ECRONICON OPEN ACCESS

EC MICROBIOLOGY
Research Article

Don't Waste the 'Wastes', they are Ways to Wealth

Abiodun Olusola Salami and Faith A Bankole*

Department of Crop Production and Protection, Obafemi Awolowo University, Ile-Ife, Nigeria

***Corresponding Author**: Faith A Bankole, Department of Crop Production and Protection, Obafemi Awolowo University, Ile-Ife, Nigeria. bankolefaitha@gmail.com, 2347037285891.

Received: May 14, 2018; **Published:** August 16, 2018

Abstract

Nigerian agriculture generates a huge quantity of waste resources, which can be converted to useful renewable energy to augment the country's primary energy needs, assist in reducing waste management problems and serve as opportunity to reap a range of benefits. Lignocellulosic residues present in these abundant agro-wastes, can be converted into edible food source by the action of saprophytic fungi. The cultivation of mushrooms which uses biotechnological procedure of bioconversion of different agro-wastes to edible mushrooms of high nourishing as well as recycling the agro-wastes into useful agricultural input is gaining relevance.

This study was carried out in the Mycology Laboratory of the Department of Crop Production and Protection, O.A.U. Ile-Ife and evaluated the response of different agro-wastes combinations in the cultivation of edible mushroom. Agro-wastes, for example, Corncob (CC), sugarcane bagasse (SB) were utilized as the main substrates while rice grain (RB) and groundnut shell (GS) were utilized as supplements to the main substrates. The agro-wastes used were pasteurized, pressed to remove excess water and then treated with $CaCO_3$ and $CaSO_4$ before inoculation. 300g of each CC: GS, CC: RB, SB: RB, SB: GS combinations were mixed together properly in a container in different percentages which were 80:20, 70:30, 60:40 were weighed, filled into transparent nylon, inoculated with spawns of *P. florida* (Oyster mushroom) and were kept in dark cupboards till complete ramification. Data were collected on number of days for complete ramification, days to pin head formation, days to maturity of fruiting body, number of fruiting bodies, weight of fruiting bodies, number of flushes and biological efficiency which were subjected to analysis of variance and significant means were separated using LSD at $p < 0.05$. Combination of different agro-wastes increased the yield of *P. florida* and substrate CCRB with ratio 80:20, was best for the production of *P. florida* fruiting bodies. CC based substrates had higher yield when compared to SB based substrates. In addition, supplementing the main substrates with additives increased growth rate, yield and the nutritional content of *P. florida*. This study concluded that the use of agro-wastes provides a viable means of producing edible mushroom. It is thus recommended that mushroom production should be increased with the use of agro-wastes from farm produce and that combination of different agro-wastes' substrates should be used in production of mushroom in place of single substrates. Thus, agro-wastes are efficiently managed and recycled.

Keywords: *Agro-Wastes; P. florida; Corncob (CC); Sugarcane Bagasse (SB); Rice Grain (RB); Groundnut Shell (GS)*

Introduction

Fungi has boundless significance in the terrestrial biological system and thus in man's life. In nature, apart from providing food to human and other animals, fungi also assume a critical part in the cycling of carbon and different components, by breaking down the lignocellulosic buildups and animal wastes thus, serving as substrates for saprophytic fungi. By this process, saprophytes play an imperative ecological part alongside other organisms, enhancing the cycling of plants and animal. At the same time, they produce different enzymes that breakdown complex substances which allows the uptake of soluble substances utilized for their own particular growth [1-3].

Mushroom is a fleshy, spore-producing fruiting body of some higher fungi [4]. These are saprophytes of the family basidiomycota and some members of the ascomycota [5]. Mushrooms are thought to be special and supernatural in origin [6]. They belong to class Basidiomycetes, subclass Hollobasidiomycetidae, order Agricale. They feed by secreting extracellular enzymes thereby digesting food externally and absorb the nutrients in net-like chain called hyphae. The hypha, act as a conscious intellect and respond to stimuli when exposed in their ecological niche [5]. Mushrooms are achlorophyllous, and are not autotrophic like the green plants [7]. Instead, during their vegetative growth stage, mushroom mycelia secrete enzymes that break down compounds such as cellulose and lignin present in the substrate. The degraded compounds are then absorbed by the hyphae and the mycelium enlarges [7]. The cultivation of mushroom is in two phases which are; spawn running phase (i.e. mycelia growth period) and fructification phase (development of the fruiting bodies) which are both dependent on external factors such as temperature and humidity [8]. They are of different types which include: *Agaricus bisporus* (white button mushroom), *Pleurotus* spp. (Oyster mushrooms), *Volvariella volvacea* (paddy straw mushroom), *Lentinus edodes* (shiitake mushrooms) and *Auricus laria* (black ear mushroom) [6]. *Pleurotus* species are characterized by a white spore pint attached to recurrent gills, often with an eccentric (off center) stipe, or no stipe at all. The common name "oyster mushroom" originated from the white shell-like appearance of the fruiting body [9]. Agro-wastes, are rich in carbon compounds, which are readily available sources of carbohydrates. This accelerates colonization and the subsequent breakdown of the substrate, which decreases the time of fruiting since the mycelium effortlessly utilizes these simple sugars for the fructification, thus, increasing productivity [10]. Some carbon rich agro-wastes are low in protein and cannot sufficiently support the growth and development of mushrooms. Such agro-waste therefore needs additional mineral nutrient such as nitrogen, phosphate, potassium and vitamins to augment the deficiencies in the main substrates for cultivation [11]. Several authors have reported the extensive use of agro-wastes such as sawdust, paddy straw, sugarcane bagasse, corn stalk, corn cobs, waste cotton, leaves and pseudo stem of banana, water hyacinth, duck weed, rice straw etc which does not require expensive processing methods neither enrichment material [4,8,12].

Sugar and starch which are readily available in these substrates tend to reduce the time of fruiting since the mycelium easily converts these carbohydrates in reserve for the fructification [10]. Supplements like limestone ($CaCO_3$) or gypsum ($CaSO_4$) is added to the cultivation medium, in order to obtain the right pH condition that is favourable for the growth of the fungus. Various substrates have different effects on the growth, yield and quality of mushrooms [13]. The commercial production of mushrooms is largely determined by the availability and utilization of cheap and locally organic materials of which are agricultural wastes [14]. The cultivation of mushrooms which uses biotechnological procedure of bioconversion of different agro-wastes to edible mushrooms of high nourishing value as well as recycling the agro-wastes into useful agricultural input is gaining relevance. Also, the residual substrate (spent mushroom compost) obtained from the cultivation of edible mushrooms can likewise be used as soil conditioner, natural fertilizer, or food for animals, thereby recycling of raw materials [15], which today is called "zeri" technology [16]. In developing countries mushrooms is an important crop that can fetch farmers a substantial income to alleviate poverty and provide employment opportunities [17]. Over the years, man has realized the nutritional value of mushrooms, as well as their healthy properties compared to other foods. Mushrooms are more beneficial and imperative as they are incredible wellsprings of carbohydrates, proteins, mineral salts, vitamins and essential amino acids, which can help to maintain a good nutritional balance [18,19]. Proximate analyses have shown the nutritional importance of mushrooms as they possess higher protein content than conventional vegetables. The cholesterol and fat content of the conventional sources of protein such as meat and chicken are of high level, which are known to cause increase in weight and cardiovascular diseases. Consequent to this, the proteins from other sources such as fungi, algae, bacteria and yeast became more popular in recent years [20]. The substance of essential amino acids in mushroom is high and near the need of the human body as mushroom is easily digestible with no cholesterol content. Sales-Campos Ceci., *et al.* [11], reported that 200g of mushrooms (dry weight) are adequate to nourish an individual weighing around 70 Kg, giving a decent dietary adjust. Hence, these macro-fungi are a nutritionally adequate food source.

