Research Paper
Nwoko et al., V-9; I (1): 1-21 (2021)

Corresponding author
magnuswhitemush@gmail.com

DARSGAH-E-AHLEBAIT

Journal of biotechnological sciences (ISSN: 2308-0043)

Influence of pH on the Fructification productivity and Mycochemical compositions of *Pleurotus ostreatus* (Jacq: Fr) Kumm. sporophores cultivated on HCl-induced substrate

Nwoko, M. C[1]., Okwulehie I.C[2]., Achufusi, J.N[3] and Ehumadu, C. R[2]

[1]Department of Biology, Federal University of Technology, Owerri, Imo State, Nigeria.
[2]Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike Abia State, Nigeria.
[3]Department of Biology, Nwafor-Orizu College of Education, Nsugbe, Anambra State, Nigeria

ABSTRACT

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 frutification and some mycochemicals 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 48hrs, 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 – 0.5% reduced substrate contamination due to *Coprinus cinerius* and enhanced fruit body number, yield and biological efficiency (BE%) as well as primordia 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 golden opportunity and ensure effective utilization of OPB incorporated with HCl acid for higher fruit body production as well as profit maximization.

KEY WORDS: HCL, *Pleurotus ostreatus*, *Fruitbodies*, OPB

INTRODUCTION

Mushrooms belong to the class Basidiomycota and order *Agaricales*. They do not possess chlorophyll like green plants; for manufacturing their food but for their growth and development, they require pre-formed food like smaller broken down molecules of lignin, cellulose and starch (Banjo, 1998). Chang (1999) defined mushroom as "a macro-fungus with 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 up by hand. Ideally, the word mushroom refers only to the fruit body of a macro-fungus. Most mushroom species are either under the Basidiomycota or Ascomycota; the two phyla are under the kingdom Fungi (Cho, 2004). *Pleurotus ostreatus* is the scientific name for Oyster mushroom. In many parts of India; it is known as Dhin (Elliott, 1991; Ogundana *et al*., 1982). It used to belong to the family Tricholomataceae, but now Pleurotaceae, which includes many species such as *P. flobellotus P. sojar - caju, P. eryngii, P. ostreatus, P. florida* and *P. sapidus*.

EFFECT OF pH ON MUSHROOM FRUIT BODY FORMATION

Fungal (mushroom) mycelia obtain nutrients from substrates at specific level of pH (Sarker *et al*., 2007), lime is used in cultivation of mushroom to enhance the pH of substrate. Rapid mycelia growth of mushroom (*Pleurotus sajor-caju*) takes place at pH 6.4-7.8 (Iqbal and Shah, 1989). Oyster mushroom (*Pleurotus* spp.) can grow and utilize various kinds of substrate materials than any other mushrooms (Cohen *et al.*, 2002). The increase in soil pH by oil palm bunch ash, oil palm bunch husk and wood ash could be due to the fact that they are liming materials. Liming has been reported to be important for soil physical and chemical properties and nutrient availability (Forth, 2004). 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 found in the works of Moyin-Jesu, (2007) and Ojeniyi, (1990) which reported that K and Ca components of wood ash were very high and this could be responsible for the increase in the soil pH which subsequently enhanced 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 & Okogbo, (2009) also reported that oil palm bunch fibres were good substrates for *Pleurotus tuber-regium.* The optimal temperatures for growth of the mycelium are around 25-28°C and the range of pH is about 5.5 to 6.5.
The tolerance of mycelia for CO_2 is rather strong. The mycelia of *Pleurotus* spp. can still grow flourish at the carbon dioxide concentration of 15 to 20%. Only when the concentration of CO_2 is raised to 30% does the growth of mycelia rapidly decrease (Chang & Miles, 2004).

MUSHROOM AND FOOD SECURITY

Human population expands 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). Mushroom along with yeast are referred to as alternative source of food (Chang, 1999; Anyankorah, 2002; James, 1995). According to James, (1995), edible mushrooms (dry) contain about 19-40% protein; that is, its protein content is twice that of vegetables and four times that of oranges, and they are rich in vitamins and minerals, low in unsaturated fatty acid and carbohydrate which makes it so ideal for diabetic and the obesed patients.

