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Original Research Article

Substrate Effects on the Yield, Proximate, Phytochemical and Vitamin Attributes of *Pleurotus pulmonarius* Mushroom

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ABSTRACT

The yield attributes and nutrient compositions of *Pleurotus pulmonarius* under four different substrates (maize-kernel, Empty Palm Fruit Bunch (EPFB), Dry Banana Leaf (DBL), and the Control) were studied in a humid tropical environment. The experimental design was a completely randomized design with nine replications. Variations in the morphological, proximate, vitamin, and phytochemical attributes of the mushrooms were recorded. The data were subjected to statistical analysis. The result revealed a significant substrate effect on the studied attributes. The DBL substrate significantly improved the pileus circumference (39.07 cm), pileus area (124.6 cm), length of stipe (6.37 cm), pileus diameter (12.43 cm), and moisture content in *P. pulmonarius*, while the maize-kernel substrate had significantly higher protein (4.6%) and flavonoids (14.42%) contents compared to other substrates. The substrate from EPFB gave significantly higher levels of vitamin B1 (3.8 mg/100 g), vitamin B2 (5.7 mg/100 g), vitamin C (114 mg/100 g), and vitamin E (17.4 mg/100 g) than the other substrates. The correlation results reveal that the concentrations of phytochemicals in *P. pulmonarius* tend to decrease as the morphological attributes expand. This substrate effect can be harnessed to produce mushrooms for specific needs. EPFB substrates can be used to enhance dietary supplementation and antioxidant activity in human nutrition. Mushrooms grown on the substrate formulated with maize kernel could serve as an alternative for animal protein because of their high protein content, while mushrooms produced on the DBL substrate with the highest yield in most attributes are recommended for commercial cultivation of *P. pulmonarius*.

***Keywords*:** Agricultural waste, hidden hunger, mushroom production, oyster mushroom, substrate effects, and yield

Introduction

Pleurotus pulmonarius is a nutrient-dense oyster mushroom of global recognition with a lot of therapeutic benefits. It has umbrella-like fruiting bodies and belongs to the class of Basidiomycetes and order of Agaricales.[1] *P. pulmonarius* contains proteins, dietary fibre, macro- and micro-minerals, and other medicinal phytochemicals of pharmaceutical importance. Mushrooms contain antioxidants, which protect against cancer formation and free radical generation in human and animal cells.[2,3] When consumed regularly, mushrooms may have therapeutic effects by preventing some dietary aliments associated with other food nutrient deficiencies.

P. pulmonarius is an edible fungus that grows in both tropical and subtropical rainforests.[4] Beyond their wild existence, cultivated mushrooms are extensively distributed. Mushrooms can be grown from a variety of agricultural wastes.[5] The use of agro-waste substrate as a raw material in *P. pulmonarius* production not only solves waste management concerns, but it also generates revenue and creates jobs.[6]

Successful conversion of agricultural wastes into reusable resources is a strategic means for sustainable agriculture.[7] Profitable mushroom production should imbibe the use of low-cost inputs like agro-waste for substrate formulation. The inability of mushrooms to photosynthesize entails that they must rely on their growth substrate for their nutrient needs. Hence, nutrient availability in the substrate can influence the composition of the mushroom grown on it. Studies have demonstrated the influence of substrate on mushroom growth and composition.[2,8-10] Conventional substrates for mushroom production often contain *Gmelina arborea* sawdust mixed with rice bran, wheat bran, lime ($CaCO_3$), and gypsum ($CaSO_4$). Most components of the conventional substrate are byproducts or waste from local agricultural production processes. Wheat production in Nigeria, especially in the southern region, is very low, translating to low wheat bran production. Hence, wheat bran can constitute a limiting factor in commercial mushroom production in the region. This study postulates that the replacement of wheat bran with locally available agro-wastes can influence the growth and composition of *P. pulmonarius* differently. Hence, the objective of this study is to assess the influence of substrate composition on the proximate, phytochemical, vitamin and yield attributes of *P. pulmonarius*. Interestingly, research in the area of mushroom substrate formulation or development is still in a nascent phase in Nigeria. Therefore, the findings of this study will add to literature on substrate effect on mushroom. It will also be of interest to mushroom consumers, growers, dietitians, pharmacists and environmentalists.

