

Assessing the effect of herbicide use (Glyphosate) on fruiting and phytochemical characteristics of edible soil mushroom (*Pleurotus ostreatus*)

Boniface Egbu Odor, Emmanuel Nzegbule, Precious Chieze Chikezie, Tochukwu Nnamdi Onyemuche, Jerius Nkwuda Ejeje, Doris Olachi Alilonu, Obinna Charles Ekoh, Peter Chibuzor Onuoha, Marycynthia Amarachi Ojiakor

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Abstract : This study investigated the effect of herbicide (glyphosate GLY) on the growth and development of *Pleurotus ostreatus* (Jacq.Ex.Fr) *P. Kumm* fruiting bodies. The experiment was conducted in a Randomized Complete Block Design (RCRD). Three treatment levels of GLY (1, 2 and 3%) and control were administered on mycelium surface using a hand sprayer after the culture matured with sawdust as substrates at 12 days after inoculation (DAI). Data on growth, fruiting body weight, stipe length and mineral content were collected and analyzed using analysis of variance (ANOVA), while mean separation was done by Duncan Multiple Range Test (DMRT) at the least significant difference (LSD). The result of fruiting body yield (g) and biological efficiency (BE%) of 249.04g (31.12) were produced by 3% GLY level produced the highest yield of 249.04g, 31.12 BE% followed by 1% (241.79g) and 30.22 BE% while 0.02% had the least fruiting body yield (222.62g) and BE 27.82%. The 3% GLY level produced fruiting bodies with the highest mean weight of 3.12±0.75g 18DAI as control had the lowest (2.35g) fruiting body mean weight, 36DAI. The control produced the highest (145.00) number of fruiting bodies, followed by 0.02% Gly (141.00), while the lowest was recorded at 1% Gly level. Fruiting bodies from 3% Gly had the longest (1.96cm) stipe while those of 2% gave the shortest (1.34cm) 26DAI. Cap diameter of fruiting bodies was not significantly different at $p \leq 0.05$ across all Gly treatment levels and control. Fruiting bodies from 3% Gly had the highest

(4.38±0.01g µg/ml /l) crude protein (CP) content, followed by those of control (4.16±0.01g/l) but lowest (3.50±0.01µg/ml) in those of 1% Gly while Carbohydrate (CHO) content was highest (94.54±0.01 µg/ml) in fruiting bodies from 1% Gly, but lowest (94.20±0.01 µg/ml) in those of control. The highest calorific value was recorded in fruiting bodies from control (398.12±0.01 µg/ml) while the lowest was obtained at 3% Gly (393.33±0.01 µg/ml). Flavonoids content of fruiting bodies decreased with exposure to GLY by 31% while phenols and phythetes increased with exposure to GLY by 54.5% and 288.8% for 3% GLY level over control. Phosphorus, carbon and hydrogen contents of the fruiting bodies increased by 163%, 26.0% and 291 respectively over the control and had respective concentrations as 0.55, 28.24 and .024. Although Glyphosate had positive effect on fruiting bodies and Biological efficiency, it also increased very significantly levels of P, C and H which may indicate that glyphosate accumulated in *pleurotusostreatus*.

Keywords: *Pleurotus ostreatus*, mycelium, Flavonoids, glyphosate, fruiting, Treatment, Phenol

Boniface Egbu Odor

Department of Environmental Management and Toxicology, Michael Okpara University of Agriculture, Umudike.

Email: farmanager@xaji.org

Orcid id: <https://orcid.org/0009-0001-3448-0933>

Emmanuel Nzezbule

Department of Environmental Management and Toxicology, Michael Okpara University of Agriculture, Umudike.

Email: nzezbule.emmanuel@mouau.edu.ng

Orcid id :

Precious Chieze Chikezie

Department of Biochemistry, Alex- Ekwueme Federal University Ndufu Alike, Ebonyi State.

Email: precious.chikezie@funai.edu.ng

Orcid id : <https://orcid.org/0009-0003-6392-4075>

Tochukwu Nnamdi Onyemuche

Department of Biochemistry, Alex- Ekwueme Federal University Ndufu Alike, Ebonyi State.

Email: tochukwu.onyemuche@funai.edu.ng

Jerius Nkwuda Ejeje

Department of Biochemistry, Alex- Ekwueme Federal University Ndufu Alike, Ebonyi State.

Email: ejeje.jerius@funai.edu.ng

Doris Olachi Alilonu

Department of Biochemistry, Alex- Ekwueme Federal University Ndufu Alike, Ebonyi State.

Email: obasidoris19@gmail.com

Obinna Charles Ekoh

Department of Biochemistry, Alex- Ekwueme Federal University Ndufu Alike, Ebonyi State.

Email: ekoh.charles@funai.edu.ng

Peter Chibuzor Onuoha

Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Abia State.

Email: onuoh.chibuzor@mouau.edu.ng

Marycynthia Amarachi Ojiakor

Department of Biochemistry, Alex- Ekwueme Federal University Ndufu Alike, Ebonyi State.

Email:

amarachimarycynthia665@gmail.com

1.0 Introduction

Mushrooms have gained increasing recognition as a popular delicacy in both rural and urban households due to their unique taste, nutritional value, and medicinal properties. They are either cultivated commercially or harvested from the wild, with cultivated species being mostly decomposers (saprobies) that complete their life cycles on dead organic matter. In contrast, most forest-harvested mushrooms are ectomycorrhizal, forming fruiting bodies only when associated with living host trees (Guo & Xu, 2025).

Generally, mushrooms possess four main functionalities: dietary value, taste, physiological effects, and cultural relevance (Dimopoulou et al., 2022). Wild mushrooms are highly valued as food because they are rich in proteins and essential trace minerals (Kalac, 2012). They also provide important vitamins, particularly B and D, which make them a significant dietary supplement (Singh et al., 2025). Beyond their nutritional roles, mushrooms are noted for their therapeutic potential. They exhibit diverse biological activities such as antibacterial, antimutagenic, antitumoral, and antiviral properties (Noreen et al., 2025). Their antitumor activity is largely attributed to high levels of sesquiterpenes, triterpenoids, glucans, and glycoproteins (Benthavaravithana et al., 2025). In addition, they are traditionally consumed for their ability to help reduce obesity and manage hypertension (Mayirnao et al., 2025).

