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Variability effect of pH on yield optimization and Mycochemical compositions of *Pleurotus ostreatus* sporophores cultured on HCl-induced substrate

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Contamination due to *Coprinus cinerius* has been identified as a major drawback in the use of oil palm bunch (OPB) for mushroom cultivation. OPB is a common agro-waste in the South-eastern part of Nigeria with high alkalinity which does not support the growth of oyster mushrooms. A study on the fructification and some mycochemical contents of *Pleurotus ostreatus* fruitbodies cultivated on hydrochloric acid (HCl)-induced OPB substrate was conducted. OPB was steeped in solutions (0.1%-0.5%) of HCl for 48 h, to optimize its pH. Experiment was conducted in a Completely Randomized Design (CRD). One way analysis of variance (ANOVA) was used for data analysis and means separation by Duncan Multiple Range Test (DMRT) at $p<0.05$. Results showed that increase in the concentration of HCl acid from 0.1 to 0.5% reduced substrate contamination due to *Coprinus cinerius* and enhanced fruit body number, yield and biological efficiency (BE%) as well as primordial initiation. Vitamins, bioactive compounds, heavy metals and other mycochemicals of nutritional importance were recorded in various fruit body samples at different concentrations. Therefore, commercial mushroom growers should avail themselves of this unique opportunity to ensure effective utilization of OPB incorporated with HCl acid for high fruit- body production as well as profit maximization.

Key words: Hydrochloric acid (HCl), *Pleurotus ostreatus, Fruitbodies,* oil palm bunch (OPB).

INTRODUCTION

Mushrooms are members of the class Basidiomycota and order *Agaricales*. Unlike green plants, they do not possess chlorophyll; for manufacturing of their own food. For their growth and development, they require pre-formed food such as smaller broken down molecules of lignin, cellulose and starch (Stamets, 2000). Chang (2012) defined mushroom as “a macro-fungus that has a distinctive fruiting body which can either be epigeous (growing on or close to the ground) or hypogenous (growing under the ground)”. The macro-fungi have

fruiting bodies large enough to be seen with unaided eye and to be picked by hand. Ideally, the word mushroom refers only to the fruit body of a macro-fungus. Most mushroom species are taxonomically categorized either under the Basidiomycota or Ascomycota; the two phyla are in the kingdom fungi (Cho, 2004).

Pleurotus ostreatus is the scientific nomenclature for Oyster mushroom. In many parts of India; it is known as Dhin (Elliott, 1982; Ogundana and Fagade, 1982). It was formally in the family Tricholomataceae, but now Pleurotaceae, which includes many species such as *P. flobellotus P. sojar-caju*, *P. eryngii, P. ostreatus, P. florida*, *P. sapidus* etc.

Effect of pH on mushroom fruit body formation

Fungal (mushroom) mycelia derive nutrients from substrates within a certain pH range (Saker et al., 2007); lime is often times incorporated in mushroom cultivation to optimize the pH of substrate substrates perceived to be acidic (Stamets, 2000). According to Chang and Miles (2004), rapid mycelia growth of *Pleurotus sajor-caju* takes place at pH 6.4-7.8. Oyster mushroom (*Pleurotus* spp.) can grow and utilize nutrients from various kinds of substrate materials than any other mushrooms (Cohen et al., 2002).

The observed increase in soil alkalinity caused by oil palm bunch ash, oil palm bunch husk and wood ash could be the main reason for their use as liming materials. Liming has been reported to be important for soil physical and chemical properties and nutrient availability. The better performance of wood and oil palm bunch ashes in improving growth and yield value of mushrooms (*Pleurotus* spp.), soil pH, K, Ca, and Mg could be due to the fact that the ash component is more soluble than other residues. This was reported by Moyin-Jesu (2007) and Ojeniyi (1990) who found that K and Ca components of wood ash were very high, and this could be responsible for their ability to enhance soil pH, which increases quick absorption of nutrient such as P, K, Ca and Mg that are essential for good growth and yield parameters of many humiculouse mushrooms. In addition, Okhuoya and Okogbo (2009) also reported that oil palm bunch fibres were good substrates for *Pleurotus tuber-regium.* The optimal temperature range for growth of the mycelium is within 25-28°C; while that of pH is between 5.5 and 6.5.

The CO_2 tolerance of mycelia is rather strong. For instance, mycelia of *Pleurotus* spp. can still flourish at carbon dioxide concentrations of 15 to 20%. But when the concentration of CO2 is raised to 30%, mycelia growth suddenly decreases (Chang and Miles, 2004).

MUSHROOMS AND FOOD SECURITY

Human population grows by 2.1%, representing a rise of about 75 million people per year, thus food production has to keep pace with population increase (Sharma, 2003). Mushrooms and yeast are referred to as important alternative sources of food (Chang, 1999; Anyankorah, 2002; James and Panter, 1995). According to James and Panter, (1995), edible mushrooms (dry) contain up to 19-40% protein; which is twice that of vegetables, four times that of oranges. Further, a mycochemical analytical profile showed that mushrooms are rich in vitamins and minerals, low in unsaturated fatty acid and carbohydrate, which makes them ideal for diabetic and obese patients (Chang and Miles, 2004).

Most mushrooms have exceptional medicinal and prophylactic properties, especially in diseases such as high blood pressure, asthma, respiratory tracts infection, anaemia, hepatitis, cancer, tumour, etc. (Ogundana and Fagade, 1982). Mushroom cultivation also represents the most efficient and economically viable biotechnological approach for the conversion of ligno-cellulosic waste materials into high-quality protein food; and, this will naturally open up new job opportunities, especially in rural areas, which can be pre-packaged by food industries and exported to other countries as food and for revenue generation.

According to Eno (2020), Nigeria produces nearly 500 metric tons of mushrooms, which is far less than its production potential; as its full production capacity has been estimated at 1.500MT per annum. This production gap has been attributed to a dearth of mushroom scientists and farmers. Osemwegie and Dania (2016) noted that data are either scarce or unavailable, on the contribution of mushroom production and commercialization to Nigeria's Gross Domestic Product (GDB); as information on the volume of mushroom tonnage produced annually for export or local consumption as well as commercial scale cottage mushroom industries are staggering (Celik and Parker, 2009; Marshall and Nair, 2009; and Barmon et al., 2012). The few available mushroom growers in Nigeria use a variety of substrates, such as sawdust, grass straws, rice bran/husks etc (Anoliefo et al., 2006; Obodai and Odamtten, 2012).

Therefore, this work aims to utilize an abundant agro-waste component (oil palm bunch) as a substrate for the

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Figure 1. Young fruit bodies of uncontaminated OPB**.**
Source: Authors

production of oyster mushroom in Nigeria.

MATERIALS AND METHODS

Study area

Mushroom cultivation stage

The experiment was conducted at the mushroom Research Section of the Michael Okpara University of Agriculture Umudike, Abia State. Umudike is located between longitude 7^0 and 70^005^0E and latitude 5^0 and 5^025^0N with a humid tropical climate. Rainfall is bi-modally distributed, with peaks between July and September of each year. Annual rainfall is approximately 170mm, spread between April and November each year (Achufusi, 2016).

Source of spawn culture

Pure culture (mycelia) of *P. ostreatus* was obtained from Dilomat farms Nigeria Limited, Rivers State University, Rivers State, Nigeria.

Spawn production/multiplication

Spawn of *P. ostreatus* was prepared using red variety sorghum grains. *Sorghum* grains were first washed in 3 changes of tap water before soaking over- night. The grains were subsequently boiled in tap water for 10-15 min using a gas-burning flame. Grains were completely drained of water before mixing with 2% (w/w) $CaCo_3$ and 4% $CaSO_4$ to optimize pH and prevent grain clumping, respectively; as recommended by Muhammad et al. (2007). They were further packed (2/3) in heat resistant transparent bottles, tightly sealed with aluminium foil held with rubber band and sterilized in an autoclave at 121°C for 30 min. After sterilization, bottles were allowed to cool at room temperature. Then, they were aseptically inoculated with actively growing mother mycelia culture of *P. ostreatus,* by grain-to-grain transfer. Subsequently they were incubated in the dark at 27 ± 2°C until grains were fully colonized by *P. ostreatus* mycelia (Shyam et al., 2010).

