Urban Green Waste Delignification by Pleurotus florida (White Oyster) Production: A Pilot Scale Study

Nomore Rukara
National Forensic Sciences University
SHALINI GUPTA
shalinigupta13jan@gmail.com

National Forensic Sciences University https://orcid.org/0000-0002-6441-5876

Research Article

Keywords: P. florida, Mycology, Urban Zone, Green Waste, Delignification

Posted Date: June 10th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-6673748/v1

Abstract

Present research work embraces mycology expertise for lignocellulosic green waste valorisation and management. Meticulous experimental cultivation of *Pleurotus florida* species mushroom on five soaked and autoclaved, axenic lignocellulosic urban green waste substrates, was conducted in triplicates. The research findings indicate substantial variability in substrate characteristics, specifically carbon, nitrate, and lignin contents, that exert control over mushroom growth dynamics yields and nutritional outcomes. Substrate carbon content was maximum in *Duranta erecta* (43±0.11%) followed by mixed substrate (35±0.12%), *Triticum aestivum* (34±0.12%), *Zoysia japonica* (28±0.15%) and lastly *Plumeria obtusa* (28±0.38%). Nitrate content was at peak in *Zoysia japonica*(196.96±1.48mg/L) followed by Mixed substrate (175.06±1.21mg/L), *Duranta erecta* (170.67±1.13mg/L), *Triticum aestivum* (168.94±0.97mg/L), and lastly Plumeria obtusa (164.14±1.23mg/L). The most lignified substrate was *Duranta erecta* (34.78±0.23%), followed by *Triticum aestivum* (30.8±0.2%), mixed substrate (27.08±0.93%), Plumeria obtusa (24.43±0.28%) and lastly *Zoysia japonica* (21.15±0.2%). Biological efficiency varied significantly (P<0.05) across substrates, being at peak on mixed substrate (75%), followed by *Duranta erecta* (67.67%), *Plumeria obtusa* (58.33%), *Triticum aestivum* (45.67%) and lastly *Zoysia japonica* (39%). There was a positive correlation between substrate lignin content and both mycelium colonization rate and biological efficiency. Highest delignification was witnessed on mixed substrate (36%), followed by *Duranta erecta* (18%), *Zoysia japonica* (13%), *Triticum aestivum* (11%) and lastly *Plumeria obtuse*. Study concludes that mushroom cultivation serves as a viable and effective biotechnological approach for lignocellulosic urban green waste biodegradation, carbon cycling, and enhancing food and nutrition security.

1. Introduction

Indian equatorial thermal constancy proffers an opulent milieu for vigorous proliferation and ornamental verdancy of terrestrial tropical ecosystem flora [1]. Moreover, when designing urban greening species collections, botanical specialists meticulously select fast growing ornamental plants which exhibits high photosynthetic alacrity and remarkable phenotypic adaptability [2]. Such species assemblages beget prodigious conversion of solar radiation into floriferous splendour and lush foliage. Consequently, boulevard arboriculture, parkland silviculture, and ornamental horticulture lignocellulosic green waste generation rate surpasses it’s intrinsic natural putrefaction capacity. Green waste accumulation induced rambunctious urban vermination, proliferates hazmat zoonotic contacts [3]. An upsurge of zoonotic and new infectious diseases has been observed since the onset of the new millennium [4]. Green waste accumulation potentially increases zoonosis and parasitosis risk, as it nourishes and shelters synanthropic vertebrates and arthropods (which are potential pathogen hosts and virus reservoirs) [3] Click or tap here to enter text.. Green waste dumps, therefore, becomes a cogent human-animal interface in the urban ecosystem [5] Click or tap here to enter text.. Consequently, it is critical to coin-up and implement a comprehensive, efficient, and sustainable green waste management strategy. Such an approach is crucial for upholding public health, promoting environmental sustainability, and supporting socio-economic development [6]. The current tempo of urban green waste management strategies

renders the notion of sustainability questionable. Most of lignocellulosic urban green waste disposed through open burning which pollutes and stymie air quality management efforts [7]. Such unorthodox green waste disposal method (open burning) is under reported [8], yet it emits significant measures of carcinogenic, teratogenic and toxic compounds such as polycyclic aromatic hydrocarbons (PAHs) [9], polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/F) [10]

Accordingly, this study implores for sustainable, multi-faceted lignocellulosic green waste management praxis, promoting green waste conversion into wealth [11], through mushroom production [12] Click or tap here to enter text., thus roping in mycological expertise to green waste management. The crux of the matter is capitalizing on strong saprophytic ligninolytic enzymatic mycelium secretions to degrade recalcitrant lignocellulosic green waste [13]. Such waste valorization aligns with bioeconomy creation and promotes the transition to the circular economy [14]. There is need for embracing productive, self-sufficient, ecofriendly and sustainable green waste management strategies [15]. Therefore, mushroom cultivation on lignocellulosic green waste emerges as one of such valuable ideas [16], as it degrades lignocellulosic green waste into manure efficiently, while producing protein rich food [17]. It also serves as a pre-runner to biogas production and vermicomposting [14] thereby maximizing biomass resource utilisation [18] (Pawar & Kakde, 2021). Mycologists have long broadened the scope of mushroom beyond food value of mushroom when the dimension of ethnomycology was pursued [19]. Such a productive waste management strategy economic growth can save third word urban local governments from the prevailing budgetary constraints which impedes service delivery [20]. Thence, there is need for urban green waste valorisation through mushroom production, for which proper implementation saves municipalities and panchayats from financially drowning [21]. Present research work aims to assess the pragmatism of harnessing mushroom production for sustainable green waste management, economic growth and nutritional enhancement through mushroom production on lignocellulosic urban green waste.

2. Material and Methods

2.1 Substrate Collection

Green waste from NFSU Gandhinagar campus was collected, segregating the waste by species and was shredded to a smaller size. The green waste was air dried (as shown in fig 1 a,b below) for three weeks before the subsequent substrate preparation processes. Mushroom substrates were made from four ornamental angiosperms *Duranta erecta* (hedge)*, Plumeria obtusa* (frangipani tree) leaves)*, Zoysia japonica* (lawn) trimming. Whilst *Triticum aestivum* as conventional biomass was collected from Kranti Research Center, Gandhinagar, Gujarat. The fifth substrate, mixed substrate was made by mixing equal portions of biomass from three urban green waste species.

2.2 Mushroom Cultivation

Mushroom Cultivation was performed in Kranti Research Center, Gandhinagar, Gujarat. Overnight soaking and thermal sterilization (autoclaving) method [22] was used for the preparation of axenic substrates. 5 types of substrates were prepared, out of which 4 are of urban green waste origin and one was *Triticum aestivum* straw (the conventional agricultural biomass). *Pleurotus florida* (White Oyster) spawn imported from Canada at Research Centre. Spawning was done using solid spawn inoculation, at the rate of 5% of dry weight of substrate. Inoculated substrate was incubated in a thermocol box at 27˚C, to facilitate mycelium colonization as shown below fig 2, 3, 4, 5. Mycelium colonization time was observed and recorded for each sample. After full mycelium colonization, 5 days mycelium consolidation time was allowed, before bag opening for mushroom fruiting.

Whole bag opening procedure was followed for basidiocarp production. After bag opening the mycelium colonized substrate was placed in a 1.2m X 0.5m X 1.6m mushroom fruiting chamber, in which temperature, humidity and CO_2 concentration were kept optimal using air conditioner, humidifier and ventilation fans (inlet and extraction fans) (table 1).

Table 1 Extrinsic environmental parameters which were maintained during mushroom cultivation.

Extrinsic Parameters	During Mycelium colonization	During Fruiting
Humidity	76% ± 8.5%	77% ± 6%
CO_2 concentration	1422 ± 140 ppb	506 ± 22 ppb
Temperature	27˚C ± 2˚C	23˚C ± 3˚C

Three flashes of mushroom basidiocarp were harvested, weighed and preserved through oven drying at 60˚C for 24 hours. Then the dried mushroom was ground to a powder using an electric grinder for further analysis.

2.3 Substrate Characterization

For substrate characterization elemental composition was assessed by XRF and carbon, nitrate , lignin content was also estimated simultaneously using respective methods given below.

2.4 Organic Carbon and Nitrate Nitrogen conten estimation

Substrate carbon content before and after mushroom cultivation was estimated using the chromic wet acid oxidation method. Nitrate Nitrogen was estimated using Phenol disulphonic method. The experiment was done in triplicate for each substrate. Variation in carbon, and Nitrate content among the substrates was authenticated by single factor one way ANOVA.

2.5 Lignin Content Estimation

Lignin content estimation was done using the klason mathod [23]. Acid insoluble lignin content for each sample was then calculated using the formular below.

$$Acid\ insoluble\ lignin\ conte = \frac{weight\ of\ extracted\ oven\ dried\ lignin}{weight\ of\ sample\ used\ for\ lignin\ estimation} \times 100\%$$

Paired two samples t-test was performed to ascetain the significance of lignin content variation before and after mushroom cultivation, whereas lignin content variation among substates species was authenticated by single factor one way ANOVA.

For each substrate delignification was calculated and expressed as a percentage using the formular below:

$$Delignification\ \% = \frac{initial\ lignin\ content - final\ lignin\ content}{initial\ lignin\ content} \times 100$$

Qualitatively lignin estimation among substrates was determined by FTIR showed characterstic peak observed in varied ranges such as Aromatic C=C stretching: around 1600 cm^{-1}, C-H stretching: around 2900-3000 cm^{-1}, O-H stretching: around 3400 cm^{-1}, C-O stretching: around 1260 cm^{-1} and 1030 cm^{-1}.

2.6 Nutritional value estimation of *P. florida*

Total lipid content percentage was estimated by Folch method. Carbohydrate content estimation by the Anthrone sulfuric acid method [24]. Mushroom protein content variation was estimated using the Lowry method [25]. Ash content was estimated using dry combustion method, [26]. The experiment was done in tripplicates and single factor one way ANOVA was used to ascertain significance of the differences in all values among mushroom samples harvested from the different substrates.

2.7 P. florida Biological efficiency on various substrates

Biological efficiency (the ratio of *Pleurotus florida* sporephore/ basidiocarp fresh weight as to substrate dry weight, was calculated and expressed as a percentage using the equation given below. The weight of the three flashes which were harvested were added up to makeup the total fresh weight of mushroom from each substrate sample. Each substrate had three replications.

$$Biological\ efficiency\ (\%) = \frac{Weight\ of\ fresh\ mushroom}{Dry\ weight\ of\ the\ substrate} \times 100\%$$

3. Results

3.1 Substrate elemental chemical composition

XRF data reveals notable major and minor elemental concentration differences among substrates (table 2). Calcium (Ca) is markedly high in Plumeria and Mixed substrates. Potassium (K) and Sulfur (S) are prevalent among the five experimental substrates, Phosphorus (P) levels remain moderate, Zoysia shows elevated Iron (Fe). Silicon (Si) varies significantly ($P<0.05$) among substrates. Negligible concentrations of trace elements (Manganese (Mn), Copper (Cu), and Zinc (Zn)) were detected. These elemental profiles collectively indicate substrate suitability and safety for cultivation. Low levels of toxic heavy metals like Lead (Pb), Chromium (Cr), and Nickel (Ni) minimize mushroom contamination risks. Enriching components such as Calcium, Potassium, and Sulphur, Silicon enhances substrate structural integrity.

