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QUANTITATIVE ASSESSMENT OF *PLEUROTUSOSTREATUS* (JACQ.EX FR.) P. KUMMPRODUCED FROM DIFFERENT SUBSTRATES.

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ABSTRACT
The cultivation of mushroom due to its importance both nutritional and medicinal and to avoid inadvertent picking of poisonous ones by harvesting the wild ones has raised mushroom science to become a highly profitable agricultural business. This study investigated the cultivation of *Pleurotusostreatus* using different local grasses as substrates to evaluate their effects on their growth and quantitative yield of the fruit bodies of this mushroom. The substrates used were *Andropogongayanus*(A.G), *Pennisetumpurpureum*(P.P), *Dactylischlorometa* (D.C), *Andropogongayanus*+ *Pennisetumpurpureum*(A.G+P.P), *Andropogongayanus* + *Dactylischlorometa* (A.G + D.C), *Pennisetumpurpureum* + *Dactylischlorometa*(P.P + D.C). Completely randomized design with six treatments and four replicates were used. The highest fruit-body number was obtained from A.G (40.25±32.07^{a}) in 1st flush and D.C (21.67±14.15^{a}) in 2nd flush while the least was obtained from A.G+P.P (10.25±8.14^{a} and 9.67±4.73^{a}) from both flushes . The largest cap diameter was obtained from A.G + P.P (5.45±0.68^{ab}cm and 4.96±0.30^{a}) respectively from both flushes while the least was from A.G + D.C (3.65±0.16^{a} cm and 3.89±1.58^{a}) respectively from both flushes. The longest average stipe length in 1st flush was produced in D.C (2.85±0.37^{b}cm) and 2nd flush was in A.G + P.P (2.69±0.52^{a} cm) while the lowest in both flushes was produced in A.G + D.C (2.03±0.25^{a} cm and 2.09±1.07^{a} cm) respectively. The highest fresh weight was obtained from A.G (85.75±33.19^{b}) in first flush and in P.P (67.33±12.22^{a}) in second flush while the least was obtained in P.P + D.C (26.67±14.47^{a} and 39.50±6.25^{a}) in both flushes. Flush A.G had the highest biological efficiency (50.2%) while P.P+D.C had the lowest biological efficiency (18.3%). The use of these substrates are highly encourages as all of them performed reasonably in the production of *P. ostreatus.*

Keywords: mushroom, *Pleurotus ostreatus*, fruit bodies, cultivation, substrates.

INTRODUCTION
Mushrooms are fungi that belong to the division Basidiomycetes, as well as a few members of thedivision Ascomycotina.Fungi play a significant part in the degradation of organic materials, as well as nutrient cycling and exchange (Kevin, 2005). Mushrooms which are macro-fungi are saprophytes, which mean they can survive in a wide range of

environments and substrates. Nevertheless, some substrates in a habitat are ideal for mushroom growth than others(Adesina*et al.,* 2011). Most mushrooms have an umbrella-shaped fruiting body, with a central stalk (stipe) supporting a cap (pileus) with gills (lamellae) on the underside that generate spores. The stipe is absent in some *Pleurotus* species, particularly those that grow on wood. (Jose *et al.,*2002).Mushroom cultivation can be considered as a cost-effective approach to extract bio-resources from agricultural leftovers as well as a source of environmental protection. The utilization of residues in bioprocesses could be one of the strategies for converting inedible biomass residues into nutrient-dense, protein-rich food in the form of edible mushrooms (Chui *et al.,* 2003). Mushroom cultivation has gained more significance because of its versatility and profitability in agricultural business all over the world (Ikeji, 2010).