Determination of substrate pH

The pH of solution containing 5% of raw sample substrate in 50ml of distilled water was determined using a Jenway3070 portable automatic digital pH meter with temperature compensation; it was calibrated with buffer 7-4 and 10. The pH value was read on the digital scale.

Experimental design

The experiment was conducted in a Completely Randomized Design (CRD). Six levels of HCl acid-induced OPB substrate; including control were replicated seven times, each. Each replicate contained 200g dry weight of OPB substrate, which made a total of 1400g/level of HCl-OPB substrate.

Substrate inoculation

Thirty (30g) of grain-based spawn of *P. ostreatus* was spread across each replicate of 200g of substrate during inoculation (Okwulehie and Okwujiako, 2008). All the inoculated substrates were placed on wooden racks in the cropping room and covered during the spawn run. Humidity of the cropping room was optimized by constantly sprinkling and flooding with tap water prior to primordial initiation (Figures 1 and 2).

Measurement of morphological characters

Stipe sizes of fruit bodies

The effect of substrate on pileus and stipe sizes of fruit bodies was

Figure 2. OPB contaminated by *C. cinerius*.
Source: Authors

determined at maturity. The mushrooms were harvested accordingly while pileus and stipe sizes were measured in cm using a meter rule (Okwulehie and Okwujiako, 2008).

Cap diameter

This was obtained by placing a transparent ruler across the centre of the pileus and reading off the diameter (Okwulehie and Okwujiako, 2008).

Effect of substrates on fruit body number of the mushrooms

The effect of substrate on fruit body number of the mushroom was determined by harvesting the mushrooms, counting and recording their number for each and later comparing their values (Okwulehie and Okwujiako, 2008).

Yield and biological efficiency

During fruiting, mushroom fruit bodies were harvested at maturity; wet weight of fresh fruit bodies was determined using digital weighing scale, while biological efficiency (BE) was determined using the modified method of Chang and Miles (2004) as:

$$BE = \frac{Freshwtofmushroom}{drywtofsubstrate} \times \frac{100}{1}$$

Proximate analysis

Proximate analysis was conducted on each of the six (6) fruit body samples. The protein, ash, fat and crude fibres were determined by the method of AOAC (2000).

Determination of crude protein

Crude protein of different samples was estimated by the Kjedahl method. Total nitrogen content was determined first, and the value was multiplied by 0.25 coefficients (Maurizio et al., 2005). Two g of dry powdered sample was digested in 5ml of concentrated sulphuric acid (H_2SO_4) and a tablet of selenium catalyst was added in a fume cupboard. The digest was made up to 250ml with the acid. Ten ml of the digest was distilled and titrated with 0.2 NH_2SO_4. The crude protein was finally obtained by multiplying total nitrogen by 0.25.

Determination of moisture content

Moisture contents (MC) was determined by placing 2 g of the powdered dry samples on clean dry glass Petri dishes of known weight and placed in an electric oven at 75^{o}C for 7-8 h (AOAC, 2000 and Konuk et al., 2006). The oven-dried samples were kept at constant weight. The percentage moisture content (PMC) was determined thus:

$$PMC = \frac{\text{wt of fresh} - \text{wt of dry sample}}{\text{wt of fresh sample}} X \frac{100}{1}$$

Determination of ash contents

Ash contents were determined by burning dried samples of the fruit bodies. Five g of the powdery samples of mushrooms was burnt at 500^{o}C overnight in a crucible. The crucible was allowed to cool and later weighed again (Mattila et al., 2002; Oei, 2003). The percentage Ash content (PAC) was determined as:

$$PAC = \frac{\text{wt of crucible+lid+Ash} - \text{wt of crucible+lid}}{\text{weight of sample}} X \frac{100}{1}$$

Determination of carbohydrate (CHO)

Carbohydrate contents were determined by difference; that is, % CHO= 100 – (5 Ash + % protein + % fat + % moisture).

Determination of ether extract

Ether extract component of mushroom samples was determined following the established method of AOAC, (1980; 2000). Two grams (2g) of each sample was inserted into an ether extracting thimble and placed on the soxhlet reflux flask channelled into a round bottom flask of unknown weight. The apparatus was filled with 250ml of petroleum ether and placed on a heating apparatus. The oil was extracted by a reflux system. After repeated refluxing, a clear solution was obtained in the flask and the sample removed. Further heating was done to separate the ether from the extracted oil. A round-bottom flask containing the oil was dried in an oven at 70^{o}C; fats and oils were determined by the gravimetric method as follows: weight of oil =weight of flask + oil –weight of flask (after drying). This was expressed as sample percentage as follows:

$$\% \text{ fats and oils} = \frac{\text{wt of oil}}{\text{wt of sample}} \text{ X } \frac{100}{1}$$

Determination of crude fibre

Total crude fibre of the samples was calculated by the Weende method (AOAC, 1980; 2000).Two g of each sample was inserted into a 250-ml beaker and hydrolyzed by adding 20ml of dilute sulphuric acid; it was boiled for 30 min on a hot plate. The mixture was filtered off through a piece of clean white nylon cloth and rinsed with hot distilled water. The residue was further boiled with 50ml of 2.5% sodium hydroxide (NaOH) for 30 min and also filtered off before rinsing with distilled water. The residue was finally collected and transferred into a crucible before it was dried in an oven to a constant weight. Finally, the sample was ashed in a muffle furnace and the weight of the crude fibre was determined and expressed as:

$$\%\text{crude fibre} = \frac{\text{wt of fibre}}{\text{wt of sample}} \text{ X } \frac{100}{1}.$$

Determination of vitamins

Vitamin content of the mushroom samples was determined by a spectrophotometric method, as described by AOAC (1980).

Determination of Vitamin A (Retinol)

The retinol content of each sample was estimated by the method of Shyam et al. (2010). Five gram (5 g) of each fruit bodies sample was homogenized using acetone solution and filtered off using Whatman filter No.1. The filtrate was later extracted with petroleum spirit using a separating funnel; two layers of both aqueous and solvent layer were obtained. The upper layer which contains vitamin A was washed with distilled water to remove residual water. This was later poured out into a volumetric flask through the discharge point of the separating funnel and made up to mark. The absorbance (A) of the solution was read using a spectrophotometer at a wave length of 450 nm and calculated as:

Mg /g = A x vol x 104= A x 12cm x sample weight.

Determination of Vitamin B_1 (Thiamin)

Five grams (5g) of each mushroom sample was homogenized with ethanolic sodium hydroxide (50ml) and filtered into a 100-ml flask. Ten ml of the filtrate was pipetted and the colour development was read at the same time. Thiamine was used to get 100 ppm and serial dilution of 0.0, 0.2, 0.6 and 0.8ppm was made. This was used to plot the calibration curve (AOAC, 2000; Shyam et al*.,* 2010).

Determination of Vitamin B_3 (Niacin)

Niacin composition was determined following the König spectrophotometric method (AOAC, 2000). Dry powdered mushroom sample of 0.5g each, was extracted with 50ml of 1 N HCl in a shaking water bath kept at 30^{o}C for 35 min. The mixture was filtered using Whatman filter paper. $KMnO_4$ (0.5g) was added to the filtrate and made up to mark. Ten ml of the extract was pipetted into a 50-ml flask, while 10ml of phosphate solution was added as buffer. The pH was adjusted with 5ml of 1 NHCl, and the solution was made up to mark with distilled water. After 15min, the extract was read by spectrophotometry at 470nm wave length.