Agricultural wastes include horticultural and forestry wastes such as, crop residues, animal manure, diseased carcasses etc. It has been established, that along the food production chain, several agricultural products are wasted and has been underutilized which has resulted

to the proliferation of such materials in the environment. Buswell., *et al.* [21] described edible mushroom cultivation as an economically-viable processes for the bioconversion of agro-wastes. Chang [1] depicted that mushroom cultivation was utilized as a result of their ecological role in the bioconversion of solid wastes released by industry as well as agriculture into palatable biomass, which are can be considered as edible food or as a wellspring of medications and pharmaceuticals. About 50% of land produce such as straws, leaves, stems, roots etc. remains waste and are not utilized, these wastes can be bio-converted into food thereby leaving behind a healthy and safe environment [8]. It is therefore important to develop a protocol that allows the cultivation of edible mushrooms using these abundant, readily available agro-wastes at low cost, which is the thrust of this study.

Materials and Methods

Location of the Study

The study was carried out at the Mycology Laboratory, Department of Crop Production and Protection, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife. The tissue culture procedure was done following Bankole and Salami [8]. The experiment was a (4 x 3 x 4) factorial experiment laid out in a randomized complete block design. The Data collected were subjected to statistical analyses using SAS version 9.0 (SAS, 2002), analysis of variance was carried out and significant means were separated using least significant difference (LSD) at probability level of 0.05, graphs and charts were plotted using Microsoft Excel (MS package, 2016).

Spawns of *Pleurotus florida* were collected from the Mycology Laboratory of the Department of Crop Production and Protection, Obafemi Awolowo University, Ile-Ife.

Fruiting body production

Corn cob (CC) and sugarcane bagasse (SB) were used as the main agro-waste while rice bran (RB) and groundnut shell (GS) were used as additives for the production of fruiting bodies in this study. These agro-wastes were combined in different ratio such as: (CC: GS, CC : RB), (SB : RB, SB : GS). Three hundred grams of the substrates used were weighed and subjected to the same treatment above (for spawn production). The quantity of calcium sulphate and calcium carbonate added depended on the weight of the substrate (300g) used. The substrates were also inoculated with the spawn and then transferred into transparent nylon. The inoculated bags of substrates were transferred into the dark chamber for incubation and monitored until full ramification of the substrates was attained. After full ramification of the substrate with the mycelia of the mushroom, they were brought out from the dark chamber to the fruiting body chamber for pin head formation. At this stage, the fully ramified substrates needed light and water, so they were set in the mushroom house for wetting and observation for pin heads formation leading to fruiting body production to form macro-fungi/mushroom. The pH of the substrate was maintained at a range of 6.30 - 7.10.

Data Collection

Data were collected on number of days for complete ramification, days for pin head formation, number of days to maturity of fruiting body, number of fruiting bodies, number of flushes at intervals; as well as weight of fruiting bodies, average growth rate and biological efficiency. (Data on days were based on counts while the weight was measured with the weighing balance).

The biological efficiency of the mushroom was calculated according to Salami., *et al.* [4]:

$$\text{Biological efficiency} = \frac{w \text{ X } 100}{W \text{ X } 1}$$

Where w = Total weight of fruit bodies

W = Total weight of substrate of spawning.

Results

All the substrates used for this study, significantly supported the growth of the Oyster mushroom (*Pleurotus florida*) at different stages of production. This is revealed in a growth flow-chart of the production cycle of *P. florida* (Plate 1). Although, there were observable differences in the level at which the substrates supported the growth of *P. florida.*

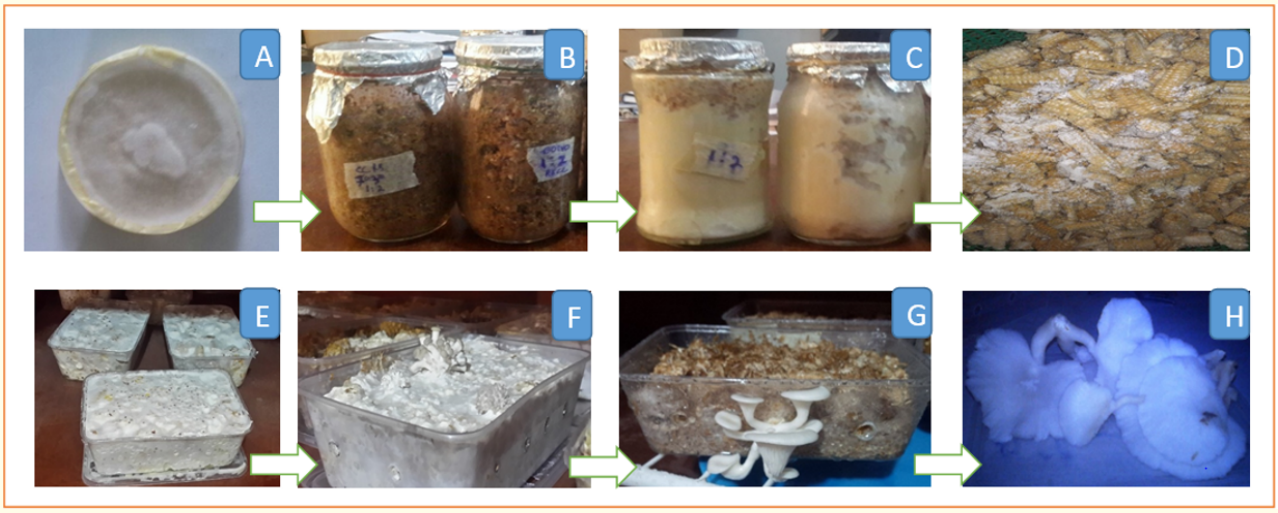

Plate 1: *Different stages in Mushroom production.*

A: Tissue Culture; B: Sterilized Bottle of Agro: Wastes; C: Completely Ramified Spawn Bottles; D: Chopped Agro: Wastes Substrates; E: Completely Ramified Substrates; F: Pin Head Formation on Ramified Substrate; G&H: Matured Fruiting Bodies.

All the substrates (which are agro-wastes) used in this study were found to contain essential elements needed for the growth and development of Oyster mushroom. These elements were found present in varied amount in the agro-wastes used in this study. The main substrates (corncobs and sugarcane bagasse) had the highest percentages of carbon content while the additives (rice bran and Groundnut shell) had lower percentages of carbon (Table 1). Also, the percentage of nitrogen was found higher in the additives when compared to the nitrogen content of the main substrates (Table 1). Groundnut shell had the highest percentage of potassium while the least potassium content was in corncobs. The phosphorus level was found to be generally low in all the substrates although, comparatively found to be highest in rice bran.

Substrates (Agro-wastes)	Nitrogen (%)	Carbon (%)	Calcium (%)	Phosphorus (ppm)	Potassium (Cmol/kg)
Corn cobs	1.60	58.00	2.04	0.17	11.25
Sugarcane bagasse	1.68	30.00	6.08	0.17	14.25
Rice bran	2.45	4.99	3.23	0.32	11.98
Groundnut shell	2.24	6.63	6.55	0.18	20.70

Table 1: *Elemental composition of the substrates.*

Fruiting body production of Oyster Mushroom (*P. florida*)

Analysis of variance for the growth parameters of *P. florida* grown on different agro-wastes combination is showed that the applied treatments (except the replication) were found to be highly significant on improving the production of Oyster mushroom (*P. florida*). This is revealed in the significant treatment effect on all the growth parameters of *P. florida* measured at probability level 0.05. These growth parameters include days to complete substrate ramification (DTCSR), days to pin head formation (DPHF), days to complete fruiting bodies maturation (DCFM), weight of the fruiting bodies (WTFB), number of fruiting bodies (NOFB), length of stipe (LOS), width of the stipe (WIDTH), diameter of cap (DOC), number of fruiting bodies (NOF), flush interval (FI) and biological efficiency (BE). The effect of

different combinations of agro-wastes on the growth parameters (DTCSR, DPHF, DCFM, WTFB, NOFB, LOS, WIDTH, DOC, NOF, FI and BE) of *P. florida* is presented in table 2. There were conspicuous differences in the measured growth parameters of *P. florida* as a result of the different combinations of agro-wastes (CCGS, CCRB, SBGS and SBRB) used in this study (Table 2). *P. florida* inoculated on CC, CCGS and SBGS substrates reached full growth in form of ramification of the substrates after 14 days of inoculation. This number of days spent by *P. florida* for the full growth in form of ramification of the substrates for fruiting body production was found to be significantly different from the observation on CCRB, SB and SBRB substrates where the full ramification by *P. florida* was recorded after 18 days, 19 days and 22 days respectively (Table 2). *P. florida* grown on substrates with GS as additives, had shorter days for complete ramification when compared with substrates having RB as additives (Table 2). Pinheads of *P. florida* were observed on substrates CC and CCGS 21 days after inoculation of the substrates with the completely ramified spawns of *P. florida* (Table 2). The number of days for the pinhead formation on substrates CC and CCGS were not significantly different from each other but significantly different from CCRB where pinheads of *P. florida* were observed 23 days after inoculation of the substrate with the completely ramified spawns of *P. florida*. However, pinheads of *P. florida* were observed on substrates SB and SBRB after 25 and 28 days of inoculation respectively. This observed number of days for pinhead formation of *P. florida* were significantly different from each other and the aforementioned substrates (Table 2).