Pleurotusostreatus is a common edible mushroom which was firstly grown in Germany as subsistence measure during the First World War and is now grown commercially around the world for food.*P. ostreatus* can be grown in all the countries specifically in temperate, sub-tropical zones (Won-sik, 2004). The colour of the caps turns light brown whenever the temperature is low but at increase in temperature, it turns pale (Chandy, 2010). *P. ostreatus* could be harvested in warmer temperature because the range of its fruiting temperature is wider than other *Pleurotus* species and does not require fruiting induction (cold shock) (Won-sik, 2004).*P. ostreatus* is used in Japanese, Korean and Chinese cookery as a delicacy. It is made into a source sometimes (Rop*et al.,* 2009).*P. ostreatus* is utilized extensively for catalyzation of large chemical conversions and bio-bleaching in the paper industry, dye de-colorization in textile industry and bio-remediation (Park *et al.,* 2007).*P. ostreatus* has high saprophytic ability to thrive on a wide variety of cellulosic substrates (Okwulehie and Okwujiako, 2008).

In Nigeria, there are many agro-wastes which have high cellulosic content that could be deployed to support to quantitative production of edible mushrooms like *P. ostreatus*. Utilizing them for mushroom farming ultimately make them useful, empowering farmers and can serve as food security. This research aimed toproduce *Pleurotusostreatus* on three different grass straws, determining the suitability of these substrates for mushroom production and yield

MATERIALS AND METHODS

The study was carried out in the mushroom house and laboratory of the department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike in Umuahia, Abia State.

SOURCE OF MATERIALS

Pleurotusostreatus spawns were obtained from the Department of Plant Science And Biotechnology, Michael Okpara University Of Agriculture, Umudike. Spawns were preserved at room temperature, in the Postgraduate laboratory (PG) of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike,

for three (3) days before inoculation. (Staments, 2002). The *Pennisetumpurpurium* and *Andropogongayanus* were collected from farmlands within Umudike area while *Dactylischlorometa* was obtained from water melon section of UbaniIbeku main market, Umuahia in Abia State; and were further prepared following the methods of Chang and Miles (2004); Okwulehie and Okwujiako (2008) and Onyeizu*et al.,* (2017).

PREPARATION OF THE SUBSTRATES FOR CULTIVATION

The substrates, (straws of *Andropogongayanus, Pennisetumpurpurium, Dactylischlorometa)*were chopped into pieces of about 5cm average lengths and soaked 48hrs in clean tap water according to the method of Sharma, (2003).The substrates were drained the next day and separately poured into a drum and pasteurized by heating on a gas for 2h at 100^{o}C. (Okwulehie and Okoro, 2013).After cooling, 1kg of the substrates (straws of *Andropogongayanus, Pennisetumpurpurium, Dactylischlorometa*) and there mixtures were poured in 2.5 lit transparent plastic buckets perforated from top to bottom. The substrates used are:

1. *Pennisetumpurpurium* straw (P.P)
2. *Andropogongayanus* straw (A.G)
3. *Dactylischlorometa* straw (D.C)
4. *Andropogongayanus* straw and*Pennisetumpurpurium* straw and (A.G + P.P)
5. *Andropogongayanus* straw and *Dactylischlorometa* straw (A.G + D.C).
6. *Pennisetumpurpurium* straw and *Dactylischlorometa* straw (P.P + D.C)

The mixtures were 50:50 with replicates of four (4).

INOCULATION AND INCUBATION

All instruments used were sterilized during inoculation with alcohol while the perforated buckets were rinsed with dilute solution of hypo-chloric acid. 30g of spawn were inoculated at the bottom of the sterilized perforated bucket followed by filling the same bucket with pasteurized substrate to the depth of 4cm. Another 30g of spawn were spread again on the surface of the substrate. Again, the substrate were filled to cover the spawn and pressed lightly. This method was repeated until the perforated buckets were filled to the depth. The inoculated/ perforated buckets were covered. The holes were made on the buckets for aeration. After the inoculation, the buckets were kept in a ventilated mushroom house of the department of plant science and biotechnology and were covered with black polyethylene bag to serve as dark incubation for ramification to take place. Ramification lasted for 14 days from the day of inoculation and the black polythene were removed on the 16th day during which the immature mushroom started to emerge as tuff of small buttons or premodials on the 17th day.