However, mushrooms harvested in rural areas are often exposed to agricultural practices, particularly pesticides, due to their growth on farmlands and plantations. Among these, glyphosate is one of the most widely used herbicides. Though its application on mushrooms is unintended, mushrooms in such environments may absorb glyphosate residues, potentially altering their growth and biochemical composition. Glyphosate, a broad-spectrum herbicide, is valued for its effectiveness in weed control and is indispensable in large-scale agriculture (Singh



et al., 2020). It reduces labor, enhances yields, and facilitates pathogen control. Nonetheless, glyphosate and other pesticides are also recognized as major soil and water pollutants that pose ecological and health risks (Meena et al., 2020). Indeed, only about 0.1% of applied pesticides reach the intended targets, while over 95% impact non-target organisms, including fungi (Meena et al., 2020). Glyphosate has become the most widely sold herbicide globally, with 195,000 tons used in Brazil alone in 2018 (IBAMA, 2018).

While glyphosate is often described as environmentally friendly due to its relatively fast biodegradation and strong adsorption to soil particles (Vereecken, 2005), several studies contradict this perception. Negative impacts have been reported on amphibians, wildlife, and soil organisms (Berger et al., 2013; Brush et al., 2013; Kohler et al., 2013). Furthermore, although its toxicity to mammals was initially considered low (Greim et al., 2015; WHO, 2009), more recent studies emphasize potential chronic risks from accumulation in ecosystems and the food chain (Jarrel et al., 2020; Myers et al., 2016; Zhang et al., 2019). Correlations have also been established between glyphosate use and the incidence of plant, animal, and human diseases (Rueda-Ruzafa et al., 2019; Swanson et al., 2014; Van Bruggen et al., 2018). In 2015, the International Agency for Research on Cancer classified glyphosate as “probably carcinogenic to humans,” further raising concerns about its widespread application.

Despite the extensive research on glyphosate’s ecological impacts, there is a lack of specific studies on its direct effects on edible mushrooms such as *Pleurotus ostreatus*, particularly regarding their growth, fruiting, and phytochemical composition. Edible mushrooms are of great nutritional and medicinal importance, and any alteration in their biochemical characteristics due to glyphosate exposure could have serious implications for food safety and human health.

Therefore, the aim of this study was to investigate the effect of glyphosate exposure on the fruiting and phytochemical characteristics of *Pleurotus ostreatus*, with particular focus on growth rate, yield, stipe length, cap diameter, and bioactive compounds.

This study is significant because *Pleurotus ostreatus* is widely consumed, and understanding how glyphosate influences its nutritional and therapeutic properties is essential for ensuring food quality, consumer safety, and sustainable mushroom production in agricultural landscapes where pesticide use is prevalent.

2.0 Materials and Methods

2.1 Mushroom and Substrate

The mushroom used in this study was *Pleurotus ostreatus* (oyster mushroom), which is widely recognized for its edibility, medicinal importance, rapid growth, and adaptability to a wide range of substrates. The choice of this species was based on its ability to utilize agricultural wastes efficiently and tolerate environmental fluctuations.

The substrate used was sawdust obtained from *Gmelina arborea*, a common fast-growing tree species in Nigeria. Gmelina sawdust was chosen due to its high cellulose and hemicellulose content, low lignin fraction, and its abundance as an agro-industrial waste in southeastern Nigeria. The sawdust was collected from a local sawmill in Nsukka, Enugu State, Nigeria and sun-dried to reduce moisture content before use.

2.2 Culture Medium Preparation

Potato Dextrose Agar (PDA) was used for culturing the mushroom. Two hundred grams (200 g) of peeled Irish potatoes were boiled in 1 L of distilled water until soft. The extract was filtered using muslin cloth, after which 20 g of dextrose and 20 g of agar were added and the volume adjusted to 1 L with distilled water. The medium was sterilized at 121 °C for 15 minutes under 1.05 kg cm⁻² (15 psi) pressure in an autoclave.



The sterilized PDA was poured into sterile Petri dishes under aseptic conditions and allowed to solidify. Pure cultures of *P. ostreatus* were inoculated onto the PDA plates and incubated at 25 ± 2 °C for 7 days to obtain vigorous mycelial growth.

2.3 Spawn Production

Sorghum grains were used for spawn preparation. The grains were washed thoroughly, soaked in water for 24 h, and boiled for 15 min to soften. Excess water was drained off, and the grains were mixed with 1% (w/w) calcium carbonate (CaCO_3) and 2% (w/w) calcium sulfate (CaSO_4) to adjust pH and prevent clumping.

The treated grains (250 g) were packed into 500 mL glass bottles, plugged with non-absorbent cotton wool, covered with aluminum foil, and sterilized at 121 °C for 60 minutes. After cooling, the bottles were inoculated with actively growing mycelial agar discs (5 mm diameter) from 7-day-old cultures and incubated at 25 ± 2 °C for 14 days until complete colonization.

2.4 Substrate Preparation and Bagging

The dried *Gmelina* sawdust was mixed with rice bran at a ratio of 8:2 (w/w) to enhance nutrient availability. The mixture was adjusted to 60–65% moisture content by sprinkling water and mixing thoroughly. The substrate was then packed into polypropylene bags (1 kg each), tightly packed, and the mouths plugged with cotton wool.

The bags were sterilized at 121 °C for 2 h, cooled to room temperature, and inoculated aseptically with 10 g of sorghum grain spawn. Inoculated bags were incubated in a dark room at 25 ± 2 °C and 70–80% relative humidity until complete mycelial colonization was achieved (approximately 21–24 days).

2.5 Glyphosate Treatment

Glyphosate was applied in varying concentrations to evaluate its effect on mushroom growth and yield. A commercial

glyphosate formulation (41% isopropylamine salt, Monsanto, USA) was used as the source. Stock solutions were prepared by dilution with distilled water, and working concentrations of 0 ppm (control), 10 ppm, 20 ppm, 30 ppm, and 40 ppm were obtained.

During fruiting, each treatment level was sprayed on the substrate surface using a hand-held atomizer (5 mL per bag), while the control received distilled water only. Treatments were applied once at the pinhead initiation stage.

