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## THE EFFECT OF DIFFERENT SUBSTRATES FOUND IN ZIMBABWE ON THE GROWTH AND YIELD OF OYSTER MUSHROOM *PLEUROTUS OSTREATUS*

G. Zhou<sup>6</sup> and W. Parawira

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### Abstract

The research was carried out to investigate the effect of different substrates on the growth and yield of *P. ostreatus*. Locally available agricultural wastes such as saw dust (S1), cotton waste (S2), wheat straw (S3) and corncob (S4) were tested for parameters such as days required for spawn run, primordial formation, harvest days, total yield and biological efficiency. Biological Efficiency (BE) was calculated as the ratio of fresh fruiting body weight (g) per dry weight of substrates (g), expressed as a percentage. Before substrates were used in this study they were subjected to nutritional (C, N, P, K, Ca, Mg and Zn) analysis. The highest yield of 1275.45 g was obtained in saw dust and the lowest yield of 1058.7 g was obtained in cotton waste. The highest carbon to nitrogen (C/N) ratio was found in saw dust (53:0.1) and the least C/N ratio was found in cotton waste (39:1). There were 19 spawn run days in saw dust and 24 spawn run days in cotton waste. Stem width (2.6 cm) and cap diameter (9.7 cm) were greatest in cotton waste and low in saw dust with stem width (2.3 cm) and cap diameter (7.4 cm). Substrates with a higher C/N ratio had the greatest yield and biological efficiency. The higher C/N ratio favoured mycelium growth and lower carbon to nitrogen ratio favored fruiting body growth. In this study saw dust had the highest C/N ratio and it had the greatest yield and low spawn run days yet cotton

waste had the least C/N ratio but its fruiting body measurements were very high. There was no significant difference at  $p \leq 0.05$  between wheat straw and corn cob in terms of growth parameters and yield as their C/N was significantly high at (44:1) and (49:1) respectively. The results signifies that apart from soya beans and maize stalk which were widely used by farmers as substrates of choice, saw dust, cotton waste, corn cob and wheat straw were good alternatives for the growth of *P. ostreatus* mushrooms. Saw dust was very good in the total yield obtained but cotton waste had the best quality of mushrooms with very big stipes and cap diameter. These locally available substrates in Zimbabwe were recommended for use by small scale farmers for sustainable production of oyster mushrooms as they produced good yields at low cost.

**Keywords:** Lignocellulosic, Substrates, Oyster mushroom, *Pleurotus ostreatus*, Growth, Yield, and Biological Efficiency.

### Introduction

Oyster mushroom (*Pleurotus species*) belongs to the family of Tricholomataceae and is the second widely cultivated mushroom worldwide following the *Agaricus bisporus* (Sanchez, 2010 and Kües *et al.*, 2000). *Pleurotus* species are popular and widely cultivated throughout the world mostly in Asia, America and Europe because of the simple,

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<sup>6</sup> Corresponding Author: G. Zhou  
Department of Biological Sciences,  
Bindura University of Science Education,  
P Bag 1020,  
Bindura, Zimbabwe.

low cost production technology used to produce them and their high biological efficiency (BE) (Mane *et al.*, 2007). The interest in oyster mushroom is also increasing largely due to its rich taste and nutritional composition of protein, minerals (P, Ca, Fe, K, and Na) and vitamin (thiamine, riboflavin, folic acid, and niacin) (Szabová *et al.*, 2013). Apart from food value, their medicinal value for diabetics and in cancer therapy has been emphasized (Shah *et al.*, 2004 and Zireva *et al.*, 2007). Several species of oyster mushrooms act as strong antioxidant while *Pleurotus ostreatus* possesses antitumor activity (Li and Chang, 2007). Oyster mushrooms can efficiently degrade agricultural wastes and they grow at a wide range of temperatures (Sanchez, 2010). In comparison to other edible mushrooms, *Pleurotus* species has a short growth time and their fruiting bodies are not often attacked by diseases and pests (Tesfaw *et al.*, 2015 and Baysal *et al.*, 2003). Small scale farmers in Zimbabwe produce oyster mushroom because it is cheap to grow and it uses agricultural wastes such as maize straw and corn cobs which are readily available since maize is a staple food crop (Chitamba, 2007).

In Zimbabwe, agricultural waste, if not utilized is either left to rot in the field or disposed through burning and thus constitute environmental hazards and hide out for pests. According to Mutema *et al* (2019) research findings showed that small scale producers specialises in oyster mushroom production and it accounted for 60% of total annual production, while large scale farmers specialise in white button mushroom of which 75% were commercialized (Chiroro, 2004). Due to the nutritional and medicinal importance of oyster mushrooms and its ability to utilize agricultural wastes there is need to upscale its production.

Oyster mushrooms require carbon, nitrogen and inorganic compounds as their nutritional sources. The main nutrients are less nitrogen and more carbon so materials containing cellulose, hemicellulose and lignin (i.e., rice and wheat straw, cotton seed hulls, sawdust, waste paper, leaves, and sugarcane residue among others) can be used as mushroom substrates (Chitamba, 2007). While oyster mushroom can grow on a wide variety of substrates, the yield and the quality

of oyster mushroom depend on the chemical and nutritional content of substrates (Badu *et al.*, 2011 and Patil *et al.*, 2010).

