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Proximate composition and heavy metal content of *Pleurotus tuberregium* mushroom grown on different substrates in (Aba) Nigeria

Musadiq Oladimeji Usman [1], Ogechi Ozioma Anyanwu [2,*], Innocent Chidi Nnorom [3] and Innocent Oghale Ajawobu [2]

[1] *Department of Chemical Sciences, Rhema University, Campus Box 7021, Aba, Abia State Nigeria.*
[2] *Department of Pharmaceutical and Medicinal Chemistry, Nnamdi Azikiwe University, Awka, Anambra state Nigeria.*
[3] *Department of Pure and Industrial Chemistry, Abia State University Uturu, Nigeria.*

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Abstract

Mushrooms have been widely used as food and food supplements in Nigeria and other countries of the world for millennia. They are highly nutritious so they contain good quality carbohydrates, proteins, vitamins and minerals. This study determined the proximate, the effects of some substrates (best medium) on the yield and the heavy metal content of mushrooms of *Pleurotus tuberregium* (Fr.) Sing., grown in the laboratory. Four different substrates were used in the experiment to grow the mushrooms namely: topsoil, sawdust, riversand, and mixture of riversand and sawdust respectively. The quality of the fruit body produced was measured with the use of ruler and vernier calipers. Some heavy metals (Cd, Cr and Pb) were analyzed using Atomic Absorption Spectrophotometer. The parameters measured were: number of fruiting bodies, height of stipe, fresh weight, diameter of pileus and diameter of stipe. The result of the experiment on production of mushroom revealed that mushrooms on riversand substrate had the highest mean stipe height, the widest mean pileus and the significantly ($p<0.05$) highest yield. Nutritional parameters were measured and it was found that highest crude fiber content was found in the mushroom grown in mixture of riversand and sawdust substrate; mushrooms on riversand substrate had the highest carbohydrate percentage content and ash content was highest in *Pleurotus tuberregium* mushroom grown on topsoil substrate. The contents of Cd, Cr and Pb were all below the detection limit of the instrumentation for *Pleurotus tuberregium* mushroom grown on all substrates. From this study, the choice of substrate for the growth of mushroom for human and industrial use should be considered to enhance yield and prevent exposure to heavy metal contaminants.

Keywords: *Pleurotus tuberregium*; Mushroom; Proximate composition; Different substrate; Heavy metals; Fungi

1. Introduction

Mushrooms are heterotrophic fungi that depend on other organisms for food. They depend on the substrate in which they grow for all their nutritional requirements; like carbon, water, nitrogen and minerals. It is an important food item for human health, nutrition and disease prevention. However, mushrooms have been widely used as food and food supplements for millennia [1]. Chang (1991) defined mushroom as "a macrofungus with a distinctive fruiting body which can either be epigeous (growing on or close to the ground) or hypogeous (growing under the ground)" [2]. The macrofungi have fruiting bodies large enough to be seen with the naked eye and to be picked up by hand. Ideally, the word mushroom refers only to the fruit body. Unlike green plants, mushrooms are heterotrophs, and without chlorophyll, they cannot generate nutrients by photosynthesis, but instead take nutrients from outer sources. Most mushroom species are either under the Basidiomycota or Ascomycota; the two phyla are under the kingdom Fungi [3]. Several mushrooms are especially tasty and many are rich in nutrients. Mushrooms are highly nutritious so they contain

* Corresponding author: O.O. Anyanwu
Department of Pharmaceutical and Medicinal Chemistry, Nnamdi Azikiwe University, Awka, Anambra state Nigeria.

good quality proteins, vitamins and mineral. Mushrooms have low calori and are highly suitable for fat persons. Stemets (2001) observed that the fungus is often found growing around the African bread fruit tree (*Treculia africana*) [4]. It attacks dead woods, on which it produces globose or ovoid sclerotia [5].

African herbalists have used *P. tuberregium* sclerotia to solve a variety of health problems, ranging from skin diseases to small pox and even in embalmment of bodies [6; 11]. Badalyan *et al*. (2008) reported that the antifungal activity of *P. tuberregium* against filamentous fungi is utilized in treating mycoses in mammals [7]. Many studies have reported the use of *Pleurotus* species in bioremediation exercises. *P. tuberregium* (a white-rot fungus) has been reported to ameliorate crude oil polluted soils and the resulting soil sample supported the germination and seedling of *Vigna unguiculata* [8]. Yongabi (2004) confirmed that the sclerotium of *P. tuberregium* is a good coagulant and disinfectant, which can be used in natural water and waste water purification [9].

