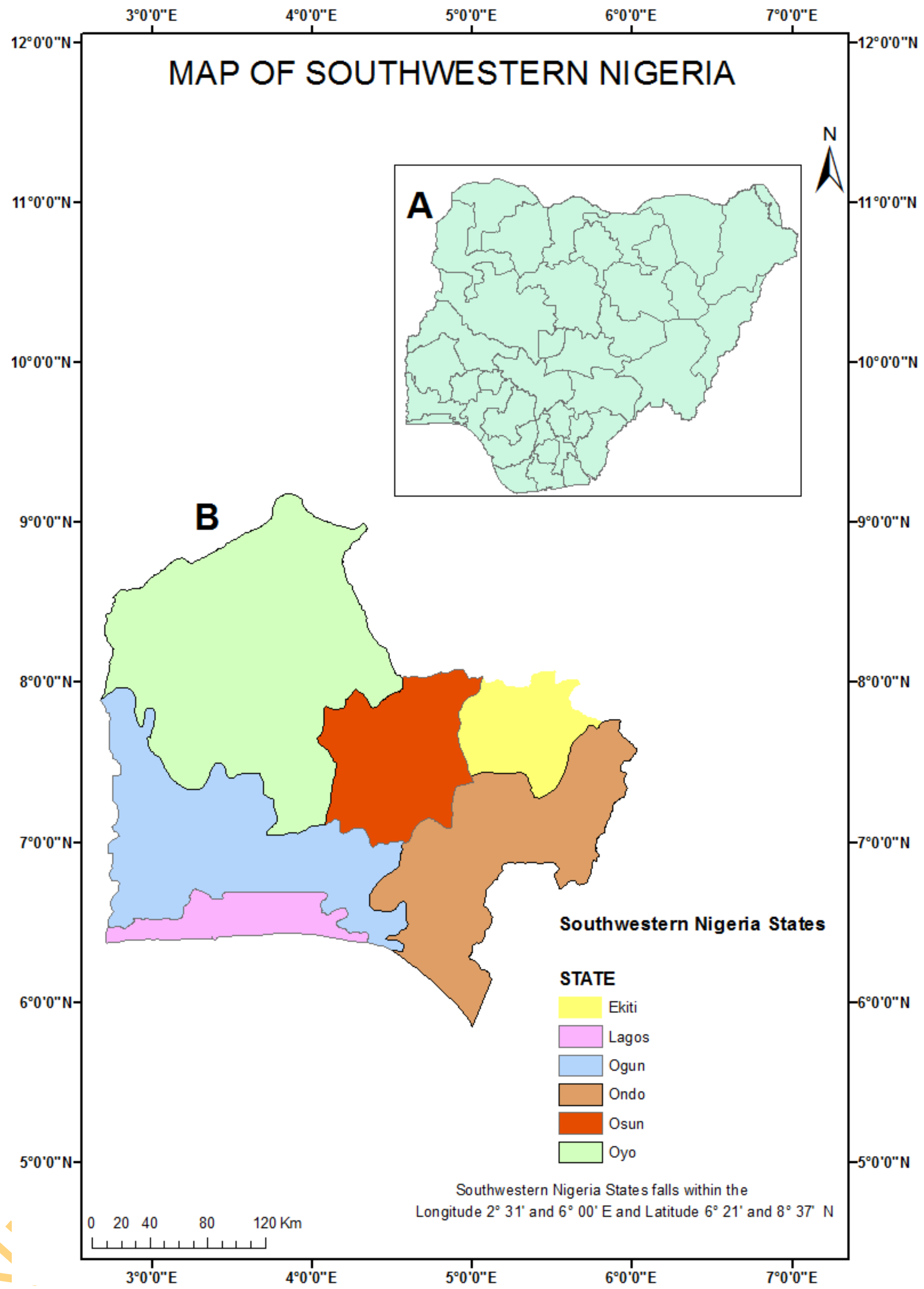

Fig. 3.1: Locations of *Auricularia* spp. found in Southwestern Nigeria

Plate 3.1 Photograph of *Auricularia* spp growing on log at Botany Department of University of Ibadan 5 x 4

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Plate 3.2 Photograph showing woods of *Gliricidia sepium* for the cultivation of *Auricularia* species. 12 x 10

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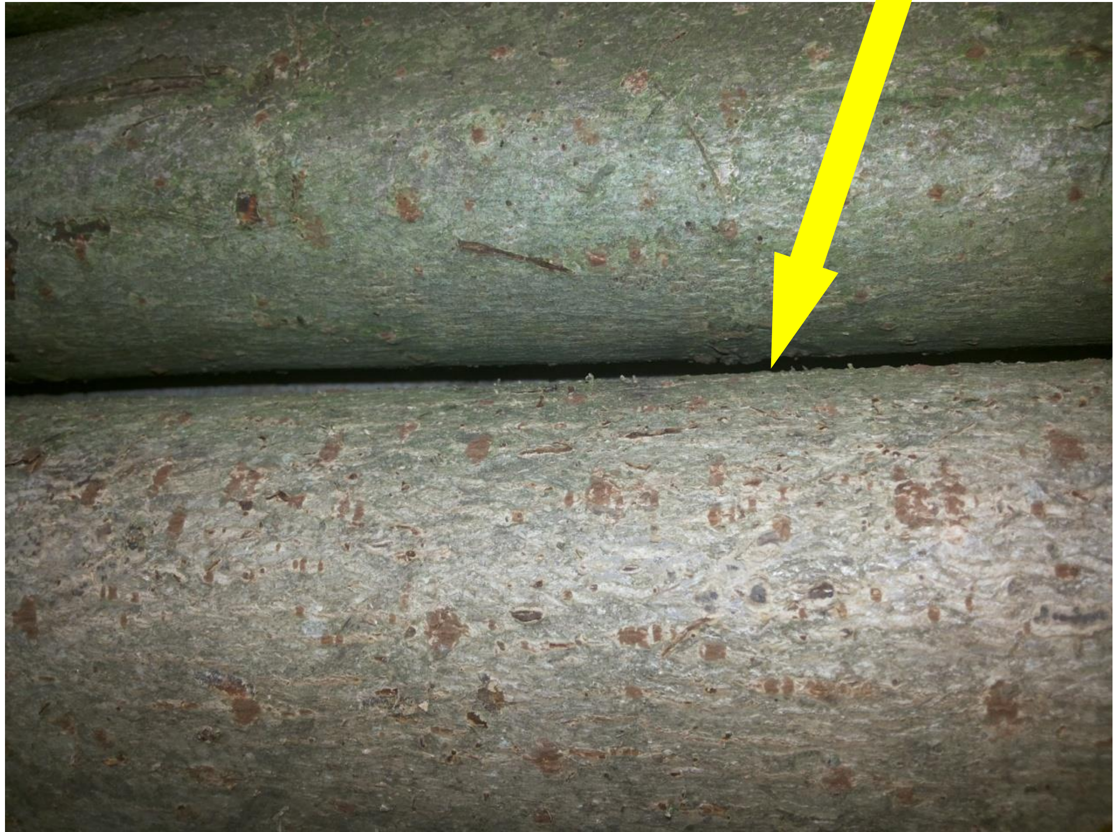

Plate 3.3 Photograph of woods of *Cedrela odorata* for the cultivation of *Auricularia* species 13 x 10

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Plate 3.4 Photograph of the woods of *Mangifera indica* for the cultivation of *Auricularia* species.13 x 10

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3.3 Morphological characterisation procedure for Mushroom.

The basidiocarps were rehydrated by soaking in water for ten minutes before analyzing their morphology. Qualitative characters such as color, shape, and presence of hymenia was evaluated by physical observation while texture was determined by touching the back and top surfaces using fingers (Onyango *et al*., 2011). For microscopic characters, free hand transverse sections of approximately 0.1 mm thick were made from rehydrated basidiocarps with the aid of a sharp surgical blade. The sections were immersed in a diluted solution of methyl blue stain and left for 10 minutes. The thinnest sections were selected and placed on glass slides and covered with cover slips. Low power (×40) objectives of a standard light microscope was used to observe the sections. Internal basidiocarp zones of the mushroom was obtained by mounted photography.

3.4 Tissue culture of basidiocarps

Fruit body of the mushroom was collected. The method used for tissue culture was derived from Weber and Webster, (2006). A laminar flow hood equipment was used to perform this procedure in the laboratory. The lamina flow hood was thoroughly cleansed using cotton swabs soaked in 80% ethanol after which the fan and UV light were set for 30 minutes to sterilize the working chamber. Petri plates were washed thoroughly with ordinary detergents and then autoclaved at 121 °C for 15 minutes. Sterilized plates were transferred to an oven for drying at 140 °C for 30 minutes. Preparation of culture media was done by weighing 25 grams of 2% malt extract agar which was dissolved in 500 milliliters of distilled water and then sterilized by autoclaving at 121 °C for 15 minutes. The media was poured in sterile Petri dishes and quickly covered using Petri dish lids and allowed to solidify. Re-hydrated basidiocarps were washed thoroughly in sterile water and 5% sodium hypochlorite. A sharp surgical blade was dipped in 80% ethanol and flamed until it was red-hot then allowed to cool for 10 seconds. Cleaned mushroom sections were broken lengthwise and sterilized surgical blades used to remove fragments (about 2×2 mm^2) from inner surfaces of the basidiocarps. Cut fragments were placed in the middle surface of the media, covered with a Petri - dish lid and tightly sealed with a parafilm.

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3.5 Mycelia Growth in Plates

Ten grams of saw dust-supplement mixture (for each supplement, 5, 10, and 25%) was weighed and filled into petri dishes, soaked in water and sterilized in an autoclave at 121° C for 15 minutes. Each plate was inoculated with a 5-mm mycelium disc from a vigorously growing culture of *Auricularia* and incubated at room temperature (28 ±2° C). Measurement of mycelia growth and density was taken at 5, 7 and 9 days when the plates were fully covered (Jonathan *et al* 2012).

3.6 Spawn Preparation

The spawn was prepared using the method described by Jonathan and Fasidi (2001). The rice straw was soaked in water for an hour to leach out herbicides. Thereafter, water was squeezed out using a muslin cloth, the moist rice straw was then placed on a slab that has been previously disinfected using cotton wool soaked with ethanol. Wheat bran was added to the rice straw and thoroughly mixed. The mixture was put into 350cm^3 (13 x 8 x 8) bottles, covered with aluminium foil and autoclaved at 15ibs pressure, 121° C for 20 minutes. After 20 minutes, the bottles were brought out and allowed to cool, then the bottles were inoculated in an aseptic condition with the mycelia of *Auricularia spp.* All the bottles were then incubated at a temperature of 28 ± 2ºC for 3 weeks, until the rice straw was completely ramified with the mycelia.

Sorghum grains were also used to prepare the mother spawn. The grains were soaked in water overnight and then drained of excess water. Aliquots (500g) of the soaked grains were weighed, mixed with 1% of calcium carbonate and 10% rice bran and filled into polypropylene bags and sterilized at 121ºC for 15 minutes. After cooling, the bags were inoculated with the mycelia of the pure cultures of *Auricularia auricula* in a laminar flowhood (Fekadu, 2014).

3.7 Substrate Collection and preparation

Wood ear mushroom cultivation was done at NIHORT Ibadan. The cultivation procedure was conducted according to the methods of Oei (2005). Fresh substrates of Sawdust (*Massona altissima*), Rice husk ((*Oryza spp*) and Cotton waste (*Gossypium spp*) were watered slightly and divided into lots of 400g each and packed into heat resistant polypropylene bags (commonly called Santana) with a diameter of 12 cm and a length of 20 cm.

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The open ends of the substrate bags (Santana nylon) which were purchased from Bodija market were tied using sterile cotton strings and the bags and steamed in a drum for 4 hours. The substrate bags were cooled to room temperature for 30 min and inoculated using grain spawns obtained from mycelia cultured from a single strain of wood ear mushrooms. Grain spawn was prepared using the standard methods (Oei, 2005). The inoculated substrates were labeled and kept in the incubation room to allow complete colonization of the substrates. Upon completion of spawn run, five holes 10 mm diameter were made on each bag. The inoculated substrate bags of *Auricularia* spp in 2014 is presented in Plate 3.10.

3.8 Log technology method for the cultivation of *Auricularia* species

The wood of *Mangifera indica, Cedrela odorata and Gliricidium sepium* used for the cultivation were identified at the herbarium of the Botany Department, University of Ibadan.

Inoculation of wood of *Cedrela odorata, Mangifera indica* and *Gliricidia sepium,* were cut to size of 40cm with the aid of chisel or an electric drill. Holes of about 4-6cm apart were drilled on the wood, spawn of *Auricularia* was inoculated into each hole, then covered with a nylon sheet after inoculation with spawn. The woods were incubated in conditions that were suitable for the mycelium and they were watered regularly. After about three months the mushrooms were ready for cropping. (Cheng and Tu, 1978 : Irawati *et al* 2012).

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Plate 3.5 a) Spawn samples of *Auricularia* species prepared from sorghum grains. on test tubes 22 x 24

b) Spawn samples bottle of *Auricularia* species prepared from sorghum grains.18 x 20

Plate 3.6 Photogragh of Spawn of *Auricularia* species in bottles of Sorghum grains 14 x 11

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Plate 3.7 Photogragh of substrate bags of *Auricularia* species during incubation.11 x 8

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Plate 3.8 Photograph of drilled holes of *Mangifera indica* ready for inoculation.11 x 9

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3.9 Data collection

The yield of *Auricularia* on the different substrates was determined by recording the number and size of the fruit bodies after sprouting. The measurements from the various substrates and their mean values were calculated.

The following parameters of growth/yield were measured..Number of fruit bodies: This was done by directly counting the number of fruit bodies on each substrate. Height of fruit bodies: The height was measured in centimeters using meter rule from the base of the stripe to the pileus.Diameter of the pileus: This was measured in centimeter with ruler from one edge of the pileus across the stipe to the other edge.Fresh weight of fruit bodies**:** This was done using an electrical weighing balance.The yield of *Auricularia* on the different substrates was determined by recording: Spawn run (days), Pin head formation (days) Fruit body formation (days) Yield (g) Width of the Pileus (cm)

3. 10 Proximate Analysis/composition

Proximate composition was determined immediately after drying of the harvested *Auricularia*. Moisture content, total ash, crude fibre, and crude fat of the *Auricularia* samples were determined according to Association of Analytical Chemists, AOAC (2012).

3.10.1 Determination of Nitrogen and crude protein content

The crude protein was determined using micro Kjedahl method as described by AOAC, (2012). Approximately 1g of each sample was weighed into the digestion tube of Kjeltec 2200 Foss Tector Digestion unit (Foss Tecator Analytical AB Hoganans, Sweden). Two tablets of a catalyst mixture containing 5g of K_2SO_4 and 5mg of Selanium were added as well as 6ml of concentrated H_2S0_4 and conc. orthophosphoric acid. Digestion was done for an hour at 420ºC. The distillation was done using 2200 FOSS distillation unit with 25ml of 40% NaOH. The distillate was collected using 25ml of 4% boric acid prepared with bromocresol green and methyl red indicators. Finally, the distillate was titrated with standardized 0.1N sulphuric acid to a reddish color. The crude protein content was estimated using the formula:

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Total Nitrogen, percent by weight (N) = $\frac{(V2-V1) \times N \times 14.007 \times 100}{W}$

Where V_2 = Volume in ml of standard sulphuric acid solution used in the titration for the sample

V_1 =Volume in ml of the standard acid solution used in the titration for the blank determination

N= Normality of standard sulphuric acid (0.01)

W= Weight in grams of the test material

% Crude protein = N X 6.25 (correction factor)

3.10.2 Determination of Crude fat content

The fat content was determined using the method of AOAC (2012). A clean and dried extraction thimble containing 5g of the dried sample plugged with grease-free cotton wool was placed in the extraction chamber (Foss Soxtec extraction method) set at a temperature of 135°C. Then extraction was carried out for at least 4hrs according to AOAC (2012). The cups were cooled in a desiccator and weighed. The crude fat was determined by the formula

Weight of fat (W_f) = W_a-W_b

Where, W_a = weight of extraction cup after extraction

W_b = weight of extraction cup before extraction

W_D = weight of dried sample

% Crude Fat content = $\frac{Wfx\ (100-moisture\%)}{WD}$

3.10.3 Determination of crude fiber content

Crude fiber analysis was determined using the method of AOAC (2012). Two grams of defatted sample was weighed into 500ml conical flask and 200ml of 1.25% (0.255M) H_2SO_4 was added and boiled for 30mins, maintaining a constant volume (using reflux condenser) rotating the flask every few mins to mix content and remove particles from the sides. Recording was done by placing a watch glass over the mouth of the beaker. After 30min heating by gently keeping the level constant with distilled water, 20ml 28% KOH was added and again boiled for further 30 min. Subsequently, washing was carried out with a mixture of 1% H_2SO_4 and 1.25% (0.313M) NaOH solution then filtered and dried in an electric oven at 130° C to a constant weight for 2

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hrs. Furthermore, it was cooled at room temperature for 30 min in a dessicator and weighed before been transferred into a crucible and placed in a murffle furnace (Surgifriend Medicals England, SM9080) for 30 min at 550ºC until completely ashed. It was cooled again in a dessicator and re-weighed. The crude fiber content was determined by using the formula.

$$\text{Crude fiber content} = \frac{(W1-W2)(100-M)}{W3}$$

Where,

W_1 = crucible weight after drying

W_2 = crucible weight after ashing

W_3 = dry weight

M= % moisture of sample

3.10.4 Determination of total ash content

The ash content was determined by the method of AOAC (2012). Crucibles were washed and dried in the laboratory hot air oven (Surgifriend Medicals England, SM9053) maintained at 105ºC. It was allowed to cool and weighed. Five grammes portion of the samples were then weighed into the dried crucibles. The samples were placed in the Murffle furnace (Surgifriend Medicals England, SM9080) maintained at 550ºC for 6hrs and the crucibles were transferred directly to a desiccator, cooled and weighed immediately. The ash content was calculated as follows:

$$\% \text{ Total ash} = \left\{ \frac{(\text{weight of crucible+ ash}) - (\text{weight of empty crucible})}{(\text{Weight of crucible + dried Sample}) - (\text{weight of empty crucible})} \times 100 \right\}$$

3.10.5 Determination of moisture content

The moisture content was determined by the method described by AOAC (2012) using the official method 925.09. Five grammes portion of each sample was weighed into a pre-weighed clean dried dish. The dish was placed in a well ventilated laboratory hot air oven (Surgifriend Medicals England, SM9053) maintained at 105ºC. The weight of the sample plus the drying dish was checked at hourly intervals after the first 2hrs until the decrease in mass between successive weighing did not exceed 0.05mg per g of sample. The loss in weight was reported as the moisture content and calculated as follows:

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% Moisture content = $\frac{\text{(Weight of fresh sample-Weight of dry sample) x 100}}{\text{Weight of fresh sample}}$

3.10.6 Determination of total carbohydrate content

Carbohydrate % = [100 – (moisture + protein + fat +fiber + ash)]

3.10.7. Determination of the mineral composition of *Auricularia* species

The mineral (potassium, calcium and magnesium, sodium) contents of *Auricularia* spp. were determined using atomic absorption spectrophotometer (Alpha 4-Chem. Tech analytical). The ash obtained from the muffle was dissolved in 10ml of 3N HCl. The mixture was heated on a steam bath to effect complete dissolution and the dissolved ash was filtered into a 100ml volumetric flask and made up to volume with distilled water. The phosphorus (P) in the sample filterate was determined by using Vanadomolybdate reagent at 470nm using colorimetric method (Kilgour, 1987) (Colorimeter SP20, Baush and Lam).

% P = $\frac{\text{ppm P x 50 x 0.0001}}{\text{Weight}}$

Potassium was determined using Jenway Digital Flame photometer (PFP7 model) according to the method of Novozamsky *et al.* (1983). About 0.1g of *Auricularia* samples were weighed and placed into 50ml digestion tubes (6 digestion tubes without plant samples were used for the preparation of the standards). About 2.5ml of sulphuric acid/salicylic acid-selenium mixture was placed into each tube. Sulphuric acid/salicylic acid-selenium mixture was prepared by dissolving 3.50g of selenium only in 1 liter concentrated (98%) sulphuric acid and 72g Salicylic acid mixture. The sample was mixed with the acid on a vortex mixer. The tubes were placed on a preheated digester at 100ºC for one hour. It was removed from the digester and 1ml of 30% hydrogen peroxide (H_2O_2) added to each tube. After the reaction has subsided an additional 2ml of 30% hydrogen peroxide (H_2O_2) was again added into each tube. The tubes were returned to the digester and the temperature increased to 300ºC. After the water has boiled off, the condensing bottles were placed over each digest tube. The temperature was increased to 320ºC and digestion continued for 45mins after the digest was clear. The samples were removed from the digester and allowed to cool.

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The samples were diluted to 50ml with distilled water and sediments allowed to settle overnight before analysis. The standard and samples were then run on autoanalyzer.

Calcium and Magnesium

Minerals content (Calcium, Magnesium,) of the mushroom were determined by employing the AOAC (2012) methodology by digestion of the sample with a mixture of concentrated nitric acid, sulphuric acid and perchloric acid (10:0:5:2, v/v) using an atomic absorption spectrophotometer (GBC 904AA; Germany).

Sodium

One gram of dry powdered sample was placed in a porcelain crucible and ashed at 450°C for 5-6 h; then the ash was dissolved in 2 mL concentrated HNO3 (Merck), and heated on a low heat for 1 min. Then, it was cooled and filtered through Whatman No. 42 filter paper to a 50 mL volumetric flask and was made to volume with triple distilled water. A blank was also prepared using similar experimental procedure (AOAC, 2012). Three such replicates were maintained for the mushroom species studied.

Aliquot of the ash solution was aspirated to the instrument (AAS/ICP-AES) for the determination of metals/minerals. Each value is the mean of three replicate determination ± standard deviation.

3.10.9.1 Data analysis

The data obtained was analyzed using one-way analysis of variance (ANOVA).Tests of significance was carried out using Tukey method at $P \leq 0.05$. Data were analysed using descriptive statistics, clustering and Pricipal Component Analyses (PCA) for the cultivation characteritics of all the specimens collected from the six states. Statistical package of Social Sciences (SPSS) software was also used for statistical analysis.

3.10.9.2 DNA extraction

A modified CTAB (Cetyltrimethylammonium Bromide) method by Abashi *et al.* (2010) was used for the DNA isolation. Pileus tissue of 4 days old *Auricularia* was collected and 200mg weighed prior to DNA extraction. The sample was thoroughly ground with 800ml of CTAB buffer (20 mM EDTA, 1.4 mM NaCl, 100 mM Tris-HCl pH 8.0, SDS (1.25%, 2% CTAB and 0.2% β-mercaptoethanol (v/v)), incubated at

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65°C for 15 min using water bath with occasional mixing, allowed to cool for approximately 1 minutes before adding equal volume of phenol, chloroform and iso-amyl alcohol at the ratio of 25:24:1. It was vortexed and centrifuged at 12000 revolutions per minute (rpm) for 15 min, the supernatant was transferred to fresh sterile tubes without disturbing the pellets. About 400 µl of ice-cold isopropanol was added to the supernatant and mixed by inverting the tubes 2-5 times to precipitate the DNA and subsequently kept at -80°C for 1h. The DNA was pelleted down by centrifugation at 12000 rpm for 10 min and the dried DNA pellets obtained were re-suspended in 100 µl of Grand Island Biological Company (GIBCO) water (Invitrogen, Carlsbad, CA, USA) and 2 µl of 10 mg/ml RNase (Qiagen Valencia, CA, USA) was added to each of the samples and kept at 4°C for 30 minutes to get rid of RNA.

3.10.9.3 Quantification of the extracted DNA and preparation of working dilution.

The extracted DNA samples were quantified using a NanoDrop spectrophotometer (ND-1000). About 2µl of the extracted DNA sample was used to obtain 1.8-2.0 ratio at OD 260/280 absorbance level and concentration through which working dilutions were prepared for polymerase chain reaction (PCR). On 1.5% agarose gel for electrophoresis, and 2.5µl of the stock DNA samples were loaded and visualized under UV light (Model-2, Upland, CA, USA) to check the quality of the extracted DNA samples. Following the high level of concentration of the extracted DNA samples, dilution of each DNA sample was uniformly made to 100ng/uL DNA prior to setting up PCR. Table 3.2 shows the concentration of DNA Products extracted from *Auricularia spp.*

Nucleic acid concentration determines the sample purity using 260/280nm ratio of absorbance on a. spectrophotometer.

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Table 3.2: Concentration of DNA Products extracted from *Auricularia* spp.

S/N	Sample Code	Nucleic Acid Conc. (ng//ul	OD 260/280
1	OG1	190.6	2.03
2	OG2	79.7	1.78
3	OG3	250.0	1.87
4	OG4	118.0	1.95
5	OG5	107.9	1.87
6	OG6	189.0	1.76
7	OG7	92.1	2.02
8	OG8	1548.2	1.89
9	LA1	79.0	2.08
10	LA2	282.3	2.14
11	LA3	309.1	2.12
12	LA4	137.8	2.13
13	LA5	890.0	1.83
14	LA6	96.9	2.11
15	LA7	94.0	2.11
16	LA8	187.7	2.03
17	OY1	110.6	2.06
18	OY2	1507.5	1.92
19	OY3	96.7	2.07
20	OY4	1118.0	1.96
21	OY5	543.5	1.99
22	OY6	193.8	2.10
23	OY7	490.7	2.01
24	OY8	239.3	2.10
25	EK1	867.5	2.04
26	EK2	87.3	1.74
27	EK3	80.8	1.75
28	EK4	120.3	1.95
29	EK5	100.6	2.11
30	EK6	450.2	1.99
31	EK7	125.0	2.09
32	EK8	138.0	2.39
33	OD1	190.6	2.03
34	OD2	107.9	1.67
35	OD3	92.1	2.02
36	OD4	105.0	2.08
37	OD5	309.1	2.12
38	OD6	56.5	1.83
39	OD7	94.0	2.11
40	OD8	1507.5	1.62
41	OS1	543.5	1.99

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Table 3.2: Continued

42	OS2	490.7	2.01
43	OS3	867.5	2.04
44	OS4	87.3	1.75
45	OS5	80.8	1.85
46	OS6	120.3	1.95
47	OS7	100.6	2.11
48	OS8	193.8	2.10

KEY: OS1-OS8= OSUN STATE, EK1-EK8= EKITI STATE, LA1-LA8= LAGOS STATE, OG1-OG8= OGUN STATE, OYO-OY8= OYO STATE, OD1-OD8= ONDO STATE.

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3.10.9.4 Random Amplified Polymorphic DNA (RAPD) PCR amplification of Mushroom.

A total of twenty five primers were subjected to screening for polymorphism with the *Auricularia* species out of which fifteen were polymorphic**.** The fifteen arbitrary RAPD decamer primers obtained from Operon Technology (Almeda, CA, USA) were used for PCR amplification (Table 3.3). PCR amplification was performed in 25µl which consisted of 2.0µl of 100ng DNA, 2.5µl of 10 x Buffer (Bioline), 1.25µl of 50mM $MgCl_2$ (Bioline), 2.0µl of 2.5mM dNTPs (Bioline), and 0.2µl 500U *Taq* DNA polymerase (Bioline), 1.0µl DMSO (dimethyl sulfoxide), 1.0µl of 10uM each primer and 16.05µl of 500ml DEPC-treated water (Invitrogen Corporation). PCR amplifications were performed using Applied Biosystems thermocycler with a cycling profile of an initial step of 94°C for 2 min., 40 cycles of 94°C for 20 s, 72°C for 1min, and 54°C for 2 min., and a 5-min final extension at 72°C. Amplified fragments were separated electrophoretically on 1.5% (w/v) agarose (Sigma Aldrich, USA) gels with 1X TBE (Tris-Boric acid-EDTA) buffer and stained with ethidium bromide (0.5mg/ml). The molecular fragments were estimated using 100-bp step DNA marker (Biolabs, New England). (Elder and Southern 1987).The RAPD primers used for the amplification of DNA samples of *Auricularia* was presented in Table 3.3.

3.10.9.5 Analysis of RAPD profiles

Data matrix generated from the RAPD profiles for fragments of similar molecular weight from each individual were scored as present (1) or absent (0). The data obtained from scoring the RAPD bands were used for genetic dissimilarity matrix

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using Jaccard's similarity coefficient (Jaccard 1908). Phylogenetic relations were determined by cluster analysis using UGPMA (unweighted pair-group method with arithmetic averages) with the NTSYS-pc software version 2.02 (Rohlf 1998) using Bootstrap analysis of 1000 for accurate result generations (randomly multiplying or repeating samples to get a 95% accurate value). Multivariate grouping was done using principal coordinate analysis (PCA) with Darwin software version 5.0.0.157 while polymorphic information content (PIC) was calculated using the method of Botstein *et al*. (1980).

Primers were retrieved by downloading sequences from the Nucleotide database of the National Center for Biotechnology Information.(NCBI;//www.ncbi.nlm.nih .gov).Twenty five primers were initially assayed or screened for their ability to detect more than one allele (polymorphic) loci among the set of the cultivated forty eight *Auricularia* samples (see appendix). The amplified fragments were separated on agarose gel and visualized with ethidium bromide staining.Only fifteen primers were recorded to have polymorphic effects on the cultivated samples.

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Table 3.3 RAPD primers used for the amplification of DNA samples from *Auricularia* species

S/No	RAPD primer	Primer sequence (5'-3')	Melting temperature (Tm°C)
1	OPB-11	GTAGACCCGT	34
2	OPB-12	CGTTGACGCA	34
3	OPB-15	GGAGGGTGTT	32
4	OPB-20	GGACCCTTAC	34
5	OPB-21	CGACCCTTAC	34
6	OPH-3	AGACGTCCAC	34
7	OPH-5	AGTCGTCCCC	32
8	OPH-10	CCTACGTCAG	32
9	OPH-15	GCTTCGTCAG	34
10	OPT-1	GGGCCACTCA	34
11	OPT-5	GGGTTTGGCA	32
12	OPT-7	GGCAGGCTGT	34
13	OPT-10	CCTTCGGAAG	32
14	OPT-19	GATGCCAGAC	32
15	OPD-18	GAGAGCCAAC	32

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CHAPTER FOUR

RESULTS

4.1 Morphological Characteristics of *Auricularia* spp

A total of fifty four (54) *Auricularia* species were randomly collected from Osun (11), Oyo (10), Ondo (9), Ekiti (8), Ogun (8) and Lagos (8). Mycelial tissues cultured from the samples were used to prepare Spawns in an aseptic condition.

