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Use Of *Paspalum Viginatum* As An Alternative Growth Substrate For Oyster Mushroom Cultivation

Francis Aboagye-Nuamah[1]*; Joseph Bekoe Oppong[2]; Bernard Bempah[3]; Augustine A. Akerimowo[1], Lawrence Nayenkun[1]
[1]Department of Agriculture & Agribusiness, Methodist University College Ghana, Wenchi, Ghana
[2]Department of Food Science & Technology, Methodist University College Ghana, Wenchi, Ghana
[3]Bencom Youth Training & Development Centre, Techiman, B/A, Ghana

* E-mail of the corresponding author: faboagye-nuamah@mucg.edu.gh

Abstract
The increasing demand for mushroom consumption worldwide calls for alternative growth substrates to ensure sustainable production. The study was conducted to evaluate the use of *Paspalum vaginatum* straw as an alternative growth substrate for the cultivation of oyster mushrooms (*Pleurotus ostreatus*), and to assess the effect of the growth substrate on the nutritional composition of the mushrooms. Six substrates were evaluated for the growth of the mushrooms. These were 100% *Paspalum* straw, *Paspalum* + sawdust (1:1), *Paspalum* + 5% rice bran, *Paspalum* + 10% rice bran, *Paspalum* + 20% rice bran, and 100% sawdust. The diameter of the mushroom cap, length of the stalk, total number and weight of fruiting bodies harvested were recorded. Proximate analysis was conducted to determine the moisture content, crude protein, total carbohydrates, fat, crude fibre, ash, Iron, Zinc, Calcium, Potassium, and Sodium contents of the mushrooms. The results indicated that the substrates significantly ($p<0.01$) influenced the number of days to first harvest, the size of the mushroom cap and the length of the stalk. The total number and weight of fruiting bodies harvested from the various substrates were not significantly different ($p\leq0.05$). This indicates that the yield of oyster mushrooms cultivated on the *Paspalum* was comparable to that of the sawdust which is widely used. The various substrates significantly affected the nutritional composition of the oyster mushrooms. Out of the six growth media, mushrooms cultivated on *Paspalum* straw substrate had the highest protein (61.3%) and Na (0.65mg) contents, but low in fat (0.1mg) and carbohydrates (14%). The mushrooms cultivated on sawdust recorded the highest fat content (0.59mg), Fe (10mg), Zn (6.9 mg) and Ca (0.77mg). Since the performance of the Paspalum in terms of the parameters studied were comparable to the sawdust, which has been the main substrate used for mushroom production, it can be concluded that *P. vaginatum* is a promising alternative substrate for the mushroom industry. The use of Paspalum for mushroom cultivation will reduce the dependence on sawdust.
Keywords: Oyster mushroom, Paspalum grass, Substrate, Yield, Proximate, Nutrients.

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1. Introduction

Mushrooms are fleshy, spore-bearing reproductive structures of the kingdom fungi that grow saprophytically on organic substrates. Edible mushrooms have for a long time played an important role in the diet of humans for thousands of years due to its nutritional and medicinal properties (Ng'etich, Nyamangyoku, Rono, Niyokuri, & Izamuhaye, 2013; Dundar, Acay, & Yildiz, 2008; Sánchez, 2010).

Mushrooms are quite rich in protein, with an important content of essential amino acids and fibre, poor in fat, a source of many different nutraceuticals such as unsaturated fatty acids, phenolic compounds, tocopherols, ascorbic acid and carotenoids (Pereira, Barros, Martins, & Ferreira, 2012); Barros, Venturini, Baptista, Estevinho, & Ferreira, 2008). Edible mushrooms also provide a nutritionally significant content of vitamins B1, B2, B12, C, D, and E (Heleno, Barros, Sousa, Martins, & Ferreira, 2010; Dundar, Acay, & Yildiz, 2008; Mattila, Könkö, Eurola, … & Piironen, 2001).

The cultivation of edible mushrooms carries great relevance in todays' world, in the context of a burgeoning population growth and extreme pressure on the environment (Chakravarty, 2011). It can best serve to reduce hunger in developing nations, and to revitalize rural economies. It provides ample opportunities for gainful employment for women, landless labourers and unemployed youth (Kushwah & Chaudhary, 2016). It can also play an important role in managing organic wastes which have become problematic for disposal and ensure

sustainable agriculture.