Despite its nutritional value, mushrooms cultivation is not widespread; many mushrooms are considered to be healthy food because they contain large enough protein needs of the rural especially during the rains [10]. It is also rich in some essential vitamins (B_1, B_2, and C) and essential minerals than most plants. They also have low fat content and hence high fibre content that enhances digestion of food. This mushroom is of economic importance in food and medicine preparation [11]. It is used to treat heart problem in the eastern part of Nigeria especially among the Igbos and Edos, and it is used in the treatment of asthma, cough and obesity [10]. Therefore, there is a need to ensure a balanced daily diet through the consumptions of *Pleurotus tuberregium* which is used in preparation of different kinds of delicacies and to improve businesses and income of Nigerians, there is a need to grow and cultivate this mushroom in commercial quantities outside its natural habitat using different substrates that support maximum yield. However, mushrooms are known wildly to have the ability to accumulate heavy metals and these heavy metals have detrimental effects.

2. Material and methods

2.1. Study Location and Sample Area

The studies were conducted at the Laboratory of the Department of Chemical Sciences, Rhema University of Nigeria, and Springboard Research Laboratory Awka, Anambra State, respectively. The sclerotia used in this study were obtained from Ahia-Ohuru, Aba South local government area of Abia State, Nigeria. Fresh wood sawdust was collected from Urata timber-shed, Aba North in Abia State. Riversand substrate was collected from Ogbor Hill in Aba North local government area of Abia State. Topsoil substrates used were collected from the premises of the school of preliminary Studies, Rhema University Nigeria.

Figure 1 Map Showing Aba the study location

2.2. Preparation of Substrates

The single substrates (saw dust, top soil and riversand) and a mixture of Riversand and Sawdust were mixed on ratio 1:1 bases. Then the four substrates were sprinkled with distilled water moderately and the water content tested by pressing the substrates with hand to allow water drip. It was tested and it dripped showing that there was moderate amount of water. 500 g of each substrate was weighed on a weighing balance and poured in four (4) replicates into plastic container, all together were sixteen (16) replicates. Medium sized sclerotia of *Pleurotus tuberregium* was then planted on each substrate while the relative humidity was maintained at 75% to 80% required for fructification of the mushroom. The substrates were: top soil (D1), sawdust (D2), riversand (D3), mixture of riversand and sawdust (D4).

2.3. Inoculation and Incubation

Five hundred grams (500 g) of each substrate was placed in a plastic container. The sclerotia were soaked in water for 4hrs. It was then seeded into the plastics containing the substrates and watered enough to create a humid environment required for fructification. The containers of the inoculated substrates were placed on the floor in the laboratory at room temperature for observation of fungal growth for 3 weeks. These were slightly watered daily to keep them damp.

2.4. Data Collection from the Growing Mushroom Fruiting Bodies

The growth of the mushrooms in the different substrates was recorded weekly. The height of fruiting bodies was measured in centimeters using a ruler from the base of the stipe to the pileus. The Diameter of the pileus was measured in centimeters with a ruler and calipers from one edge of the pileus across the stipe to the other edge. Length of the stipe was measured using a ruler from the base of pileus to the point where it was harvested at the base. Fresh weight of fruiting bodies after harvesting was measured using an electric weighing balance and Dry weight was measured after oven drying at 105 ℃ for 15 minutes. Total number of fruiting bodies was done by counting the number of fruiting bodies on each substrate.

2.5. Proximate Analysis of Harvested Mushroom Fruiting Bodies

Moisture content, carbohydrate and crude fibre of the *Pleurotus tuberregium* mushroom was determined following the method described by [12]. Ash content of the *Pleurotus tuberregium* mushroom was determined by the method described by the Association of Analytical Chemists [13]. The fat content and crude protein content (as estimated by the kjeldahl method) of the mushroom was determined by Official method of analysis of Association of Analytical Chemists [14].

2.6. Acid Digestion and Metal Determination of Samples

Heavy metal Lead (Pb), Chromiun (Cr), Cadmium (Cd) analysis was conducted at Springfield Research Institute, Awka, Anambra State using Agilent FS240AA Atomic Absorption Spectrophotometer according to the method of American Public Health Association [15].