Table 2. Elemental chemical composition of experimental substrates

Analyte	% concentration in *Duranta*	% concentration in *Triticum*	% concentration in *Zoysia*	% concentration in *Plumeria*	% concentration in Mixed
Ca	41.914	10.279	25.846	82.298	69.934
K	39.93	36.612	21.335	6.19	1.205
Cl	13.363	19.45	-	-	0.961
S	1.599	1.513	3.372	1.268	2.388
P	1.142	0.843	3.044	0.058	0.892
Fe	1.075	1.375	11.288	3.386	5.395
Mn	0.214	0.174	0.383	0.398	0.384
Cu	0.145	0.081	0.346	0.249	0.117
Sr	0.098	0.022	0.121	0.528	0.236
Zn	0.089	0.044	0.113	0.132	0.115
Br	0.032	0.012	0.055	0.042	0.023
Ni	0.024	-	0.024	0.005	0.017
Cr	-	0.043	0.09	0.061	0.083
Ti	-	0.307	1.653	0.444	0.812
Si	-	29.224	30.756	4.904	19.267
Ba	0.376	-	-	-	0.142
Os	-	0.01	-	0.037	0.028
Rb	-	0.008	0.024	-	0.012
Zr	-	0.003	0.034	-	0.015
Pb	-	-	0.07	-	0.021
Ag	-	-	0.043	-	0.002

V	-	-	0.058	-	0.023
Al	-	-	1.343	-	0.491

3.2 Substrate Carbon and Nitrate content

Substrate carbon content was significantly different among substrates (P<0.05) highest *Duranta erecta* (43%), followed by Mixed substrate (34%), *Triticum aestivum* (34%), *Plumeria obtusa* (29%) and lastly *Zoysia japonica* (27%) (fig 6 below). Statistically significant (P < 0.05) variation in substrate carbon content signifies inherent biological. High carbon content of *Duranta erecta* at 43%, suggests a notably greater carbon fixation capacity. On the other hand, mixed substrate and *Triticum aestivum* exhibit fair carbon contents of 34%. Mixed substrate resulting in an intermediate carbon content indicative of fused biomass traits. Relatively lower carbon contents in *Plumeria obtusa* (29%) and *Zoysia japonica* (27%).
Nitrate nitrogen content was significantly different among substrates (P<0.05) with the highest nitrate content being in *Zoysia japonica* 196.96mg/L, mixed substrate 175mg/L, *Duranta erecta* 170.67mg/L, *Triticum aestivum* 168.94mg/L, and lastly *Plumeria obtusa* 164.14mg/L as shown on fig 6 below.

3.3 Lignin estimation in substrate

Considering the chemical structure and multiple functional groups, lignin content was estimation by FTIR-ATR spectroscopy considering 5 five peaks; around 1000nm and 1200nm ether group, around 1600nm aromatic C=C group, around 2900nm methoxyl group, and around 3400nm hydroxyl group. All the five peaks proved *Duranta erecta* to be more lignified, followed by *Triticum aestivum* straw, *Plumeria obtusa* and lastly *Zoysia japonica* trimmings as shown by the FTIR spectra in fig 8 below. Substrate lignin content was significantly different among substrates. The most lignified substrate is *Duranta erecta* (34.78%), followed by *Triticum aestivum* (30.8%), mixed substrate (27.08%), *Plumeria obtusa* (24.43%), and *Zoysia japonica* (21.15%) (fig 7, 8 below).

3.4 Mushroom production and its analysis

3.4.1 Mycelium colonization time

Mycelium colonization time was significantly different among substrates (P□0.05) (table 3) shortest on *Plumeria obtusa* (frangipani) leaves (21 days), followed by *Triticum aestivum* (wheat) straw (21.3 days), mixed substrate (22.3days), *Duranta erecta* (golden dewdrop) (23 days) and lastly *Zoysia japonica* (lawn) trimmings (32.3days).

Table 3. Mycelium growth time factors (value indicate the average of triplicate samples with standard deviation)

Substrate	Spawning rate	Colonization time (days)	Primordial initiation time	Total crop duration (days)	No of flashes
Mixed	5%	22.3 ± 1.15	11.33 ± 3.2	54.33 ± 2.51	3
Duranta	5%	23.0 ± 0.0	8 ± 2	51.67 ± 2.52	3
Plumeria	5%	21.0 ± 1.0	12 ± 2	54.67 ± 1.15	3
Triticum	5%	21.33 ± 0.58	9.66 ± 0.58	57.33 ± 3.51	3
Zoysia	5%	32.33 ± 8.32	15.0±1.0	60.0 ± 3.0	3

3.4.2 Pleurotus florida Productivity and Biological Efficiency

Three basidiocarp flashes were harvested from each substrate sample and the yields were as presented in table 5 below. *Pleurotus florida* productivity revealed distinct yield patterns across substrates and flushes. The mixed substrate consistently outperformed other substrates, achieving the highest total yield of 150g and a biological efficiency (BE) of 75%. Yield peaked in the first flush, gradually declining through the second and third, reflecting typical nutrient depletion dynamics, thereby concurring with the trending patterns in literature. *Duranta erecta* followed, with a total yield of 135.34g and biological efficiency of 67.67%, showing a similar decreasing trend but maintaining relatively higher productivity in the second flush compared to others. *Plumeria obtusa* exhibited moderate yield (116 g) and biological efficiency (58.33%), with improved performance in later flushes, unlike *Triticum aestivum* and *Zoysia japonica*, which recorded lower yields and efficiencies overall.

Biological efficiency varied significantly ($P<0.05$) across substrates. The mixed substrate exhibited the highest biological efficiency at 75%, indicating a higher propensity of synergistic interactions among substrate intrinsic factors (Latif et al., 2023) which likely optimizes nutrient availability thereby promoting mycelium hyphae growth and mushroom fruiting (Karpagavalli et al., 2024). Among single species-based substrates, *Duranta erecta* demonstrated superior performance with a biological efficiency of 67.67%, surpassing *Plumeria obtusa* which recorded 58.33%. This suggests that, *Duranta erecta's* chemical composition, lignin content and physical structure is more supportive to *Pleurotus florida* mycelium hyphae growth and basidiocarp fruiting. Conversely, *Triticum aestivum* straw and *Zoysia japonica* trimmings showed lower biological efficiencies at 45.67% and 39%, respectively. These reduced values could be attributed to less favourable substrate intrinsic parameter (nutrient content and textural properties) that limit mycelial hyphae apical cells penetration (during growth) and nutrient absorption.

Overall, these results highlight the critical role substrate choice plays in maximizing *Pleurotus florida* productivity. Utilizing mixed substrates can significantly enhance *Pleurotus florida*'s biological efficiency and yield, making it advantageous for commercial cultivation to optimize production, viability and profitability. Duranta erecta or a combination of other woody substrates are recommended for higher

productivity. Further research on substrate combinations is warranted, while using lignocellulosic urban green waste supports sustainable, environmentally friendly mushroom farming. There was a positive correlation (r=0.617) between lignin content and biological efficiency (fig 9). Based on the aforementioned yields and biological efficiency (table 4) of *Pleurotus florida* on different urban green waste-based substrates.

There was a statistically significant variation (P<0.05) in nitrate nitrogen content among substrates, indicating a clear gradient in nutrient availability. There is no correlation (r=0.3) between nitrate concentration and biological efficiency. A strong positive corelation (r=0.72) between substrate nitrates concentration and mushroom protein content found (fig 10 below).

Table 4 Mycelium yield and biological efficiency of Pleurotus florida (value indicate the average of triplicate samples with standard deviation)

Substrate	Yield				
	First flash yield (grams)	Second flash yield (grams)	Third flash yield (grams)	Total yield per sample (grams)	Biological efficiency %
Duranta erecta	54±8.08g	46.1±6.16g	35.32±12.62g	135.34±17.75g	67.67±8.9%
Plumeria obtuse	32.68±7.06g	37.13±3.48g	46.2±3.06g	116±5.86g	58.33±2.91%
Triticum aestivum	21.34±0.68g	46±3.22g	23.64±2.9g	91±0.58g	45.67±0.3%
Zoysia japonica	25±3.79g	25±4.93g	28±10.15g	78±13.11g	39±6.56%
Mixed substrate	66.59±14.53g	42.1±4.04g	41.32±19.01g	150±7.02g	75±3.51%

3.4.3 Nutritional composition of *Pleurotus florida* mushroom cultured on different substrates

Protein and carbohydrate content significantly varied among substrates (P<0.05). There was no significant variation (P>0.05) in lipid content in mushroom from different substrates (table 5, 6).

Table 5 Pleurotus florida nutritional content (value indicate the average of triplicate samples with standard deviation)

Substate	Protein content %	Carbohydrates content %	Lipids content %	Ash content %	Metabolizable energy (Kcal/100g)
Mixed	15.37±1.86	16.20±1.75	7.103±2.06	7	105.3605
Duranta	14.93±1.84	11.77±1.11	4.53±0.71	14	95.02513
Plumeria	10.34±1.86	14.40±0.72	5.15±1.11	7	88.87462
Triticum	13.01±1.86	22.17±1.98	4.96±0.64	6	128.0597
Zoysia	17.14±1.9	13.44±1.20	7.35±1.48	8	109.3496

Table 6. Mineral content of Pleurotus florida mushroom from different substrates

Mineral element	Mixed substrate (%)	*Duranta erecta (%)*	*Plumeria obtuse (%)*	*Triticum aestivum (%)*	*Zoysia japonica (%)*
Potassium	72.742	77.785	70.588	74.669	71.128
Phosphate	11.013	10.996	11.87	10.125	11.06
Calcium	10.103	6.532	12.923	8.234	11.516
Sulphur	4.34	3.445	2.999	4.503	4.368
Iron	1.01	0.592	0.774	1.416	0.921
Zinc	0.376	0.361	0.405	0.69	0.532
Copper	0.258	0.152	0.192	0.176	0.312
Manganese	0.111	0.113	0.131	0.179	0.118

3.5 Delignification of Lignocellulosic waste by Mushroom

Duranta erecta substrate spectra flaunted a noticeable decrease peak height around 1000nm, 1200nm, 1600nm and 3300nm, after mushroom cultivation (fig 11). Suggesting a considerable delignification i.e degradation of ether (C-O-C), aromatic (C=C), hydroxyl (OH) functional groups respectively. A small variation on the same functional groups was observed before and after cultivation as shown in *Zoysia japonica* see fig 12 below. Accurately assessing delignification in *Triticum aestivum* and *Plumeria obtusa* proved to be an exceptionally challenging endeavour due to the complexity and indistinct nature of the spectral data obtained (fig 13, 14). The spectral profiles were convoluted and lacked clarity, rendering precise analysis unattainable by conventional methods. Consequently, the irregular and ambiguous spectral signals impeded reliable quantification and understanding of the lignin degradation processes. Therefore, overcoming the spectral complications remains essential for advancing the study of delignification mechanisms in *Triticum aestivum* and *Plumeria obtusa*. *Pleurotus florida* induced substrate delignification, and the findings were as shown in fig 15 below. Substrate delignification was significantly different among substrates ($P<0.05$). Highest delignification was witnessed on mixed

substrate (36%), followed by *Duranta erecta* (18%), *Zoysia japonica* (13%), *Triticum aestivum* (11%) and lastly *Plumeria obtusa* 4%. As shown on the graph above, this research found a decrease in substrate carbon content across all substrate with the highest decrease in carbon content being in *Zoysia japonica* followed by *Duranta*, mixed, *Triticum* and lastly *Plumeria*. There was a moderate positive correlation between substrate % carbon loss and % delignification (r = 0.59) (fig16) below.