The mushroom industry is expanding globally, with world production being greater than two million tonnes annually (Ragunathan & Swaminathan, 2003). This increasing trend is not an exception in Ghana, as mushroom production has grown steadily from 120 tons in 2010 to approximately 300 metric tons in 2014 (Obodai, Ofori, Dzomeku, … & Sonnenberg, 2014). In Ghana, mushrooms are a delicacy (Quarcoo & Adotey, 2013) and the total amount consumed is on the rise although the variety of mushrooms consumed is heavily biased towards *Pleorotus ostreatus* (Quarcoo, Adotey, & Gordon, 2014).

The most cultivated mushrooms worldwide are *Agaricus bisporus*, *Lentinula edodes*, *Pleurotus spp* and *Flammulina velutipes* (Miles & Chang, 2004). *Pleurotus* spp rank second to *Agaricus bisporus* (Sánchez, 2010). These species require shorter growth time, demand few environmental controls, can also be cultivated with simple and low cost production technology, and have higher biological efficiency(Sánchez, 2010; Bonatti, Karnopp, Soares, & Furlan, 2004). They grow on most lignocellulosic materials such as rotten or rotting wood, wood residues and most agricultural wastes (Straatsma, Gerrits, Thissen, Amsing, Loeffen, & Van Griensven, 2000; Yildiz & Karakaplan, 2003).

Although mushroom cultivation has not been a traditional venture as compared to its harvest from the wild (Lee, Chang, & Noraswati, 2009; Obodai et al., 2014), oyster mushrooms have been cultivated in Ghana since 1990 (Obodai *et al*., 2014). In Ghana, mushroom cultivation is usually carried out on composted sawdust of *Triplochiton scleroxylon* or a combination of *T. scleroxylon* and *Chlorophora excels*a (Obodai *et al*., 2014). Despite the increasing demand for mushrooms, the scarcity and increasing cost of sawdust will cause a decline in successful production. Obodai *et al*., (2014) suggest that there are other agricultural by-products available that can be used in mushroom production in both urban and peri-urban areas of Ghana and the subtropics. In the search for alternative substrate for the commercial mushroom cultivation, the use of some agro-wastes as substrates have been evaluated in Ghana (Adjapong, Ansah, Angfaarabung, & Sintim, 2015; Obodai *et al*., 2014).

The objective of this study, therefore, was to evaluate the use of *Paspalum* grass as an alternative substrate for oyster mushroom cultivation, assess its effect on the yield, and the effect of the growth substrate on the nutritional composition of oyster mushrooms.

2. **Materials And Methods**

The experimental setup was made up of six treatments in a randomized complete block design with four replications. The treatments were as follows: *Paspalum* grass (100%), *Paspalum* grass + Sawdust (1:1), *Paspalum* grass + 20% Rice bran, *Paspalum* grass + 10% Rice bran, *Paspalum* grass + 5% Rice bran, and Sawdust (traditional method).

2.1 Spawn preparation:

Sorghum seeds were washed in clean water to remove the chaff, dust and other particles. It was then soaked in water for 12 h for maximum absorption of water. It was drained with a mesh and then put into a spawn bottle measuring 300ml. Each spawn bottle was mixed with calcium carbonate and sterilized in an autoclave at 121^{o}C for 2h after which it was cooled for 24 hours. The cooled bottles were placed under aseptic condition in a dark place and incubated at 27^{o}C.

2.2 Sawdust:

The sawdust was prepared according to the composting method (Ryckeboer et al., 2003). Fifteen kilograms of sawdust from *T. scleroxylon* was spread on a cemented floor and 0.5kg each of both lime and rice bran were added and thoroughly mixed together as additives. Water was sprinkled on the mixture to moisten and enhance decomposition. The substrate was gathered in a heap form and covered with a polythene sheet. It was stirred every 4 days to ensure uniform fermentation for a 30-day period.

2.3 Grass straw:

The grass was soaked in a barrel overnight to moisten it and then wash thoroughly. The barrel was ⅔ filled with water to enhance proper absorption. The soaked grass was spread on a polythene sheet to drain excess moisture. The required amount of rice bran and lime were thoroughly mixed with the grass straw as additives, with the

exception of the 100% grass straw substrate and where no additive was added.