Morphologically, 31 samples of *A. auricula*, (yellow brown, auriform, leathery texture) and 12 samples of *A. polytricha* (dark brown, discoid, gelatinous) were identified, while 5 samples were unidentified and 6 samples did not grow. *Auricularia* species samples cultivated on sawdust, cotton waste, rice straw in bags produced mycelial growth, but did not fructify while samples on drilled logs had mycelial growth and fructified.

Morphological characteristics of *Auricularia* spp cultivated on log wood from 48 local government areas in the southwestern part of Nigeria are presented in Table 4. Three *Auricularia* species were identified morphologically in this study, namely *Auricularia polythrica*, *A. auricula* and *A.* sp which a mixed characters.The identity of the species were based on the color, texture and shape of the mushroom while the mycelia color and nature of the tissues were also considered.

Three external shapes observed were discoid, flattened and Auriform as presented in Table 4. In terms of their texture they were the gelatinous, rubbery and leathery and mycelia colour was mostly white and off white.

Cotton waste, sawdust and rice straw were initially employed for the cultivation using plastic bag method. The bags gave mycelial growth but did not fructify, hence the log method was adopted as presented in Plate 4.1.

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Table 4.0: Result of the Morphological characteristics of *Auricularia spp* surveyed from 48 local government areas in the South western Nigeria

KEY	EXTERNAL COLOUR	EXTERNAL SHAPE	EXTERNAL TEXTURE	MYCELIAL COLOUR	MYCELIAL TYPE	PROBABLE IDENTITY
OG1	Dark brown	Discoid	Gelatinous	White	Cottony	*Auricularia sp*
OG2	Dark brown	Discoid	Gelatinous	White	Cottony	*A. sp*
OG3	Yellowish brown	Auriform	Leathery	Off white	Cottony	*Auricularia. auricula*
OG4	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*
OG5	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*
OG6	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*
OG7	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*
OG8	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*
LA1	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*
LA2	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*
LA3	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*
LA4	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*
LA5	Brown	Flattened	Rubbery	Off white	Scanty	A.sp
LA6	Dark brown	Discoid	Gelatinous	White	Cottony	*A.polytricha*
LA7	Dark brown	Discoid	Gelatinous	White	Cottony	*A.polytricha*
LA8	Dark brown	Discoid	Gelatinous	White	Cottony	*A.polytricha*
OY1	Dark brown	Discoid	Gelatinous	White	Cottony	*A.polytricha*
OY2	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A.auricula*
OY3	Brown	Flattened	Rubbery	Off white	Scanty	*A.sp*
OY4	Brown	Flattened	Rubbery	Off white	Scanty	*A.sp*
OY5	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*
0Y6	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*
OY7	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A.Auricula*
OY8	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*

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Table 4: Continued.

KEY	EXTERNAL COLOUR	EXTERNAL SHAPE	EXTERNAL TEXTURE	MYCELIAL COLOUR	MYCELIAL TYPE	PROBABLE IDENTITY
EK1	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*
EK2	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*
EK3	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A.auricula*
EK4	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A.auricula*
EK5	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*
EK6	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*
EK7	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*
EK8	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A.auricula*
OD1	Dark brown	Discoid	Gelatinous	White	Cottony	*A.polytricha*
OD2	Dark brown	Discoid	Gelatinous	White	Cottony	*A. polytricha*
OD3	Dark brown	Discoid	Gelatinous	White	Cottony	*A.polytricha*
OD4	Dark brown	Discoid	Gelatinous	White	Cottony	*A. polytricha*
OD5	Dark brown	Discoid	Gelatinous	White	Cottony	*A.polytricha*
OD6	Dark brown	Discoid	Gelatinous	White	Cottony	*A. polytricha*
OD7	Dark brown	Discoid	Gelatinous	White	Cottony	*A.polytricha*
OD8	Dark brown	Discoid	Gelatinous	White	Cottony	*A. polytricha*
OS1	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*
OS2	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*
OS3	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*
OS4	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*
OS5	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A.auricula*
OS6	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*
OS7	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*
OS8	Yellowish brown	Auriform	Leathery	Off white	Cottony	*A. auricula*

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From the morphological features, there were thirty-one (31) samples of *Auricularia auricula* (external color, yellow brown,shape is auriform and the texture leathery) while the mycelia color and nature were off white and cottony respectively. The other samples were were twelve (12) *Auricularia polythrica*, dark brown,discoid in shape, while mycelia color and nature are white and cottony respectively. *Auricularia* sp that have mixed morphological character were five (5) samples and six (6) samples did not grow.

Plate 4.1a is a photograph of *Auricularia* species growing on inoculated log of *Mangifera indica*.

Plate 41b. is a photograph of *Cedrela odorata* logs which were used for the cultivation of *Auricularia* species but did not give as much growth as *M. indica*

Plate 4.1c present the picture of the logs of *Gliricidium sepium* that were used for the cultivation of *Auricularia* species .

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Plate: 4.1a. Photograph showing *Auricularia* species growing on inoculated wood of *Mangifera indica.* 13 x 10

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Plate 4.1b Photograph showing *Cedrela odorata* wood, used for the cultivation of *Auricularia* species. 32 x 24

Plate 4.1c Photograph showing *Gliricidia sepium* logs, used for the cultivation of *Auricularia* species. 33 x 24

TABLE 4.1: GROWTH CHARACTERISTICS OF *AURICULARIA* SPECIES ON WOOD IN SOUTHWEST NIGERIA

S/n	Key	Locations	Substrates	Spawn Run (days)	Pin head Formation (days)	Fruit Body formation (days)	Colour of Mushroom	Average yield (g)	Dry wt (g)	Width of Pileus(cm)	fruit body (D)
1	**OYI**	Akinyele	Wood of *M.indica*	25	33	34	Dark brown	11.28	**2.85**	2.2	5
			Wood of *G.sepium*	24	30	32	Dark brown	11.1	**2.88**	3.5	3
			Wood of *C.odorata*	22	28	30	Dark brown	12	**3.02**	3.4	4
2	**OY2**	Egbeda	Wood of *M.indica*	29	39	40	Dark brown	11.8	**3.01**	4.4	3
			Wood of *G.sepium*	22	34	36	Brown	10.48	**3.05**	5.6	4
			Wood of *C.odorata*	26	35	37	Brown	8.76	**3.12**	4.8	4
3	**OY3**	Ido	Wood of *M.indica*	28	34	37	Dark brown	9.14	**2.64**	5.3	6
			Wood of *G.sepium*	23	31	33	Brown	12.2	**3.04**	4.5	2
			Wood of *C.odorata*	26	30	32	Brown	8.71	**2.87**	3.8	2
4	**OY4**	Iseyin	Wood of *M.indica*	28	31	33	Dark brown	7.95	**2.96**	5.1	3
			Wood of *G.sepium*	23	30	32	Brown	8.9	**3.11**	5.4	3
			Wood of *C.odorata*	25	28	30	Brown	11.23	**3.07**	3.8	4
5	**OY5**	Ogbomosho North	Wood of *M.indica*	24	30	33	Brown	9.75	**2.64**	4.3	5
			Wood of *G.sepium*	22	28	30	Dark brown	8.23	**2.94**	2.7	4
			Wood of *C.odorata*	24	30	30	Brown	11.89	**3.14**	4	2
6	**OY6**	Oluyole	Wood of *M.indica*	29	32	34	Brown	8.7	**2.85**	5.8	3
			Wood of *G.sepium*	22	27	31	Brown	9.12	**3.16**	4.3	4
			Wood of *C.odorata*	26	29	30	Brown	8.8	**3.11**	2.6	4
7	**OY7**	Oyo	Wood of *M.indica*	28	32	33	Brown	10.05	**2.92**	3.8	5
			Wood of *G.sepium*	23	30	32	Brown	8.64	**2.69**	4.4	3
			Wood of *C.odorata*	34	40	41	Dark brown	7.99	**2.77**	3.4	2
8	**OY8**	Olorunsogo	Wood of *M.indica*	20	26	28	Brown	8.5	**2.88**	5.2	2
			Wood of *G.sepium*	18	23	26	Brown	9.04	**3.03**	3.4	3
			Wood of *C.odorata*	16	25	27	Brown	8.21	**3.05**	3	2

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TABLE 4.1: Continued

S/n	Key	Locations	Substrates	Spawn Run (days)	Pin head Formation (days)	Fruit Body formation (days)	Colour of Mushroom	Average yield (g)	Dry wt (g)	Width of Pileus(cm)	fruit body (D)
9	**LA1**	Agege	Wood of *M.indica*	12	21	23	Brown	8.9	**3.05**	3.3	2
			Woodof *G.sepium*	8	14	16	Brown	11.23	**2.55**	4.7	2
			Wood of *C.odorata*	10	17	19	Brown	9.75	**3.14**	5.1	3
10	**LA2**	Ojo	Wood of *M.indica*	28	31	33	Brown	8.23	**3.12**	3.2	3
			Wood of *G.sepium*	23	29	30	Brown	11.89	**3.1**	2.8	6
			Wood of *C.odorata*	26	30	32	Brown	8.7	**2.96**	3.7	4
11	**LA3**	Apapa	Wood of *M.indica*	28	32	34	Dark brown	8.76	**3.08**	4	3
			Wood of *G.sepium*	23	26	28	Brown	9.14	**2.64**	4.8	4
			Wood of *C.odorata*	25	28	30	Brown	12.2	**2.96**	2	3
12	**LA4**	Badagry	Wood of *M.indica*	24	29	31	Brown	8.71	**2.97**	3	3
			Woodof *G.sepium*	22	27	29	Dark brown	7.95	**2.85**	3	4
			Wood of *C.odorata*	24	26	28	Brown	9.04	**3.01**	5	2
13	**LA5**	Epe	Wood of *M.indica*	29	31	33	Brown	8.21	**2.88**	4	2
			Wood of *G.sepium*	22	25	27	Brown	8.9	**3.05**	3.7	2
			Wood of *C.odorata*	24	28	31	Dark brown	11.23	**3.12**	5.2	2
14	**LA6**	Shomolu	Wood of *M.indica*	29	33	35	Dark brown	9.75	**3.02**	4.3	6
			Wood of *G.sepium*	23	27	29	Dark brown	8.23	**2.97**	3	3
			Wood of *C.odorata*	26	29	31	Dark brown	9.01	**2.87**	5.2	3
15	**LA7**	Ikorodu	Wood of *M.indica*	28	31	32	Dark brown	10.05	**3.14**	3	3
			Wood of *G.sepium*	25	29	31	Dark brown	8.64	**2.94**	4	3
			Wood of *C.odorata*	24	28	31	Dark brown	7.99	**2.86**	4.5	4
16	**LA8**	Mushin	Wood of *M.indica*	22	24	26	Dark brown	8.5	**3.05**	2.1	4
			Wood of *G.sepium*	24	27	29	Dark brown	9.04	**2.64**	3.6	4
			Wood of *C.odorata*	29	31	33	Dark brown	8.21	**2.96**	4.4	4
17	**ODI**	Idanre	Wood of *M.indica*	23	27	29	Dark brown	8.7	**2.89**	5.8	3

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TABLE 4.1: Continued

S/n	Key	Locations	Substrates	Spawn Run (days)	Pin head Formation (days)	Fruit Body formation (days)	Colour of Mushroom	Average yield (g)	Dry wt (g)	Width of Pileus(cm)	Fruit body (D)
			Wood of *G.sepium*	25	27	29	Dark brown	8.76	**2.92**	4.3	3
			Wood of *C.odorata*	24	30	32	Dark brown	9.14	**2.88**	2.6	3
18	**OD2**	Ilaje	Wood of *M.indica*	22	26	28	Dark brown	12.2	**3.02**	3.8	3
			Woodof *G.sepium*	26	28	31	Dark brown	8.71	**3.11**	4.4	2
			Wood of *C.odorata*	28	29	31	Dark brown	7.95	**3.07**	3.4	2
19	**OD3**	Ile Oluji/Okeigbo	Wood of *M.indica*	23	26	29	Dark brown	9.14	**3.04**	5.2	2
			Wood of *G.sepium*	26	28	30	Dark brown	12.2	**3**	4.3	2
			Wood of *C.odorata*	28	31	32	Dark brown	8.71	**2.98**	2.6	3
20	**OD4**	Odigbo	Wood of *M.indica*	23	25	28	Dark brown	7.95	**2.87**	3.8	3
			Wood of *G.sepium*	25	28	30	Dark brown	9.04	**3.1**	4.4	6
			Wood of *C.odorata*	24	27	29	Dark brown	8.21	**2.96**	3.4	4
21	**OD5**	Okitipupa	Wood of *M.indica*	24	27	30	Dark brown	7.46	**2.95**	4	4
			Woodof *G.sepium*	22	28	31	Dark brown	7.84	**3.08**	5.8	4
			Wood of *C.odorata*	24	26	28	Dark brown	7.52	**2.97**	4.3	4
22	**OD6**	Ose	Wood of *M.indica*	29	31	33	Dark brown	8.25	**2.96**	2.6	4
			Wood of *G.sepium*	25	28	31	Dark brown	7.55	**2.85**	3.8	3
			Wood of *C.odorata*	24	27	29	Dark brown	7.6	**3.02**	4.4	3
23	**OD7**	Owo	Wood of *M.indica*	22	25	28	Dark brown	7.41	**3.11**	3.4	6
			Wood of *G.sepium*	24	26	28	Dark brown	7.35	**3.16**	5.2	3
			Wood of *C.odorata*	29	31	33	Dark brown	8.37	**3.11**	3.4	3
24	**OD8**	Ifedore	Wood of *M.indica*	22	25	27	Dark brown	9.58	**3.07**	5.2	3
			Woodof *G.sepium*	26	29	31	Dark brown	8.9	**3.14**	3	3
			Wood of *C.odorata*	28	31	32	Dark brown	8.7	**3.08**	4	3

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TABLE 4.1: Continued

S/n	Key	Locations	Substrates	Spawn Run (days)	Pin head Formation (days)	Fruit Body formation (days)	Colour of Mushroom	Average yield (g)	Dry wt (g)	Width of Pileus(cm)	fruit body (D)
25	**EK 1**	Ado Ekiti	Wood of *M.indica*	24	27	29	Dark brown	8.76	**3**	5.8	3
			Wood of *G.sepium*	22	25	28	Dark brown	9.14	**3.12**	4.3	2
			Wood of *C.odorata*	24	26	29	Dark brown	12.2	**3.11**	2.6	2
26	**EK2**	Ijero	Wood of *M.indica*	29	31	33	Dark brown	8.71	**2.98**	3.8	2
			Wood of *G.sepium*	23	25	27	Dark brown	7.95	**3.02**	4.4	2
			Wood of *C.odorata*	25	27	29	Dark brown	10	**3.04**	3.4	2
27	**EK3**	Ikole	Wood of *M.indica*	24	26	28	Dark brown	8.37	**3.1**	5.2	2
			Woodof *G.sepium*	22	24	26	Dark brown	6.62	**2.92**	5.2	2
			Wood of *C.odorata*	26	28	30	Dark brown	8.21	**2.88**	3	4
28	**EK4**	Oye	Wood of *M.indica*	28	30	32	Dark brown	7.46	**2.96**	4	4
			Wood of *G.sepium*	23	27	29	Dark brown	7.84	**3.12**	4.5	2
			Wood of *C.odorata*	26	29	31	Dark brown	7.52	**3.08**	2.1	2
29	**EK5**	Irepodun/Ifelodun	Wood of *M.indica*	28	31	33	Dark brown	12	**2.94**	3.6	5
			Wood of *G.sepium*	23	27	29	Dark brown	8.25	**3.02**	4.4	4
			Wood of *C.odorata*	25	30	32	Dark brown	7.55	**3.14**	5.8	3
30	**EK6**	Ikere	Wood of *M.indica*	24	29	31	Dark brown	7.6	**2.86**	4.3	3
			Woodof *G.sepium*	29	32	34	Dark brown	9.04	**3.11**	2.6	3
			Wood of *C.odorata*	25	29	31	Dark brown	8.21	**3.07**	3.8	3
31	**EK7**	Ilejemeje	Wood of *M.indica*	24	28	30	Dark brown	7.46	**2.55**	4.4	3
			Wood of *G.sepium*	22	26	28	Dark brown	7.84	**2.87**	3.4	3
			Wood of *C.odorata*	24	27	29	Dark brown	7.52	**2.95**	5.2	2
32	**EK8**	Emure	Wood of *M.indica*	29	31	33	Brown	8.25	**3.14**	5.6	2
			Wood of *G.sepium*	22	26	28	Dark brown	7.55	**2.97**	4.8	2
			Wood of *C.odorata*	26	30	32	Brown	7.6	**2.88**	5.3	2

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TABLE 4.1: Continued

S/n	Key	Locations	Substrates	Spawn Run (days)	Pin head Formation (days)	Fruit Body formation (days)	Colour of Mushroom	Average yield (g)	Dry wt (g)	Width of Pileus(cm)	fruit body (D)
33	**OS1**	Bolunduro	Wood of *M.indica*	28	31	33	Brown	10	**3.05**	4.5	2
			Woodof *G.sepium*	34	36	38	Brown	11.23	**3.12**	3.8	4
			Wood of *C.odorata*	20	24	26	Brown	9.75	**3.11**	5.1	4
34	**OS 2**	Ejigbo	Wood of *M.indica*	18	23	26	Brown	8.23	**3.16**	3	4
			Wood of *G.sepium*	16	22	25	Brown	11.89	**2.84**	4.9	3
			Wood of *C.odorata*	12	19	23	Brown	8.7	**2.97**	4.5	3
35	**OS3**	Ifedayo	Wood of *M.indica*	8	16	18	Dark brown	9.12	**3.11**	3.9	4
			Wood of *G.sepium*	10	18	22	Dark brown	8.8	**2.69**	4.8	3
			Wood of *C.odorata*	28	31	32	Brown	10.05	**2.77**	4.2	3
36	**OS4**	Ifelodun	Wood of *M.indica*	17	24	26	Dark brown	8.64	**2.97**	3.8	3
			Woodof *G.sepium*	29	31	33	Brown	7.95	**2.94**	4.4	2
			Wood of *C.odorata*	22	25	27	Brown	10	**3.14**	3.8	2
37	**OS5**	Ila	Wood of *M.indica*	26	29	31	Brown	8.37	**2.87**	4.3	2
			Wood of *G.sepium*	28	31	33	Dark brown	6.62	**3.08**	5.3	4
			Wood of *C.odorata*	34	36	38	Dark brown	7.52	**3.12**	3	4
38	**0S6**	Irepodun	Wood of *M.indica*	22	25	27	Brown	8.25	**2.94**	4	5
			Wood of *G.sepium*	26	29	31	Dark brown	8.76	**3.16**	4.5	4
			Wood of *C.odorata*	28	31	33	Brown	9.14	**3.11**	2.1	3
39	**OS7**	Iwo	Wood of *M.indica*	34	37	39	Dark brown	12.2	**3.14**	3.6	3
			Woodof *G.sepium*	20	24	26	Brown	8.71	**2.96**	4.4	3
			Wood of *C.odorata*	24	27	29	Brown	7.95	**2.64**	5.8	5
40	**OS8**	Obokun	Wood of *M.indica*	12	27	30	Dark brown	10	**3.08**	4.3	2
			Wood of *G.sepium*	8	15	17	Brown	8.37	**2.97**	2.6	2
			Wood of *C.odorata*	10	19	21	Brown	6.62	**2.88**	3.8	4

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TABLE 4.1: Continued

S/n	Key	Locations	Substrates	Spawn Run (days)	Pin head Formation (days)	Fruit Body formation (days)	Colour of Mushroom	Average yield (g)	Dry wt (g)	Width of Pileus(cm)	fruit body (D)
41	**OG1**	Abeokuta North 1	Wood of *M.indica*	28	31	33	Dark brown	7.52	**3.12**	4.4	2
			Wood of *G.sepium*	22	25	27	Dark brown	8.25	**3.05**	3.4	2
			Wood of *C.odorata*	26	29	31	Dark brown	7.55	**3.14**	5.2	2
42	**OG2**	Ewekoro	Wood of *M.indica*	28	32	34	Dark brown	7.6	**3.11**	4.8	4
			Woodof *G.sepium*	23	27	29	Dark brown	7.41	**2.97**	5.3	3
			Wood of *C.odorata*	26	29	31	Dark brown	7.35	**3.08**	4.5	3
43	**OG3**	Ifo	Wood of *M.indica*	28	31	33	Brown	8.37	**3.03**	3.8	3
			Wood of *G.sepium*	23	27	29	Dark brown	9.58	**2.94**	5.1	3
			Wood of *C.odorata*	25	28	29	Brown	7.52	**3.14**	3	3
44	**OG4**	Ijebu Ode	Wood of *M.indica*	24	26	28	Dark brown	8.25	**3.05**	4.9	3
			Wood of *G.sepium*	29	31	33	Dark brown	7.55	**2.69**	4.5	3
			Wood of *C.odorata*	25	28	30	Dark brown	7.6	**2.77**	3.9	3
45	**OG5**	Ikenne	Wood of *M.indica*	24	27	29	Dark brown	11.23	**2.84**	5.6	3
			Wood of *G.sepium*	18	24	26	Dark brown	9.75	**3.12**	4.8	2
			Wood of *C.odorata*	17	24	27	Brown	8.23	**3.11**	5.3	2
46	**OG6**	Shagamu	Wood of *M.indica*	14	28	30	Brown	11.89	**2.97**	4.5	4
			Wood of *G.sepium*	11	18	22	Brown	8.7	**2.87**	3.8	4
			Wood of *C.odorata*	14	21	23	Brown	9.12	**2.94**	5.1	4
47	**OG7**	Odeda	Wood of *M.indica*	23	27	29	Dark brown	8.8	**2.69**	3.8	5
			Wood of *G.sepium*	12	19	22	Dark brown	10.05	**3.08**	4.4	5
			Wood of *C.odorata*	29	31	33	Brown	8.64	**3.12**	3.8	5
48	**OG8**	Odogbolu	Wood of *M.indica*	25	28	30	Brown	8.23	**2.77**	4.3	3
			Wood of *G.sepium*	24	27	29	Dark brown	11.89	3.14	5.3	5
			Wood of *C.odorata*	18	24	26	Brown	9.75	3.05	5.0	3

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4.2. Morphological characters of *Auricularia* spp on wood substrates in Oyo state of Southwestern Nigeria

The growth parameters of *Auricularia spp* on Log wood substrates in Oyo state of South Western Nigeria are presented (Table 4.2). The highest days of spawn run was (26.38 ± 1.12 days) for *M.indica,* while the lowest was for *G. sepium* which was (22.13± 0.64 days). While the highest fruit body formation for for *M.indica* was (34.00± 1.23 days) and lowest was for *G. sepium* (31.50± 1.00 days) .

There was no significant difference in the days to spawn running, pin head formation, fruiting body formation and days to mature fruiting body of Auricularia sp on the three substrates evaluated; *M indica, G. sepium* and *C. odorata.* Similarly, the average yield, biological efficiency, width of pileus and growth index showed no significant difference in the three substrates evaluated. Nevertheless, for the dry weight, of *Auricularia* species grown on *C. odorata* had a significantly higher ($p \leq 0.05$) dry weight (3.02 ± 0.05 g) compared to *G. sepium* and *M. indica.*While *M. indica* had the least dry weight (2.84 ± 0.05g).

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Table 4.2 Morphological characteristics of *Auricularia spp* on wood substrates in Oyo state of Southwestern Nigeria

Substrate	Spawnrun (days)	Pin head Formation (days)	Fruitbody formation (days)	Average yield (g)	Dry weight (g)	Width of pileus (cm)	Days to mature fruit body (D)
M. indica	26.38 ± 1.12^{a}	32.13 ± 1.30^{a}	34.00 ± 1.23^{a}	9.65 ± 0.48^{a}	2.84 ± 0.05^{ab}	4.51 ± 0.40^{a}	4.00 ± 0.50^{a}
G. sepium	22.13 ± 0.64^{a}	29.13 ± 1.14^{a}	31.50 ± 1.00^{a}	9.71 ± 0.49^{a}	2.99 ± 0.05^{b}	4.23 ± 0.35^{a}	3.25 ± 0.25^{a}
C. odorata	24.88 ± 1.77^{a}	30.63 ± 1.67^{a}	32.13 ± 1.62^{a}	9.70 ± 0.60^{a}	3.02 ± 0.05^{a}	3.60 ± 0.24^{a}	3.00 ± 0.38^{a}

Values are means ± Standard error. Means with same letters along the column are not significantly different according to Duncan Multiple Range Test ($p \leq 0.05$) (N=24. 3 replicates each, from 8 areas)

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Plate 4.2: Photograph showing *Auricularia auricula* 8 x 6

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Plate 4.3 Photograph of *Auricularia polythricha*. 9 x 6

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Plate 4.4 Photograph of dark brown sample of *Auricularia* sp. 10 x 7

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Plate 4.5 Photograph of *Auricularia* species growing on inoculated wood of *Mangifera indica.* 5 x 4

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4.3. Morphological characteristics of *Auricularia* on wood substrate in Lagos state

The growth characteristics of *Auricularia* spp on wood substrates in Lagos state are presented (Table 4.3). The spawn run was highest in *M .indica* (25.00± 2.06 days) and lowest in *G. sepium* (21.25± 1.93 days).While the width of pileus was highest in *C. odorata* (4.39±0.39 cm) and lowest for *M. indica* (3.36± 0.25 cm). There was no significant difference in the days to spawn running, pin head formation, fruiting body formation and days to mature fruiting body for the three substrates evaluated; *M. indica, G. sepium* and *C. odorata.* Similarly, the average yield and width of pileus, showed no significant difference in the three substrates evaluated. Nevertheless, for the dry weight, *M. indica* had a significantly higher ($p \leq 0.05$) dry weight (3.04 ± 0.03 g) compared to *G. sepium* and *C. odorata. G. sepium* had the least dry weight (2.84 ± 0.07 g).

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Table 4.3 Morphological characteristics of *Auricularia* on wood substrate in Lagos state of Southwestern Nigeria

Substrate	Spawn run (days)	Pin head Formation (days)	Fruit body formation (days)	Average yield (g)	Dry weight (g)	Width of pileus (cm)	Days to mature fruit body (D)
M. indica	25.00 ± 2.06 [a]	29.00 ± 1.50 [a]	30.88 ± 1.48 [a]	8.89 ± 0.24 [a]	3.04 ± 0.03 [a]	3.36 ± 0.25 [a]	3.25 ± 0.45 [a]
G. sepium	21.25 ± 1.93 [a]	25.50 ± 1.71 [a]	27.38 ± 1.68 [a]	9.38 ± 0.50 [a]	2.84 ± 0.07 [ab]	3.70 ± 0.27 [a]	3.50 ± 0.46 [a]
C. odorata	23.50 ± 2.02 [a]	27.13 ± 1.54 [a]	29.38 ± 1.57 [a]	9.52 ± 0.52 [a]	2.99 ± 0.04 [b]	4.39 ± 0.39 [a]	3.13 ± 0.30 [a]

Values are means ± Standard error. Means with same letters along the column are not significantly different according to Duncan Multiple Range Test ($p \leq 0.05$) (N=24. 3 replicates each, from 8 areas)

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4.4 Morphological characteristics of *Auricularia* spp on wood substrates in Ondo state

The growth parameters of *Auricularia spp* on wood substrates in Ondo state is presented in Table 4.4. Growth on *C.odorata* had 26.13±0.81 days of spawn run which was the highest and *M.indica* had the lowest (23.50±0.82 days) of spawn run. The width of pileus was highest in *G.sepium* (4.40±0.30 cm) and lowest in *C.odorata* (3.51±0.24 cm)

There was no significant difference in the days to spawn running, fruiting body formation and days to mature fruiting body for the three substrates evaluated; *M indica, G. sepium* and *C. odorata.* Similarly, the average yield, dry weight and width of pileus showed no significant difference in the three substrates evaluated. On the other hand, the number of days taken for pin head formation was significantly higher ($p \leq 0.05$) in *C. odorata* (29.00 ± 0.73 days) compared to *M. indica* and *G. sepium. M. indica* had the least days to pin head formation (26.50 ± 0.71 days)

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Table 4.4 Morphological characteristics of *Auricularia* on wood substrates in Ondo state

Substrate	Spawn run (days)	Pinhead Formation (days)	Fruit body formation (days)	Average yield (g)	Dryweight (g)	Widthof pileus (cm)	Days to mature fruit body (D)
M. indica	23.50 ± 0.82 [a]	26.50 ± 0.71 [ab]	29.00 ± 0.66 [a]	8.84 ± 0.55 [a]	2.99 ± 0.03 [a]	4.23 ± 0.38 [a]	3.50 ± 0.42 [a]
G. sepium	24.88 ± 0.48 [a]	27.75 ± 0.31 [b]	30.13 ± 0.40 [a]	8.79 ± 0.54 [a]	3.05 ± 0.04 [a]	4.40 ± 0.30 [a]	3.25 ± 0.45 [a]
C. odorata	26.13 ± 0.81 [a]	29.00 ± 0.73 [a]	30.75 ± 0.65 [a]	8.28 ± 0.20 [a]	3.01 ± 0.03 [a]	3.51 ± 0.24 [a]	3.13 ± 0.23 [a]

Values are means ± Standard error. Means with same letters along the column are not significantly different according to Duncan Multiple Range Test ($p \leq 0.05$)

(N=24. 3 replicates each, from 8 areas)

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4.5 Morphological characteristics of *Auricularia* spp on wood substrates in Ekiti state

The growth parameters of *Auricularia spp* on wood substrates in Ekiti state of Southwestern Nigeria are presented in Table 4.5. *C. odorata* was the highest (8.60 ± 0.59 g) and *G.sepium* had the lowest (8.03 ± 0.29 g). *M.indica* had 4.59 ± 0.30 cm as the highest width of pileus and *C. odorata* (3.90 ± 0.49 cm) had the lowest. There was no significant difference in the average yield, dry weight and width of pileus. Days to mature fruiting body were also not significantly different for the three substrates evaluated; (*M indica, G. sepium* and *C. odorata*). On the other hand, number of days taken for spawn running and pin head formation were significantly higher ($p \leq 0.05$) in *M. indica* (26.25 ± 0.86 days) and (29.13 ± 0.69 days) respectively while *G. sepium* had the least number of days to spawn running and pin head formation (23.25 ± 0.84days) and (26.50 ± 0.87 days), respectively. Days to fruiting body formation was significantly higher ($p \leq 0.05$) in *M. indica* (31.13 ± 0.69 days) and least in *G. sepium* (28.63 ± 0.84 days).