Plate 2: *Ramified substrate bags for P. florida (Oyster mushroom).*

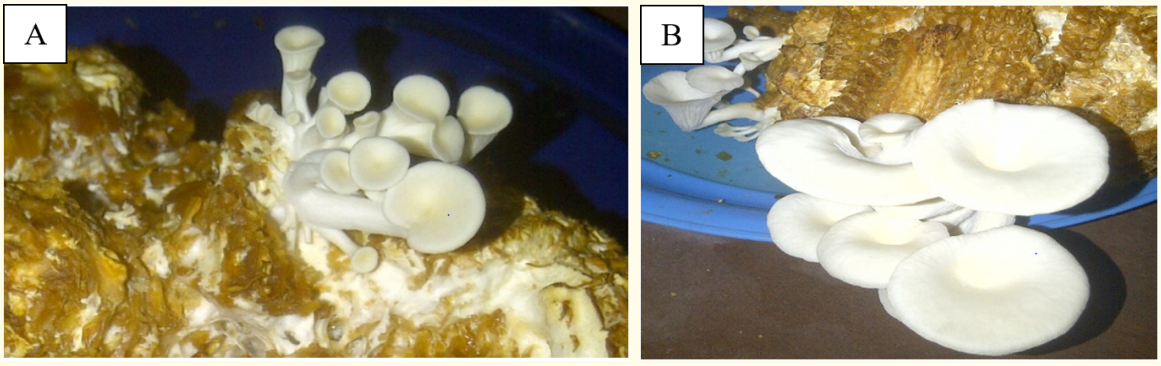

Plate 3: *Pinheads and matured fruiting bodies of Oyster Mushroom.*
Plate 3a: Pinheads of Oyster mushroom (P. florida), Plate 3b: Matured fruiting bodies of Oyster mushroom (P. florida).

The number of days taken for the complete maturation of the fruiting bodies (DCFM) of *P. florida* was also recorded for all the substrates used in this study (Table 2). The matured fruiting bodies of *P. florida* were first harvested on CC 24 days after inoculation of the substrate with the completely ramified spawns of *P. florida*. Whereas, the matured fruiting bodies of *P. florida* were harvested after 25, 26, 27, and 30 days after inoculation on substrates CCGS, SBGS, CCRB and SB respectively (Table 2). The matured fruiting bodies of *P. florida* were harvested on SBRB after 31 days of inoculation with the fully ramified spawns of *P. florida.* These recorded values of DTCM of the fruiting bodies of *P. florida* were significantly different from each other at P< 0.05 (Table 2). It was also observed that mushrooms harvested from substrates with CC as the main substrate matured earlier than the other substrates with SB as the main substrate (Table 2). Using the days of pinhead formation as the benchmark, a range of 3-5 days was recorded for the maturation of the pinheads into harvested fruiting bodies (Table 2). The highest weight (35.11g) of matured fruiting bodies of *P. florida* was recorded on CCRB, this was followed by CCGS, CC, SBGS, SBRB with corresponding values of 24.65g, 23.72g, 20.69g and 13.51g respectively. These recorded weights of the matured fruiting bodies of *P. florida*, were found to be significantly different from each other at P < 0.05 (Table 2). The matured fruiting bodies of *P. florida* was found to be lowest on SB with a recorded weight of 11.85g. The matured fruiting bodies of *P. florida* harvested from substrates with CC as the main substrate had significant higher weight than those harvested where SB was used as the main substrate in this study. In addition, the matured fruiting bodies of *P. florida* harvested from the combination of CC and RB had higher weight when compared with CC (as single substrate without additives) (Table 2). Similar trend was observed in the biological efficiency of oyster mushroom (*P. florida*). *Pleurotus florida* grown on CCRB was found to have the highest biological efficiency of 17.55%. This was followed by *P. florida* grown on CCGS, CC, SBGS and SBRB which were found to have 8.22%, 7.90%, 6.89%, and 6.75% respectively while the lowest biological efficiency was recorded *P. florida* grown on SB alone (4.74%) (Table 2). The average number of matured fruiting bodies of *P. florida* harvested on CCRB and SBGS was 4.19 and 4.17 respectively. This was found not to be significantly different from each other but was significantly different from average number of matured fruiting bodies of *P. florida* harvested the other substrates (SB, SBRB, CCGS and CC) with 1.83, 2.56, 3.81, 3.92 values respectively. The number of fruiting bodies of *P. florida* harvested on SB based substrates was found to be relatively higher than the number of fruiting bodies of *P. florida* harvested from other substrates. Also, the number of matured fruiting bodies of *P. florida* harvested from the substrates with additives/supplements were found to be higher than main substrates without additives/supplements (Table 2). The length of the stipe of *P. florida* harvested from CCRB was found to have an average length of 5.77 cm which was found to be significantly longer than length of the stipe of *P. florida* harvested from the other substrates (SB, SBRB, CCGS and CC) used in this study. The width of stipe of *P. florida* harvested on SBGS was found to be 2.86 cm, 4.56 cm, 4.99 cm, 3.89 cm, 2.73 cm and 2.76 cm for SBRB, CCRB, SBGS, CCGS, CC and SB respectively (Table 2). *Pleurotus florida* harvested on CCRB had the highest average diameter of the cap (8.11 cm), this was followed by SBGS, CCGS, SBRB, SB and the lowest was recorded on CC alone (Table 2).

Combination	DTCSR (Days)	DPHF (Days)	DCFM (Days)	WTFB (g)	NOFB	LOS (cm)	WIDTH (cm)	DOC (cm)	NOF	FI	BE (%)
SBRB	22.00	28.00	31.00	13.51	2.56	3.59	2.86	5.58	1.33	1.83	6.75
CCRB	18.00	23.00	27.00	35.11	4.19	5.77	4.56	8.11	1.75	3.58	17.55
SBGS	14.00	22.00	26.00	20.69	4.14	4.69	4.99	7.43	1.75	3.92	6.89
CCGS	14.00	21.00	25.00	24.65	3.81	4.05	3.89	6.00	1.42	2.08	8.22
CC	14.00	21.00	24.00	23.72	3.92	2.50	2.73	3.71	1.75	3.00	7.90
SB	19.00	25.00	30.00	11.85	1.83	3.37	2.76	5.07	1.00	1.50	4.74
$LSD_{0.05}$	0.18	0.33	0.35	0.72	0.28	0.22	0.16	0.34	0.06	0.28	0.34

Table 2*: Effect of different combinations of agro-wastes on the growth parameters of P. florida.*

DTCSR: Days to Complete Substrate Ramification; DPHF: Days to Pin Head Formation; DCFM: Days to Complete Fruiting Body Maturation; WTFB: Total Weight of Fruiting Body; NOFB: Number of Fruiting Body; LOS: Length of Stipe; WIDTH: Width of the Stipe; DOC: Diameter of Cap; NOF: Number of Flushes; I: Flushing Interval; BE: Biological Efficiency; CCGS: Corncobs + Groundnut Shell; CCRB: Corncobs + Rice Bran; SBGS: Sugarcane Bagasse + Ground Nut Shell; SBRB: Sugarcane Bagasse + Rice Bran