4. Discussion

During elemental analysis of selected substrates, Calcium (Ca) is markedly high in Plumeria and Mixed substrates, essential for basidiomycetes cell wall stability [27]. Potassium (K) and Sulfur (S) are prevalent among the five experimental substrates, supporting metabolic functions [28]. Phosphorus (P) levels remain moderate, contributing to energy transfer within mushrooms. Zoysia shows elevated Iron (Fe), which can enhance enzymatic activity but requires monitoring due to potential oxidative effects. Silicon (Si) varies significantly ($P<0.05$) among substrates, its presence improves substrate structure and water retention. Negligible concentrations of trace elements (Manganese (Mn), Copper (Cu), and Zinc (Zn)) were detected. These were within safe limits, supporting mushroom growth without toxicity concerns. Low levels of toxic heavy metals like Lead (Pb), Chromium (Cr), and Nickel (Ni) minimize mushroom contamination risks, guaranteeing environmental and consumer safety. Enriching components such as Calcium, Potassium, and Sulphur enhance mushroom nutrition and substrate stability. Conclusively, the XRF spectroscopy findings confirm that the five experimental substrates provide a balanced elemental chemical profile conducive to healthy mushroom growth and pose minimal toxicity concerns.

Statistically significant ($P < 0.05$) variation in substrate carbon content signifies inherent biological or physiological distinctions among the biomass source species [29]. High carbon content of *Duranta erecta* at 43%, suggests a notably greater carbon fixation capacity, as compared to the other substrates studied. Such a high carbon content echoes structural tissue complexity with higher lignin, hemicellulose and cellulose. On the other hand, mixed substrate and *Triticum aestivum* exhibit fair carbon contents of 34%. For *Triticum aestivum* (wheat), this aligns with its known biomass composition [30], balancing carbon allocation between structural carbohydrates and metabolic compounds. The higher nitrate content of *Zoysia japonica* renders it a more nitrogen-rich environment conducive to fungal metabolism and growth, which is critical for mushroom mycelial development and fruiting body formation. However, if nitrates content exceeds the optimum level it inhibits basidiocarp formation and reduces the yield [31]. Relatively lower nitrate nitrogen in *Duranta erecta* trimmings, *Triticum aestivum* straw, and *Plumeria obtusa* leaves based substrates suggests limited nitrogen availability, but not necessarily below the optimum range for production of *Pleurotus florida*. These nitrate content variations potentially affect mushroom yield and quality due to suboptimal nutrient supply [32].

Lignin is a three-dimensional amorphous, branched complex organic polymer of phenyl-propane units heterogeneously bonded by several inter-linkage components [33]. Lignin content comparison at one wavelength impossible when using FTIR [34]. Substrate lignin content has been proved as paramount

parameter, transcending other factors regulating mycelium colonization rate [35]. There was a moderate negative correlation (r = -0.534) between mycelium colonization time and lignin content, which proves mycelium colonization rate to be higher on more lignified substrates. This resonates well with the findings from [36]. Densely lignified substrates were proved to promote two-dimensional (surface) mycelium growth in *Ganoderma lucidum [56]* (Yang et al., 2024) and *Trametes versicolor [37]*, whereas less lignified substrate allows easy three-dimensional spreading of the mycelium [36].

Higher substrate lignin content induced faster pace of two-dimensional mycelium growth enhances three-dimensional mycelium colonization rate as well, as mycelial hyphae will penetrate the substrate from a larger surface area. Mycelium colonization rate is hinged on substrate nutrient availability and accessibility, hyphae extension, and branching at the substrate tips [38], all of which are depended on lignin content and complexity. As proven by [39] increasing lignin content increases rate of mycelium growth up until a certain optimum level is reached. In ligninolytic fungal species, higher substrate lignin content stimulates the secretion of lignin-degrading auxiliary enzymes and lignin-modifying enzymes [40]. Subsequently, these ligninolytic enzymes catalyse saprophytic nutrition, promotes mycelium growth [41] and basidiocarp production [40]. This research found that the higher the lignin content the higher the rate of mycelium colonisation. But it was limited to a comparison of the rate of mycelium colonization among substrates of various lignin content without a closer look on the optimum lignin content which gives the best results. Therefore, further studies to detect the optimum lignin content for shortening mycelium colonization time for *Pleurotus florida* mushroom is crucial, for closing the knowledge gap.

The Supremes of mixed substrate echoes earlier research findings which backs the notion of optimal conditions proffered by mixtures [42]. These patterns demonstrate that substrate composition profoundly determines not only total production but also yield distribution across flushes. When conjugating the findings of this study with literature proves that, different substrates provide varying nutrient profiles [43] and physical conditions [44], impacting mycelium growth and fruiting [45]. Overall, the data reinforce lignin's critical role but also highlight the complexity of optimizing biological efficiency.

Fungal enzyme (lignin peroxidase) oxidizes both phenolic and non-phenolic aromatic nuclei by electron displacement, which bares phenoxy radicals and cation-radicals [46]. Cation-radicals then spontaneously react with water, other nucleophiles and molecular oxygen [47]. The resultant enzymatic combustion cleaves C-C and C-O linkages, depolymerize and opens aromatic rings, from which a numerous aromatic and aliphatic products are formed. Vanillin, syringaldehyde, and guaiacol are part of the aromatic products of fungal lignin degradation [48]. Resultantly, some of the functional groups present in lignin remains even after delignification. Therefore, slight variations were observed in FTIR-ATR spectroscopy spectra. Therefore, most studies on lignin content and biomass delignification authenticate FTIR-ATR spectroscopy data by information from other analysis techniques such SEM EDX.

A positive correlation between delignification and biological efficiency (r = 0.647) as shown on the graph below. Which resonates well with other scholars, who found strong positive correlation between

delignification and biological efficiency *Pleurotus* on wheat straw [49] on oil palm empty fruit bunch [50] and *Hypsizygus ulmarius* mushroom cultivated on bean straw, corn silage and wheat straw [51]. The higher the biological efficiency the higher the delignification of substrate. Substrate carbon loss occurs through methene production and saprophytic foraging of *Pleurotus florida* mycelium [52].

Conclusion

This study concludes and confirms that mushroom cultivation serves as a lucrative and effective biotechnological approach for lignocellulosic urban green waste biodegradation, carbon cycling, and enhancing food and nutrition security. The integration of mycology with urban green waste management turns service delivery into a profitable entrepreneurship and presents a promising avenue for sustainable food production, smart cities, circular economy initiatives. Further research is needed to identify the optimal lignin concentration in substrates that maximizes mycelium colonization rate and mushroom yield. Controlled studies varying lignin levels could clarify the threshold beyond which lignin benefits plateau or decline. Given the superior performance of mixed green waste substrates in yield and biological efficiency, scaling up their use in commercial and community-level mushroom farming is advised. This approach could amplify economic returns while promoting effective biodegradation of diverse waste streams. Embedding mushroom cultivation into urban green waste management infrastructures can reduce urban green waste repletion and urban vermination. It also reduces landfill volumes, accelerate lignocellulosic urban green waste recycling, and generate nutritious food, contributing to urban sustainability goals, Smart city mission. Broadening the scope of urban green waste to include varied lignocellulosic biomasses beyond those species studied here could identify new substrates suitable for different species of edible mushroom species, thereby supporting food and nutrition security across both urban peri-urban and rural contexts.

Declarations

Acknowledgement

The authors are thankful to National Forensic Science University for the laboratory and instrumental facility. The authors wish to thank Kranti Mushroom Farm, Gandhinagar, Gujarat for his essential support throughout the research process.

Author Contribution All authors contributed to the research conception and design. Material preparation, data collection, analysis, and result interpretation were performed by Nomore Rukara, and Shalini Gupta. The manuscript was written by Nomore Rukara and correction, finalization of manuscript was done by Shalini Gupta. Both the authors read and approved the final manuscript.

Data Availability All data generated or analysed during this study are included in this manuscript.

Ethics Approval No ethical approval required.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

Funding No funding is available.