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Table 4.5: Morphological characteristics of *Auricularia* spp on wood substrates in Ekiti state

Substrate	Spawn run (days)	Pinhead Formation (days)	Fruit body formation (days)	Average yield (g)	Dryweight (g)	Widthof pileus (cm)	Days to mature fruit body (D)
M. indica	26.25 ± 0.86 [a]	29.13 ± 0.69 [a]	31.13 ± 0.69 [a]	8.58 ± 0.52 [a]	2.94 ± 0.06 [a]	4.59 ± 0.30 [a]	3.00 ± 0.38 [a]
G. sepium	23.25 ± 0.84 [ab]	26.50 ± 0.87 [ab]	28.63 ± 0.84 [b]	8.03 ± 0.29 [a]	3.02 ± 0.03 [a]	4.20 ± 0.29 [a]	2.50 ± 0.27 [a]
C. odorata	25.13 ± 0.30 [b]	28.25 ± 0.53 [b]	30.38 ± 0.46 [ab]	8.60 ± 0.59 [a]	3.02 ± 0.04 [a]	3.90 ± 0.49 [a]	2.50 ± 0.27 [a]

Values are means ± Standard error Means with same letters along the column are not significantly different according to Duncan Multiple Range Test ($p \leq 0.05$)

(N=24. 3 replicates each, from 8 areas)

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4.6 Morphological characteristics of *Auricularia* on wood substrates in Osun state

The growth parameters of *Auricularia spp* on wood substrates in Osun state of Southwestern Nigeria are presented (Table 4.6). The highest days of spawn run was found in *C. odorata* (22.25 ± 2.89 days) with the lowest in *M. Indica* (20.63 ± 3.04 days) Also, the fruit body formation was highest in *M. indica* (28.75 ± 2.17 days) while the lowest was found in *G. sepium* (28.13 ± 2.42 days). There were no significant differences ($p \geq 0.05$) in the number of days taken for spawn running, pin head formation, fruit body formation, days to mature fruit body, average yield, dry weight and width of pileus for the three substrates evaluated; (*M. indica, G. sepium* and *C. odorata*)

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Table 4.6: Morphological characteristics of *Auricularia* on wood substrates in Osun state

Substrate	Spawn run (days)	Pinhead Formation (days)	Fruit body formation (days)	Average yield (g)	Dryweight (g)	Widthof pileus (cm)	Days to mature fruit body (D)
M. indica	20.63 ± 3.04 [a]	26.50 ± 2.19 [a]	28.75 ± 2.17 [a]	9.35 ± 0.48 [a]	3.04 ± 0.04 [a]	3.93 ± 0.17 [a]	3.13 ± 0.40 [a]
G. sepium	21.38 ± 3.33 [a]	25.75 ± 2.55 [a]	28.13 ± 2.42 [a]	9.04 ± 0.61 [a]	2.97 ± 0.05 [a]	4.34 ± 0.29 [a]	3.13 ± 0.30 [a]
C.odorata	22.25 ± 2.89 [a]	26.50 ± 2.12 [a]	28.63 ± 1.97 [a]	8.72 ± 0.44 [a]	2.97 ± 0.07 [a]	4.04 ± 0.41 [a]	3.50 ± 0.33 [a]

Values are means ± Standard error Means with same letters along the column are not significantly different according to Duncan Multiple Range Test ($p \leq 0.05$) (N=24. 3 replicates each from 8 areas)

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4.7: Morphologica characteristics of *Auricularia* spp on wood substrates in Ogun state

Table 4.7 showed the growth characteristics of *Auricularia* on log wood substrates in Osun state of South Western Nigeria are presented. The spawn run was highest in *M. indica* (24.25± 1.63 days) and lowest in *G.sepium* (20.25 ± 2.19 days). Also, the highest pin head formation was in *M. indica* (28.75± 0.80 days) and the lowest in G.sepium (24.75± 1.54 days). There were no significant differences ($p \geq 0.05$) in the number of days taken for spawn run, pin head formation, fruit body formation, days to mature fruiting body, average yield, dry weight, and width of pileus for the three substrates evaluated; *M. indica, G. sepium* and *C. odorata.*

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Table 4.7: Morphological characteristics of *Auricularia* spp on wood substrates in Ogun state

Substrate	Spawn run (days)	Pinhead Formatio n (days)	Fruit body formation (days)	Average yield (g)	Dry weight (g)	Width of pileus (cm)	Days to mature fruit body (D)
M. indica	24.25 ± 1.63 [a]	28.75 ± 0.80 [a]	30.75 ± 0.80 [a]	8.99 ± 0.58 [a]	2.95 ± 0.06 [a]	4.51 ± 0.21 [a]	3.38 ± 0.32 [a]
G. sepium	20.25 ± 2.19 [a]	24.75 ± 1.54 [a]	27.13 ± 1.33 [a]	9.15 ± 0.53 [a]	2.98 ± 0.05 [a]	4.58 ± 0.25 [a]	3.38 ± 0.42 [a]
C. odorata	22.50 ± 1.90 [a]	26.75 ± 1.19 [a]	28.75 ± 1.15 [a]	8.22 ± 0.31 [a]	3.04 ± 0.05 [a]	4.48 ± 0.29 [a]	3.13 ± 0.35 [a]

Values are means ± Standard error Means with same letters along the column are not significantly different according to Duncan Multiple Range Test ($p \leq 0.05$) (N=24. 3 replicates each, from 8 areas)

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4.8: Overall effects of wood substrates on morphological characteristics of *Auricularia* spp in Southwestern Nigeria

The growth parameters of *Auricularia* on wood substrates in Southwestern Nigeria is presented in Table 4.8.The highest fruit body formation (30.75± 0.56 days) found in *M. indica* with the lowest as 28.81± 0.60 days in *G. sepium*. On the other hand, the average yield was highest for *M. indica* (9.05± 0.20 g) and lowest for *C. odorata* (8.84± 0.20g). There were no significant differences ($p \geq 0.05$) in the number of days taken for spawn running, fruiting body formation, days to mature fruiting body, average yield, dry weight and width of pileus for the three substrates evaluated; *M indica, G. sepium* and *C. odorata* except for number of days to pin head formation which was significantly higher ($p \leq 0.05$) in *M. indica* (28.67 ± 0.58 days) and least in *G. sepium* (26.56 ± 0.63 days).

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Table 4.8: Overall Morphological characteristics of *Auricularia* spp on wood substrates in Southwestern Nigeria

Substrate	Spawn run (days)	Pinhead Formation (days)	Fruit body formation (days)	Average yield (g)	Dryweight (g)	Widthof pileus (cm)	Days to mature fruit body (D)
M. indica	24.33 ± 0.74 [a]	28.67 ± 0.58 [a]	30.75 ± 0.56 [a]	9.05 ± 0.20 [a]	2.97 ± 0.02 [a]	4.19 ± 0.13 [a]	3.38 ± 0.17 [a]
G. sepium	22.19 ± 0.75 [a]	26.56 ± 0.63 [b]	28.81 ± 0.60 [a]	9.02 ± 0.21 [a]	2.97 ± 0.02 [a]	4.24 ± 0.12 [a]	3.17 ± 0.15 [a]
C. odorata	24.06 ± 0.73 [a]	28.04 ± 0.58 [ab]	30.00 ± 0.55 [a]	8.84 ± 0.20 [a]	3.01 ± 0.02 [a]	3.99 ± 0.15 [a]	3.06 ± 0.13 [a]

Values are means ± Standard error Means with same letters along the column are not significantly different according to Duncan Multiple Range Test ($p \leq 0.05$) (N=144. 3 replicates each, from 8 areas in six states)

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4.9: Overall effects of states on Cultivated *Auricularia* spp in Southwestern Nigeria

The growth parameters of cultivated *Auricularia* in Southwestern Nigeria are presented in Table 4.9. The highest spawn run was found in Ekiti state (24.88 ± 0.47 days) while the lowest was in Osun state with 21.42± 1.71 days.The days to maturity was highest in Oyo state (3.42 ± 0.23 days) and lowest was in Ekiti state (2.67 ± 0.18 days) There were no significant differences ($p \geq 0.05$) in the number of days taken for spawn running, days to mature fruiting body, dry weight, width of pileu for the three substrates evaluated; *M. indica, G. sepium* and *C. odorata* in the six South Western States. Number of days to pin head formation and fruiting body formation was highest in Oyo state (30.63 ± 0.81 days) and (32.54 ± 0.75 days) respectively and was significantly different ($p \leq 0.05$) from the other states. The number of days to pin head formation and fruiting body formation were not significantly different in the other five states; Lagos, Ondo, Ekiti, Osun and Ogun States evaluated. The average yield was significantly highest ($p \leq 0.05$) for *Auricularia spp* cultivated in Oyo State (9.69 ± 0.29 g) compared to other states, with the least occurring in Ekiti state (8.40 ± 0.27 g).

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Table 4.9: Overall Morphological characteristics of Cultivated *Auricularia on Mangifera indica* in Southwestern Nigeria

Substrate	Spawn run (days)	Pinhead Formation (days)	Fruit body formation (days)	Average yield (g)	Dryweight (g)	Widthof pileus (cm)	Days to mature fruit body (D)
Oyo	24.46 ± 0.79 [a]	30.63 ± 0.81 [a]	32.54 ± 0.75 [a]	9.69 ± 0.29 [a]	2.95 ± 0.03 [a]	4.11 ± 0.20 [a]	3.42 ± 0.23 [a]
Lagos	23.25 ± 1.15 [a]	27.21 ± 0.93 [b]	29.21 ± 0.92 [b]	9.26 ± 0.25 [ab]	2.96 ± 0.03 [a]	3.82 ± 0.19 [a]	3.29 ± 0.23 [a]
Ondo	24.83 ± 0.46 [a]	27.75 ± 0.40 [b]	29.96 ± 0.35 [b]	8.64 ± 0.26 [abc]	3.01 ± 0.02 [a]	4.05 ± 0.19 [a]	3.29 ± 0.21 [a]
Ekiti	24.88 ± 0.47 [a]	27.96 ± 0.45 [b]	30.04 ± 0.44 [b]	8.40 ± 0.27 [c]	2.99 ± 0.03 [a]	4.23 ± 0.21 [a]	2.67 ± 0.18 [a]
Osun	21.42 ± 1.71 [a]	26.25 ± 1.27 [b]	28.50 ± 1.21 [b]	9.04 ± 0.29 [bc]	2.99 ± 0.03 [a]	4.10 ± 0.17 [a]	3.25 ± 0.19 [a]
Ogun	22.33 ± 1.11 [a]	26.75 ± 0.75 [b]	28.88 ± 0.69 [b]	8.78 ± 0.28 [bc]	2.99 ± 0.03 [a]	4.52 ± 0.14 [a]	3.29 ± 0.20 [a]

Values are means ± standard error Means with same letters along the column are not significantly different according to Duncan Multiple Range Test ($p \leq 0.05$) (N=144. 24 replicates from each state).

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Fig: 4.5 as presented shows the morphological relationship among species of *Auricularia* at locations in Southwestern Nigeria in the Pricipal component analysis (PCA) scattered diagram.

The PCA consist of the locations which were broadly grouped into six categories based on their morphological relationship for the seventeen (17) characters and forty-eight (48) samples.

Group1:
OS4, OS6, OS8, OS1, OS7, OS2, OS3

Group2:
OG6,OG7,OG5,OG4,OG8,OG3,LA3,LA4,LA2,LA1,EK7,EK8,EK4,EK5,EK6, EK2,EK3,OY6,OY7,OY2,OY5,EK1

Group3:
OG1, OG2, OY3, OY4

Group4
OY1, OD2, OD4, OD3, OD1, OD8, OD7, OD6, OD5

Group 5:
LA8, **LA7**, LA6.

Group 6:
LA5.

Key: OS=OSUN, OG=OGUN,LA=LAGOS, EK=EKITI,OY=OYO,OD=ONDO

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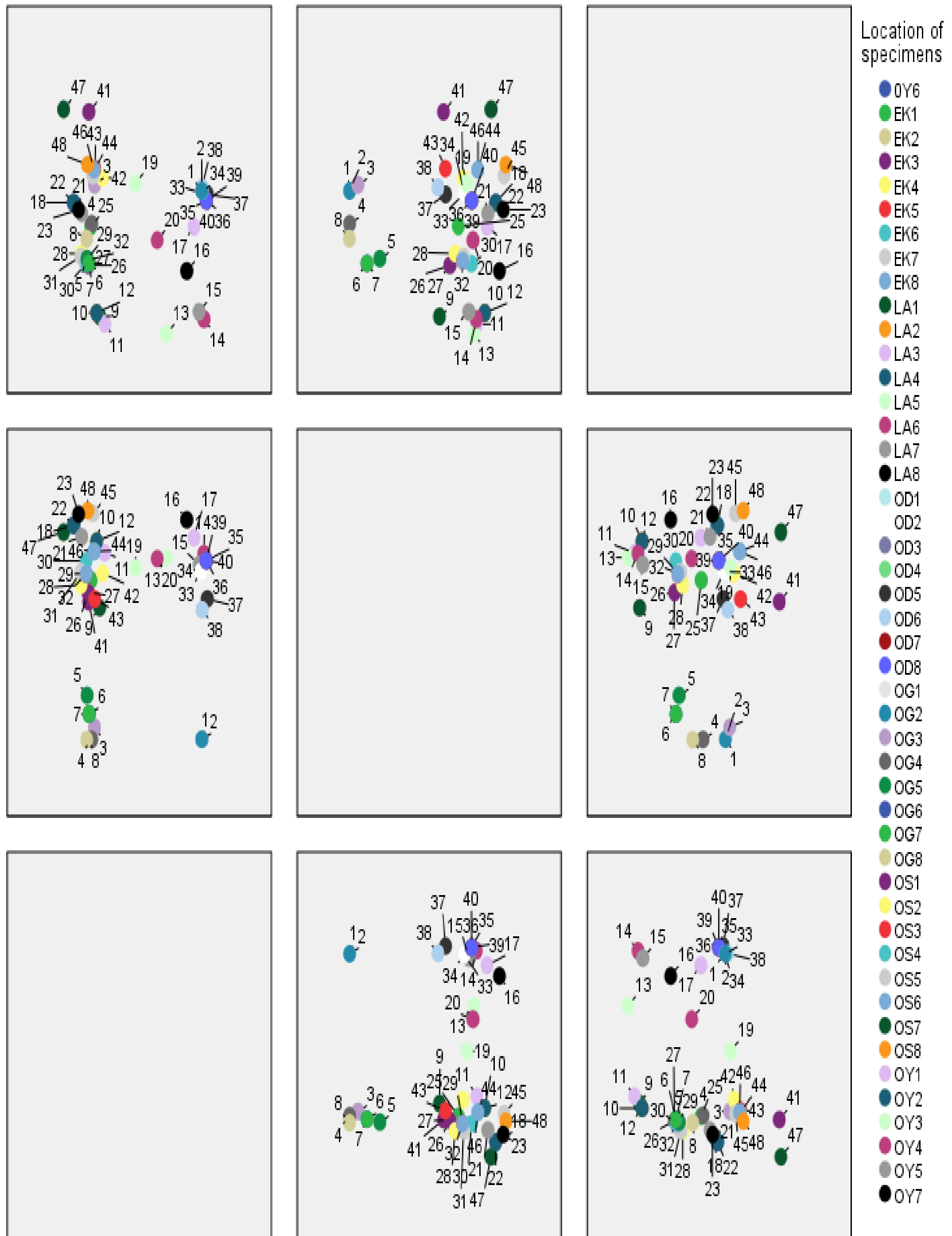

Fig 4.5. Scatter diagram obtained from principal component axes I, II and III for principal component analysis (PCA) of 48 x 17 morphological and nutrient composition data matrix.

4.10 Rotated components matrix of Principal Component Analysis (PCA)

To have a good diagram, seventeen characters were used for the construction of the scattered diagram.They were:

I. Morphological (External color,shape texture and the mycelium color and type

II. Proximate analysis (protein, ash, moisture, fat, crude fiber and carbohydrate

III. Nutrient composition (nitrogen, phosphorous, sodium, potassium calcium and magnesium)

The PCA analysis of the morphological characters of the *Auricularia species* gave eigen values with absolute values that are equal to or higher than 0.025 and the values are 23.0%, 16.0%, 11.0%, 10.0% and 9.0% in a corresponding order of the component 1-6 on the table.

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Table 4.10: Rotated components matrix of the 17 x 48 characters of the *Auricularia* species

Variables	Component					
	1	2	3	4	5	6
Colour	0.987	-	-	-	-	-
Shape	0.813	-	-	0.502	-	-
Texture	0.987	-	-	-	-	-
My.colour	-0.942	-	-	-	-	-
My.type	-	-	-	0.819	-0.375	0.256
Nitrogen	-0.272	-	-0.833	-	-	-
Phosphorus	0.282	-	0.754	-	0.297	0.307
Sodium	-	0.825	-	-	-	0.384
Potassium	-0.293	-	-	-	-	0.756
Calcium	-	0.367	-	-	0.703	0.257
Magnesium	-	0.918	-	-	-	-
Protein	-	-	-	-	0.772	-
Ash	-	0.463	-	-	-	0.530
Moisture	-	0.804	-	-	-	-
FAT	-	-0.350	0.568	-	0.361	-
C.Fiber	-	-	0.805	0.253	-	-
CHO	-	-	-0.317	-0.795	-.0267	0.256
Eigen Values %	23	16	14	11	10	9
Cummulative %	23	39	54	65	74	83

Only Eigen values with absolute values equal to or greater than 0.025 are shown

Key-My.-Mycelium C.-Crude

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4.11: *Auricularia spp* cultivated on wood substrate of *M. indica* in Southwestern Nigeria

The growth parameters of cultivated *Auricularia spp* cultivated on log wood substrate of *M indica* in Southwestern Nigeria is presented in Table 4.10. There were no significant differences (p≥0.05) in the number of days taken for spawn running, fruiting body formation, days to mature fruiting body, average yield, biological efficiency, and growth index for *M. indica,* in the six Southwestern States. Number of days to pin head, and fruit body formation was highest in Oyo state (30.63 ± 0.81days) and (32.54 ± 0.75 days), respectively and was significantly different (p≤0.05) from the other states. The number of days to pin head formation was significantly highest (p≤0.05) in Oyo state (32.13 ± 1.30 days) than the other five states; Lagos, Ondo, Ekiti, Osun and Ogun States evaluated. The least number of days to pin head formation was observed in Ondo state (26.50 ± 0.71 days). The dry weight was significantly highest (p≤0.05) in *Auricularia spp* cultivated on *M. indica* in Lagos State (3.04 ± 0.03g) and Osun (3.04 ± 0.04g) states compared to the other states, with the least occurring in Oyo state (2.84 ± 0.05g).

4.12: *Auricularia spp* cultivated on wood substrate of *G. sepium* in Southwestern Nigeria

The growth parameters of *Auricularia spp* cultivated on log wood substrate of *G. sepium* in Southwestern Nigeria is presented in Table 4.11. There were no significant differences (p≥0.05) in the number of days taken for spawn running, pin head formation, fruiting body formation, days to mature fruiting body, average yield, dry weight and width of pileus in *G. sepium,* in the six Southwestern States evaluated. Nevertheless days to spawn running was highest in Ondo state (24.88 ± 0.48 days), days to pin head and fruit body formation were highest in Oyo state (29.13±1.14 days) and (31.50±1.00 days), respectively. Average yield was highest in Oyo state (9.71±0.49g), dry weight in Ondo state (3.05±0.04g), width of pileus in Ogun state (4.58±0.25cm), days to mature fruiting body in Lagos state (3.50±0.46days).

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Table 4.11: *Auricularia spp* cultivated on wood substrate of *M. indica* in Southwestern Nigeria

Substrate	Spawn run (days)	Pinhead Formation (days)	Fruit body formation (days)	Average yield (g)	Dryweight (g)	Widthof pileus (cm)	Days to mature fruit body (D)
Oyo	26.38 ± 1.12 [a]	32.13 ± 1.30 [a]	34.00 ± 1.23 [a]	9.65 ± 0.48 [a]	2.84 ± 0.05 [b]	4.51 ± 0.40 [a]	4.00 ± 0.50 [a]
Lagos	25.00 ± 2.06 [a]	29.00 ± 1.50 [ab]	30.88 ± 1.48 [a]	8.89 ± 0.24 [a]	3.04 ± 0.03 [a]	3.36 ± 0.25 [b]	3.25 ± 0.45 [a]
Ondo	23.50 ± 0.82 [a]	26.50 ± 0.71 [b]	29.00 ± 0.66 [a]	8.84 ± 0.55 [a]	2.99 ± 0.03 [a]	4.23 ± 0.38 [ab]	3.50 ± 0.42 [a]
Ekiti	26.25 ± 0.86 [a]	29.13 ± 0.69 [ab]	31.13 ± 0.69 [a]	8.58 ± 0.52 [a]	2.94 ± 0.06 [ab]	4.59 ± 0.30 [a]	3.00 ± 0.38 [a]
Osun	20.63 ± 3.04 [a]	26.50 ± 2.19 [b]	28.75 ± 2.17 [a]	9.35 ± 0.48 [a]	3.04 ± 0.04 [a]	3.93 ± 0.17 [ab]	3.13 ± 0.40 [a]
Ogun	24.25 ± 1.63 [a]	28.75 ± 0.80 [ab]	30.75 ± 0.80 [a]	8.99 ± 0.58 [a]	2.95 ± 0.06 [ab]	4.51 ± 0.21 [a]	3.38 ± 0.32 [a]

Values are means ± standard error Means with same letters along the column are not significantly different according to Duncan Multiple Range Test ($p \leq 0.05$) (N=48. 8 replicates per state)

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Table 4.12: *Auricularia spp* cultivated on wood substrate of *G.sepium* in Southwestern Nigeria

Substrate	Spawn run (days)	Pinhead Formation (days)	Fruit body formation (days)	Average yield (g)	Dryweight (g)	Widthof pileus (cm)	Days to mature fruit body (D)
Oyo	22.13 ± 0.64 [a]	29.13 ± 1.14 [a]	31.50 ± 1.00 [a]	9.71 ± 0.49 [a]	2.99 ± 0.05 [a]	4.23 ± 0.35 [a]	3.25 ± 0.25 [a]
Lagos	21.25 ± 1.93 [a]	25.50 ± 1.71 [a]	27.38 ± 1.68 [a]	9.38 ± 0.50 [a]	2.84 ± 0.07 [a]	3.70 ± 0.27 [a]	3.50 ± 0.46 [a]
Ondo	24.88 ± 0.48 [a]	27.75 ± 0.31 [a]	30.13 ± 0.40 [a]	8.79 ± 0.54 [a]	3.05 ± 0.04 [a]	4.40 ± 0.30 [a]	3.25 ± 0.45 [a]
Ekiti	23.25 ± 0.84 [a]	26.50 ± 0.87 [a]	28.63 ± 0.84 [a]	8.03 ± 0.29 [a]	3.02 ± 0.03 [a]	4.20 ± 0.29 [a]	2.50 ± 0.27 [a]
Osun	21.38 ± 3.33 [a]	25.75 ± 2.55 [a]	28.13 ± 2.42 [a]	9.04 ± 0.61 [a]	2.97 ± 0.05 [a]	4.34 ± 0.29 [a]	3.13 ± 0.30 [a]
Ogun	20.25 ± 2.19 [a]	24.75 ± 1.54 [a]	27.13 ± 1.33 [a]	9.15 ± 0.53 [a]	2.98 ± 0.05 [a]	4.58 ± 0.25 [a]	3.38 ± 0.42 [a]

Means with same letters along the column are not significantly different according to Duncan Multiple Range Test ($p \leq 0.05$) (N=48. 8 replicates per state)

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4.13: *Auricularia* spp cultivated on wood substrate of *C. odorata* in Southwestern Nigeria

The growth parameters of cultivated *Auricularia spp* cultivated on wood substrate of *C. odorata* in Southwestern Nigeria is presented in Table 4.12. There were no significant differences ($p \geq 0.05$) in the number of days taken for spawn running, pin head formation, fruiting body formation, days to mature fruiting body, average yield, dry weight, biological efficiency, width of pileus and growth index for *G. sepium,* in the six South Western States evaluated. Nevertheless, days to spawn running was highest in Ondo state (26.13 ± 0.81), days to pin formation and days to fruiting body formation were highest in Oyo state (30.63 ± 1.67 days) and (32.13 ± 1.62days respectively. Average yield was highest in Oyo state (9.70 ± 0.60g), dry weight in Ogun state (3.04 ± 0.05g), width of pileus in Ogun state (4.48 ± 0.29 cm), days to mature fruiting body in Osun state (3.50 ± 0.33days).

4.14: Nutrient contents of *Auricularia* spp cultivated on wood Substrates in Southwestern Nigeria

Table 4.13 shows the nutritional composition of *Auricularia* spp cultivated on wood Substrates in South Western Nigeria. There were significant differences ($p \leq 0.05$) in the nutritional composition observed in the six states. The nitrogen content was highest in Lagos state (13.59±0.68mg/kg) and least in Oyo state (8.85 ± 0.56mg/kg). Phosphorous was highest in Ogun state (39.25±7.61mg/kg)and least in Lagos state (17.25 ± 1.45mg/kg) while sodium content was highest in Lagos state (70.49 ± 3.00mg/kg) and least in Oyo state (15.41 ± 0.16mg/kg)· Potassium content ranged from 331.50±14.89mg/kg (Ondo state) to 1511.63 ± 168.65mg/kg (Osun state). Also, calcium content ranged from 33.31 ± 1.78mg/kg (Ekiti state) to 61.90 ± 3.57mg/kg (Lagos state). Magnesium content was highest in Osun state (116.73 ± 6.07ng/kg) and least in Ogun state (56.74 ±1.02mg/kg).

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Table 4.13: *Auricularia spp* cultivated on wood substrate of *C. odorata* in Southwestern Nigeria

Substrate	Spawn run (days)	Pinhead Formation (days)	Fruit body formation (days)	Average yield (g)	Dryweight (g)	Widthof pileus (cm)	Days to mature fruit body (D)
Oyo	24.88 ± 1.77 [a]	30.63 ± 1.67 [a]	32.13 ± 1.62 [a]	9.70 ± 0.60 [a]	3.02 ± 0.05 [a]	3.60 ± 0.24 [a]	3.00 ± 0.38 [a]
Lagos	23.50 ± 2.02 [a]	27.13 ± 1.54 [a]	29.38 ± 1.57 [a]	9.52 ± 0.52 [a]	2.99 ± 0.04 [a]	4.39 ± 0.39 [a]	3.13 ± 0.30 [a]
Ondo	26.13 ± 0.81 [a]	29.00 ± 0.73 [a]	30.75 ± 0.65 [a]	8.28 ± 0.20 [a]	3.01 ± 0.03 [a]	3.51 ± 0.24 [a]	3.13 ± 0.23 [a]
Ekiti	25.13 ± 0.30 [a]	28.25 ± 0.53 [a]	30.38 ± 0.46 [a]	8.60 ± 0.59 [a]	3.02 ± 0.04 [a]	3.90 ± 0.49 [a]	2.50 ± 0.27 [a]
Osun	22.25 ± 2.89 [a]	26.50 ± 2.12 [a]	28.63 ± 1.97 [a]	8.72 ± 0.44 [a]	2.97 ± 0.07 [a]	4.04 ± 0.41 [a]	3.50 ± 0.33 [a]
Ogun	22.50 ± 1.90 [a]	26.75 ± 1.19 [a]	28.75 ± 1.15 [a]	8.22 ± 0.31 [a]	3.04 ± 0.05 [a]	4.48 ± 0.29 [a]	3.13 ± 0.35 [a]

Means with same letters along the column are not significantly different according to Duncan Multiple Range Test ($p \leq 0.05$) (N=48. 8 replicates per state).

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Table 4.14: Nutrient composition of *Auricularia spp* cultivated on wood Substrates in Southwestern Nigeria

State	Dry Weight (mg/kg)					
	Nitrogen	Phosphorous	Sodium	Potassium	Calcium	Magnesium
Oyo	8.85 ± 0.56^{c}	22.29 ± 1.93^{bc}	15.41 ± 0.16^{e}	878.25 ± 75.56^{b}	35.89 ± 1.86^{b}	73.40 ± 4.53^{c}
Lagos	13.59 ± 0.68^{a}	17.25 ± 1.45^{c}	70.49 ± 3.00^{a}	502.88 ± 204.72^{c}	61.90 ± 3.57^{a}	104.46 ± 4.40^{b}
Ondo	9.56 ± 0.52^{c}	18.86 ± 0.63^{c}	18.20 ± 2.28^{de}	331.50 ± 14.89^{c}	36.66 ± 0.52^{b}	69.96 ± 2.99^{c}
Ekiti	9.06 ± 0.14^{c}	32.83 ± 3.58^{ab}	45.03 ± 1.58^{b}	1199.63 ± 94.52^{ab}	33.31 ± 1.78^{b}	70.29 ± 4.90^{c}
Osun	8.96 ± 0.53^{c}	32.69 ± 5.07^{ab}	24.34 ± 4.07^{cd}	1511.63 ± 168.65^{a}	55.01 ± 4.35^{a}	116.73 ± 6.07^{a}
Ogun	11.57 ± 0.80^{b}	39.25 ± 7.61^{a}	29.85 ± 2.19^{c}	478.50 ± 3.33^{c}	40.03 ± 1.37^{b}	56.74 ± 1.02^{d}

Means with same letters along the column are not significantly different according to Duncan Multiple Range Test ($p \leq 0.05$) (N=48. 8 replicates per state).

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4.15 Proximate Analysis of *Auricularia spp* cultivated on wood Substrates in Southwestern Nigeria

The proximate composition of *Auricularia* spp cultivated on wood substrates in Southwestern Nigeria is presented in Table 4.14). There was no significant difference in the protein and ash content in the various states evaluated. Nevertheless, Protein content was highest in *Auricularia* spp cultivated in Lagos state (6.98 ± 0.81%) while ash content was highest in Ondo state (4.83 ± 0.42%). The highest moisture content was observed in *Auricularia* cultivated in Ondo state (16.22 ± 0.65%) and the least in Ogun state (10.43 ± 0.19%)· Fat content was highest in Ekiti state (6.60 ± 0.14%) which was significantly different ($p \leq 0.05$) from the other states. The crude fibre was highest in Ogun state (22.54 ± 0.49%) and lowest in Lagos state (18.49 ± 0.37%)· Carbohydrate content was highest in Ogun state (54.23 ± 1.03%) and least in Osun state (46.19 ± 2.48%) There was no significant difference in the ash content in the various states evaluated.