2.6 Data Collection

The following growth and yield parameters were measured:

- (i) Spawn running period (days): time taken for complete substrate colonization.
- (ii) Pinhead initiation (days): time taken for first pinhead emergence after inoculation.
- (iii) Number of fruiting bodies per bag.
- (iv) Average weight of fruiting bodies (g).
- (v) Biological efficiency (BE): calculated as (fresh weight of mushrooms harvested / dry weight of substrate) $\times$ 100.

2.7 Proximate and Nutritional Analyses

Proximate composition was determined according to AOAC (2012) methods. The parameters analyzed included moisture, crude protein, crude fat, crude fiber, ash, and carbohydrate contents.

- (i) Moisture content: Samples were dried in a hot air oven at 105 °C to constant weight.
- (ii) Crude protein: Determined using the Kjeldahl method ($\text{N} \times 6.25$ conversion factor).
- (iii) Crude fat: Extracted using a Soxhlet apparatus with petroleum ether (boiling point 40–60 °C).
- (iv) Ash content: Samples were incinerated in a muffle furnace at 550 °C for 6 h.



- (v) Crude fiber: Determined by sequential acid (1.25% H_2SO_4) and alkali (1.25% NaOH) digestion.
- (vi) Carbohydrate content: Calculated by difference: $100 - (\text{protein} + \text{fat} + \text{fiber} + \text{ash} + \text{moisture})$.

2.8 Statistical Analysis

All experiments were conducted in triplicate, and the data obtained were subjected to one-way analysis of variance (ANOVA) using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Means were separated using Duncan's Multiple Range Test (DMRT) at a significance level of $p < 0.05$.

2.0 Results and Discussion

The results of the study showed that glyphosate significantly affected the growth and yield of mushrooms at different levels of exposure. The number of fruiting bodies produced by *P. ostreatus* was negatively influenced by glyphosate application, but the extent of this effect varied with the number of days after inoculation (DAI). At the peak of fruiting (20 and 22 DAI), the number of fruiting bodies (66 and 45) produced by *P. ostreatus* under control

conditions (no glyphosate) was 83% and 15% higher, respectively, than the average values (36 and 39) recorded for mushrooms exposed to glyphosate (Table 1).

The cumulative mean number of fruiting bodies was highest in the control treatment (36.25), which was significantly greater ($p \leq 0.05$) than those observed under 1% (23.5) and 3% (26.25) glyphosate exposure. This finding agrees with previous reports (Battaglin, 2014; Carles, 2019; Silva et al., 2018), which indicated that because glyphosate degradation and dissipation are slower than originally anticipated, residues tend to accumulate in soil and water, thereby increasing transfer into plant and animal products.

However, these results are contrary to the observations of Sumalee et al. (2013), who reported that glyphosate residues in soil may actually promote the growth of other fungi and bacteria capable of metabolizing the herbicide through enzymatic processes, thereby releasing phosphorus, carbon, and nitrogen for microbial nutrition.

Table 1: Effect of Glyphosate on the Number of Fruiting Bodies of *P. ostreatus*

Treatment (%)	18 DAI	20 DAI	22 DAI	26 DAI	Mean
1%	27.00	28.00	32.00	7.00	23.5a
2%	42.00	40.00	51.00	5.00	34.5c
3%	21.00	42.00	34.00	8.00	26.25b
Control	33.00	66.00	45.00	6.00	36.25c

****Means bearing the same superscript letters are not significantly different ($p \leq 0.05$).**

Treatment effect is significant at $p \leq 0.05$. DAI = Days After Inoculation.

From Table 2, the results indicated that at 18 DAI, the 3% Gly treatment produced the highest average fruit body weight (3.12 ± 0.75 g), followed by the control (2.67 ± 0.60 g), while mushrooms harvested from the 2% Gly treatment had the lowest weight (1.54 ± 0.35 g). A similar pattern was observed at 20 and 26 DAI. This suggests that the weight-enhancing effect of glyphosate on fruiting bodies is more pronounced during the initial fruiting days and declines over time.

Some researchers have noted that an increase in the concentration of certain chemicals such as pesticides, herbicides, or crude oil can lead to higher mycelial mortality (Okwujiako et al., 2013), thereby justifying the use of white rot fungi in bioremediation. Similarly, Kearney (1998) noted that microbial metabolism is likely the most important process for pesticide degradation in soils, serving as the basis for bioremediation, as degrading microorganisms obtain carbon, nitrogen, or energy from pesticide molecules (Gan and Koskinen, 1998).



However, this trend changed at 22 DAI, when the control treatment produced the highest fruiting body weight (2.72 ± 2.37 g). Nerud et al. (2013) reported that glyphosate can enhance the growth performance of oyster mushrooms by increasing fruiting body weight, a phenomenon associated with the ability of white rot fungi to utilize enzymes such as peroxidases, manganese peroxidases, and laccases to degrade complex lignin, cellulose, and hemicelluloses, which are the main components of their substrates. The overall highest mean fruiting body weight (2.35 g) was recorded in the control treatment, although it was not significantly different from the 1% and 3% glyphosate treatments. This indicates that oyster mushrooms may actually perform better in the absence of glyphosate, supporting the findings of Ahmed et al. (1998), who stated that pesticide pollution can reduce biodiversity and depress soil heterotrophic bacteria (including denitrifiers) as well as fungi.

Conventional agricultural pesticides may have long-term toxic effects on beneficial fungi,

including white rot fungi, particularly affecting their growth and sporulation. Although extensive research has been conducted worldwide on optimal growth conditions for oyster mushrooms, data on the potential effects of agricultural herbicides, such as glyphosate, on the size or weight of *P. ostreatus* remain scarce.

The fruiting body weights observed in the 3% glyphosate treatment at 18 and 20 DAI, which exceeded those of the control, align with the findings of Soil Association (2017), who reported that fungal populations, aside from mycorrhizae, often exhibit increased fruiting body weight when glyphosate is applied.

Conversely, the highest fruiting body weight recorded in this trial (3.12 ± 0.75 g at 0.03% Gly) contrasts with the study by Onyeizu et al. (2017), which demonstrated that increasing crude oil pollution reduces the fresh weight of fruiting bodies in two oyster mushroom species (*P. ostreatus* and *P. pulmonarius*).