Most mushrooms have exceptional medicinal potentials and properties; curative and prophylactic, especially in diseases such as high blood pressure, asthma, respiratory tracts infection, anaemia, hepatitis, cancer, tumor, etc (Ogundana *et al*., 1982). Mushroom cultivation also serves as the most efficient and economically viable biotechnology for the conversion of long-cellulosic waste materials into high-quality protein food and this will naturally open up new job opportunities especially in rural areas and may be pre-package by food industries and exported to other countries as food and for revenue generation.

MATERIALS AND METHODS

STUDY AREA

Mushroom cultivation stage

This stage of the study was conducted at the screen house 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 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 mycelia culture 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 sorghum grains. *Sorghum* grains were washed in 3 changes of water and soaked over- night. The grains were boiled in tap water for 10-15minutes using gas cooker as a local heat source. Grains were also completely drained of water before mixing with 2% (w/w) $CaCo_3$ and 4% $CaSo_4$ to optimize pH and prevent them from clumping respectively as recommended by Muhammad *et al.,* (2007). They were subsequently packed in 2/3 in heat resistant transparent bottles, tightly sealed with Aluminum foil and sterilized in an autoclave at 121°C for 30minutes. After sterilization, bottles were allowed to cool before they were aseptically inoculated with actively growing mycelia of *P. ostreatus* by grain-to-grain transfer and incubated in the dark (at 27 ± 2°C) until grains were fully colonized by *P. ostreatus* mycelia (Shyam *et al.,* 2010).

DETERIMINATION OF SUBSTRATE pH

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

EXPERIMENTAL DESIGN

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 & Okwujiako, 2008). All inoculated substrates were placed on wooden rack in the culture room and covered during spawn run. Humidity of the

cropping room was optimized by constantly flooding with tap water. Inoculated substrate was regularly checked to ensure optimum moisture content prior to primordial initiation.

Plate 1: Young fruit bodies from Uncontaminated OPB

Plate 2: OPB contaminated by *C. cinerius*

MEASUREMENT OF MORPHOLOGICAL CHARACTERS

Stipe Sizes of Fruit Bodies

The effect of substrate on Pileus and stipe sizes of fruit bodies was determined at maturity. The mushrooms were harvested accordingly while Pileus and stipe sizes were measured in cm using meter rule (Okwulehie & Okwujako, 2008).

Cap Diameter

This was done by placing a transparent ruler across the center of the pileus and reading off the diameter.

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 & Okwujako, 2008).

YIELD AND BIOLOGICAL EFFICIENCY

During fruiting, mushroom fruit bodies were harvested at maturity, wet of fresh fruit bodies was determined using digital weighing balance while Biological efficiency (B.E) was determined using the method of Chang & Miles (2004) as;

$$\text{BE} = \frac{Fresh\ wt\ of\ mushroom}{dry\ wt\ of\ substrate} \times \frac{100}{1}$$

Proximate Analysis

Proximate Analysis was carried out on each of the 6 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 determined by Kjedahl method. Total nitrogen content was determined first and the value was multiplied by 0.25 coefficients (Maurizio *et*

al., 2005). 2g of dry powdered sample was digested in 5ml of concentrated sulphuric acid (H_2SO_4) and a tablet of selenium catalyst added in a fume cupboard, the digest was made up to 250ml with the acid. 10ml 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 was determined by placing 2g 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 7-8hrs (AOAC, 2000 & Konuk *et al.*, 2006). The oven-dried samples were maintained at constant weight. The percentage moisture content (PMC) was determined thus:

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

Determination of Ash Contents

Ash contents were determined by burning dried samples of the fruit bodies. 5g of the powdery samples of mushrooms were burnt at 500^{o}c overnight in a crucible. Crucible was allowed to cool and later weighed again (Mattila *et al.*, 2002; Oei, 2003). The percentage Ash content

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

Determination of Carbohydrate (CHO)

Carbohydrate contents were determined by difference i.e. (% CHO= 100 – (5 Ash + % protein + % fat + % moisture).