FRUITING AND HARVESTING

Fruiting of the mushroom commenced on the different concentration of the substrates from the 17th day of the inoculation. The first flushes were harvested on the 21st day

from the substrates of A.G, P.P, A.G + D.C and on 22nd day from D.C and A.G + P.P then on 26th day from P.P + D.C due to different nutrients concentration of the substrates. The second flush was harvested on 32nd day from A.G, P.P, A.G + P.P, and A.G + D.C, on 35th day from D.C and on 36th day from P.P + D.C. The third flush was harvested on 43rd day from A.G, A.G + P.P, and A.G + D.C and D.C on the 53rd day. P.P and P.P + D.C did not produce third flush due to differences in nutrient concentration.

MEASUREMENTS OF PARAMETERS

The yield of the *Pleurotusostreatus* on different substrates concentrations were determined by recording the number and size of the fruiting bodies. Data were collected from the different replicates and the mean of each set of data were calculated. The following parameters of growth/yield were taken.

Stipe lenght (Height): The height of *Pleurotusostreatus*were measured in centimeters from the stipe base using transparent rule

Cap (pileus) diameter: The diameter of the pileuswas measured in centimeter (cm) with transparent plastic rule from one edge of the pileus across the stipe to the other edge.

Fresh weights: The fruit-bodies were weighed immediately after harvest using electronic balance. And their mean weights were recorded.

Dry weight: Dry weight of the fruit-bodies were weighed using a digital scale (Model, 2000) made in U.S.A.

Number of fruit bodies: These were determined by counting the number of fruit bodies produced on each bucket.

Biological efficiency

The biological efficiency (yield of mushroom per kg substrates on dry wt. basis) was determined using the following formula (Okwulehie*et al.,* 2017):

$$B.E\ (\%) = \frac{\text{Fresh Weight of mushroom}}{\text{Dry Weight of substrate}} \times \frac{100}{1}$$

Figure 1: Fruit-bodies of *P. ostreatus*

A cluster of harvested fruit bodies of *P. ostreatus*

RESULTS AND DISCUSSION

RESULTS

Morphological characteristics of the fruit-bodies and the yield of *P. ostreatus* on different substrates and substrate combinations

Table 1 The morphological characteristicsand the effects of the substrates and substrates combination on the*P. ostreatus*

First flush

TREATMENTS	NF	CD	SL	F wt	Dry wt
A. G.	40.25±32.07^{a}	4.30±1.07^{ab}	2.62±0.48^{ab}	85.75±33.19^{b}	4.25±3.50^{a}
P. P.	32.50±16.66^{a}	4.24±0.53^{ab}	2.32±0.41^{ab}	68.00±10.07^{ab}	5.00±1.16^{a}
D. C.	32.25±10.69^{a}	3.79±0.49^{a}	2.85±0.37^{b}	61.50±12.79^{ab}	4.50±0.58^{a}
A. G. + D. C.	21.25±14.64^{a}	3.65±0.16^{a}	2.03±0.25^{a}	42.75±34.74^{a}	4.25±2.22^{a}
A. G. + P. P.	10.25±8.14^{a}	5.45±0.68^{ab}	2.59±0.24^{ab}	47.00±13.88^{a}	4.25±1.26^{a}
P. P. + D. C.	16.00±10.42^{a}	5.07±1.81^{b}	2.60±0.46^{ab}	39.50±6.25^{a}	4.25±0.96^{a}
Total	25.42±18.70	4.42±1.07	2.50±0.43	57.42±25.30	4.42±1.69
P≤0.05	0.178^{NS}	0.094^{NS}	0.094^{NS}	0.055^{NS}	0.990^{NS}
Second flush					
	NF	CD	SL	F wt	Dry wt
A. G.	15.33±13.58^{a}	3.91±0.21^{a}	2.32±0.22^{a}	32.33±30.17^{a}	3.33±1.16^{a}
P. P.	13.67±1.53^{a}	4.66±0.26^{a}	2.36±0.65^{a}	67.33±12.22^{a}	4.00±0.00^{a}
D. C.	21.67±14.15^{a}	4.45±0.89^{a}	2.30±0.44^{a}	26.33±12.10^{a}	3.00±1.00^{a}
A. G. + D. C.	9.67±7.37^{a}	3.89±1.58^{a}	2.09±1.07^{a}	50.33±38.28^{a}	2.67±2.08^{a}
A. G. + P. P.	9.67±4.73^{a}	4.96±0.30^{a}	2.69±0.52^{a}	32.00±18.74^{a}	3.33±1.16^{a}
P. P. + D. C.	10.67±7.23^{a}	4.61±1.12^{a}	2.56±0.15^{a}	26.67±14.47^{a}	2.33±0.58^{a}
Total	13.44±8.93	4.41±0.85	2.39±0.54	39.17±24.82	3.11±1.13
P≤0.05	0.602^{NS}	0.632^{NS}	0.850^{NS}	0.264^{NS}	0.611^{NS}

