African Scientist Vol. 24, No. 1 March 31, 2023
Printed in Nigeria

1595-6881/2023 $80.00 + 0.00

https://africansciientistjournal.org

AFS2023006/24112

The Growth of *Pleurotus ostreatus* Using Potato Dextrose Agar Supplemented with Waste Human Hair Broth (WHHB)

Obuekwe I.S.*and Odemwingie O.S.

Department of Microbiology, Faculty of Life Sciences, University of Benin, Edo State, Nigeria.

*Corresponding author Email: ifeyinwa.obuekwe@uniben.edu . Tel: +234 802 941 9741

(Received March 14, 2023; Accepted in revised form March 18, 2023)

ABSTRACT: Waste human hair causes environmental problems such as flooding, as a result of clogged drainages. It slowly breaks down over time to yield macro and trace minerals that are eventually returned to nature. *Pleurotus ostreatus* is cultivated worldwide, and it's known to grow on several carbon and nitrogen sources. This study assessed the growth of *P. ostreatus* using potato dextrose agar (PDA) supplemented with Waste Human Hair Broth (WHHB). The growth of *P. ostreatus* mycelium was studied for 15 days on PDA supplemented with different concentrations of WHHB (0, 25, 50, 75 and 100% v/v) which was obtained by modified hydrolysis technique. Subsequent *P. ostreatus* growth was analysed using 0 and 100% v/v WHHB in grain spawn for 5 days. The physicochemical parameters and nutrient content of WHHB was analysed using standard methods, and mycelia growth was measured using standard methods. WHHB had a pH of 7.20 ± 0.02, temperature of 25ºC and Protein content of 421.33 ± 3.06 mg/dL. Mycelia growth diameter and growth rate were significantly higher ($p < 0.05$) in the 100% v/v WHHB relative to the control (0% v/v WHHB) after 15 days, and mycelium in 100% v/v WHHB grew luxuriously during spawn production after 5 days relative to 0% v/v WHHB. The pH and temperature values reported are optimal for the growth of *P. ostreatus.* Higher growth in WHHB is attributed to protein, macro and micro nutrients availability in the broth. The presence of nutrients in WHHB allowed for improved mycelium growth in the 100% v/v WHHB.

Keywords: Waste human hair, *Pleurotus ostreatus*, PDA, Mycelia, Growth

Introduction

Hair fibre is the non-living part of hair that is trimmed from our scalps. Keratin is its major component and constitutes about 65%-95% of the hair weight, as well as others which include water, lipids, pigment and trace elements (Cruz et *al.*, 2016). In urban areas, hair waste is part of Municipal Solid Waste (MSW) generated from barbing salons, where it accumulates in barbershops and its often mixed with other waste materials like shampoo sachets, artificial colouring agents and plastics (Gupta, 2014). They eventually find their way into dumpsites where they sometimes remain for years because of their recalcitrant nature or leak into the drainage systems and cause blocking or clogging of drainage pipes ensued with flooding (Shruti *et al*., 2022). Furthermore, burning of human hair fibre or the waste piles containing them is practiced in many parts of the world (Gupta, 2014). This leads to the production/release of foul odour and toxic gases such as ammonia, carbonyl sulphides, hydrogen sulphides, phenols and pyridines (Gupta, 2014).

Keratin rich human hair waste do not breakdown easily and persist for a long time in the environment. However, in the presence of an alkali as well as heat and pressure, it is possible to break the bonds present in hair and make them tender (Shruti *et al*., 2022). Keratins in chicken feathers and other origins have been found to be soluble in calcium hydroxide solution (Singh and Prasad, 2019). Urea has also been discovered to disperse

keratins in solution with the aid of disulfide splitting agents such as sodium bisulfite or monothioglycol (Singh and Prasad, 2019). These treatments result in amino acid residues and polypetides that can be applied as liquid fertilizers which causes improved plant growth due to rapid release of nitrogen in the soil (Sobucki *et al*., 2019; Singh and Prasad, 2019). Indeed, macronutrients such as nitrogen, phosphorus and potassium in feather hydrolysate are similar to those found in waste human hair (Sobucki *et al*., 2019). These nutrients are essential for the growth of mushrooms (Tesfay *et al.,* 2020).