Determination of Ether Extract

Ether extract or Fats and oil content were estimated following the method of AOAC, (1980; 2000). 2g of each sample was inserted into an Ether extracting thimble and placed on the soxhlet reflex flask channeled into a round bottom flask of unknown weight. The apparatus was filled with 250ml of petroleum ether and placed on a heating mantle. The oil was extracted by a reflex system. After a repeated refluxing, a clear solution was obtained in the flask and the sample removed. Further heating was done to separating the ether from the extracted oil. Round-bottom flask containing the oil was dried in an oven at 70^{o}c, fats and oils 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.

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

Determination of Crude Fibre

Total crude fibre of the samples were determined by the Weende method (AOAC, 1980; 2000) 2g of each sample was inserted into a 250ml beaker and hydrolyzed by adding 20ml of dilute sulphuric acids and boiled for about 30mins on a hot plate. The mixture was filtered off through a piece of clean white nylon clothing and rinsed with hot distilled water. The residue was further boiled with 50ml of 2.5% sodium hydroxide (NaOH) for 30mins 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 determined and expresses as:

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

Determination of Vitamins

Vitamins were determined using spectrophotometric method, according to AOAC, (1980).

Determination of Vitamin A (Retinol)

The vitamin A content in each sample was determined by the method of Shyam *et al.*, (2010). About 5g of each sample was first homogenized using acetone solution and filtered off using Whatman filter No. 1. The filtrate was then extracted with petroleum spirit using separating funnel, two layers of both aqueous and solvent layer were obtained. The upper layer which contains vitamin A was washed with diluted water to remove residual water. It was later poured out to the volumetric flask through the tap of the separating funnel and made up to mark. The absorbance of the solution was read using a spectrophotometer at wave length of 450 nanometer (nm) and was calculated as:

Mg /g = A x vol x 104

= A x 12cm x sample weight.

Determination of Vitamin B_1 (Thiamin)

5g of each mushroom sample was homogenized with ethanol sodium hydrozide (50ml). It was filtered into a 100ml flask. 10ml of the filtrate was pipetted and the colour development read at the same time. Thiamin acid was used to get 100ppm and serial dilution of 0.0, 0.2, 0.6 and 0.8ppm was made. This was used to plot the calibration curve.

Determination of Vitamin B_3 (Niacin)

Niacin content was determined following konig spectrophotometric method. 0.5g of dry powdered sample of each mushroom was extracted with 50ml of INHCl in a shaking water bath kept at 30^oc for 35mins. The mixture was filtered using whatman filter paper. $KmnO_4$ (0.5g) was added to the filtrate and made up to mark. 10ml of the extracts was pipetted into a 50ml flax and 10ml of phosphate solution was added as buffer. The pH was adjusted with 5ml of INHCl and the solution was made up to mark with distilled water. After 15mins, the extract was read by spectrophotometry at 470nm wave length.

Determination of Vitamin C (Ascorbic Acid)

Vitamin C content of each sample was determined by the method of Kamman *et al.*, (1980). Five grams (5g) of each sample was homogenized in a 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 to remove the colour. The volume of the filtrate was adjusted to 100ml of water by washing with more of the extraction solution. 20ml of each filtrate was measured into a conical flask. 10mls of 2% potassium iodide solution was added to each of the flasks followed by 5mls of starch solution (indicator). The mixture was titrated against 0.01 mol $CuSO_4$ solution, titration of the

brink of the mixture; the vitamin C content was given by the relationship that 1ml of 0.01 mol CuSO4, 0.88n vitamin C. (Shyam *et al.*, 2010).

Therefore, vitamin mg/100g sample = $\frac{100}{Va}$ x vf x 0.88T.

where: Vf = volume of filtrate analyzed

Va = volume of acid analyzed

0.88T = constant.

Determination of Vitamin K (Phylloquinone).

Determination of vitamin K followed the method of Careri *et al*. (1996). Powdery mushroom sample of 1.0 g was weighed out, quantitatively transferred into a 40-ml brown glass flask and ultrasonically shaken with 10 ml methanol for 15 min the amount of material analyzed was increased to 5.0 g. The extract was centrifuged at 1000 g 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 the mixture was heated at 80 °C for 1 h. The hydrolysate was extracted by partition of the alkaline solution with 4 ml n-hexane on a Vortex for 1 min and centrifuged at 2000 g for 10 min; after the upper hexane layer had been carefully separated from the aqueous phase, two additional 4 ml n-hexane were 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 sm membrane.