2.4 Bagging, Spawning, Incubation, and Culturing:
The substrates were packed into heat resistant polythene bags (1kg each) and labelled. A wooden mallet was used to compress the bagged substrates to make them compact. To avoid the flow back from the polythene bag, a 2.0cm wide and 2.5cm long polyvinyl chloride (PVC) pipe was inserted at the neck of each bag and held in place with an elastic band. The bagged substrates were sterilized in a well cleaned barrel for 2h at 121^{o}C. The substrate bags were then inoculated with the spawn and a sterilized cotton wool was inserted at the necks of the PVC pipe to prevent contamination. The bags were then incubated for 30 days at 23^{o} - 27^{o}C and 95% relative humidity. The elastic band, and the cotton wool were removed after the incubation. Round-shaped openings were made at the top of the spawn bags with a sharp knife to allow for the passage of fruiting bodies. Water was sprinkled on the openings 3 times a day (morning, afternoon and evening) to maintain a relative humidity of 80-85%.

2.5 Harvesting, Data collection and Analysis
The fruiting bodies of the mushrooms were harvested as soon as the gills were formed and the edge still curled under. Harvesting was done by gently pulling the mushrooms from the substrates. Harvesting continued as long as the mycelium remained white and firm on the substrates.

The following parameters were recorded per substrate:
a) number of days to first harvest;
b) number of days between harvests (harvest interval);
c) total number of harvests per substrate;
d) diameter of the mushroom cap;
e) length of the mushroom stalk;
f) number of fruiting bodies harvested; and
g) fresh weight of mushrooms harvested.

2.6 Proximate Analysis
All samples of the oyster mushroom used for this study were freshly harvested from a series of flushes of cultivated oyster mushroom in the University's mushroom house. Fruiting bodies for each of the six growth substrates were harvested from the respective bags. Each of the six treatments had four replications. The freshly harvested samples were carefully cleaned with a soft brush to remove foreign inedible materials. Randomly collected samples were then put into well labelled transparent polypropylene bags and sent to the University laboratory for analytical determination of moisture content. Weighed samples were dried in an electric oven at 105^{o}C for 4 hours. The dried samples were then milled into fine powder and stored in air-tight polythene bags for the proximate analysis. Analysis was carried out at the Soil Science laboratory of the Kwame Nkrumah University of Science and Technology. The analyses were carried out according to the guidelines of the Association of Official Analytical Chemist (AOAC, 2002) and on a dry weight basis. Parameters analysed were carbohydrate, crude protein, crude fat, crude fibre, ash, moisture content, iron, zinc, calcium, potassium and sodium.

2.7 Statistical Analysis
The data collected on the harvest indices were subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS) version 16. The Analysis of Variance (ANOVA) was computed and the means were separated using the Least Significant Difference (LSD) ($p<0.05$).

3. Results And Discussion

3.1 Growth and Yield of Oyster Mushroom
The effect of the substrates on the growth and yield of oyster mushrooms measured by the number of days to first harvest, number of times harvested, diameter of the mushroom cap, length of the stalk, total number, fresh weight of the mushrooms harvested per substrate and harvest interval are presented in Table 1.

Each mushroom species prefers a particular growing medium although some species can grow on a wide range of materials (Royse, Rhodes, Ohga, & Sanchez, 2004). *Pleurotus* species grow on a wider array of forest and agricultural wastes than any other mushroom group. The growth and fruiting of an individual mushroom species on a particular waste material will depend largely on the ability of that mushroom to produce the enzymes

essential to degrade the major components of the waste and thereafter absorb it as food (Royce *et al*, 2004).

The number of days to first harvest was significantly ($p<0.05$) affected by the type of substrates. Mushrooms cultivated on the *Paspalum* grass substrate with 5% rice bran were harvested earlier (6 days) than that of the sawdust, which took about 10 days for the first mushrooms to be harvested (Table 1). The average number of days between harvests ranged from 5 to 8 days. The harvest interval was significantly ($p<0.05$) shorter in the sawdust substrate (5.65 days) than the others, which took more than seven days.

The length of the stalk is an indication of the how long the mushroom is. The size of the cap also indicates how big the mushroom is. The size of the cap together with the length of the stalk measure the overall size of the mushroom. The results revealed significant differences ($p<0.05$) in the length of the stalks and cap size of mushrooms harvested from the various substrates. The stalks of the mushrooms cultivated on the sawdust substrate were significantly longer (6.53 cm) than those from substrates made from grass with rice bran additives. Mushrooms harvested from the sawdust had significantly bigger caps (7.67 cm) than those cultivated on the grass substrates (5.03 cm). However, mushrooms harvested from substrates consisting of grass-sawdust and grass-rice bran combinations were not significantly different from each other (Table 1).