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Materials and Methods

Materials

The following materials were used in the experiments: mushroom spores, mushroom spawns, sawdust, polyethylene bags, an autoclave (Model 18 L, Life Case Medical, China), cotton wool, potato dextrose agar, a hot air oven (Model BS-2648, Baid & Tatlock, England), a UV-Visible spectrophotometer (Model UV-7504, Cole-Parmer, USA), and analar-grade chemicals: lime (CaCO3) and gypsum (CaSO4).

Mushroom Collection and Identification

The mushroom spores and spawns of *P. pulmonarius* (identification number is UNN/13002-FUN) used for this study were obtained from the Department of Crop Science, University of Nigeria, Nsukka (6.8651340, 7.4129414) in June 2022. Nsukka is located in the derived savannah ecological zone and is 447.26 m above sea level. The maize kernel, empty palm fruit bunch, and dry banana leaf were obtained from the Department of Crop Science, University of Nigeria, Nsukka Farm.

Culture of Mushrooms

Fresh mushrooms were taken, and the surfaces were sterilized. Then, a small piece of the mushroom's inner tissues containing the spores was placed on potato dextrose agar medium using sterile forceps. The inoculated dishes were incubated at 27 °C for four days. Three sub-culturing were produced.[11]

Spawn Preparation

The spawn preparation was carried out with red sorghum grain. About 250 g of red sorghum was washed in clean water three times to remove chaff, dust, and other particles. The grains were boiled in clean water for 45 minutes and allowed to stay for 24 hours for maximum absorption of water. Soaked grains were washed in water, drained, and put into spawn bottles. Two-thirds (2/3) of each spawn bottle was filled with grains and mixed with 5 g of calcium carbonate ($CaCO_3$); thereafter, they were autoclaved at 121 °C (at a pressure of 1 kg/cm^2) for 2 hours each day for 3 consecutive days.[11] The grains in the bottles were inoculated with three 9-mm mycelium discs per bottle (in triplicate of each grain type) under aseptic condition.

Experimental design and treatments

The experiment was carried out to determine the effect of four different substrates on the *P. pulmonarius* mushrooms. The experimental design was a completely randomized design replicated nine times. The four substrate formulations (treatments) were the conventional substrate (control), maize-kernel substrate, Empty Palm Fruit Bunch (EPFB) substrate, and Dry Banana Leaf (DBL) substrate. The contents of the conventional (control) substrate consists of 10 kg of *G. arborea* sawdust mixed with 3 kg of rice bran, 2 kg of wheat bran, 200 g of lime ($CaCO_3$), and 30 g of gypsum ($CaSO_4$). The dry banana leaf substrate was made up of 10 kg of *G. arborea* sawdust mixed with 3 kg of rice bran, 2 kg of dry banana leaves, 200 g of lime, and 30 g of gypsum. The maize kernel substrate consists of 10 kg of *G. arborea* sawdust mixed with 3 kg of rice bran, 2 kg of maize kernel, 200 g of lime, and 30 g of gypsum. The empty palm fruit bunch substrate consists of 10 kg of *G. arborea* sawdust mixed with 3 kg of rice bran, 2 kg of empty palm fruit bunch, 200 g of lime, and 30 g of gypsum.

Mushroom cultivation from spawns

The mushrooms were cultivated from the spawn.[12] The formulated substrates were sterilized at 121 °C for 15 minutes in an autoclave. After the sterilization, mushroom spawn was introduced into 12 x 24 cm polypropylene bags containing 170 g of the substrate. The spawn was spread over the substrate. Small holes were perforated on the bags for aeration. The bag and its contents were placed in the dark room and maintained in a moist condition until the mushrooms were matured for harvest.