Determination of Vitermin E (Tococpherol)

The estimation of Tococpherol was done by colorimeter method of Baker and Frank, (1949). The tocopherol is determined by Emmerie-Emmerie. Engel reaction which is based on the reduction by tocopherol of ferric to ferrous ions which is then form a red complex with α,α'Dipyridyl. Tocopherols and carotene are first extracted into xylene and the extinction 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 measure 1.5 ml serum, 1.5 standard, and 1.5 ml water (blank) respectively. Then in test and blank 1.5 ml. Xylene was added to all the tubes, stopper mixed well, and centrifuged, 1ml of the xylene layers was transferred in to other stopered tubes taking care not to include any ethanol or protein.1ml α,α'-Dipyridyl reagents was added to each tube was stoppered and mixed 1.5 ml of the mixture was pipetted into colorimeter cuvettes and extinction of test and standard was read against the blank at 460 nm. Then in turn beginning with the blank 0.33 ml ferric chloride Oxidative stress, lipoproteins in cardiovascular dysfunctions solution was added mixed and after exactly 1.5 min extinction test and standard was read against the blank at 520 nm. Tocopherol was calculated in mg/l by following formula-

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

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. About 2g of the sample was defatted with 100ml of diethyl ether, using soxhlet apparatus for 2 hours. To extract the phenols component of the sample, the fat-free sample was boiled with 50ml of either for 15mins. 5mls of the extract was Pipette into a 50ml flask into which 10ml of distilled water, 2ml of ammonium hydroxide (NH_4OH) solution and 5ml of concentrated amyl alcohol had been added.

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

The % Phenols were calculated as follows: $$\frac{100 \times Au \times C \times VF \times D}{W \times AS \times 1000 \times Va}$$

Where:

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

Determination of Tannins.

Tannins were determined according to the method of Okeke & Elekwa, (2003). 0.5g of the sample in 10ml of 2m HCl was shaken for 5mins and transferred into a volumetric flask and made up to 50ml. the mixture was filtered and 5ml of the filtrate was introduced into a test tube. 3ml of 0.1 NHCl and 3ml of 0.008m of potassium ferro-cynide ($K_3F[CN]_3$) were added. The absorbance was read at 720nm within 10mins.

Determination of Sterols

The crude fat analysis was carried out by the method of AOAC, (2006b). 250mlbextraction flask was dried in the oven at 105^0C, 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 labeled porous thimble; 200ml of the petroleum ether was then measured and added to the 250ml conical flask. The covered porous thimble with the sample was placed in the condenser of the soxhlet extractor. The sample was extracted for (5) five hours. 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 105^0C for one (1) hour. The flask containing the dried oil was cooled in the desiccator and the weight and the weight of the cooled flask and the dried oil was measured.

Determination of Alkaloids

For quantitative estimation of Alkaloids, the method of Maxwell *et al.*, (1995) was followed. The alkaloids were extracted from 20g of each of the dried powdered mushroom samples using 100ml of 10% acetic acid. The extracts were filtered to remove cellular debris and then concentrated to a quarter of the original volume. 1% NH_40 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 alkaloids 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 by 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 15minutes. 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 & Kobayashi, (1995). Equal parts of fehling's solution 1 and 11 (5ml) were added to 5ml of the dry mushroom sample. A brick red precipitate shows the presence of a reducing sugar.

Hyrolysis test.

About 5ml dilute sulphuric acid was added to about 0.1g of the mushroom extract and neutralized with 20% potassium hydroxide solution. About 10ml of a mixture of equal parts of fehling's solution 1 and 11 was added and boiled for minutes. A more dense brick red precipitate indicates the presence and amount of glycosides.

Determination of Minerals

Mineral compositions of dried mushroom samples were determined by 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 and 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) was estimated using flame photometer

Determination of Heavy Metals

The concentrations of Fe, Cu and Zn in the sample were determined by Energy Dispersive X-ray Fluorescence (EDXRF) technique according to the method of Stihi *et al.*, (2011) and Ghisa *et al*., (2008). Using the Elvax spectrometer having an x-ray tube with Rh anode, operated at 50kv and 100µA. Samples were excited for 300sec and the characteristic x-rays were detected by a multichannel spectrometer based on a solid state si-pin-diode x-ray detector with a 140µm Be- window and an energy solution of 200ev at 5.9 Kev. Elvax software was used to interpret the EDXRF spectra. The accuracy of the results as evaluated by measuring a certified reference sample good results were achieved between certified values and data obtained.