Pleurotus spp., commonly known as oyster mushrooms, are edible, basidiomycetous fungi cultivated as food worldwide especially in south East Asia, India, Europe and Africa (Mahadevan and Shanmugasundaram, 2018). They show good adaptability to a wide range of temperature, making it possible to grow this mushroom almost all year round without controlled climatic conditions (Besufekad *et al.,* 2020). They contain protein, amino acids, vitamins, crude fibre, lipids, sugars, glycogen and other bioactive compounds which are essential for normal functioning of the human body (Mahadevan and Shanmugasundaram, 2018). Oyster mushroom are the easiest and least expensive commercial mushrooms to grow because they are well known for conversion of crop residues to food protein (Tesfay *et al.,* 2020). The consumption of oyster mushrooms has many advantages which include prevention of different diseases such as diabetes, heart disease, high blood cholesterol level, gastric cancer, hepatitis B, liver illness, kidney problems and hypertension, (Tesfay *et al.,* 2020).

Oyster Mushroom is capable of utilising several substrate types such as organic wastes due to the secretion of hydrolysing and oxidizing enzymes in mycelium hyphae (Shruti *et al*., 2022). In addition, the use of low-cost agricultural by-products (feather flour, chicken manure, and cereal meals and brans) is a promising approach to oyster mushroom cultivation (Carrasco *et al.,* 2018). Oyster mushroom cultivation can play an important role in managing organic wastes like human hair waste because its disposal has become a problem globally. Innovative systems which utilize waste materials as resources can contribute immensely to economic growth and waste reduction. This work therefore, compared the growth of *Pleurotus ostreatus* in potato dextrose agar (PDA) using waste human hair broth (WHHB) or distilled water.

Materials and methods

Hair sample collection: Waste human hair (WHH) from male scalps were collected from a hair salon situated in Benin City, Edo State, Nigeria. A Large plastic sterile bin was used for hair sample collection.

Preparation of WHHB: The waste human hair samples (50 g) were washed and rinsed thoroughly with hexane (70% n-hexane) and methanol (99% methanol) (1:1) and subsequently rinsed with 70% (v/v) ethanol. The samples were allowed to air dry for 24 h to remove impurities (Singh and Prasad, 2019). Using a modified hydrolysis technique, 5 L of water, 10 g of calcium hydroxide $Ca(OH)_2$ and 50 g of waste human hair were added to an airtight pot (Stainless steel, 10 litre capacity). The pot was heated at 121°C for 60 min and allowed to cool slightly, before the resulting broth was filtered with a Whatman filter paper (Grade 42) (Sobucki *et al*., 2019).

Physicochemical analyses of waste human hair broth (WHHB): Five physicochemical parameters of WHHB were analysed using standard techniques. pH was determined with a pH (Ocean Star Technologies) meter after submerging the pH probe in the WHHB sample (Ullah *et al*, 2013). A thermometer (Ocean Star Technologies) was used to assess temperature by placing it in the WHHB for some minutes to achieve a stable reading. (Ullah *et al*, 2013). Electrical conductivity (EC) and Total Dissolved Solids (TDS) were determined using an EC/TDS meter (Mettler Toledo Instruments). EC/TDS meter was switched on and then introduced directly into the WHHB and the value for EC recorded while a shift of button to TDS recorded TDS values (Ullah *et al*, 2013). Turbidity was determined using turbidity meter (Newtry Laboratories) (Ullah *et al*, 2013).

Nutrient Analyses of WHHB: Nitrogen content was determined according to the Kjeldahl method while nitrogen value was multiplied by 6.25 as a conversion factor to yield the amount of protein present in WHHB (Bremner and Mulvaney, 1982; Weichselbaum, 1946). Fat determination was based on enzymatic hydrolysis and oxidation analysis which was done with semi-automatic RX monza analyzer (Richmond, 1973). Sulphur content was based on Gravimetric method (AOAC, 1992) while phosphorus was according to the Gravimetric Quinolinium Molybdophosphate method (AOAC, 1992)**.** Determination of Potassium, Manganese, Calcium, Copper, Iron, Magnesium and Zinc were done using Atomic Absorption Spectrophotometry (AAS). A 100 ml of the WHHB sample was acid digested with HCL (10 mL) and spectrophotometric reading for each element was subsequently done (AOAC, 1992)**.**

Mushroom species: Viable mycelium culture of *P. ostreatus* (Oyster mushroom) already grown in Petri dishes were obtained from Mycofarms and Allied Synergy Limited, Benin City, Edo State, Nigeria.