Data Collection

The data were collected on the morphological, nutritional, and phytochemical attributes of the harvested mushrooms from the four substrates. The length of the stipe (LS) and pileus diameter (PD) of three fruiting bodies from each mushroom sample were measured using a meter rule. The LS and PD were used to compute the stipe-pileus ratio (SPR), while the PD was used to calculate the pileus circumference (PC) and pileus area (PA).

Laboratory Analysis

The proximate content of the harvested *P. pulmonarius* was determined following the standard method of the Association of Official Analytical Chemists.[13] The proximate compositions are comprised of percentage ash, protein, fibre, moisture, fat, and carbohydrate contents.

Determination of ash content

Ash content was determined using a muffle furnace. A silica dish was heated to 450 °C, cooled, and weighed. The sample (2 g) was transferred into the dish and weighed. The dish was placed in the muffle furnace and ashed (heated at 450 °C) in a furnace for 3 hours and allowed to cool. The percentage ash content was calculated using the formula below:

$$\%Ash = \frac{Weight\ of\ ash}{Weight\ of\ fresh\ sample} X \frac{100}{1} \qquad equation\ 1$$

Determination of crude protein content

Crude protein content was determined using the micro-Kjeldahl apparatus. Oven-dried ground plant material (1 g) was carefully weighed into a 30 ml Kjeldahl flask, and 25 ml of concentrated H_2SO_4 was added. The catalyst (a mixture of selenium oxide, potassium sulphate, and copper sulphate) (1 g) was also added. This was heated cautiously on the digestion rack under the fume hood until a clear greenish solution appeared. After the digestion had cleared, the mixture was heated for another 30 minutes and allowed to cool. About 10 ml of distilled water was added to avoid caking and then transferred to the Kjeldahl distillation apparatus. About 10 ml of 40% NaOH was added to the mixture and allowed to distill. The distillate was titrated to a first pink colour with 0.01 HCl, and the concentration of proteins was calculated using the formula shown below:

$$\%Nitrogen = \frac{Titre\ value\ x\ 14.1\ x\ 0.01\ x\ 100\ x\ s}{1000\ x\ weight\ of\ sample} \qquad equation\ 2$$

$$\%Protein = \%Nitrogen\ x\ 6.25 \qquad equation\ 3$$

Determination of crude fat content

This was done using a Soxhlet extractor. An extraction flask was thoroughly washed and dried in a hot oven for 30 minutes. It was placed in a desiccator to cool. A 2 g of ground sample was weighed and placed inside the extractor thimble, which was put inside the Soxhlet extractor. Some petroleum ether, or about three-quarters of the volume of the flask, was added. The apparatus was set up, then heated and allowed to run for 4 hours. The petroleum ether was recovered at the end of the extraction before the thimble was removed. The oil collected in the flask was dried at 100 °C in an oven and then weighed. The percentage (%) fat content was calculated using the following formula:

$$\%Fat = \frac{(Weight\ of\ flask + oil\ after\ drying) - Weight\ of\ sample}{Weight\ of\ empty\ flask} X \frac{100}{1} \qquad equation\ 4$$

Determination of crude fibre

This was done by adding preheated H_2SO_4 (150 ml) to 2 g of the ground mushroom and then boiling for 30 minutes before the solution was filtered. The residue was washed three times with hot water. To this was added 150 ml of pre-heated KOH and heated to boiling. Some drops of anti-forming agent were added, boiled slowly for 30 minutes, and then filtered. The residue was washed three times with hot water and another three times with acetone. It was dried at 1030 °C for 1 hour, weighed,

heated at 500 °C, and then weighed again. Percentage fibre was calculated using the formula shown below:

$$\%Fibre = \frac{(Weight\ of\ residue\ after\ drying) - Weight\ of\ ash}{Weight\ of\ ground\ sample\ used} X \frac{100}{1} \quad equation\ 5$$

Determination of moisture content

Two grams (2 g) of the ground extract were dried to a constant weight at 105 °C in a hot air-circulating oven for 24 hours. The moisture content was calculated as the difference in weight after drying, as shown below:

$$\%Moisture = \frac{Weight\ of\ dried\ sample - Weight\ of\ ground\ sample\ used}{Weight\ of\ ground\ sample\ used} X \frac{100}{1} \quad equation\ 6$$

Carbohydrate Content Determination

The carbohydrate content was determined by the difference method.[13] The percentages of protein, fat, ash, moisture, and crude fibre are summed up and subtracted from 100%.

