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BIOCONVERSION OF *PROSOPIS AFRICANA* BY SELECTING HIGHER FUNGI THROUGH SOLID-STATE FERMENTATION INTO VALUE-ADDED PRODUCTS

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ABSTRACT

Agricultural residues such as *Prosopis africana* pods are abundant yet underutilized due to their high lignocellulosic content and resistance to degradation. This study evaluated their bioconversion through solid-state fermentation (SSF) using *Pleurotus ostreatus*, *Pleurotus pulmonarius*, and a mixed inoculum over 28 days. The combined fungal culture achieved the greatest lignin reduction (33.5%) while maintaining the lowest organic matter loss (2.04%), demonstrating efficient nutrient conservation and selective degradation. Proximate composition improved significantly, with crude protein increasing from 8.19% to 10.89% and crude fat also enhanced, while crude fiber declined sharply from 25.17% to 3.4%, indicating improved digestibility. Mineral analysis revealed enrichment of iron and manganese, while reductions in calcium and potassium suggested fungal utilization during metabolism. Fermentation also reduced anti-nutritional factors such as tannins, flavonoids, and phenols, with tannins declining by up to 87%, while secondary metabolites including saponins and terpenoids increased. Lignocellulose degradation was most pronounced in the mixed culture, reflecting synergistic enzyme production and confirming the efficiency of *Pleurotus spp*. in breaking down complex substrates. Protease activity increased consistently throughout fermentation, correlating with improved protein content and hydrolysis of complex biomolecules. Collectively, these transformations highlight the potential of Pleurotus-based SSF to convert *P. africana* pods into nutritionally enriched, bioactive-rich, and more digestible products. The synergistic inoculum proved particularly effective, highlighting the application of mixed fungal cultures as a sustainable strategy for developing value-added feed resources from agro-waste.

Keywords: *Pleurotus ostreatus*, *Pleurotus pulmonarius*, *Prosopis africana*, Solid-State Fermentation, Lignin Degradation, Bioconversion, Agro-Waste

INTRODUCTION

The improper disposal of agricultural waste through burning, landfilling, and dumping contributes significantly to environmental degradation and greenhouse gas emissions (Chilakamarry et al., 2022; Awogbemi et al., 2022). The agricultural sector accounts for 21–37% of global emissions (Lynch et al., 2021). Agro-wastes are rich in nutrients and bioactive compounds (Dwivedi et al., 2022) and can be transformed into enzymes, biofuels, feed, and pharmaceuticals through microbial and biotechnological processes (Chojnacka et al., 2019; Reshmy et al., 2022; Balan et al., 2022; Bala et al., 2022; Gupta et al., 2022). However, their complex lignocellulosic nature requires effective pretreatment for efficient bioconversion (Verma et al., 2011).

Prosopis africana pods contain proteins, carbohydrates, and minerals, including valuable ash content (Abdulganiyu et al., 2024). Fungi are diverse organisms capable of producing extracellular enzymes that degrade organic matter into assimilable nutrients (Bahram & Netherway, 2022). Their filamentous growth forms mycelium, which serves as a source of mycoproteins. Mixed fungal cultures can optimize enzyme production for effective biodegradation, and combining *P. africana* pods with other substrates can enhance microbial activity (Oni, Oke, & Sani, 2020).

Biodegradation via fungi provides a sustainable method for converting agro-wastes into value-added products. Solid-state fermentation (SSF) using fungi like *Aspergillus niger* and *Lactobacillus plantarum* has been shown to improve the nutritional and physicochemical properties of substrates (Wang et al., 2024), though challenges remain in scale-up, particularly heat and mass transfer (Finkler et al., 2021; Chilakamarry et al., 2022). The nutrient and phytochemical composition of *P. africana* seeds and pods supports fungal growth and metabolite production (Ajiboye et al., 2013).

SSF parameters, such as temperature and moisture, significantly influence enzyme production and bioactive metabolite yield (Carboue et al., 2020). Higher fungi, such as *Pleurotus* species, have successfully transformed lignocellulosic materials into value-added products (Croan, 2000), and agro-industrial wastes like jackfruit have been similarly utilized (Pathak et al., 2022).