Nevertheless, Protein content was highest in *Auricularia* spp cultivated in Lagos state (6.98 ± 0.81) while ash content was highest in Ondo state (4.83 ± 0.42%). The highest moisture content was observed in *Auricularia* cultivated in Ondo state (16.22 ± 0.65%) and the least in Ogun state (10.43 ± 0.19%)· Fat content was highest in Ekiti state (6.60 ± 0.14) which was significantly different ($p \leq 0.05$) from the other states. The crude fibre was highest in Ogun state (22.54 ± 0.49%) and lowest in Lagos state (18.49 ± 0.37%)·. Carbohydrate content was highest in Ogun state (54.23 ± 1.03%) and least in Osun state (46.19 ± 2.48%)

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Table 4.15: Proximate Analysis of *Auricularia* spp cultivated on wood substrates in Southwestern Nigeria

State	% Protein	% Ash	% Moisture	% Fat	% Crude Fibre	% CHO
Oyo	6.26 ± 0.41^{a}	4.40 ± 0.63^{a}	12.49 ± 0.59^{b}	3.60 ± 0.16^{bc}	19.31 ± 0.35^{c}	53.95 ± 1.08^{a}
Lagos	6.98 ± 0.81^{a}	4.29 ± 0.52^{a}	14.10 ± 0.32^{b}	2.76 ± 0.08^{c}	18.49 ± 0.37^{c}	53.38 ± 0.89^{a}
Ondo	6.24 ± 0.10^{a}	4.83 ± 0.42^{a}	16.22 ± 0.65^{a}	3.41 ± 0.15^{c}	20.51 ± 0.42^{bc}	48.80 ± 0.94^{bc}
Ekiti	6.70 ± 0.17^{a}	3.87 ± 0.72^{a}	12.82 ± 0.68^{b}	6.60 ± 0.14^{a}	18.66 ± 0.28^{c}	51.35 ± 0.88^{ab}
Osun	6.55 ± 0.16^{a}	4.72 ± 0.43^{a}	13.01 ± 1.28^{b}	4.41 ± 0.32^{b}	25.13 ± 2.48^{a}	46.19 ± 2.48^{c}
Ogun	5.31 ± 0.14^{a}	3.14 ± 0.12^{a}	10.43 ± 0.19^{c}	4.36 ± 0.59^{b}	22.54 ± 0.49^{ab}	54.23 ± 1.03^{a}

Means with same letters along the column are not significantly different according to Duncan Multiple Range Test ($p \leq 0.05$) (N=48. 8 replicates per state).

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4.16 Nutrent composition of *Auricularia* across six Southwestern States

Nitrogen content was least in Oyo state (8.85 ± 0.56 mg/kg) and Lagos with the highest (13.59 ± 0.68) while *Auricularia* species found in Osun state recorded the highest magnesium content (116.73 ± 6.07) followed by that of Lagos state (104.46 ±4.40). Phosphorus content was highest in Ogun State (39.25 ± 7.61) sodium content (70.49 ± 3.00) and calcium contents highest (61.90 ± 3.57) in Lagos State. Potassium content was highest (1511.63±168.65) in Osun State followed by Ekiti State (1199.63± 94.52) This is presented in Table 4.15.

4.17 Molecular characterisation of *Auricularia spp*

The major allele frequency, number of alleles, genetic diversity and polymorphic information content (PIC) obtained from the 48 accessions of *Auricularia* Mushroom collected from South West of Nigeria is presented in Table 4.16. Allele frequency ranged from 0.3542 (OPB-15) to 0.6042 (0PH-15), gene diversity from 0.5930 (0PH-15) to 0.7977 (OPB-12) and polymorphic information content from 0.5594 (OPH-15) to 0.7819 (OPB-12).The percentage of polymorphic amplicons varied from 56% to 78.2%. OPB-12 RAPD primer gave the highest level of polymorphism (%) while OPH-5 gave the least (%). Nevertheless, the polymorphisms revealed by the 14 decamer primers indicate that they are good and reliable for genetic diversity assessment in Mushroom and there is a high degree of diversity in the species studied.The banding profiles of the twenty-four *Auricularia* genotypes using RAPD primers OPB-06, OPB-7, OPB-12 and OPH-15 are shown in Plates 4.5-4.8.

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Table 4.16 Nutrient composition of *Auricularia spp* cultivated on wood Substrates in Southwestern Nigeria

State	Dry Weight (mg/kg)					
	Nitrogen	Phosphorus	Sodium	Potassium	Calcium	Magnesium
Oyo	8.85 ± 0.56^{c}	22.29 ± 1.93^{bc}	15.41 ± 0.16^{e}	878.25 ± 75.56^{b}	35.89 ± 1.86^{b}	73.40 ± 4.53^{c}
Lagos	13.59 ± 0.68^{a}	17.25 ± 1.45^{c}	70.49 ± 3.00^{a}	502.88 ± 204.72^{c}	61.90 ± 3.57^{a}	104.46 ± 4.40^{b}
Ondo	9.56 ± 0.52^{c}	18.86 ± 0.63^{c}	18.20 ± 2.28^{de}	331.50 ± 14.89^{c}	36.66 ± 0.52^{b}	69.96 ± 2.99^{c}
Ekiti	9.06 ± 0.14^{c}	32.83 ± 3.58^{ab}	45.03 ± 1.58^{b}	1199.63 ± 94.52^{ab}	33.31 ± 1.78^{b}	70.29 ± 4.90^{c}
Osun	8.96 ± 0.53^{c}	32.69 ± 5.07^{ab}	24.34 ± 4.07^{cd}	1511.63 ± 168.65^{a}	55.01 ± 4.35^{a}	116.73 ± 6.07^{a}
Ogun	11.57 ± 0.80^{b}	39.25 ± 7.61^{a}	29.85 ± 2.19^{c}	478.50 ± 3.33^{c}	40.03 ± 1.37^{b}	56.74 ± 1.02^{d}

Means with same letters along the column are not significantly different according to Duncan Multiple Range Test ($p \leq 0.05$) (N=48. 8 replicates per state)

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Table 4.18: The major allele frequency, number of alleles, genetic diversity and polymorphic information content (PIC) obtained from the 48 accessions of *Auricularia* spp

Primers	Major Allele Freq.	Sample Size	No. of observations	Allele No	Availability	Genetic diversity	PIC
OPB-11	0.4375	48	48	14	1	0.7752	0.7615
OPB-12	0.3958	48	48	13	1	0.7977	0.7819
OPB-15	0.3542	48	48	11	1	0.7891	0.7644
OPB-20	0.4375	48	48	14	1	0.776	0.7627
OPB-21	0.5417	48	48	16	1	0.6892	0.6788
OPH-3	0.4583	48	48	12	1	0.7526	0.7358
OPH-5	0.5625	48	48	6	1	0.6337	0.6005
OPH-10	0.4375	48	48	5	1	0.7188	0.6791
OPH-15	0.6042	48	48	5	1	0.592	0.5594
OPT-1	0.4583	48	48	11	1	0.737	0.713
OPT-5	0.5417	48	48	8	1	0.6528	0.6195
OPT-7	0.4583	48	48	7	1	0.7196	0.6872
OPT-10	0.4583	48	48	16	1	0.7648	0.7536
OPT-19	0.5208	48	48	14	1	0.7023	0.6874
Mean	0.4762	48	48	10.86	1	0.7215	0.6989

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4.18 Phylogenetic relationship

From the phylogenetic reconstruction, six distinct groups were identified. The first group was formed by 8 genotypes (OD1,OD8,OY1,OG1,OG2,LA6,LA7,LA8) second group by 6 genotypes (OD2,OD3,OD4,OD5,OD6 and OD7) third group consisted of 22 genotypes (OG3, OG4, OG5, OG6, OS5, OG7, EK8, EK6, OG8, EK4,EK3,OS1,EK1,OS8,OS7,OS6,EK7,OS4,OS3,OS2,EK2,EK5) while the fourth group was made of 1 genotype (LA5). The fifth group (group 6) had 9 gentypes and finally the sixth group 6 was made up of 2 genotypes (OY3 and OY4).The dendrogram of 48 samples of *Auricularia* from 6 States in South Western Nigeria is presented in Fig. 4.2

4.19 Principal component analysis

Principal component analysis (PCA) of 48 accessions of *Auricularia* collected from 6 States in South West Nigeria is shown in Fig 4.3. The PCA also placed the 48 genotypes of mushroom into 6 groups as was observed in the dendrogram. Group 1 consist of 8 locations (OD1, OD8, OY1, OG1, OG2, LA6, LA7, LA8) Group 2 consist of 6 locations (OD2, OD3, OD4, OD5, OD6 and OD7). Group 3 this variety of mushroom was found in 22 locations. OG3, OG4, OG5, OG6, OS5, OG7, EK8, EK6, OG8, EK4, EK3, OS1, EK1, OS8, OS7, OS6, EK7, OS4, OS3, OS2, EK2, EK5) Group 4 consist of only one genotype which is LA5. While Group 5 genotypes were found in only nine locations (OY2, LA1, LA2, LA3, LA4, OY5, OY6, OY7 and OY8) and predominantly in Lagos state. Finally the Group 6 was found in two locations of Oyo state. (OY3 and OY4|) as observed in the dendrogram.

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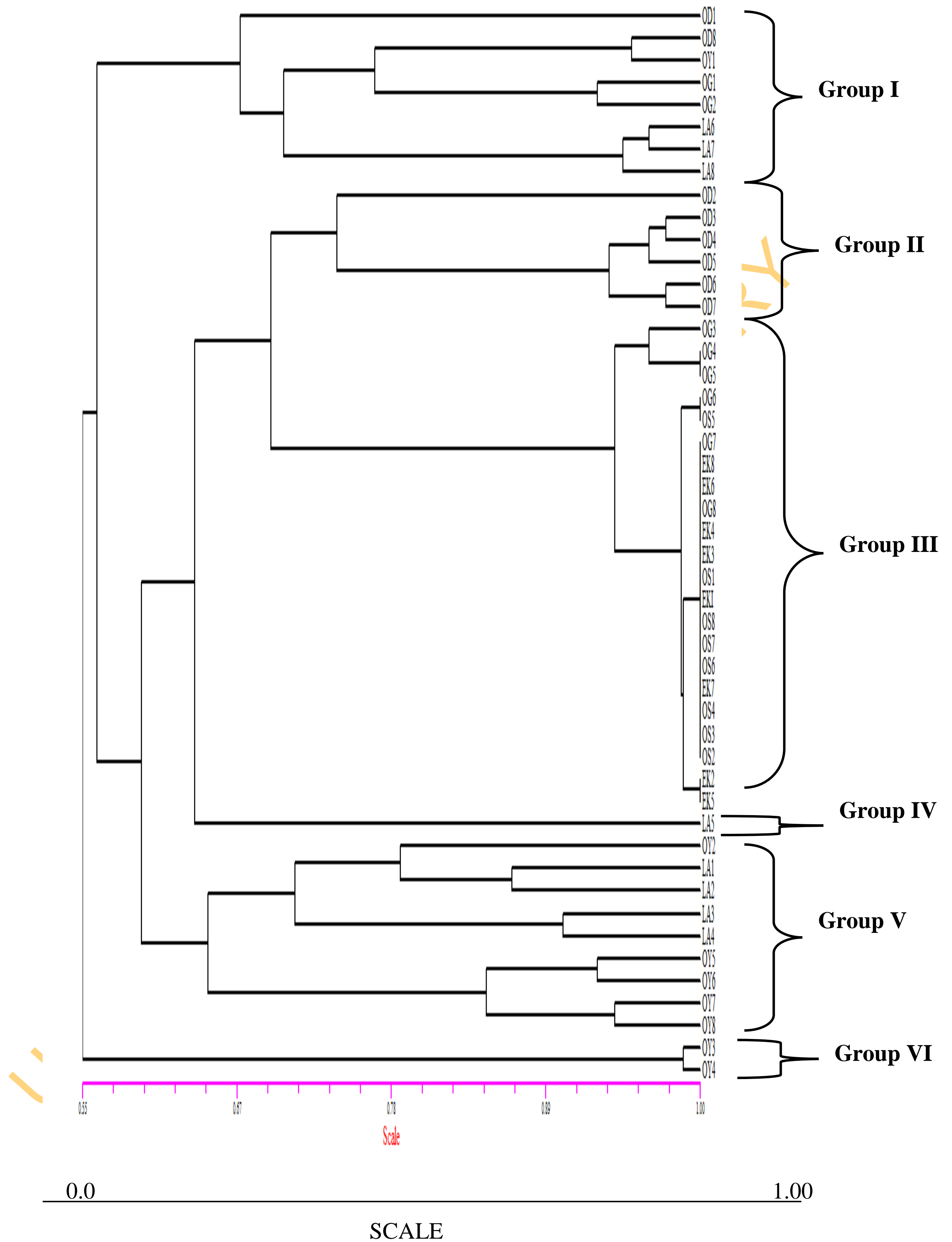

Fig: 4.2 Dendrogram of 48 accessions of *Auricularia* species from 6 states in Southwestern Nigeria.

Group 1 consisted of 8 locations (OD1, OD8, OY1, OG1, OG2, LA6, LA7, LA8) this must have been due to the traders moving the mushroom from one place to the other. Group 2 consisted of 6 locations (OD2,OD3,OD4,OD5,OD6 and OD7) from this group his particular variety is localized in Ondo State but the Group 3 of this variety of mushroom was found in 22 locations (OG3, OG4, OG5, OG6, OS5, OG7, EK8,EK6,OG8,EK4,EK3,OS1,EK1,OS8,OS7,OS6,EK7,OS4,OS3,OS2,EK2,EK5).

This report shows that the variety in Group 3 is prominent in three states and might be due to the similarity in the soil and weather conditions. *Auricularia* species obtained from Ekiti state EK2 and EK5 were the most closely related specie with 100% similiarity compared to the others analyzed using the unweighted pair group method of arithmetic means (UPGMA) as shown on the dendrogram. In Group 4, the variety was found in only one location (LA5) that means that it has not spread to other locations. *Auricularia* in Group 5 was found in only nine locations (OY2, LA1, LA2, LA3, LA4, OY5, OY6, OY7 and OY8) predominant in Lagos state. Finally, the Group 6 was only found in two locations of Oyo state. In addition, the 6 distinct molecularly characterized *Auricularia* spp mushrooms showed a similarity to the morphological characterization of *Auricularia* spp under investigation.

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Figure 4.3: Principal component analysis of 48 samples of *Auricularia* collected from 6 States in Southwestern Nigeria.

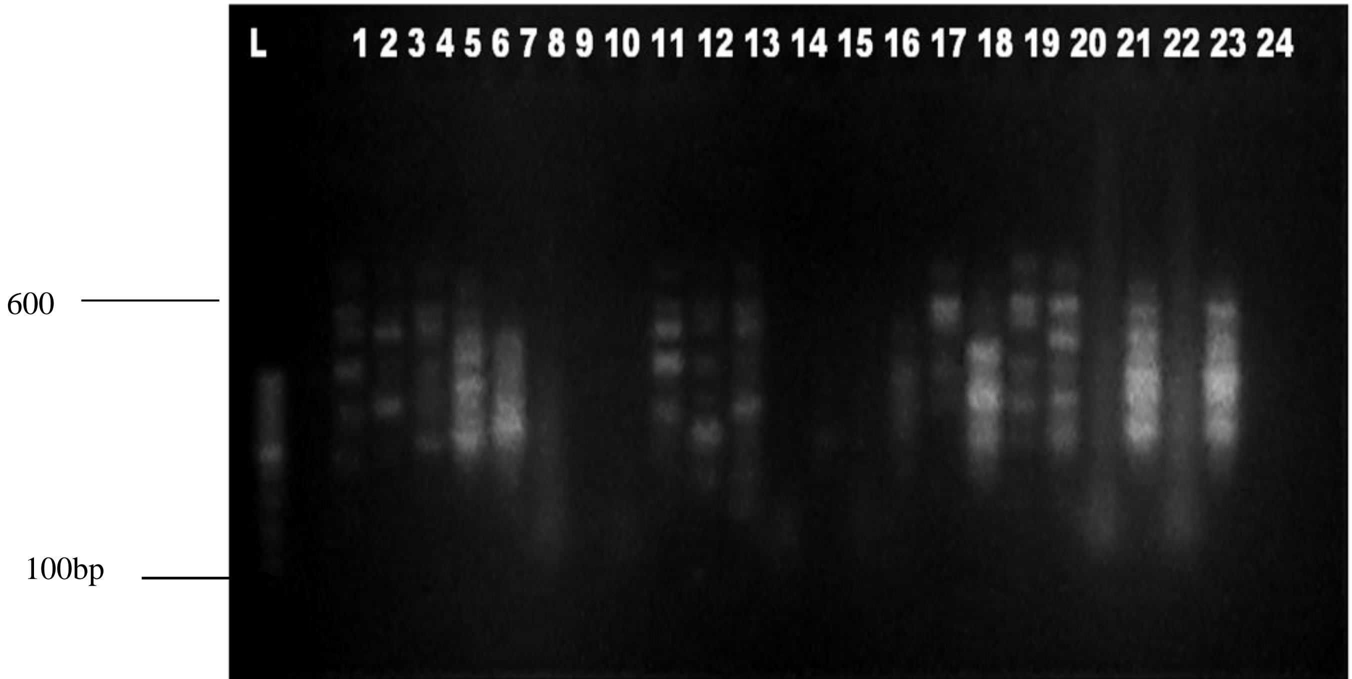

Plate 4.5 :Banding profiles of twenty-four Mushroom genotypes using RAPD primer OPB-06 L= 100 bp DNA ladder,

L = Ladder

1= OG1,2= OG2, 3= OG3,4= OG4, 5= OG5, 6= OG6, 7= OG7,

8=OG8, 9= LA1, 10= LA2, 11=LA3, 12= LA4, 13= LA5, 14= LA6, 15= LA7, 16= LA8, 17 = OY1,

18= OY2,19= OY3, 20= OY4, 21= OY5 ,22= OY6, 23= OY7, 24= OY8

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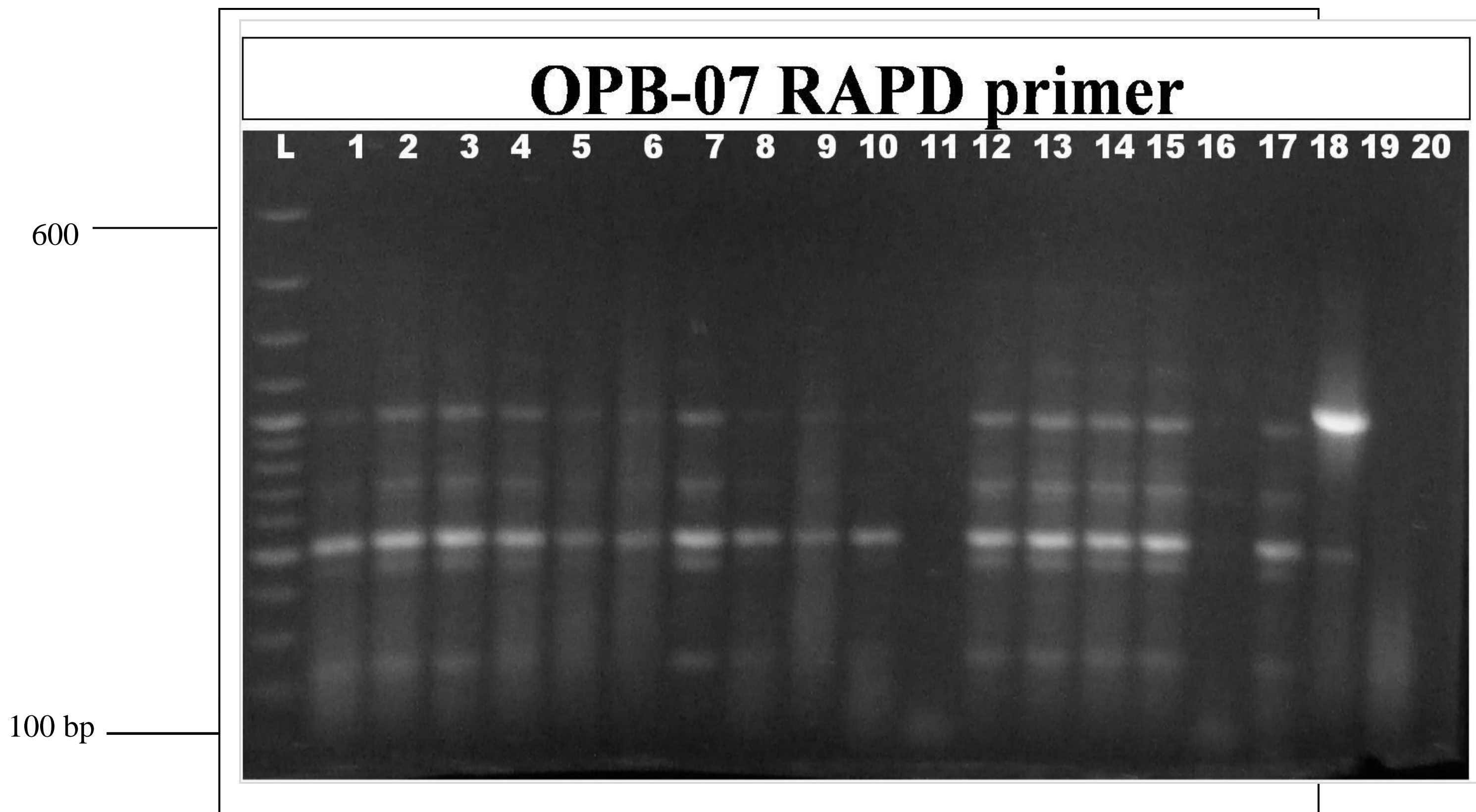

Plate 4.6: Banding profiles of seventeen out of twenty-four Mushroom genotypes using RAPD primer

OPB-07 L= 100 bp DNA ladder,

L = Ladder

1= OG1,2= OG2,3=OG3,4=OG4,5=OG5,6=OG6,7=OG7

,8= OG8, 9= LA1, 10= LA2, 11= LA3, 12= LA4, 13= LA5, 14= LA6, 15= LA7,

16= LA8, 17 = OY1,18= OY2, 19= OY3, 20= OY4, 21= OY5, 22= OY6, 23= OY7,

24= OY8

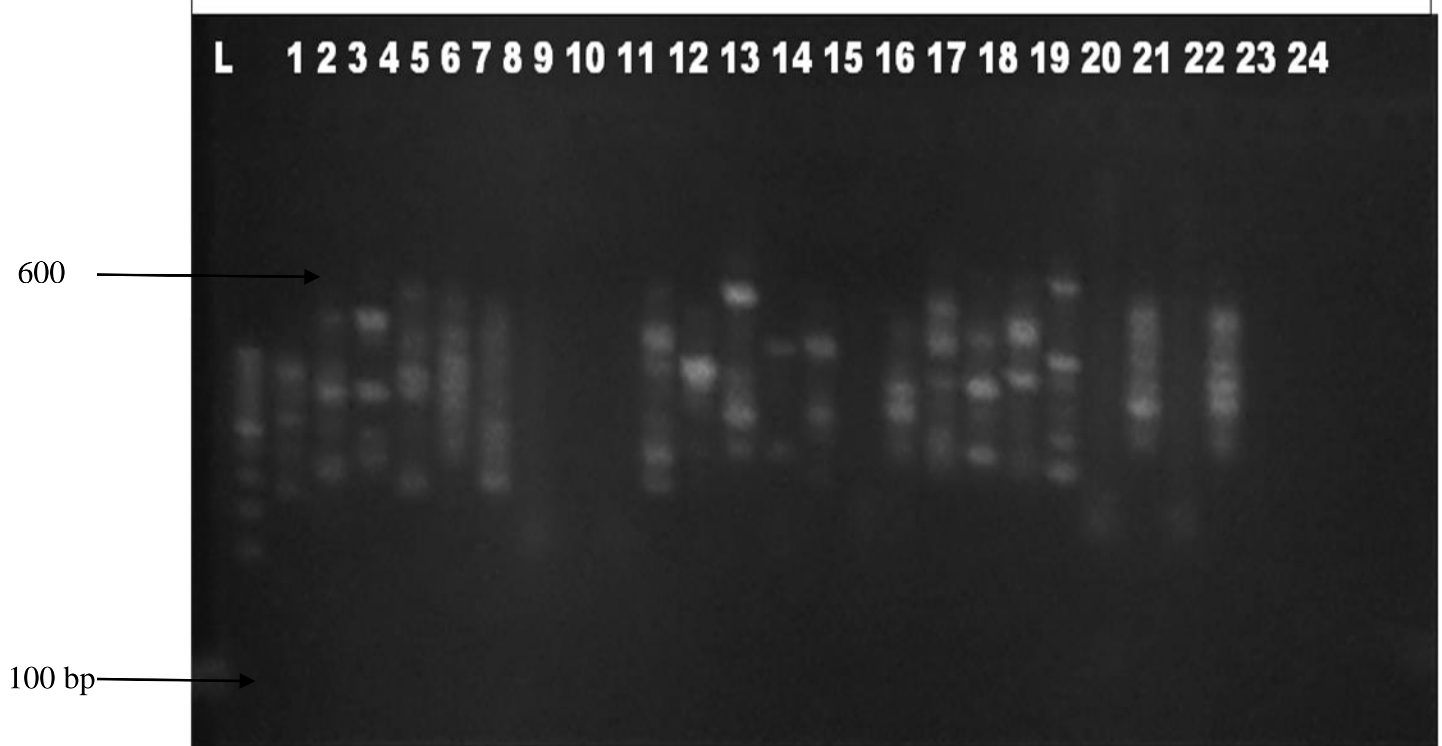

Plate: 4.7 Banding profiles of twenty-four Mushroom genotypes using RAPD primer OPB-12 .L= 100 bp DNA ladder,

L = Ladder

1= OG1,2= OG2,3= OG3,4= OG4,5= OG5,6= OG6,7= OG7, 8= OG8,9= LA1,10= LA2,11= LA3,12= LA4,13=LA5,14= LA6,15=LA7,16= LA8,17=OY1,18= OY2,19= OY3,20= OY4,21= OY5,22= OY6,23= OY7,24= OY8

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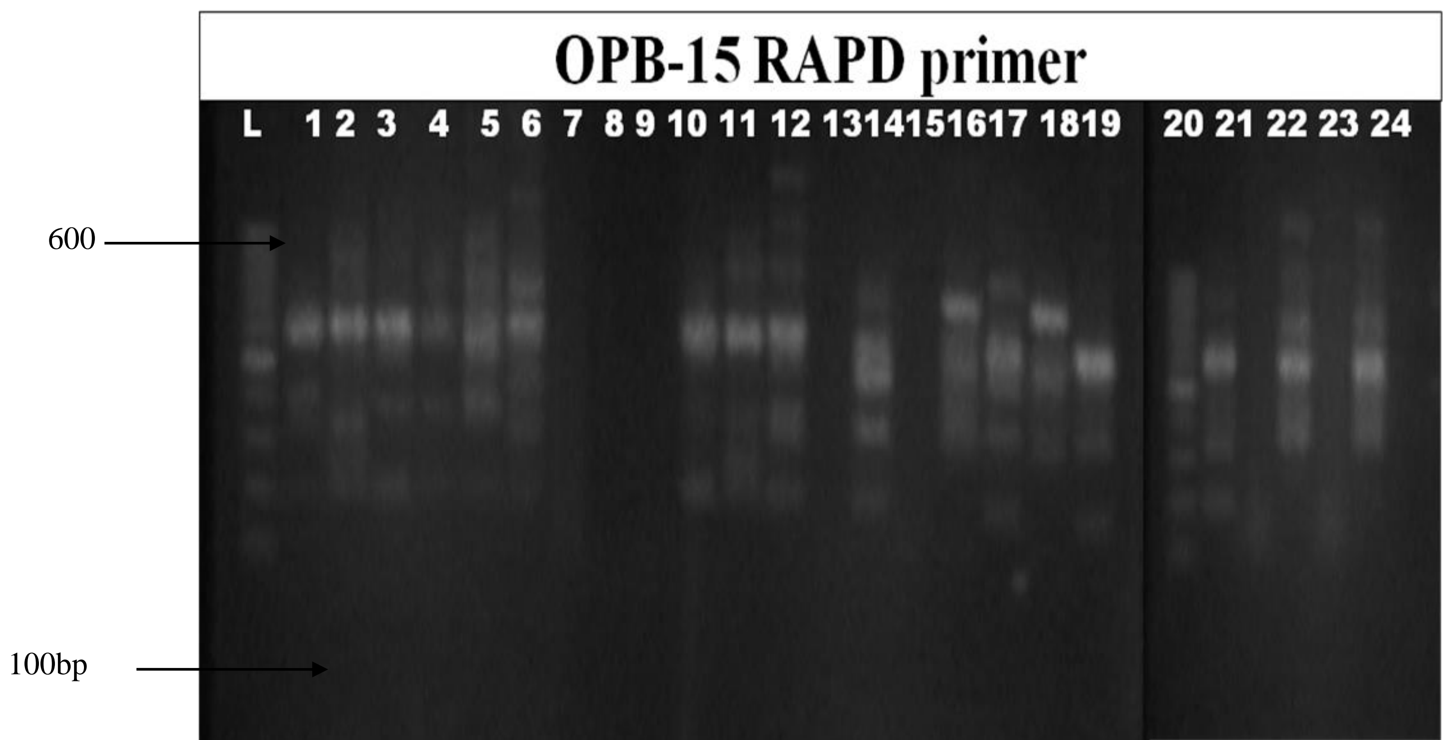

Plate 4.8: Banding profiles of twenty-four Mushroom genotypes using RAPD primer OPB-15 L= 100 bp DNA ladder,

Ladder = Ladder

1= OG1,2= OG2,3= OG3,4= OG4,5= OG5,6= OG6,7= OG7, 8= OG8,9= LA1,10= LA2,11= LA3,12= LA4,13= LA5,14= LA6,15= LA7,16= LA8,17= OY1,18= OY2,19= OY3,20= OY4,21= OY5,22= OY6,23= OY7,24= OY.