Determination of Vitamin C (ascorbic acid)

Vitamin C content of each mushroom sample was estimated by the method of Kamman et al. (1980). Five grams (5g) of each sample was homogenized in 100ml of EDTA/TCA extraction solution. The homogenate was filtered and the filtrate was used for the analysis. Each sample filtrate was passed through a packaged cotton wool containing activated charcoal for discolouration. The volume of the filtrate was adjusted to 100ml of water by washing with more of the extraction solution. Twenty ml of each filtrate was measured into a conical flask. 10 ml of 2% potassium iodide solution was added to each of the flasks followed by 5 mls of starch solution (indicator). The mixture was titrated against 0.01 molar $CuSO_4$ solution; titration of the brink of the mixture. Vitamin C content of the samples was calculated as 1ml of 0.01 mol CuSO4 at 0.88n, according to the formula of Shyam et al. (2010):

$$\text{vit mg/100g sample} = \frac{100 \text{ x vf x } 0.88T}{\text{va}}$$

Where: Vf = volume of filtrate analysed; Va = volume of acid analysed; 0.88T = constant.

Determination of Vitamin K (Phylloquinone)

Determination of vitamin K followed the method of Careri et al. (1996). Powdery mushroom sample of 1.0g was weighed out, transferred into a 40-ml brown glass flask and ultrasonically shaken with 10 ml methanol for 15 min. The amount of mushroom sample was increased to 5.0 g and was centrifuged at 1000 rpm for 5 min (ALC 4236 centrifuge, ALC, Milan, Italy). A 2-ml aliquot of the methanol phase was mixed with 4 ml of sodium carbonate solution (5 g/100 ml), and heated at 80°C for 1 h. The hydrolysate was extracted by partitioning of the alkaline solution with 4 ml n-hexane on a Vortex mixer for 1 min and centrifuged at 2000 rpm for 10 min; after the upper hexane layer had been carefully separated from the aqueous phase, two additional 4 ml of n-hexane was added to the aqueous phase and processed as before. The combined extracts (12 ml) were collected in a 50-ml round-bottom flask and concentrated to a low volume in a rotary evaporator at 35 °C (Biichi, Brinkman Instruments, Inc., Chicago, IL, USA) and then evaporated to dryness under a stream of nitrogen. The final residue was re-dissolved in methanol (1 ml) and analyzed by HPLC after filtration through a 0.2-mm membrane.

Determination of Vitamin E (Tococpherol)

Tocopherol estimation was done by the colorimetric method of Baker and Frank (1968). The tocopherol is determined by Emmerie-Emmerie Engel reaction which is based on the reduction by tocopherol of ferric to ferrous ions; it latter formed a red complex with α,α'-dipyridyl. Tocopherol and carotene were first extracted into xylene and the extract read at 460 nm to measure carotenes. A correction is made for these after adding ferric chloride and reading at 520 nm.

Into three stopper centrifuge tubes were measured 1.5ml and 1.5 ml standard sample solution and water (blank), respectively. Then, in the test and blank 1.5 ml of xylene was added to all the tubes, stoppered mixed, and centrifuged; thereafter, 1ml of the xylene layer was transferred into other stoppered tubes taking care not to include any ethanol or protein. One ml of α, α'-dipyridyl reagent was added to each tube that was stoppered and mixed; then1.5 ml of the mixture was pipetted into colorimeter corvettes and extraction of test and standard samples were read against the blank at 460 nm. Tocopherol was calculated as mg/l by the following formula:

$$\frac{(\text{Extinction of unknown at 520 nm- Extn at 460 nm} \times 0.29)}{\text{Extn of standard at 520 nm}} \times 10$$

Determination of percentage bioactive compounds

Determination of phenolics content

To determine the phenolics content of the powdered sample of the mushroom, a fat-free sample was used. Two grams (2g) of the sample was defatted with 100 ml of diethyl ether, using a soxhlet apparatus for 2 h. To extract the phenols component of the sample, the fat-free sample was boiled with 50ml of ether for 15 min. Five ml of the extract was pipetted into a 50-ml flask into which 10ml of distilled water, 2ml of ammonium hydroxide (NH_4OH) solution and 5ml of concentrated amyl alcohol were added.

The mixture was made up to mark and left to react for 30 min for colour development. The absorbance of solution was read using a spectrophotometer at 505nm wave length (Harborne, 1973). The % phenol was calculated as follows:

$$\frac{100 \text{ x Au x C x VF x D}}{\text{W x AS x 1000 xVa}}$$

Where:W = weight of sample of analysed; Au = absorbance of the test sample; As = absorbance of standard solution; C = concentration of standard in mg/ml; VF = volume of filtrate analysed; VA = volume of acid analysed; D =dilution factor, where applicable.

Determination of tannins

Tannin content of the mushroom samples was estimated following the modified method of Okeke and Elekwa (2003). 0.5g of the sample in 10ml of 2-MHCl was vigorously shaken for 5 min and transferred into a volumetric flask and made up to 50ml. The mixture was filtered, and 5ml of the filtrate was transferred into a test tube. Three ml of 0.1 NHCl and 3ml of 0.008 M potassium ferrocynide ($K_3F[CN]_3$) were added. The absorbance was read at 720 nm within 10 min.

Determination of sterols

The crude fat analysis was carried out by the method of AOAC (2006b). A 250-ml extraction flask was dried in the oven at 105^{o}C, transferred to the desiccator to cool at room temperature and the weight of flask measured. Exactly 0.25g of the sample was weighed into a labelled porous thimble; 200ml of petroleum ether was subsequently measured and added to the 250-ml conical flask. The covered porous thimble with the sample was placed in the condenser of the soxhlet extractor. The sample was extracted for 5 h. The porous thimble was removed with care and the petroleum ether in the top container (tube) was collected by recycling for reuse. The extraction flask was removed from the heating mantle when it was almost free of petroleum ether. The extraction flask containing the oil was oven-dried at 105^{o}C for one (1) h. The flask containing the dried oil was cooled in the desiccator and the weight of the cooled flask and the dried oil were measured.

Determination of alkaloids

The method of Maxwell et al. (1995) was followed to estimate the quantity of alkaloids in the mushroom samples. The alkaloids were extracted from 20g of each of the dried powdered mushroom sample using 100ml of 10% acetic acid. The extracts were filtered to remove cellular debris before being concentrated to a quarter of the original volume. One % NH_4OH was added to the concentration in drops until no precipitate was formed. The alkaloids, thus obtained were dried to a constant weight at 65^{o}C in an oven. The weight was used to calculate the percentage of alkaloids in the mushroom samples, using the formula:

$$\text{Alkaloids (\%)} = \frac{\text{weight of residue}}{\text{weight of sample}} \text{ X } \frac{100}{1}$$

Determination of terpenes

The extraction was carried out following the method of Ortan et al. (2009). The dried ethanol and aqueous extracts were made to be free of water by drying to constant weight for a period of time in the laboratory and the terpenes constituents extracted with redistilled chloroform. The terpenes were removed with 10ml of the solvent for 15 min. The mixture was filtered and concentrated to 1ml in the vial for gas chromatography analysis and 1ml was injected into the injection port.

Determination of glycosides

Glycosides were determined by the method of Peng and Kobayashi (1995). Equal parts of Fehling's solution I and II(5ml) were added to 5ml of the dry mushroom sample. A brick red precipitate shows the presence of a reducing sugar.

Hyrolysis test

Five mls (5ml) of dilute sulphuric acid was added to about 0.1g of the mushroom extract and neutralized with 20% KOH solution. Ten grams (10ml) of a mixture of equal parts of Fehling's solutions I and II were added and boiled for 10 min. A more dense brick red precipitate indicates the presence and amount of glycosides.

Determination of minerals

Mineral contents of dried mushroom samples were estimated by a wet-ashing method. The solutions of ash obtained from the samples were dissolved in a drop of trioxonitrate (v) acid made up to 50ml with deionized water. They were analyzed for calcium (Ca) using vanadate ethyldiamine-tetra acetic acid (EDTA) complexometric titration method according to MFA, (1982). Sodium (Na) Chlorine (Cl) and Potassium (K) were estimated using a flame photometer.