In Nigeria's agro-based economy, converting *P. africana* pods through SSF supports waste valorization and sustainable agriculture (Arvanitoyannis & Varzakas, 2008; Belewu & Banjo, 1999). This study investigates the bioconversion of *P. africana* pods using *P. ostreatus* and *P. pulmonarius*, focusing on changes in proximate, phytochemical, and mineral compositions, and ligninolytic enzyme activity, providing insights into sustainable agro-waste management.

MATERIALS AND METHODS

Materials

Dried *Prosopis africana* pods, jam bottles, measuring tools, sterile Petri dishes, aluminum foil, cotton wool, distilled water, PDA media, and 70% ethanol were used. All glassware was sterilized in a hot air oven, and surfaces were disinfected with ethanol.

As shown in Plate 1, the seed dimensions of mature *Prosopis africana* pods vary significantly between samples (Ogunjimi et al., 2006)

Plate 1: Seed of mature *Prosopis africana* pods

Collection and Preparation of Substrate

Mature, naturally fallen *P. africana* pods were collected from the University of Ilorin, Nigeria. Seeds were manually removed, and pods were crushed, ground into powder, and sieved. The powder was stored dry before use. Samples were analyzed for proximate and phytochemical composition.

Fungal Cultures and Solid-State Fermentation (SSF)

Pleurotus ostreatus and *P. pulmonarius* strains were obtained from the Mycology Laboratory, University of Ilorin, and maintained on PDA at 4°C. For SSF, 100 g of powdered substrate was moistened with sterile distilled water, autoclaved, and inoculated with agar plugs of the fungi. Inoculated bottles were incubated at approximately 28°C for 28 days. Samples were collected at 14 and 28 days, oven-dried at 60°C, and stored for subsequent analysis.

Analytical Methods

Mineral content was determined after digestion with a mixture of nitric and hydrochloric acids (3:1), with calcium and magnesium measured by atomic absorption spectrophotometry, potassium and sodium by flame photometry, and phosphorus via colorimetry (AOAC, 2005). Proximate composition, including moisture, ash, crude protein, crude fat, and crude fiber, was determined using AOAC methods, and carbohydrate content was calculated by difference (AOAC, 2005).

Phytochemical screening was conducted for alkaloids, glycosides, terpenoids, phenolics, tannins, flavonoids, saponins, and steroids using standard spectrophotometric and colorimetric methods (Harborne, 1998). Protease activity was assessed by incubating crude enzyme extracts with hemoglobin at pH 3.0 and 50°C, with tyrosine release measured at 280 nm using a standard curve (Wong et al., 1988).

Lignocellulosic components were analyzed with cellulose determined by difference, hemicellulose extracted with 0.5 M NaOH at 80°C for 3.5 hours, and lignin quantified via sulfuric acid treatment with residual weight recorded (Van Soest, 1963). The calorific value of samples was measured using a Leco AC350 oxygen bomb calorimeter (AOAC, 2005).

Statistical Analysis

All data were analyzed using one-way ANOVA at $p < 0.05$ in SAS software. Mean separation was conducted using Duncan's Multiple Range Test (DMRT), and correlation analyses were performed to evaluate relationships among variables (Steel, Torrie, & Dickey, 1997).

RESULTS AND DISCUSSION

Loss of Organic Matter (LOM)

Bioconversion of *Prosopis africana* pods using *Pleurotus ostreatus* (P.O), *Pleurotus pulmonarius* (P.P), and their combination (P.O + P.P) significantly influenced organic matter loss (LOM). After 14 days, LOM ranged from 1.59% (P.O + P.P) to 2.67% (P.P), increasing by day 28 to 2.04–4.61%. Two-way ANOVA showed organism type ($p < 0.0001$), incubation time ($p < 0.0001$), and their interaction ($p < 0.0001$) significantly affected LOM. P.P induced the highest degradation at 28 days, while the combination consistently exhibited the lowest LOM.