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4.20 RAPD profiles

Data generated from the RAPD profiles for Forty eight fragments of similar molecular weight from each individual were scored as present (1) or absent (0). RAPD primers measured the polymorphisms between the genomes of the different samples of the *Auricularia* species. The percentage of polymorphic amplicons varied from 56% to 78.2%. OPB-12 RAPD primer gave the highest level of polymorphism (78.2%) while OPH-5 gave the least (56%). Nevertheless, the polymorphisms revealed by the 15 decamer primers indicate that they are good for genetic diversity assessment in Mushroom and there is a high degree of diversity in the species studied

The forty eight fragments of data obtained from scoring the RAPD bands were used for genetic dissimilarity matrix using Jaccard's similarity coefficient (Jaccard 1908). Phylogenetic relations were determined by cluster analysis using UGPMA (unweighted pair-group method with arithmetic averages) with the NTSYS-pc software version 2.02 (Rohlf 1998) suing Bootstrap analysis of 1000 for accurate result generations. Multivariate grouping was done using principal component analysis (PCA) while polymorphic information content (PIC) was calculated using the method of Botstein *et al*. (1980).

The highest dissimilarity matrix (0.719) was revealed among the Mushroom samples from Oyo State, while the least value (0.000) was recorded among those collected from Ogun and Ondo States. Those from Osun, Ekiti and Lagos States shared dissimilarity values of 0.575 and 0.649 but 0.649 value is the most predominant dissimilarity value among those from Lagos State.

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Fig 4 .4 : Dissimilarity matrix based on Jaccard coefficients among various *Auricularia* species using RAPD data.

CHAPTER FIVE

DISCUSSION

This study evaluated the cultivation of *Auricularia* spp on three log wood substrates (*Cedrela odorata, Gliricidia sepium* and *Mangifera indica*) as well as the assessment of its nutritional status in six Southwestern States of Nigeria (Lagos, Ogun, Ondo, Osun, Oyo and Ekiti States) and the degree of genetic diversity in the *Auricularia* obtained from these states. Most researchers in Nigeria identify mushroom by examining it based on phenotypic characters. It is relatively impossible to distinguish between genetically related species by the morphological characters.. Morphologically, mushrooms belong to the same and even different genera may look similar. Onyango *et al*. (2010) carried out an investigation on *the* morphological identification of *Auricularia* species.

Edible mushrooms can be easily grown and consumed either in fresh, dried or processed form. Mushrooms are known to be excellent sources of protein, besides having a low fat content and are free of cholesterol. Cultivation of edible and medicinal mushroom presents an economically important biotechnological industry that has developed all over the world. In order to ensure the success of *Auricularia* spp cultivation, a good quality spawn is required. Zadrazil *et al.* described that spawn or mycelia run phase involved the growth of mycelium through substrate following inoculation, biodegradation of the substrates ingredients by the mycelium and at the same time the mycelia supports the formation of fruiting bodies (Dang, 2013).

The substrates used in spawn production may be different from the materials used in the cultivation or fruiting of the mushroom. Substrates may be used singly or in combination. Some of the popular and widely used substrates used in the spawn making include various grains such as wheat and sorghum (Chang, 2001). The most crucial factor in domesticating wild mushrooms is the development of appropriate protocols for spawn production (Oei, 2005). Production of spawn is considered as a difficult and fastidious task and is often regarded as non-practical for the common mushroom grower. Therefore, it is usually produced by specialist in spawn manufacturing using microbiological sterile techniques. Having a good understanding of the nutritional and physiological preferences of mushroom mycelia has been

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reported by Zervakis *et al*. (2001) as being essential to its domestication. Grain spawns derived from sorghum and millet has successfully been used in the mushroom industry as the 'seed' for bulk inoculation of substrates (Royse *et al.,* 1997; Stamets; Oei, 2005). Hence the use of sorghum for *A. polytricha* spawn production in this research offered the opportunity to utilise local resources in production of edible, protein-rich mushroom that will sustain food security for the rural populace.

Spawn production was effectively achieved with the use of sorghum grains. Rapid mycelia growth observed in this study may be attributed to a greater food reservoir in large sorghum grains. According to Narain *et al.* (2008), high rates of colonization may be attributed to mycelia getting the most suitable ratio of mixture with a high reservoir of energy and all the nutritional ingredients such as carbon, nitrogen, lipids and minerals. Another factor that may have influenced the rate of mycelia growth is quality of the inoculants used and aeration of the grains.

The quality and yield of mushroom as well as mycelium growth are affected by the nutrient composition as well as the physical nature of the substrate used (Baldrian and Valaskova, 2008). The formation of fruiting bodies or sporophores is critically influenced by the nutritional state as well as physiological condition of the mycelium (Dang 2013). The beginning of fruiting body development is in correlation with nutritional deprivation of the growth substrates (Fan *et al.,* 2008

In a research conducted by Irawati *et al.* (2012) on the cultivation of the edible mushroom *Auricularia polytricha* using sawdust based substrate made of three Indonesian commercial plantation species, *Falcataria moluccana, Shorea sp*., and *Tectona grandis*, biological efficiency (BE)was least when cultivated on *T. grandis* (5.6 ± 1.9) and highest when cultivated in *F. moluccana* (15.6 ± 1.7). The fastest mycelia growth was found in the substrate made of *Shorea sp*. These tree species gave higher BE than the three log wood species utilised in the present study. Mycelial growth and fruiting body formation are greatly affected by tree species and quality (Ohga, 2000). Significant differences were observed in the cultivation of *Auricularia* spp on the log wood substrates from the different states

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Out of the three wood species in Nigeria used for this study, *Mangifera indica* gave the highest average yield of *Auricularia* spp during cultivation when compared to *Cedrela odorata* and *Gliciridium sepium*. This is in agreement with Adenipekun *et al*., (2015), who reported that organic substrates and supplements tested were significantly different ($p<0.05$) in suitability for wood ear mushroom cultivation. The best performance was obtained from maize cobs and wheat straw substrates supplemented with wheat bran and this combination is recommended to *Auricularia* growers.

Agricultural wastes generated from food processing industries and farmers' activities have adverse environmental effects related to their disposal (Gateri *et al.,* 2009) but with the application of appropriate bioconversion technologies like biogas production, these wastes are also potentially useful substrates for the production of mushrooms (Chang and Buswell, 2003). These solid agro-industrial residues are made up of cellulose, hemicelluloses and lignin and also pectin, starch and other polysaccharides and are insoluble in water. *Auricularia polytricha* are commonly produced on a synthetic medium consisting of sawdust, cotton seed hulls, bran, and other cereal grains as nutrient supplements (Dang, 2013). Adenipekun *et al.,* (2015) evaluated different agro-wastes: wheat straw (WS), rice straw (PS), Maize stalk (MS), WS + 4% wheat bran (WB), PS + 4% wheat bran, RS + 4% wheat bran and MS + 4% Wheat bran in an attempt to obtain suitable substrates for cultivation of *Auricularia polytricha.* However, maximum average weight per fruit body was recorded in WS+PS (1:1).

About 60 million tons of rice bran, a by-product of the rice milling process is produced yearly (Pourali *et al.,* 2009) and is usually used as animal feed. Rice bran naturally contains proteins, fibres, vitamins, minerals and antioxidant. Its nutritional property, which can support the growth and development of mushroom, makes it suitable as a supplement source used in mushroom cultivation. In this study, agricultural wastes cotton, sawdust and rice straw were initially used for the cultivation of the mushroom but they failed to produce mycelia growth. The

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procedure was discontinued since fruting bodies needed for this research could not be cropped. This prompted the use of the log wood for the cultivation which gave a good harvest of the *Auricularia* spp.

Three main strains (brown, dark brown and yellow brown) occurring in the forest were previously identified through characterisation using morphological markers (Onyango *et al.,* 2010). Li *et al*. (2011) reported that similar clustered patterns, reveals that all the tested strains could be divided into three distinct groups, each of which was correlated with different geographical regions. More recently, Musngi *et al.,* (2005) used phenotypic differentiations to classify the strains of *Auricularia* in the Philipines. Morphologically three external basidiocarp colours were observed in this study. These were yellowish brown, brown and dark brown. With regard to basidiocarp shapes, there occurred significant variations ranging from auriform, discoid, and flattened. Three external shapes discoid, flattened and auriform and three textures gelatinous, rubbery and leathery types were also observed. The mycelia colour was mostly white and off white in the *Auricularia* spp evaluated in this study.

There are also varying occurrence in the texture of the basidiocarps. Yellowish brown basidiocarps had tougher leathery texture and were auriform in shape. Brown strains were rubbery with flattened shape while the dark brown had soft gelatinous texture. Similar observations were made by Onyango *et al.,* (2011) morphologically characterised Kenyan native wood ear mushroom (*Auricularia auricula).* Nevertheless, they reported yellowish brown basidiocarps of *Auricularia auricula* with soft gelatinous feel and brown basidiocarps with tougher leathery feels which was different in *Auricularia polytricha* investigated in this study. The mycelia structure for the yellowish brown and dark brown *Auricularia polytricha* was cottony while the brown *A. polytricha* was scanty. The brown *A. auricula* was reported to have velvety type mycelia structure by Onyango *et al.,* (2011). This differentiates the specie *auricula* from *polytricha* which had scanty type mycelia structure in this study.

In general, the fruiting bodies of mushrooms contain about 56.8% carbohydrate, 25.0% protein, 5.7% fat and 12.5% ash on a dry weight basis (Ouzouni *et al.,* 2009).

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There was no significant difference in the protein and ash content of *Auricularia* spp in the various states evaluated. The protein content obtained in the present study was higher than that reported in *Auricularia judae* (3.72±0.15%) by Adedotun and Adeniyi (2014). It was however lower than that reported by Usha and Suguna (2014) who investigated the nutritional value of edible mushrooms viz., *Auricularia polytricha* and *Pleurotus ostreatus* in India (36.0% and 33.3%) respectively and in *Auricularia polytricha* (7.2 ± 0.1%) by Hung and Nhi, (2012). However, the carbohydrate contents obtained in the present study was significantly higher (46.19 ± 2.48% and 54.23 ± 1.03%) than that reported by Usha and Suguna, (2014) in *Auricularia polytricha* and *Pleurotus ostreatus* (28.5% and 44.7%) respectively, but it was lower than 88.6 ± 0.2% that was reported by Hung and Nhi, (2012).

Protein is an important constituent of mushrooms. Protein content of mushrooms depends on the composition of the substratum, size of pileus, harvest time and species of mushrooms (Bano and Rajarathnam, 1982). Protein content in *Pleurotus sp.* has been documented to range between 8.9 and 38.7% on dry weight basis (Bano and Rajarathnam, 1982). Rai and Sohi also reported protein content of *Agaricus bisporus* to be 29.3% on dry weight basis (Thatoi and Singdevsachan, 2014). Manjunathan *et al.* (2011) reported the proximate compostion of four wild mushrooms from Tamil Nadu, India in which *A. polytricha* had the highest concentration of protein (37%) and *Clitocybe sp.* had the least (24.8%). The highest protein content observed in this study from *Auricularia* spp from Lagos State (6.98 ± 0.81%) was lower than that reported by Manjunathan *et al.* (2011). The difference in protein contents of mushroom could be due to a number of factors, namely the type of mushroom, the stage of development, the part of the samples, level of nitrogen available and the location (Longvah and Deosthale, 1998).

The bulk of fruiting bodies of mushrooms are made up of carbohydrate content, accounting for 50 to 65% on dry weight basis Manjunathan *et al.* (2011). Pushpa and Purushothama (2010) have analysed the nutrient content of five mushroom species and found 49.20%, 28.38%, 32.08%, 34.88%, 34.36% carbohydrate content in *Agaricus bisporus and Pleurotus florida,* respectively. Manjunathan and Kaviyarasan

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(2011) analysed the nutrient composition of *Lentinus tuberregium* in both wild and cultivated type and found 58.05% and 55.8% carbohydrate in cultivated and wild varieties respectively. Johnsy *et al.* (2011) also studied the nutritional values of wild mushrooms and found good source of carbohydrates ranged from 33.23% in *A. auricula* to 50.2% in *Lentinus tuberregium.* The carbohydrate content obtained in this study ranged from 46.19 ± 2.48% (Osun state) to 54.23 ± 1.03% (Ogun State) in the *Auricularia* spp evaluated from the six south western states of Nigeria which was higher than the values reported by Usha and Suguna, (2014) in *Pleurotus ostreatus* (28.5%) and *Auricularia polytricha* (44.7%).respectively.

Fat content was the lowest proximate parameter in most of the states from which *Auricularia* spp was collected except Ogun (4.36±0.39%) and Ekiti (6.60±0.40%) states with higher fat contents. In mushrooms, the fat content is very low as compared to proteins and carbohydrates (Thatoi and Singdevsachan, 2014). Kavishree *et al*., (2008) have analyzed twenty-three species of naturally grown and collected mushroom fruiting bodies from different geographic locations of India for their total fat and fattyacid contents and mushroom species were found to contain 0.6-4.7% total fat. These mushroom species were also high in unsaturated fatty acids (52-87%), compared to saturated fatty acids. According to proximate composition of four wild mushrooms studied by Manjunathan *et al.,* (2011), the fat contents were less and ranged from 0.74% to 2.25%.

According to Kurtzman (1997), the moisture content of most edible mushrooms ranges from 85-94% but Chang and Miles (1989) reported that the moisture content ranged from 70-94% and for tough edible mushroom, 50-75%. Johnsy *et al.,* (2011) observed that the moisture content of collected mushroom samples (*Pleurotus roseus, Pleurotus ostreatus, Pleurotus sajor caju, Termitomyces microcarpus, Termitomyces heimii, Auricularia auricular, Volvariella volvacea, Lentinus squarrosulus, Lentinus tuberegium and Grifola frondosa*) ranged from 87.13% to 95.17%. The moisture content obtained in this study from *Auricularia* spp cultivated from six Southwestern states ranged from 10.43 ± 0.19 % (Ogun State) to 16.22 ± 0.65% (0ndo State). In the the study of edible mushrooms namely., *Auricularia polytricha* and *Pleurotus*

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ostreatus by Usha and Suguna, (2014), the moisture contents were 90.6% and 93.3% respectively. Gbolagade *et al.* (2006) also reported high moisture content of 97.1% in *Auricularia polytricha*. Mushrooms cultivated in this study had lower moisture contents.

Khan *et al*. (2008) reported the fibre content in some edible mushrooms range from 26.2% *Pleurotus sajor-caju*, 27%, *Pleurotus ostreatus* while *Pleurotus florida* had 26.8%, *Pleurotus cystidiosus* was 25.5%, and in *Pleurotus geestaranus* 26.3% respectively. Adedotun and Adeniyi, (2014) evaluated the nutritional and anti-nutritional characteristics of some dominant fungi species in South Western Nigeria and observed the following for crude fibre content *Pleurotus sajorcajor* (11.53±0.27), *Auricularia judae* (2.49±0.27%), *Xylaria hypoxylon* (36.81±0.27%) *Coltricia perennis* (22.54±0.27%), *Xylaria polymorpha* (29.16±0.27) *Trametes vesicolor* (24.22±0.27%). Fibre contents observed from cultivated *auricularia* spp in this study ranged from 18.49 ± 0.37% (Lagos state) to 25.13 ± 2.48% (Osun state).

Major mineral constituents in mushrooms are Na, K, Ca, Mg, P, S and elements like As, Cd, Cr, Co, Cu, Fe, Mo, Mn, Ni, Pb, Se, Zn among others form minor constituents (Bano and Rajarathanum, 1982). Mattilla *et al.* (2001) reported that the mineral content of wild edible mushrooms were higher than cultivated ones. Micronutrient profile of seven wild edible mushrooms were analyzed by Agrahar-murugkar and Subbulakshmi (2005) which are commonly consumed in the Khasi hills of Meghalaya and reported that the calcium (g) content ranged from 0.42 in *Clitocybe cibarius* to 1.91 in *Clitocybe cineria*. Phosphorus (g) levels were the highest in *Clitocybe cibarius* (0.58g).

Manjunathan *et al.,* (2011) studied the maco and micro mineral contents of four wild mushrooms and reported that the calcium content was 208 mg/g for *Clitocybe* sp., and 195 mg/g for *Macrolepiota rhodocus*. The highest sodium and potassium content (858.4 and 1369.1 mg/g respectively) was found in *Clitocybe* sp. whereas *M. rhodocus* had the highest magnesium content (250 mg/g). Furthermore, iron content

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varied from 16.3 mg/g (*A. polytricha*) to 85.6 mg/g (*Macrolepiota rhodocus*) while copper content ranged from 0.3 mg/g (*A. polytricha*) to 9.0 mg/g (*M.rhodocus*).

Singdevsachan *et al.* (2013) recently reported the mineral contents of two wild mushrooms (*Lentinus sajor-caju* and *Lentinus torulosus*) from Similipal Biosphre Reserve, Odisha, India. *L. torulosus* showed the highest iron (2.94 mg/kg), potassium (0.85 mg/kg) and phosphorus (0.24 mg/kg) contents whereas *Lentinus sajor-caju* showed the highest manganese (0.12 mg/kg) and nickel (0.05 mg/kg) contents. The levels of macro minerals, potassium, phosphorous manganese and magnesium contents were higher in the *Auricularia* spp evaluated from the six south western states of Nigeria than in *L. torulosus* reported by Singdevsachan *et al.* (2013). The mineral proportions of mushrooms vary according to the species, age and the diameter of the fruiting body as well as on the type of the substratum (Demirbas, 2001).

Molecular markers such as rDNA sequencing, Restriction fragment length polymorphism (RFLP), Random amplified polymorphic DNA (RAPD) and genotyping have been used to discriminate mushroom species or strains of *Agaricus, Auricularia, Ganoderma, Lentinula, Stropharia,* and *Volvariella.* All of these technologies provided data for mushroom strain identification and protection (Chandra *et al.,* 2010). Mushrooms genetic diversity studies have been previously determined using molecular markers especially RAPD (Ravash *et al.,* 2009; Staniaszek *et al.,* 2013). Analysis of genetic diversity is very important in fungi in practical pharmacology and cultivation programs, and RAPD technique is a useful tool to analyze the genetic diversity among *Auricularia. polytricha* strains.

The genetc diversity of *A. polytricha* was assessed using 15 RAPD primers. RAPD primers can measure the polymorphisms between the genomes of two organisms of the same species. The highest polymorphism was observed in primer OPB-12 (PIC value 0.7819) and primer OPB-15 (PIC value 0.7644). The values of the major alleles ranged from 0.3542 -0.6042. The genetic diversity ranged from 0.592 (OPH-5) to 0.798 (OPB-12). The percentage of polymorphic amplicons varied from 56% (OPH-5) to 78.2% (OPB-12). OPB-12 was the most informative primer for diversity studies

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in *Auricularia* spp evaluated in this study. The highest number of alleles (alternate form of a gene) was recorded in primer OPB-21 and OPT-10 (16.0000) while the least was found to be (5.0000) in primers OPH-10 and OPH-15. Nevertheless, the polymorphisms revealed by the 14 decamer primers indicate that they are good and reliable for genetic diversity assessment in mushrooms and there is a high degree of diversity in the species studied. An average polymorphism of 69.9% was obtained.

This report was in accordance with the discovery of Khan *et al.,* (2011) who conducted molecular characterization of Oyster mushroom *(Pleurotus* spp.) using 14 RAPD primers and obtained the highest polymorphism by primers OPL3 (72.70 %) and OPL11 (70%). Two species (P-56 and P-17) were found to be genetically similar having a similarity value of 86%. The result obtained in this study also agrees with the report of Ravash *et al.,* (2009) who used RAPD markers to confirm the similarity or dissimilarity of genetic relationship of *Pleurotus* spp.

The genes in DNA molecule are known to carry the information that controls the organism. In essence, the information on the difference between the genetic makeup of some macrofungi indigenous to Nigeria and the genes of their close relatives has a lot of implication on the type(s) of bioactive combines they can produce. But in this study, all the *Auricularia* species found in the Southwestern states could be grouped into six cluster using the result of the molecular characterisation

As earlier reported, the Groups 1 and 2 *Auricularia* spp were dark brown, discoid in shape and gelatinous in texture while Groups 3 and 5 were yellowish brown, auriform in shape and leathery in texture. On the other hand, Groups 4 and 6 were brown in colour with flattened shape and rubbery in texture. Both the dendrogram and the principal component analysis grouped the accessions into 6 distinct groups based on states and morphological characters. The RAPD analysis in this study has proven to be useful in discrimination, characterization and differentiation of *Auricularia* varieties and grouped them according to similarity.

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CHAPTER SIX

CONCLUSIONAND RECOMENDATIONS

6.1 Conclusion

There is an increase in the cultivation of edible mushroom for research and consumption. Most of these mushrooms are collected from the wild, secondary forests and local markets which are later cultivated to prevent extinction. In this report, *Auricularia auricula* mushroom is generally accepted by the three major tribes in Nigeria as food and for medicinal purposes.

Characterisation of these mushrooms which leads to helped in identification purpose is very imperative for both the consumers and sellers. Biotechnology has made it possible to differentiate mushrooms that are phenotypically similar to be quite different. The use of molecular tools like Random Amplified Polymorphic DNA (RAPD) has been useful in the identification of *Auricularia* spp. From the Phylogenetic tree obtained in this study, the samples collected are related and can be grouped into six categories. The areas and locations that *Auricularia* samples were collected from in Southwestern Nigeria were different and have uncommon barriers.

From this report, it is obvious that morphological traits alone may not be solely used to classify fungi and the result also show that in Southwestern Nigeria, *Auricularia* spp can be grouped genomically into 6 groups in terms of molecular relationship and evolutionary trend.

Dendrograms were constructed by Unweighted Pair-group Method with Arithmetic Averages (UPGMA) method, and the principal component analysis (PCA) exhibited similar clustered patterns, revealing that all the tested strains could be divided into six distinct groups, each of which correlated with different geographical regions.

6.2 RECOMMENDATION

1. Mass production of *Auricularia* spp is achievable when large quantities of *Mangifera indica* wood are collected and inoculated with the seed of *Auricularia* species.

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2. In order to have a good report of mushroom classification, morphological and molecular characteristics are very important in the classification of *Auricularia* spp. It is imperative to know that there might be a wide genetic diversity or relationship of a growing *Auricularia* mushroom that have similar ecological proximity or different locations in a given area.
3. At the tissue culture stage of spawn production, the seed of mushroom (spawn) should be subjected to molecular screening so as to be sure of the genotype of mushroom that is to be cultivated. This is because, it has been proved that mushroom that have similar morphological or physiological characteristics may not necessarily be the same, and this can lead to growing unidentified mushroom.
4. When considering Mushroom cultivation, it is important to ensure that the need of the expanding population is met through the availability of edible mushroom and the seed(s)/spawn and also, to double the profit margin realized at the end bearing in mind that mushroom cultivation is a business entity.
5. Mushroom farmers are in great need of spawn (seed) without which mushroom cannot be cultivated because of the laboratory items needed. Research results on mushroom cultivation should be transferred to the farmers through extension workers and this will definitely create employment for the unemployed youth and adults.
6. The cultivation of *Auricularia* species on logs of wood is well recommended in areas where trees are in abundance and also readily accessible. The preferred period to use log is when the leaves are just beginning to dry (autumn).

6.3 Contributions To Knowledge

1. *Mangifera indica* log wood was the best substrate for the cultivation of *Auricularia* spp for commercial production in Southwestern Nigeria.It can be used to grow and domesticate wild *Auricularia* species.
2. *Auricularia polythricha* from Lagos state had the highest protein (6.98 ± 0.81%) and moisture contents (14.10±0.32%) with low levels of fat (2.76±0.08%). Hence consumption of this variety is recommended as an

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alternative source of protein and can be used for low cholesterol required food.

3. Primer OPB-12 which showed the highest range of genetic diversity is the most useful RAPD primer for diversity studies in *Auricularia* spp.

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APPENDIX I

SCREENING OF RAPD PRIMERS

			OPH-15				OPT1						OPT-5				OPT-7					OPT-10							OPT-19										
0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
0	1	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	1	0	0	0	0	0	0	0	1	1	1	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
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1	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0
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APPENDIX II

Results for chemical analysis of mushroom (mg/kg) Dry Weight

S/N	Sample code	Nitrogen	Phosphorus	Sodium	Potassium	Calcium	Magnesium
1	EK-1	9.37	48.3	38.5	1630	30.3	93.5
2	EK-2	9.14	36.5	47.2	1248	33.1	91.5
3	EK-3	8.37	37.7	46.3	1330	28.5	63.2
4	EK-4	8.63	35.8	47.7	1372	30.4	64.4
5	EK-5	8.99	37.4	46.3	1139	27.7	63.5
6	EK-6	9.10	29.6	40.6	842	41.8	63.0
7	EK-7	9.41	18.3	41.5	833	37.2	64.8
8	EK-8	9.44	19.0	52.1	1203	37.5	58.4
9	LA-1	11.2	19.3	64.4	274	61.3	78.7
10	LA-2	16.3	19.6	68.9	388	61.7	103
11	LA-3	12.3	21.0	67.4	283	66.4	112
12	LA-4	12.7	18.7	58.7	296	37.8	117
13	LA-5	15.3	18.4	84.3	1933	69.3	112
14	LA-6	15.7	19.5	77.4	284	66.4	105
15	LA-7	13.4	10.4	77.5	286	66.8	94.1
16	LA-8	11.8	11.1	65.3	279	65.5	111
17	OG-1	8.94	52.1	27.4	475	41.8	54.8
18	OG-2	8.93	65.4	26.2	487	31.0	55.4
19	OG-3	9.07	66.6	26.7	468	44.2	54.0
20	OG-4	13.4	50.7	26.8	491	40.3	56.8
21	OG-5	13.7	18.3	37.2	464	40.6	63.2
22	OG-6	13.0	19.9	24.3	486	41.6	57.4
23	OG-7	13.9	20.4	28.4	480	40.0	55.0
24	OG-8	11.6	20.6	41.8	477	40.7	57.3

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Results for chemical analysis of mushroom (mg/kg) Dry Weight CONT

S/ N	Sample code	Nitrogen	Phosphorus	Sodium	Potassium	Calcium	Magnesium
25	OD-1	10.4	15.3	18.4	326	38.4	78.5
26	OD-2	11.4	20.0	17.8	366	36.7	73.6
27	OD-3	10.2	21.1	26.3	384	36.8	74.4
28	0D-4	10.8	18.4	10.6	375	34.1	74.7
29	OD-5	7.39	18.4	11.4	295	36.5	76.5
30	OD-6	9.44	19.6	14.3	275	37.0	58.9
31	OD-7	9.37	20.1	18.3	288	35.3	67.4
32	OD-8	7.48	18.0	28.5	343	38.5	55.7
33	OS-1	8.38	48.4	22.1	1842	55.6	82.8
34	OS-2	7.39	47.9	17.4	1836	55.3	103
35	OS-3	8.74	48.9	17.4	1684	53.0	112
36	OS-4	10.5	31.4	22.5	1773	51.4	117
37	OS-5	11.9	19.5	52.4	1830	53.7	131
38	OS-6	8.40	32.2	21.0	1631	39.6	127
39	OS-7	8.41	14.8	21.2	749	82.7	133
40	OS-8	7.98	18.4	20.7	748	48.8	128
41	OY-1	10.5	28.6	15.3	736	37.5	83.2
42	OY-2	6.38	27.0	15.7	744	37.9	78.4
43	OY-3	6.98	25.9	16.3	830	31.0	78.3
44	OY-4	8.38	27.6	15.4	1264	26.7	78.5
45	OY-5	8.51	19.3	15.0	1173	39.5	78.6
46	OY-6	10.3	16.3	14.8	789	43.8	81.0
47	OY-7	10.4	16.8	15.6	746	36.5	64.3
48	OY-8	9.33	16.8	15.2	744	34.2	44.9

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APPENDIX III

PROXIMATE ANALYSIS

Lab No	Sample	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
20142053	**OYI**	6.58	6.96	12.37	3.11	19.24	51.74
20142054	**OY2**	4.59	3.76	11.56	2.87	20	57.22
20142055	**OY3**	7.43	5.39	14.24	3.54	21.34	48.06
20142056	**OY4**	5.87	6.06	10.56	3.83	18.66	55.02
20142057	**OY5**	7.86	3.72	11.47	4.06	18.21	54.68
20142058	**OY6**	4.94	2.32	12.38	3.47	19.35	57.54
20142059	**OY7**	5.87	5.1	11.64	4.11	19	54.28
20142060	**OY8**	6.93	1.9	15.66	3.81	18.66	53.04
20142061	**LA1**	7.57	5.96	14.74	2.57	17.64	51.52
20142062	**LA2**	6.22	6.11	14.33	2.68	17.23	53.43
20142063	**LA3**	9.13	4.38	12.87	2.51	18.11	53,00
20142064	**LA4**	7.07	3.77	14.56	2.49	18.56	53.55
20142065	**LA5**	2.74	4.16	13.34	2.98	17.84	58.94
20142066	**LA6**	5.09	5.23	14.62	2.84	19.64	52.58
20142067	**LA7**	9.7	1.94	12.98	3.11	18.55	53.72
20142068	**LA8**	8.35	2.79	15.34	2.89	20.34	50.29
20142069	**ODI**	6.11	3.11	14.16	3.87	22.04	50.71
20142070	**OD2**	6.09	4.12	15.72	3.47	18.67	51.93
20142071	**OD3**	6.24	4.57	16.26	4.11	21.31	47.51
20142072	**OD4**	5.97	6.13	18.12	2.99	19.33	47.46
20142073	**OD5**	6.33	5.44	19.1	3.28	21.44	44.41
20142074	**OD6**	6.71	3.47	16.46	3.52	19.64	50.20
20142075	**OD7**	5.91	5.79	13.51	2.96	20.37	51.46
20142076	**OD8**	6.55	6.02	16.4	3.11	21.24	46.68
20142077	**APEK 3**	7.04	2.75	14.32	6.84	18.96	50.09
20142078	**EK7**	7.11	2.5	11.11	6.17	19.35	53.76
20142079	**EK8**	6.97	7.65	10.97	7.04	17.66	49.71
20142080	**EK4AP**	5.89	1.9	10.77	6.66	19.64	55.14
20142081	**EK5**	6.11	4.79	11.28	6.59	17.39	53.84
20142082	**APEK 2**	7.23	2.43	14.67	7.04	18.63	50

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PROXIMATE ANALYSIS (Continued)