In Pakistan, it was discovered that sawdust was the best among others with respect to yield, quality and efficiency (Shah *et al.*, 2004). In separate studies it was also found that pinhead formation, fruiting body maturation and time of spawn running were earlier on mushrooms growing on sugarcane bagasse (Shah *et al.*, 2004). In Turkey, the shortest mycelium growing time, the shortest harvest time and total harvested amount were realized on soybean stalk, while the longest for harvesting and growing times for mycelium and the total lowest harvested amount were obtained with cotton stalk (Nunes *et al.*, 2012). In Nigeria, it was discovered that agricultural wastes produced higher yields as compared to other substrates like saw dust (Adedokun, 2014). According to Mutema *et al.*, (2019) mushroom producers in Zimbabwe used substrates such as maize stalk (53.3%), followed by Soya bean (43%), then wheat straw (36.7%). Other substrates included cotton hulls (10%) and banana leaves (6%) and saw dust (3%).

Information such as the nutritional composition of substrates, shortest mycelium growing time, the shortest harvest time and total harvested amount of mushrooms per each substrate used in mushroom production and the effects of different locally available substrates on the growth and yield of *P. ostreatus* is lackings yet vital if oyster mushrooms were to be produced at an industrial scale in Zimbabwe. Muposa (2013) noted that lack of knowledge by Agriculture Extension Workers was one of the problems faced by mushroom producers in Zimbabwe. The objective of the study was to compare the effects of different locally available agro-wastes on the growth and yield of Oyster mushroom *Pleurotus ostreatus* with the view of increasing the substrate options and providing knowledge on the best substrate and alternative substrates for effective commercial cultivation of oyster mushrooms. Since Zimbabwe is an agro based economy, it was necessary to explore the potential use of the abundant agro-wastes as additional substrates for mushroom production.

## Materials and Methods

A mixed approach method was employed in gathering and analysing data. The experiment was conducted in a custom made growth room in a backyard in Masvingo, Zimbabwe (House Number 2654, Rujeko). Mushroom house conditions were partially controlled. Relative humidity and room temperature were monitored and maintained with a hydrometer and six's thermometer respectively. Relative humidity was maintained between 80 and 85 % by spraying fine mist of water occasionally (Oei, 2003). Response variables such as days of spawn run, days of primordial formation, harvest days, stem length, stem width and cap diameter were compared based on the different types of the substrates used, that is, sawdust (S1), cotton waste (S2), wheat straw (S3) and corncob (S4) in the production of oyster mushroom.

### Spawn source

*Pleurotus ostreatus* 10kg spawn was obtained from Mustella spawn laboratories situated in Marlborough, Harare. The prices were \$6/Kg (RTGS) or \$1.4/kg (USD). The minimum quantity sold was a 10kg spawn.

### Substrate sources

Four different substrates namely, sawdust (S1), cotton waste (S2), wheat straw (S3) and corncob (S4) were evaluated for growing oyster mushroom in this study. Saw dust was collected from a local sawmill at Rank timber market in Masvingo. The maize residues, that is, the corncob was abundantly available from local farms. Cotton waste was purchased from farmers in Zambia since it was not available locally during the time of the study. Wheat straw was obtained from a local mushroom farmer in Mashava area

### Nutritional Analysis of the Substrates

Nutritional analysis for all the four substrates used in the investigation was conducted at the University of Zimbabwe, Department of Biological Sciences in Harare. The nitrogen, carbon, phosphorus, calcium, magnesium, and zinc contents for each of the four substrates that is wheat straw, corn cob, cotton waste and saw dust was determined. Thirty samples of each substrate were obtained from different randomly chosen

sources. All the samples were ground into a fine powder using pestle and mortar before being analyzed for their nutritional content, that is, according to (Onyango *et al.*, 2011). After grinding to powder, 100g of each sample was mixed to make a composite sample where after thorough homogenization or mixing 100g sample for each substrate was taken for analysis. The nutritional analysis was done according to standard methods in Table 1.

**Table 1 Summary of the method used in the nutritional analysis of substrates**

Parameter	Method used	Instrument
Nitrogen	Wet digestion-Calorimetry	Spectrophotometer
Carbon	Modified Walkly-Black	Spectrophotometer
Phosphorus	Wet digestion-Ascorbic Acid	Spectrophotometer
Ca, Mg. K and Zn	Atomic Absorption	Spectrophotometer

### Digestion Mixture for Nitrogen and Phosphorous

Selenium dust (0.42 g) and lithium sulphate (14 g) were added to 350 ml of 30% hydrogen peroxide. The mixture was slowly added with care to 420 ml of concentrated sulphuric acid while cooling in an ice bath. The mixture was stored at temperatures of 2°C for 4 weeks.