Values followed by the same superscript within the same column are not significantly different ($p > 0.05$)

ABBREVIATIONS: NF: Number of Fruit-bodies, CD: Cap diameter, SL: Stipe length, Fwt: fresh weight, Dry wt: Dry weight.

The results of morphological characteristics of the fruit-bodies and the yield of *P. ostreatus* on different substrates and substrate combinationsare presented in Table 1.

The results showed that the total number of fruit-bodies produced, the highest was recorded in substrate A.G in 1st flush having 40.25±32.07^{a} g followed by substrates P.P with 32.50±16.66^{a}gand D.C with 32.25±10.69^{a}g while the lowest was recorded in substrate A.G + P.P having 10.25±8.14^{a}g. In the 2nd flush, the highest number of fruit-bodies was recorded in substrate D.C having 21.67±14.15^{a}g followed by substrates A.G with 15.33±13.58^{a} g and substrates P.P having 13.67±1.53^{a} g. The lowest was recorded

in substrate A.G + P.P having 9.67±4.73[a] g. The substrates D.C was recorded the highest in the number of the fruit-bodies in 2nd flush because the cellulosic contents in A.G in the 1st flush were absorbed quantitatively by the mushroom. The numbers of fruit-bodies produced in both flushes were not significantly different as shown in the table 1. The result Table 1 showed that the largest average size of the cap diameter in 1st flush was recorded in substrate A.G + P.P as 5.45±0.68[ab] cm followed by substrate P.P + D.C as 5.07±1.81[b] and substrate P.P + D.C as 5.07±1.81[b] while the lowest average cap diameter was recorded in substrate A.G + D.C as 3.65±0.16[a] cm. In the 2nd flush, the largest average size of the cap diameter was recorded in substrate A.G + P.P as 4.96±0.30[a] cm followed by P.P as 4.66±0.26[a] cm and P.P + D.C as 4.66±0.26[a] cm while the lowest average cap diameter was recorded in substrate A.G + D.C as 3.89±1.58[a] cm. Cap diameter in 1st flush showed no significant different in substrates of A.G, P.P, A.G + P.P and in D.C, A.G + D.C except in substrate P.P + D.C while Cap diameter in 2nd flush shows no significant difference.

Table 1 also showed the result of the stipe length of *P. ostreatus* recorded by different substrates and substrates combination. The longest average stipe length in 1st flush was produced in substrate D.C having 2.85±0.37[b]cm followed by substrate A.G having 2.62±0.48[ab]cm. The smallest average stipe length in 1st flush was recorded in substrate A.G + D.C having 2.03±0.25[a] cm. In the 2nd flush, the longest average stipe length was recorded in substrate A.G + P.P having 2.69±0.52[a] cm followed by substrate P.P + D.C having 2.56±0.15[a] cm while the smallest average stipe length was recorded in substrate A.G + D.C having 2.09±1.07[a] cm. The stipe length was not significantly different except in D.C and A.G + D.C in 1st flush but there was no significant difference in 2nd flush.