The type of substrates used did not significantly affect the number of fruiting bodies harvested. The grass substrate recorded the highest number of mushrooms as against the traditional sawdust substrate with means of 49.33 and 33.67 respectively (Table 1). Substrates composed of grass and sawdust, and those with 5 – 10% rice bran additives grew more mushrooms than the traditional sawdust substrate. The means were, however, not significantly different from each other. The number of times mushrooms were harvested was not significantly ($p<0.05$) influenced by the substrates. Again, there were no significant differences in the total weight of fruiting bodies harvested from the various substrates (Table 1).

Table 1: Effect of Substrates on the Number of days to first harvest, number of harvests, total number and weight of mushrooms harvested, cap diameter and stalk length

Treatment	No. of Days to First Harvest	Harvest Interval (Days)	Mean Number of Harvests	Total No. of Fruiting Bodies Harvested	Total Weight (kg)	Cap Diameter (cm)	Stalk length (cm)
Grass only	7.25 ± 0.65ab	7.21 ± 0.32bc	3.75±0.33	49.33±9.47	109.33±14.36	5.03±0.23b	5.89±0.25b
Sawdust only	10.16 ±1.16c	5.65 ± 1.20a	4.00±0.00	33.67±8.30	155.92±25.04	7.67±0.64a	10.88±0.65a
Grass+ Sawdust	8.08 ± 1.10abc	6.32 ± 0.81b	4.08±0.29	39.83±7.07	99.50±15.68	5.33±0.13b	6.13±0.20b
Grass+20%Ricebran	9.50 ± 1.16bc	8.00 ± 0.01c	3.92±0.31	34.92±5.41	106.00±18.54	5.04±0.19b	5.80±0.18b
Grass+10%Ricebran	7.25± 0.39ab	7.92 ± 0.11c	4.33±0.28	40.58±5.78	137.17±21.40	5.04±0.18b	5.78±0.20b
Grass+5%Ricebran	6.17 ± 0.84a	7.87 ± 0.45c	3.58±0.40	43.08±8.15	108.08±17.81	4.77±0.45b	5.51±0.53b

Means with the same letter in a column are not significantly different at $p<0.05$

Table 2: Proximate (%) and Mineral composition (mg/g) of mushrooms as influenced by the growth substrate

Treatment	Protein (%)	Ash (%)	Fat (%)	Fibre (%)	Moisture (%)	Carbohydrate (%)	Fe (mg/g)	Zn (mg/g)	Ca (mg/g)	K (mg/g)	Na (mg/g)
Grass only	61.30d	6.83d	0.20a	14.59c	2.50d	14.58a	8.00a	5.20a	0.11c	3.43b	0.65f
Sawdust only	35.27a	4.99a	0.50b	11.01a	2.51d	45.75e	10.10c	6.90e	0.77d	3.59c	0.36b
Grass+ Sawdust	58.67c	5.16ab	0.48b	14.09c	3.86e	17.79b	7.47b	6.50d	0.09b	3.20a	0.44d
Grass +20%Ricebran	49.71b	5.33b	0.59c	14.20c	0.43a	30.01cd	7.20b	5.40b	0.09b	3.75d	0.35a
Grass +10%Ricebran	48.50b	5.77c	0.50b	16.15d	1.41c	28.64c	7.93a	6.37c	0.06a	3.89e	0.41c
Grass +5%Ricebran	49.74b	5.12ab	0.58c	13.10b	0.85b	30.51d	8.30a	6.60d	0.11c	3.75d	0.50e

Means in a column with the same letter are not significantly different at p<0.05

Although mushrooms produced on the sawdust substrate were heavier than the other substrates, the mean weights were not significantly different. This is an indication that the substrates did not significantly influence the weight of mushrooms harvested. The results revealed significant positive correlations ($p<0.01$) among the parameters measured. The length of the stalk and size of the cap were significantly positively correlated ($r=0.91$). The total weight of the mushrooms harvested was positively correlated with the stalk length ($r = 0.53$) and cap size ($r=0.48$). The number of fruiting bodies harvested was also positively correlated ($p<0.01$) with the total weight of the mushroom. The harvest interval was also positively correlated ($p<0.01$) with the cap size ($r=0.61$) and stalk length ($r=0.57$).