Lab No	Sample	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
20142083	**APEK 1**	6.74	3.07	15.11	6.55	18.61	49.92
20142084	**EK6**	6.54	5.9	14.32	5.88	19.05	48.31
20142085	**OS4AP**	5.77	4.13	15.67	5.36	21.49	47.58
20142086	**OS 8**	6.44	2.96	18.94	4.47	24.87	42.32
20142087	**OS 5**	6.38	3.77	14.33	5.62	18.59	51.31
20142088	**OS 7**	6.97	3.82	15	4.98	35.76	33.47
20142089	**OS 6**	7.12	5.33	12.37	3.66	13.76	57.76
20142090	**APOS 3**	6.71	5.96	9.77	4.42	28.62	44.52
20142091	**APOS2**	6.83	6.43	8.76	2.98	27.32	47.68
20142092	**APOS1**	6.18	5.33	9.24	3.77	30.59	44.89
20142093	**OG1**	5.33	3.74	10.34	6.84	22.34	51.41
20142094	**OG2**	5.14	2.96	11.12	6.16	24.56	50.06
20142095	**OG3**	5.07	3.11	9.75	4.88	23.57	53.62
20142096	**OG4**	4.95	3.04	10.37	4.00	21.11	56.53
20142097	**OG5**	4.78	2.74	11.21	2.58	20.94	57.75
20142098	**OG6**	5.97	3.48	10.27	2.8	21.37	56.11
20142099	**OG7**	5.44	3.15	9.78	2.48	22.44	56.71
20142100	**OG8**	5.78	2.88	10.56	5.16	23.98	51.64

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APPENDIX IV DESCRIPTIVE STATISTICS

Descriptives[a]									
		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Spawn running (days)	C. indica	8	26.38	3.159	1.117	23.73	29.02	20	29
	G. sepium	8	22.13	1.808	0.639	20.61	23.64	18	24
	C. odorata	8	24.88	4.998	1.767	20.7	29.05	16	34
	Total	24	24.46	3.856	0.787	22.83	26.09	16	34
Pin head Formation (days)	C. indica	8	32.13	3.682	1.302	29.05	35.2	26	39
	G. sepium	8	29.13	3.227	1.141	26.43	31.82	23	34
	C. odorata	8	30.63	4.719	1.668	26.68	34.57	25	40
	Total	24	30.63	3.954	0.807	28.96	32.29	23	40
Fruiting body formation (days)	C. indica	8	34	3.464	1.225	31.1	36.9	28	40
	G. sepium	8	31.5	2.828	1	29.14	33.86	26	36
	C. odorata	8	32.13	4.581	1.619	28.3	35.95	27	41
	Total	24	32.54	3.695	0.754	30.98	34.1	26	41
Average yield (gms)	C. indica	8	9.6463	1.35353	0.47855	8.5147	10.7778	7.95	11.8
	G. sepium	8	9.7137	1.38942	0.49123	8.5522	10.8753	8.23	12.2
	C. odorata	8	9.6988	1.70063	0.60126	8.277	11.1205	7.99	12
	Total	24	9.6863	1.42345	0.29056	9.0852	10.2873	7.95	12.2
Dry weight (gms)	C. indica	8	2.8438	0.137	0.04844	2.7292	2.9583	2.64	3.01
	G. sepium	8	2.9875	0.14907	0.0527	2.8629	3.1121	2.69	3.16
	C. odorata	8	3.0188	0.13141	0.04646	2.9089	3.1286	2.77	3.14

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	Total	24	2.95	0.15424	0.03148	2.8849	3.0151	2.64	3.16
Biological efficiency	C. indica	8	3.1875	0.45481	0.1608	2.8073	3.5677	2.6	3.9
	G. sepium	8	3.2025	0.47467	0.16782	2.8057	3.5993	2.7	4.06
	C. odorata	8	3.2175	0.59151	0.20913	2.723	3.712	2.6	4
	Total	24	3.2025	0.48802	0.09962	2.9964	3.4086	2.6	4.06
Width of pileus (cm)	C. indica	8	4.513	1.1344	0.4011	3.564	5.461	2.2	5.8
	G. sepium	8	4.225	0.9939	0.3514	3.394	5.056	2.7	5.6
	C. odorata	8	3.6	0.6676	0.236	3.042	4.158	2.6	4.8
	Total	24	4.113	0.9896	0.202	3.695	4.53	2.2	5.8
Days to mature fruiting body (D)	C. indica	8	4	1.414	0.5	2.82	5.18	2	6
	G. sepium	8	3.25	0.707	0.25	2.66	3.84	2	4
	C. odorata	8	3	1.069	0.378	2.11	3.89	2	4
	Total	24	3.42	1.139	0.232	2.94	3.9	2	6
Growth Index (%)	C. indica	8	132.88	72.379	25.59	72.36	193.39	44	260
	G. sepium	8	136.81	48.447	17.129	96.31	177.32	68	225
	C. odorata	8	134.38	50.599	17.889	92.07	176.68	65	200
	Total	24	134.69	55.594	11.348	111.21	158.16	44	260

a. State = Oyo

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ANOVA[a]

		Sum of Squares	Df	Mean Square	F	Sig.
Spawn running (days)	Between Groups	74.333	2	37.167	2.916	0.076
	Within Groups	267.625	21	12.744		
	Total	341.958	23			
Pin head Formation (days)	Between Groups	36	2	18	1.168	0.33
	Within Groups	323.625	21	15.411		
	Total	359.625	23			
Fruiting body formation (days)	Between Groups	27.083	2	13.542	0.991	0.388
	Within Groups	286.875	21	13.661		
	Total	313.958	23			
Average yield (gms)	Between Groups	0.02	2	0.01	0.005	0.995
	Within Groups	46.583	21	2.218		
	Total	46.603	23			
Dry weight (gms)	Between Groups	0.139	2	0.07	3.588	0.046
	Within Groups	0.408	21	0.019		
	Total	0.547	23			
Biological efficiency	Between Groups	0.004	2	0.002	0.007	0.993
	Within Groups	5.474	21	0.261		
	Total	5.478	23			
Width of pileus (cm)	Between Groups	3.482	2	1.741	1.92	0.171
	Within Groups	19.044	21	0.907		
	Total	22.526	23			
Days to mature fruiting body (D)	Between Groups	4.333	2	2.167	1.784	0.192
	Within Groups	25.5	21	1.214		
	Total	29.833	23			
Growth Index (%)	Between Groups	63.188	2	31.594	0.009	0.991
	Within Groups	71022.719	21	3382.034		
	Total	71085.906	23			

a. State = Oyo

UNIVE

Descriptives[a]

		N	Mean	Std. Dev.	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
	C. indica	8	25	5.831	2.062	20.13	29.87	12	29
	G. sepium	8	21.25	5.445	1.925	16.7	25.8	8	25
	C. odorata	8	23.5	5.707	2.018	18.73	28.27	10	29
Spawn running (days)	Total	24	23.25	5.636	1.15	20.87	25.63	8	29
	C. indica	8	29	4.243	1.5	25.45	32.55	21	33
	G. sepium	8	25.5	4.84	1.711	21.45	29.55	14	29
Pin head Formation (days)	C. odorata	8	27.13	4.357	1.54	23.48	30.77	17	31
	Total	24	27.21	4.53	0.925	25.3	29.12	14	33
	C. indica	8	30.88	4.19	1.481	27.37	34.38	23	35
	G. sepium	8	27.38	4.749	1.679	23.4	31.35	16	31
Fruiting body formation (days)	C. odorata	8	29.38	4.438	1.569	25.66	33.09	19	33
	Total	24	29.21	4.511	0.921	27.3	31.11	16	35
	C. indica	8	8.8888	0.6745	0.23847	8.3249	9.4526	8.21	10.05
	G. sepium	8	9.3775	1.41629	0.50073	8.1935	10.5615	7.95	11.89
	C. odorata	8	9.5163	1.48224	0.52405	8.2771	10.7554	7.99	12.2
Average yield (gms)	Total	24	9.2608	1.22196	0.24943	8.7448	9.7768	7.95	12.2
	C. indica	8	3.0388	0.08374	0.02961	2.9687	3.1088	2.88	3.14
	G. sepium	8	2.8425	0.20797	0.07353	2.6686	3.0164	2.55	3.1
	C. odorata	8	2.985	0.10254	0.03625	2.8993	3.0707	2.86	3.14
Dry weight (gms)	Total	24	2.9554	0.16016	0.03269	2.8878	3.023	2.55	3.14
Biological efficiency	C. indica	8	2.925	0.21876	0.07734	2.7421	3.1079	2.7	3.3
	G. sepium	8	3.075	0.47132	0.16664	2.681	3.469	2.6	3.9
	C. odorata	8	3.1375	0.48385	0.17107	2.733	3.542	2.6	4
	Total	24	3.0458	0.40215	0.08209	2.876	3.2156	2.6	4

	C. indica	8	3.363	0.715	0.2528	2.765	3.96	2.1	4.3
	G. sepium	8	3.7	0.7653	0.2706	3.06	4.34	2.8	4.8
	C. odorata	8	4.388	1.0947	0.387	3.472	5.303	2	5.2
Width of pileus (cm)	Total	24	3.817	0.9426	0.1924	3.419	4.215	2	5.2
	C. indica	8	3.25	1.282	0.453	2.18	4.32	2	6
	G. sepium	8	3.5	1.309	0.463	2.41	4.59	2	6
Days to mature	C. odorata	8	3.13	0.835	0.295	2.43	3.82	2	4
fruiting body (D)	Total	24	3.29	1.122	0.229	2.82	3.77	2	6
	C. indica	8	116.05	48.362	17.098	75.62	156.48	53	200
	G. sepium	8	123.11	61.251	21.656	71.91	174.32	47	235
	C. odorata	8	154.36	71.872	25.41	94.28	214.45	67	260
Growth Index (%)	Total	24	131.18	60.95	12.441	105.44	156.91	47	260

a. State = Lagos

ANOVA[a]

		Sum of Squares	df	Mean Square	F	Sig.
Spawn running (days)	Between Groups	57	2	28.5	0.889	0.426
	Within Groups	673.5	21	32.071		
	Total	730.5	23			
Pin head Formation (days)	Between Groups	49.083	2	24.542	1.219	0.316
	Within Groups	422.875	21	20.137		
	Total	471.958	23			
Fruit body formation (days)	Between Groups	49.333	2	24.667	1.237	0.31
	Within Groups	418.625	21	19.935		
	Total	467.958	23			
Average yield (gms)	Between Groups	1.738	2	0.869	0.56	0.58
	Within Groups	32.605	21	1.553		
	Total	34.343	23			
Dry weight (gms)	Between Groups	0.165	2	0.082	4.061	0.032
	Within Groups	0.425	21	0.02		
	Total	0.59	23			
Biological efficiency	Between Groups	0.191	2	0.095	0.568	0.575
	Within Groups	3.529	21	0.168		
	Total	3.72	23			
Width of pileus (cm)	Between Groups	4.366	2	2.183	2.853	0.08
	Within Groups	16.068	21	0.765		
	Total	20.433	23			
Days to mature fruiting body (D)	Between Groups	0.583	2	0.292	0.216	0.808
	Within Groups	28.375	21	1.351		
	Total	28.958	23			
Growth Index (%)	Between Groups	6651.438	2	3325.719	0.886	0.427
	Within Groups	78792.528	21	3752.025		
	Total	85443.965	23			

a. State = Lagos

Descriptives[a]

		N	Mean	Std. Dev.	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Spawn running (days)	C. indica	8	23.5	2.33	0.824	21.55	25.45	22	29
	G. sepium	8	24.88	1.356	0.479	23.74	26.01	22	26
	C. odorata	8	26.13	2.295	0.811	24.21	28.04	24	29
	Total	24	24.83	2.239	0.457	23.89	25.78	22	29
Pin head Formation (days)	C. indica	8	26.5	2	0.707	24.83	28.17	25	31
	G. sepium	8	27.75	0.886	0.313	27.01	28.49	26	29
	C. odorata	8	29	2.07	0.732	27.27	30.73	26	31
	Total	24	27.75	1.962	0.4	26.92	28.58	25	31
Fruit body formation (days)	C. indica	8	29	1.852	0.655	27.45	30.55	27	33
	G. sepium	8	30.13	1.126	0.398	29.18	31.07	28	31
	C. odorata	8	30.75	1.832	0.648	29.22	32.28	28	33
	Total	24	29.96	1.732	0.353	29.23	30.69	27	33
Average yield (gms)	C. indica	8	8.8363	1.56005	0.55156	7.532	10.1405	7.41	12.2
	G. sepium	8	8.7938	1.52238	0.53824	7.521	10.0665	7.35	12.2
	C. odorata	8	8.275	0.56853	0.20101	7.7997	8.7503	7.52	9.14
	Total	24	8.635	1.2698	0.2592	8.0988	9.1712	7.35	12.2
Dry weight (gms)	C. indica	8	2.9888	0.08543	0.0302	2.9173	3.0602	2.87	3.11
	G. sepium	8	3.045	0.1111	0.03928	2.9521	3.1379	2.85	3.16
	C. odorata	8	3.0088	0.07605	0.02689	2.9452	3.0723	2.88	3.11
	Total	24	3.0142	0.09112	0.0186	2.9757	3.0526	2.85	3.16
Biological efficiency	C. indica	8	2.8875	0.51944	0.18365	2.4532	3.3218	2.4	4
	G. sepium	8	2.9	0.4957	0.17525	2.4856	3.3144	2.4	4
	C. odorata	8	2.725	0.19086	0.06748	2.5654	2.8846	2.5	3

	Total	24	2.8375	0.41788	0.0853	2.661	3.014	2.4	4
	C. indica	8	4.225	1.0767	0.3807	3.325	5.125	2.6	5.8
	G. sepium	8	4.4	0.8401	0.297	3.698	5.102	3	5.8
Width of pileus (cm)	C. odorata	8	3.513	0.6917	0.2445	2.934	4.091	2.6	4.4
	Total	24	4.046	0.9311	0.1901	3.653	4.439	2.6	5.8
	C. indica	8	3.5	1.195	0.423	2.5	4.5	2	6
	G. sepium	8	3.25	1.282	0.453	2.18	4.32	2	6
Days to mature fruiting body (D)	C. odorata	8	3.13	0.641	0.227	2.59	3.66	2	4
	Total	24	3.29	1.042	0.213	2.85	3.73	2	6
	C. indica	8	137.68	68.5	24.219	80.41	194.94	57	260
	G. sepium	8	149.56	51.64	18.257	106.39	192.73	73	220
	C. odorata	8	116.11	31.443	11.117	89.83	142.4	85	170
Growth Index (%)	Total	24	134.45	52.351	10.686	112.34	156.56	57	260

a. State = Ondo

UNIVERSITY OF IB ARY

ANOVA[a]

		Sum of Squares	Df	Mean Square	F	Sig.
Spawn running (days)	Between Groups	27.583	2	13.792	3.301	.057
	Within Groups	87.750	21	4.179		
	Total	115.333	23			
Pin head Formation (days)	Between Groups	25.000	2	12.500	4.134	.031
	Within Groups	63.500	21	3.024		
	Total	88.500	23			
Fruiting body formation (days)	Between Groups	12.583	2	6.292	2.344	.121
	Within Groups	56.375	21	2.685		
	Total	68.958	23			
Average yield (gms)	Between Groups	1.562	2	.781	.462	.636
	Within Groups	35.522	21	1.692		
	Total	37.085	23			
Dry weight (gms)	Between Groups	.013	2	.007	.767	.477
	Within Groups	.178	21	.008		
	Total	.191	23			
Biological efficiency	Between Groups	.153	2	.076	.414	.666
	Within Groups	3.864	21	.184		
	Total	4.016	23			
Width of pileus (cm)	Between Groups	3.536	2	1.768	2.263	.129
	Within Groups	16.404	21	.781		
	Total	19.940	23			
Days to mature fruiting body (D)	Between Groups	.583	2	.292	.251	.780
	Within Groups	24.375	21	1.161		
	Total	24.958	23			
Growth Index (%)	Between Groups	4600.417	2	2300.209	.827	.451
	Within Groups	58433.723	21	2782.558		
	Total	63034.140	23			

a. State = Ondo

UNIVERS

Descriptives[a]

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Spawn running (days)	C. indica	8	26.25	2.435	.861	24.21	28.29	24	29
	G. sepium	8	23.25	2.375	.840	21.26	25.24	22	29
	C. odorata	8	25.13	.835	.295	24.43	25.82	24	26
	Total	24	24.88	2.309	.471	23.90	25.85	22	29
Pin head Formation (days)	C. indica	8	29.13	1.959	.693	27.49	30.76	26	31
	G. sepium	8	26.50	2.449	.866	24.45	28.55	24	32
	C. odorata	8	28.25	1.488	.526	27.01	29.49	26	30
	Total	24	27.96	2.216	.452	27.02	28.89	24	32
Fruiting body formation (days)	C. indica	8	31.13	1.959	.693	29.49	32.76	28	33
	G. sepium	8	28.63	2.387	.844	26.63	30.62	26	34
	C. odorata	8	30.38	1.302	.460	29.29	31.46	29	32
	Total	24	30.04	2.136	.436	29.14	30.94	26	34
Average yield	C. indica	8	8.5763	1.48169	.52386	7.3375	9.8150	7.46	12.00

	G. sepium	8	8.0287	.81044	.28653	7.3512	8.7063	6.62	9.14
	C. odorata	8	8.6012	1.67586	.59251	7.2002	10.0023	7.52	12.20
	Total	24	8.4021	1.34002	.27353	7.8362	8.9679	6.62	12.20
Dry weight (gms)	C. indica	8	2.9413	.18114	.06404	2.7898	3.0927	2.55	3.14
	G. sepium	8	3.0188	.09493	.03356	2.9394	3.0981	2.87	3.12
	C. odorata	8	3.0188	.10218	.03613	2.9333	3.1042	2.88	3.14
	Total	24	2.9929	.13153	.02685	2.9374	3.0485	2.55	3.14
Biological efficiency	C. indica	8	2.8125	.51944	.18365	2.3782	3.2468	2.40	4.00
	G. sepium	8	2.6500	.26186	.09258	2.4311	2.8689	2.20	3.00
	C. odorata	8	2.8375	.54232	.19174	2.3841	3.2909	2.50	4.00
	Total	24	2.7667	.44689	.09122	2.5780	2.9554	2.20	4.00
Width of pileus (cm)	C. indica	8	4.588	.8391	.2967	3.886	5.289	3.6	5.8
	G. sepium	8	4.200	.8229	.2909	3.512	4.888	2.6	5.2
	C. odorata	8	3.900	1.3763	.4866	2.749	5.051	2.1	5.8
	Total	24	4.229	1.0390	.2121	3.790	4.668	2.1	5.8
Days to mature	C. indica	8	3.00	1.069	.378	2.11	3.89	2	5

	G. sepium	8	2.50	.756	.267	1.87	3.13	2	4
	C. odorata	8	2.50	.756	.267	1.87	3.13	2	4
	Total	24	2.67	.868	.177	2.30	3.03	2	5
Growth Index (%)	C. indica	8	173.15	72.534	25.645	112.51	233.79	72	280
	G. sepium	8	183.74	68.463	24.205	126.50	240.97	87	260
	C. odorata	8	165.61	69.946	24.730	107.14	224.09	75	265
	Total	24	174.17	67.635	13.806	145.61	202.73	72	280

a. State = Ekiti

UNIVERSITY OF IBADAN

ANOVA[a]

		Sum of Squares	df	Mean Square	F	Sig.
Spawn running (days)	Between Groups	36.750	2	18.375	4.493	.024
	Within Groups	85.875	21	4.089		
	Total	122.625	23			
Pin head Formation (days)	Between Groups	28.583	2	14.292	3.557	.047
	Within Groups	84.375	21	4.018		
	Total	112.958	23			
Fruiting body formation (days)	Between Groups	26.333	2	13.167	3.517	.048
	Within Groups	78.625	21	3.744		
	Total	104.958	23			
Average yield (gms)	Between Groups	1.675	2	.838	.444	.647
	Within Groups	39.625	21	1.887		
	Total	41.300	23			
Dry weight (gms)	Between Groups	.032	2	.016	.919	.414
	Within Groups	.366	21	.017		
	Total	.398	23			
Biological efficiency	Between Groups	.166	2	.083	.393	.680
	Within Groups	4.427	21	.211		
	Total	4.593	23			
Width of pileus (cm)	Between Groups	1.901	2	.950	.870	.433
	Within Groups	22.929	21	1.092		
	Total	24.830	23			
Days to mature fruit body(D)	Between Groups	1.333	2	.667	.875	.432
	Within Groups	16.000	21	.762		
	Total	17.333	23			
Growth	Between Groups	1326.466	2	663.233	.134	.875

Within Groups	103886.108	21	4946.957		
Total	105212.573	23			

a. State = Ekiti

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Descriptives[a]

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Spawn running (days)	C. indica	8	20.63	8.601	3.041	13.43	27.82	8	34
	G. sepium	8	21.38	9.425	3.332	13.50	29.25	8	34
	C. odorata	8	22.25	8.172	2.889	15.42	29.08	10	34
	Total	24	21.42	8.387	1.712	17.88	24.96	8	34
Pin head Formation (days)	C. indica	8	26.50	6.188	2.188	21.33	31.67	16	37
	G. sepium	8	25.75	7.206	2.548	19.73	31.77	15	36
	C. odorata	8	26.50	6.000	2.121	21.48	31.52	19	36
	Total	24	26.25	6.208	1.267	23.63	28.87	15	37
Fruiting body formation (days)	C. indica	8	28.75	6.135	2.169	23.62	33.88	18	39
	G. sepium	8	28.13	6.854	2.423	22.39	33.86	17	38
	C. odorata	8	28.63	5.579	1.972	23.96	33.29	21	38
	Total	24	28.50	5.942	1.213	25.99	31.01	17	39
Average yield (gms)	C. indica	8	9.3513	1.36011	.48087	8.2142	10.4883	8.23	12.20
	G. sepium	8	9.0413	1.71784	.60735	7.6051	10.4774	6.62	11.89
	C. odorata	8	8.7163	1.25856	.44497	7.6641	9.7684	6.62	10.05
	Total	24	9.0363	1.41893	.28964	8.4371	9.6354	6.62	12.20
Dry weight	C. indica	8	3.0400	.10337	.03655	2.9536	3.1264	2.87	3.16

	G. sepium	8	2.9700	.15446	.05461	2.8409	3.0991	2.69	3.16
	C. odorata	8	2.9675	.18805	.06649	2.8103	3.1247	2.64	3.14
	Total	24	2.9925	.14985	.03059	2.9292	3.0558	2.64	3.16
Biological efficiency	C. indica	8	3.0625	.45650	.16140	2.6809	3.4441	2.70	4.00
	G. sepium	8	2.9750	.56252	.19888	2.5047	3.4453	2.20	3.90
	C. odorata	8	2.8750	.40620	.14361	2.5354	3.2146	2.20	3.30
	Total	24	2.9708	.46483	.09488	2.7746	3.1671	2.20	4.00
Width of pileus (cm)	C. indica	8	3.925	.4773	.1688	3.526	4.324	3.0	4.5
	G. sepium	8	4.338	.8280	.2927	3.645	5.030	2.6	5.3
	C. odorata	8	4.038	1.1600	.4101	3.068	5.007	2.1	5.8
	Total	24	4.100	.8480	.1731	3.742	4.458	2.1	5.8
Days to mature fruiting body (D)	C. indica	8	3.13	1.126	.398	2.18	4.07	2	5
	G. sepium	8	3.13	.835	.295	2.43	3.82	2	4
	C. odorata	8	3.50	.926	.327	2.73	4.27	2	5
	Total	24	3.25	.944	.193	2.85	3.65	2	5
Growth	C. indica	8	144.26	63.853	22.575	90.88	197.64	75	225

G. sepium	8	144.99	38.032	13.446	113.19	176.78	95	220
C. odorata	8	120.44	40.392	14.281	86.67	154.21	70	190
Total	24	136.56	48.098	9.818	116.25	156.87	70	225

a. State = Osun

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ANOVA[a]

		Sum of Squares	df	Mean Square	F	Sig.
Spawn running (days)	Between Groups	10.583	2	5.292	.069	.933
	Within Groups	1607.250	21	76.536		
	Total	1617.833	23			
Pin head Formation (days)	Between Groups	3.000	2	1.500	.036	.965
	Within Groups	883.500	21	42.071		
	Total	886.500	23			
Fruiting body formation (days)	Between Groups	1.750	2	.875	.023	.978
	Within Groups	810.250	21	38.583		
	Total	812.000	23			
Average yield (gms)	Between Groups	1.613	2	.807	.379	.689
	Within Groups	44.694	21	2.128		
	Total	46.307	23			
Dry weight (gms)	Between Groups	.027	2	.014	.581	.568
	Within Groups	.489	21	.023		
	Total	.516	23			
Biological efficiency	Between Groups	.141	2	.070	.306	.739
	Within Groups	4.829	21	.230		
	Total	4.970	23			
Width of pileus (cm)	Between Groups	.728	2	.364	.483	.624
	Within Groups	15.813	21	.753		
	Total	16.540	23			
Days to mature fruiting body (D)	Between Groups	.750	2	.375	.399	.676
	Within Groups	19.750	21	.940		
	Total	20.500	23			
Growth Index (%)	Between Groups	3122.290	2	1561.145	.655	.530
	Within Groups	50086.126	21	2385.054		
	Total	53208.416	23			

a. State = Osun

UNIVER

Descriptives[a]

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Spawn running (days)	C. indica	8	24.25	4.621	1.634	20.39	28.11	14	28
	G. sepium	8	20.25	6.182	2.186	15.08	25.42	11	29
	C. odorata	8	22.50	5.372	1.899	18.01	26.99	14	29
	Total	24	22.33	5.451	1.113	20.03	24.63	11	29
Pin head Formation (days)	C. indica	8	28.75	2.252	.796	26.87	30.63	26	32
	G. sepium	8	24.75	4.367	1.544	21.10	28.40	18	31
	C. odorata	8	26.75	3.370	1.191	23.93	29.57	21	31
	Total	24	26.75	3.686	.752	25.19	28.31	18	32
Fruiting body formation (days)	C. indica	8	30.75	2.252	.796	28.87	32.63	28	34
	G. sepium	8	27.13	3.758	1.329	23.98	30.27	22	33
	C. odorata	8	28.75	3.240	1.146	26.04	31.46	23	33
	Total	24	28.88	3.366	.687	27.45	30.30	22	34
Average yield (gms)	C. indica	8	8.9863	1.65038	.58350	7.6065	10.3660	7.52	11.89
	G. sepium	8	9.1475	1.48625	.52547	7.9050	10.3900	7.41	11.89
	C. odorata	8	8.2200	.87893	.31075	7.4852	8.9548	7.35	9.75

	Total	24	8.7846	1.38101	.28190	8.2014	9.3677	7.35	11.89
Dry weight (gms)	C. indica	8	2.9475	.16184	.05722	2.8122	3.0828	2.69	3.12
	G. sepium	8	2.9825	.15021	.05311	2.8569	3.1081	2.69	3.14
	C. odorata	8	3.0438	.12861	.04547	2.9362	3.1513	2.77	3.14
	Total	24	2.9912	.14671	.02995	2.9293	3.0532	2.69	3.14
Biological efficiency	C. indica	8	2.9500	.54248	.19180	2.4965	3.4035	2.50	3.90
	G. sepium	8	3.0000	.48697	.17217	2.5929	3.4071	2.40	3.90
	C. odorata	8	2.7000	.28284	.10000	2.4635	2.9365	2.40	3.20
	Total	24	2.8833	.45173	.09221	2.6926	3.0741	2.40	3.90
Width of pileus (cm)	C. indica	8	4.513	.5963	.2108	4.014	5.011	3.8	5.6
	G. sepium	8	4.575	.6964	.2462	3.993	5.157	3.4	5.3
	C. odorata	8	4.475	.8311	.2938	3.780	5.170	3.0	5.3
	Total	24	4.521	.6840	.1396	4.232	4.810	3.0	5.6
Days to mature fruiting body (D)	C. indica	8	3.38	.916	.324	2.61	4.14	2	5
	G. sepium	8	3.38	1.188	.420	2.38	4.37	2	5
	C. odorata	8	3.13	.991	.350	2.30	3.95	2	5
	Total	24	3.29	.999	.204	2.87	3.71	2	5
Growth Index	C. indica	8	143.54	45.445	16.067	105.54	181.53	76	220

(%)	G. sepium	8	149.45	51.284	18.132	106.58	192.32	88	240
	C. odorata	8	159.39	69.465	24.560	101.31	217.46	76	265
	Total	24	150.79	54.242	11.072	127.89	173.70	76	265

a. State = Ogun

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ANOVA[a]

		Sum of Squares	df	Mean Square	F	Sig.
Spawn running (days)	Between Groups	64.333	2	32.167	1.091	.354
	Within Groups	619.000	21	29.476		
	Total	683.333	23			
Pin head Formation (days)	Between Groups	64.000	2	32.000	2.704	.090
	Within Groups	248.500	21	11.833		
	Total	312.500	23			
Fruiting body formation (days)	Between Groups	52.750	2	26.375	2.664	.093
	Within Groups	207.875	21	9.899		
	Total	260.625	23			
Average yield (gms)	Between Groups	3.929	2	1.965	1.033	.373
	Within Groups	39.936	21	1.902		
	Total	43.865	23			
Dry weight (gms)	Between Groups	.038	2	.019	.872	.433
	Within Groups	.457	21	.022		
	Total	.495	23			
Biological efficiency	Between Groups	.413	2	.207	1.014	.380
	Within Groups	4.280	21	.204		
	Total	4.693	23			
Width of pileus (cm)	Between Groups	.041	2	.020	.040	.961
	Within Groups	10.719	21	.510		
	Total	10.760	23			
Days to mature fruiting body (D)	Between Groups	.333	2	.167	.155	.858
	Within Groups	22.625	21	1.077		
	Total	22.958	23			
Growth Index (%)	Between Groups	1026.491	2	513.245	.162	.852
	Within Groups	66644.988	21	3173.571		
	Total	67671.478	23			

a. State = Ogun

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Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Spawn running (days)	C. indica	48	24.33	5.130	.740	22.84	25.82	8	34
	G. sepium	48	22.19	5.221	.754	20.67	23.70	8	34
	C. odorata	48	24.06	5.076	.733	22.59	25.54	10	34
	Total	144	23.53	5.195	.433	22.67	24.38	8	34
Pin head Formation (days)	C. indica	48	28.67	4.002	.578	27.50	29.83	16	39
	G. sepium	48	26.56	4.341	.627	25.30	27.82	14	36
	C. odorata	48	28.04	4.037	.583	26.87	29.21	17	40
	Total	144	27.76	4.195	.350	27.07	28.45	14	40
Fruiting body	C. indica	48	30.75	3.856	.557	29.63	31.87	18	40