Determination of Flavonoids

Flavonoids were determined using a gravimetric method.[14] Five grams of the mushroom were weighed into a flask, and the flavonoids were extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. Thereafter, it was filtered with Whatman filter paper No. 43 (125 mm), and the filtrate was transferred into a weighed beaker and evaporated to dryness to get the weight of the flavonoids. The percentage of flavonoids was calculated using the formula below.

$$Flavonoid(\%) = \frac{(Weight\ of\ beaker + flavonoid) - Weight\ of\ beaker}{Weight\ of\ ground\ sample\ used} X \frac{100}{1} \quad equation\ 7$$

Determination of Tannins

Tannin was determined using the spectrophotometric method.[14] One gram of the mushroom sample was weighed, and 10.0 ml of distilled water was added. This was shaken at a 5-minute interval for 30 minutes. The solution of the sample was centrifuged or filtered to get the extract. Then, 2.5 ml of the supernatant was transferred into a test tube. This was added to 1.0 ml of Folin-Denis reagent, followed by 2.5 ml of saturated Na_2CO_3 solution. About 2.5 ml of a standard tannic acid solution and blank were also prepared along with the test sample, and the absorbance was measured after 90 minutes of incubation at room temperature. The percentage tannin was calculated as follows:

$$Tannin(\%) = \frac{Absorbance\ of\ test\ sample}{Absorbance\ of\ standard\ solution} X \frac{Conc.\ of\ standard\ solution}{1} X \frac{100}{weight\ of\ sample} X \frac{Total\ vol.\ of\ extract}{Vol.\ of\ extract\ analyzed} \quad equation\ 8$$

Determination of Saponin

The determination of the saponin in the mushroom sample was carried out using a gravimetric method.[14] Two grams of the sample were weighed into a 100-ml conical flask. This was added to 20 ml of 20% aqueous ethanol. The solution of the sample was then heated in a hot water bath for 4 hours with continuous stirring at about 55 °C. This was filtered and re-extracted with another 20 ml of 20% ethanol. The extract was concentrated in the water bath to 40 ml at 90 °C before 20 ml of diethyl ether was added and shaken vigorously to separate the aqueous layer. The aqueous layer was recovered, 60 ml of n-butanol was added, and it was washed twice with 10 ml of 5% aqueous sodium chloride. This was evaporated in the oven and then quantified in percentage.

Determination of alkaloid

The alkaloids present in the mushrooms were determined by weighing 5 g of the test sample into a 100-ml beaker before 50 ml of 10% acetic acid in ethanol was introduced into it. The beaker and its contents were covered and allowed to stand for 4 hours, after which they were filtered. Thereafter, 10 ml of ammonium hydroxide was introduced into the filtrate to precipitate the alkaloids. The precipitate was filtered, weighed, and dried, and the percentage alkaloid calculated.[14]

$$Alkaloid(\%) = \frac{(Weight\ of\ filterpaper + Alkaloid) - Weight\ of\ filter\ paper}{Weight\ of\ ground\ sample\ used} X \frac{100}{1} \quad equation\ 9$$

Determination of Total Phenol

Total phenol was determined using the spectrophotometric method.[15] Two grams of the test sample were weighed into a flask. This was defused with petroleum ether and allowed to stand for a few minutes to air dry. The residues were boiled with 50 ml of diethyl ether for 15 minutes to extract the phenol, and 5 ml of the aliquots of the boiled sample were transferred into the test tube. This was added to 2 ml of NH_4OH solution and 5 ml of amyl alcohol and allowed to stand for 30 min. for colour development. The standard and the blank were prepared the same way, and the concentration of phenol was calculated.