The number and fresh weight of fruiting bodies of oyster mushroom harvested is an indication of yield. The current study showed that the yield of the mushrooms cultivated on the *Paspalum* grass was not significantly ($p<0.05$) different from that of the other substrates used. Although, the sawdust substrates produced mushrooms with bigger caps, longer stalks and heavier weight, the Paspalum straw substrate recorded the greatest number of fruiting bodies compared to the other substrates. Thus, the *Paspalum* straw has the potential to produce yields comparable to that of the sawdust which has been the main substrate used for mushroom production in Ghana. Unlike Royse *et al.*, (2004) who concluded that switch grass does not appear to be an economically viable alternative for growers currently using cotton seed hulls/wheat straw substrates, this study indicated the *Paspalum* grass could be a suitable substrate for use in oyster mushroom production. According to Royse *et al.*, (2004), cost effective production of oyster mushrooms depends on the reliability, availability and cost of substrate ingredients. Yang *et al*., (2016) have stated that selecting an economic and efficient substrate material to reduce production cost has been an important consideration in mushroom cultivation. Although sawdust is widely used, the rising price as a result of the increasing need by mushroom producers creates the need for alternative substrates. In Ghana, this *Paspalum* spp is a perennial native grass. It is fast growing, can withstand harsh environmental conditions, and could be obtained almost everywhere in the country. The grass is currently being used as lawn, hence its use in the mushroom industry will be cost effective. This makes it an economically viable alternative for growers currently using sawdust as the main substrate.

3.2 Proximate Analysis

The nutritional composition of the *Pleurotus ostreatus* as influenced by the various growth substrates is presented in Table 2. The findings of this study confirm that mushrooms are generally highly nutritious foods. The nutritional composition of mushrooms has been compared to that of eggs, milk and meat (Oei, 2003). Mushrooms are naturally low in sodium, fat, cholesterol, and calories and have often been referred to as functional foods. In addition to the provision of basic nutrition, mushrooms help prevent chronic disease due to the presence of antioxidants and beneficial dietary fibers such as chitin and beta-glucans. They also promote a healthy complexion and hair, increased energy, and overall lower weight.

Generally, high protein content ranging from 35.27 – 61.30% was recorded from the mushrooms with significant differences among the substrates used (Table 2). This confirms earlier reports. For example Barros, Venturini, Baptista, Estevinho, & Ferreira, (2008) stated that the protein content could be as high as 54% - 59% dm in some species of mushrooms. Among the six substrates, *P. ostreatus* produced on the *Paspalum* substrate had the highest protein content of 61.30% on a dry weight basis. The nutritional value of mushrooms is primarily related to their protein content. It is reported that mushrooms have higher quality protein than that of plant proteins (FAO, 1991). Digestible protein on dry weight basis normally ranges between 20% and 40% which is better than many leguminous sources like soybeans, peanuts, and other protein-yielding vegetable foods (Chang & Mshingeni, 2001; Chang & Buswell, 1996). In the present study, the higher protein content recorded in mushrooms produced on grass substrate is an indication that the grass has the potential to produce nutritious oyster mushrooms.

The type of substrate used significantly ($p<0.05$) affected the ash content of the mushrooms. The highest ash content was recorded in mushrooms grown on grass (6.83%), and the least was recorded in mushrooms grown on sawdust (4.99%). This result is different from the observation of (Manzi, Aguzzi, & Pizzoferrato, 2001) who reported an ash content of 0.8 - 0.9g in 100g of edible part of *P. ostreatus*.

The amount of fat recorded ranged from 0.20% - 0.59% with mushrooms produced on *Paspalum* substrate recording the lowest. The fat content is low when compared to that of carbohydrates and proteins. This agrees with earlier reports of fat content ranging from 0.2 to 8g per 100g of dried fruiting bodies from different studies with different species of *Pleurotus* (Hossain, Alam, Amin, Basunia, & Rahman, 2007).