Table 1: Mean Loss of Organic Matter (%)

Organism	Day 14	Day 28
P. ostreatus	2.42 ± 0.05	2.87 ± 0.05
P. pulmonarius	2.67 ± 0.05	4.61 ± 0.05
P.O + P.P	1.59 ± 0.05	2.04 ± 0.05

Note: P.P = *Pleurotus pulmonaris* P.O = *Pleurotus ostreatus* P.P + P.O = *Pleurotus pulmonaris + Pleurotus ostreatus*

Mineral Composition (mg/100g)

Fermentation reduced calcium, potassium, and magnesium while increasing iron and manganese. P.O recorded the highest iron at 28 days (8.108 mg/100g) and P.P the highest manganese (0.498 mg/100g).

Table 2: Summary of Mineral Composition Across Treatments

Mineral	Control 1d	P.O 14d	P.O 28d	P.P 14d	P.P 28d	P.O+P.P 14d	P.O+P.P 28d
Calcium	5.414	2.570	1.267	1.944	1.502	2.570	1.388
Magnesium	1.933	1.921	5.911	1.904	5.840	1.904	5.841
Iron	0.298	1.159	8.108	0.820	3.890	0.267	3.606
Copper	0.150	0.006	0.350	0.147	0.279	0.006	0.296
Zinc	1.898	1.895	2.100	2.078	1.753	1.792	1.727
Potassium	11.222	10.042	8.493	9.404	7.658	9.648	7.513
Manganese	ND	0.011	0.561	0.069	0.498	0.015	0.453

Note: P.O = *Pleurotus ostreatus*; P.P = *Pleurotus pulmonarius*; P.O + P.P = combination of P.O + P.P; ND = Not Detected, d=d=day(s)

Proximate Composition (%)

Crude protein, fat, and carbohydrates increased while crude fiber decreased sharply in all treatments. P.O + P.P had the highest protein (10.886%) and fat (6.776%) at 28 days.

Table 3: Summary of Proximate Composition Across Treatments

Component	Control 1d	P.O 14d	P.O 28d	P.P 14d	P.P 28d	P.O+P.P 14d	P.O+P.P 28d
Moisture	11.931	12.640	12.923	12.206	12.686	14.480	14.706
Ash	3.908	3.916	3.973	3.816	3.936	3.970	3.983
Protein	8.188	9.713	9.906	9.433	9.646	10.506	10.886
Fat & Oil	2.304	4.933	5.343	4.900	5.213	5.823	6.776
Crude Fiber	25.171	3.233	3.346	3.243	3.276	3.376	3.360
Carbohydrate	48.498	65.565	64.509	66.402	65.143	61.845	60.289

Note: P.O = *Pleurotus ostreatus*; P.P = *Pleurotus pulmonarius*; P.O + P.P = combination of P.O + P.P; Control = unfermented *Prosopis africana* pods,d=d=day(s)

Phytochemical Composition Analysis

Flavonoids, glycosides, tannins, and phenols decreased while alkaloids, saponins, steroids, and terpenoids increased significantly ($p < 0.05$). Saponins rose from 6.42 mg/L to above 98 mg/L in all treatments.

Table 4: Summary of Phytochemical Composition Across Treatments

Component	Control 1d	P.O 14d	P.O 28d	P.P 14d	P.P 28d	P.O+P.P 14d	P.O+P.P 28d
Alkaloids	3.655	10.132	10.136	10.126	10.134	10.145	10.149
Saponins	6.420	98.763	98.743	98.670	98.666	98.786	98.790
Tannins	67.307	8.869	8.873	8.860	8.863	8.881	8.887
Phenols	18.859	9.246	9.248	9.239	9.241	9.261	9.263
Flavonoids	11.179	1.112	1.112	1.112	1.112	1.113	1.113
Glycosides	65.410	55.623	55.633	55.593	55.610	55.666	55.683
Terpenoids	8.380	30.366	30.383	30.296	30.316	30.450	30.483
Steroids	4.523	7.266	7.350	7.216	7.250	7.400	7.433

Note: P.O = *Pleurotus ostreatus*; P.P = *Pleurotus pulmonarius*; P.O + P.P = combination of P.O + P.P; Control = unfermented *Prosopis africana* pods,d=day(s).