References

1. Matsuo, T., Poorter, L., van der Sande, M. T., Mohammed Abdul, S., Koyiba, D. W., Opoku, J., de Wit, B., Kuzee, T., & Amissah, L. (2025). Drivers of biomass stocks and productivity of tropical secondary forests. *Ecology*, *106*(1). https://doi.org/10.1002/ecy.4488
2. Khosravi Mashizi, A., Sharafatmandrad, M., & Alizadeh, R. (2024). Exploring plant diversity and aesthetic service of mountain and plain landscapes in semiarid ecosystems. *Journal for Nature Conservation*, *82*, 126740. https://doi.org/10.1016/J.JNC.2024.126740
3. Sangkachai, N., Gummow, B., Hayakijkosol, O., Suwanpakdee, S., & Wiratsudakul, A. (2024). A review of risk factors at the human-animal-environmental interface of garbage dumps that are driving current and emerging zoonotic diseases. *One Health*, *19*, 100915. https://doi.org/10.1016/J.ONEHLT.2024.100915
4. Mphasa, M., Ormsby, M. J., Mwapasa, T., Nambala, P., Chidziwisano, K., Morse, T., Feasey, N., & Quilliam, R. S. (2025). Urban waste piles are reservoirs for human pathogenic bacteria with high levels of multidrug resistance against last resort antibiotics: A comprehensive temporal and geographic field analysis. *Journal of Hazardous Materials*, *484*, 136639. https://doi.org/10.1016/J.JHAZMAT.2024.136639
5. Othman, N. M. I., Shamsuddin, A. S., Abu Sari, N., Sapak, Z., Mustaffha, S., Syed Ismail, S. N., & Saili, A. R. (2024). Types and factors of waste accumulation. *Advances in Energy from Waste*, 43–80. https://doi.org/10.1016/B978-0-443-13847-8.00002-6
6. Thanvisitthpon, N., Kallawicha, K., & Chao, H. J. (2024). Effects of urbanization and industrialization on air quality. *Health and Environmental Effects of Ambient Air Pollution: Volume 1: Air Pollution, Human Health, and the Environment*, *1*, 231–255. https://doi.org/10.1016/B978-0-443-16088-2.00003-X
7. Gutberlet, J., & Bramryd, T. (2025). Reimagining urban waste management: Addressing social, climate, and resource challenges in modern cities. *Cities*, *156*, 105553. https://doi.org/10.1016/J.CITIES.2024.105553
8. Prakash, J., Choudhary, S., Raliya, R., Chadha, T. S., Fang, J., & Biswas, P. (2021). Real-time source apportionment of fine particle inorganic and organic constituents at an urban site in Delhi city: An IoT-based approach. *Atmospheric Pollution Research*, *12*(11), 101206. https://doi.org/10.1016/J.APR.2021.101206

9. Sawyer, W.E., Sylva, L., Aigberua, A.O., Akram, M. (2024). Call to Action for a Sustainable Air Pollution-Free World. In: Ogwu, M.C., Izah, S.C. (eds) Sustainable Strategies for Air Pollution Mitigation. The Handbook of Environmental Chemistry, vol 133. Springer, Cham. https://doi.org/10.1007/698_2024_1123
10. Mohankumar, T. *et al.* (2024). Source and Risk Assessment of Polychlorinated Biphenyls (PCBs) in Ambient Air and Its Human Health Implications. In: Gautam, S., Kumar, R.P., Samuel, C. (eds) Aerosol Optical Depth and Precipitation. Springer, Cham. https://doi.org/10.1007/978-3-031-55836-8_5
11. Priya, K., Rani, J., & Gwal, S. (2025). Transforming agricultural residues to value-added products: waste to wealth. *Sustainable Management of Agro-Food Waste: Fundamental Aspects and Practical Applications*, 69–85. https://doi.org/10.1016/B978-0-443-23679-2.00006-9
12. Koul, B., Yakoob, M., & Shah, M. P. (2022). Agricultural waste management strategies for environmental sustainability. *Environmental Research*, *206*, 112285. https://doi.org/10.1016/J.ENVRES.2021.112285
13. Shinde, R., Shahi, D. K., Mahapatra, P., Naik, S. K., Thombare, N., & Singh, A. K. (2022). Potential of lignocellulose degrading microorganisms for agricultural residue decomposition in soil: A review. *Journal of Environmental Management*, *320*, 115843. https://doi.org/10.1016/J.JENVMAN.2022.115843
14. Grimm, D., & Wösten, H. A. B. (2018). Mushroom cultivation in the circular economy. In *Applied Microbiology and Biotechnology* (Vol. 102, Issue 18). https://doi.org/10.1007/s00253-018-9226-8
15. Hajam, Y. A., Kumar, R., & Kumar, A. (2023). Environmental waste management strategies and vermi transformation for sustainable development. *Environmental Challenges*, *13*, 100747. https://doi.org/10.1016/J.ENVC.2023.100747
16. Pérez-Chávez, A. M., Mayer, L., & Albertó, E. (2019). Mushroom cultivation and biogas production: A sustainable reuse of organic resources. *Energy for Sustainable Development*, *50*, 50–60. https://doi.org/10.1016/J.ESD.2019.03.002
17. Viriato, V., Carvalho, S. A. D. de, Santoro, B. de L., & Bonfim, F. P. G. (2024). A Business Model for Circular Bioeconomy: Edible Mushroom Production and Its Alignment with the Sustainable Development Goals (SDGs). *Recycling*, *9*(4). https://doi.org/10.3390/recycling9040068
18. Pawar, J., & Kakde, U. (2021). *International Journal of Botany Studies www.botanyjournals.com Mushroom cultivation on agricultural wastes for environmental sustainability and food security*. https://doi.org/10.20546/ijcmas.2016.505.0
19. Nosheen, S., Ajmal, I., & Song, Y. (2021). Microbes as biofertilizers, a potential approach for sustainable crop production. In *Sustainability (Switzerland)* (Vol. 13, Issue 4, pp. 1–20). MDPI AG. https://doi.org/10.3390/su13041868
20. [20] Liu, L., & Zhao, S. (2025). Local government debt, financing constraints and firms' green total factor productivity. *International Review of Financial Analysis*, *97*, 103874. https://doi.org/10.1016/J.IRFA.2024.103874

21. Zhou, M., Jiang, K., & Chen, Z. (2023). The side effects of local government debt: Evidence from urban investment bonds and corporate pollution in China. *Journal of Environmental Management*, *344*, 118739. https://doi.org/10.1016/J.JENVMAN.2023.118739
22. Chen, F., Grimm, A., Eilertsen, L., Martín, C., Arshadi, M., & Xiong, S. (2021). Integrated production of edible mushroom (Auricularia auricular-judae), fermentable sugar and solid biofuel. *Renewable Energy*, *170*, 172–180. https://doi.org/10.1016/J.RENENE.2021.01.124
23. Bajpai, P. (2018). Pulp Bleaching. *Biermann's Handbook of Pulp and Paper*, 465–491. https://doi.org/10.1016/B978-0-12-814240-0.00019-7
24. Hodge, J.E. and Hofreiter, B.T. (1962) Determination of reducing sugars and carbohydrates. In: Whistler, R.L. and Wolfrom, M.L., Eds., Methods in Carbohydrate Chemistry, Academic Press, New York, 380-394.
25. Kumar Maurya, A., & John, V. (2022). *Nutritional Estimation of Edible Mushroom (Pleurotus sp.)*. https://www.researchgate.net/publication/366175799
26. Raghuramulu, N., Madhuvan, N.K. and Kalyanasundharam, S. (2003) A Manual of Laboratory Techniques. National Institute of Nutrition KMR, Hyderabad, 319-320
27. Freitas, V. V., Borges, L. L. R., Vidigal, M. C. T. R., dos Santos, M. H., & Stringheta, P. C. (2024). Coffee: A comprehensive overview of origin, market, and the quality process. *Trends in Food Science & Technology*, *146*, 104411. https://doi.org/10.1016/J.TIFS.2024.104411
28. Sangeeta, Sharma, D., Ramniwas, S., Mugabi, R., Uddin, J., & Nayik, G. A. (2024). Revolutionizing Mushroom processing: Innovative techniques and technologies. *Food Chemistry: X*, *23*, 101774. https://doi.org/10.1016/J.FOCHX.2024.101774
29. [29] Hu, C., Sun, B., Liu, M., Yu, J., Zhou, X., Chen, L. (2022) Fecal transplantation from young zebrafish donors efficiently ameliorates the lipid metabolism disorder of aged recipients exposed to perfluoro butane sulfonate. The Science of the total environment. 823:153758.
30. Azizi, Se., Dalli, M., Idrissi Yahyaoui, M. *et al.* Unraveling the phytochemical complexity and antimicrobial potency of introduced versus native *Argania spinosa* trees in Eastern Morocco. *Chem. Pap.* **78**, 9223–9234 (2024). https://doi.org/10.1007/s11696-024-03739-1
31. Siti Maryam Salamah Ab Rhaman, Laila Naher; Evaluating carbon, nitrogen and heavy metal content in different agriculture biomass for mushroom substrate. *AIP Conf. Proc.* 9 June 2022; 2454 (1): 020023. https://doi.org/10.1063/5.0078403
32. Han J, Feng J, Kang J, Chen JM, Du XY, Ding SY, Liang L, Wang W. Fast growth of single-crystal covalent organic frameworks for laboratory x-ray diffraction. Science. 2024 Mar;383(6686):1014-1019. doi: 10.1126/science.adk8680. Epub 2024 Feb 29. Erratum in: Science. 2024 Apr 5;384(6691):eadp4377. doi: 10.1126/science.adp4377. PMID: 38422145.
33. Nabipour, H., & Hu, Y. (2022). Introduction to flame retardants for polymeric materials. *Bio-Based Flame-Retardant Technology for Polymeric Materials*, 1–27. https://doi.org/10.1016/B978-0-323-90771-2.00018-3

34. Zhang, L., Larsson, A., Moldin, A., & Edlund, U. (2022). Comparison of lignin distribution, structure, and morphology in wheat straw and wood. *Industrial Crops and Products*, *187*, 115432. https://doi.org/10.1016/J.INDCROP.2022.115432
35. Suwannarach, N., Kumla, J., Zhao, Y., & Kakumyan, P. (2022). Impact of Cultivation Substrate and Microbial Community on Improving Mushroom Productivity: A Review. In *Biology* (Vol. 11, Issue 4). MDPI. https://doi.org/10.3390/biology11040569
36. Yang, J., Cen, C., Wang, Z., & Jian, M. (2024). Impacts of spatiotemporal urban expansion on the species richness and functional traits of adults and sapling woody trees and shrubs of urban remnant forest patches. *Ecological Indicators*, *166*, 112498. https://doi.org/10.1016/J.ECOLIND.2024.112498
37. Desisa, B., Muleta, D., Jida, M., Dejene, T., Goshu, A., Negi, T., & Martin-Pinto, P. (2024). Improvement of nutritional composition of shiitake mushroom (Lentinula edodes) using formulated substrates of plant and animal origins. *Future Foods*, *9*, 100302. https://doi.org/10.1016/J.FUFO.2024.100302
38. Napitupulu, T. P. (2025). Agricultural relevance of fungal mycelial growth-promoting bacteria: Mutual interaction and application. *Microbiological Research*, *290*, 127978. https://doi.org/10.1016/J.MICRES.2024.127978
39. Wu, Y., Li, D., Hu, Y., Li, H., Ramstein, G. P., Zhou, S., Zhang, X., Bao, Z., Zhang, Y., Song, B., Zhou, Y., Zhou, Y., Gagnon, E., Särkinen, T., Knapp, S., Zhang, C., Städler, T., Buckler, E. S., & Huang, S. (2023). Phylogenomic discovery of deleterious mutations facilitates hybrid potato breeding. *Cell*, *186*(11), 2313-2328.e15. https://doi.org/10.1016/J.CELL.2023.04.008
40. Knežević, A., Milovanović, I., Stajić, M., Lončar, N., Brčeski, I., Vukojević, J., & Ćilerdžić, J. (2013). Lignin degradation by selected fungal species. *Bioresource Technology*, *138*, 117–123. https://doi.org/10.1016/J.BIORTECH.2013.03.182
41. Elsacker, E., Vandelook, S., Van Wylick, A., Ruytinx, J., De Laet, L., & Peeters, E. (2020). A comprehensive framework for the production of mycelium-based lignocellulosic composites. *Science of The Total Environment*, *725*, 138431. https://doi.org/10.1016/J.SCITOTENV.2020.13843
42. Latif, N., Rafeeq, R., Safdar, N., Younas, K., Gardezi, M. A., & Ahmad, S. (2023). Unraveling the Nexus: The impact of economic globalization on the environment in Asian economies. *Research in Globalization*, *7*, 100169. https://doi.org/10.1016/J.RESGLO.2023.100169
43. Koutrotsios, G., Tagkouli, D., Bekiaris, G., Kaliora, A., Tsiaka, T., Tsiantas, K., Chatzipavlidis, I., Zoumpoulakis, P., Kalogeropoulos, N., & Zervakis, G. I. (2022). Enhancing the nutritional and functional properties of Pleurotus citrinopileatus mushrooms through the exploitation of winery and olive mill wastes. *Food Chemistry*, *370*, 131022. https://doi.org/10.1016/J.FOODCHEM.2021.131022
44. Karpagavalli, S., Manisha, R., Mageshwari, S., & Sowbharnika, M. (2024). Influence of growth substrates on bioactive compounds and yield of oyster mushroom (Pleurotus florida). *Scientia Horticulturae*, *329*, 112959. https://doi.org/10.1016/J.SCIENTA.2024.112959