Culture media preparation: This was based on five different treatments, the first treatment (control media; 0 % v/v WHHB) had 7.8 g of PDA powder (Manufacturers composition) that was mixed in 200 mL of distilled

water. Second treatment (25% v/v WHHB) had 7.8 g of PDA powder in 50 mL of waste human hair broth and made up to 200 mL by adding distilled water. Third treatment (50% v/v WHHB) had 7.8 g of PDA powder in 100 mL of waste human hair broth and made up to 200 mL by adding distilled water. Fourth treatment (75% v/v WHHB) had 7.8 g of PDA powder in 150 mL of waste human hair broth and made up to 200 mL by adding distilled water. The fifth treatment (100% v/v WHHB group) had 7.8 g of PDA powder in 200 mL of waste human hair broth (Shruti *et al*., 2022). All treatment flasks were sterilized at 121°C at 15 psi for 30 min; they were then allowed to cool for 10 min and streptomycin was added at 1g/L. The different treatment flasks at 20 mL each were then poured in 90 mm Petri dishes and allowed to solidify (Shruti *et al*., 2022). All treatments were done in replicates.

Inoculation of P. ostreatus in culture media and mycelial measurement: A flamed spatula was used to cut 7 mm by 7 mm square plugs into mycelia colonised agar and used to inoculate the freshly prepared agar of the different treatments. The plates were then incubated at 30^{o}C and radial growth of mycelium was measured at every 3 days intervals for 15 days (Shruti *et al*., 2022). All treatments were done in triplicates.

Mycelial growth (radial measurement) was done every 3 days using a metre rule across the Petri dish horizontally for 15 days (Mahadevan and Shanmugasundaram, 2018) and growth rate calculated by dividing the colony diameter on the last day (cm) of measurement by number of days of growth. Mycelial density was also measured visually and rated thus:

+ = Very Scanty mycelial density
2+ = Scanty mycelial density
3+ = Moderate mycelial density
4+ = Abundant mycelial density and
5+ = Very abundant mycelial density (Mahadevan and Shanmugasundaram, 2018).

Spawn production: Guinea corn was cleaned manually to remove inert matter, stubble and debris. Two (2) kg of the cleaned grains were soaked in 0.5% $CuSO_4$ for 10 min, thoroughly washed twice with 10 L of tap water and soaked in 6 L of tap water for 2 h. Thereafter, the grains were drained and the following additives added: rice bran at the rate of 10% and chalk ($CaCO_3$) at the rate of 2% on dry weight basis of the grains. The additives were thoroughly and evenly mixed with the grains. The grain medium was filled halfway into 500 mL glass bottles. A stopper of cotton wool and foil paper was used to plug the mouth of the bottles and covered by a piece of foil paper by tying a rubber band around the neck. The bottles (6) containing the grains and additives were autoclaved at 121°C at 15 psi for 30 min after which they were allowed to cool for 24 h. The first set of bottles (3) were inoculated with mycelial culture of *P. ostreatus* maintained on PDA exclusively (Control 0% v/v WHHB), and the second set of bottles (3) were inoculated with mycelial culture of *P. ostreatus* maintained on 100% v/v WHHB-supplemented PDA (Tesfay *et al.,* 2020). Mycelial growth was then observed for 5 days in inoculated bottles.

Data analyses: All experiments were performed in replicates and values were reported as standard deviations. Differences in growth were analysed using IBM SPSS statistics package 20 (SPSS Inc. and IBM Company, Chicago, USA) where $P < 0.05$ implies significant difference between values obtained.

Results

Growth of *P. ostreatus* was assessed on PDA supplemented with different concentrations of WHHB. Some physicochemical parameters of WHHB were analysed and shown in Table 1. The broth (WHHB) was slightly basic with a temperature of 25 °C, and had low electrical conductivity and Total dissolved solids (TDS).