The explanation given on cellulose, hemicellulose, lignin content of degraded substrate, as well as the enzyme activity.

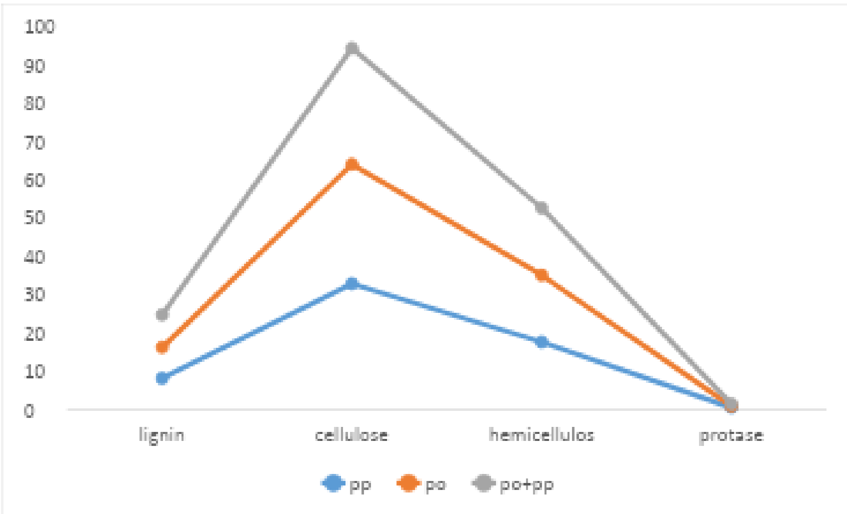

Figure 1: Effect of *Pleurotus ostreatus, Pleurotus pulmonaris,* and their Combination on Enzyme Activity and Lignin Content on Degraded Substrates (14 Days)

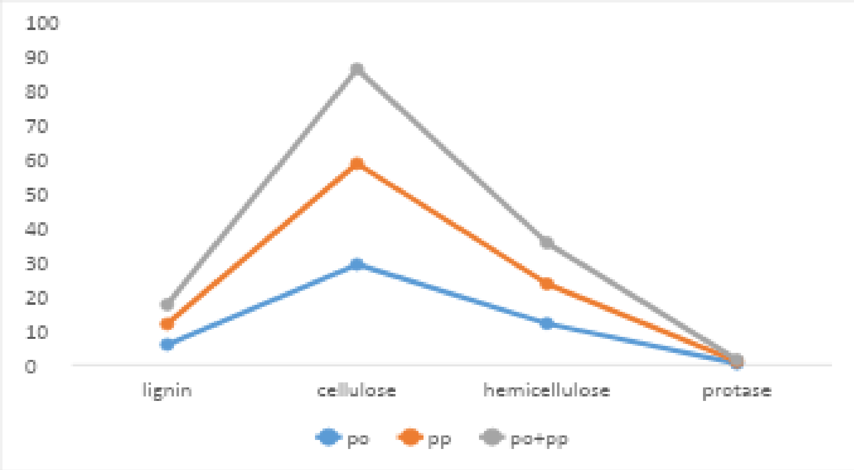

Figure 2: Effect of *Pleurotus ostreatus, Pleurotus pulmonaris,* and Their Combination on Enzyme Activity and Lignin Content on Degraded Substrates (28 days)

Enzyme Activity and Lignocellulose Content

Lignin, cellulose, and hemicellulose decreased significantly over 28 days, while protease activity increased. The combination (P.O + P.P) recorded the highest protease activity increase (0.563 → 0.570), indicating enhanced protein hydrolysis. Lignin declined from 8.510% to 5.660%, with parallel declines in cellulose and hemicellulose.

Discussion

The research confirms the ability of *Pleurotus ostreatus* and *P. pulmonarius* to degrade lignocellulosic agro-waste (*Prosopis africana* pods) through solid-state fermentation. Their rapid colonization reflects their saprophytic nature and production of ligninolytic enzymes, including laccase, peroxidases, cellulases, and β-glucosidases (Da Luz et al., 2012; Fen et al., 2014; Kumla et al., 2020). Mechanized processing of *Prosopis africana* pods can improve their suitability as substrates for fungal bioconversion (Tajudeen et al., 2011). Weber et al. (2008) reported on the growth and survival of *Prosopis africana* across different rainfall gradients, providing context for the selection of robust plant material for bioconversion studies.