The concentration of Pb and Se in the sample was determined 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 absorber concentration.

Several standard solutions of different known concentrations were prepared and the elemental concentration in unknown sample was determined by extrapolation from the calibration curve. All sample concentrations were reported as mg/kg dry weight of material.

Statistical analysis

Data obtained were statistically analyzed using Analysis of Variance (ANOVA) mean separation and tests of significance were carried out by Duncan Multiple Range Test (DMRT) at $p<0.05$ (Steel & Torie, 1984).

RESULTS AND DISCUSSION

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

HCl OPB Levels	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 ($p\leq0.05$), means ±SEM (n=3).

pH variations in substrate and formation of mushroom fruit bodies

Result revealed that the naturally obtained OPB substrate that was neither soaked in water nor acid solution had a pH of 10.10. 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 hrs while control was found at a pH of 9.0. This variation also conforms to the work of Okwulehie *et al.*, (2018) which reported a direct proportionate increase in acidity of OPB substrate with with increased HCl solution after steeping for 48 hrs.

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). Result 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 & Verma, (1992), Okwulehie *et al.*, (2006), Okwulehie & Okwujiako, (2008) which maintained that culture media of pH between 6.0 and 8.0 recorded significantly greater mycelia extension than those above the range. In this investigation, the time for primordial initiation, apparently preceded by fruit body production was shorter compared to the result obtained by Shah *et al.*, (2004) which 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 & Miles (2004), Nwoko *et al* (2017), Okoi & Iboh (2015) and Hassan *et al* (2010).

Table 2: Morphological characters of fruit bodies

HCl OPB Level (%)	FBN	C.D (cm)	S.L (cm)	WT (g)
Control	**424^{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).

Morphological Characteristics of Fruit Bodies

Results of some of the morphological characteristics of fruit bodies revealed that 0.5% HCl OPB substrate produced the highest (705.00) number of fruit bodies. Result 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 was 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 implication of HCl. That could probably be due to high alkaline level of the substrate which does not support mycelia growth as well as fruit body production (Bilgrama & Verma, 1992; Okwulehie *et al.*, 2006; Okwulehie & Okwujiako, 2008). In This experiment, the mean cap diameter and weight of fruit bodies from all levels of HCl OPB substrates; including control was 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 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-wastes components, Okwulehie & Okwujiako, (2008) observed that *Pennisetum* straw significantly increased the stipe length of *P. ostreatus*, followed by *A. gayanus* straw, *Oryza* straw. They also noted that *Panicum* straw causes a reduction of the cap diameter. Okoi & Iboh, (2015) maintained that different substrates have effect on the pileus diameter, stipe length and stipe girth. Other factors can also affect the general size of a mushroom fruit body. Ogbo & Okhuoya, (2009) reported that crude oil has significant effect on the macro morphological characteristics such as pileus diameter, stipe height, stipe girth and fresh weight of mushroom fruit bodies. 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 (**Table 3)**, HCl did not significantly affect other morphological characters studied.

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).

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 to a significant yield increase as well as biological efficiency of the mushroom fruit bodies. As the lowest fruit body yield was recorded in 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 & Verma, (1992), Chang & Miles, (2004), Shah *et al.,* (2004), Okwulehie *et al.,* (2006) and Khan *et al.,* (2013) which reported that oyster mushrooms grow and perform well at pH near to neutral of slightly acidic at 6.1.