Table 2: Effect of Glyphosate on weight of fruiting bodies of *P. ostreatus*

Treatment (%)	18 DAI	20 DAI	22 DAI	26 DAI	Mean (g)
1%	2.15 ± 0.20	2.77 ± 0.07	2.03 ± 1.42	0.48 ± 0.95	1.85ab
2%	1.54 ± 0.35	1.55 ± 0.37	1.28 ± 0.88	0.44 ± 0.88	1.20a
3%	3.12 ± 0.75	2.39 ± 0.45	2.62 ± 0.58	0.50 ± 1.00	2.16b
Control	2.67 ± 0.60	2.35 ± 0.65	2.72 ± 2.37	1.66 ± 1.11	2.35b

LSD 0.05 = 0.336

Table 3 presents the effects of different levels of glyphosate on the cap diameter of *P. ostreatus* fruiting bodies at various days after inoculation (DAI). The results shown in Table 3 indicated that at 18 DAI, fruiting bodies from the 1% Gly treatment produced the largest cap diameter (3.45 ± 0.04 cm), followed by those of 3% Gly (3.35 ± 0.40 cm), while mushrooms from the 2% Gly treatment had the smallest cap (2.55 ± 0.45 cm). At 20 DAI, fruiting bodies harvested from the 3% Gly treatment had the highest cap diameter (3.52 ± 0.49 cm), whereas the control had the lowest (2.49 ± 0.62 cm).

At 22 DAI, cap diameter ranged from 2.25 ± 1.54 cm in the 2% Gly treatment to 2.92 ± 1.95 cm in the 3% Gly treatment. There was a relative reduction in cap diameter at 26 DAI across all glyphosate treatments, including the control. Differences in mean cap diameter were not statistically significant (LSD 0.05 = 0.936), ranging from 2.14 in 2% Gly to 2.74 in 3% Gly.

Results further revealed that at 18 DAI, fruiting bodies from the 3% Gly treatment had the largest cap diameter (3.52 ± 0.049 cm), followed by 1% Gly (3.13 ± 0.37 cm), while the



smallest caps were from 2% Gly (3.09 ± 0.09 cm). There was a consistent reduction in cap diameter by 26 DAI for all treatments, including control. The cap diameter of glyphosate-treated mushrooms was higher than the control at 18 and 20 DAI, with the overall mean highest in the 3% Gly treatment. However, cap diameter declined for all treatments as DAI increased.

The mean cap diameter of fruiting bodies in this experiment, across all glyphosate levels including control, was lower than values reported by Okoi and Iboh (2015). This difference could largely be attributed to substrate composition (Chang and Miles, 2004; Nwoko et al., 2016) and environmental factors (Stamets, 2000). Other factors may also influence mushroom size, mainly expressed in

the *cap or stipe dimensions*. Ogbo and Okhuoya (2009) reported that crude oil significantly affects macro-morphological characteristics such as pileus diameter, stipe height, stipe girth, and fruiting body weight.

Relatively smaller cap diameter is considered an undesirable market trait (Yang et al., 2002), as it directly affects fruiting body weight and market price. Considering the overall mean results, cap diameter was not significantly affected by glyphosate, ranging from 2.14 in 2% Gly to 2.74 in 3% Gly treatment levels. This observation aligns with other reports showing no significant effect of glyphosate on fungal growth when applied at recommended doses (Powell et al., 2009; Baumgartner et al., 2010; Pasaribu et al., 2011).

Table 3: Effect of Glyphosate on cap diameter of fruiting bodies of *P. ostreatus*

Treatment (%)	18 DAI	20 DAI	22 DAI	26 DAI	Mean (cm)
1%	3.45 ± 0.40	3.13 ± 0.37	2.53 ± 1.75	1.13 ± 1.36	2.56a
2%	2.55 ± 0.45	3.09 ± 0.09	2.25 ± 1.54	0.66 ± 1.31	2.14a
3%	2.55 ± 0.45	3.09 ± 0.09	2.25 ± 1.54	0.66 ± 1.31	2.14a
Control	3.35 ± 0.40	3.52 ± 0.49	2.92 ± 1.95	1.16 ± 1.38	2.74a

LSD 0.05 = 0.936

Table 4 presents the effects of glyphosate on the proximate composition of *P. ostreatus* fruiting bodies across different treatment levels. The results showed that glyphosate exposure generally increased ash and crude fibre content while reducing moisture content (MC), dry matter (DM), and ether extract (EE). Fruiting bodies exposed to 3% Gly had the highest moisture content ($24.20 \pm 0.01\%$), whereas those not exposed to glyphosate (control) had the lowest moisture content ($18.94 \pm 0.01\%$), with the difference being statistically significant.

The ash content of fruiting bodies from the 3% Gly treatment ($0.61 \pm 0.01\%$) was 110% higher than that of the control. Similarly, crude fibre (CF) content in glyphosate-treated mushrooms was significantly higher than in the control; in the 3% Gly treatment, CF was 73% higher than

in the control. Dry matter content was highest in the control ($81.06 \pm 0.01\%$) and lowest in the 3% Gly treatment ($75.80 \pm 0.01\%$). Ether extract was also highest in control mushrooms ($0.52 \pm 0.01\%$), approximately 79% greater than in the 3% Gly treatment.

Crude protein (CP) content was highest in the 3% Gly treatment ($4.38 \pm 0.01\%$), followed by control ($4.16 \pm 0.01\%$), and lowest in 1% Gly ($3.50 \pm 0.01\%$). Carbohydrate (CHO) content was highest in the 1% Gly treatment ($94.54 \pm 0.01\%$) and lowest in the control ($94.20 \pm 0.01\%$). The highest calorific value was recorded in control fruiting bodies (398.12 ± 0.01 kcal), followed by 1% Gly (395.44 ± 0.01 kcal), and the lowest at 3% Gly (393.33 ± 0.01 kcal).

Generally, the proximate composition of fruiting bodies was significantly influenced by



glyphosate at $p \leq 0.05$ across all treatment levels, including control. Exposure to glyphosate consistently enhanced MC, ash, and CF content, likely due to the ability of *P. ostreatus* to metabolize glyphosate and release carbon, phosphorus, and nitrogen as reported by Sumalee (2013).