Loss of organic matter (LOM) provides insights into fungal efficiency during fermentation. In this study, *P. pulmonarius* caused the greatest LOM, while the mixed inoculum maintained the lowest despite achieving significant lignin reduction. This suggests that combined cultures exhibit selective degradation, conserving nutrients while still enhancing digestibility. Such outcomes align with previous reports showing that synergistic fungal interactions often increase efficiency without excessive nutrient loss (Chilakamarry et al., 2022).

Solid-state fermentation (SSF) is recognized as a sustainable method for upgrading low-value biomass into bioactive and enriched products (Yazid et al., 2017). Its efficiency, however, depends on key parameters such as temperature and moisture, which strongly influence fungal metabolism and metabolite yield (Carboue et al., 2020). This supports the application of SSF in the bioconversion of *Prosopis africana* by higher fungi into value-added products.

Proximate composition was significantly influenced by fermentation. Crude protein increased post-fermentation, consistent with fungal biomass accumulation and the action of extracellular proteases, though still lower than legumes such as pigeon peas (Coe et al., 2005). Fungi can transform low-value biomass, such as *Prosopis africana,* into nutritionally and functionally enriched products through enzyme-mediated processes (El-Bakry et al., 2015). Enhanced digestibility and amino acid availability offer feed benefits (Carpenter et al., 2004; Zhao et al., 2019; Qin et al., 2015; Esteves et al., 2021). Crude fat also increased, possibly due to lipid accumulation in fungal cells, while crude fiber declined markedly, reflecting hydrolysis of cellulose and hemicellulose. Carbohydrate values fluctuated but remained high, suggesting potential as an energy-dense feed. Moisture increased slightly but remained below safe storage limits (<15%) (Ogbemudia et al., 2017). Ash content was stable, though lower than in some plant materials, likely due to volatilization losses (Mole & Waterman, 1982). These findings agree with earlier reports on the nutrient richness of *P. africana* seeds and pods (Helen & Moctar, 2007; Barminas, 1998; Ajiboye et al., 2013).

Fermentation also affected mineral composition. Iron and manganese contents increased significantly, consistent with reports of fungal-mediated solubilization and release of bound minerals (Bajpai et al., 2005). Calcium and potassium levels, however, declined, suggesting utilization by fungi during metabolism. The increase in iron and manganese is nutritionally important, as these elements support hemoglobin synthesis and enzymatic functions, while the reduction in calcium and potassium suggests the need for supplementation if fermented pods are used in feed. These results mirror earlier findings on fermented legumes and cereals (Lee et al., 1993; Jenkins & Atwal, 1994; Coulibaly et al., 2011; Simwaka et al., 2017).

Phytochemical composition also shifted during fermentation. Anti-nutritional factors such as tannins, phenols, flavonoids, and glycosides declined, while alkaloids, saponins, terpenoids, and steroids increased. Tannin reduction is beneficial since tannins limit protein digestibility and mineral absorption (Reed et al., 1982; Philip et al., 2018; Qianqian et al., 2018; Abhishek et al., 2019). Decreases in flavonoids and phenols may result from fungal metabolism, while increased saponins and terpenoids suggest secondary metabolite biosynthesis by *Pleurotus spp*. Although high saponin levels reduce palatability, moderate levels have cholesterol-lowering and immunostimulatory effects (Lalitha et al., 1990). These compositional changes highlight the potential of SSF to detoxify anti-nutrients while enriching substrates with beneficial bioactive compounds.

Lignocellulose degradation was evident across treatments, with lignin, cellulose, and hemicellulose all declining significantly. The mixed fungal culture achieved the greatest lignin reduction, consistent with reports that synergistic cultures enhance ligninolytic enzyme production (Adenipekun & Fasidi, 2005; Kuforiji & Fasidi, 2004). Lignin breakdown improves feed digestibility, while reductions in cellulose and hemicellulose reflect carbon metabolism by fungi. Optimizing culture conditions and inducers can further enhance enzyme yields in *Pleurotus spp*., thereby improving substrate transformation (Potu, Thadikamala, & Moses, 2014). Similar applications of microbial biotechnology in SSF have been reported for other agro-industrial wastes (Yafetto, 2022).