Table 4.1 showed the results of the fresh weight of *P. ostreatus* recorded by different substrates and substrates combination in 1st and 2nd flushes. The highest fresh weight in 1st flush was recorded in substrate A.G having 85.75±33.19[b]g followed by substrate P.P having 68.00±10.07[ab]g while the lowest was recorded in substrate P.P + D.C having 39.50±6.25[a]g. In 2nd flush, the highest fresh weight was recorded in P.P having 67.33±12.22[a]g, followed by A.G + D.C having 50.33±38.28[a]g while the lowest fresh weight was recorded in D.C having 26.33±12.10[a] g. The fresh weight of the mushroom was not significantly different except in each of the substrates except in substrates in A.G and P.P, D.C in 1st flush while 2nd flush showed no significant difference in each of the substrates.

Table 1 showed the result of the dry weight of *P. ostreatus* recorded by different substrates and substrates combination. The highest dry weight in 1st flush was recorded in substrate P.P having 5.00±1.16[a] g followed by substrate D.C having 4.50±0.58[a]g while the lowest dry weight was recorded in substrate P.P + D.C having 4.25±0.96cm . In the 2nd flush, the highest fresh weight was recorded in substrate P.P having 4.00±0.00[a]g followed by substrates A.G and A.G + P.P having 3.33±1.16[a] g while the lowest dry weight in 2nd flush was recorded in substrates P.P + D.C having 2.33±0.58cm. The dry weight of the mushroom in both flushes was not significantly different in each of the substrates.

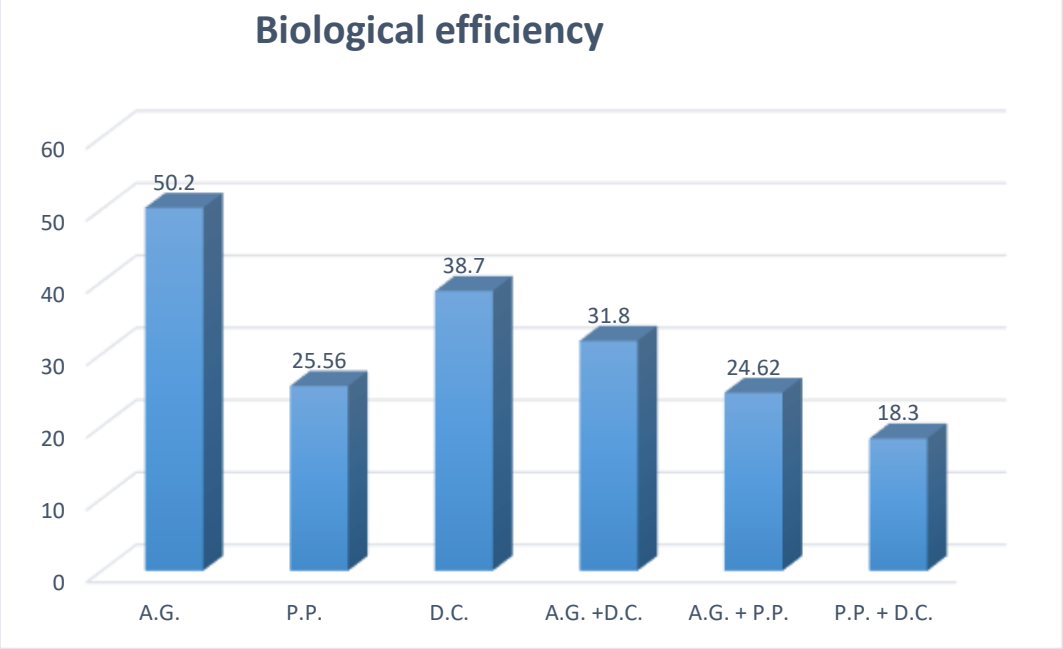

Fig 2 presents the effects of substrates and substrates combinations on the biological efficiency of the *P. ostreatus*.