Table 1: Physicochemical parameters of hydrolysed WHHB

Parameters	Units	Values
pH		7.20±0.02
Temperature	°C	25.03±0.06
Electrical Conductivity (EC)	µs/cm	408.00±0.00
Total Dissolved Solid (TDS)	Ppm	272.00±1.73
Turbidity	NTU	23.00±0.10

Nutrients obtained from the WHHB is shown in Table 2. They include both macro and micro nutrients

Table 2: Nutrients content of the hydrolyzed WHHB

Parameters	Values (mg/l)
Protein (mg/dl)*	421.33±3.06
Fat (mg/dl)*	25.13±0.03
Nitrogen (N)*	27.00±0.03
Sulphur (S)*	12.94±0.54
Phosphorus (P)*	7.86±0.67
Potassium (K)*	21.67±0.00
Manganese (Mn)^	3.41±0.45
Calcium (Ca)^	21.26±0.61
Copper (Cu)^	0.67±0.02
Iron (Fe)^	9.30±0.55
Magnesium (Mg)*	17.93±0.64
Zinc (Zn)^	2.61±0.06

*****Indicates macro nutrients, ^ Indicates micro nutrients

Mycelia growth diameter (mm) in the different treatments at days 0, 3, 6, 9, 12 and 15 is shown in Table 3. Mycelia growth was consistently higher ($p < 0.05$) in 100% (v/v) WHHB relative to the control and other treatments but was least in 50% v/v WHHB.

Table 3: Mycelia growth of *P. ostreatus* in the different treatments for 15 days

Culture Medium	Mycelium growth diameter (mm)					
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
0% WHHB	7.00±0.42	16.00±0.82	30.00±0.82	49.00±0.82	68.00±0.47	88.00±0.82
25% WHHB	7.00±0.93	8.67±0.47	21.00±0.82	33.33±0.47	49.00±0.82	64.33±1.70
50% WHHB	7.00±0.39	0.33±0.47	1.00±0.82	2.33±0.47	11.00±4.97	23.00±5.89
75% WHHB	7.00±0.68	17.00±1.63	29.00±2.45	42.00±1.63	59.33±0.47	74.00±0.82
100% WHHB	7.00±0.82	19.33±0.47	39.00±0.82	54.00±0.82	80.67±1.25	99.00±0.82

Key: WHHB - Waste Human Hair Broth, Trts: Treatments. Each value is expressed as a mean + SD (n = 3).

Mycelial growth rate was also assessed in all treatments (Table 4) and was faster ($p < 0.05$) in the 100% v/v WHHB relative to the control and other treatments but was least in 50% v/v WHHB. Mycelia growth was also denser in 100% v/v WHHB.

Table 4: Mycelial growth rate and density of *P. ostreatus* in the different treatments for 15 days

Culture medium	Mean colony diameter (mm)	Mycelial growth rate (mm/day)	Mycelial density
0% WHHB	88.00±0.82	5.86±0.04	+4
25% WHHB	64.33±1.70	4.4±0.00	+5
50% WHHB	23.00±5.89	1.53±0.32	+2
75 % WHHB	74.00±0.82	4.93±0.05	+5
100% WHHB	99.00±0.82	6.60±0.04	+5

Key:
+ = Very scanty mycelial density
+2 = Scanty mycelial density
+3 = Moderate mycelial density
+4 = Abundant mycelial density
+5 = Very abundant mycelial density

Plate I is a visual presentation of all treatments after 15 days of *P. ostreatus* growth

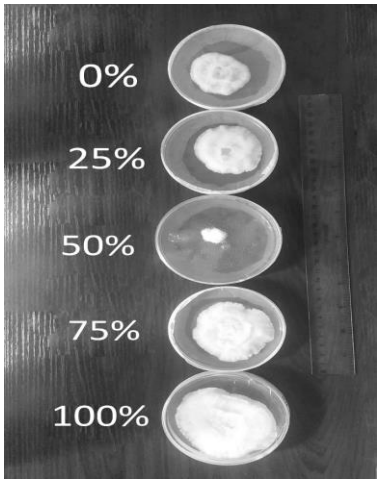

Plate 1: Mycelial growth of *P. ostreatus* in different treatments after 15 days

During spawn production, *P. ostreatus* mycelium in 100% v/v WHHB grew luxuriously on guinea corn after 5 days when compared to mycelium from the 0% (v/v) WHHB treatment (Plate 2).