Determination of heavy metals

The amount of Fe, Cu and Zn in the sample was estimated by Energy Dispersive X-ray Fluorescence (EDXRF) technique according to the method of Stihi et al. (2011) and Ghisa et al. (2008). A Elvax spectrometer was used, having an x-ray tube with Rh anode, operated at 50kv and 100μA. Mushroom samples were excited for 300 s and the characteristic x-rays were detected by a multi-channel spectrometer based on a solid state Si-PIN photodiode x-ray detector with a 140μm Be- window and an energy level of 200ev at 5.9 keV. Elvax software was used to interpret the EDXRF spectra. The accuracy of the results was evaluated by measuring a certified reference sample, and good results were achieved between certified values and data obtained.

The amount of Pb and Se in the sample was estimated by Atomic Absorption Spectrometry (AAS) (Wagner, 1999; Petisleam et al., 2007; Dima et al., 2006), using the AVANTA GBC spectrometer with flame and hollow cathode lamps (HCl). Pb and Se were determined by the method of calibration curve according to the absorption concentration. Several standard solutions of different known concentrations were prepared and the elemental concentration in the unknown sample was determined by extrapolation from the calibration curve. All fruit body sample concentrations were reported as mg/kg dry weight of material.

Statistical analysis

Data obtained in the course of this investigation were statistically analyzed using Analysis of Variance (ANOVA), and mean separation was carried out by a Duncan Multiple Range Test (DMRT) at $p<0.05$ level of significance (Steel and Torie, 1984).

RESULTS AND DISCUSSION

pH variations in substrate and formation of mushroom fruit bodies

The results revealed that the naturally obtained OPB substrate that was neither soaked in water nor acid solution had a pH of 10.1 (Table 1). This value is relative to those obtained by Achufusi (2016) and Okwulehie et al. (2018) who reported pH values of 10.3 and 9.5, respectively, on raw OPB substrate during mushroom cultivation. It was observed that HCl acid solution of 0.1% - 0.5% reduced pH of the substrate from 8.2 – 6.1, respectively, after steeping for 48 h; while the control was found at a pH of 9.0. This variation also was in line with the work of Okwulehie et al. (2018), who reported a direct proportionate increase in acidity of OPB substrate with increased HCl solution after steeping for 48 h.

pH is generally considered to be one of the most important environmental factors that seriously affects the fruiting, growth and extension of fungal mycelia (kang et al., 2006). Our results indicated that HCl acid delayed primordial formation, revealing that concentration of the acid delayed primordial formation, but increased fruit body production. This observation was in line with the works of Bilgrama and Verma (1992), Okwulehie et al. (2006), and Okwulehie and Okwujiako (2008), who reported that culture media of pH between 6.0 and 8.0 recorded significantly greater mycelia extension than those above the range. In our investigation, the time for primordial initiation, apparently preceded by fruit body production was shorter compared to the result obtained by Shah et al. (2004), who reported a fruiting duration of oyster mushroom within 3-6 weeks after spawn inoculation. Contrarily, Khan et al. (2001) investigated oyster mushroom cultivation and observed that primordial formation took place after 8 days of spawn running; while spore carp formation took place after 10-12 days of spawn running. Early fruit body formation recorded in this experiment could be due to certain factors such as HCl acid, substrate and cultivation technique according to Chang and Miles (2004), Nwoko et al. (2017), Okoi and Iboh (2015), and Hassan et al. (2010).

Morphological characteristics of fruit bodies

Results of some of the morphological characteristics of fruit bodies showed that 0.5% HCl OPB substrate produced the highest (705.00) number of fruit bodies (Table 2). The results also showed that as the percentage of HCl in the OPB substrate increased from 0.1-0.5, the number of fruit bodies increased from 541-705; while control had the lowest (424.00). This observation is in line with the work of Okwulehie et al. (2018), who recorded the highest number of fruit bodies of *P. ostreatus* at 0.5%HCl OPB substrate, and got the lowest in control. Achufusi (2016) did not observe the growth of any *P. ostreatus* fruit body from OPB substrate without the addition of HCl. That could probably be as a result of high alkaline level of the substrate which does not support mycelia growth as well as fruit body production (Bilgrama and Verma, 1992; Okwulehie et al., 2006; Okwulehie and Okwujiako, 2008). In this experiment, the mean cap diameter and weight of fruit bodies from all levels of HCl OPB substrates, including control, were higher than the values reported by Okwulehie et al. (2018); unlike in stipe length where they obtained higher values. This could be due to variation in the oyster mushroom species.

Substrate variation has been recorded as another

Table 1. pH of substrates and fruiting duration of *P. ostreatus*.

HCl OPB level	pH of OPB	Fruiting duration/days
Raw OPB(%)	10.1[a]	-
Control	9.0[b]	17[c]
0.1	8.2[c]	16[d]
0.2	7.8[d]	17[c]
0.3	7.4[e]	18[b]
0.4	7.1[f]	18[b]
0.5	6.1[g]	19[a]

Means followed by the same alphabet within column are not significantly different by DMRT (p≤0.05), means ±SEM (n=3).
Source: Authors

Table 2. Morphological characters of fruit bodies.

HCl OPB Level (%)	FBN	C.D (cm)	S.L (cm)	WT (g)
Control	24[f]	6.94±0.41[c]	2.83±0.16[b]	8.93±1.53[b]
0.1	541[e]	5.96±0.23[e]	2.44±0.07[e]	5.23±0.52[d]
0.2	591[d]	7.75±0.29[a]	3.00±0.12[a]	9.02±0.79[a]
0.3	621[c]	7.08±0.23[b]	2.66±0.09[c]	7.28±0.55[c]
0.4	687[b]	6.39±0.23[d]	2.42±0.08[e]	5.98±0.50[d]
0.5	705[a]	7.14±0.20[b]	2.53±0.08[d]	7.34±0.54[c]

FBN= Fruits Body Number, CD= Cap Diameter, SL= Stipe Length, Wt=Weight, Means followed by the same alphabet within column are not significantly different by DMRT (p≤0.05), means ±SEM (n=3).
Source: Authors

Table 3. Productivity and biological efficiency of fruit bodies.

HCL OPB Levels (%)	Dry with substance	Productivity	Biological efficiency (%)
Control	1400[a]	865.02±0.00[f]	61.79±0.00[f]
0.1	1400[a]	1002.56±0.00[e]	71.64±0.00[e]
0.2	1400[a]	1428.42±0.00[d]	102.03±0.00[d]
0.3	1400[a]	1661.66±0.00[c]	118.69±0.00[c]
0.4	1400[a]	1735.10±0.00[b]	132.41±0.00[b]
0.5	1400[a]	1799.10±0.00[a]	137.97±0.00[a]

Means followed by the same alphabet within column are not significantly different by DMRT (P≤0.05), means ±SEM (n=3).
Source: Authors

important factor that can influence the morphological characteristics of mushroom fruit bodies. For instance, in an experiment to determine the yield of *P. ostreatus*on other agro-waste components, Okwulehie and Okwujiako (2008) reported that *Pennisetum* straw significantly raised the stipe length of the studied oyster mushroom, followed by *A. gayanus* and *Oryza* straws. They also noted that *Panicum* straw causes a reduction of the cap diameter. Okoi and Iboh (2015) noted that different substrates have an effect on the pileus diameter, stipe length and stipe girth. Other factors can also affect the general size of a mushroom fruit body. Ogbo and Okhuoya (2009) reported that crude oil has a significant effect on the macro-morphological characteristics such as pileus diameter, stipe height, stipe girth and fresh weight of mushroom carpophores. A relatively smaller mushroom cap is an undesirable market quality (Yang et al., 2002). Apart from number of fruit bodies which had a direct correlation with productivity, HCl did not significantly affect other morphological characters studied (Table 3).

Table 4. Vitamin concentrations (mg/100g) of fruit bodies.