Fig. 2 above presents the results of the biological efficiency of the *P. ostreatus* recorded by the substrate and substrates combination. The results were 50.2%, 25.56%, 38.7%, 31.8%, 24.62%, and 18.3% for A.G, P.P, D.C, A.G + D.C, A.G + P.P and P.P + D.C respectively. Substrate A.G (50.2%) had the highest percentage of biological efficiency followed by substrate D.C with 38.7% and A.G + D.C with 31.8% while P.P+D.C (18.3%) had the lowest percentage of biological efficiency. The statistical analysis showed no significant difference between the treatments except in A.G.

DISCUSSION

*P. ostreatus*commonly known as oyster mushroom is an excellent edible mushroom which can serve both for commercial purposes and home consumption, the epidermal constituent like chitosan can serve immensely for industrial and agricultural purposes. The result of the investigation generally indicates that the grass substrates support the appreciable growth of *P.ostreatus*.Fructification occurred in all the substrates and substrate combinations. This indicates that these substrates contained nutrients that supported the growth of the mushroom. This is in line with the report of Wabali and Wocha (2013) that nutrient concentration of substrates has effect on yield of *P. ostreatus*. Table 4.1 shows that *Andropogongayanus*(A.G) substrate yielded the highest number of fruit-bodies. This report supported the earlier report of Okwulehie and Okwujiako (2008) that *Pleurotus*spp. have a high saprophytic ability and can grow well in a variety

of cellulosic substrates (Thambidurai et al., 2006). The result from table 1 also shows that the number of fruit-bodies yielded is lower than what was reported by Okwulehie*et al.,* (2017) but higher than the earlier report by Okwulehie and Okwujiako (2008).
Cap diameter and stipe length depends on the amount of aeration and light (Kivaisi*et al.,* 2003). It was observed during this study that the two parameters also depend on the length of time taken from the primordial formation to harvesting in addition to the substrate type. It was also observed that the pileus diameter was very much dependent on the number of caps per cluster. The fewer they were, the wider the diameter of the cap and this was due to lower competition for space and nutrient availability. The cap diameter and the stipe length reported in table 1 were lower than what was reported by Okwulehie*et al.,* (2017) and Ogundele*et al.,* (2014) but similar to what was reported by Okwulehie and Okwujiako (2008).
The fresh weight in table 1 was lower to what was reported by Okwulehie*et al.,* (2017) and andOgundele*et al.,* (2014) but similar to the report by Okwulehie and Okwujiako (2008).
Fig 2 indicated that A.G substrates had the highest biological efficiency more than other substrates. Biological efficiency of this study was higher than the report by Okwulehie*et al.,* (2017) but similar to the reports by Falemara*et al.,* (2016) who reported on higher biological efficiency of two *pleurotus* species produced on concorbs and sawdust substrates.

CONCLUSION AND SUGGESTION

The results of these findings established that grass straws (*Andropogongayanus, Dactylis*chlorometa and *Penisetumpurpurieum*) and their combinations are good for the growth of mushrooms. It shows that *P. ostreatus* can be obtained quantitatively from the substrates named above and supports commercial production of mushrooms; therefore farmers are advised to use these substrates and their combination in mushroom production especially for commercial purposes. Furthermore, the growth of mushroom from these agro waste especially grass straws shows that mushroom can be used as waste recycling and good for bioremediation therefore burning of these agro waste may not be necessary for burning contributes negatively to the ecosystem, encourages desertification and releases carbon to the atmosphere.*Andropogangayanus* producing the highest fruit-bodies supports the recommendation of Okwulehie and Okwujiako (2008) to use *Andropogon* straw as a preffered straw production of *Pleurotusostreatusvarflorida* mushroom.

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