### Digestion Procedure

A sample of 0.1g of each substrate was weighed into a digestion tube, and the weight was recorded. After that, 4.4 ml of digestion mixture was added to each tube. Blanks were included for standard compensation. Heat was applied in the digester block at 360°C for 3 hours until the solution was colourless and free from plant materials. The mixture was removed from the block and allowed to cool to room temperature. Distilled water 25ml was added and was mixed well with a vortex mixer, until no more sediment dissolved and it was allowed to cool. Distilled water was added to make up to 100 ml level. The supernatant was allowed to settle so that a clear solution was taken from the top of the tube for

analysis of Nitrogen and Phosphorous using standard methods mentioned above.

### ***Preparation and Pasteurisation of Substrates***

Wheat straw was cleaned and excess soil was removed by dipping the straw in clean tap water in a 200 litre drum. The procedure was also done with corn cob, cotton waste, and saw dust. Wheat straw and corn cob were then chopped into small pieces of five to seven centimeters using a machete and a wooden block. A digital balance (Ohaus Scout SPX2201) was used to weigh 415g of powdered hydrated lime as well as 20kg of each substrate used in this investigation. The hydrated lime powder was dissolved in 50 litre tap water in a 100 litre plastic drum. The wheat straw (20kg) was added immediately, completely submerged and the drum covered with a lid. The straw was left to soak for 12 hours, unloaded onto a sterilised plastic sheet and allowed to drain excess water. The procedure was repeated in preparing all the three substrates, that is, sawdust, cotton waste and corn cob.

### ***Sterilisation of spawning equipment***

A sodium hypochlorite solution for sterilisation of apparatus was made by mixing 50ml of 5% sodium hypochlorite with 20 litre of water in a 20 litre plastic bucket. All equipment and working surfaces were sterilised by soaking in or washing with the sodium hypochlorite solution. The spawn bags were also dipped in the solution for five minutes and placed on a sterilised surface prior to spawning.

### ***Spawning***

This is the process of adding the 'mushroom seed' to the pasteurized substrate. The recommended rate of inoculation (spawning) used was 3-7% of substrate (dry weight) (Stamets, 1993). Heat resistant (10 kg) polythene bags were filled with a bottom layer of pasteurized substrate followed by uniform distribution of the spawn. The bottom ends of the bags were folded to enable them to stand without any form of support. An empty bottle was used to compress the substrate and spawn mixture as more was added to a final weight of 10 kg. A piece of cotton wool was plugged at the neck of the bags. About (6–8) holes were punched on the sides of the plastic bags to

facilitate cross-sectional ventilation. Finally, a total of 6 polythene bags from each substrate type were inoculated with a spawn and the experiment was done in duplicates. After the holes were punched, two openings were cut at the base of the bags to improve drainage.

The bags were then incubated for spawn running and tied in a mushroom growing house under complete darkness at controlled temperature of 25°C. The humidity of the bags was maintained constant by spraying with water twice a day. The thickening of the mycelia in the bags (colonization of the bags) was an indication of the end of the incubation period and that the bags were to be opened for fruiting. The procedure was done for all the substrates on the same day so as to maintain uniform conditions during the investigation.

### ***Maintenance during spawn run***

The following conditions were maintained for the success of this stage; little aeration, or windows closed, darkness, high humidity and optimum temperature range of 23-28°C (SIRDC, 2017). Air humidity was maintained between 80-90% with the use of a hygrometer to monitor the moisture. Watering of the floor was done at least once daily using a horse pipe. The mushroom house temperature was measured using a six's thermometer at 6am, 12pm and 6pm daily.

### ***Maintenance during fruiting***

After the substrate has been fully colonized and has become white, free circulation of air was maintained by opening the door and louvers on the sides of the mushroom house. High humidity was attained through watering daily and misting the bags (SIRDC, 2017)

### ***Maintenance during harvesting***

Good ventilation was maintained by opening the windows. A lot of light was required at this stage and it was achieved by opening the doors and windows during the day. High humidity was obtained through watering and misting of the mushrooms daily.

### ***Experimental design***

A completely randomized design (CRD) with four replicates, that is, (saw dust (S1), cotton waste (S2), wheat straw (S3) and corn cob (S4) was used.

CRDs were used for studying the effects of one primary factor without the need to take other nuisance variables into account. It simply compared the values of a response variable based on the different levels of that primary factor. The response variables such as days of spawn run, days of primordial formation, harvest days, stem length, stem width and cap diameter were compared based on the different types of the substrates used.

## Data collection

### ***Determination of the number of days to complete spawn run***

The date of spawning was marked as day one for the mycelia to begin colonizing the straw. Mycelial growth was observed daily and the number of days taken to completely cover the substrate was recorded for each bag. The numbers of days for complete spawn run were determined by counting from day one to the day when all substrate was completely covered by mycelia

### ***Determination of the number of days to complete primordia formation***

The days of completion of colonisation of substrate were noted. The number of days taken to produce first visible primordia was determined by counting the number of days from completion of colonisation to the day that the first primordia was observed, for each bag.

### ***Determination of the number of harvesting days***

The days of primordial emergence were noted. The number of days for the first flush was determined for each bag by counting from day one to the last day that the fruit was harvested on the bag and that was repeated for all the four treatments.

### ***Length, thickness of stem and diameter of cap***

The growth parameters were measured in centimetres using a tap measure and the measurements were done for all the four treatments. The findings were recorded in the table of results.