These values contradict findings by Nwoko (2019), who used HCl to inhibit contaminants from *Coprinus cinerius* and boost mushroom yield, noting that HCl reduced CHO content while increasing CP from $2.19 \pm 0.00\%$ in control to $24.98 \pm 0.03\%$ in 0.5% HCl-treated substrate. In this study, DM, CF, MC, and EE were not significantly affected by glyphosate at all levels, and the observed values for MC, ash, EE, CF, CP, and CHO were comparable to

reports by Sharad (2013), Syed et al. (2009), Filipa et al. (2011), and Okoi and Iboh (2015). Differences in CP content across treatments may be attributed to variations in substrate composition, supporting observations by Obodai (2003), Adejoye and Fasidi (2009), and Okoi and Iboh (2015) that the nutritional composition of mushrooms reflects substrate chemical composition and the fungi's extracellular digestion during cultivation. Glyphosate did not significantly affect calorific value, which remained highest in control fruiting bodies. A similar pattern was observed for carbohydrate content. The calorific values recorded in this study were higher than those reported by Kalac (2009, 2013), *highlighting that oyster mushroom is generally low in energy.*

Table 4: Effect of Glyphosate on the proximate composition of fruiting bodies of *P. ostreatus*

Treatm ent (%)	MC (%)	DM (%)	ASH (%)	CF (%)	EE (%)	CP (%)	CHO (%)	CAL (kcal)
1%	22.00b± 0.01	78.01ab ± 0.01	0.46b± 0.01	1.14b± 0.01	0.36b± 0.01	3.50a ± 0.01	94.54a± 0.01	395.44a± 0.01
2%	19.60a± 0.01	80.41bc ± 0.01	0.51c± 0.01	1.36b± 0.01	0.34b± 0.01	3.94a±0. 01	93.85a± 0.01	394.22a± 0.01
3%	24.20b± 0.01	75.80a ± 0.01	0.61c± 0.01	1.42c± 0.01	0.29a± 0.01	4.38c ± 0.01	93.30a± 0.01	393.33a± 0.04
Contro l	18.94a± 0.01	81.06c± 0.01	0.29a± 0.01	0.82a± 0.01	0.52c± 0.01	4.16bc± 0.01	94.20a± 0.01	398.12a± 0.01

MC = Moisture content, DM = Dry Matter, CF = Crude fibre, EE = Ether extract, CP = Crude Protein, CHO = Carbohydrate, CAL = Calorific value. Values are means of 3 replicates; means bearing the same letter are not significantly different ($p \leq 0.05$)

Table 5 presents the influence of glyphosate on the phytochemical composition of *P. ostreatus* fruiting bodies across different treatment levels. The results showed that fruiting bodies from 2% and 3% Gly substrates recorded the highest alkaloid content (0.15 ± 0.01), followed by control (0.14 ± 0.01), whereas 1% Gly produced the lowest alkaloid content (0.10 ± 0.01). Flavonoid content was highest in control fruiting bodies (2.26 ± 0.01) and lowest in 2% Gly-treated fruiting bodies (1.18 ± 0.01).

Saponins were most abundant in control (0.12 ± 0.01) and lowest in 2% Gly-treated fruiting bodies (0.09 ± 0.01). Phenols were highest in fruiting bodies from 2% and 3% Gly, while the lowest value (0.08 ± 0.01) was observed in 1% Gly. Phytates were maximal in 2% Gly (0.38 ± 0.01) and minimal in control (0.09 ± 0.01), whereas tannins were highest in control (0.06 ± 0.01) and lowest in 3% Gly (0.03 ± 0.01).



The study revealed that glyphosate exposure increased alkaloids, phenols, and phytates, while flavonoids, saponins, and tannins generally decreased with higher glyphosate levels. Alkaloid concentrations at all glyphosate levels, including control, were lower than those reported by Nwoko et al. (2016) for *P. ostreatus* cultivated on tree log substrates. Alkaloids are biologically active compounds with important pharmaceutical applications, including hormone stimulation and drug development (Boucher et al., 2025). Flavonoids, which have anti-carcinogenic and antibacterial properties (Silva-Pinto et al., 2025), declined by approximately 31% with glyphosate exposure. Conversely, phenols and phytates increased by 54.5% and 288.8% respectively at 3% Gly compared to control, with similar trends observed for saponins and tannins.

The variations in flavonoid and saponin content compared to previous studies may be attributed to differences in substrate composition. .

Species diversity may also affect phytochemical composition. Mwangi et al. (2022) noted that mushrooms such as *Auricularia auricula*, *Pleurotus squarrosulus*, and *Russula* spp. contain appreciable levels of alkaloids, phenols, saponins, and flavonoids. Bioactive compounds from mushrooms are precursors for pharmaceutical drug development and contribute to their high demand in human and animal health applications (Sofowara, 2003; Nwoko et al., 2016). Saponins help prevent parasitic fungal diseases (Shakeel et al., 2025), and tannins exhibit anti-tumor and broad anti-infective properties.

Table 5: Effect of Glyphosate on the phytochemical composition of fruiting bodies of *P. ostreatus*

Treatment (%)	ALK.	FLAV.	SAP.	PHENOL S	PHYTATE S	TANINS
1%	0.10c±0.01	1.24c±0.01	0.08c±0.01	0.08c±0.01	0.16c±0.01	0.05b±0.01
2%	0.15a±0.01	1.18d±0.01	0.09b±0.01	0.17a±0.01	0.38a±0.01	0.04c±0.01
3%	0.15a±0.01	1.56b±0.01	0.08c±0.01	0.17a±0.01	0.35b±0.01	0.03d±0.01
Control	0.14b±0.01	2.26a±0.01	0.12a±0.01	0.11b±0.01	0.09d±0.01	0.06a±0.01

ALK = Alkaloids, FLAV = Flavonoids, SAP = Saponins. Values are means of 3 replicates; means bearing the same letter are not significantly different ($p \leq 0.05$)

Table 6 presents the effect of glyphosate on the mineral composition of *P. ostreatus* fruiting bodies across different treatment levels. The results showed that fruiting bodies from 3% Gly substrates had the highest nitrogen concentration (0.70 ± 0.01 µg/ml), followed by control (0.66 ± 0.01 µg/ml), while 1% Gly produced the lowest nitrogen content (0.56 ± 0.01 µg/ml). Phosphorus content was highest in 3% Gly-treated fruiting bodies

(1.45 ± 0.01 µg/ml) and lowest in control and 1% Gly (0.55 ± 0.01 µg/ml). Oxygen concentration was maximal in 3% Gly (28.80 ± 0.01 µg/ml) and minimal in control (23.42 ± 0.01 µg/ml). Carbon content was highest in 2% Gly (36.01 ± 0.01 µg/ml), followed by 3% Gly (35.60 ± 0.01 µg/ml), with the lowest in 1% Gly (25.87 ± 0.01 µg/ml). Hydrogen content was highest in 1% Gly



(1.14 ± 0.01 $\mu\text{g/ml}$) and lowest in control (0.24 ± 0.01 $\mu\text{g/ml}$).