HCL OPB Levels (%)	Vitamin A	Vitamin B_1	Vitamin B_3	Vitamin K	Vitamin C	Vitamin E
Control	0.97±0.07^a	9.51±1.16^c	0.42±0.05^c	0.42±0.05^d	1.84±0.07^a	0.27±0.01^a
0.1	0.63±0.05^d	1.57±0.39^e	1.68±0.16^a	0.47±0.02^d	1.78±0.06^b	0.26±0.02^b
0.2	0.38±0.05^f	5.62±0.03^d	1.14±0.09^d	0.57±0.03^c	1.65±0.03^c	0.22±0.01^c
0.3	0.81±0.07^b	9.43±0.90^c	1.51±0.29^b	0.36±0.04^e	1.65±0.04^c	0.27±0.02^c
0.4	0.76±0.06^c	16.72±0.57^a	1.18±0.06^d	1.80±0.05^b	1.65±0.04^c	0.24±0.01^c
0.5	0.45±0.07^c	11.41±0.56^b	1.53±0.12^b	2.90±0.98^a	1.65±0.01^c	0.22±0.01^d

Means followed by the same alphabet within column are not significantly different by DMRT (p≤0.05), means ±SEM (n=3).
Source: Authors

Productivity and biological efficiency of *P. ostreatus* fruit bodies

Influence of HCl acid on the productivity and biological efficiency of the studied oyster mushroom indicated that increase in the percentage concentration of HCl acid from 0.1- 0.5% resulted in a significant yield increase as well as biological efficiency of the mushroom fruit bodies. As the lowest fruit body yield was recorded in the control (865.02g), OPB substrate induced with 0.5% HCl solution produced the highest quantity (1799.10g) of fruit bodies with biological efficiency of 137.97%. Rip (2010) reported that only experienced mushroom growers have been able to produce mushrooms with biological efficiency of 100% and above and this was obtained between 0.2 – 0.5% HCl OPB substrates. This result justifies the claims by Bilgrama and Verma (1992), Chang and Miles (2004), Shah et al. (2004), Okwulehie et al. (2006) and Khan et al. (2013),who reported that oyster mushrooms grow and perform optimally at slightly acidic pH6.1.

The overall yield and biological efficiency of the oyster mushroom as observed in this experiment were significantly higher than those obtained by Shah et al. (2004), who cultivated *P. ostreatus* on saw dust amended with different agro-waste, Nwoko et al. (2017), who grew *P. ostreatus* on trees logs, Okwulehie and Okwujiako (2008) and Okoi and Iboh (2015), who in their separate investigations cultivated oyster mushrooms on different agro-waste components.

The fact here is that HCl acid optimized the pH of OPB substrate, which was initially found to be alkaline, to support the growth and productivity of the mushroom studied. High alkalinity of OPB substrate could be the major reason Achufusi (2016) could not record any fruit body production when he attempted to grow *P. ostreatus* on the substrate, but instead observed heavy contamination by *C. cinerius.* Although there were traces of contamination by *C. cinerius* in our study, which was high in control, it reduced gradually until none was found in 0.4 and 0.5% HCl concentrations. This is in line with Okwulehie et al. (2018), who reported no contamination due to *Coprinus* spp. at 0.4% HCl acid-induced OPB substrate during cultivation of *P. pulmonarius.*

Vitamin concentrations (mg/100g) of *P. ostreatus* fruit bodies

Results of the effect of HCl acid on the vitamin concentrations of *P. ostreatus* fruit bodies grown on OPB substrate are significantly (p≤ 0.05) different across the various levels of HCl acid concentration solution. Vitamin A content of the oyster mushroom was lower than the values reported by Nwoko et al. (2017) in *P. ostreatus cultivated* on various wood logs, as well as Okwuelehie and Okwujiako (2008) in *P. ostreatus* cultivated on different substrates and substrate supplementations (Table 4).

On the other hand, vitamin B_1 values as observed in this experiment were higher than those reported by the above mentioned researchers. Results of vitamin B_3, K, C and E observed in this study were richer than those reported by Okwulehie et al. (2009), but lower than those reported by Okwulehie et al. (2008). The variations in the concentration of vitamins could be due to substrate variations, age of fruit bodies and other factors inherent in the species; since most of them could be varieties of the same species (Chang, 2013; Nwoko et al., 2017; Okwulehie et al., 2009).

The appreciable vitamin contents, especially vitamin B_1, B_3 and C, are in line with the report of Maltila et al. (2004), Shibata and Demiale (2003) and Okwulehie et al. (2008), who maintained that mushrooms are rich in nutrients such as vitamins, protein, minerals, etc. In the current investigation, HCl acid did not significantly affect the vitamin concentrations of the mushrooms, compared to the control, and suggests no possible health risk when consumed by humans. Similarly, Nwoko et al. (2017) and Bobek and Galbary (2001) stated that the recommended dietary intake (RDI) of vitamins such as Retinol or Vitamin A is 200 mg; an indication that these mushroom samples meet the nutrient requirement by humans for a

Table 5. Bioactive (%) compound composition of fruit bodies.

HCL OPB levels (%)	Phenolics	Tannins	Steroids	Alkaloids	Terpenoids	Glycosides
Control	110.43±2.68^{d}	5.58±0.87	0.49±0.01^{a}	39.52±0.46^{c}	9.37±0.39^{a}	1.70±0.00^{d}
0.1	188.96±6.01^{a}	5.89±0.83^{d}	0.32±0.01^{b}	36.32±0.68^{d}	8.21±0.30^{b}	1.93±0.01^{b}
0.2	106.56±1.76^{e}	5.47±0.19^{e}	0.19±0.00^{c}	46.87±0.43^{q}	4.43±0.64^{e}	1.98±0.00^{a}
0.3	11.32±18.93^{c}	101.06±2.09^{c}	0.25±0.09^{d}	36.32±0.39^{d}	6.29±0.24^{c}	1.83±0.00^{c}
0.4	107.63±8.37^{f}	131.83±0.62^{b}	0.07±0.02^{e}	41.46±2.21^{b}	5.37±0.38^{d}	1.79±0.01^{d}
0.5	158.87±8.33^{b}	134.68±2.77^{a}	0.05±0.13^{e}	24.09±0.94^{e}	3.75±0.25^{f}	1.67±0.00^{e}

Means followed by the same alphabet within column are not significantly different by DMRT (p≤0.05), means ±SEM (n=3).
Source: Authors

healthy diet.

Bioactive compound concentrations of the fruit bodies

Results showed the effect of HCl acid on the bioactive compounds composition of *P. ostreatus* fruit bodies grown on OPB substrate. It was observed that phenolics were in appreciable quantities in the fruit bodies harvested across all the treatment levels including control, followed by alkaloids. Alkaloids have a remarkable effect in animal physiology and are important in pharmaceutical companies, for drug production (Edeoga and Erieta, 2001). Edeoga and Erieta (2001) also recorded that alkaloids are stimulants and act by slowing down the action of several hormones. Phenolic, tannin, alkaloid and terpenoide concentrations in all fruit body samples were higher than those reported by Okwulehie et al. (2007). Flavonoids serve as anti- carcinogens, anti-bactarials (Hilang and Feraro, 1992); saponins are used inthe prevention of parasitic fungal diseases (Edeoga and Erieta, 2001); while tannins have been reported as anti-tumour agents and perform a wide range of anti-infective actions (Haslam, 1996). The high concentrations of these important bioactive compounds in *P. ostreatus* fruit bodies from various percentage HCl-OPB substrates show that these mushroom samples may be useful in the production of certain pharmaceutical active ingredients (Okwulehie et al., 2007) (Table 5).