Enzyme activity, particularly protease, increased consistently throughout fermentation, with the highest activity recorded in the combined inoculum. Protease activity plays a key role in hydrolyzing complex proteins into more digestible forms, contributing to the observed increase in crude protein content. This also supports the potential use of fermented pods as protein-enriched animal feed (Wong et al., 1988).

Overall, this study demonstrates that *Pleurotus ostreatus* and *P. pulmonarius* can effectively convert *Prosopis africana* pods into nutritionally improved, bioactive-rich products. Improvements in protein, fat, iron, manganese, and bioactive compounds, combined with reductions in fiber, tannins, and lignin, emphasize the potential application of fermented *P. africana* as sustainable animal feed. The synergistic culture of both fungi proved particularly effective, balancing nutrient preservation with efficient lignocellulose degradation.

CONCLUSION

This study demonstrates that *Pleurotus ostreatus* and *P. pulmonarius* can efficiently bioconvert *Prosopis africana* pods through solid-state fermentation, resulting in significant nutritional enhancement, reduction of anti-nutritional factors, and effective lignin degradation. These transformations highlight the potential of fermented pods as a sustainable and protein-enriched feed resource. Notably, the synergistic interaction of both fungi produced superior outcomes, underscoring the value of mixed cultures in maximizing substrate utilization. Future research should focus on optimizing fermentation parameters and evaluating large-scale applications to fully harness the biotechnological potential of this process.

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APPENDIX
Loss of Organic Matter

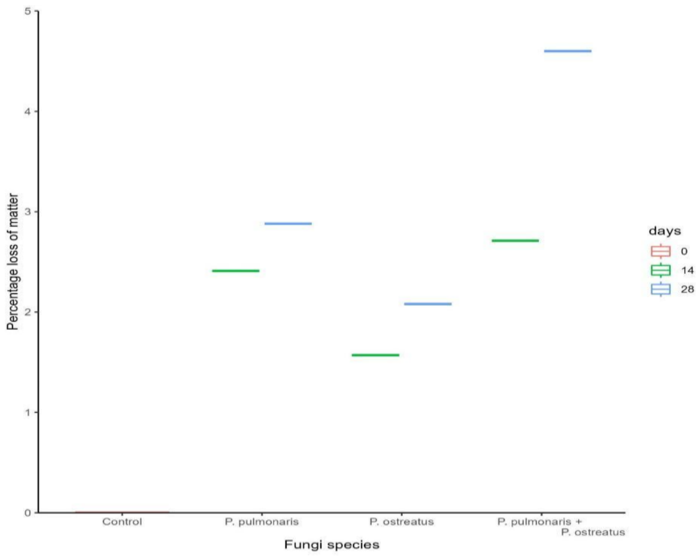

Figure 3**:** The Percentage Mean of Loss of Organic Matter (LOM)