### ***Determination of the yield of first flush (YFF) and biological efficiency (BE)***

The fresh weight of mushrooms was determined using a digital balance (Ohaus Scout SPX2201) and the results were recorded for each bag on each harvest day. The mean weight of mushrooms harvested was calculated for each substrate used and they were tabulated. Total yields and biological efficiencies were calculated for each substrate at the end of the first fruiting flush.

## Data Analysis

To evaluate the growth performance of mushroom on different substrates, yield and biological efficiency was calculated. Accordingly, biological yield (g) was determined by weighing the whole cluster of fruiting bodies without removing the base of stalks, and Economic yield (g) was determined by weighing all the fruiting bodies on a substrate after removing the base of stalks. After the last harvest the dry weight of the spent substrate was determined by exposing it to direct sunlight until there was no further change in weight for 3 consecutive days. The data collected was subjected to statistical analysis using Analysis of Variance (ANOVA) on Genstat version 9 (Hilbe, 2007). Mean values of all the parameters and the standard errors of each parameter were separated using LSD at 5 % level of significance using Duncan's multiple range test.

### ***Biological efficiency (BE)***

Biological efficiency is the percentage measurement of the yield of fresh mushrooms from the dry weight of the substrate. BE was calculated as follows:

$$\%BE = \frac{FW_m}{DW_s} * 100 \%;$$

Where, BE = Biological Efficiency (%);

FW<sub>m</sub> = Total fresh weight (g) of mushroom yield across all flushes, and

DW = Substrate dry weight (g).

### ***Validity***

The data obtained was analysed using one way ANOVA. For normality testing, the ShapiroWilk

or Kolmogorov-Smirnov test was used to test for a normally distributed population within the samples. In Equal Variance Testing, SigmaPlot was used to test for equal variance by checking the variability about the group means. For the *P* values for normality and equal variance, the *P* value determined the probability of being incorrect in concluding that the data was not normally distributed (*P* value was the risk of falsely rejecting the null hypothesis that the data is normally distributed). If the *P* computed by the test was greater than the *P* set here, the test passed. To strictly adhere to normality and or equal variance, the *P* value was low. The parametric statistical methods were relatively robust in terms of detecting violations of the assumptions, the suggested value in SigmaPlot was 0.050. Larger

values of *P* (for example, 0.100) required less evidence to conclude that the data was normal.

### Consistency

Internal consistency reliability was a way to gauge how well a test or investigation was actually measuring what it was meant to measure. In this study all other conditions for oyster mushroom production were maintained at their optimum levels. The type of the substrate was the only varied factor among all the treatments.

## Results and Discussions

### Nutritional analysis of substrates

**Table 2: Nutritional analysis of substrates used in the study.**

Parameter tested	Wheat straw (dry weight)	Corn cob (dry weight)	Cotton Waste (dry weight)	Saw Dust (dry weight)
% Nitrogen	0.763	0.752	1.06	0.071
% Carbon	43.89	49.09	39.07	53.46
<b>Carbon: Nitrogen</b>	<b>44:1</b>	<b>49:1</b>	<b>39:1</b>	<b>53:0.1</b>
Total Phosphorus mg/kg	0.866	15.41	52.46	312.55
Calcium mg/kg	6.64	17.99	375.76	144.57
Magnesium mg/kg	30.76	63.69	32.49	85.77
Potassium mg/kg	130.45	630.63	370.91	907.05
Zinc mg/kg	0.411	0.146	0.209	0.572

The results of the nutritional analyses of the different substrates are shown in Table 2. The highest carbon to nitrogen ratio was found in saw dust (53:0.1) followed by corn cob (49:1) and wheat straw (44:1). The least carbon to nitrogen ratio was found in cotton waste (39:1) as indicated in Table 2. All the substrates used in the growth of oyster mushroom in this investigation were all suitable since they all exhibited a significantly higher ratio of carbon to nitrogen at ( $P=0.029$ ). According to Chang and Miles (2002), *Pleurotus* species require carbon, nitrogen and inorganic compounds as their nutritional sources. Oyster mushroom can grow on a wide variety of substrates, however, the yield and the quality of oyster mushroom depend on the chemical and nutritional content of substrates (Badu *et al.*, 2011 and Patil *et al.*, 2010). The main mineral components (Ca, K, Mg, P, Zn) were present in the substrates however, in significantly different amounts. Saw dust contained the highest

phosphorus, magnesium, and potassium as indicated in Table 2. Cotton waste contained the highest calcium amount. There was no significant difference in the mean values of zinc across all the substrates. The mean amount of zinc in all the substrates was extremely very low at 0.334mg/kg. Generally saw dust was the substrate with the highest carbon to nitrogen ratio as well as the highest percentage of mineral components. The second best substrate after saw dust was corn cob with the highest carbon to nitrogen ratio as well as magnesium and potassium amounts. Cotton waste was the third substrate with high phosphorus and potassium amounts however, its carbon to nitrogen ratio (39:1) was the least as compared to all the four substrates but significant for the growth of *P. ostreatus*. According to the studies conducted by Hoa *et al* (2015), it was confirmed that the mineral elements (Ca, Cu, Fe, K, Mg, Mn, P, and Zn) were naturally present in all the substrate used in this study, that is, saw dust,

cotton waste, wheat straw, corn cob. Among substrate formulas used saw dust contained the maximum amount of Ca (521.28 mg/100 g). Calcium content in corn cob was the second highest and not significantly different with Ca content in sugarcane bargasse. In general, Cu and Zn contents of all substrate formulas were low.