2 g of the dried mushroom sample was weighed out into a digestion flask and 20 ml of the digestion acid mixture (650 ml conc. HNO_3; 80 ml perchloric acid and 20 ml conc. H_2SO_4) was added. The flask was then heated until a clear digest was obtained. The digest was diluted with distilled water to the 100 ml mark and appropriate dilutions were then made for each element.

The sample was thoroughly mixed by shaking, and 100 ml of it was transferred into a glass beaker of 250 ml volume, to which 5 ml of conc. nitric acid was added in increments of 5 ml till all the residue was completely dissolved and heated to boil till the volume was reduced to about 15-20 ml. The mixture was cooled, transferred and made up to 100 ml using metal free distilled water. The sample was aspirated into the oxidizing air-acetylene flame. When the aqueous sample was aspirated, the sensitivity for 1% absorption was observed.

2.7. Statistical Analysis

The results were analyzed using analysis of variance (ANOVA), and test of significance were carried out by Duncan's multiple range test.

3. Results and discussion

Seven days after planting the sclerotia, it was observed that white mycelia had colonized all the substrates. Fruiting bodies were first observed on riversand (D3), followed by mixture of riversand and sawdust (D4), followed by topsoil (D1). However, the sawdust substrate (D2) supported the poorest growth. The result of the experiment on production of mushroom showed that riversand (D3) substrate had the highest mean stipe height while the least was from the sawdust (D2) substrate. It was observed that the sawdust (D2) substrate produced only one fruiting body (Table 1) but rather, extensive mycelial ramifications were observed in that substrate. The riversand (D3) substrate produced the highest number of fruiting bodies (13) while the topsoil (D1) substrate and mixture of riversand and sawdust (D4) substrate produced (8) and (10) fruiting bodies respectively. Fruit bodies with the widest mean stipe were those grown riversand (D3) substrate (Table 1). The effect of the different substrate on pileus diameter, stipe height and stipe diameter are also shown in Table 1. Mushrooms grown on riversand (D3) substrate had the widest mean pileus diameter (13.50 cm) while the sawdust (D2) substrate produced mushrooms with the least (1.40 cm) respectively.

Table 1 Effects of different substrates on *P. tuberregium* stipe height, stipe diameter, pileus diameter and number of fruiting bodies

Substrates	Diameter of the pileus (cm)	Height of the stipe (cm)	Diameter of the stipe (cm)	Number of fruiting bodies
D1	5.70^{b}	9.75^{a}	1.80^{a}	8
D2	1.40^{b}	2.50^{b}	0.65^{b}	1
D3	13.50^{a}	13.25^{a}	2.80^{a}	13
D4	13.40^{a}	11.40^{a}	2.55^{a}	10

kEY; a is significantly different from b

Each value is a mean of four (4) replicates. Values in the same column that do not share a common superscript are significantly different at $P \leq 0.05$ (one way ANOVA then LSD post hoc comparison)

The different stages of growth of *Pleurotus tuberregium* mushroom on day 12 is shown in the slides below. Figure 2 shows the stage of growth of *Pleurotus tuberregium* mushroom growing on riversand (D3) substrate, Figure 3 shows the stage of growth of *Pleurotus tuberregium* mushroom growing on sawdust (D2) substrate, Figure 4 shows the stage of growth of *Pleurotus tuberregium* mushroom growing on mixture of riversand and sawdust (D4) substrate while Figure 5 shows the stage of growth of *Pleurotus tuberregium* mushroom growing on topsoil (D1) substrate.

Figure 2 *Pleurotus tuberregium* mushroom growing on Riversand Substrate

Figure 3 *Pleurotus tuberregium* mushroom growing on sawdust substrate

Figure 4 *Pleurotus tuberregium* mushroom growing on mixture of riversand and sawdust

Figure 5 *Pleurotus tuberregium* mushroom growing on topsoil substrate

The weights of the fruiting bodies of the cultivated mushrooms on harvest are presented in Figure 6. Figure 6 shows that the highest mean fresh weight of the mushroom fruiting body grown 24.75 g were those grown on mixture of riversand and sawdust (D4) substrate and the least 13.27 g were from mushrooms grown on topsoil (D1) substrate.