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 & Okwujiako, (2008), Sharad & Naigaon (2013), Okoi & 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 why Achufusi, (2016) could not record any fruit body production when attempted to grow *P. ostreatus* on the substrate, but instead observed heavy contamination by *Coprinus cinerius.* Although there was traces of contamination by *Coprinus cinerius* which was high in control, but reduced gradually until none was found in 0.4 and 0.5% HCl concentrations. This conforms to the report by Okwulehie *et al.,* (2018) which reported no contamination due to *Coprinus* spp. at 0.4% HCl acid-induced OPB substrate during cultivation of *P. pulmonarius.*

Table 4.4: Vitamins concentration (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 ($p \leq 0.05$), means ±SEM (n=3)

Vitamins Concentrated (mg/100g) of *P. ostreatus* fruit bodies

The results of the effect of HCl acid on the vitamins concentrations of *P .ostreatus* fruit bodies grown on OPB substrate are significantly ($p \leq 0.05$) 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, Okwuelehie & Okwujiako, (2008) in *P. ostreatus* cultivated on different substrates and substrates supplementations.

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 vitamins contents especially vitamin B_1, B_3 and C are in line with the report of Maltila *et al.,* (2004), Shibata & 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 vitamins concentrate of the mushroom, compared to the control and suggests no possible health risk when consumed by humans. Similarly Nwoko *et al.,* (2017) and Bobek & Galbary, (2001) stated that the recommended dietary intake (RDI) of vitamins such as Retinol or Vitamin A is 200mg; an indication that these mushroom samples meet the nutrient requirement by man for a healthy living.

Table 4.5: Bioactive (%) compounds 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).

Bioactive compounds concentration of the fruit bodies

Results showed the effect of HCl acid on the bioactive compounds composition of *P. ostreatus* fruit bodies cultivated 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 powerful effect in animal physiology and are important in pharmaceutical industries, for drug manufacturing (Edeoga & Erieta, 2001). Edeoga & Erieta (2001) also recorded that alkaloids are stimulants and acts by prolonging the action of several hormones. Phenolics, tannins, alkaloids and terpenoides concentrations in all fruit body samples were higher than those reported by Okwulehie *et al.*, (2007). Flavonoids act as anti-carcinogens, anti-bactarials (Hilang & Feraro, 1992); saponins are implicated in the prevention of parasitic fungal diseases (Edeoga & Erieta, 2001) while tannins have been used as anti- tumor agents and perform a wide range of anti-infective actions (Haslam, 1996). The high concentrations of these important bioactive compounds in fruit bodies from various percentage HCl-OPB substrates shows that these mushroom samples may be considered useful in the production of certain pharmaceutical chemicals (Okwulehie *et al.,* 2007).

The obtained values were significantly higher than those reported by Onyeizu *et al.,* (2017) and Okwulehie *et al.,* (2009) in experiments involving *Pleurotus 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 & Miles, (2004) which asserts 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 – 0.5% HCl concentration, there seems to be a gradual increase in the quantity of Tannins from 101.06±2.09 – 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 of grazing on plants by herbivorous (Okwulehie *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).

Table 4.6: Proximate Composition (%) of fruit bodies

HCLOPBLevel(%)	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).

Proximate composition of fruit bodies

Proximate composition of fruit bodies of *P. ostreatus* showed that there was 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), Patil *et al.,* (2008), Syed *et al.,* (2009), Filipa *et al.,* (2011) and Okoi & Iboh, (2015). Hydrochloric acid appeared to have 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% HCl OPB substrate. These are eventually higher than the values obtained by Okwulehie *et al.,* (2008), Okoi & Iboh (2015) and filipa *et al.,* (2011). Chang & Miles (2004) maintained that the high CHO contents of mushrooms is due to the high lignacellulosic compositions in the substrate where they grow; which mushrooms were able to break down using extra cellular enzymes.

The appreciable amounts of CF protein and CHO in *P. ostreatus* fruit bodies as generally observed in this study has been attributed to the nature of substrate and to a large extent, mushroom species (Nwoko *et al*., 2016). This further substantiates the claims by Obodai, (2003), Adejoye & Fasidi, (2009) and Okoi & Iboh, (2015) which in separate experiments noted that the nutritional composition of mushrooms could reflect the chemical composition of the substrate used, as mushrooms are able to carry out extra-cellular digestion of the decomposed substrate during cultivation.

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.

Table 4.7: Minerals concentration 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 ($p \leq 0.05$), means ±SEM (n=3).

Minerals concentration 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.