The amount of calcium obtained in saw dust according to Hoa *et al* 2015 were significantly higher as compared to the calcium content of the substrates used in this study. Thus the mineral content of substrates might vary from one region to another thus a research like this one would be important to inform farmers to choose the best substrate types before mushroom cultivation. The results of zinc contents of substrates were significantly very low and the results were the same with that of Hoa *et al.*, (2015). Zinc is not required in large amounts for the growth of mushrooms. In terms of mineral composition, corn cob was the second best substrate followed by cotton waste and lastly wheat straw.

Studies conducted by Adenipekun (2006) suggested that *P. ostreatus* grew best on the medium containing macro nutrients. It was concluded that the medium without Ca and Mg gave lowest mycelial dry weight (Adenipekun *et*

*al*, 2006). In this study cotton waste contained the highest calcium content whereas corncob was the second best in magnesium content from sawdust. Calcium has the better ability to support growth of *P. ostreatus* and that was largely attributed to its role in the fungus metabolic processes such as glycolysis and respiration (Adenipekun *et al.*, 2006). The mineral components of substrates plays an important role in the growth of *P. ostreatus* mushrooms and the mineral composition of these substrates vary from one place to another. In terms of mineral composition, corn cob was the second best substrate followed by cotton waste and lastly wheat straw.

### Growth parameters of *P. ostreatus*

#### **Spawn run, Primordia formation and first harvest days for *P. ostreatus***

**Table 3: Growth Parameters of *P. ostreatus* under different substrates in the study**

Treatments	Spawn Run (days)	Primordia Formation (days)	Harvest (days)	Stem Length (cm)	Stem Width (cm)	Cap diameter (cm)
Sawdust	19	19	22	4.1	2.3	7.4
Cotton waste	24	26	30	7.2	2.6	9.7
Wheat straw	23	25	28	6.6	2.1	6.6
Corn cob	27	23	25	4.6	1.9	7.1

The substrates showed significant differences in terms of days required for spawn run as shown in Table 3. The days required for spawn run in saw dust was 19 days, which was followed by 23 days in wheat straw and 24 days cotton waste. Maximum days required for spawn run were 27 days obtained in corn cob. Minimum days required for primordial formation were 19 days in sawdust, which was followed by 23 days, 25 days and 26 days in corncob, wheat straw and cotton waste, respectively.

The minimum duration required for first harvest was 22 days obtained in saw dust followed by 25 days in corn cob and 28 days in wheat straw. Maximum days required for first harvest were on cotton waste that is, 30 days based on the results in Table 3. Duncan's One Way Analysis of Variance at 5% level of significance was used to compare the means of the growth parameters of *P. ostreatus*. The differences in the mean values among the substrates groups were not good enough to exclude the possibility that the differences were due to random sampling

variability; there was not a statistically significant difference for ( $P = 0.394$ ). According to Hoa *et al* (2015) the results can be explained by the differences in carbon to nitrogen ratio of the substrates. In the study, saw dust had the highest carbon to nitrogen ratio followed by corn cob. The fastest rate of mycelial growth was obtained in sawdust and wheat straw as indicated by least number of spawn run days of 19 and 23 respectively. The results were similar to the findings of Alborés *et al.*, (2006) who revealed that there was a positive correlation between the carbon and nitrogen ratio of substrate and mycelium growth rate. Naraian *et al.*, (2009) also reported that mycelium growth and primordial development of *Pleurotus ostreatus* were dependent on the lignocellulosic materials, especially the carbon to nitrogen ratio. Mycelium growth patterns were also in agreement with the findings of Dahmardeh *et al.*, (2010) that colonization period of oyster mushroom took three weeks and fruiting bodies appeared after 2 to 5 days when saw dust, cotton waste and wheat straw were used as substrates. The results of this study were also similar with the finding of Hoa *et al.*, 2015 who found that the first fruiting body occurred on different days depending on the type of substrate used. For instance, in this study the preordia formation days were 19 in saw dust but 26 in cotton waste, thus the formation of fruiting bodies depended on the type of substrate used.

Based on the results of Duncan's one way ANOVA at 5% level of significance, the differences in the mean values of days to spawn run, primordial formation days and first harvest days among the substrates groups were not great enough to exclude the possibility that the differences were due to random sampling variability; there was no significant difference for ( $P = 0.394$ ). The result implied that the effect of different substrates on the growth parameters of *P. ostreatus* were the same.