Mineral Analysis

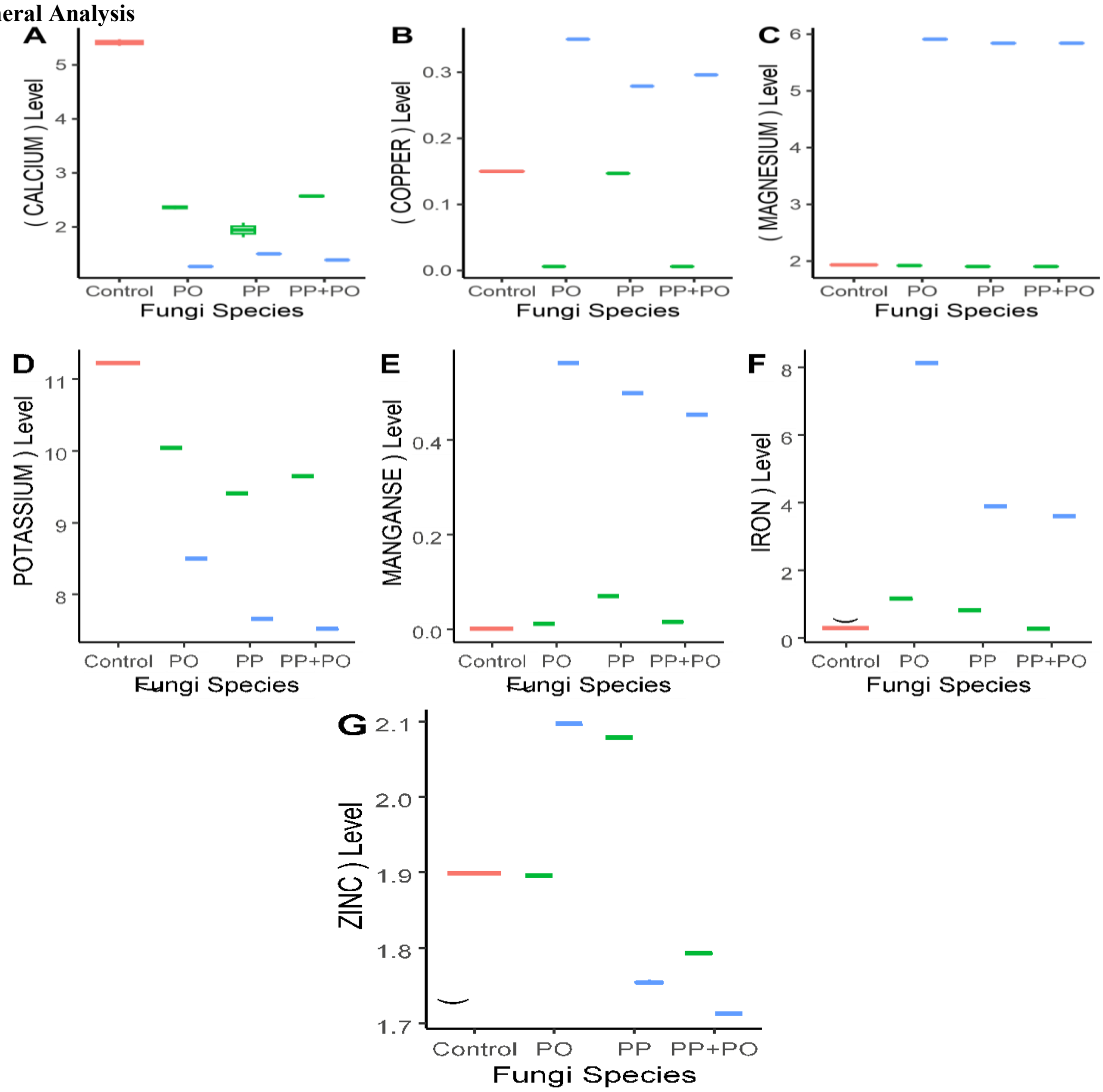

Figure 4**:** Effect of *Pleurotus pulmonaris* and *Pleurotus ostreatus* on Nutrient Content of the Degraded Substrate and the Undegraded Substrate