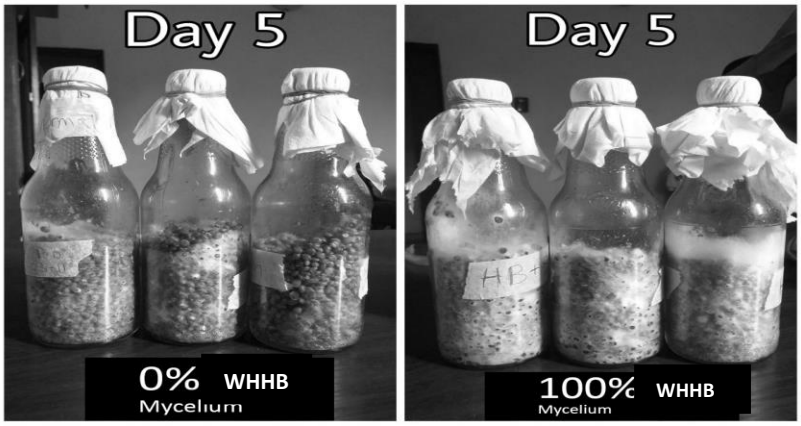

Plate 2: Grain spawn with mycelium in 0% and 100% WHHB on Day 5.

Discussion

The physicochemical properties and nutrient content of WHHB was determined, and the impact of different concentrations of WHHB on *P. ostreatus* grown in PDA was assessed over a period of 15 days. Growth rate was based on mycelia colony diameter, growth rate and density after 15 days of observation. Growth was also observed in grain spawn from 0% v/v and 100% v/v WHHB treatments after a period of 5 days. Generally, fungal growth is affected by different physicochemical characteristics such as pH and temperature of the growth media (Shruti *et al*, 2022). pH and temperature have been reported to affect the growth of fungi generally and in particular *Pleurotus spp*. (Shruti *et al*, 2022). The WHHB used for this study was slightly basic with a temperature of 25ºC. These have been reported as optimal growth pH and temperature for *Pleurotus* spp (Khan *et al*., 2013; Kumar *et al*., 2020; Shruti *et al*., 2022). For example, Khan *et al*. (2013) observed that *P. ostreatus* grew favourably at a slightly alkaline pH of 7.8. Similarly, *P. ostreatus* grew maximally in PDA at pH of 7 while minimal growth was observed at pH of 9 (Kumar *et al.* 2020). Conversely, Shruti *et al.* (2022) observed increased mycelial growth at pH of 5 relative to 5.5 and 6.5 for *P. eryngii* (King Oyster Mushroom). The growth of *P. ostreatus* at room temperature (25℃) has been reported by other authors (Kumar *et al*., 2020; Shruti *et al*., 2022). *P. ostreatus* grew maximally when incubated at 25℃ in PDA (Kumar *et al.*, 2020). Again, fast mycelial growth of *Pleurotus eryngii* was seen in Potato Palm sugar media (PPSM) at 25 ℃ (Shruti *et al*., 2022). The moderate EC value obtained from WHHB highlight the moderate presence of ions like magnesium, potassium and iron in WHHB solution (Nurdiawati *et al.*, 2019). Human hair is an organic compound and is likely to yield

a solution of organic constitution and very little free ions. The high turbidity and distinctive amber colouration of WHHB could be as a result of the presence of colloidal particles (Ullah *et al*, 2013).