### **Stem Length, Width and Cap diameter**

The greatest stem length of 7.2 cm was recorded in cotton waste (S2), followed by 6.6 cm in wheat straw (S3) and 4.6 cm corn cob (S4). In saw dust minimum stem length of 4.1 cm was recorded. The results showed the maximum stem width of 2.6 cm on cotton waste and minimum width of 1.9 in corn

cob. Mushrooms growing in saw dust had the best stem width of 2.3cm followed by stem width of 2.1cm in wheat straw. The highest cap diameter of 9.7 cm was recorded in cotton waste which was followed by 7.4cm in saw dust and 7.1cm in corn cob (S4). Lowest cap diameter of 6.6 cm was recorded in wheat straw as shown in Table 3. Based on Duncan's One Way Analysis of Variance at 5% level of significance, there was significant difference between the means of cap diameter versus stem width, cap diameter versus stem length and stem length versus stem width in all the four substrates used in the *P. ostreatus* mushroom.

The greatest stem length and cap diameter of *P. ostreatus* was obtained in cotton waste yet from the nutritional analysis of substrates cotton waste had the least carbon to nitrogen ratio. Saw dust had the minimum stem length yet its carbon: nitrogen ratio was largest as compared to all substrates. These findings were similar to the results reported by Yang (2000) that higher carbon to nitrogen ratio favored the mycelium growth, and lower carbon to nitrogen ratio favored the fruiting body growth. The results from this study showed that apart from saw dust which was widely used by farmers as the substrate of choice, cotton waste, corn cob, and wheat straw were also possible agro-waste materials for oyster mushroom production.

### **Yield and Biological efficiency parameters of *P. ostreatus***

The result on total yield and biological efficiency of *Pleurotus ostreatus* by using different substrate were presented in Table 4. The differences in the BE and yield mean values among the substrate groups were greater than would be expected by chance at  $p < 0.001$ ). The highest yield of 1275.45 g was obtained by using saw dust as substrate (S1) which was followed by a yield of 1208.8g in corn cob (S4) and (1178.5 g) was obtained in wheat straw (S3). Cotton waste (S2) had the lowest yield of 1058.7 g. Maximum BE that is 115.9 % was obtained in saw dust and minimum B.E. of 94.3 % was obtained in wheat straw as illustrated in Table 4.



**Table 4: Yield and biological efficiency of *P. ostreatus* per substrate used.**

Treatment	First Harvest (g)	Second Harvest (g)	Third Harvest (g)	Total Yield	Biological Efficiency (%)
Saw Dust	757	336.45	181	1274.45	115.9
Cotton Waste	657	267.3	134.4	1058.7	70.6
Wheat Straw	702	302.7	156	1178.5	94.3
Corn Cob	715	320	173.8	1208.8	100.7

**One way ANOVA of the first, second and third harvest of *P. ostreatus*.**

The differences in the mean values of the first, second and third harvest among the substrate groups were greater than would be expected by chance; there was a statistically significant

difference ( $P = <0.001$ ) as indicated in Table 5. According to Duncan's multiple comparison procedures, the critical differences between first harvests versus third harvest, first harvest versus second harvest and second harvest versus third harvest across all the substrates was significantly high.

**Table 5: One way ANOVA for the first, second and third harvest on each substrate.**

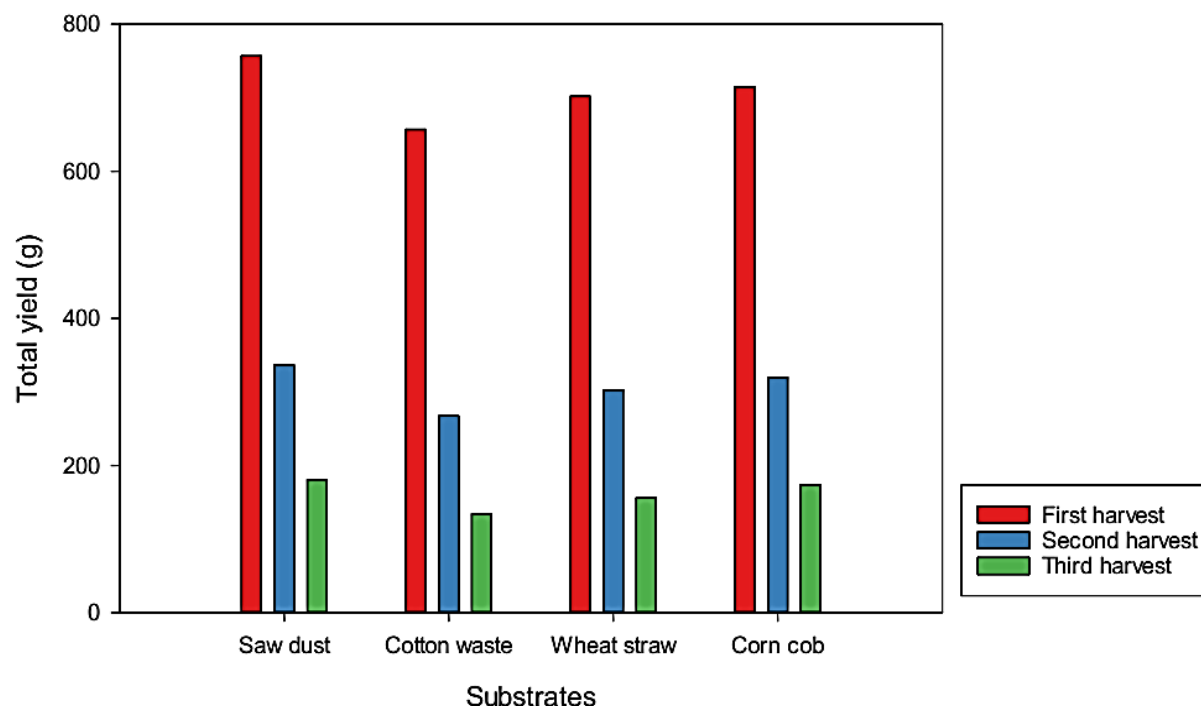
Group Name	N	Missing	Mean	Std Dev	SEM
First harvest (gm)	10	6	707.75	41.177	20.589
Second harvest	10	6	306.613	29.61	14.805
Third harvest	10	6	161.3	20.785	10.392
Source of Variation	DF	SS	MS	F	P
Between Groups	2	640846	320423	319.958	<0.001
Residual	9	9013.07	1001.45		
<b>Total</b>	<b>11</b>	<b>649859</b>			
Comparison	Diff of Means	p	q	p	p<0.050
First harvest vs Third harvest	546.45	3	34.535	<0.001	Yes
First harvest vs Second harvest	401.137	2	25.352	<0.001	Yes
Second harvest vs Third harvest	145.313	2	9.184	<0.001	Yes