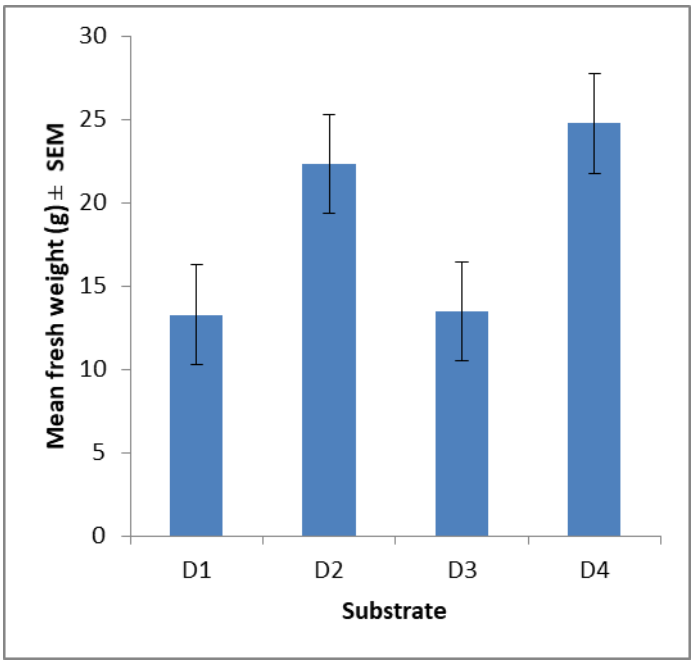

Figure 6 Mean fresh weight of *P. tuberregium* grown on different substrates

Growth of the stipe height, pileus diameter and stipe diameter of *P. tuberregium* on the different substrates were measured progressively. Figures 7, 8 and 9 compare the growth of *P. tuberregium* on the different substrates from day7 to day 12.

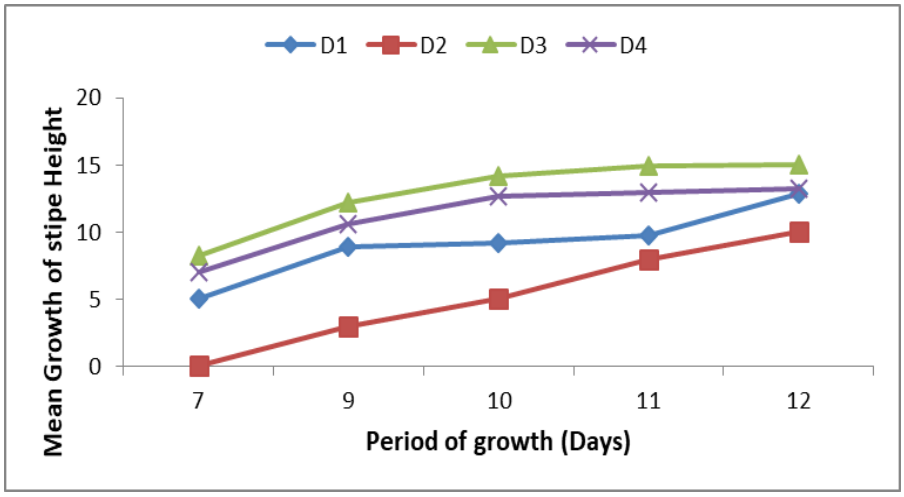

Figure 7 Growth of the Stipe Height of *Pleurotus tuberregium* from Day 7 to Day 12 on Different Substrates

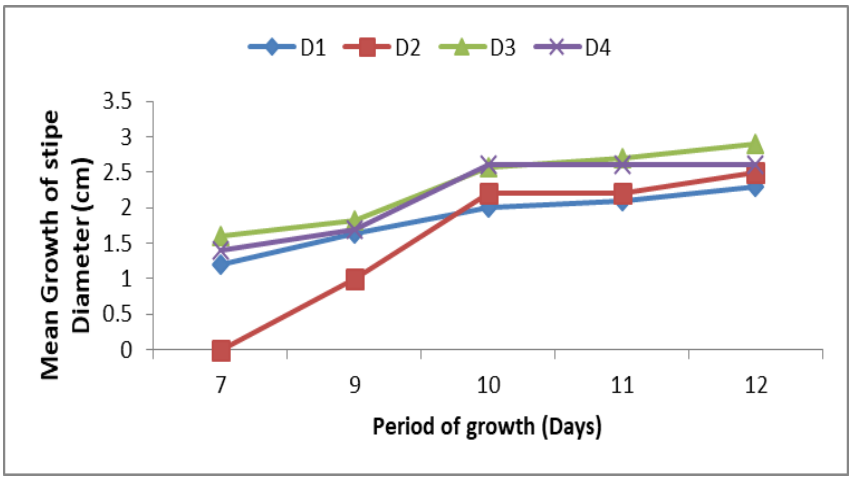

Figure 8 Growth of the Stipe Diameter of *Pleurotus tuberregium* from Day 7 to Day 12 on Different Substrates