$$Phenol\ (mg/100g) = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} X \frac{Conc.\ of\ standard}{1} \quad equation\ 10$$

Determination of Thiamin

The thiamin content of the mushrooms was determined using a UV spectrophotometer.[16] One gram of the sample was extracted with 100 ml of 0.2 N HCL, boiled for 30 minutes, and the resultant solution was treated with 5 ml of phosphatase enzyme. This was incubated at 37 °C to liberate free thiamin. The sample was filtered, and 3 g of anhydrous Na_2SO_4 was added. Five ml of the solution was pipetted into a 5-ml stopped flask, and 3 ml of 15% NaOH was added. The absorbance was taken at the 435 nm wavelength, and the concentration of the thiamin was calculated.

$$Thiamin = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} X \frac{Conc.\ of\ standard}{weight\ used} \quad equation\ 11$$

Determination of Riboflavin

The riboflavin content of the mushrooms was also determined using a spectrophotometric method.[16] Five grams of the sample were extracted with 50 ml of 0.2 NHCL and boiled in a water bath for one hour. This was cooled, and the pH was adjusted to 6.0 using NaOH. Thereafter, 1N HCL was added to lower the pH to 4.5, and this was filtered into a 100-ml measuring flask, and the volume was made up to mark. Then, a 10 ml aliquot from the 100 ml volume was added to 1 ml of acetic acid (glacial) in a test tube, mixed, and 0.5 ml of 3% $KMnO_4$ solution. This was left for 2 minutes before 0.5 ml of 3% H_2O_2 was added and mixed before the absorbance reading was taken at 470 nm. The concentration of riboflavin was calculated as follows:

$$Riboflavin = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} X \frac{Conc.\ of\ standard}{weight\ used} \quad equation\ 12$$

Determination of Pro-Vitamin A

A standard procedure was used for the determination of pro-vitamin A.[16] One gram of the sample was weighed into a test tube. Then, the proteins were first precipitated with 3 ml of absolute ethanol before the extraction of pro-vitamin A with 5 ml of heptane. The test tube containing this was shaken vigorously for 5 minutes. On standing, 3 ml of the heptane layer was taken up in a cuvette and read at 450 nm against a blank of heptane. The standard was prepared and read at 450 nm wavelength, and pro-vitamin A was calculated from the standard.

Analysis of Vitamin C

The determination of vitamin C was carried out by weighing five grams of mushroom into a 100-ml volumetric flask. This was added to 25 ml of 20% meta-phosphoric acid as a stabilizing agent and diluted to the volume with distilled water. Ten ml of the solution was pipetted into a small flask, and 2.5 ml of acetone was added. This was filtered, and the absorbance was read in a UV spectrophotometer at a wavelength of 264

nm using water as the blank. The concentration of vitamin C was calculated from the standard.[17]

Determination of Vitamin E

The vitamin E content of the mushrooms was determined by weighing five grams of the test sample into a 100 ml beaker containing 50 ml of 0.1 N H_2SO_4. This was boiled in a water bath and then filtered. Thereafter, 5 ml of the filtrate was mixed with 5 ml of ethanol before the mixture was distilled and the distillate collected. The standard curve and the blank were prepared, and the absorbance was taken at 470 nm wavelength in a UV-visible spectrophotometer. The concentration of the test sample was calculated from curve curve.[16]

Statistical Analysis

The data collected were subjected to analysis of variance (ANOVA) using GenStat Release 10.3 Discovery Edition statistical software (VSN Int. Ltd., Hempstead, UK). The correlation coefficient was used to determine the relationship among the morphological and phytochemical attributes determined.