**Yield per flush of *P. ostreatus* in all the four substrates**

Fig 1 show the different yield per each flush/harvest for all the four substrates used in the production of oyster mushroom. Saw dust produced the best first flush yield of 757 g followed by corncob with 715 g and 702 g on wheat straw. Cotton waste had the least first flush yield of 657 g. There was a gradual decrease in the

yield of mushrooms from the first harvest to the third harvest in all the four substrates. In cotton waste for instance, the first harvest was 657g followed by second harvest of 267.3g and lastly the third harvest of 134.4 g. The trend was the same in all the four substrates. The gradual decrease in the yield of mushroom was clearly shown in Fig 1.

**Fig 1: The yield per flush of *P. ostreatus* in all the four substrates.**



**One way ANOVA analysis for yield and biological efficiency of *P. ostreatus***

Duncan’s ANOVA was used at 5% significance level to test the variance of means for yield and Biological efficiency for all the four substrates and the results were outlined in Table 6 below. The differences in the mean values among the substrate groups were greater than would be expected by

chance; there was a statistically significant difference at  $p < 0.001$ . As indicated in Table 6, the critical difference between yield and biological efficiency was very high at  $p < 0.05$ .

**Table 6: One way ANOVA for the yield and biological efficiency of *P. ostreatus***

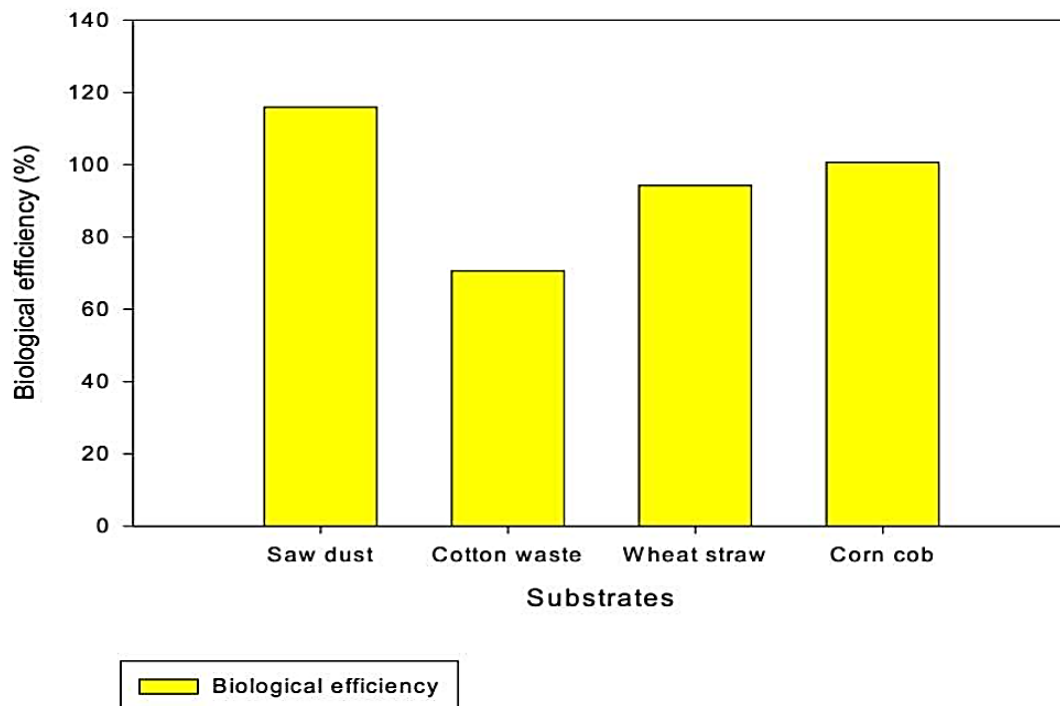
Group Name	N	Missing	Mean	Std Dev	SEM
Yield (gm)	10	6	1180.113	90.307	45.154
B.E %	10	6	95.375	18.838	9.419
Source of Variation	DF	SS	MS	F	P
Between Groups	1	2353310.89	2353310.89	553.054	<0.001
Residual	6	25530.719	4255.12		
Total	7	2378841.61			
Comparison	Diff of Means	p	q	p	p<0.050
Yield vs B.E	1084.738	2	33.258	<0.001	Yes