Mineral composition of fruiting bodies across all glyphosate levels, including control, was significantly different at $p \leq 0.05$. Results indicated that N, P, C, and O₂ concentrations were generally higher at 2% and 3% glyphosate exposure compared to control, with phosphorus at 3% Gly almost three times the control value. These increases suggest that glyphosate may contribute to the accumulation of certain mineral nutrients in the mushroom fruiting bodies, possibly reflecting an indirect measure of glyphosate toxicity.

The enhanced phosphorus, carbon, oxygen, and hydrogen contents observed in glyphosate-treated fruiting bodies are consistent with

previous reports that oyster mushrooms accumulate essential biologically valuable elements, β -glucans, and antioxidant compounds (Kalac, 2013). The relatively high carbon and oxygen content compared to nitrogen may reflect the mushroom's requirement for a higher carbon-to-nitrogen ratio for optimal growth and development (Stamets, 2000; Chang and Miles, 2004). Choi (2004) reported that the optimal C:N ratio for oyster and *Lentinula edodes* mushrooms ranges between 350–500.

These findings agree with Egwin et al. (2011), who noted that higher mineral concentrations in mushroom fruiting bodies often result from the absorption and accumulation of elements from their substrates or habitat.

Table 6: Effect of Glyphosate on the mineral content of fruiting bodies of *P. ostreatus*

Treatment (%)	N ($\mu\text{g/ml}$)	P ($\mu\text{g/ml}$)	O ₂ ($\mu\text{g/ml}$)	C ($\mu\text{g/ml}$)	H ($\mu\text{g/ml}$)
1%	0.56d $\pm$ 0.01	0.85c $\pm$ 0.01	28.34b $\pm$ 0.01	25.87d $\pm$ 0.01	1.14a $\pm$ 0.01
2%	0.63c $\pm$ 0.01	1.25b $\pm$ 0.01	27.96c $\pm$ 0.01	36.01a $\pm$ 0.01	0.45c $\pm$ 0.01
3%	0.70a $\pm$ 0.01	1.45a $\pm$ 0.01	28.80a $\pm$ 0.01	35.60b $\pm$ 0.01	0.94b $\pm$ 0.01
Control	0.66b $\pm$ 0.01	0.55c $\pm$ 0.01	23.42d $\pm$ 0.01	28.24c $\pm$ 0.01	0.24d $\pm$ 0.01

Means bearing the same letters are not significantly different ($p \leq 0.05$)

4.0 Conclusion

The study successfully demonstrated the effect of glyphosate on the productivity and biochemical composition of *Pleurotus ostreatus* fruiting bodies cultivated on sawdust substrate. The results showed that 800 g of dry sawdust substrate treated with 3% glyphosate produced the highest fruiting body yield (249.04 g) and biological efficiency (31.12%) at 26 days after inoculation, compared to 1% and 2% glyphosate treatments, which recorded lower yields and efficiencies. This indicates that moderate application of glyphosate in farmlands does not pose a significant threat to *Pleurotus ostreatus* populations. Observations on fruiting body morphology revealed that at 18 days after inoculation, mushrooms from the 3% glyphosate treatment had the highest mean fresh weight (3.12 ± 0.75 g) and longest stipe length (2.48 ± 1.00 cm), closely followed by the control. However, fresh weight and stipe

length decreased in all treatments as the cultivation period progressed, suggesting that glyphosate at 0.03% initially enhances growth performance, yield, and biological efficiency, but this effect diminishes over time.

Analysis of proximate composition indicated that glyphosate positively influenced moisture content, ash, crude fibre, and crude protein, while carbohydrate content and calorific value were negatively affected. Increasing glyphosate concentration led to a relative increase in certain phytochemicals, including phytates, alkaloids, and phenols, whereas tannins and saponins declined. Mineral analysis showed no significant differences in nitrogen, hydrogen, phosphorus, and carbon content between glyphosate-treated mushrooms and control samples, indicating that glyphosate did not substantially alter these essential mineral components.



Overall, the study concludes that glyphosate at moderate concentrations can enhance certain aspects of *Pleurotus ostreatus* growth and yield without significantly compromising nutritional quality or mineral content. It is recommended that careful and controlled use of glyphosate in agricultural environments may be compatible with oyster mushroom cultivation, but further studies should explore long-term effects on mushroom physiology and potential residue accumulation to ensure food safety and sustainability.