Results and Discussion

Morphological Attributes

The data presented in Table 1 highlights the significant impact of substrate composition on the morphological attributes of *P. pulmonarius*. Among the substrates tested, the dry banana leaf proved to be the most effective, producing mushrooms with the highest values in pileus circumference, pileus area, diameter of pileus, length of stipe, and stipe-pileus ratio. This suggests that the dry banana leaf substrate provides a more conducive environment for the growth and development of these morphological traits compared to the maize kernel and the conventional (control) substrates. In contrast, the control substrate consistently yielded the lowest values across all measured attributes, indicating its relative inefficacy in supporting robust mushroom growth. Substrate compositions have been assumed to influence mushroom quantity and quality[2]. Notably, while the empty palm fruit bunch substrate resulted in the highest mushroom weight, this difference was not statistically significant when compared to the weights produced by the other substrates. This indicates that, although there is variability in the effectiveness of different substrates, several can support similar levels of mushroom weight under the conditions tested. This points to the importance of substrate selection in mushroom cultivation, as the nutritional and physical properties of the substrate can greatly influence its growth performance and morphological characteristics. Earlier studies recommended banana tree wastes as a promising raw material for the cultivation of *P. ostreatus*.[18] Optimizing substrate composition could be a key strategy for enhancing yield and quality in mushroom production.

Proximate Compositions

The proximate compositions of *P. pulmonarius* grown on different substrates, as shown in Table 2, reveal considerable variations attributable to substrate composition. Mushrooms cultivated on dry banana leaf substrate exhibited the highest moisture content (88.73%), ash content (3.00%), and fiber content (1.500%), indicating that this substrate may enhance water retention and mineral absorption. Conversely, mushrooms grown on maize kernel substrate had the highest protein (4.60%) and carbohydrate (14.549%) contents, suggesting that maize kernel may provide more nitrogen and energy sources essential for protein and carbohydrate synthesis. The control substrate resulted in moderate moisture content (79.97%) and ash content (2.55%) but lower protein (2.35%) and carbohydrate (13.705%) levels, underscoring its lesser efficacy compared to other substrates. These findings align with Chukwurah et al.,[19] who demonstrated that mixed agricultural waste substrates significantly influence the growth and nutritional quality of mushrooms. Similarly, Adebayo et al.[20] and Jimoh et al.[21] emphasized that specific substrates, such as palm fruit husk and rice straw, optimize yield and nutritional content, particularly protein and carbohydrate levels. Furthermore, Garuba et al.[22] and Adebayo et al. [20] noted that substrates like banana leaves and cassava peels could enhance proximate constituents such as starch and fiber, reflecting the results observed with dry banana leaf substrate in this study. These insights suggest that the choice of substrate is crucial for optimizing the nutritional quality of *P. pulmonarius*, with different substrates providing distinct advantages depending on the desired nutritional profile. Utilizing a diverse range of agro-wastes, as recommended by these studies, can enhance both the yield and the nutritional content of cultivated mushrooms, making them more beneficial for consumers and profitable for growers.

Vitamin Compositions

The vitamin composition of *P. pulmonarius* grown on different substrates reveals significant variations influenced by substrate type, as presented in Table 3. Mushrooms cultivated on empty palm fruit bunch substrate demonstrated the highest levels of vitamins B1 (3.80 mg/100g), B2 (5.70 mg/100g), C (114.00 mg/100g), and E (17.40 mg/100g), suggesting that this substrate is particularly effective in enhancing the vitamin content of mushrooms. In contrast, those grown on dry banana leaf substrate exhibited the lowest levels of these vitamins, indicating that this substrate may not be as conducive for vitamin enrichment. The control substrate yielded moderate vitamin levels, demonstrating its relative efficacy compared to the other substrates used. These findings are consistent with Elattar et al.,[23] who reported that substrate composition significantly affects the nutritional profile of mushrooms, including vitamin content. Similarly, Dunkwal and Jood[24] highlighted that substrate variations, such as wheat and brassica straw, influence the vitamin B1 and B2 content in *Pleurotus sajor-caju*. Garuba et al.[22] and Jimoh et al.[21] observed that different agricultural waste substrates can markedly alter the vitamin composition of *Pleurotus* species, emphasizing the importance of substrate selection in mushroom cultivation. Rózsa et al.[25] further confirmed that substrate enhancements, like the addition of proteinaceous materials, can lead to higher vitamin concentrations in mushrooms. These studies collectively underscore the critical role of substrate choice in optimizing the vitamin content of cultivated mushrooms, with implications for both nutritional value and commercial viability. The evidence suggests that utilizing substrates such as empty palm fruit bunch can significantly enhance the vitamin profile of *P. pulmonarius*, thereby improving its health benefits and market appeal.