The effect of different ratio of combinations (80:20, 70:30, and 60:40) of the main substrates to the additives on the growth parameters (DTCSR, DPHF, DCFM, WTFB, NOFB, LOS, WIDTH, DOC, NOF, FI and BE) of *P. florida* is presented in table 3. *P. florida* grown on substrates' combination (SBRB, CCRB, SBGS, CCGS) with 80:20 (80% main substrates to 20% additives) combination ratio for fruiting bodies production was found to completely ramify the substrates combination 16 days after inoculation with the fully ramified spawns (Table 3). This number of days recorded for *P. florida* grown on substrates combination (SBRB, CCRB, SBGS, CCGS) with 80% main substrates and 20% additives was found to be significantly different from the other ratio (70:30 and 60:40) of combinations with 17 and 18 days of complete ramification of the substrates respectively (Table 3). *P. florida* was found to ramify faster on substrates (SBRB, CCRB, SBGS, CCGS) with combination ratio 80:20 when compared to the other ratio (70:30 and 60:40) used in this study. Pinheads of *P. florida* were observed on the combinations with ratio 80:20 (80% main substrates to 20% additives) after 22 days of inoculation the completely ramified spawns of *P. florida*. Whereas, pinheads of *P. florida* were observed on the combinations (SBRB, CCRB, SBGS, CCGS) with ratio 70:30 (70% main substrates: 30% additives) and 60: 40 (60% main substrates to 40% additives) 23 and 24 days after inoculation the completely ramified spawns of *P. florida* (Table 3). The same trend as aforementioned for DTCSR and DPHF applied also for DCFM. An increasing trend was observed in the DTCSR, DPHF and DCFM of *P. florida* as the ratio of additives increased from 20 - 40% (Table 3).

The matured fruiting bodies of *P. florida* harvested from substrates' combination (SBRB, CCRB, SBGS, CCGS) with ratio 80:20 (80% main substrates to 20% additives) was found to have the highest weight of 27.54g, this was followed by substrates combination with ratio 70:30 (70% main substrates: 30% additives) and 60:40 (60% main substrates to 40% additives) with 25.27g and 17.65g respectively (Table 3). These recorded weights of the matured fruiting bodies of *P. florida*, were found to be significantly different from each other at $P < 0.05$ (Table 3). It was also observed that as the proportion additives increased, the weight of the harvested fruiting bodies of *P. florida* decreased. Similar trend was observed in the biological efficiency of oyster mushroom (*P. florida*). *P. florida* grown on substrates' combination (SBRB, CCRB, SBGS, and CCGS) with ratio 80:20 was found to have the highest biological efficiency of 11.49%. This was followed by substrates' combination (SBRB, CCRB, SBGS, CCGS) with ratio 70:30 (70% main substrates: 30% additives) and 60: 40 (60% main substrates to 40% additives) having 10.33% and 7.40% respectively (Table 3). The highest number of harvested fruiting bodies of *P. florida* (4.39) was also recorded on substrates' combination (SBRB, CCRB, SBGS, CCGS) with ratio 80:20, whereas, substrates' combinations with ratio 70:30 and 60:40 were found to have 3.10 and 3.52 harvested fruiting bodies respectively (Table 3). The largest diameter of cap (7.37 cm) of the harvested fruiting bodies of *P. florida* was recorded on the substrates with ratio 80:20. This was significantly different from the substrates' combination (SBRB, CCRB, SBGS, CCGS) with ratio 70:30 and 60:40 (Table 3). The length of the stipe of *P. florida* harvested from substrates (SBRB, CCRB, SBGS, CCGS) with ratio 70:30 was found to have an average length of 4.69 cm which was significantly longer than the length of stipe of *P. florida* harvested from the other substrates' combination (SBRB, CCRB, SBGS, CCGS) with ratio (80:20 and 60:40) having 4.54 cm and 4.33 cm respectively (Table 3). The width of stipe of *P. florida* harvested on substrates (SBRB, CCRB, SBGS, CCGS) with ratio 80:20, 70:30 and 60:40 was found to be 4.39 cm, 3.64 cm and 4.19 cm respectively (Table 3).

Ratio	DTCSR (Days)	DPHF (Days)	DCFM (Days)	WTFB (g)	NOFB	LOS (cm)	Width (cm)	DOC (cm)	NOF	FI	BE (%)
80:20	16.00	22.00	26.00	27.54	4.39	4.54	4.39	7.37	1.68	3.43	11.49
70:30	17.00	23.00	27.00	25.27	3.10	4.69	3.64	6.43	1.43	2.06	10.33
60:40	18.00	24.00	28.00	17.65	3.52	4.33	4.19	6.54	1.56	3.06	7.74
$LSD_{0.05}$	0.15	0.28	0.31	0.62	0.24	0.18	0.15	0.29	0.05	0.24	0.29

Table 3: *Effect of different ratio of combinations of agro-wastes on growth parameters of P. florida.*

DTCSR: Days to Complete Substrate Ramification; DPHF: Days to Pin Head Formation; DCFM: Days to Complete Fruiting Body Maturation; WTFB: Total Weight of Fruiting Body; NOFB: Number of Fruiting Body; LOS: Length of Stipe; WIDTH: Width of the Stipe; DOC: Diameter of Cap; NOF: Number of Flushes; FI: Flushing Interval; BE: Biological Efficiency; 80:20: 80% Main Substrates: 20% Additive; 70:30: 70% Main Substrates: 30% Additive; 60:40: 60% Main Substrates: 40% Additive.

The effect of different percentages of calcium derivatives (1% $CaCO_3$: 1% $CaSO_4$, 1% $CaCO_3$: 2% $CaSO_4$, 1% $CaCO_3$: 3% $CaSO_4$, 1% $CaCO_3$: 4% $CaSO_4$) added to the substrates (SBRB, CCRB, SBGS, CCGS) on the growth parameters (DTCSR, DPHF, DCFM, WTFB, NOFB, LOS, WIDTH, DOC, NOF, FI and BE) of *P. florida* is presented in table 4. *Pleurotus florida* grown on substrates (SBRB, CCRB, SBGS, CCGS) with calcium augmentation ratio 1:1 (1% $CaCO_3$: 1% $CaSO_4$), was found to completely ramify the substrates after 18 days of inoculation (Table 4). Whereas, the *P. florida* inoculated on substrates (SBRB, CCRB, SBGS, CCGS) with calcium augmentation ratio 1:2 (1% $CaCO_3$: 2% $CaSO_4$), 1:3 (1% $CaCO_3$: 3% $CaSO_4$) and 1:4 (1% $CaCO_3$: 4% $CaSO_4$) was found to have completely ramify the substrates after 17 days of inoculation with the fully ramified spawns of *P. florida* for fruiting bodies production (Table 4). Gradual increase in the percentage of calcium sulphate with respect to the weight of the substrate used in this study decreased the numbers taken for full ramification of the substrates (SBRB, CCRB, SBGS, and CCGS) (Table 4).

A different pattern was observed for the days to pin head formation as the lowest and the highest percentage of calcium carbonate and calcium sulphate (1% $CaCO_3$: 1% $CaSO_4$ and 1% $CaCO_3$: 4% $CaSO_4$) used in this study were found to have the lowest day to pinhead formation of 23 days and were not significantly different from each other (Table 4). The weight of the harvested fruiting bodies of *P. florida* increased significantly with the gradual increase in the percentages of the calcium derivatives. The highest weight of the fruiting bodies of *P. florida* was harvested from the substrates (SBRB, CCRB, SBGS, and CCGS) with the highest percentage of the calcium derivative. *Pleurotus florida* fruiting bodies harvested from substrates (SBRB, CCRB, SBGS, and CCGS) with 1% calcium carbonate: 4% calcium sulphate were found to have the highest weight and biological efficiency of 25.18 g and 10.21% respectively (Table 4). A regression equation further revealed that a unit increase in the calcium derivatives, increases the weight of the fruiting bodies with 0.86 unit in this study (Figure 1). Fruiting bodies harvested from substrates (SBRB, CCRB, SBGS, and CCGS) augmented with the highest calcium derivatives (1:4), had the shortest length of stipe, lowest width of stipe and large diameter. Hence, a well-developed fruiting body of *P. florida*. This is unlike the fruiting bodies of *P. florida* harvested from substrates augmented with the lowest calcium derivatives (1:1) which was found to have the longest stipe and a deformed cap (Table 4 and Plate 4).