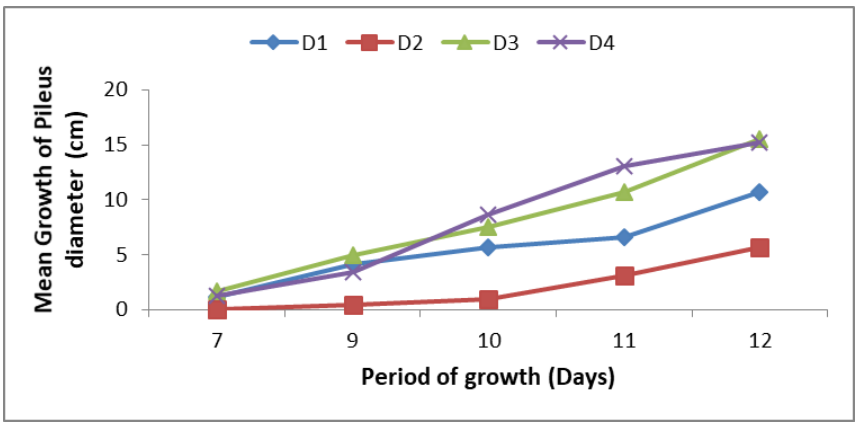

Figure 9 Growth of the Pileus Diameter of *Pleurotus tuberregium* from Day 7 to Day 12 on Different Substrates

3.1. Correlation Studies of Results

The relationships between the parameters measured while the *Pleurotus tuberregium* mushrooms were growing were analyzed through regression analysis. Correlation study of the yield of *Pleurotus tuberregium* mushrooms; stipe height,

stipe diameter, pileus diameter and number of fruiting bodies on the four substrates were compared. The correlation studies carried out was between Pileus diameter and number of fruiting bodies which gave R2 value of 0.8535; that between pileus diameter and stipe diameter R2 value of 0.9459; that of pileus diameter and stipe height R2 value of 0.8334; that of stipe height and stipe diameter R2 value of 0.9656; that of stipe diameter and number of fruiting bodies R2 value of 0.9711 and that of stipe height and number of fruiting bodies R2 value of 0.9880 respectively. These regression studies showed that as the mushroom grew from day 1 to day 12, the pileus diameter increased as the number of fruiting bodies was increasing. This is also the case for all the other correlations respectively indicating a true relationship between parameters measured.

3.2. Proximate Analysis of *P. tuberregium* grown on different substrates

Table 2 Proximate analysis of *P. tuberregium* mushrooms grown on different substrates

Substrate	Moisture (%)	Protein (%)	Crude Fibre (%)	Ash (%)	Lipid (%)	Carbohydrate (%)
D1-fruit body from topsoil	24.190	17.150	13.110	6.680	5.150	33.720
D3-fruit body from riversand	25.160	16.100	15.260	3.680	1.100	38.700
D4-fruit body from mixture of riversand and sawdust	19.090	16.450	29.930	4.120	8.800	21.610

Several nutritional parameters were measured for the *P. tuberregium* mushrooms grown on different substrates. The moisture contents of *P. tuberregium* (Table 2) on topsoil (D1), riversand (D3) and mixture of riversand and sawdust substrates were 24.19%, 25.16 % and 19.09% respectively. Percentage protein content ranged from 16.10 % for riversand (D3) substrate to 17.15 % for topsoil (D1) substrate. Table 2 shows that mushroom that grew on riversand (D3) substrate had the highest percentage carbohydrate content. On the other hand the lipid content is significantly lower (about 1.10 %). The protein, fibre, lipid and carbohydrate contents of *P. tuberregium* on mixture of riversand and sawdust were found as 16.45 %, 29.93 %, 8.80 % and 21.61 % respectively. The highest crude fibre content was observed in the mixture of riversand and sawdust (D4) substrate and the least in topsoil (D1) substrate. These were found as 29.93 % in mixture of riversand and sawdust (D4) substrate, 15.26 % in riversand (D3) substrate and 13.11 % in topsoil (D1) substrate respectively (Table 2). The total ash content found in *P. tuberregium* produced on the different substrates varied from 3.68 % to 6.68 %. These were found as 3.68 % in riversand (D3) substrate, 4.12 % in mixture of riversand and sawdust (D4) substrate and 6.68 % in topsoil (D1) substrate as shown in Table 2 respectively.