Table 1: Substrate effect on the morphological attributes of *P. pulmonarius*

Substrates	WT (g/hypha)	CL (cm)	AR (cm^2)	DI (cm)	LS (cm)	SPR
Maize Kernel	40.40	24.30	56.50	8.40	4.83	0.58
Empty Palm Fruit Bunch	54.80	29.52	77.80	9.80	5.20	0.54
Dry banana leaf	41.80	39.07	124.6	12.43	6.37	1.09
Control Substrate	25.60	19.20	32.70	7.00	3.50	0.61
LSD ($p<0.05$)	n.s.	11.64	58.08	3.83	1.35	0.89

Mushroom Weight (WT), pileus circumference (CL), pileus area (AR), diameter of pileus (DI), length of stipe (LS), stipe-pileus ratio (SPR) and n.s. = non-significant.

Table 2: Proximate compositions of *P. pulmonarius* grown in the four different substrates

Substrates	Moisture (%)	Ash (%)	Protein (%)	Fibre (%)	Fat (%)	Carbohydrate (%)
Maize Kernel	76.33	2.80	4.60	1.400	0.321	14.549
Empty Palm Fruit Bunch	81.35	2.05	3.50	1.025	0.543	11.532
Dry banana leaf	88.73	3.00	2.45	1.500	0.500	3.820
Control Substrate	79.97	2.55	2.35	1.225	0.200	13.705
LSD ($p<0.05$)	9.41	1.44	1.88	0.520	0.330	7.290

Table 3: Vitamin compositions of *P. pulmonarius* grown with four different substrates

Substrates	Vitamin B_1 (mg/100g)	Vitamin B_2 (mg/100g)	Vitamin C (mg/100g)	Vitamin E (mg/100g)
Maize Kernel	2.00	3.00	60.00	8.40
Empty Palm Fruit Bunch	3.80	5.70	114.00	17.40
Dry banana leaf	0.80	1.20	24.00	4.80
Control Substrate	1.60	2.40	48.00	6.60
LSD ($p<0.05$)	1.12	1.99	7.65	4.98

Phytochemical Compositions

The mushroom grown in the substrate formulated with maize kernel was significantly higher in percentage flavonoids content when compared with other substrates. Mushroom grown in the conventional substrate (Control) was significantly higher in phenol and alkaloid, saponnin and glycosides when compared with mushrooms grown with other substrates. The tannin content of the mushroom grown in the substrate formulated with empty palm fruit bunch was significantly higher, although statistically the same with that grown with the conventional substrate (Table 4).

The mushroom grown in the substrate formulated with maize kernel that had higher flavonoids content when compared with other substrates could be harnessed as one of the safe healthy natural sources of spices because of the flavour. Flavonoids are also considered important in the antioxidant activities.[26] Antioxidants are substances that can neutralize oxidative activities in the body by donating an electron or hydrogen atom to the free radicals.[27]

Correlation coefficient

Table 5 presents correlation coefficients examining the relationships between selected morphological and phytochemical attributes of *P. pulmonarius*. Among the phytochemical attributes, significant correlations were observed between flavonoid and phenol ($r = -0.526$), indicating a moderate negative relationship. Similarly, phenol showed positive correlations with Alkaloid ($r = 0.409$) and saponnin ($r = 0.573$), suggesting that mushrooms with higher phenolic content tend to also contain increased levels of alkaloids and saponins. Conversely, glycosides exhibited negative correlations with flavonoid ($r = -0.537$), indicating an inverse relationship between these two phytochemical attributes. The negative correlation between mushroom weight and phenol suggests that mushroom size may influence phenolic content, potentially due to dilution effects or metabolic differences. Additionally, the positive correlations between phenol and alkaloid as well as saponnin suggest potential co-regulation or metabolic pathways that influence the production of these compounds.