The obtained values were significantly higher than those reported by Onyeizu et al. (2017) and Okwulehie et al. (2009) in experiments involving *P. pulmonarius* cultivated on different wood logs and agro-waste, respectively. Tannins, terpenoids, and glycosides were also in moderate quantities, but higher than the values obtained by Okwulehie et al. (2007) in an investigation to determine the pharmaceutical and nutritional benefit of two wild macro-fungi found in Nigeria. Nwoko et al. (2016) also obtained lower concentrations of bioactive compounds in *P. ostreatus* cultivated on deciduous trees logs. The high quantities of these physiologically important compounds in the fruit bodies were not due to HCl acid, which served as a buffer to the substrate; but could, however, be attributed to variation in substrates. This justifies the position of Change and Miles (2004) who assert that the nutritional composition of mushrooms to a large extent depends on the substrate where the mushroom was grown. A considerable pharmacological activity of mushrooms is the major reason for their high demand for drug development in pharmaceutical industries (Okwulehie et al., 2007, 2008). Nwoko et al. (2016) further asserted that most bioactive compounds, which play essential roles in human and animal physiology, have been found in many mushrooms. This observation alone has justified the resources committed to this investigation. From 0.3 to 0.5% HCl concentration, there seems to be a gradual increase in the quantity of tannins from 101.06±2.09 to 134.68±2.77%. This could be particularly due to increase in the concentration of HCl acid. Tannins inhibit pathogenic fungi and also reduce the rate at which herbivores graze on plants (Okwuehie et al., 2007; Haslam, 1996). This could no doubt be attributed to the reason for a constant increase in fruit body production as the concentration of HCl acid increased from 0.1 – 0.5% (Okwulehie et al., 2018).

Proximate composition of fruit bodies

Proximate composition of fruit bodies of *P.ostreatus* showed that there was a significant (p≤0.05) difference among different levels of treatment compared to control. Values obtained in all the studied parameters (MC, Ash, EE, CF, protein and CHO) were relative to the values obtained by various researchers such as Okwulehie et al. (2008), Sharad (2013), Pathmashini et al. (2008), Syed et al. (2009), Araujo Silva et al. (2011) and Okoi and Iboh (2015). Hydrochloric acid appears to have a reduction effect on the CHO content of the oyster mushroom, but tends to increase protein from 2.19±0.00% low, in control to 24.98±0.03% high, in fruit bodies obtained from 0.5%

Table 6. Proximate composition (%) of fruit bodies.

Hcl OPB level (%)	MC	Ash	E.E	C.F	Protein	CHO
Control	8.44±0.01^{c}	2.97±0.04^{a}	2.44±0.01^{b}	3.56±0.03^{bc}	2.19±0.00^{e}	80.09±0.09^{a}
0.1	8.45±0.01^{c}	2.62±0.03^{c}	2.39±0.02^{c}	3.42±0.08^{c}	3.10±0.02^{d}	79.93±0.16^{b}
0.2	8.84±0.27^{bc}	2.21±0.04^{d}	2.16±0.04^{d}	2.98±0.03^{e}	19.98±0.0^{c}	63.83±0.29^{c}
0.3	8.94±0.26^{bc}	2.64±0.26^{bc}	2.59±0.06^{a}	3.38±0.14^{d}	21.95±0.06^{bc}	60.50±0.69^{d}
0.4	8.81±0.15^{b}	2.60±0.01^{bc}	2.45±0.06^{b}	3.58±0.02^{b}	21.13±0.02^{b}	61.42±0.25^{e}
0.5	9.07±0.15^{a}	2.77±0.06^{b}	2.52±0.04^{a}	3.94±0.06^{a}	24.98±0.03^{a}	56.72±0.33^{f}

Means followed by the same alphabet within column are not significantly different by DMRT (p≤0.05), means ±SEM (n=3).
Source: Authors

Table 7. Mineral concentrations of fruit bodies

HCL OPB level (%)	Na	K	Cl	Ca
Control	140.78±4.19^{d}	4.13±0.10^{cd}	111.00±3.39^{c}	11.23±0.20^{d}
0.1	146.13±2.09^{c}	4.23±0.15^{c}	103.25±2.14^{d}	11.50±0.60^{cd}
0.2	147.18±1.28^{b}	5.00±0.18^{a}	112.00±3.39^{bc}	11.98±0.43^{c}
0.3	140.73±1.41^{d}	4.87±0.22^{bc}	112.35±0.06^{b}	12.35±0.07^{b}
0.4	149.85±6.32^{a}	4.90±0.65^{b}	112.45±0.49^{a}	12.45±0.50^{a}
0.5	147.30±5.89^{b}	5.07±0.46^{a}	112.18±0.13^{b}	12.18±0.13^{ab}

Means followed by the same alphabet within column are not significantly different by DMRT (p≤0.05), means ±SEM (n=3).
Source: Authors

HCl OPB substrate. These are eventually higher than the values obtained by Okwulehie et al. (2008), Okoi and Iboh (2015) and Araujo Silva et al. (2011). Chang and Miles (2004) maintained that the high CHO contents of mushrooms is due to the high lignacellulosic compositions in the substrate where they grow; in which mushrooms were broken down using extra cellular enzymes.

The high amounts of CF protein and CHO in *P. ostreatus* fruit bodies as generally observed in this study have been attributed to the type of substrate and to a large extent, mushroom species (Nwoko et al., 2016). This further elucidates the claims by Obodai et al (2003), Adejoye and Fasidi (2009) and Okoi and Iboh (2015), who in separate investigations noted that the nutritional composition of mushrooms could reflect the chemical composition of the substrate used, as mushrooms are capable of carrying out extra-cellular digestion of the decomposed substrate during cultivation (Table 6).

The high nutritional composition of oyster mushroom cultivated on HCl induced OPB substrate does not only reveal the readily available agro-waste as a good substrate for mushroom cultivation (Lisdar et al., 2011), but also suggests that HCl can help build up the protein composition of the fruit bodies.

Mineral concentrations of fruit bodies

Results showed that fruit bodies harvested from 0.4 (149.85 ±6.32)-0.5% (147.30±5.89) HCl OPB substrates had appreciable concentrations of sodium while control (140.78±4.19) was among the lowest (Table 7).

The concentration of potassium was highest (5.07±0.46) in fruit bodies from 0.5%HC;l while control (4.13±0.10mg/ 100g) was the lowest. Although there was an irregular trend in the concentration of all the studied minerals with respect to percentage HCl; nonetheless, a general consideration could infer that increase in the concentration of HCl in the OPB substrate had a positive effect on the concentration of the studied minerals. These observations conform the report of Egwin et al. (2011) who maintained that the relative higher concentration of mineral nutrients in mushroom fruit bodies may be due to the absorption and accumulation of elements from their habitat. This is contrary to the observations by Adam and Duncan (2002), who noted that crude oil, had a decreasing effect on the minerals studied in mushroom fruit bodies implicated in a mycoremediation experiment. They noted that the observed effect could be due to crude oil which acts as a physical barrier preventing or reducing access of fruit bodies to nutrients. Sudheep and

Table 8. Heavy metal concentrations of fruit bodies.

HCL OPB level (%)	Zinc	Fe	S e	Pb	Cu
Control	154.79±1.15^{c}	167.43±2.11^{f}	0.90±0.02^{a}	0.08±0.01^{b}	0.52±0.02^{d}
0.1	150.97±3.55^{f}	197.70±2.10^{a}	0.84±0.00^{bc}	0.08±0.04^{b}	0.61±0.02^{c}
0.2	159.00±1.45^{d}	176.70±3.20^{e}	0.86±0.02^{b}	0.09±0.03^{b}	0.77±0.01^{b}
0.3	166.63±1.29^{c}	191.23±4.47^{c}	0.81±0.02^{cd}	0.12±0.11^{ab}	0.79±0.02^{b}
0.4	169.10±1.44^{b}	184.70±0.62^{d}	0.82±0.0^{c}	0.17±0.10^{a}	0.81±0.03^{ab}
0.5	181.07±1.22^{a}	194.30±1.01^{b}	0.94±0.01^{ab}	0.14±0.04^{a}	0.86±0.02^{a}

Means followed by the same alphabet within column are not significantly different by DMRT (p≤0.05), means ±SEM (n=3).
Source: Authors

Sridhar (2014) reported that mushrooms gave high potassium content while sodium, calcium and phosphorus contents were low, also in an experiment involving crude oil. It is generally believed that mushrooms are rich in mineral elements and this largely depends on the substrate where the mushroom was cultured (Okwulehie et al., 2009; Chang, 2013; Nwoko et al., 2017). HCl alone may not be responsible for the observed increase in the concentration of certain minerals studied in this investigation; since in some cases, the control is either equal or higher than other treatment groups. This, therefore, suggests that the fruit bodies could be safe for human consumption.