45. Kumar, V., Chhetri, A. Evaluation of five different species of *Pleurotus* for their yield potential and growth parameters under Tripura's conditions of North East India. *Indian Phytopathology* **77**, 1103–1107 (2024). https://doi.org/10.1007/s42360-024-00802-5
46. Esakkimuthu, E. S., Ponnuchamy, V., Mikuljan, M., Schwarzkopf, M., & DeVallance, D. (2024). Fungal enzyme degradation of lignin-PLA composites: Insights from experiments and molecular docking simulations. *Heliyon*, *10*(1), e23838. https://doi.org/10.1016/J.HELIYON.2023.E23838
47. Gupta, A., Tiwari, A., Ghosh, P., Arora, K., & Sharma, S. (2023). Enhanced lignin degradation of paddy straw and pine needle biomass by combinatorial approach of chemical treatment and fungal enzymes for pulp making. *Bioresource Technology*, *368*, 128314. https://doi.org/10.1016/J.BIORTECH.2022.128314
48. Luo, J., Molbay, M., Chen, Y., Horvath, I., Kadletz, K., Kick, B., Zhao, S., Al-Maskari, R., Singh, I., Ali, M., Bhatia, H. S., Minde, D. P., Negwer, M., Hoeher, L., Calandra, G. M., Groschup, B., Su, J., Kimna, C., Rong, Z., … Erturk, A. (2025). Nanocarrier imaging at single-cell resolution across entire mouse bodies with deep learning. *Nature Biotechnology*. https://doi.org/10.1038/s41587-024-02528-1
49. Niu, D., Zuo, S., Ren, J., Li, C., Zheng, M., Jiang, D., & Xu, C. (2020). Effect of wheat straw types on biological delignification and in vitro rumen degradability of wheat straws during treatment with Irpex lacteus. *Animal Feed Science and Technology*, *267*, 114558. https://doi.org/10.1016/J.ANIFEEDSCI.2020.114558
50. Dimawarnita, F., Perwitasari, U., Marsudi, S., Faramitha, Y., & Suharyanto. (2022). The Utilization of Oil Palm Empty Fruit Bunches for Growth of Oyster Mushroom (Pleurotus ostreatus) and Biodelignification Process During Planting Cycle. *Agrivita*, *44*(1), 165–177. https://doi.org/10.17503/agrivita.v44i1.2311
51. Öztürk, C., & Atila, F. (2021). Changes in lignocellulosic fractions of growing substrates during the cultivation of Hypsizygus ulmarius mushroom and its effects on mushroom productivity. *Scientia Horticulturae*, *288*, 110403. https://doi.org/10.1016/J.SCIENTA.2021.110403
52. Rahman, T., Nehar, L., Prodhan, Y., Shahed, S., Hasib, S. Al, Rahman, M. S., & Tuly, S. S. (2025). Enhancing solar still performance using external condensers and floating fins: A comparative study. *Cleaner Chemical Engineering*, *11*, 100167. https://doi.org/10.1016/J.CLCE.2025.100167

Figures

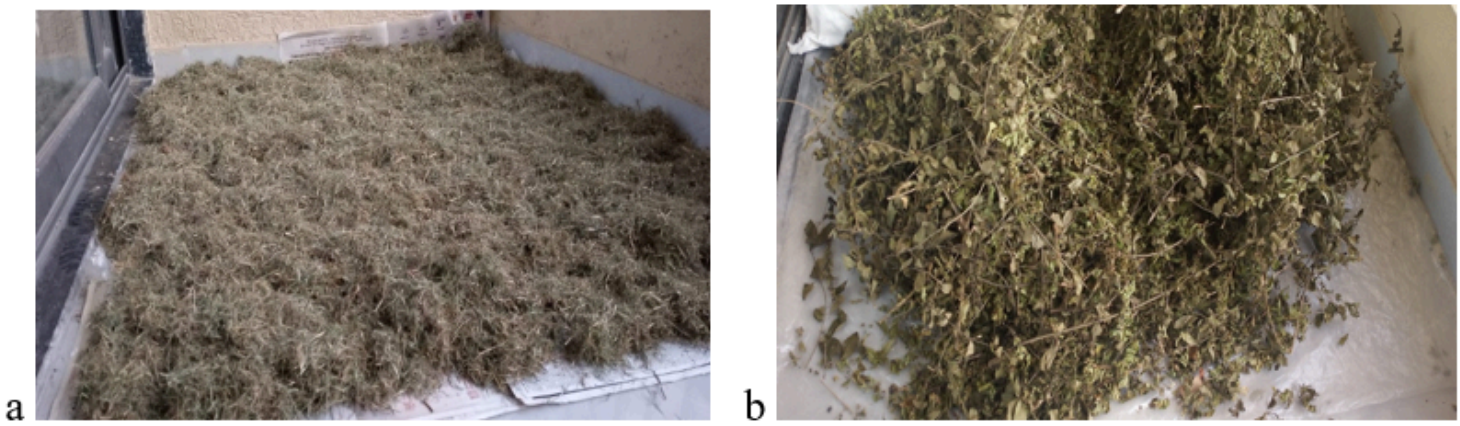


Figure 1

(a) Zoysia japonica trimmings being air dried (b) Duranta erecta substrate being air dried

Figure 2

a) Duranta erecta being dried b) Shredding c) Substrate hydration by soaking in water over night

Figure 3

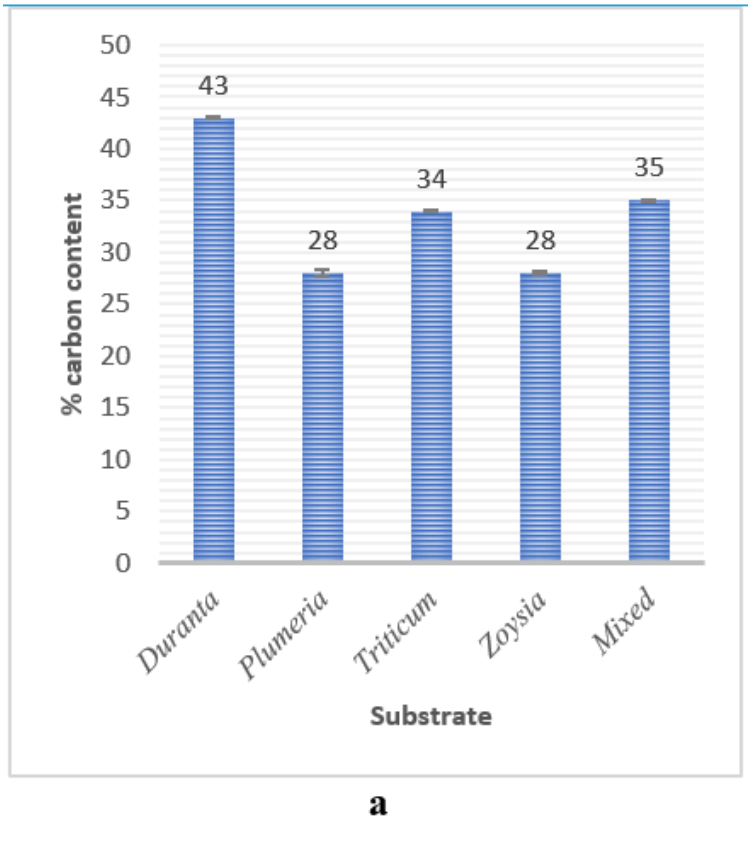
d) Inoculated substrate incubation e) full colonized substrate d) fully colonized substrate

Figure 4

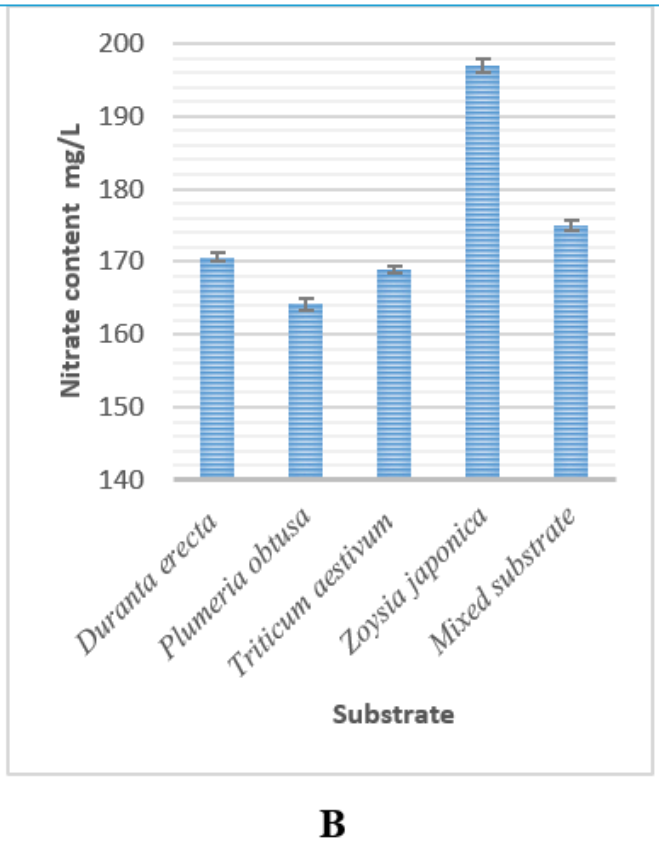
g) Mushroom fruiting chamber h) Substrates arrangement under control condition