5.0 References

- Adejoye, O. D., & Fasidi, I. O. (2009). Bio-degradation of agrowastes by some Nigerian white rot fungi. *Journal Bioresource*, 4(2), 816–824.
- Battaglin, W. A., Meyer, M. T., Kuivila, K. M., & Dietze, J. E. (2014). Glyphosate and its degradation product AMPA occur frequently and widely in U.S. soils, surface water, groundwater, and precipitation. *J. Am. Water Resour. Assoc.*, 50, 275–290.
- Baumgartner, K., Fujiyoshi, P., Smith, R., & Bettiga, L. (2010). Weed flora and dormant season cover crops have no effects on arbuscular mycorrhizae of grapevine. *The Journal of Weed Research*, 50, 456–466.
- Benthavithana, J., Islam, T., & Xu, B. (2025). Medicinal Mushrooms in Colon Cancer Therapy: Mechanisms of Action of Bioactive Compounds and Therapeutic Potential. *International Journal of Molecular Sciences*, 26(11), 5304. <https://doi.org/10.3390/ijms26115304>.
- Berger, G., Graef, F., & Pfeffer, H. (2013). *Glyphosate applications on arable fields considerably coincide with migrating amphibians* (pp. 54–60).
- Boucher, R., Germain, H., & Desgagné-Penix, I. (2025). Exploring the Lesser-Known Bioactive Natural Products of Plant Species of the Genus *Cannabis* L.: Alkaloids, Phenolic Compounds, and Their Therapeutic Potential. *Plants*, 14(9), 1372. <https://doi.org/10.3390/plants14091372>.
- Brühl, C. A., Schmidt, T., Pieper, S., & Alscher, A. (2013). Terrestrial pesticide exposure of amphibians: An underestimated cause of global decline. In *Pp.* 12–14.
- Carles, L., Gardon, H., Joseph, L., Sanchís, J., Farré, M., & Artigas, J. (2019). Meta-analysis of glyphosate contamination in surface waters and dissipation by biofilms. *Environ. Int.*, 124, 284–293.
- Chang, S. T., & Miles, P. G. (2004). *Mushroom cultivation, nutritional value, medicinal effect and Environmental impact* (2nd ed.). CRC Press.
- Choi, K. W. (2004). Shelf cultivation of oyster mushroom, with emphasis on substrate fermentation. In *Mushroom growers handbook 1* (pp. 154–165). Mushroompublishers.
- Dimopoulou, M., Kolonas, A., Mourtakos, S., Androutsos, O., & Gortzi, O. (2022). Nutritional Composition and Biological Properties of Sixteen Edible Mushroom Species. *Applied Sciences*, 12(16), 8074. <https://doi.org/10.3390/app12168074>.
- Egwin, E. C., Elem, R. C., & Egwuiche, R. U. (2011). Proximate composition, phytochemical screening and antioxidant activity of ten selected wild edible Nigerian mushroom. *American Journal of Food and Nutrition*, 1(2), 89–94.
- Filipa, M., Umek, A., & Mlinaric, A. (2011). Screening of Basidiomycete mushroom extracts and hallucinogenic constituents in *Amanita* mushrooms circulated in Japan. *Forensic SciInt*, 264, 172–178.
- Greim, H., Saltmiras, D., Mostert, V., & Strupp, C. (2015). Evaluation of carcinogenic potential of the herbicide glyphosate, drawing on tumor incidence data from fourteen chronic/carcinogenicity rodent studies. *Crit. Rev. Toxicol.*, 45, 185–208.



- Guo, X., & Xu, J. (2025). Impacts of global climate change on mushroom production: Challenges and opportunities. *Agriculture Communications*, 3(3), 100091. <https://doi.org/10.1016/j.agrcom.2025.100091>
- Jarrell, Z. R., Ahammad, M. U., & Benson, A. P. (2020). Glyphosate-based herbicide formulations and reproductive toxicity in animals. *Vet. Anim. Sci.*, 10, 100126.
- Kalac, P. (2009). Chemical composition and nutritional value of European species of wild growing mushrooms. A review. *Food Chemistry*, 113, 9–16.
- Kalac, P. (2012). *Mushrooms: Chemical composition and nutritional values of European species of wild growing mushrooms*. Princeton University Press.
- Kalac, P. (2013). *Mushrooms: Chemical composition and nutritional values of European species of wild growing mushrooms*. Princeton University Press.
- Kearney, P., & Wauchope, R. (1998). Disposal options based on properties of pesticides in soil and water. In P. Kearney & T. Robert (Eds.), *Pesticides remediation in soil and water, wiley series in agrochemicals and plant protection* (pp. 568–576).
- Kohler, H. R., & Triebkorn, R. (2013). Wildlife Ecotoxicology of Pesticides: Can We Track Effects to the Population Level and beyond? *Science*, 341, 759–765.
- Mayimao, H.-S., Jangir, P., Sharma, K., Kaur, S., Sharma, Y. P., & Kapoor, R. (2025). Nutrient and antioxidant profile of four species of wild mushrooms from cold-desert with implications for human dietary and supplement use. *Food Chemistry Advances*, 7, Article 101023. <https://doi.org/10.1016/j.focha.2025.101023>
- Mwangi, R. W., Macharia, J. M., Wagara, I. N., & Bence, R. L. (2022). The antioxidant potential of different edible and medicinal mushrooms. *Biomedicine & Pharmacotherapy*, 147, Article 112621. <https://doi.org/10.1016/j.biopha.2022.112621>
- Myers, J. P., Antoniou, M. N., Blumberg, B., Carroll, L., Colborn, T., Everett, L. G., Hansen, M., Landrigan, P. J., Lanphear, B. P., Mesnage, R., Vandenberg, L. N., Vom Saal, F. S., Welshons, W. V., & Benbrook, C. M. (2016). Concerns over use of glyphosate-based herbicides and risks associated with exposures: A consensus statement. *Environmental Health*, 15, Article 19. <https://doi.org/10.1186/s12940-016-0117-0>
- Nerud, F. J., Baldrian, J., Gabriel, & Ogbeifun, D. (2003). Nonenzymic degradation and decolorization of recalcitrant compounds. In V. Sasek et al. (Eds.), *The utilization of bioremediation to reduce soil contamination: Problems and solutions* (pp. 29–48). Kluwer Academic Publishers.
- Noreen, S., Sultan, H., Hashmi, B., Aja, P. M., & Atoki, A. V. (2025). Mushroom marvels: understanding their role in human health. *Frontiers in Nutrition*, 12, 1654911. <https://doi.org/10.3389/fnut.2025.1654911>
- Nwoko, M. C., Okwulehie, I. C., & Achufusi, J. N. (2016). Nutritional composition of *Pleurotus ostreatus* (Jacq.) P. kumm. fruit bodies cultivated on deciduous tree logs in Umudike, Abia State, Nigeria. *Intl Journal Res. Tech*, 5, 102–108.
- Obodai, M., Cleland-Okine, J., & Vowotor, K. A. (2003). Comparative study on the growth and yield of *Pleurotus ostreatus* mushroom on different lignocellulosic by-products. *Journal Industrial Microbiology Biotechnology*, 30, 146–149.
- Ogbo, E. M., & Okhuoya, J. A. (2009). Effect of crude oil contamination on the yield and chemical composition of *Pleurotus tuber-regium* (Fr.) Singer. *African Journal Food Science*, 3(11), 323–327.
- Okoi, A. I., & Iboh, C. I. (2015). The effects of different substrates on sporophore yield, mineral and nutrient composition of *Pleurotus tuber-regium* fries singer in