In terms of morphological attributes, mushroom weight displayed a negative correlation with both phenol ($r = -0.488$) and glycosides ($r = -0.445$), suggesting that heavier mushrooms may tend to have lower concentrations of these phytochemicals. Circumference, area, and diameter of pileus also showed significant correlations with various phytochemical attributes. Length of stipe displayed a strong negative correlation with Tannin ($r = -0.600$), indicating that mushrooms with longer stipes tend to have lower tannin content. The negative correlations between flavonoid and glycosides with various morphological attributes indicate complex relationships where certain morphological features may influence the biosynthesis or accumulation of specific phytochemicals. Understanding these correlations can aid in optimizing cultivation practices to enhance desired phytochemical profiles in *P. pulmonarius*. Optimizing the production protocol of plants will help maximize its benefits.[28] Further research is warranted to elucidate the underlying genetic and environmental factors that contribute to these relationships, which could inform breeding and cultivation strategies aimed at producing mushrooms with tailored phytochemical compositions for potential health benefits.

Table 4: Phytochemical compositions of *P. pulmonarius* grown with four different substrates

Substrates	Flavonoids (%)	Phenol (%)	Tannin (%)	Alkaloid (%)	Saponnin (%)	Glycosides (%)
Maize Kernel	14.42	0.11	0.005	2.5	2.0	0.28
Empty Palm Fruit Bunch	3.73	0.23	0.009	2.0	1.5	1.00
Dry banana leaf	4.14	0.45	0.003	2.5	2.0	0.65
Control Substrate	4.00	1.02	0.008	3.0	3.00	1.25
LSD ($p<0.05$)	4.02	0.05	0.002	ns	1.49	0.94

n.s. = non-significant

Table 5: Correlation coefficients among selected morphological and phytochemical attributes of *P. pulmonarius*

	Fla	Phe	Tan	Alk	Sap	Gly	WT	CL	AR	DI	LS	SPR
Fla	1											
Phe	-0.526	1										
Tan	-0.396	0.227	1									
Alk	0.126	0.409	-0.285	1								
Sap	-0.212	0.573	0.081	0.168	1							
Gly	-0.537	0.526	0.312	0.513	0.530	1						
WT	-0.028	-0.488	0.073	-0.232	-0.445	-0.108	1					
CL	-0.174	-0.333	-0.548	0.050	-0.187	0.088	0.424	1				
AR	-0.161	-0.322	-0.517	-0.011	-0.208	-0.001	0.397	.969**	1			
DI	-0.162	-0.310	-0.513	0.113	-0.175	0.123	0.404	.948**	.958**	1		
LS	-0.037	-0.508	-.600*	-0.029	-0.252	-0.035	0.443	.822**	.771**	.862**	1	
SPR	-0.144	0.039	-0.365	-0.048	0.033	-0.091	-0.040	0.084	0.020	0.082	0.426	1

"*" and "**" = Correlation is significant at 0.05 and 0.01 probability levels, respectively, Fla = Flavonoid, Phe = Phenol, Tan = Tannin, Alk = Alkaloid, Sap = Saponnin, Gly = Glycosides, WT = mushroom weight, CL = circumference, AR = area, DI = diameter of pileus, LS = length of stipe and SPR = stipe-pileus ratio

Conclusion

The findings of this study highlight the significant impact of substrate composition on the morphological, proximate, and vitamin compositions of *P. pulmonarius*. Substrates like empty palm fruit bunch and maize kernel were particularly effective in enhancing vitamin and flavonoid contents, respectively, while dry banana leaf substrate supported superior morphological growth attributes. These results underscore the critical role of substrate selection in optimizing both the yield and nutritional quality of cultivated mushrooms. Practical applications include the potential use of specific substrates to produce mushrooms with targeted nutritional profiles, offering enhanced health benefits and market appeal. These insights can inform cultivation practices, leading to more efficient and profitable mushroom production.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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