Figure 5

j) Pin head formation, h) P. florida mushroom on Duranta erecta substrate.



a



B

Figure 6

Substrate a. Carbon and b. Nitrate content (Data presented are the mean of triplicates with standard error (5%))

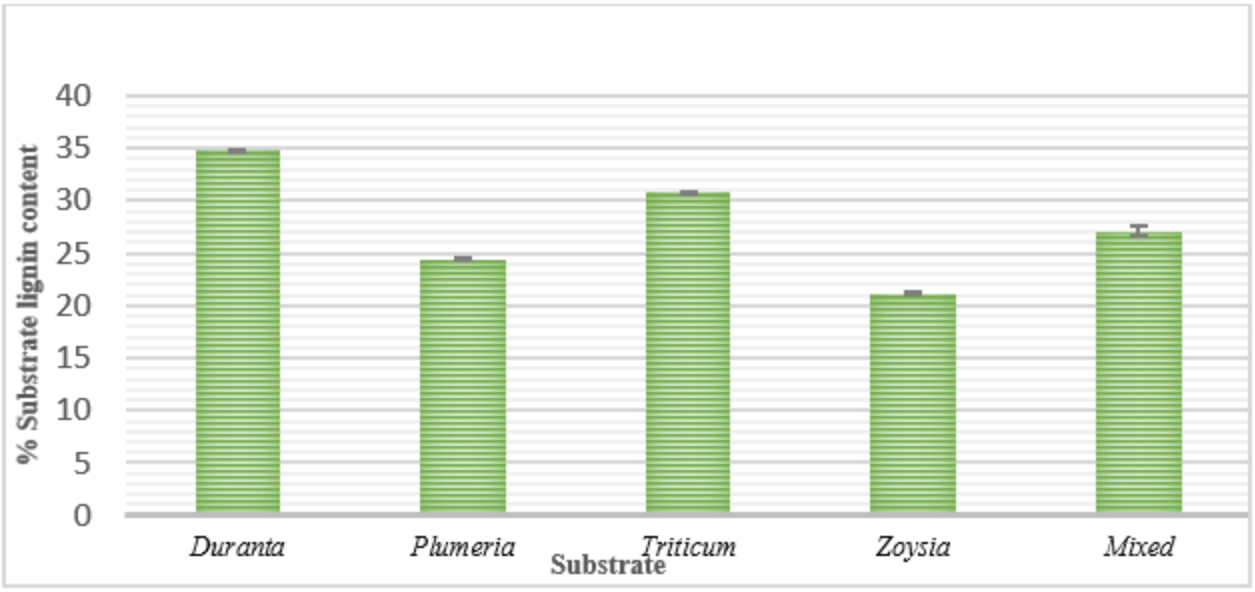


Figure 7

Percentage Substrate lignin content Data presented are the mean of triplicates with standard error (5%))