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	G. sepium	48	28.81	4.139	.597	27.61	30.01	16	38
	C. odorata	48	30.00	3.815	.551	28.89	31.11	19	41
	Total	144	29.85	3.993	.333	29.20	30.51	16	41
Average yield (gms)	C. indica	48	9.0475	1.35584	.19570	8.6538	9.4412	7.41	12.20
	G. sepium	48	9.0171	1.44230	.20818	8.5983	9.4359	6.62	12.20
	C. odorata	48	8.8379	1.38231	.19952	8.4365	9.2393	6.62	12.20
	Total	144	8.9675	1.38728	.11561	8.7390	9.1960	6.62	12.20
Dry weight (gms)	C. indica	48	2.9667	.14119	.02038	2.9257	3.0077	2.55	3.16
	G. sepium	48	2.9744	.15503	.02238	2.9294	3.0194	2.55	3.16
	C. odorata	48	3.0071	.12211	.01762	2.9716	3.0425	2.64	3.14
	Total	144	2.9827	.14022	.01168	2.9596	3.0058	2.55	3.16
Biological efficiency	C. indica	48	2.9708	.45659	.06590	2.8383	3.1034	2.40	4.00
	G. sepium	48	2.9671	.47475	.06852	2.8292	3.1049	2.20	4.06
	C. odorata	48	2.9154	.46044	.06646	2.7817	3.0491	2.20	4.00
	Total	144	2.9511	.46143	.03845	2.8751	3.0271	2.20	4.06
Width of pileus (cm)	C. indica	48	4.188	.9073	.1310	3.924	4.451	2.1	5.8

	G. sepium	48	4.240	.8305	.1199	3.998	4.481	2.6	5.8
	C. odorata	48	3.985	1.0173	.1468	3.690	4.281	2.0	5.8
	Total	144	4.138	.9217	.0768	3.986	4.289	2.0	5.8
Days to mature fruiting body (D)	C. indica	48	3.38	1.160	.167	3.04	3.71	2	6
	G. sepium	48	3.17	1.038	.150	2.87	3.47	2	6
	C. odorata	48	3.06	.885	.128	2.81	3.32	2	5
	Total	144	3.20	1.035	.086	3.03	3.37	2	6
Growth Index (%)	C. indica	48	141.26	61.836	8.925	123.30	159.21	44	280
	G. sepium	48	147.94	54.380	7.849	132.15	163.73	47	260
	C. odorata	48	141.71	57.997	8.371	124.87	158.56	65	265
	Total	144	143.64	57.824	4.819	134.11	153.16	44	280

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ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Spawn running (days)	Between Groups	131.097	2	65.549	2.479	.088
	Within Groups	3728.792	141	26.445		
	Total	3859.889	143			
Pin head Formation (days)	Between Groups	112.097	2	56.049	3.287	.040
	Within Groups	2404.396	141	17.052		
	Total	2516.493	143			
Fruiting body formation (days)	Between Groups	91.625	2	45.813	2.952	.055
	Within Groups	2188.313	141	15.520		
	Total	2279.938	143			
Average yield (gms)	Between Groups	1.231	2	.616	.317	.729
	Within Groups	273.978	141	1.943		
	Total	275.209	143			
Dry weight (gms)	Between Groups	.044	2	.022	1.126	.327
	Within Groups	2.767	141	.020		
	Total	2.811	143			
Biological efficiency	Between Groups	.092	2	.046	.214	.808

	Within Groups	30.356	141	.215		
	Total	30.448	143			
Width of pileus (cm)	Between Groups	1.730	2	.865	1.019	.364
	Within Groups	119.747	141	.849		
	Total	121.477	143			
Days to mature fruiting body (D)	Between Groups	2.431	2	1.215	1.137	.324
	Within Groups	150.729	141	1.069		
	Total	153.160	143			
Growth Index (%)	Between Groups	1339.288	2	669.644	.198	.821
	Within Groups	476791.455	141	3381.500		
	Total	478130.742	143			

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Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Spawn running (days)	Oyo	24	24.46	3.856	.787	22.83	26.09	16	34
	Lagos	24	23.25	5.636	1.150	20.87	25.63	8	29
	Ondo	24	24.83	2.239	.457	23.89	25.78	22	29
	Ekiti	24	24.88	2.309	.471	23.90	25.85	22	29
	Osun	24	21.42	8.387	1.712	17.88	24.96	8	34
	Ogun	24	22.33	5.451	1.113	20.03	24.63	11	29
	Total	144	23.53	5.195	.433	22.67	24.38	8	34
Pin head Formation (days)	Oyo	24	30.63	3.954	.807	28.96	32.29	23	40
	Lagos	24	27.21	4.530	.925	25.30	29.12	14	33
	Ondo	24	27.75	1.962	.400	26.92	28.58	25	31
	Ekiti	24	27.96	2.216	.452	27.02	28.89	24	32
	Osun	24	26.25	6.208	1.267	23.63	28.87	15	37
	Ogun	24	26.75	3.686	.752	25.19	28.31	18	32
	Total	144	27.76	4.195	.350	27.07	28.45	14	40
Fruiting body formation (days)	Oyo	24	32.54	3.695	.754	30.98	34.10	26	41
	Lagos	24	29.21	4.511	.921	27.30	31.11	16	35
	Ondo	24	29.96	1.732	.353	29.23	30.69	27	33
	Ekiti	24	30.04	2.136	.436	29.14	30.94	26	34
	Osun	24	28.50	5.942	1.213	25.99	31.01	17	39
	Ogun	24	28.88	3.366	.687	27.45	30.30	22	34
	Total	144	29.85	3.993	.333	29.20	30.51	16	41
Average yield (gms)	Oyo	24	9.6863	1.42345	.29056	9.0852	10.2873	7.95	12.20
	Lagos	24	9.2608	1.22196	.24943	8.7448	9.7768	7.95	12.20
	Ondo	24	8.6350	1.26980	.25920	8.0988	9.1712	7.35	12.20
	Ekiti	24	8.4021	1.34002	.27353	7.8362	8.9679	6.62	12.20
	Osun	24	9.0362	1.41893	.28964	8.4371	9.6354	6.62	12.20
	Ogun	24	8.7846	1.38101	.28190	8.2014	9.3677	7.35	11.89

	Total	144	8.9675	1.38728	.11561	8.7390	9.1960	6.62	12.20
Dry weight (gms)	Oyo	24	2.9500	.15424	.03148	2.8849	3.0151	2.64	3.16
	Lagos	24	2.9554	.16016	.03269	2.8878	3.0230	2.55	3.14
	Ondo	24	3.0142	.09112	.01860	2.9757	3.0526	2.85	3.16
	Ekiti	24	2.9929	.13153	.02685	2.9374	3.0485	2.55	3.14
	Osun	24	2.9925	.14985	.03059	2.9292	3.0558	2.64	3.16
	Ogun	24	2.9912	.14671	.02995	2.9293	3.0532	2.69	3.14
	Total	144	2.9827	.14022	.01168	2.9596	3.0058	2.55	3.16
Biological efficiency	Oyo	24	3.2025	.48802	.09962	2.9964	3.4086	2.60	4.06
	Lagos	24	3.0458	.40215	.08209	2.8760	3.2156	2.60	4.00
	Ondo	24	2.8375	.41788	.08530	2.6610	3.0140	2.40	4.00
	Ekiti	24	2.7667	.44689	.09122	2.5780	2.9554	2.20	4.00
	Osun	24	2.9708	.46483	.09488	2.7746	3.1671	2.20	4.00
	Ogun	24	2.8833	.45173	.09221	2.6926	3.0741	2.40	3.90
	Total	144	2.9511	.46143	.03845	2.8751	3.0271	2.20	4.06
Width of pileus (cm)	Oyo	24	4.113	.9896	.2020	3.695	4.530	2.2	5.8
	Lagos	24	3.817	.9426	.1924	3.419	4.215	2.0	5.2
	Ondo	24	4.046	.9311	.1901	3.653	4.439	2.6	5.8
	Ekiti	24	4.229	1.0390	.2121	3.790	4.668	2.1	5.8
	Osun	24	4.100	.8480	.1731	3.742	4.458	2.1	5.8
	Ogun	24	4.521	.6840	.1396	4.232	4.810	3.0	5.6
	Total	144	4.137	.9217	.0768	3.986	4.289	2.0	5.8
Days to mature fruiting body (D)	Oyo	24	3.42	1.139	.232	2.94	3.90	2	6
	Lagos	24	3.29	1.122	.229	2.82	3.77	2	6
	Ondo	24	3.29	1.042	.213	2.85	3.73	2	6
	Ekiti	24	2.67	.868	.177	2.30	3.03	2	5
	Osun	24	3.25	.944	.193	2.85	3.65	2	5
	Ogun	24	3.29	.999	.204	2.87	3.71	2	5
	Total	144	3.20	1.035	.086	3.03	3.37	2	6
Growth Index (%)	Oyo	24	134.69	55.594	11.348	111.21	158.16	44	260
	Lagos	24	131.18	60.950	12.441	105.44	156.91	47	260
	Ondo	24	134.45	52.351	10.686	112.34	156.56	57	260
	Ekiti	24	174.17	67.635	13.806	145.61	202.73	72	280

Osun	24	136.56	48.098	9.818	116.25	156.87	70	225
Ogun	24	150.79	54.242	11.072	127.89	173.70	76	265
Total	144	143.64	57.824	4.819	134.11	153.16	44	280

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APPENDIX V

Results for chemical analysis of mushroom (mg/kg) Dry Weight

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Oyo	8	8.8475	1.57626	.55729	7.5297	10.1653	6.38	10.50
Lagos	8	13.5875	1.93201	.68307	11.9723	15.2027	11.20	16.30
Ondo	8	9.5600	1.46990	.51969	8.3311	10.7889	7.39	11.40
Ekiti	8	9.0563	.38485	.13607	8.7345	9.3780	8.37	9.44
Osun	8	8.9625	1.48523	.52511	7.7208	10.2042	7.39	11.90
Ogun	8	11.5675	2.25189	.79616	9.6849	13.4501	8.93	13.90
Total	48	10.2635	2.34210	.33805	9.5835	10.9436	6.38	16.30
Oyo	8	22.288	5.4554	1.9288	17.727	26.848	16.3	28.6
Lagos	8	17.250	4.0887	1.4456	13.832	20.668	10.4	21.0
Ondo	8	18.862	1.7824	.6302	17.372	20.353	15.3	21.1
Ekiti	8	32.825	10.1351	3.5833	24.352	41.298	18.3	48.3
Osun	8	32.688	14.3480	5.0728	20.692	44.683	14.8	48.9
Ogun	8	39.250	21.5292	7.6117	21.251	57.249	18.3	66.6
Total	48	27.194	13.7987	1.9917	23.187	31.200	10.4	66.6
Oyo	8	15.412	.4643	.1641	15.024	15.801	14.8	16.3
Lagos	8	70.488	8.4744	2.9962	63.403	77.572	58.7	84.3
Ondo	8	18.200	6.4489	2.2800	12.809	23.591	10.6	28.5
Ekiti	8	45.025	4.4685	1.5799	41.289	48.761	38.5	52.1
Osun	8	24.338	11.5038	4.0672	14.720	33.955	17.4	52.4
Ogun	8	29.850	6.1908	2.1888	24.674	35.026	24.3	41.8

ANOVA

Total	48	33.885	20.3156	2.9323	27.986	39.784	10.6	84.3
Oyo	8	878.25	213.724	75.563	699.57	1056.93	736	1264
Lagos	8	502.88	579.044	204.723	18.78	986.97	274	1933
Ondo	8	331.50	42.119	14.891	296.29	366.71	275	384
Ekiti	8	1199.63	267.333	94.517	976.13	1423.12	833	1630
Osun	8	1511.63	477.004	168.646	1112.84	1910.41	748	1842
Ogun	8	478.50	9.426	3.333	470.62	486.38	464	491
Total	48	817.06	534.468	77.144	661.87	972.26	274	1933
Oyo	8	35.887	5.2621	1.8604	31.488	40.287	26.7	43.8
Lagos	8	61.900	10.0955	3.5693	53.460	70.340	37.8	69.3
Ondo	8	36.663	1.4628	.5172	35.440	37.885	34.1	38.5
Ekiti	8	33.313	5.0278	1.7776	29.109	37.516	27.7	41.8
Osun	8	55.013	12.3172	4.3548	44.715	65.310	39.6	82.7
Ogun	8	40.025	3.8799	1.3717	36.781	43.269	31.0	44.2
Total	48	43.800	12.8787	1.8589	40.060	47.540	26.7	82.7
Oyo	8	73.400	12.8225	4.5334	62.680	84.120	44.9	83.2
Lagos	8	104.100	12.4558	4.4038	93.687	114.513	78.7	117.0
Ondo	8	69.963	8.4799	2.9981	62.873	77.052	55.7	78.5
Ekiti	8	70.288	13.8579	4.8995	58.702	81.873	58.4	93.5
Osun	8	116.725	17.1674	6.0696	102.373	131.077	82.8	133.0
Ogun	8	56.738	2.8928	1.0228	54.319	59.156	54.0	63.2
Total	48	81.869	24.2825	3.5049	74.818	88.920	44.9	133.0

		Sum of Squares	df	Mean Square	F	Sig.
Nitrate	Between Groups	147.195	5	29.439	11.177	.000
	Within Groups	110.620	42	2.634		
	Total	257.815	47			
Phosphate	Between Groups	3196.837	5	639.367	4.668	.002
	Within Groups	5752.211	42	136.957		
	Total	8949.048	47			
Sodium	Between Groups	17268.249	5	3453.650	68.108	.000
	Within Groups	2129.751	42	50.708		
	Total	19398.000	47			
Potassium	Between Groups	8652994.688	5	1730598.938	15.229	.000
	Within Groups	4772826.125	42	113638.717		
	Total	13425820.813	47			
Calcium	Between Groups	5528.960	5	1105.792	20.491	.000
	Within Groups	2266.560	42	53.966		
	Total	7795.520	47			
Magnesium	Between Groups	21506.962	5	4301.392	29.109	.000
	Within Groups	6206.201	42	147.767		
	Total	27713.163	47			

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APPENDIX VI **Proximate Analysis**

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	Minimum	Maximum
					Lower Bound	Upper Bound	
% Protein							
	Oyo	8	6.2588	1.15361	.40786	5.2943	7.2232
	Lagos	8	6.9838	2.27976	.80602	5.0778	8.8897
	Ondo	8	6.2388	.27936	.09877	6.0052	6.4723
	Ekiti	8	6.7038	.48826	.17263	6.2956	7.1119
	Osun	8	6.5500	.44587	.15764	6.1772	6.9228

	Ogun	8	5.3075	.40896	.14459	4.9656	5.6494
	Total	48	6.3404	1.16568	.16825	6.0019	6.6789
% Ash	Oyo	8	4.4012	1.78196	.63002	2.9115	5.8910
	Lagos	8	4.2925	1.46642	.51846	3.0665	5.5185
	Ondo	8	4.8313	1.18198	.41789	3.8431	5.8194
	Ekiti	8	3.8738	2.03475	.71939	2.1727	5.5748
	Osun	8	4.7163	1.21679	.43020	3.6990	5.7335
	Ogun	8	3.1375	.32657	.11546	2.8645	3.4105

	Total	48	4.2088	1.47852	.21341	3.7794	4.6381
% Moisture	Oyo	8	12.4850	1.66864	.58995	11.0900	13.8800
	Lagos	8	14.0975	.91237	.32257	13.3347	14.8603
	Ondo	8	16.2163	1.84521	.65238	14.6736	17.7589
	Ekiti	8	12.8188	1.93048	.68253	11.2048	14.4327
	Osun	8	13.0100	3.60804	1.27563	9.9936	16.0264

	Ogun	8	10.4250	.53764	.19008	9.9755	10.8745
	Total	48	13.1754	2.58774	.37351	12.4240	13.9268
% Fat	Oyo	8	3.6000	.44113	.15596	3.2312	3.9688
	Lagos	8	2.7588	.23055	.08151	2.5660	2.9515
	Ondo	8	3.4138	.41455	.14657	3.0672	3.7603
	Ekiti	8	6.5962	.40606	.14356	6.2568	6.9357
	Osun	8	4.4075	.90334	.31938	3.6523	5.1627

	Ogun	8	4.3625	1.67190	.59111	2.9648	5.7602
	Total	48	4.1898	1.46084	.21085	3.7656	4.6140
% Crude Fiber	Oyo	8	19.3075	.98231	.34730	18.4863	20.1287
	Lagos	8	18.4888	1.04406	.36913	17.6159	19.3616
	Ondo	8	20.5050	1.19122	.42116	19.5091	21.5009
	Ekiti	8	18.6613	.78330	.27694	18.0064	19.3161
	Osun	8	25.1250	7.02191	2.48262	19.2545	30.9955

	Ogun	8	22.5387	1.37522	.48622	21.3890	23.6885
	Total	48	20.7710	3.74347	.54032	19.6841	21.8580
% CHO	Oyo	8	53.9475	3.06529	1.08374	51.3849	56.5101
	Lagos	8	53.3788	2.53131	.89495	51.2625	55.4950
	Ondo	8	48.7950	2.66476	.94213	46.5672	51.0228
	Ekiti	8	51.3463	2.50024	.88397	49.2560	53.4365

	Osun	8	46.1913	7.02532	2.48383	40.3179	52.0646
	Ogun	8	54.2288	2.92249	1.03326	51.7855	56.6720
	Total	48	51.3146	4.67594	.67491	49.9568	52.6723

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ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
% Protein	Between Groups	13.390	5	2.678	2.228	.069
	Within Groups	50.474	42	1.202		
	Total	63.864	47			
% Ash	Between Groups	15.591	5	3.118	1.503	.210
	Within Groups	87.152	42	2.075		
	Total	102.743	47			
% Moisture	Between Groups	146.344	5	29.269	7.300	.000
	Within Groups	168.387	42	4.009		
	Total	314.731	47			
% Fat	Between Groups	70.930	5	14.186	20.286	.000
	Within Groups	29.370	42	.699		
	Total	100.300	47			
% Crude Fiber	Between Groups	271.636	5	54.327	5.896	.000
	Within Groups	387.002	42	9.214		
	Total	658.638	47			
% CHO	Between Groups	418.266	5	83.653	5.766	.000
	Within Groups	609.362	42	14.509		
	Total	1027.628	47			

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Dissimilarity matrix based on Jaccard coefficients among the varieties of Mushroom using RAPD markers

	OD1	OD2	OD3	OD4	OD5	OD6	OD7	OD8	OY1	OY2	OY3	OY4	OY5	OY6	OY7	OY8	LA1	LA2	LA3	LA4	LA5	LA6	LA7	LA8
OD1	0.696	0.696	0.696	0.696	0.696	0.635	0.635	0.696	0.719	0.719	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.635	0.635	0.635	0.635	0.635
OD2		0.537	0.537	0.537	0.537	0.696	0.696	0.649	0.719	0.719	0.575	0.575	0.575	0.575	0.649	0.649	0.649	0.649	0.624	0.696	0.696	0.696	0.696	0.696
OD3			0.051	0.123	0.123	0.696	0.696	0.649	0.719	0.719	0.575	0.575	0.575	0.575	0.649	0.649	0.649	0.649	0.624	0.696	0.696	0.696	0.696	0.696
OD4				0.123	0.123	0.696	0.696	0.649	0.719	0.719	0.575	0.575	0.575	0.575	0.649	0.649	0.649	0.649	0.624	0.696	0.696	0.696	0.696	0.696
OD5					0.051	0.696	0.696	0.649	0.719	0.719	0.575	0.575	0.575	0.575	0.649	0.649	0.649	0.649	0.624	0.696	0.696	0.696	0.696	0.696
OD6						0.696	0.696	0.649	0.719	0.719	0.575	0.575	0.575	0.575	0.649	0.649	0.649	0.649	0.624	0.696	0.696	0.696	0.696	0.696
OD7							0.167	0.696	0.719	0.719	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.566	0.566	0.566	0.459	0.459
OD8								0.696	0.719	0.719	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.566	0.566	0.566	0.459	0.459
OY1									0.719	0.719	0.649	0.649	0.649	0.649	0.475	0.475	0.546	0.546	0.649	0.696	0.696	0.696	0.696	0.696
OY2										0.206	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719
OY3											0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719
OY4												0.193	0.308	0.308	0.649	0.649	0.649	0.649	0.624	0.696	0.696	0.696	0.696	0.696
OY5													0.308	0.308	0.649	0.649	0.649	0.649	0.624	0.696	0.696	0.696	0.696	0.696
OY6														0.122	0.649	0.649	0.649	0.649	0.624	0.696	0.696	0.696	0.696	0.696
OY7															0.649	0.649	0.649	0.649	0.624	0.696	0.696	0.696	0.696	0.696
OY8																0.365	0.546	0.546	0.649	0.696	0.696	0.696	0.696	0.696
LA1																	0.546	0.546	0.649	0.696	0.696	0.696	0.696	0.696
LA2																		0.270	0.649	0.696	0.696	0.696	0.696	0.696
LA3																			0.649	0.696	0.696	0.696	0.696	0.696
LA4																				0.696	0.696	0.696	0.696	0.696
LA5																					0.167	0.167	0.566	0.566
LA6																						0.152	0.566	0.566
LA7																							0.566	0.566
LA8																								0.162

	OG1	OG2	OG3	OG4	OG5	OG6	OG7	OG8	OS1	OS2	OS3	OS4	OS5	OS6	OS7	OS8	EKI	EK2	EK3	EK4	EK5	EK6	EK7
OD1	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696
OD2	0.537	0.537	0.537	0.537	0.537	0.537	0.537	0.537	0.537	0.537	0.537	0.537	0.537	0.537	0.537	0.537	0.537	0.537	0.537	0.537	0.537	0.537	0.575
OD3	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.575
OD4	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.575
OD5	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.575
OD6	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.448	0.575
OD7	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696
OD8	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696
OY1	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649
OY2	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719
OY3	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719	0.719
OY4	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.193
OY5	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.118
OY6	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.308
OY7	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.575	0.308
OY8	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649
LA1	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649
LA2	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649
LA3	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649	0.649
LA4	0.624	0.624	0.624	0.624	0.624	0.624	0.624	0.624	0.624	0.624	0.624	0.624	0.624	0.624	0.624	0.624	0.624	0.624	0.624	0.624	0.624	0.624	0.624
LA5	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696
LA6	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696
LA7	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696
LA8	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696
OG1	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696	0.696
OG2		0.213	0.213	0.213	0.213	0.213	0.213	0.213	0.213	0.213	0.213	0.213	0.213	0.213	0.213	0.213	0.213	0.213	0.213	0.213	0.213	0.213	0.575
OG3			0.000	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.575
OG4				0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.575
OG5					0.026	0.026	0.026	0.026	0.026	0.026	0.000	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.575
OG6						0.000	0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.023	0.000	0.575
OG7							0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.023	0.000	0.575
OG8								0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.023	0.000	0.575
OS1									0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.023	0.000	0.575
OS2										0.000	0.026	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.023	0.000	0.575
OS3											0.026	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.023	0.000	0.575
OS4												0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.575
OS5													0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.023	0.000	0.575
OS6														0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.023	0.000	0.575
OS7															0.000	0.023	0.000	0.000	0.000	0.000	0.023	0.000	0.575
OS8																0.023	0.000	0.000	0.000	0.000	0.023	0.000	0.575
EKI																	0.023	0.023	0.023	0.023	0.000	0.023	0.575
EK2																		0.000	0.000	0.000	0.023	0.000	0.575
EK3																			0.000	0.000	0.023	0.000	0.575
EK4																				0.000	0.023	0.000	0.575
EK5																					0.023	0.000	0.575

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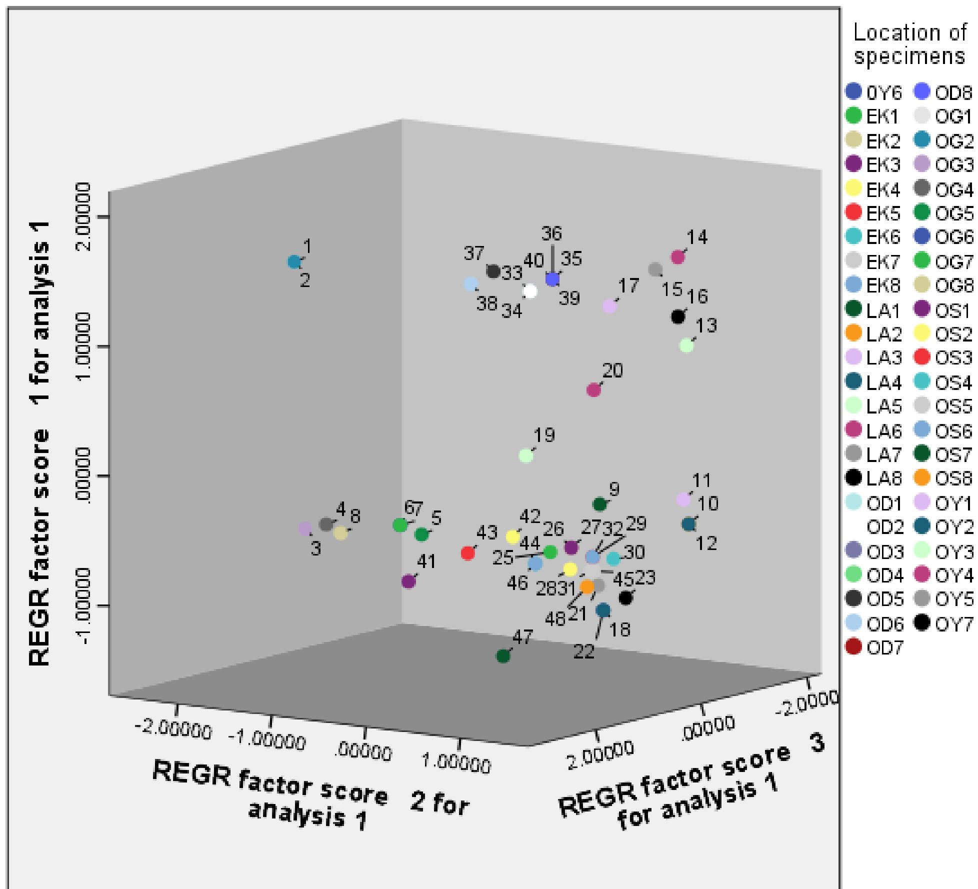

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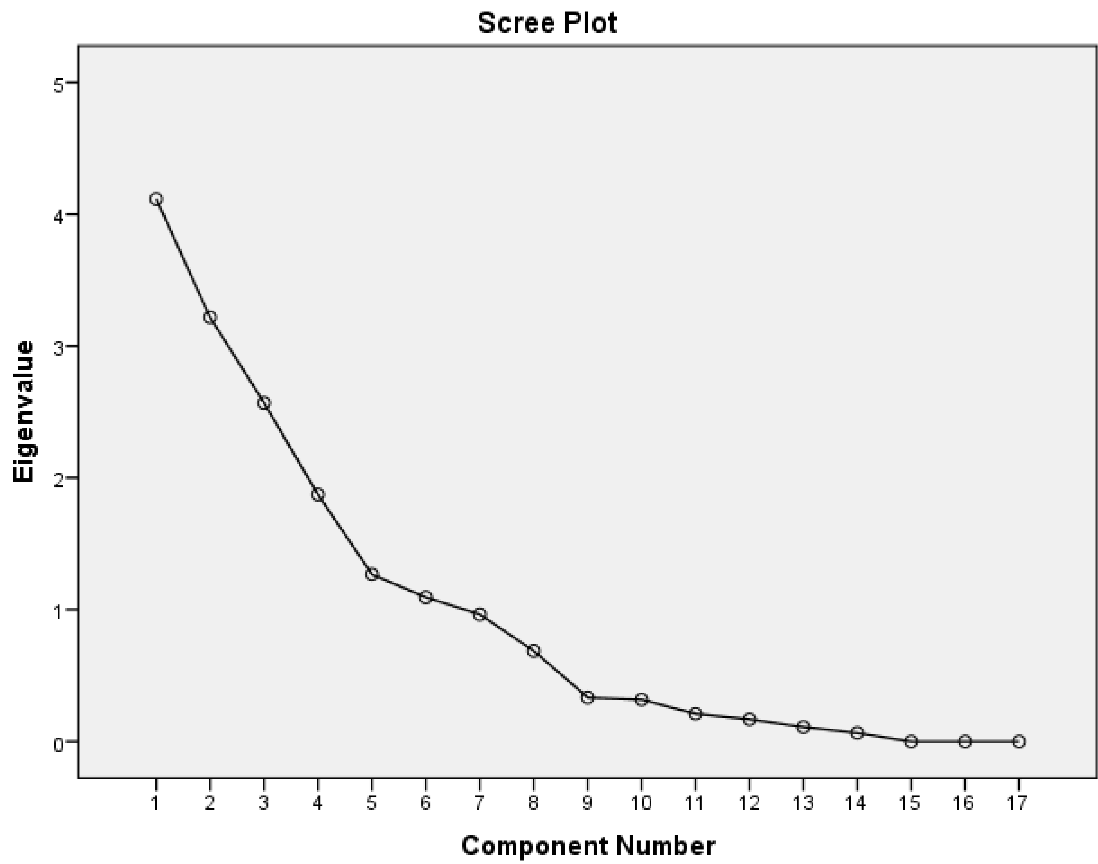

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Table 5 :GROWTH PARAMETERS OF *AURICULARIA* SPP ON LOG WOODS IN OYO STATE

	SPAWN RUN (DAYS)			PIN FORMATION (DAYS)			FRUIT BODY FORMATION (DAYS)			WIDTH OF PILEUS (mm)			YIELD (GRAMS)		
	M.indica	*G.sepium*	*C.odorata*	*M.indica*	*G.sepium*	*C.odorata*	*M.indica*	*G.sepium*	*C.odorata*	*M.indica*	*G.sepium*	*C.odorata*	*M.indica*	*G.sepium*	*C.odorata*
OY1	25	24	22	33	30	28	34	32	30	2.2	3.5	3.4	11.28	11.1	12
OY2	29	22	26	39	34	35	40	30	28	4.4	5.6	4.8	12	10.48	8.76
OY3	28	23	26	34	31	30	37	33	32	5.3	4.5	3.8	9.14	12.2	7.71
OY4	28	23	25	31	30	28	33	32	30	5.1	5.4	3.8	9	8.9	8.23
OY5	24	22	24	30	28	30	33	30	30	4.3	2.7	4	10	8.23	11.89
OY6	29	22	26	32	27	29	34	31	30	5.8	4.3	2.6	9	9.12	8.8
OY7	28	23	34	32	30	40	33	32	41	3.8	4.4	3.4	10.05	8.64	6.99
OY8	20	18	16	26	23	25	28	26	27	5.2	3.4	3	9.5	9.04	7.21
AVG	26.4	22.1	24.8	32.1	29.1	30.6	34	30.75	31	4.5	4.2	3.6	9.9	9.7	8.9