The concentration of Potassium was highest (5.07±0.46) in fruit bodies from 0.5%HCl while control (4.13±0.10mg/100g) was the lowest. Although there was an irregular trend on the concentration of all the studied minerals with respect to percentage HCl, but 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 to the report of Egwin *et al.,* (2011) which 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 & Duncan, (2002) which 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. Naga & Kandikere, (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, control is either equal or higher than other treatment groups. This therefore suggests that the fruit bodies could be safe for human consumption.

Table 4.8: Heavy metals concentration 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 ($p \leq 0.05$), means ±SEM (n=3).

Heavy metals concentration of fruit bodies

Results showed that increased in the concentration of HCl acid tends to increase the amount of Zinc in the fruit bodies. 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 (Obodai, 2003; Adejoye & Fasidi, 2009; Okoi & Iboh, 2015). Stihi *et al.*, (2011), Das (2005), 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 compered to those reported by Demirbas, (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, dichlorodiphenyltrichloethane (DDT), trinitrotoluene and synthetic dyes (Eggen &Vaclav, 2002).

Selenium concentrations in 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 & Beelman, (2002) noted that many mushrooms are now being fortified with genes capable of synthesizing 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 were similar to those reported by Stihi, (2011), Nwoko *et al.*, (2017) and Demirbas, (2001).

Heavy metals concentrations obtained in this investigation are relative to 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 admitted maximum level for Pb and Cd is set about 2 and 3 mg/1kg d.w, in cultivated mushrooms. Apart from Pb, other heavy metals studied in this research are of high nutritional importance. Although HCl slightly excited 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

Experiment was successfully conducted to evaluate the influence of pH on the fruiting duration, some macro-morphological characters and productivity of *Pleurotus ostreatus* fruit bodies cultivated on acid-induced oil palm bunch substrate.
Hydrochloric acid induced changes on the pH of the substrate towards acidity i.e. 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 *Coprinus 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 ($p \geq .0.05$) 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 ($p \geq .0.05$) 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 studies 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 researches 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 without much stress involved in acid dilution and pH optimization.

REFERENCES

Achufusi, J. N. (2016). *Yield, heavy metals, nutritional evaluation, amino acid profile and lignocellulosic evaluation of Pleurotus ostreatus var florida (eger) fruit bodies cultivatedon three supplemented palm substrates in Umudike, Abia State. An unpublished Ph.D. Dissertation* of the Dept. of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Abia State. Nigeria. p40.

Anyankorah, C. H. (2002). Cultivation of edible mushroom. *African Journal of Biotectnology*. 30:399-401.

Bano, k., Kale, R. D., & Satyavathi, G. P. (1998). Vermicompost as fertilizer for ornamental plants. *Proceeding of IV National Symposum on Soil Biology and Ecology* in: D. Rajagopal., R.D. Kale and K. Bano. (eds): pp 165 – 168.

Bilgrama, K. S. & Verma, R. N. (1992). *Physiology of fungi*. Villkas publishing house, Hvt Ltd, London. Pp7

Chang S. T. 1999. World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on Lentinus edodes (Berk.) Sing. in China. *Intl. Journal of Med. Mushrooms*, 1:291-300.

Chang, S.T. & Miles, P.G. (2004). *Mushroom cultivation, nutritional value, medicinal effect and Environmental impact.* 2nd (ed), CRC p.451

Cho, S. B. (2004). *What Is Mushroom?* Mushworld, Korea Heineart Inc. pp 1-3;8 -14.

Cohen, R., Jensen, K.A., Houtman, C.J. & Hammel, K.E. (2002). Significant levels of extracellular reactive oxygen species produced by brown rot basidiomycetes on cellulose. *FEBS Lett* 531: 483–488.

Elliott, T. J. (1982). *Genetics and breeding of cultivated mushrooms*. *In "Tropical Mushrooms"* edited by S. T. Chang & T. H. Quimio. The Chinese University Press, Hong Kong..

Igbal, M. & Shah, A. A. (1989). Effect of $CaCO_3$ on substrate of *Pleurotus sajor-caju. Journal of Agric.*, **5:** 359 – 361.

James, L.F & Panter, K.E. (1995). Swainsonine-induced high mountain disease in calves. *Vet. Hum. Toxicol.*, 33:217-219.