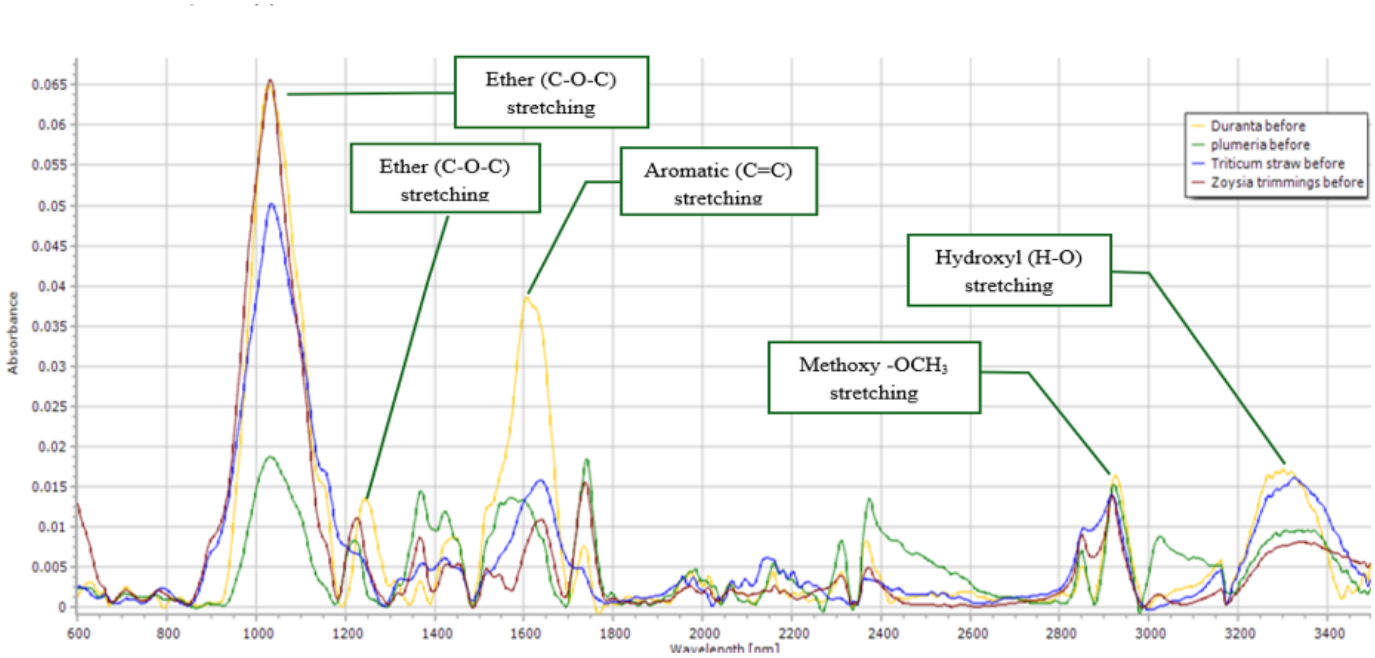


Figure 8

FTIR-ATR Spectroscopy spectra for the substrate lignin content

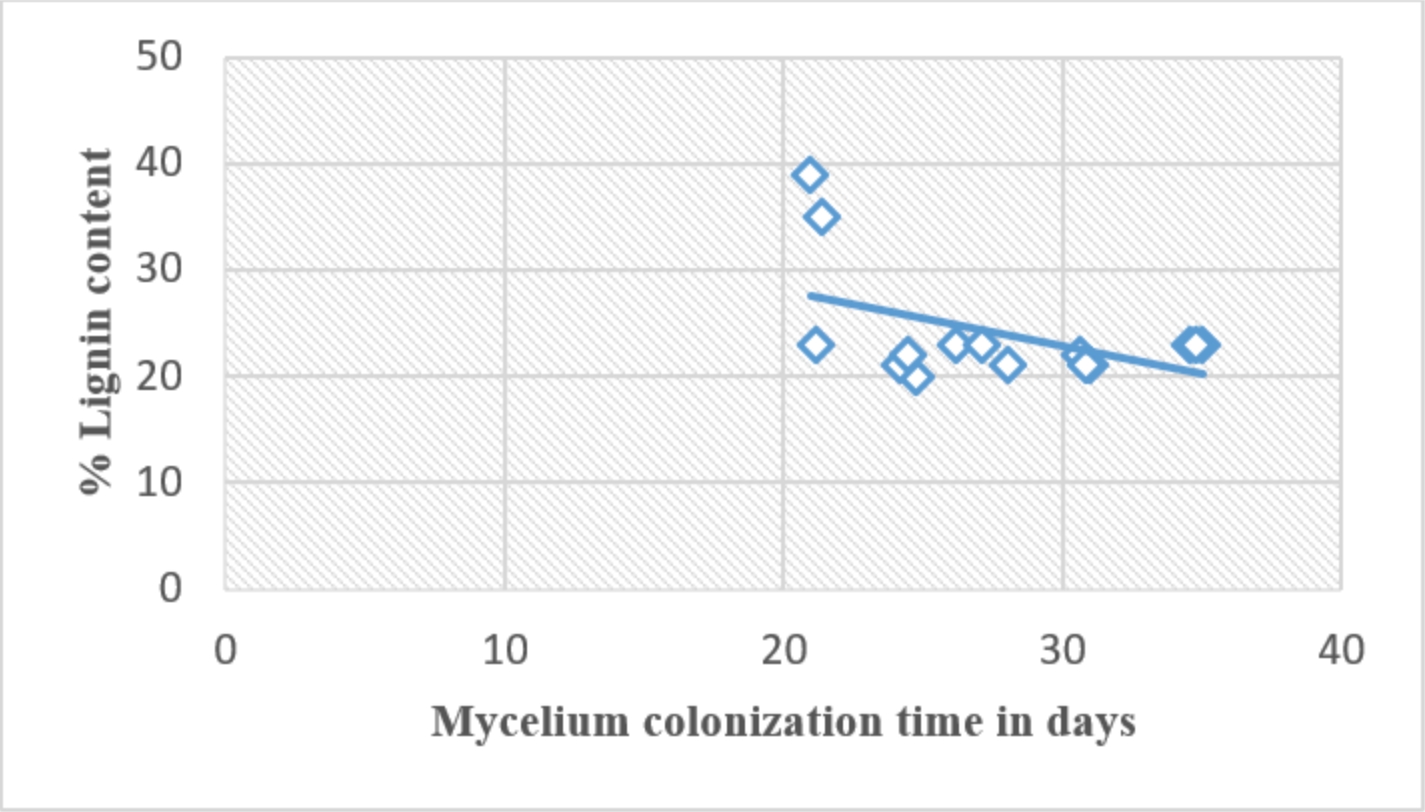


Figure 9

Correlation between mycelium colonization time and lignin content

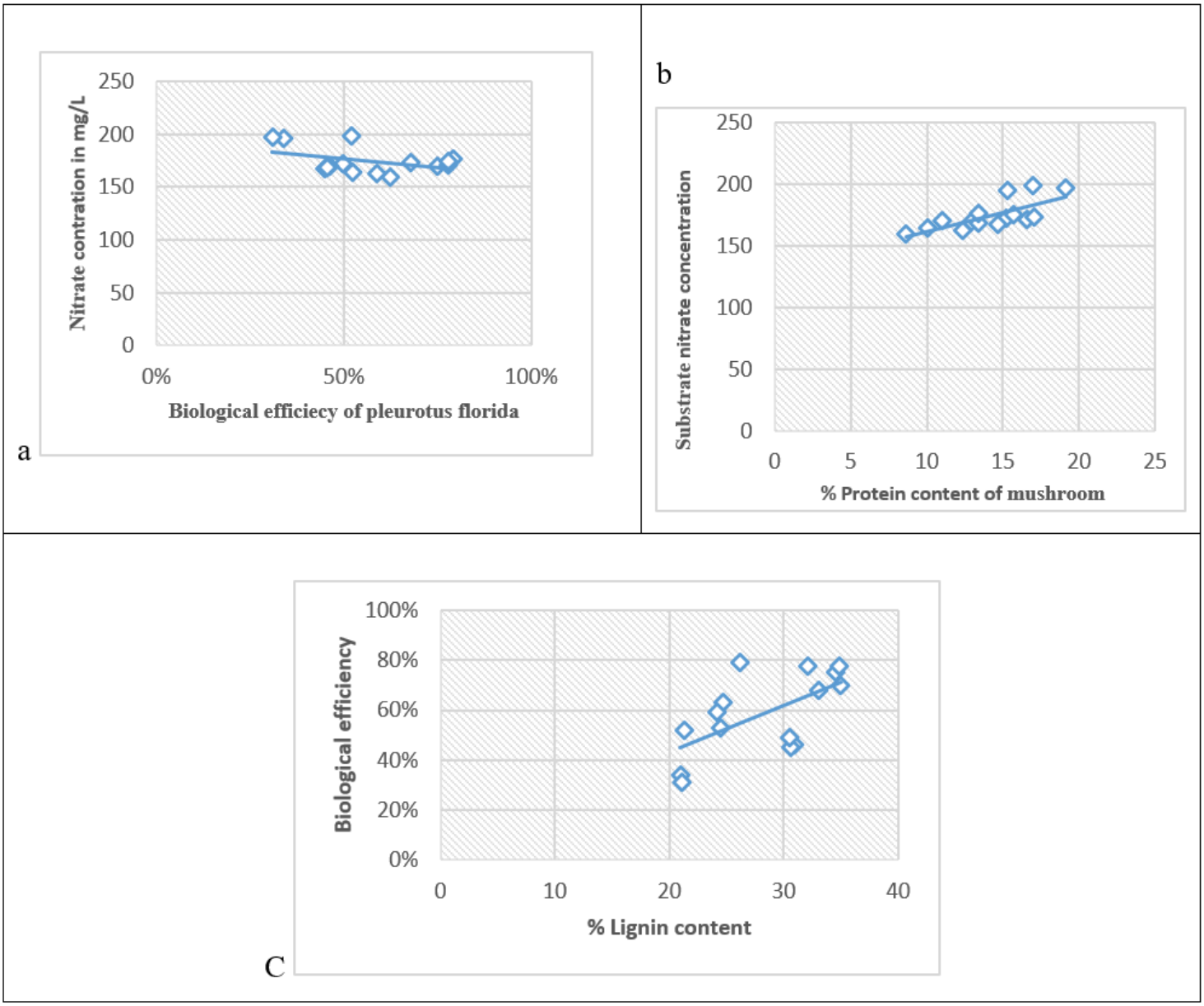


Figure 10

a. Correlation between biological efficiency and nitrates content b. mushroom protein content and substrate nitrates content, c. biological efficiency and substrate lignin content

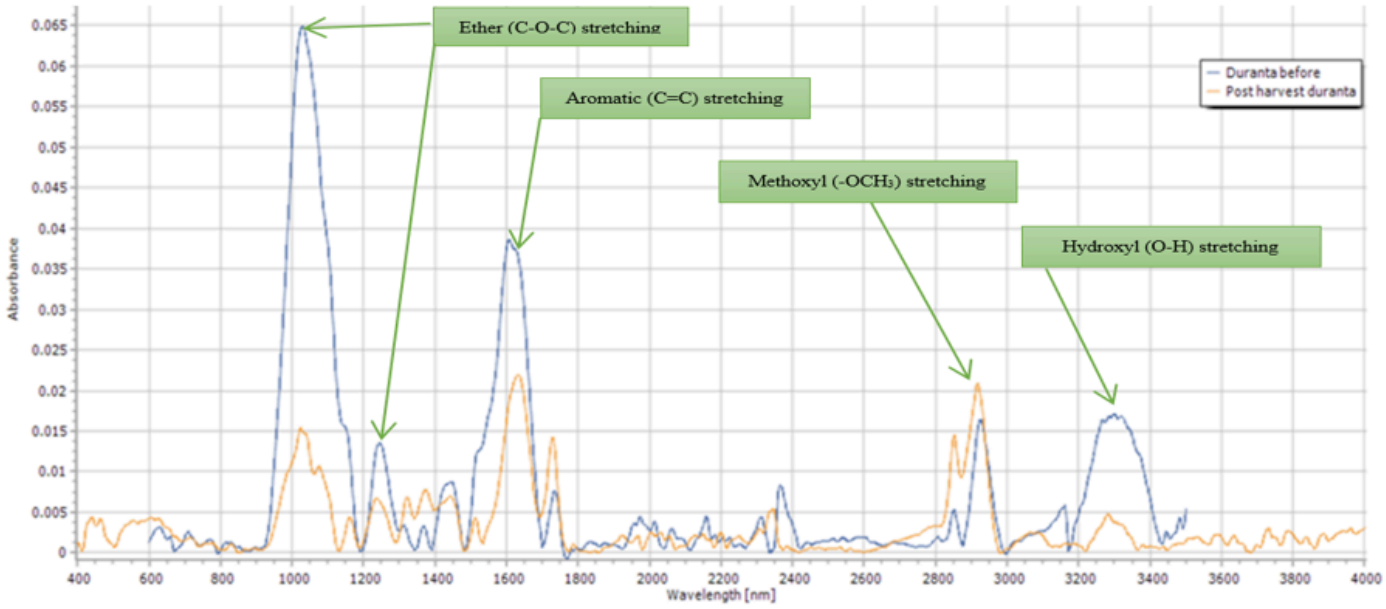


Figure 11

FTIR-ATR spectroscopy spectra showing Duranta erecta lignin content before and after mushroom cultivation.

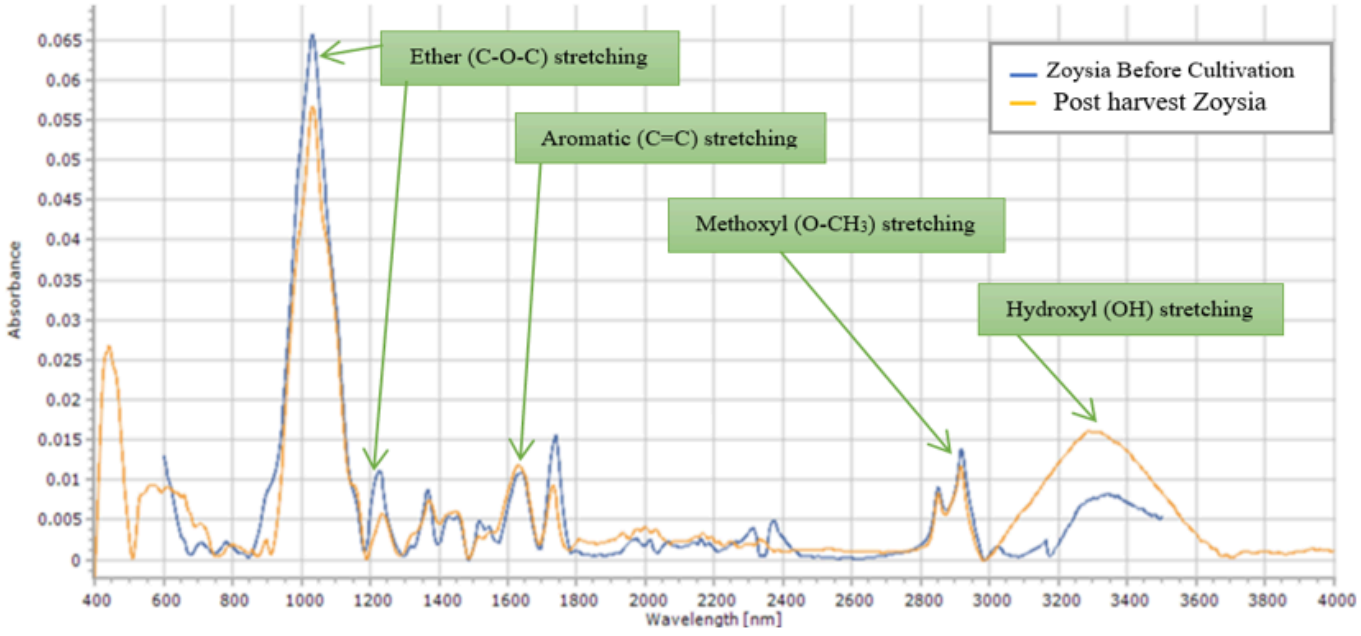


Figure 12

FTIR-ATR spectroscopy spectra showing Zoysia japonica lignin content before and after mushroom cultivation.

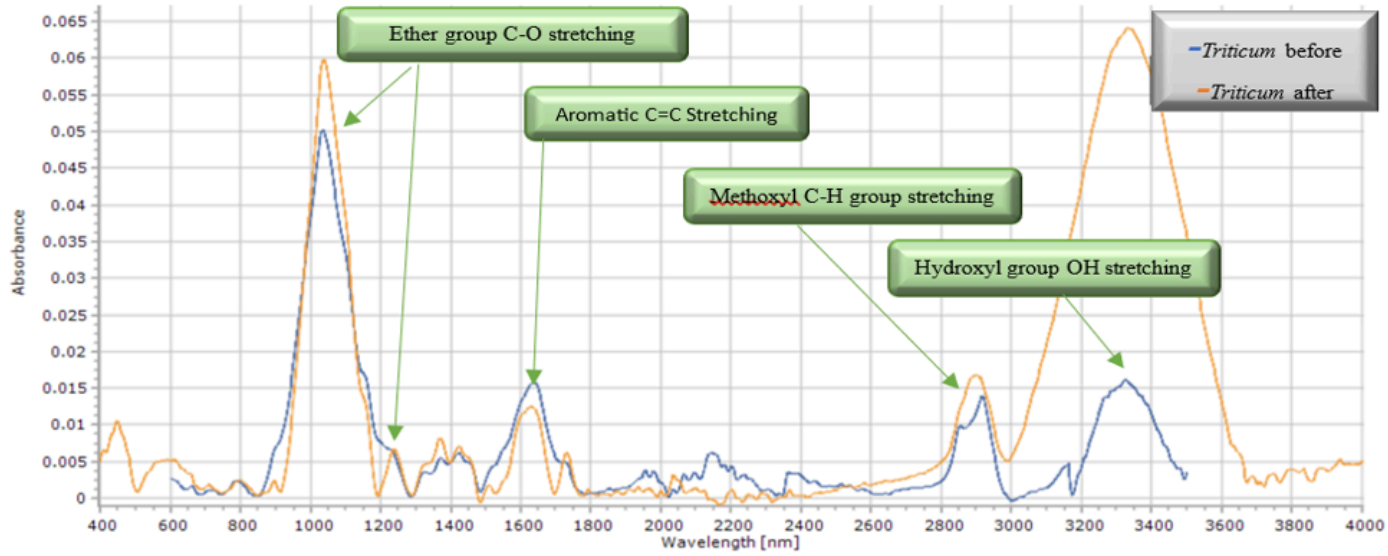


Figure 13

FTIR-ATR spectroscopy showing Triticum aestivum lignin content before and after mushroom cultivation.