Heavy metal concentrations of fruit bodies

Results showed that increased in the concentration of HCl acid tends to increase the amount of Zinc in the fruit bodies (Table 8). High concentration of zinc in fruit bodies of *P. ostreatus*is seldom reported by many scientists; but there could be variation in concentration due to substrate used during its cultivation (Oboda et al.i, 2003; Adejoye and Fasidi, 2009; Okoi and Iboh, 2015). Stihi et al. (2011), Nwoko et al*.* (2017) and Okoi and Iboh (2015) reported lower values of zinc in oyster mushrooms cultivated on different substrates. The concentration of iron in the fruit bodies was also on the increase with increase in the percentage concentration of HCl. These values were also higher compared to those reported by Demirbaş (2001), but relative to those of Nwoko et al. (2017).

The observed increase in the concentration of zinc and iron in *P. ostreatus* fruit bodies with increase in the percentage concentration of HCl could be attributed to the ability of mushrooms to break down and utilize various recalcitrant compounds including some important environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorophenols, dioxins, dichlorodiphenyltrichloroethane (DDT), trinitrotoluene and synthetic dyes (Eggen and Sasek, 2002).

Selenium contents of the fruit bodies at all levels of HCl solution were moderate and similar to the values reported by Stihi et al. (2011). This shows that HCl had no significant effect on the accumulation of these important heavy metals in the oyster mushroom. Wermer and Beelman (2002) noted that many mushrooms are now being fortified with genes capable of synthesizing compounds with selenium, regarding its importance in human nutrition.

The concentrations of lead and copper in the fruit bodies were also directly proportional to the percentage of HCl used in the optimization of the pH of the OPB substrate. The obtained values conform to those reported by Stihi et al (2011), Nwoko et al. (2017) and Demirbaş (2001).

Heavy metal contents obtained in this investigation are within the admitted maximum level of certain contaminants in foodstuffs as established by the commission of the European communities (commission Regulation [EC] No 466/2001). The acceptable maximum level for Pb and Cd is set about 2 and 3 mg/kg d.w, in cultivated mushrooms. Apart from Pb, other heavy metals studied in this research are of high nutritional importance. Although, HCl slightly elevated the concentration of Pb, but its highest concentration at 0.5% could only increase Pb to 0.14±0.01, which falls below the European commission of regulation limit.

Conclusion

The experiment was successfully conducted to evaluate the influence of pH on the fruiting duration, some macro-morphological characters and productivity of *P. ostreatus* fruit bodies cultivated on acid-induced oil palm bunch substrate.

Hydrochloric acid induced changes on the pH of the substrate towards acidity; that is, from 9.0 in control – 6.1 in 0.5%. Hydrochloric acid delayed fruit body production from 17 days in control – 19 days in 0.5%.

Increase in the concentration of HCl acid in the OPB

substrate from 0.1 - 0.5% inhibited substrate contamination due to *C. cinerius,* and enhanced fruit body yield. This indicates that HCl acid acted as a suitable buffer for the optimization of pH of the OPB substrate.

Hydrochloric acid had no significant (p≥.0.05) effect on the macro-morphological characters of the fruit bodies studied, while increase in the concentration of HCl acid supported more fruit body production as well as biological efficiency.

Hydrochloric acid had no significant (p≥.0.05) effect on the macro-morphological characters of the fruit bodies studied, while increase in the concentration of HCl acid positively affected the number of fruit bodies produced which correlates with yield and biological efficiency. Hydrochloric acid had a significant positive effect on vitamins B_1, B_3 and K concentrations in the fruit bodies. But such could not be said of vitamins A, C and E. There was a percentage increase in tannins with increase in the concentration of HCl acid, unlike other bioactive compounds studied.

Protein concentration of fruit bodies was significantly increased with increase in the concentration of HCl; while carbohydrate contents of fruit bodies decreased with increase in the concentration of HCl acid. Other studied nutrient parameters were not significantly affected by HCl acid.

All the mineral nutrients studied were not significantly affected by HCl acid when compared to their control values. The concentration of Zn, Fe, Pb and Cu increased with increase in the concentration of HCl acid; while Se was not affected. The concentration of the studied heavy metals was found within the acceptable limit for human consumption and safety as justified by the commission of the European communities (Commission Regulation [EC] No 466/2001).

Recommendations

Commercial mushroom growers should avail themselves of this golden opportunity and ensure effective utilization of OPB incorporated with HCl acid for higher fruit body production as well as profit maximization. Other mineral or organic acids should be sourced for and studied in a related experiment. This could provide a cheaper or more efficient alternative to HCl acid.

Further research studies should consider upward adjustment of the percentage concentration of HCl acid. This would help obtain the acid solution level that would give the optimum pH of the OPB substrate.

Finally, ready-to-use OPB substrate produced by this method should be commercialized to enable mushroom farmers and other intending mushroom growers produce large quantity of mushrooms involved in acid dilution and pH optimization without much stress.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Achufusi JN (2016). Yield, heavy metals, nutritional evaluation, amino acid profile and lignocellulosic evaluation of *Pleurotus ostreatus* var florida (eger) fruit bodies cultivated on three supplemented palm substrates in Umudike, Abia State.An unpublished PhD. Dissertation of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Abia State. Nigeria P 40.

Adejoye OD, Fasidi I (2009). Effect of cultural conditions on biomass and laccase production in submerged medium by Schizophyllum commune (Fr.), a Nigerian edible mushroom. Electronic Journal of Environmental, Agricultural and Food Chemistry 8(12):816-824.

Anoliefo G, Isikhuemhen O, Ohimain E (2006). Sensitivity studies of the common bean (Vigna unguiculata) and Maize (Zea mays) to different soil types from the crude oil drilling site at Kutchalli, Nigeria. Journal of Soils and Sediments 6(1):30-36.

Anyankorah CH (2002). Cultivation of edible mushroom. African Journal of Biotechnology 30:399-401.

AOAC (2000) Official Methods of Analysis. 17th Edition, The Association of Official Analytical Chemists, Gaithersburg, MD, USA.

AOAC (1980). Official Methods of Analysis. 13th Edition, Association Official Analytical Chemists, Washington DC.

Araujo Silva G, Filipa Silva N, Moderno Fortunato T (2011). Stem cell and tissue engineering therapies for ocular regeneration. Current Stem Cell Research and Therapy 6(3):255-272.

Baker H, Frank O (1968). Determination of serum tocopherol. Practical clinical biochemistry. Gowenlock AH (ed). 6th ed. London, Heinemann Medica books.

Barmon BK, Sharmin I, Abbasi PK, Mamun A (2012). Economics of mushroom (*Agaricusbisporus*) production in a selected Upazila of Bangladesh. The Agriculturists 10(2):77-89.

Bilgrama KS, Verma RN (1992). Physiology of fungi. Villkas publishing house, Hvt Ltd, London P 7.

Celik Y, Peker K (2009). Benefit/cost analysis of mushroom production for diversification of income in developing countries. Bulgarian Journal of Agricultural Science 15(3):228-237.

Chang ST (1999). World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on Lentinusedodes (Berk.) Sing. in China. International Journal of Medicinal Mushrooms 1:291-300.

Chang ST, Miles PG (2004). Mushroom cultivation, nutritional value, medicinal effect and Environmental impact. 2nd (ed), CRC. P 451.

Cho SB (2004). What Is Mushroom? Mushworld, Korea Heineart Inc. pp. 1-14.