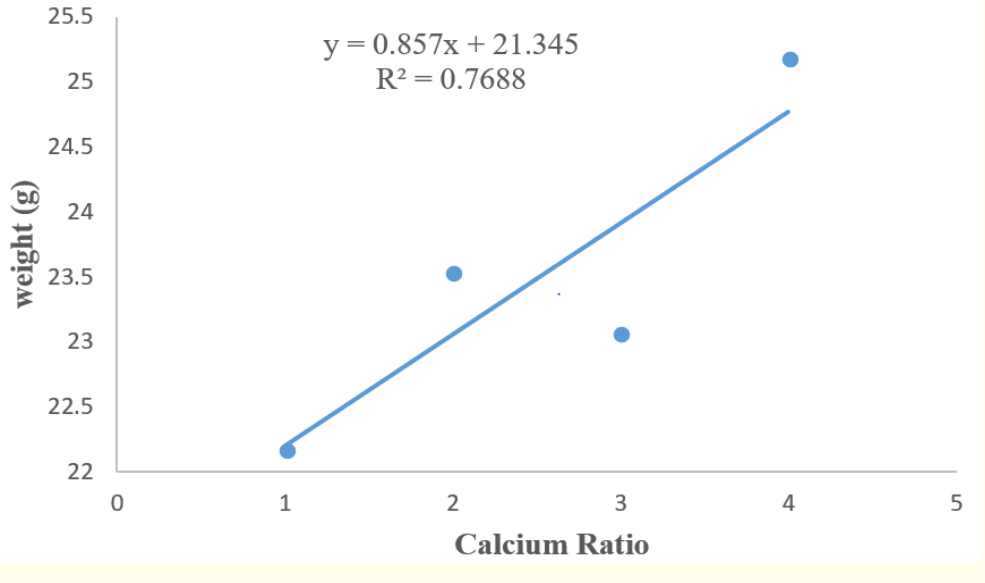

Figure 1: *Regression equation of the weight of fruiting bodies against the percentage calcium increase.*

Calcium	DTCSR (Days)	DPHF (Days)	DCFM (Days)	WTFB (g)	NOFB	LOS (cm)	Width (cm)	DOC (cm)	NOF	FI	BE (%)
1:1	18.00	23.00	27.00	22.17	3.89	4.94	4.12	6.88	1.58	3.08	9.64
1:2	17.00	24.00	27.00	23.53	3.92	4.82	4.06	6.98	1.72	3.86	10.21
1:3	17.00	24.00	28.00	23.07	4.14	4.29	4.27	6.34	1.53	2.64	9.36
1:4	17.00	23.00	28.00	25.18	3.25	4.04	3.85	6.92	1.42	1.83	10.21
$LSD_{0.05}$	0.17	0.32	0.35	0.72	0.28	0.22	0.17	0.33	0.06	0.28	0.34

Table 4: *Effect of different percentages of calcium carbonate and calcium sulphate on growth parameters of P. florida. DTCSR: Days to Complete Substrate Ramification; DPHF: Days to Pin Head Formation; DCFM: Days to Complete Fruiting Body Maturation; WTFB: Total Weight of Fruiting Body; NOFB: Number of Fruiting Body; LOS: Length of Stipe; WIDTH: Width of the Stipe; DOC: Diameter of Cap; NOF: Number of Flushes; FI: Flushing Interval; BE: Biological Efficiency; 1:1: 1% $CaCO_3$: 1% $CaSO_4$; 1:2: 1% $CaCO_3$: 2% $CaSO_4$; 1:3: 1% $CaCO_3$: 3% $CaSO_4$; 1:4: 1% $CaCO_3$: 4% $CaSO_4$*

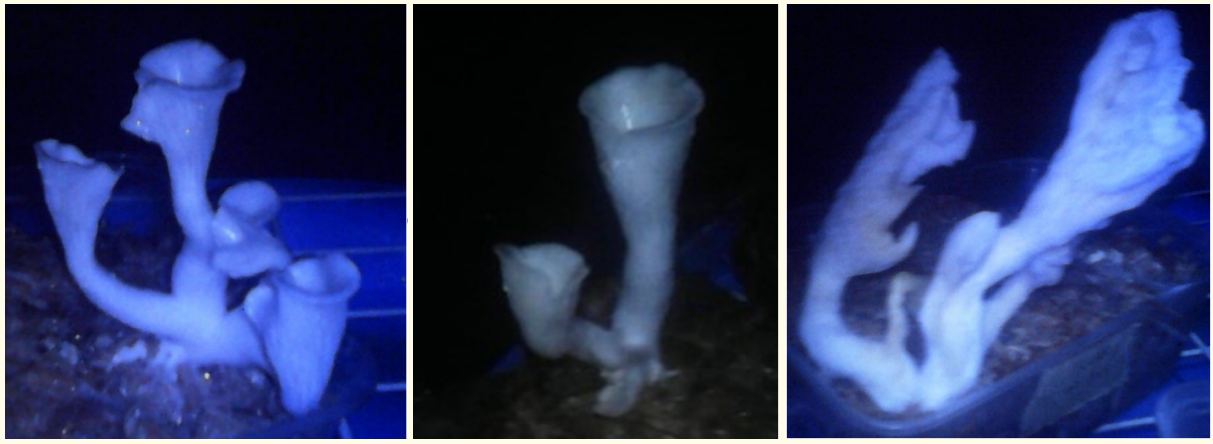

Plate 4: *Deformed fruiting bodies of P. florida (Oyster mushroom).*

Agro-wastes were used in this study for its availability, its cheap sources of essential nutrients and the ability of *P. florida* to easily breakdown the lignocellulosic constituents for its growth when compared to other substrates used for cultivation. These wastes can be recycled into food thus, eliciting a safe environment. The substrates used in this study supported the growth of *P. florida* significantly although at different levels. This is similar to the findings of Modal., *et al*. [12] and Salami., *et al*. [4], who reported that different species of Oyster mushroom performed well on different substrates containing hemicellulose and lignin such as corn cobs, sugarcane bagasse, rice bran, maize straw, banana leaves, saw dust and log of wood. The observed variation as occasioned by the different substrates can be adduced to the differences in the chemical and elemental composition of these agro-wastes [4]. Mineral elements necessary for the fructification of the mushroom are the same as those required by any cultivated plant, which are major elements and microelements [15]. The elemental composition of the substrates used in this study supported the previous report that mineral elements are necessary for the growth, development and fructification of the Oyster mushroom. All these essential elements were present and available in the substrates used for the cultivation of *P. florida* in this study. Sales-Campos Ceci., *et al.* [11] reported that carbon is the main source of energy derived from plant tissue, used by fungi for their development, these are the polysaccharides and lignin in the cell wall, although other polymeric

compounds such as lipids and proteins can also be used. The main substrates (corncobs and sugarcane bagasse) used in this study have higher percentages of carbon and contained lower percentage of nitrogen, but the supplements used as additives have higher nitrogen percentage than carbon. The complementary effect of the carbon which is higher in the main substrates and nitrogen which is also higher in the additives used sufficiently justified the fact that the supplemented substrates performed better than the single based substrates, hence, an increase in the production of oyster mushroom. It has been reported that substrate formula need the addition of nitrogen-rich supplements at the outset of production to improve the C/N ratio and to accelerate the breakdown process of the substrates [11]. The nitrogen percentage available in the groundnut shell was not significantly different than the nitrogen percentage available in rice bran and readily available for the *P. florida*. The availability of these nutrients also contributed to the rapid growth and development of *P. florida*, thus, revealed in the number of days used for the cultivation process which was shorter than using the conventional method of cultivation. The carbon content of sugarcane bagasse was lower compared to corncobs but it was easily converted to absorbable nutrient needed and readily available for the organism's use. This may be due to the presence of sucrose which has been identified as the main sugar present in sugarcane which is easily used up by the organism. This might have accounted for the rapid growth of the *P. florida* on sugarcane bagasse substrates and reduced DTCR. It took a longer time for the organism to breakdown the polysaccharides present in the corncobs' substrates but after the breakdown, it was able to support the growth of the organism for a longer period of time.