Table 3 Determination of Cd, Cr, and Pb from *P. tuberregium* mushrooms grown on different substrates

Samples	Lead ppm	Chromium ppm	Cadmium ppm
D1	0.00	0.00	0.00
D3	0.00	0.00	0.00
D4	0.00	0.00	0.00
Sample	**Cadmium**	**Chromiun**	**Lead**
D1	BDL	BDL	BDL
D3	BDL	BDL	BDL
D4	BDL	BDL	BDL

BDL*Below Detection Limit; KEY: D1 = Top soil substrate; D3 = Riversand substrate; D4 = Mixture of Riversand and Sawdust.

The differences in yield in the different substrates could be due to the nutrient status of the respective substrates). The observation that the mushroom grew successfully on different substrates indicates the potential of mushroom within the bioconversion of those materials [16].

Ayodele and Okhuoya (2007) obtained higher fresh weight of fruiting bodies (61.63 g) on sawdust compared to the 24.75 g obtained from our mixture of riversand and sawdust (D4) substrate [16]. The fact that sawdust gave the poorest

yield and lowest number of fruiting body could be attributed to the fact that it was still forming mycelia and storing food reserves in the sclerotia. In agreement with the experiment carried out by [17], the sawdust used in this study was a mixture of wood particles from different plants.

However, there may therefore be some particles from some of the wood that may tend to inhibit the growth of the fungus. This inhibition effect of sawdust mixture has been reported by [18] as cited in [17]. This is further corroborated by [19] who grew mushrooms on different pure sawdust types and obtained the best results from Eucalyptus sawdust followed by pine sawdust confirming the fact that sawdust from different trees produces different effects on the growth of mushroom. [20] as cited in [17] had also reported that supplemented oak sawdust was a poor substrate for the growth of *Pleurotus sajorcaju*.

The results obtained also showed that the mixture of riversand and sawdust produced mushrooms with highest fresh weights. This could probably be due to the riversand providing good aeration for the germination and the fructification of the mushrooms. Also sandy soils of which river sand is one offers the least resistance to enlargement of sporophores unlike compact soils like top soil that would have to combat with microbial antagonism as well [21]. The combined effects of the qualities of riversand and sawdust may have been responsible for the highest yield recorded in that substrate.

The highest percentage protein occurred in *P. tuberregium* mushroom grown on topsoil (D1) substrate, this is contrary to the report as stated by [21], however the difference in percentage of protein of analyzed mushrooms of both topsoil and riversand substrate in this study did not differ much. Also the highest carbohydrate content was found in *P. tuberregium* mushroom grown on riversand substrate. Olumide and Chiejina (2010) reported that sclerotia have already stored in them all the nutrients required for fruiting; this therefore explains why riversand with little or no fertility could produce mushrooms with the highest percentage carbohydrate while the other substrates may have to combat first with the microbial antagonists in them [21].

The contents of Cd, Pb and Cr (Table 3) were all below detection limit for mushrooms produced from the used substrates. It is therefore a good alternative to meat in dishes as it equally poses no detrimental effect.

4. Conclusion

The study revealed that sawdust (D2) substrate which happened to be a mixture of different wood shavings did not support good yield of *Pleurotus tuberregium* mushroom. However sawdust substrates could still serve better if a prior knowledge of the kind of sawdust used is ascertained. Riversand (D3) substrate was the best medium for growth as it gave the highest number of fruiting bodies, highest mean stipe height, widest mean stipe and widest pileus. There is a need for close monitoring of amounts of heavy metals in substrates before they are used for mushroom production. Our study showed that highest crude fiber content was found in the mushroom grown in mixture of riversand and sawdust substrate (D4); mushrooms on riversand substrate had the highest carbohydrate percentage content and ash content was highest in *Pleurotus tuberregium* mushroom grown on topsoil substrate. Finally, protein content was highest in mushrooms grown on topsoil (D1) substrates. The contents of Cd, Pb and Cr were all below detection limit indicating that it is safe; hence the consumption of such mushrooms would serve as a good alternative to meat in dishes as it equally poses no detrimental effect.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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