The fibre contents ranged from 11.01% - 16.15% (Table 2). These values are however within those obtained by (Manzi et al., 2001) who observed that some mushrooms were low in crude fibre of about 5% dm while others were as high as 40%. Afiukwa et al., (2013) recorded a fibre content of 29% in *P. ostreatus* which is significantly higher than the results obtained from this study. Ahmed, Kadam, Mane, Patil, & Baig, (2009) recorded a fibre value of 7.40 - 8.02%, which is lower than the results of this study. Mushrooms are valuable source of dietary fibre which plays important role in human nutrition. Fibre is an important part of a healthy balanced diet which can help prevent a number of health conditions including diabetes, weight gain, heart disease and some cancers. It also improves digestive health by improving the function of the alimentary tract and also lowering blood glucose and cholesterol levels (Manzi, Marconi, Aguzzi, & Pizzoferrato, 2004).

Moisture content ranging between 0.43% - 3.86% was obtained from mushrooms cultivated on the six substrates with significant differences ($p<0.01$) between them (Table 2). Okechukwu, Okereke, Onyedineke, & Obi, (2011) recorded moisture content of 3.0 – 10.55% which is higher than the range recorded for this study. The low moisture content could be attributed to the dried samples used in this study. Carbohydrate content of 14.58% and 45.75% were recorded in mushrooms cultivated on *Paspalum* and sawdust respectively.

The study revealed significant differences ($p<0.01$) in the mineral composition of the mushrooms cultivated on different substrates (Table 2). The study recorded an Iron (Fe) content of 7.2 - 10.10mg/100g. This is higher than those observed by Khan, (2010) which ranged between 5.5 to 6.5 mg/100g but lower than that of Ahmed *et al.*, (2009) who recorded a range of 11.87 - 12.38 mg/100g among *P. ostreotus* cultivated on various agro-wastes. The zinc content ranged between 6.6 - 6.9 mg/100g. This is higher than the 0.1 – 1.3mg/100g recorded by Afiukwa et al., (2013) but within the range of 3.0 – 27.0 mg/100g reported by Khan, (2010). The study revealed significant differences in the calcium contents of 0.1mg/100g from mushrooms cultivated on the various substrates. Significant differences ($p<0.01$) were also observed in the potassium (K) content of mushrooms with a range of 0.35 – 0.65g/mg from the various substrates. Good amounts of iron and zinc were recorded from the mushrooms. Iron is essential for the biosynthesis of the oxygen-carrying pigment of red blood cells and the cytochromes that function in cellular respiration.

Zinc is indispensable in numerous biochemical pathways as an important co-factor for certain enzymes. The zinc content obtained from this study (6.6 - 6.9mg/100g) also meets about 44% – 46% of the recommended daily intake of zinc for both men and women which is about 15mg. Potassium is very important in the maintenance of osmotic balance between cells and the interstitial fluid in animal systems (Okechukwu *et al.*, 2011). In this study, the potassium content which ranged from 3.20 – 3.89mg/100g would be an excellent food source for lowering blood pressure, reducing the risk of osteoporosis, and in maintaining bone health.

Generally, increasing consumption of mushrooms appears to decrease the risk of obesity and overall mortality, diabetes, and heart diseases. Since the growth substrate tends to have an influence on the nutritional quality of the mushrooms cultured on them, the use of substrates that will produce mushrooms of the highest quality should be promoted. In this regard, the *Paspalum* grass seems to be one of such promising substrates since it produced mushrooms with high protein and sodium contents but low in fat and carbohydrates.

4. Conclusion

The study showed that the yield of mushrooms cultivated on the *Paspalum viginatum* substrate was not significantly ($p<0.05$) different from that of the sawdust which is widely used for commercial oyster mushroom production. Although the sawdust substrate produced heavier mushrooms with bigger caps and longer stalks, the mushrooms on the *Paspalum* substrate flushed earlier and recorded the greatest number of fruiting bodies. The substrates significantly ($p<0.01$) affected the nutritional composition of the oyster mushrooms. The Paspalum straw substrate produced healthier and nutritious oyster mushrooms with high protein and zinc but low fat and carbohydrate contents than that of mushrooms cultivated on the sawdust substrate.

Since the performance of the grass in terms of the parameters studied were comparable to that of the sawdust, which has been the main substrate used for mushroom production, it can be concluded that *P. vaginatum* promises to be an economically viable and environmentally friendly alternative growth substrate for the mushroom industry. The use of grass for mushroom cultivation will reduce the dependence on sawdust. It is recommended that the harvesting stage of the grass be evaluated to ascertain the influence of the age of the grass on oyster mushroom yield. The optimum level of additives should also be evaluated.

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