Cohen R, Jensen KA, Houtman CJ, Hammel KE (2002). Significant levels of extracellular reactive oxygen species produced by brown rot basidiomycetes on cellulose. FEBS Letter 531:483-488.

Demirbaş A (2001). Concentrations of 21 metals in 18 species of mushrooms growing in the East Black Sea region. Food Chemistry 75(4):453-457.

Edeoga HO, Eriata DO (2001). Alkaloid, tannin and saponin contents of some Nigeria medicinal plants. Journal of Medicinal and Aromatic Plant Sciences 23(3):344-349.

Eggen T, Sasek V (2002). Use of edible and medicinal oyster mushroom [*Pleurotus ostreatus* (Jacq.: Fr.) Kumm.] spent compost in remediation of chemically polluted soils. International Journal of Medicinal Mushrooms 4(3):54-61.

Elliot TJ (1982). Genetics and breeding of cultivated mushrooms. In Tropical Mushrooms: Biological Nature and Cultivation Methods. Chinese University. Press Hong Kong.

Eno J (2020). Mushroom farming in Nigeria: How profitable? Romania news Bulletin.

James LF, Panter KE (1995). Swainsonine-induced high mountain disease in calves. Veterinary and Human Toxicology 33:217-219.

Kamman JF, Wanthesen JJ, Labuza TP (1980). Technique for

measuring thiamin and riboflavin in fortified foods. Journal of Food Science 45(6):1497-1499.
Kang H, Hwang S, Lee H, Park KW (2006). Effect of high concentration of plant oils and fatty acids for mycelia growth and pin head formation of *Hericiumeriaceum*. Transactions of the ASAE 45(1):257-260.
Khan HA, Alhomida AS, Sobki SH, Syed SH, Zohair AA, Adnan AK, Abdulrahman AM (2001). Serum markers of tissue damage and oxidative stress in patients with acute myocardial infarction. Biomedical Research 24(1):15-20.
Khan M W, Ali M A, Khan NA, Khan MA, Rehmna A, Javed N (2013). The effect of different levels of lime and pH on mycelia growth and production efficiency of oyster mushroom. *Pleurotus spp.* Pakistan Journal of Botany 45(1):297-302.
Marshall E, Nair NG (2009). Making money by growing mushrooms. FAO Diversification Booklet Number 7, Rome. 64p.
Moyin-Jesu EI (2007). Use of plant residue for improving soil fertility pod nutrients, root growth and pod weight of Okra *Abelmoschusesculentus* L. Bioresource Technology 98:2057-2064.
Muhammad AA, Muhammad S, Shabbier A, Asif HM (2007). Protein and fat contents of various *Pleurotus species* raised on different waste materials. Pakistan Journal of Agricultural Sciences 44(3):140-143.
Nwoko MC, Okwulehie IC, Achufusi JN (2016). Nutritional composition of *Pleurotus ostreatus* (Jacq) P. kumm. Fruit bodies cultivated on deciduous tree logs in Abia State, Nigeria. International Journal of Applied Research and Technology 5(7):102-108.
Nwoko MC, Onyeizu UR, Chukunda FA, Ukoima HN (2017). Productivity, vitamins and heavy metals analysis of *Pleurotus ostreatus* (Jacq: Fri.) kumm. Fruit bodies cultivated on wood logs. International Journal of Information Research and Review 4:3890-3894.
Obodai M, Cleland-Okine J, Vowotor KA (2003). Comparative study on the growth and yield of Pleurotus ostreatus mushroom on different lignocellulosic by-products. Journal of Industrial Microbiology and Biotechnology, 30(3), 146-149.
Obodai M, Odamtten GT (2012). Mycobiota and some physical and organic composition of agricultural wastes used in the cultivation of the mushroom Volvariella volvacea.
Ogbo EM, Okhuoya JA (2009). Effect of crude oil contamination on the yield and chemical composition of *Pleurotus tuber-regium* (Fr.) Singer. African Journal Food Science 3(11):323-327.
Ogundana SK, Fagade OE (1982). Nutritive value of some Nigerian edible mushroom. Journal Food Chemistry 8:263-268.
Ojeniyi SO (1990). Effect of bush burning and tillage method on soil physical and chemical properties of humid tropical alfisols. Soil Tillage 15:2769-2777.
Okhuoya JA, Okogbo FO (2009). Culyivation of *Pleurotus tuber-regium* (Fr.) Sing. On various farm waste product. Academic Science 71:1-3.
Okoi AI, Iboh CI (2015). The effects of different substrates on sporophore yield, mineral and nutrient composition of *Pleurotus tuber-regium* fries singer in Calabar, Nigeria. International Journal of Agricultural Science Research 4(6):126-131.
Okwulehie IC, Nwoko MC, Achufusi JN, Onyeizu UR, Ezera VN (2018). Yield and Some Macro-Morphological Characters of *Pleurotus pulmonarius* (Fries) Quel. Fruit Bodies Cultivated on HCl-Optimized Oil Palm Bunch Substrate. Journal of Environmental and Analytical Toxicology 8:538.
Okwulehie IC, Okwujiako IA (2008). The use of local Nigerian substrates for the production of *Pleurotus ostraetua*varflorida(Eger) sporophores. Global Science Books 2(2):38-40.
Okwulehie IC, Okwujiako IA, Igbojionu V (2006). Studies on nutritional requirements and growth of *Pleurotus pulmonarius* (Fries) Quelet, an exotic mushroom. Nigerian Journal of Botanical 19(2):308-316.
Osemwegie OO, Dania AT (2016). Production of Mushrooms for Agro-income. Annals of Science and Technology 1(1):19-27.
Rip S (2010). Pf substrate formula's biological efficiency. RipSnort mushroom cultivation catalogue P 34.
Saker NC, Hassain MM, Sultana N, Mian IH, Karim AJ, Amin SM (2007). Effect of different levels of pH on the growth and yield of *Pleurotus ostreatus* (Jacquin ex. Fr.) P. kummer. Bangladesh. Journal Mush 1(1):57-62.
Shah ZA, Ashraf M, Ishtiaq CHM (2004). Comparative Study on Cultivation and Yield Performance of Oyster Mushrooms *Pleurotusostraetus* on Different Substrates (Wheat Straw Leaves and saw dust). Experientia 25:1237-1238.
Sharad CM (2013). Productivity and proximate conten of plenteous sajor-caju. Bioscience Discovery 4(4):169-172.
Sharma BB (2003). Effects of different substrate (grain straws) on spawn growth and yield of Pink oyster mushroom, *Pleurotus djamour*(Fr) Boediji. Journal of Mycology and Plant Pathology 33(2):265-268.
Shyam SP, Syed AA, Sureh MT, Mirza MV (2010). The nutritional value of *Pleurotus ostreatus* (Jacq: Fr) Kumm. Cultivated on different lignocellulosic Agro-waste. Yesh want machavidya, Nanded, pp. 431-602.
Stamets PE (2000). Techniques for the cultivation of the medicinal mushroom royal sun *Agaricus-Agaricus blazei* Murr. (Agaricomycetideae). International Journal of Medicinal Mushrooms 2(2):151-160.
Stihi C, Radulescu C, Busuioc G, Popescu IV, Gheboianu A, Ene A (2011). Studies on accumulation of heavy metals from substrate to edible wild mushrooms. Romanian Journal of Physics 56(1-2):257-264.
Sudheep NM, Sridhar KR (2014). Nutritional composition of two wild mushrooms consumed by the tribals of the Western Ghats of India. Mycology 5(2):64-72.
Werner AR, Beelman RB (2002). Growing high-selenium edible and medicinal button mushrooms (*Agaricus bisporus* (J. Lge) Imbach) as ingredients for functional foods or dietary supplements. International Journal of Medicinal Mushrooms 4(2):20-25.
Yang JH, Lin HC, Mau JL (2002). Antioxidant properties of several commercial mushrooms. Food Chemistry 77(2):229-235.