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Table 11: Nutrient contents of mushroom (mg/kg) in Ekiti State

S/N	Sample code	Nitrogen	Phosphorus	Sodium	Potassium	Calcium	Magnesium
1	EK-1	9.37	**48.3**	38.5	**1630**	30.3	**93.5**
2	EK-2	9.14	36.5	47.2	1248	33.1	91.5
3	EK-3	8.37	37.7	46.3	1330	28.5	63.2
4	EK-4	8.63	35.8	**47.7**	1372	30.4	64.4
5	EK-5	8.99	37.4	46.3	1139	27.7	63.5
6	EK-6	9.10	29.6	40.6	842	41.8	63.0
7	EK-7	9.41	18.3	41.5	833	37.2	64.8
8	EK-8	**9.44**	19.0	52.1	1203	**37.5**	58.4

KEY:

EK1=Ado Ekiti,EK2=Ilemeje,EK3-Ikole,EK4=Oye,EK5=Irepodun,Ek6=Ikere,EK7=Ijero,EK8=Emure

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Table 12: Nutrient contents of mushroom (mg/kg) in Lagos State

S/N	Sample code	Nitrogen	Phosphorus	Sodium	Potassium	Calcium	Magnesium
9	LA-1	11.2	19.3	64.4	274	61.3	78.7
10	LA-2	**16.3**	19.6	68.9	388	61.7	103
11	LA-3	12.3	**21.0**	67.4	283	66.4	112
12	LA-4	12.7	18.7	58.7	296	37.8	**117**
13	LA-5	15.3	18.4	**84.3**	**1933**	**69.3**	112
14	LA-6	15.7	19.5	77.4	284	66.4	105
15	LA-7	13.4	10.4	**77.5**	286	66.8	94.1
16	LA-8	11.8	11.1	65.3	279	65.5	111

KEY:
LAl=Agege,LA2=Ojo,LA3=Apapa,LA4=Badagry,LA5=Epe,LA6=Shomolu,LA7=Ikorodu,LA8=Mushin

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Table 13: Nutrient contents of mushroom (mg/kg) in Ogun State

S/N	Sample code	Nitrogen	Phosphorus	Sodium	Potassium	Calcium	Magnesium
17	OG-1	8.94	52.1	27.4	475	41.8	54.8
18	OG-2	8.93	65.4	26.2	487	31.0	55.4
19	OG-3	9.07	**66.6**	26.7	468	**44.2**	54.0
20	OG-4	13.4	50.7	26.8	**491**	40.3	56.8
21	OG-5	13.7	18.3	37.2	464	40.6	**63.2**
22	OG-6	13.0	19.9	24.3	486	41.6	57.4
23	OG-7	**13.9**	20.4	28.4	480	40.0	55.0
24	OG-8	11.6	20.6	**41.8**	477	40.7	57.3

KEY:

OG1= Abeokuta,OG2=Ewekoro,OG3=Ifo,OG4=Ijebu Ode,OG5=Ikene,OG6=Shagamu,OG7=Odeda,OG8=Odogbolu

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Table 14:Nutrient contents of mushroom (mg/kg) in Ondo State

S/N	Sample code	Nitrogen	Phosphorus	Sodium	Potassium	Calcium	Magnesium
25	OD-1	10.4	15.3	18.4	326	38.4	**78.5**
26	OD-2	**11.4**	20.0	17.8	366	36.7	73.6
27	OD-3	10.2	**21.1**	26.3	384	36.8	74.4
28	0D-4	10.8	18.4	10.6	**375**	34.1	74.7
29	OD-5	7.39	18.4	11.4	295	36.5	76.5
30	OD-6	9.44	19.6	14.3	275	37.0	58.9
31	OD-7	9.37	20.1	18.3	288	35.3	67.4
32	OD-8	7.48	18.0	**28.5**	343	**38.5**	55.7

KEY:

OD1=Idanre,OD2=Ilaje,OD3=Ileoluji,OD4=Odigbo,

OD5=Okitipupa,OD6=Ose,OD7=Owo,OD8=Ifedore

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Table 15: Nutrient content of mushroom (mg/kg) in Osun State

S/N	Sample code	Nitrogen	Phosphorus	Sodium	Potassium	Calcium	Magnesium
33	OS-1	8.38	48.4	22.1	**1842**	55.6	82.8
34	OS-2	7.39	47.9	17.4	1836	55.3	103
36	OS-4	10.5	31.4	22.5	1773	51.4	117
37	OS-5	11.9	19.5	**52.4**	1830	53.7	131
38	OS-6	8.40	32.2	21.0	1631	39.6	127
39	OS-7	8.41	14.8	21.2	749	**82.7**	**133**
40	OS-8	7.98	18.4	20.7	748	48.8	128

KEY:

OS1=Boluwaduro,OS2=Ejigbo,OS3=Ifedayo,OS4,=Ifelodun,

OS5=Ila,OS6=Irepodun,OS7=Iwo,OS8=Obokun

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Table 16: Nutrient contents of mushroom (mg/kg) in Oyo State

S/N	Sample code	Nitrogen	Phosphorus	Sodium	Potassium	Calcium	Magnesium
41	OY-1	**10.5**	**28.6**	15.3	736	37.5	**83.2**
42	OY-2	6.38	27.0	15.7	744	37.9	78.4
43	OY-3	6.98	25.9	**16.3**	830	31.0	78.3
44	OY-4	8.38	27.6	15.4	**1264**	26.7	78.5
45	OY-5	8.51	19.3	15.0	1173	39.5	78.6
46	OY-6	10.3	16.3	14.8	789	**43.8**	81.0
47	OY-7	10.4	16.8	15.6	746	36.5	64.3
48	OY-8	9.33	16.8	15.2	744	34.2	44.9

KEY:
OY1=Akinyele,OY2=Egbeda,OY3=Ido,OY4=Iseyin,
OY5=Ogbomosho,OY6=Oluyole,OY7=Oyo,OY8=Olorunsogo

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Table 20: Proximate analysis of *Auricularia* spp cultivated on *Mangifera indica* log wood in Ondo state

Sample	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
ODI	6.11	3.11	14.16	3.87	**22.04**	50.71
OD2	6.09	4.12	15.72	3.47	18.67	**51.93**
OD3	6.24	4.57	16.26	**4.11**	21.31	47.51
OD4	5.97	6.13	18.12	2.99	19.33	47.46
OD5	6.33	5.44	**19.1**	3.28	21.44	44.41
OD6	**6.71**	3.47	16.46	3.52	19.64	50.20
OD7	5.91	5.79	13.51	2.96	20.37	51.46
OD8	6.55	**6.02**	16.4	3.11	21.24	46.68

KEY=

OD1=Idanre,OD2=Ilaje,OD3=Ile oluji,OD4=Odigbo,OD5=Okitipupa,OD6=Ose,OD7=Owo,OD8=Ifedore

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Table 21: Proximate analysis of *Auricularia* spp cultivated on *Mangifera indica* log wood in Ekiti state

Sample	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
EK 3	7.04	2.75	14.32	6.84	18.96	50.09
EK7	7.11	2.5	11.11	6.17	19.35	53.76
EK8	6.97	**7.65**	10.97	**7.04**	17.66	49.71
EK4	5.89	1.9	10.77	6.66	**19.64**	**55.14**
EK5	6.11	4.79	11.28	6.59	17.39	53.84
EK 2	**7.23**	2.43	14.67	**7.04**	18.63	50
EK 1	6.74	3.07	**15.11**	6.55	18.61	49.92
EK6	6.54	5.9	14.32	5.88	19.05	48.31

KEY:

EK1=Ado Ekiti,EK2=Ilemeje,EK3-Ikole,EK4=Oye,EK5=Irepodun,Ek6=Ikere,EK7=IjeroEK8=Emure

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Table 22: Proximate analysis of *Auricularia* spp cultivated on *Mangifera indica* log wood in Osun state

Sample	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
OS4	5.77	4.13	15.67	5.36	21.49	47.58
OS 8	6.44	2.96	**18.94**	4.47	24.87	42.32
OS 5	6.38	3.77	14.33	**5.62**	18.59	51.31
OS 7	6.97	3.82	15	4.98	**35.76**	33.47
OS 6	**7.12**	5.33	12.37	3.66	13.76	**57.76**
OS 3	6.71	5.96	9.77	4.42	28.62	44.52
OS2	6.83	**6.43**	8.76	2.98	27.32	47.68
OS1	6.18	5.33	9.24	3.77	30.59	44.89

KEY:

OS1=Boluwaduro,OS2=Ejigbo,OS3=Ifedayo,OS4,=Ifelodun,OS5=Ila,OS6=Irepodun, OS7=Iwo,OS8=Obokun

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Table 23: Proximate analysis of *Auricularia* spp cultivated on *Mangifera indica* log wood in Ogun state

Sample	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
OG1	5.33	**3.74**	10.34	**6.84**	22.34	51.41
OG2	5.14	2.96	11.12	6.16	**24.56**	50.06
OG3	5.07	3.11	9.75	4.88	23.57	53.62
OG4	4.95	3.04	10.37	4.00	21.11	56.53
OG5	4.78	2.74	**11.21**	2.58	20.94	**57.75**
OG6	**5.97**	3.48	10.27	2.8	21.37	56.11
OG7	5.44	3.15	9.78	2.48	22.44	56.71
OG8	5.78	2.88	10.56	5.16	23.98	51.64

KEY

OG1= Abeokuta,OG2=Ewekoro,OG3=Ifo,OG4=Ijebu Ode,OG5=Ikene,OG6=Shagamu,OG7=Odeda,OG8=Odogbolu

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Nutrient contents of mushroom (mg/kg) in Ekiti State

S/N	Sample code	Nitrogen	Phosphorus	Sodium	Potassium	Calcium	Magnesium
1	**EK-1**	**9.37**	**48.3**	**38.5**	**1630**	**30.3**	**93.5**
2	EK-2	9.14	36.5	47.2	1248	33.1	91.5
3	EK-3	8.37	37.7	46.3	1330	28.5	63.2
4	EK-4	8.63	35.8	**47.7**	1372	30.4	64.4
5	EK-5	8.99	37.4	46.3	1139	27.7	63.5
6	EK-6	9.10	29.6	40.6	842	41.8	63.0
7	EK-7	9.41	18.3	41.5	833	37.2	64.8
8	EK-8	**9.44**	19.0	52.1	1203	**37.5**	58.4

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Descriptive Statistics	Nitrogen	Phosphorus	Sodium	Potassium	Calcium	Magnesium
Mean	9.06	**32.83**	45.03	**1199.63**	33.31	**70,29**
Std. dev.	0.38	10.14	4.47	267.33	5.03	13.86
Std. Error	0.14	3.58	1.58	94.52	1.78	4.90
Range	1.07	30.00	**13.60**	797.00	14.10	35.10

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: Nutrient contents of mushroom (mg/kg) in Lagos State

S/N	Sample code	Nitrogen	Phosphorus	Sodium	Potassium	Calcium	Magnesium
9	LA-1	11.2	19.3	64.4	274	61.3	78.7
10	LA-2	16.3	19.6	68.9	388	61.7	103
11	LA-3	12.3	21.0	67.4	283	66.4	112
12	LA-4	12.7	18.7	58.7	296	37.8	117
13	LA-5	15.3	18.4	84.3	1933	69.3	112
14	LA-6	15.7	19.5	77.4	284	66.4	105
15	LA-7	13.4	10.4	77.5	286	66.8	94.1
16	LA-8	11.8	11.1	65.3	279	65.5	111

KEY:

LAI=Agege,LA2=Ojo,LA3=Apapa,LA4=Badagry,LA5=Epe,LA6=Shomolu,LA7=Ikorodu,LA8=Mushin.

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Descriptive Statistics	Nitrogen	Phosphorus	Sodium	Potassium	Calcium	Magnesium
Mean	13.59	**17.25**	70.49	**502.88**	61.90	**104.10**
Std. dev.	1.93	4.09	8.47	579.04	10.10	12.46
Std. Error	0.68	1.45	3.00	204.72	3.57	4.40
Range	5.10	10.60	**25.60**	1659.00	31.50	38.30

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Nutrient contents of mushroom (mg/kg) in Ogun State

S/N	KEY	Nitrogen	Phosphorous	Sodium	Potassium	Calcium	Magnesium
17	OG-1	8.94	52.1	27.4	475	41.8	54.8
18	OG-2	8.93	65.4	26.2	487	31.0	55.4
19	OG-3	9.07	**66.6**	26.7	468	**44.2**	54.0
20	OG-4	13.4	50.7	26.8	**491**	40.3	56.8
21	OG-5	13.7	18.3	37.2	464	40.6	**63.2**
22	OG-6	13.0	19.9	24.3	486	41.6	57.4

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Key:

23	OG-7	**13.9**	20.4	28.4	480	40.0	55.0
24	OG-8	11.6	20.6	**41.8**	477	40.7	57.3

OG1=Abeokuta,OG2=Ewekoro,OG3=Ifo,OG4=Ijebu Ode,OG5=Ikene,OG6=Shagamu,OG7=Odeda,OG8=Odogbolu

Descriptive Statistics	**Nitrogen**	**Phosphorus**	**Sodium**	**Potassium**	**Calcium**	**Magnesium**
Mean	11.57	**39.25**	29.85	**478.50**	40.03	**56.74**
Std. dev.	2.25	21.53	5.19	9.43	3.88	2.89
Std. Error	0.80	7.61	2.19	3.33	1.37	1.02
Range	4.97	48.30	**17.50**	27.00	13.20	9.20

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Nutrient contents of mushroom (mg/kg) in Ondo State

S/N	Sample code	Nitrogen	Phosphorus	Sodium	Potassium	Calcium	Magnesium
25	OD-1	10.4	15.3	18.4	326	38.4	78.5
26	OD-2	11.4	20.0	17.8	366	36.7	73.6
27	OD-3	10.2	21.1	26.3	384	36.8	74.4
28	0D-4	10.8	18.4	10.6	375	34.1	74.7
29	OD-5	7.39	18.4	11.4	295	36.5	76.5
30	OD-6	9.44	19.6	14.3	275	37.0	58.9
31	OD-7	9.37	20.1	18.3	288	35.3	67.4
32	OD-8	7.48	18.0	28.5	343	38.5	55.7

KEY:

OD1=Idanre,OD2=Ilaje,OD3=Ileoluji,OD4=Odigbo,

OD5=Okitipupa,OD6=Ose,OD7=Owo,OD8=Ifedore

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Descriptive Statistics	Nitrogen	Phosphorus	Sodium	Potassium	Calcium	Magnesium
Mean	9.56	**18.86**	18.20	**331.50**	36.66	**69.96**
Std. dev.	1.47	1.78	6.45	42.12	1.46	8.48
Std. Error	0.52	0.63	2.28	14.89	0.52	3.00
Range	4.01	5.80	**17.90**	109.00	4.40	22.80

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Nutrient content of mushroom(mg/kg) in Osun State

S/N	Sample code	Nigrogen	Phosphoorus	Sodium	**Potassium**	Calcium	Magnesium
33	OS-1	8.38	48.4	22.1	**1842**	55.6	82.8
34	OS-2	7.39	47.9	17.4	1836	55.3	103
36	OS-4	10.5	31.4	22.5	1773	51.4	117
37	OS-5	11.9	19.5	52.4	1830	53.7	131
38	OS-6	8.40	32.2	21.0	1631	39.6	127
39	OS-7	8.41	14.8	21.2	749	82.7	133
40	OS-8	7.98	18.4	20.7	748	48.8	128

KEY:

OS1=Boluwaduro, OS2=Ejigbo,OS3=Ifedayo,OS4,=Ifelodun,

OS5=Ila, OS6=Irepodun,OS7=Iwo,OS8=Obokun

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Descriptive Statistics	Nitrogen	Phosphorus	Sodium	Potassium	Calcium	Magnesium
Mean	8.96	**32.69**	24.34	**1511.63**	55.01	**116.73**
Std. dev.	1.49	14.35	11.50	477.00	12.32	17.17
Std. Error	0.53	5.07	4.07	168.65	4.35	6.07
Range	4.51	34.10	**35.00**	1094.00	43.10	50.20

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Nutrient contents of mushroom(mg/kg) in Oyo State

41	OY-1	**10.5**	**28.6**	15.3	736	37.5	**83.2**
42	OY-2	6.38	27.0	15.7	744	37.9	78.4
43	OY-3	6.98	25.9	**16.3**	830	31.0	78.3
44	OY-4	8.38	27.6	15.4	**1264**	26.7	78.5
45	OY-5	8.51	19.3	15.0	1173	39.5	78.6
46	OY-6	10.3	16.3	14.8	789	**43.8**	81.0
47	OY-7	10.4	16.8	15.6	746	36.5	64.3
48	OY-8	9.33	16.8	15.2	744	34.2	44.9

KEY:

OY1=Akinyele,OY2=Egbeda,OY3=Ido,OY4=Iseyin,

OY5=Ogbomosho,OY6=Oluyole,OY7=Oyo,OY8=Olorunsogo

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Descriptive Statistics	Nitrogen	Phosphorus	Sodium	Potassium	Calcium	Magnesium
Mean	8.85	**22.29**	15.41	**878.25**	35.89	**73.40**
Std. dev.	1.58	5.46	0.46	213.72	5.26	12.82
Std. Error	0.56	1.93	0.16	75.56	1.86	4.53
Range	4.12	12.30	**1.50**	528.00	17.10	38.30

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Proximate analysis of *Auricularia* spp cultivated on *Mangifera indica* log wood in Oyo state

Sample	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
OYI	6.58	**6.96**	12.37	3.11	19.24	51.74
OY2	4.59	3.76	11.56	2.87	20	57.22
OY3	7.43	5.39	14.24	3.54	**21.34**	48.06
OY4	5.87	6.06	10.56	3.83	18.66	55.02
OY5	**7.86**	3.72	11.47	4.06	18.21	54.68
OY6	4.94	2.32	12.38	3.47	19.35	**57.54**
OY7	5.87	5.1	11.64	**4.11**	19	54.28
OY8	6.93	1.9	**15.66**	3.81	18.66	53.04

KEY:

OY1=Akinyele,OY2=Egbeda,OY3=Ido,OY4=Iseyin,

OY5=Ogbomosho,OY6=Oluyole,OY7=Oyo,OY8=Olorunsogo

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Descriptive Statistics	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
Mean	6.26	**4.40**	12.49	3.60	19.31	53.95
Std. dev.	1.15	1.78	1.67	0.44	0.98	3.07
Std. Error	0.41	0.63	0.59	0.16	**0.35**	1.08
Range	3.27	5.06	5.10	1.24	3.13	9.48

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Proximate analysis of *Auricularia* spp cultivated on *Mangifera indica* log wood in Lagos state.

Sample	% Protein	% Ash	% Moisture	% Fat	% C.Fiber	% CHO
LA1	7.57	5.96	14.74	2.57	17.64	51.52
LA2	6.22	**6.11**	14.33	2.68	17.23	53.43
LA3	**9.13**	4.38	12.87	2.51	18.11	53,00
LA4	7.07	3.77	14.56	2.49	18.56	53.55
LA5	2.74	4.16	13.34	**2.98**	17.84	**58.94**
LA6	5.09	5.23	14.62	2.84	19.64	52.58
LA7	9.7	1.94	12.98	3.11	18.55	53.72
LA8	8.35	2.79	**15.34**	2.89	**20.34**	50.29

KEY:

LAI=Agege, LA2=Ojo,LA3=Apapa,LA4=Badagry,LA5=Epe,LA6=Shomolu,LA7= Ikorodu,LA8=Mushin

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Statistics	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
Mean	6.76	**4.68**	13.97	3.13	18.33	53.13
Std. dev.	2.21	1.42	0.77	1.13	0.78	2.94
Std. Error	0.78	0.50	0.27	0.40	**0.28**	1.04
Range	6.96	4.17	1.87	3.39	2.41	10.63

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Proximate analysis of *Auricularia* spp cultivated on *Mangifera indica* log wood in Ondo state

Sample	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
ODI	6.11	3.11	14.16	3.87	**22.04**	50.71
OD2	6.09	4.12	15.72	3.47	18.67	**51.93**
OD3	6.24	4.57	16.26	**4.11**	21.31	47.51
OD4	5.97	6.13	18.12	2.99	19.33	47.46
OD5	6.33	5.44	**19.1**	3.28	21.44	44.41
OD6	**6.71**	3.47	16.46	3.52	19.64	50.20
OD7	5.91	5.79	13.51	2.96	20.37	51.46
OD8	6.55	**6.02**	16.4	3.11	21.24	46.68

KEY=

OD1=Idanre,OD2=Ilaje,OD3=Ileoluji,OD4=Odigbo,OD5=Okitipupa,OD6=Ose,OD7=Owo,OD8=Ifedore

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Descriptive	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
Mean	6.24	**4.83**	16.22	3.41	20.51	48.80
Std. dev.	0.28	1.18	1.85	0.41	1.19	2.66
Std. Error	0.10	0.42	0.65	0.15	**0.42**	0.94
Range	0.80	3.02	5.59	1.15	3.37	7.61

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Proximate analysis of *Auricularia* spp cultivated on *Mangifera indica* log wood in Ekitistate

Sample	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
EK 3	7.04	2.75	14.32	6.84	18.96	50.09
EK7	7.11	2.5	11.11	6.17	19.35	53.76
EK8	6.97	7.65	10.97	7.04	17.66	49.71
EK4	5.89	1.9	10.77	6.66	19.64	55.14
EK5	6.11	4.79	11.28	6.59	17.39	53.84
EK 2	7.23	2.43	14.67	7.04	18.63	50
EK 1	6.74	3.07	15.11	6.55	18.61	49.92
EK6	6.54	5.9	14.32	5.88	19.05	48.31

KEY:

EK1=AdoEkiti,EK2=Ilemeje,EK3-Ikole,EK4=Oye,EK5=Irepodun,Ek6=Ikere,EK7=IjeroEK8=Emure

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Statistics	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
Mean	6.93	**3.49**	12.96	6.22	18.82	51.59
Std. dev.	0.75	1.88	2.07	1.38	0.98	2.24
Std. Error	0.27	0.67	0.73	0.48	**0.35**	0.79
Range	2.46	5.75	4.57	4.15	2.96	5.43

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Proximate analysis of *Auricularia* spp cultivated on *Mangifera indica* log wood in Osun state

Sample	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
OS4	5.77	4.13	15.67	5.36	21.49	47.58
OS 8	6.44	2.96	**18.94**	4.47	24.87	42.32
OS 5	6.38	3.77	14.33	**5.62**	18.59	51.31
OS 7	6.97	3.82	15	4.98	**35.76**	33.47
OS 6	**7.12**	5.33	12.37	3.66	13.76	**57.76**
OS 3	6.71	5.96	9.77	4.42	28.62	44.52
OS2	6.83	**6.43**	8.76	2.98	27.32	47.68
OS1	6.18	5.33	9.24	3.77	30.59	44.89

KEY:

OS1=Boluwaduro,OS2=Ejigbo,OS3=Ifedayo,OS4,=Ifelodun,OS5=Ila,OS6=Irepodun,OS7=Iwo,OS8=Obokun

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Statistics	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
Mean	6.55	**4.72**	13.01	4.41	25.13	46.19
Std. dev.	0.45	1.22	3.61	0.90	7.02	7.03
Std. Error	0.16	0.43	1.28	0.32	**2.48**	2.48
Range	1.35	3.47	10.18	2.64	22.00	24.29

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Proximate analysis of *Auricularia* spp cultivated on *Mangifera indica* log wood in Ogun state

OG1	5.33	**3.74**	10.34	**6.84**	22.34	51.41
OG2	5.14	2.96	11.12	6.16	**24.56**	50.06
OG3	5.07	3.11	9.75	4.88	23.57	53.62
OG4	4.95	3.04	10.37	4.00	21.11	56.53
OG5	4.78	2.74	**11.21**	2.58	20.94	**57.75**
OG6	**5.97**	3.48	10.27	2.8	21.37	56.11
OG7	5.44	3.15	9.78	2.48	22.44	56.71
OG8	5.78	2.88	10.56	5.16	23.98	51.64

KEY
OG1=Abeokuta,OG2=Ewekoro,OG3=Ifo,OG4=Ijebu Ode,OG5=Ikene,OG6=Shagamu,OG7=Odeda,OG8=Odogbolu

Statistics	**%Protein**	**%Ash**	**%Moisture**	**%Fat**	**%C.Fiber**	**%CHO**
Mean	5.31	**3.14**	10.43	4.36	22.54	54.21
Std. dev.	0.41	0.33	0.54	1.67	1.38	2.92
Std. Error	0.14	0.12	0.19	0.59	**0.49**	1.03
Range	1.19	1.00	1.46	4.36	3.62	7.69

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Proximate analysis of *Auricularia* spp cultivated on *Mangifera indica* log wood in Oyo state

Sample	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
OYI	6.58	**6.96**	12.37	3.11	19.24	51.74
OY2	4.59	3.76	11.56	2.87	20	57.22
OY3	7.43	5.39	14.24	3.54	**21.34**	48.06
OY4	5.87	6.06	10.56	3.83	18.66	55.02
OY5	**7.86**	3.72	11.47	4.06	18.21	54.68
OY6	4.94	2.32	12.38	3.47	19.35	**57.54**
OY7	5.87	5.1	11.64	**4.11**	19	54.28
OY8	6.93	1.9	**15.66**	3.81	18.66	53.04

KEY:
OY1=Akinyele,OY2=Egbeda,OY3=Ido,OY4=Iseyin,
OY5=Ogbomosho,OY6=Oluyole,OY7=Oyo,OY8=Olorunsogo

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Descriptive Statistics	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
Mean	6.26	**4.40**	12.49	3.60	19.31	53.95
Std. dev.	1.15	1.78	1.67	0.44	0.98	3.07
Std. Error	0.41	0.63	0.59	0.16	**0.35**	1.08
Range	3.27	5.06	5.10	1.24	3.13	9.48

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: Proximate analysis of *Auricularia* spp cultivated on *Mangifera indica* log wood in Lagos state.

Sample	% Protein	% Ash	% Moisture	% Fat	% C.Fiber	% CHO
LA1	7.57	5.96	14.74	2.57	17.64	51.52
LA2	6.22	**6.11**	14.33	2.68	17.23	53.43
LA3	**9.13**	4.38	12.87	2.51	18.11	53,00
LA4	7.07	3.77	14.56	2.49	18.56	53.55
LA5	2.74	4.16	13.34	**2.98**	17.84	**58.94**
LA6	5.09	5.23	14.62	2.84	19.64	52.58
LA7	9.7	1.94	12.98	3.11	18.55	53.72
LA8	8.35	2.79	**15.34**	2.89	**20.34**	50.29

KEY:

LAI=Agege, LA2=Ojo,LA3=Apapa,LA4=Badagry,LA5=Epe,LA6=Shomolu,LA7= Ikorodu,LA8=Mushin

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Statistics	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
Mean	6.76	**4.68**	13.97	3.13	18.33	53.13
Std. dev.	2.21	1.42	0.77	1.13	0.78	2.94
Std. Error	0.78	0.50	0.27	0.40	**0.28**	1.04
Range	6.96	4.17	1.87	3.39	2.41	10.63

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Proximate analysis of *Auricularia* spp cultivated on *Mangifera indica* log wood in Ondo state

Sample	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
ODI	6.11	3.11	14.16	3.87	**22.04**	50.71
OD2	6.09	4.12	15.72	3.47	18.67	**51.93**
OD3	6.24	4.57	16.26	**4.11**	21.31	47.51
OD4	5.97	6.13	18.12	2.99	19.33	47.46
OD5	6.33	5.44	**19.1**	3.28	21.44	44.41
OD6	**6.71**	3.47	16.46	3.52	19.64	50.20
OD7	5.91	5.79	13.51	2.96	20.37	51.46
OD8	6.55	**6.02**	16.4	3.11	21.24	46.68

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KEY=

OD1=Idanre,OD2=Ilaje,OD3=Ileoluji,OD4=Odigbo,OD5=Okitipupa,OD6=Ose,OD7=Owo,OD8=Ifedore

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Descriptive	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
Mean	6.24	**4.83**	16.22	3.41	20.51	48.80
Std. dev.	0.28	1.18	1.85	0.41	1.19	2.66
Std. Error	0.10	0.42	0.65	0.15	**0.42**	0.94
Range	0.80	3.02	5.59	1.15	3.37	7.61

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Proximate analysis of *Auricularia* spp cultivated on *Mangifera indica* log wood in Ekitistate

Sample	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
EK 3	7.04	2.75	14.32	6.84	18.96	50.09
EK7	7.11	2.5	11.11	6.17	19.35	53.76
EK8	6.97	7.65	10.97	7.04	17.66	49.71
EK4	5.89	1.9	10.77	6.66	19.64	55.14
EK5	6.11	4.79	11.28	6.59	17.39	53.84
EK 2	7.23	2.43	14.67	7.04	18.63	50
EK 1	6.74	3.07	15.11	6.55	18.61	49.92
EK6	6.54	5.9	14.32	5.88	19.05	48.31

KEY:

EK1=AdoEkiti,EK2=Ilemeje,EK3-Ikole,EK4=Oye,EK5=Irepodun,Ek6=Ikere,EK7=IjeroEK8=Emure

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Statistics	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
Mean	6.93	**3.49**	12.96	6.22	18.82	51.59
Std. dev.	0.75	1.88	2.07	1.38	0.98	2.24
Std. Error	0.27	0.67	0.73	0.48	**0.35**	0.79
Range	2.46	5.75	4.57	4.15	2.96	5.43

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Proximate analysis of *Auricularia* spp cultivated on *Mangifera indica* log wood in Osun state

Sample	%Protein	%Ash	%Moisture	%Fat	%C.Fiber	%CHO
OS4	5.77	4.13	15.67	5.36	21.49	47.58
OS 8	6.44	2.96	**18.94**	4.47	24.87	42.32
OS 5	6.38	3.77	14.33	**5.62**	18.59	51.31
OS 7	6.97	3.82	15	4.98	**35.76**	33.47
OS 6	**7.12**	5.33	12.37	3.66	13.76	**57.76**
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OS2	6.83	**6.43**	8.76	2.98	27.32	47.68
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Std. dev.	0.45	1.22	3.61	0.90	7.02	7.03
Std. Error	0.16	0.43	1.28	0.32	**2.48**	2.48
Range	1.35	3.47	10.18	2.64	22.00	24.29

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