For the fruiting body production of *P. florida*, the different agro-wastes' combinations, in different ratio, augmented with calcium carbonate and calcium sulphate have significant effect on improving the production of Oyster Mushroom (*P. florida*). This is due to the fact that all the necessary elements and nutrient needed for the growth of *P. florida* is present in available and absorbable form in the substrates. Sarker., *et al*. [13] opined that various substrates have different effects on the growth, yield and quality of mushrooms, this corroborates the result of this study. The differences in the days to complete ramification of the substrates for the fruiting body production was as a result of the differential elemental and nutrient composition of the substrates. Combined substrates with GS as additives, ramified faster than the other substrates. The reason for this is not far-fetched owing to the higher percentage of the essential elements such as potassium and calcium in GS than the other additive (rice bran) used in this study. Also, GS contained higher carbon percentage than the rice bran supplement. These constituents present in the combined substrates were available at required amount for the growth and proper fructification of the *P. florida*. The organism also possess an efficient and effective system to breakdown the substrates to derive necessary nutrient for its spread and fast growth in form of ramification of the substrates used. This observed trend also was also recorded for number of days to pin head formation for substrates. Although, the trend was similar to what was observed on DTCSR, it took substrates with earlier days of full ramification of the substrates longer days (about 7 days' interval) before pinhead formation was recorded on them. While the substrates that ramified later by *P. florida*, had shorter days' interval of 5 days from full ramification to pin-head formation. Thus, early ramification of the substrates does not necessarily predict pinhead formation. From this study *P. florida* primordial initiation was shortened to 21 - 28 days. This is an improvement on the study of Naraian., *et al*. [22] who reported in his study on the influence of different nitrogen rich supplements during cultivation of *Pleurotus florida* on corn cob substrate that primordial initiation was observed between 20 - 35 days. Complete maturation of the fruiting bodies took an average of 3 - 4 days after pinhead formation for all the substrates used. The earlier trend of the substrates in succession that formed pinhead also matured in that order. This further asserts that the average days of complete mushroom production can be shortened to 24 days by combining different agro-wastes. Hence, an improvement in the Oyster mushroom production process. Maturation of the *P. florida* fruiting bodies was earlier on CC based substrates because the carbon present in this substrate, remains available for easy absorption, utilization and conversion into edible products by the organism. SB also have these essential nutrients for the fructification and maturation of *P. florida*, but at lower percentage when compared to Corncobs. Higher weight was recorded generally in combined substrates than the uncombined substrates. This might be due to the additive effect of the essential nutrient available in the combined substrates when compared to the single based substrates. This explanation also holds for the biological efficiencies of the *P. florida* which was higher on combined substrates than the uncombined substrates. Although, the number of fruiting bodies harvested from SB based substrates were higher than the CC based substrates, the weight of the harvested mushroom from combined substrates where CC was the main substrate exceeded the weight of those harvested where SB

served as the main substrate. This can be alluded to the fact that SB has available nutrient elements which is easily used up unlike in CC where the nutrients was sustained and progressively released for the organism's use. The fibrous nature of CC might have also contributed to the higher weights because substrates that have higher fibre contents tends to produce fruiting bodies with higher weight when compared to the substrates with less fibre content. Also, the length and the width of stipe of the mushroom harvested from CC based substrates were higher than others. This may be as a result of the high fibre content of the substrate. Ratio of the different combinations of agro-wastes was significant on the growth parameters that was measured in this study. The ratio of combination, also had a significant effect on the days for the transition from the reproductive stage to the vegetative stage where the pinheads emerged. The days to complete substrates ramification (DTCSR), days to pin head formation (DPHF) and days to complete fruiting body maturation (DCFM) increased as the proportion of the additives increased. This is because, the additives have low carbon percentage which is an essential element necessary for spawn running. Increasing the additives beyond 20% as observed in this study will negatively affect the production cycle in terms of days to completion of production and the harvested yield. Supplementing the main substrates with the additives (although supplies other complementary nutrients) substitutes/ reduces the fibrous content of the main substrates which is highly essential for the fruiting bodies to possess higher weight. Hence, supplanting the main substrates beyond 20% of the additives may be detrimental to the production of Oyster mushroom. Eighty percent of the main substrates supplemented with 20% of the additives will supply the necessary nutrients needed for the production of the fruiting bodies of *P. florida.* This explanation also holds for the observed trend of the effect of ratio of supplementation on the weight of the harvested fruiting bodies. It was observed that as proportion additives increased, the weight of the fruiting bodies harvested decreased significantly. This is similar to the report of Naraian., *et al* [22]. The biological efficiencies of the supplemented substrates were higher than the un-supplemented substrates. Increasing the levels of the additives beyond 20% negatively affected the biological efficiency. This is similar to Naraian., *et al*. [22] who reported that the biological efficiencies of supplemented substrates increased over the un-supplemented set. Also, with increasing level of additives, the biological efficiency was negatively affected at higher levels. The observation on the weight of fruiting body is similar for the biological efficiency because they are directly proportional. Ratio 2 (70:30, 70% main substrates: 30% additives) is most appropriate for spawn production according to this study. The different percentages of calcium carbonate and calcium sulphate added to the substrates also had a highly significant effect on the growth parameters measured. Calcium has been earlier reported as one of the major elements needed for the growth of various fungi [15]. Gradual increase of the percentage of calcium sulphate with respect to the weight of the substrate used increased the numbers taken for full ramification of the substrates. This suggests that there is a limit to which calcium additives can be added to the substrates without becoming a problem to the production. The required percentages conducive to production of spawn is different from the percentage needed for the fruiting body production. This may be as a result of slightly different activities the organism undergoes at both production stages. Sales-Campos Ceci., *et al.* [11] had reported that calcium augmentation beyond 5% results in deformed fruiting bodies, this study observed similar trend. Fruiting bodies harvested from substrates augmented with the lower percentage of calcium derivatives had the longest stipe and a deformed cap which invariably represents a deformed fruiting body (Plate 1). However, fruiting bodies harvested from substrates augmented with the higher percentage of calcium derivatives (1:4), had the shortest length of stipe, moderate width of stipe and large diameter. Hence, a well-developed fruiting body. This was corroborated by Sales-Campos Ceci., *et al.* [11] in his earlier report that some minerals such as sodium chloride, magnesium, and calcium also stimulate the early formation of fruiting bodies as well as increased mycelia growth. Combination of different substrates increased the yield of *P. florida.* Substrate CCRB at ratio 80:20, and 5% calcium augmentation (1% $CaCO_3$ and 4% $CaSO_4$) is best for the production of *P. florida* fruiting bodies. Corncob based substrates had higher yield when compared to Sugarcane bagasse based substrates. Also, adding supplements to the main substrates with additives, increases the nutritional content of *P. florida.* The protein content of *P. florida* increases with increasing percentage of the additives [23-77].

Conclusion

It can be concluded from this study that agro-wastes are useful raw material in mushroom production rather than constituting a challenge to waste management problem encountered in the agricultural industry. Agro-wastes used in this study provides an alternative and profitable means of producing edible mushroom without the incorporation of edible food (cereals) in the production cycle of edible

mushroom. However, the response of other species of oyster mushroom to agro-waste should be researched while other physical factors such as temperature, humidity, different pH that can improve mushroom production should also be worked on. From the foregoing, it can be recommended that; mushroom production should be increased with the use of agro-wastes from farm produce. Agro-wastes are recommended for the entire production of *P. florida* without the use of any edible food materials such as cereals. Combination of different agro-wastes' substrates is highly recommended for the production of mushroom in place of single substrates. Augmentation of substrates formulation with calcium sulphate and calcium carbonate within the range of 4 - 5% is recommended for successful production of oyster mushroom *P. florida.*

Acknowledgement

We are grateful to TETFund Research Project Intervention 2nd batch, 2011-2014 for providing the grant to carry out this study.

Bibliography

1. Chang ST. "Mushroom biology: The impact on mushroom production and mushroom products, In: Mushroom Biology and Mushroom Products, Proceeding of the first international conference on mushroom biology and mushroom products (Eds), The Chinese University Press, Hong Kong (1993): 3-20.