Proximate Analysis

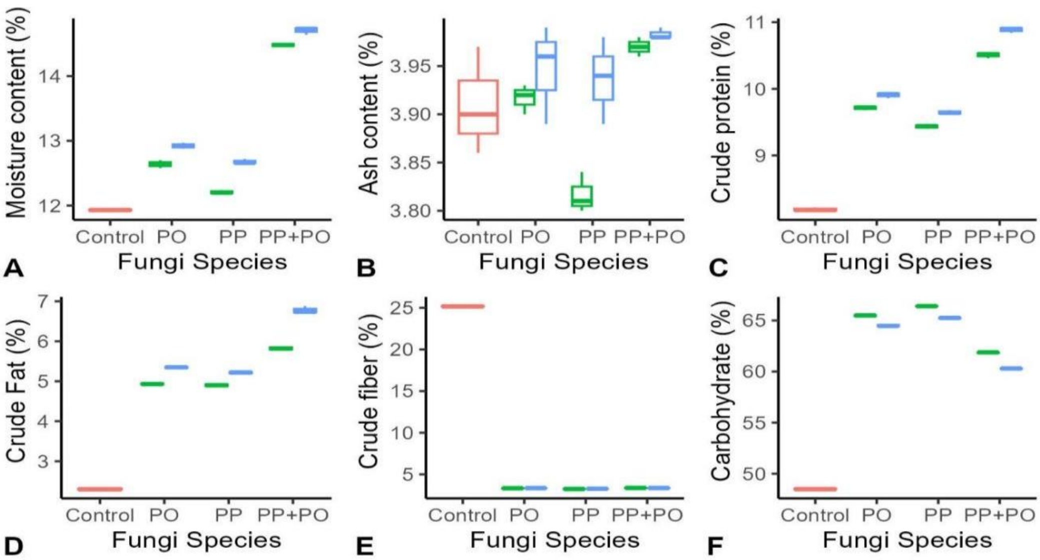

Figure 5: Effect of *Pleurotus pulmonaris* and *Pleurotus ostreatus* on Proximate Consitutent Levels in Days of the Degraded Substrate and the Undegraded Substrate

Phytochemical Analysis

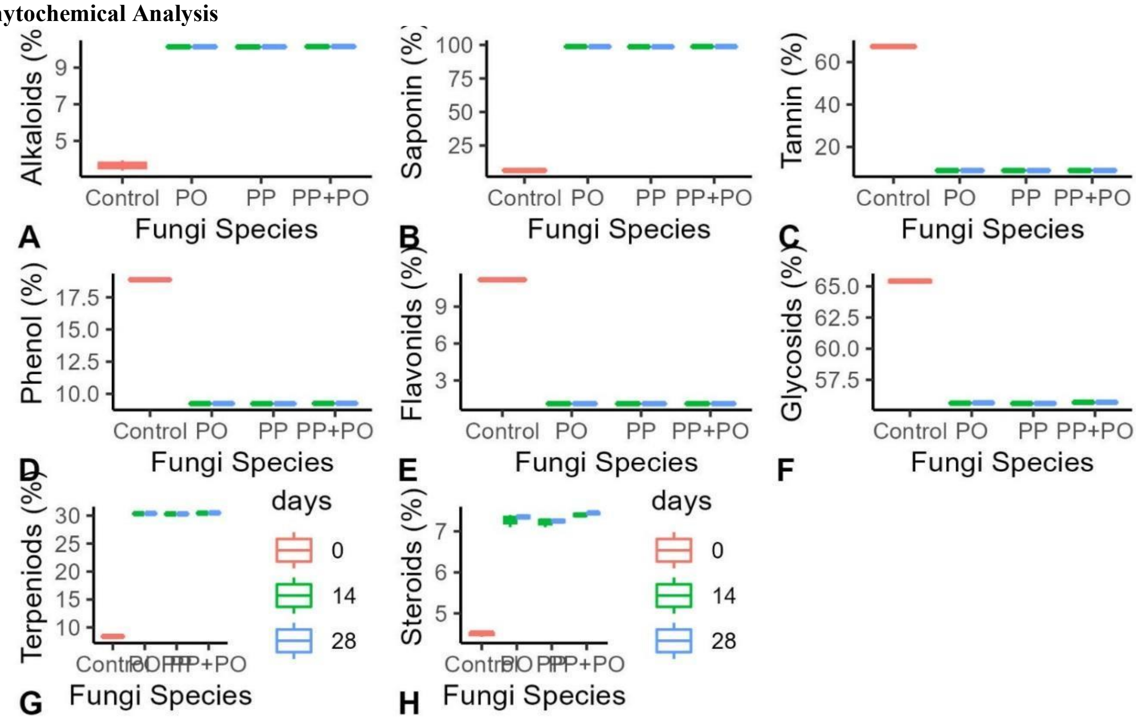

Figure 6**:** Effect of *Pleurotus pulmonaris* and *Pleurotus ostreatus* on Phytochemical Analysis of the Degraded Substrate and the Undegraded Substrate