Kang, H., Hwang, S., Lee, H. & Park, K.W. (2006). Effect of high concentration of plant oils and fatty acids for mycelia growth and pin head formation of *Hericium eriaceum*. *Transactions of the ASAE.*, **45(1):** 257-260.

Khan, H.A., Alhomida, A.S., Sobki, S.H., Syed, S.H., Zohair, A.A., Adnan, A.K. & Abdulrahman, A.M. (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, N. A., Khan, M. A., 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. Pak. Journal of Bot.*, **45(1):** 297-302.

Moyin-Jesu, E. I. (2007). Use of plant residue for improving soil fertility pod nutrients, root growth and pod weight of Okra *Abelmoschus esculentus* L. *Bioresour. Tech.,* 98:2057-2064.

Muhammad, A.A., Muhammad, S., Shabbier, A. & Asif, H.M. (2007). Protein and fat contents of various *Pleurotus species* raised on different waste materials. *Pak. Journal Agric Sci.,* **44(3):** 140 – 143.

Nwoko, M. C., Onyeizu, U. R., Chukunda, F. A. & Ukoima, H. N. (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.

Ogbo, E.M. & Okhuoya, J.A. (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, S. K. & Fagade, O. E. (1982). Nutritive value of some Nigerian edible mushroom. *Journal Food Chem.,* 8:263-2 268.

Ojeniyi, S.O. (1990). Effect of bush burning and tillage method on soil physical and chemical properties of humid tropical alfisols. *Soil Tillage,* 15, 2769-2777.

Okhuoya, J. A. & Okogbo, F. O. (2009). Culyivation of *Pleurotus tuber-regium* (Fr.) Sing. On various farm waste product. *Acad. Sci.:* **71:**1-3.

Okoi, A.I & Iboh, C. I. (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, I. C. & Okwujako, I. A. (2008). The use of local Nigerian substrates for the production of *Pleurotus ostraetua* var florida (Eger) sporophores. *Global Sci. books.* **2(2):** 38 – 40.

Okwulehie, I.C., Nwoko, M.C., Achufusi, J. N, Onyeizu, U.R & Ezera, V.N. (2018) Yield and Some Macro-Morphological Characters of *Pleurotus pulmonarius* (Fries) Quel. Fruit Bodies Cultivated on HCl-Optimized Oil Palm Bunch Substrate. *Journal of Environ. Anal. Toxicol* 8: 538. doi: 10.4172/2161-0525.1000538

Okwulehie, I.C., Okwujiako, I. A. & 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.

Patil, S. S., Sharad, S. M. & Naigoan, D. N. (2012). Cultivation of oyster mushroom *(P. ostreatus)* on sawdust. *Journal of Sci.and BioSci.* 37(2): 177 – 182.

Rip. S. (2010). Pf substrate formula's biological ffficiency. RipSnort mushroom cultivation catalog. P34.

Saker, N. C., Hassain, M. M., Sultana, N., Mian, I. H., Karim, A. J. M. S. & Amin, S. M. R. (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, Z. A., Ashraf, M., & Ishtiaq C. H. M. (2004). Comparative Study on Cultivation and Yield Performance of Oyster Mushrooms *Pleurotus ostraetus* on Different Substrates (Wheat Straw Leaves and saw dust) *Experientia.* **25:** 1237 – 1238.

Sharad, C. M. (2013). Productivity & proximate conten of plenteous sajor-caju. *Bioscience Discovery*, 4.(4): 169-172.

Sharma, B. B. (2003). Effcets of different substrate (grain straws) on spawn growth and yield of Pink oyster mushroom, *Pleurotus djamour* (Fr) Boediji. *Journal mycol. Pl. Pathol.,* **33(2):** 265-268

Shyam, S.P., Syed, A. A., Sureh, M.T. & Mirza, M.V. (2010). The nutritional value of *Pleurotus ostreatus* (Jacq: Fr) Kumm. Cultivated on different lignocellulosic Agro-waste. Yesh want machavidya, Nanded. pp. 431-602.

Yang, J. H., Lin, H. C. & Mau, J. L. (2002). Antioxidant properties of several commercial mushrooms. *Food. Chem.,* **77:** 229 – 235.