2. Salami AO and Elum EA. "Bioremediation of a Crude Oil Polluted Soil with Pleurotus pulmonarius and Glomus mosseae using Amaranthus hybridus as a Test Plant". *Journal of Bioremediation and Biodegradation* 1.3 (2010): 113.

3. Sławińska A and Kalbarczyk J. "Evaluation of Enzymatic Activity of Pleurotus ostreatus. Regarding Stages of Mycelium Development". *ACTA Scientiarum Polonorum Horticulture* 10.2 (2011): 195-202.

4. Salami AO., *et al*. "Effect of different substrates on the growth and protein content of Oyster mushroom (Pleurotus florida)". *International Journal of Biological and Chemical Sciences* 10.2 (2016): 475-485.

5. Jebapriya GR., *et al*. "Application of Mushroom Fungi in Solid Waste Management". *International Journal of Computing Algorithm* 2 (2013): 279-285.

6. Bhatti MI. "Growth, Development and Yield of Oyster Mushroom, Pleurotus ostreatus (Jacq. Ex. Fr.) Kummer as affected by different spawn rates". *Pakistan Journal of Botany* 39.7 (2007): 2685-2692.

7. Viziteu G. "Substrate-cereal straw and Corn Cobs. In: Mushroom Growers' Handbook1, Gush, R. (Ed.). P and F publishers, USA (2000).

8. Bankole FA and Salami AO. "Use of Agro-Wastes for Tissue Culture Process and Spawn Production of Oyster Mushroom (Pleurotus florida)". *Journal of Applied Life Sciences International* 14.1 (2017): 1-9.

9. Stanley HO., *et al*. "Cultivation of oyster mushroom (Pleurotus pulmonarius) on amended corncob substrate". *Agriculture and Biology Journal of North America* 2.8 (2011): 1239-1243.

10. Przybylowicz P and Donoghue J. Shiitake growers' handbook: The art and science of mushroom cultivation, Kendall/Hunt Publishing Company, Dubuque, Iowa (1990).

11. Sales-Campos., *et al*. "Productivity and Nutritional Composition of Lentinus strigosus (Schwinitz) Fries Mushroom from the Amazon Region Cultivated in Sawdust Supplemented with Soy Bran, Recent Trends for Enhancing the Diversity and Quality of Soybean Products, Prof. Dora Krezhova (Ed.), ISBN (2011).

12. Modal SR., *et al*. "Comparative study on growth and yield performance of oyster mushroom (Pleurotus florida) on different substrates". *Journal of the Bangladesh Agricultural University* 8.2 (2010): 213-220.

13. Sarker NC., *et al*. "Relationship between Nutrient Content in substrates and Economic Yield of Oyster Mushroom (Pleurotus ostreatus)". *Bangladesh Journal of Mushroom* 2.1 (2008): 27-33.

14. Stanley RP. "Enumerative combinatorics". *Cambridge university press* 49 (2011).

15. Chang ST and Miles PG. "Historical record of the early cultivation of Lentinus in China". *Mushroom Journal for the Tropics* 7 (1997): 31-37.

16. Chang STO. Mushroom Cultivation using the "Zeri" Principle: Potential for Application in Brazil, Proceeding of First International Symposium on Mushroom in food, health, technology and the environment in Brazil (2003).

17. Olumide OJ. "Economic analysis of mushroom marketing as a copping strategy for poverty reduction in Ondo State". *African Crop Science conference proceedings* (2007): 1255-1260.

18. Garcia HS., *et al*. "Nutritional importance of the mushrooms, In: Mushroom Biology and Mushroom Products, Proceeding of the first international conference on mushroom biology and mushroom products, Chang ST; Buswell JA and Chiu SW. (Eds), The Chinese University Press, Hong Kong (1993): 227-236.

19. Chang ST and Miles PG. "Mushrooms: Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact, 2nd edition CRC Press, Boca Raton, FL (2004).

20. Urben AF., *et al*. "Cultivo de cogumelos comestíveis e medicinais (apostila do curso)". EMBRAPA (2003): 169.

21. Buswell J A., *et al*. "Ligninolytic enzyme production and secretion in edible mushroom fungi". In Mushroom Biology and Mushroom Production, Royse, D. J., editor. University Park: Pan (1996): 113-122.

22. Naraian R., *et al*. "Influence of different nitrogen rich supplements during cultivation of Pleurotus florida on corn cob substrate". *The Environmentalist* 29 (2009): 1-5.

23. Adejoye O D., *et al*. "Effect of carbon, nitrogen and mineral sources on growth of Pleurotus florida, a Nigeria edible mushroom". *African Journal Biotechnology* 5.14 (2006): 1355-1359.

24. Adenipekun CO and Gbolagade JS. "Nutritional Requirements of Pleurotus florida (Mont.) Singer, A Nigerian Mushroom". *Pakistan Journal of Nutrition* 5.6 (2006): 597-600.

25. Agrahar-Murugkar D and Subbulakshmi G. "Nutritional value of edible wild mushrooms collected from the Khasi hills of Meghalaya". *Food Chemistry* 89.4 (2005): 599-603.

26. Ahmed Imtiajj., *et al*. "Comparative study of environmental and nutritional factors on the mycelial growth of edible mushrooms". *Journal of Culture Collections* 6 (2008): 97-105.

27. Akinyele BJ and Adetuyi FC. "Effect of agrowastes, pH and temperature variation on the growth of Volvariella volvacea". *African Journal of Biotechnology* 4.12 (2005): 1390-1395.

28. Akpaja EO., *et al*. "Ethnomycology and usage of edible and medicinal mushrooms among the Igbo people of Nigeria". *International Journal of Medicinal Mushroom* 5.3 (2003): 313-319.

29. Ayodele SM., *et al*. "Some edible and medicinal mushrooms found in Igala land in Nigeria and their sociocultural and ethnomycological uses". *Proceeding of the 5th International Medicinal Mushroom Conference, Nantong, China* (2009): 526-531.

30. Baysal E., *et al*. "Cultivation of oyster mushroom on waster paper with some added supplementary materials. Bioresour". *Technology* 89.1 (2003): 95-97.

31. Behnam Elhami., *et al*. "Effect of substrate type, different levels of Nitrogen and Mangenese on growth and development of Oyster Mushroom (*Pleurotus florida*)". Global Science books: Dynamic biochemistry, process biotechnology and molecular biology (2008).

32. Bernaś E., *et al*. "Edible Mushrooms as a Source of Valuable Nutritive Constituents". *Acta Scientiarum Polonorum – Technologia Alimentaria* 5.1 (2006): 5-20.

33. Bononi VL., *et al*. "Cultivo de cogumelos comestíveis". 2nd Ed., Ícone, ISBN: 85-274-0339-0, S. Paulo (1999).

34. Çağlarırmak N. "The nutrients of exotic mushrooms (Lentinula edodes and Pleurotus species) and an estimated approach to the volatile compounds". *Food Chemistry* 105.3 (2007): 1188-1194.

35. Chang ST and Miles PG. "Mushroom biology-a new discipline". *Mycology Journal* 6 (1992): 64-65.

36. Chang ST and Mshigeni KE. "Mushroom and their human health: their growing significance as potent dietary supplements". The Uni of Namibia, Windhoek (2001): 1-79.

37. Chang ST. "Training Manual on Mushroom Cultivation Technology". Asian and Pacific Centre for Agricultural Engineering and Machinery (APCAEM), A-7/F, China International Science and Technology Convention Centre, no. 12, Yumin Road, Chaoyang district, Beijing 100029, China (2010).

38. Demirer T., *et al*. "Effect of different types and doses of nitrogen fertilizers on yield and quality characteristics of mushrooms (Agaricus bisporus (Lange) Sing) cultivated on wheat straw compost. Journal of Agriculture and Rural Development in the Tropics and Subtropics, 106.1 (2005): 71-77.

39. Eira AF and Montini RMC. "Manual do cultivo do shiitake (Lentinula edodes (Berk) Pegler). Fundação de Estudos e Pesquisas Agrícolas e Florestais, Universidade Estadual Paulista FEPAF-UNESP, Botucatu, São Paulo (1997).