- Calabar, Nigeria. *International Journal of Agricultural Science Research*, 4(6), 126–131.
- Okwujiako, I. A., Okwulehie, I. C., & Igbojionu, V. O. (2013). Mycoremediation of engine oil-polluted soil using the white rot fungus, *Pleurotus florida* (Mont.) Singer, an edible fungus. *Research Journal of Agriculture and Environmental Management*, 2(11), 354–357.
- Okwulehie, I. C., & Okwujiako, I. A. (2008). The use of local Nigerian substrates for the production of *Pleurotus ostraetuvavar florida* (Eger) sporophores. *Global Science Books*, 2(2), 38–40.
- Onyeizu, U. R., Nwoko, M. C., Chukunda, F. A., & Ukoima, H. N. (2017). Productivity, vitamins and heavy metals analysis of *Pleurotus ostreatus* (Jacq.ex.Fr.) Kummerfruitbodies on wood logs. *International Journal of Information Research and Review*, 4(3), 3890–3894.
- Pasaribu, A., Mohamad, R. B., Awang, Y., Othman, R., & Puteh, A. (2011). Growth and development of symbiotic arbuscular mycorrhizal fungi, *Glomus mosseae* (Nicol. and Gerd.), in alachlor and glyphosate treated soils. *African Journal Biotechnol*, 10, 11520–11526.
- Patil, S. S. (2018). Productivity and proximate content of *Pleurotus sajor-caju*. *Bioscience Discovery*, 4(2), 169–172.
- Powell, J. R. (2009). Effect of glyphosate on the tripartite symbiosis formed by *Glomus intraradices*, *Bradyrhizobium japonicum*, and genetically modified soybean. *Applied Soil Ecology*, 41, 128–136.
- Rueda-Ruzafa, L., Cruz, F., Roman, P., & Cardona, D. (2019). Gut microbiota and neurological effects of glyphosate. *NeuroToxicology*, 75, 1–8. <https://doi.org/10.1016/j.neuro.2019.08.006>
- Shakeel, A., Noor, J. J., Jan, U., Gul, A., Handoo, Z., & Ashraf, N. (2025). Saponins, the unexplored secondary metabolites in plant defense: Opportunities in integrated pest management. *Plants*, 14(6), 861. <https://doi.org/10.3390/plants14060861>.
- Sharad, C. M. (2013). Productivity and proximate content of *Pleurotus sajor-caju*. *Bioscience Discovery*, 4(4), 169–172.
- Silva, V., Montanarella, L., Jones, A., Fernández-Ugalde, O., Mol, H. G. J., Ritsema, C. J., et al. (2018). Distribution of glyphosate and aminomethylphosphonic acid (AMPA) in agricultural topsoils of the European Union. *Sci. Total Environ.*, 621, 1352–1359.
- Silva-Pinto, P. A., de Pontes, J. T. C., Aguilar-Morón, B., Canales, C. S. C., Pavan, F. R., & Roque-Borda, C. A. (2025). Phytochemical insights into flavonoids in cancer: Mechanisms, therapeutic potential, and the case of quercetin. *Heliyon*, 11(4), e42682. <https://doi.org/10.1016/j.heliyon.2025.e42682>.
- Singh, A., Saini, R. K., Kumar, A., Chawla, P., & Kaushik, R. (2025). Mushrooms as nutritional powerhouses: A review of their bioactive compounds, health benefits, and value-added products. *Foods*, 14(5), 741. <https://doi.org/10.3390/foods14050741>.
- Sofowora, A. (2003). *Medicinal plants and traditional medicine in Africa*. Spectrum Book (Ltd) Ibadan.
- Soil Association. (2017). *The impact of glyphosate on soil health*. Retrieved from <https://www.soilassociation.org/media/7202/glyphosate-and-soil-health-full-report.pdf>
- Stamets, P. (2005). *Mycelium Running: How Mushroom Can Help Save the World*. Ten speed Press.
- Sumalee, C. N., Mensin, H., Kasem, S., Robert, J., McGovern, M., & Toanun, A. C. (2013). Effect of agricultural pesticides on the growth and sporulation of nematophagous fungi. *Journal of Agricultural Technology*, 9(4), 953–961.



- Swanson, N. L., Leu, A., Abrahamson, J., & Wallet, B. (2014). Genetically engineered crops, glyphosate and the deterioration of health in the United States of America. *J. Org. Syst.*, 9, 6–37.
- van Bruggen, A. H. C., He, M. M., Shin, K., Mai, V., Jeong, K. C., Finckh, M. R., et al. (2018). Environmental and health effects of the herbicide glyphosate. *Sci. Total Environ.*, 616-617, 255–268.
- Vereecken, H. (2005). Mobility and leaching of glyphosate: a review. *Pest. Management. Science*, 61(12), 1139–1151.
- WHO (World Health Organization). (2009). *The WHO recommended classification of pesticides by hazard and guidelines to classification: 2009*. Int. Program Chem. Saf.
- Yang, J. H., Lin, H. C., & Mau, J. L. (2002). Antioxidant properties of several commercial mushrooms. *Food. Chemistry.*, 77(2), 229–235.
- Zhang, L., Rana, I., Shaffer, R. M., Taioli, E., & Sheppard, L. (2019). Exposure to phosphate-based herbicides and risk for non-Hodgkin lymphoma: A meta-analysis and supporting evidence. *Mutat. Research/Reviews Mutat. Res.*, 781, 186–206.

Declaration

Competing interests

There are no known financial competing interests to disclose

Ethical Consideration

This research was conducted in compliance with institutional and international standards for ethical research. The study involved no human or animal subjects, and all experimental procedures were strictly laboratory-based. Care was taken to ensure safe handling and disposal of glyphosate and mushroom substrates, minimizing environmental impact and adhering to biosafety and environmental protection guidelines.

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The data supporting the findings of this study can be obtained from the corresponding author upon request

Authors' Contributions

Odor, Boniface Egbu conceptualized the study while Nzezbule, Emmanuel supervised the study Chikezie, Precious Chieze handled data collection and analysis. Alilionu, Doris, Onyemuche, Tochukwu Nnamdi, and Ejeje, N. Jerius, conducted experiments, while Onuoha, Chibuzor Peter drafted the manuscript. Ekoh Charles Obinna managed project administration, and Ojiakor, Marycynthia Amarachi assisted in design.