Generally, *P. ostreatus* Mushrooms derive their food from the substrates (macro- and micro-nutrients) on which they grow since different substrates contain varying biological and chemical components (Onyeka *et al*., 2018; Graeme and Nia, 2018). The presence of macronutrients such as nitrogen, potassium phosphorus and magnesium in WHHB is in agreement with the work of Unnikrishnan and Ramasamy, (2020) were similar nitrogen, phosphorus and magnesium values were observed in alkaline hydrolysis of waste human hair. Again, Srivastava and Bano, (1970) observed increased mycelium growth of *P. flabellatus* due to the presence of potassium, phosphorus, and magnesium in modified potato dextrose broth. Further, the presence of protein residues, potassium, sulphur and magnesium in hydrolysed waste human hair caused vigorous growth in Okra leaves when foliar spray was applied (Menon *et al.*, 2020). The crude protein in WHHB may have supplied the nitrogen needed for *P. ostreatus* growth. This is similar to the work carried out by Nurdiawati *et al.,* (2019) where crude protein (12.96%) from hydrolysed chicken feathers successfully supplemented the growth of mung bean (*Vigna radiata*) relative to NPK.

The PDA supplemented exclusively with (100% v/v WHHB) had the higher mycelia growth diameter relative to the non-supplemented group (0% v/v WHHB) while 50 % v/v WHHB had the least mycelium growth. The lower mycelium growth could be as a result of the concept of minimum effective dose (MED), at a specific lower dose, the WHHB could hamper the growth of the mycelium. But as dosage is increased, the WHHB becomes useful and spurs growth in *Pleurotus ostreatus* (Mohamadhasani and Rahimi, 2022). The high mycelia growth observed in 100% v/v WHHB could be as a result of the presence of nitrogen from the amino acid residues/protein in WHHB (Nurdiawati *et al.*, 2019). Chioza and Ohga, (2013) reported that the optimal growth of *Paecilomyces hepiali* in agar with organic nitrogen sources (peptone and beef extract) was because they supplied nitrogen to the fungi since *P. hepiali* growth was limited in media without nitrogen supplementation. Again, *Cordyceps sinensis* grew very well in broth solution containing (0.001g/L) peptone (Dong and Yao, 2005). Furthermore, mycelial growth rate day^{-1} (6.60) was again higher in the 100% v/v WHHB as compared to that of 0% v/v WHHB (5.89). This finding is similar to that of Chioza and Ohga, (2013), where *P. hepiali* grew faster on rice-based agar supplemented with either peptone or beef extract than on non-supplemented rice- based agar. Wiriya *et al.* (2014) also observed increased growth of *Termitomyces clypeatus* when supplemented with peptone (0.0016g/L), as well as favourable mycelial growth of *Ophiocordyceps longissima* in different nitrogen sources (yeast extract, peptone and tryptone) as reported by Sung *et al.* (2011). Again, mycelium from the 0% v/v and 100% v/v WHHB groups were used to inoculate grain spawn (guinea corn seeds) and were observed after 5 days. Interestingly, 100% v/v WHHB mycelium covered more grain in a shorter time than the 0% v/v WHHB treatment. It was observed that mycelium from the 100% v/v WHHB colonised the sides of the bottle while mycelium from the 0% v/v WHHB had only colonised most of the grain on day 5. This may be as a result of higher macro and micro nutrients availability in the 100% v/v WHHB that made mycelium grow better in the treatment. Chioza and Ohga, (2013) attributed the fast growth and high mushroom yield of *P. hepiali* from peptone and beef extract supplemented agar to the nitrogen content of the extracts. The findings in this work is in keeping with the suggestion of Stamets, (2000) who pointed out the need for versatility of growth media for fungal mycelia in order to retain its innate ability to digest more complex substrates (Stamets, 2000). In summary, the availability of various agar media recipes such as WHHB for *P. ostreatus* growth is beneficial to the development of the species globally.

Conclusion

Luxuriant growth of *P. ostreatus* (oyster mushroom) mycelium was observed in 100% WHHB relative to 0% WHHB as a result of the presence of macro and micro nutrients (nitrogen, phosphorous potassium, zinc and magnesium) in WHHB. Again, mycelia from the 100% v/v WHHB outperformed that from 0% v/v WHHB on grain spawn. The composition of solid growth agar media recipes could determine the extent of fungal growth. Conclusively, the growth of *P. ostreatus* in PDA supplemented with WHHB was enhanced, and this adds to the variety of mycelia growth recipes currently available across the world.

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