40. Eira AF and Minhoni MTA. "Manual do cultivo do "Hiratake" e "Shimeji" (Pleurotus spp). Fundação de Estudos e Pesquisas Agrícolas e Florestais, Universidade Estadual Paulista FEPAF-UNESP, Botucatu, São Paulo (1997).

41. Fasidi IO. "Substrate sourcing and preparation for mushroom cultivation". Training workshop on cultivation of tropical mushrooms 20th-22nd November 2006 at University of Ibadan, Oyo State (2006).

42. Ghorai S., *et al*. "Fungal biotechnology in food and feed processing". *Food Research International* 42.5-6 (2009): 577-587.

43. Godfrey EZ., *et al*. "Effects of temperature and hydrogen peroxide on mycelia growth of eight Pleurotus strains". *Scientia Horticulture* 125.2 (2010): 95-102.

44. Grodzinskaya AA., *et al*. "Cultivation of edible mushrooms using agricultural and industrial wastes". AgronomiaTropical-Maracay, 52.4 (2003): 427-447.

45. México DF., *et al*. "El cultivo de los hongos comestibles. Instituto Politécnico Nacional, ISBN (1993).

46. Hamman S. "Bioremediation capability of white rot fungi". Review article, spring 2004. *Journal of Science and Technology* 6 (2005): 18-22.

47. Hitivani N and Mecs L. "Effects of certain heavy metals, on the growth, dye decolouration and enzyme activity of Lentinula edodes". *Ectoxicology and Environmental Safety* 55.2 (2003): 199-203.

48. Ivan HR., *et al*. "Adding supplements to sugarcane bagasse with rice bran and sugarcane molasses for shiitake (Lentinula edudes) spawn production". *Brazilian Journal of Microbiology* 34.1 (2003): 61-65.

49. Jedinak A., *et al*. "Pleurotus ostreatus inhibits colitis-related colon carcinogenesis in mice". *International Journal of Molecular Medicine* 26.5 (2010): 643-650.

50. Khare BK., *et al*. "Development and Biotechnology of Pleurotus Mushroom Cultivation". *Journal of Science and Technology* 6 (2014): 24-30.

51. Martinez-Carrera D. Mushroom. McGraw-Hill Encyclopedia of Science and Technology, 9th Edition McGraw-Hill, Inc., New York (2002).

52. Mateus Dias Nunes., *et al*. "Nitrogen Supplementation on the Productivity and the Chemical Composition of Oyster Mushroom". *Journal of Food Research* 1.2 (2012): 113-119.

53. Matheus DR and Okino LK. "Utilização de Basidiomicetos em processos biotecnológicos, In: Zigomiceto, Basidiomicetos e Deuteromicetos: noções básicas de taxonomia e aplicações biotecnológicas, Bononi, V. L. R.; Grande, R. A. P. (Eds.). Instituto de Botânica/ Secretaria do Meio Ambiente, São Paulo (1999): 107- 139.

54. Matilla P., *et al*. "Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms". *Journal of Agricultural and Food Chemistry* 49.5 (2001): 2343-2348.

55. Maziero R. "Substratos alternativos para o cultivo de Pleurotus spp. (1990): Dissertação (Mestrado em Ciências Biológicas/Botânica), Instituto de Biociências USP, Universidade de São Paulo, São Paulo (1990): 136.

56. Montini RMC. "Efeito de linhagense substratos no crescimento micelial e na produtividade em cultivo axênico de shiitake (Lentinula edodes (Berk.) Pegler)". Thesis (Doctorate) Faculdade de Ciências Agronômicas. Universidade Estadual Paulista, UNESP, Botucatu, São Paulo (2001).

57. Nayana J. "Antioxidant and antitumour activity of Pleurotus florida". *Current Science* 79.7 (2000): 7-10.

58. Nwanze PI., "The effect of the interaction of various spawn grains with different culture medium on carpophores dry weights and stipe and pileus diameters of lentinus squarrosulus (Mon.) singer". *African Journal of Biotechnology* 4.7 (2005): 615- 619.

59. Obodai M., *et al*. "Yield of seven strains of oyster mushrooms (Pleurotus spp.) grown on composted sawdust of Triplochiton scleroxylon". *Tropical Science* 40.2 (2002): 95-99.

60. Oei P. "Mushroom Cultivation, Appropriate Technology for Mushroom Growers. Backhugs Publishers, Leiden". *The Netherlands* (2003).

61. Omemu AM., *et al*. "Effect of Different Processing and Supplementation on Maize Cob as Microbiological Growth Medium for Fungi". *World Journal of Agricultural Sciences* 4.5 (2008): 600-604.

62. Royse DJ. "Yield, size and bacterial blotch resistance of Pleurotus eryngii grown on cotton seed hull/ oak sawdust supplemented with manganese, copper and whole ground soybean". *Bioresource Technology* 98.10 (2007): 1898-1906.

63. Shah ZA., *et al*. "Comparative Study on Cultivation and Yield Performance of Oyster Mushroom (Pleurotus ostreatus) on Different Substrates (Wheat Straw, Leaves, Saw Dust)". *Pakistan Journal of Nutrition* 3.3 (2004): 158-160.

64. Shaiesta S., *et al*. "Efficacy of fungicides against Trichoderma spp. causing green mold disease of Oyster mushroom (Pleurotus sajor-caju)". *Research Journal of Microbiology* 8.1 (2013): 13-24.

65. Son H., *et al*. "Optimization of fermentation conditions for the production of bacterial cellulose by a newly isolated Acetobacter" 33.1 (2001): 1-5.

66. Sonali DR. "Department of Biotechnology, Walchnad college of Arts and Science Solapur, India*". Pelagia Research Library* (2012).

67. Stamets P and Chiiton JS. "The Mushroom Cultivator: A Practical Guide to Growing Mushroom at Home". Agarikon Press, Olympia, Washington (1993).

68. Stamets P. "Growing Gourmet and Medicinal Mushrooms, 3 edition Ten Speed Press", Barkeley, Ca (2000): 175-174.

69. Stamets P. "Mycelium Running. How mushroom can help save the world" Ten speed Press, Berkeley/Toronto". 1st Edition. 339 (2005).

70. Sumaira Sharif., *et al*. "Proximate Composition and Micronutrient Mineral Profile of wild Ganoderma lucidum and Four Commercial Exotic Mushrooms by ICP-OES and LIBS". *Journal of Food and Nutrition Research* 4.11 (2016): 703-708.

71. Trufem SFB. "Utilização de zigomicetos em processos biotecnológicos". In: Zigomiceto, Basidiomicetos e Deuteromicetos: noções básicas de Taxonomia e aplicações biotecnológicas, Bononi, V. L. R.; Grande, R. A. P. (Eds.), Instituto de Botânica/ Secretaria do Meio Ambiente, São Paulo (1999): 51-67.

72. Urben AF., *et al*. "Produção de cogumelos por meio de tecnologia chinesa modificada, Urben, A. F. (Ed.), Editora EMBRAPA - Recursos Genéticos e Biotecnologia, Brasília-DF (2001).

73. Brasília DF and Vedder PJC. "Cultivo moderno del champiñon. Tradução: Martinez, J. M. G. Ediciones Mundi-Prensa, Madrid (1996).

74. Won-Sik Kong. "Descriptions of commercially important Pleurotus species. In: Mushroom Growers". Handbook (2004): 1-8.

75. Wuyep PQ., *et al*. "Production and regulation of lignin degrading enzymes from Lentinus squarosulus". *African Journal of Biotechnology* 2.11 (2003): 444-447.

76. Yang CD., *et al*. "Effects of growth medium composition, iron sources and atmospheric oxygen concentrations on production of luciferase-Bacterial magnetic particle complex by a recombinant Magnetospirillum magneticum AMB-1". *Enzyme and Microbial Technology* 29.1 (2001): 13-19.

77. Zhanxi L and Zhanhua L. Juncao Technology, Xiangzhou, L. (Ed.), translated by Dongmei, L; Rui, T, China Agricultural Scientech Press, the People's Republic of China (2001).

Volume 14 Issue 9 September 2018