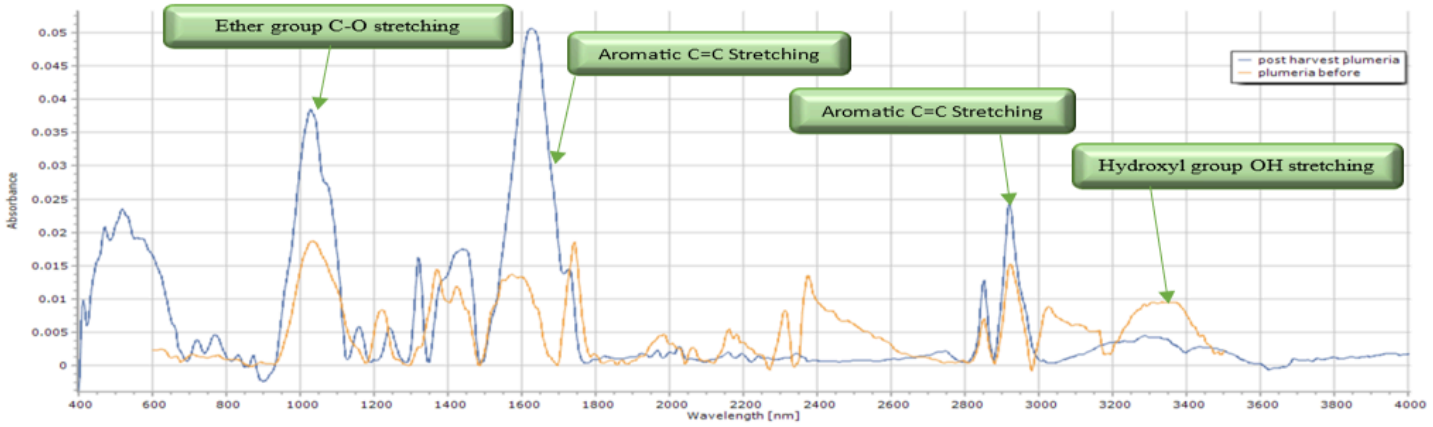


Figure 14

FTIR-ATR spectroscopy showing Plumeria obtusa lignin content before and after mushroom cultivation.

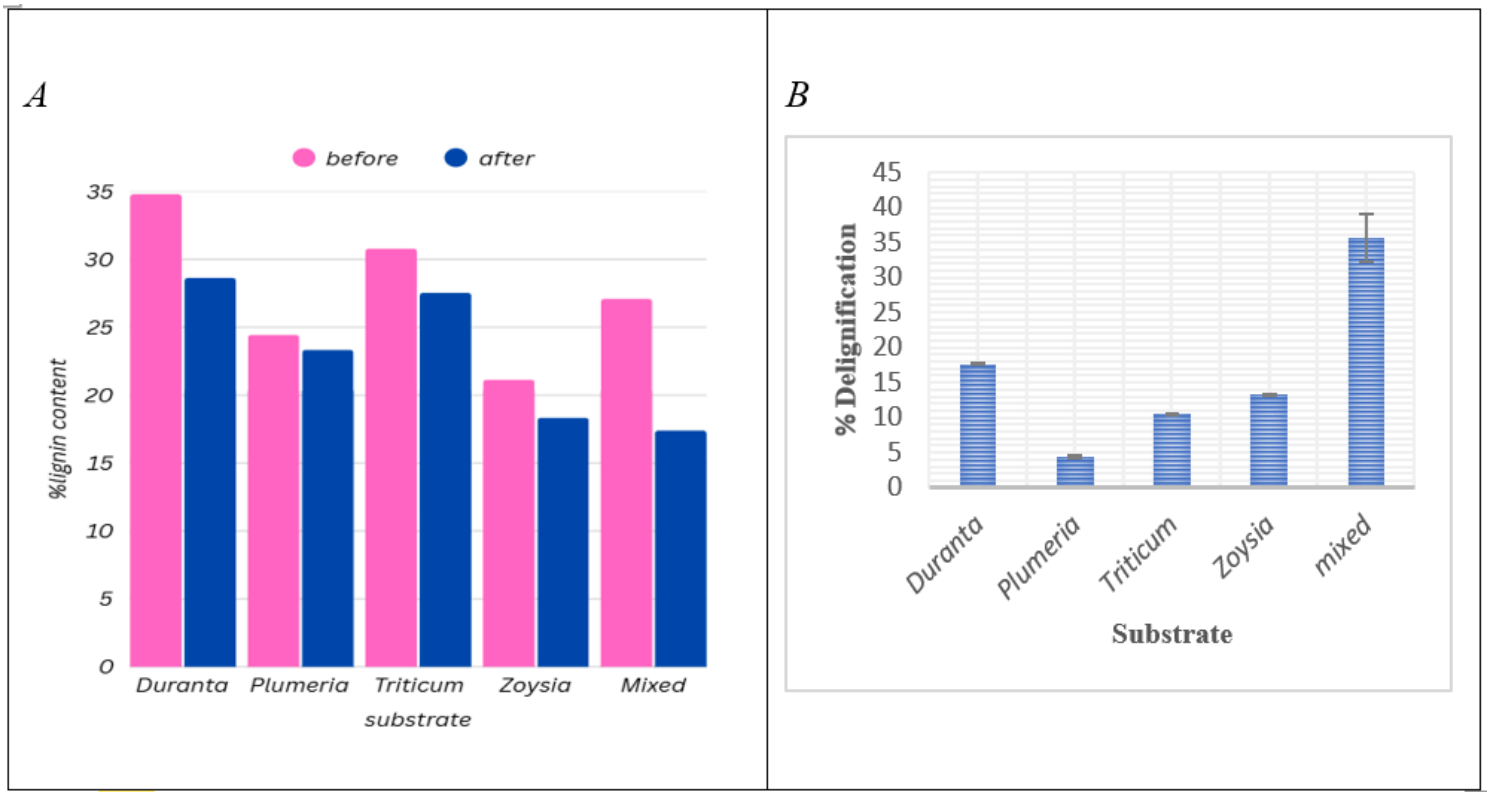


Figure 15

A. Substrate lignin content before and after mushroom cultivation B. Percentage delignification of substrate (Data presented are the mean of triplicates with standard error (5%))

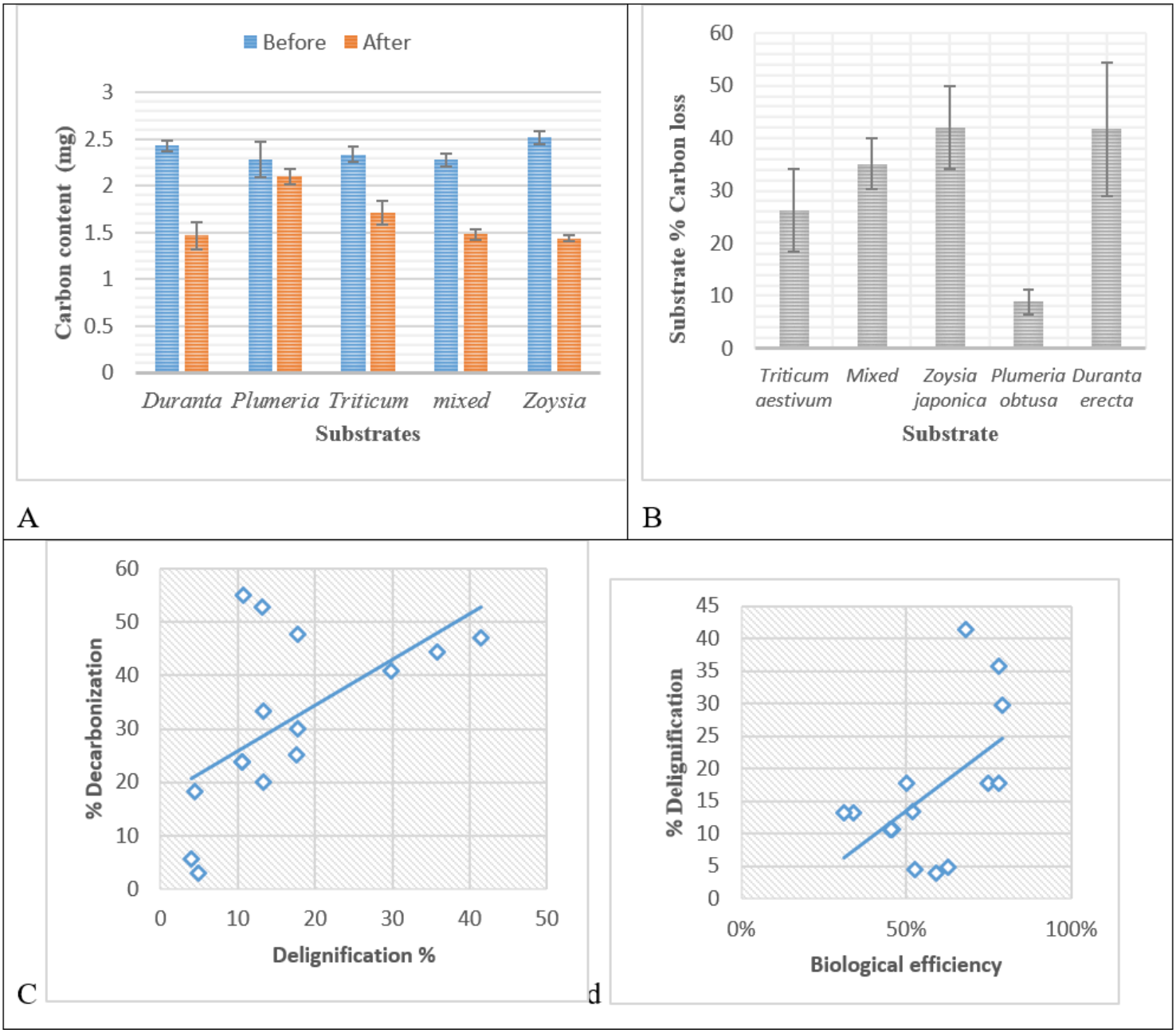


Figure 16

a. A contrast of substrate carbon content before and after mushroom cultivation b. Substrate % carbon lossc. *correlation between Substrate % delignification & % carbon loss*d. *correlation between % substrate delignification and biological efficiency*(Data presented are the mean of triplicates with standard error (5%))