### Biological efficiency of *P. ostreatus* on each substrate

Biological Efficiency (BE) is the ratio of fresh fruiting body weight (g) per dry weight of substrates (g), expressed as a percentage. In this case the mean values of BE for each substrate were used to draw the graph in Fig 2. The highest biological efficiency was obtained in saw dust followed by corncob and wheat straw. Cotton waste was the substrate with the lowest biological efficiency. The results in this study were in agreement with Hoa *et al* 2015 who obtained quite a significant high mushroom yield of *P. ostreatus* from the first flush, followed by the second flush and the trend gradually decreased at next flushes. In general, substrates that gave the higher yields also gave the higher value of BE. According to Hoa *et al.*, 2015, the total yield of *P. ostreatus* ranged from 232.54 to 270.60 g/flush. On

substrate yield, corn cob gave the highest total yield (270.60 g/bag) followed by sugarcane barge and sawdust (258.82 and 257.70g/bag, respectively). However, in this study, the total yield per flush was 757g/flush in saw dust and 715g/flush in corn cob thus the yield was too high as compared to the study by Hoa *et al.*, (2015). The differences in terms of yield and BE of both oyster mushrooms grown on different substrate types were due to the differences in physical and chemical composition of substrate formulas such as cellulose/lignin ratio and mineral contents, pH, and nutritional analysis of the carbon to nitrogen ratio.

**Fig 2. Biological efficiency of *Pleurotus ostreatus* on each substrate.**



Duncan's ANOVA was used at 5% significance level to test the variance of means for yield and Biological efficiency for all the four substrates. The differences in the mean values among the substrate groups were greater than would be expected by chance at  $p < 0.001$ . The different substrates had an effect on the growth and yield of

*P. ostreatus*. The yield obtained varied with the substrate type used in the investigation with sawdust having the highest total yield of 1274.45 g followed by corn cob with 1208.8g. Wheat straw was the next with 1178.5 g and cotton waste had the lowest yield of 1058.7g. Biological efficiency also followed the same trend; the highest

biological efficiency was obtained in saw dust followed by corncob and wheat straw. Cotton waste was the substrate with the lowest biological efficiency.

The findings on this study were similar to the results of the investigations conducted in Pakistan where the highest biological efficiency, total yield and quality of *P. ostreatus* were obtained using saw dust as substrate (Shah *et al.*, 2004). However investigations conducted in Nigeria produced different results with higher yields obtained using agricultural wastes than saw dust (Adedokun, 2014). Thus further investigations were required using a wide range of different saw dust obtained from different plant species as well as agricultural wastes to determine the most efficient substrate in the production of oyster mushrooms. Saw dust produced higher yields in this investigation however agricultural wastes were readily available, cheap and more sustainable to use since they can be obtained without causing any harm to the environment such as deforestation, global warming and climate change. Use of saw dust and agricultural wastes in mushroom production was a sustainable way of recycling dead organic matter. *Pleurotus ostreatus* was found to utilize all the agricultural wastes and the substrates were suitable for spawn run, yield and biological efficiency (Das *et al.*, 2000). The large sized fruit bodies were considered to be of good quality and rated highly in mushroom production (Onyango *et al.*, 2011).

The growth of oyster mushrooms was significantly affected by the Carbon/nitrogen ratios of the substrates. Higher yield and biological efficiency were obtained from saw dust. Low yield and BE were obtained in cotton waste which had the least carbon to nitrogen ratio of (39:1). Although the ratio of carbon to nitrogen obtained in the study was significantly high, the results were supported by the research findings of Adenipekun (2006). In their study, the carbon to nitrogen ratios significantly affected growth of *P. ostreatus* and growth at 5:1 (C/N) was the best. The least mycelia growth was 21.7mg/30cm<sup>3</sup> recorded in basal medium with C/N ratio of 1:5 which was not significantly different from the control at  $p \leq 0.01$ . If the ratio of carbon to nitrogen

was low, then the yield was also very low and vice versa. Thus there was direct relationship between the yield of mushrooms and the carbon to nitrogen ratios in the substrates.

## Conclusions

The four substrates used in the investigation had the potential to be used as alternative substrates in the production of oyster mushrooms without compromising on the yields and quality. Saw dust and cotton waste were the best substrates of choice for the mushroom farmers. Saw dust was good for faster growth rates and higher yield whereas with cotton waste it took long to reach maturity but it was good for producing mushrooms of good quality with large cap diameter and stipe length. It was recommended that further studies should be done to determine the effect of different substrates on nutritional content of the mushroom produced.

## Acknowledgements

The authors would like to thank the Bindura University of Science Education (BUSE) for availing their facilities and the farmers who participated in the research.

## Conflicts of Interest

The authors declare no conflict of interest.

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