

Article

Sustainable Bioconversion of Wetland Plant Biomass for *Pleurotus ostreatus* var. *florida* Cultivation: Studies on Proximate and Biochemical Characterization

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Citation: Elbagory, M.; El-Nahrawy, S.; Omara, A.E.-D.; Eid, E.M.; Bachheti, A.; Kumar, P.; Abou Fayssal, S.; Adelodun, B.; Bachheti, R.K.; Kumar, P.; et al. Sustainable Bioconversion of Wetland Plant Biomass for *Pleurotus ostreatus* var.

florida Cultivation: Studies on Proximate and Biochemical Characterization. *Agriculture* **2022**, *12*, 2095. <https://doi.org/10.3390/agriculture12122095>

Academic Editor: Luciano Beneduce

Received: 16 November 2022

Accepted: 5 December 2022

Published: 7 December 2022

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Abstract: The abundant biomass growth of aquatic macrophytes in wetlands is one of the major concerns affecting their residing biota. Moreover, the biomass degenerates within the wetlands, thereby causing a remixing of nutrients and emission of greenhouse gases. Therefore, it is crucial to find sustainable methods to utilize the biomass of aquatic macrophytes devoid of environmental concerns. The present study investigates the utilization of the biomass of three aquatic macrophytes, including the lake sedge (CL: *Carex lacustris* Willd.), water hyacinth (EC: *Eichhornia crassipes* Mart. Solms), and sacred lotus (NL: *Nelumbo nucifera* Gaertn.) to produce oyster (*Pleurotus ostreatus* var. *florida*) mushrooms. For this purpose, different combinations of wheat straw (WS: as control) and macrophyte's biomass (WH) such as control (100% WH), CL50 (50% WH + 50% CL), CL100 (100% CL), EC50 (50% WH + 50% EC), EC100 (100% EC), NL50 (50% WH + 50% NL), and NL100 (100% NL) were used for *P. florida* cultivation under controlled laboratory conditions. The results showed that all selected combinations of wheat straw and macrophyte biomass supported the spawning and growth of *P. florida*. In particular, the maximum significant ($p < 0.05$) growth, yield, bioefficiency, proximate, and biochemical parameters were reported using the WH substrate followed by CL, NL, and EC biomass, which corresponds to the reduction efficiency of the substrate parameters. Therefore, the findings of this study reveal that the biomass of selected aquatic macrophytes can be effectively utilized for sustainable mushroom cultivation while minimizing the risk associated with their self-degeneration.

Keywords: waste management; sustainable agriculture; mushroom cultivation; super foods; pollution reduction

1. Introduction

Wetland ecosystems have been regarded as a significant contributor to global greenhouse gas emissions [1]. Wetland vegetation serves as a major sink of carbon and CH₄ emissions, which consumes nutrients from the water and sediments. However, CH₄ release flux largely varies depending on the type of wetland plant, nutrient availability, the interaction of the root–shoot system, and the plant’s biochemical composition [2]. Moreover, wetlands have a very high capacity to store atmospheric carbon dioxide in their soils and vegetation, which counteracts the greenhouse effect and slows the rate of global warming [3]. A wide variety of aquatic and semi-aquatic plants are some of the keystone species of the wetland ecosystem that regulate its functionality. However, wetland plant biomass contributes to environmental pollution in several ways due to its rapid growth, thus causing the death of other organisms above and below the water surface [4].

Moreover, when wetland plants die, their decomposing tissues release harmful greenhouse gases and nutrients (such as nitrogen and phosphorus) that pollute both the atmosphere and nearby water bodies [5]. The released nutrients from the decayed wetland plants can re-enter the water, which promotes the growth of harmful algae blooms, thereby reducing the amount of oxygen in the water and consequently causing harmful effects on fish and other aquatic animals [6,7]. Similarly, large amounts of CO₂ and other toxic gases are released into the atmosphere if the wetland plant biomass is burnt. Therefore, it has become necessary to find sustainable methods for managing wetland plant biomass [8]. However, wetland plant biomass is rich in several nutrients and mineral elements and can be utilized for several purposes, such as biofuel and mushroom production. Several studies have reported that aquatic plants harvested from wetlands could be a renewable resource for sustainable biofuel and essential raw materials for horticulture industries [9,10].

Unlike conventional horticulture crops, mushroom cultivation has become a fast-growing agri-business that provides good income without the need for large fields. In recent years, mushroom cultivation has expanded significantly in India [11]. After China, India is currently the second-largest mushroom producer worldwide. Oyster mushrooms are India’s most commonly grown mushrooms, followed by button and shiitake mushrooms [12]. In India, approximately 0.23 million tons of mushrooms are produced annually [12]. Several small- and medium-sized businesses have recently been established to cultivate edible and medicinal mushrooms [13]. Currently, there are about 1500 mushroom farmers, of whom 60% have less than 1 hectare of land under cultivation to meet the market demands [14]. The remaining growers fall into two categories: medium and large growers, with 1–5 and 5–10 hectares of land under cultivation, respectively, which are responsible for international exports. Out of several mushroom species cultivated in India, oyster mushrooms make up about 17% of the total, followed by button and shiitake mushrooms [15].

Mushroom cultivation is very beneficial for managing plant waste. Growing mushrooms can aid in lowering greenhouse gas emissions caused by the natural decomposition of plant waste. Moreover, growing mushrooms can help recycle plant materials and lessen the amount of organic waste that would otherwise end up in landfills, which significantly contributes to plant waste management [16]. Therefore, the mushroom cultivation sector plays a significant role in the biological management of plant residues for generating foods [17]. Recent studies have shown that mushrooms can be grown using wetland waste as a substrate [9]. The wetland biomass comprises lignin, cellulose, hemicellulose, and other mineral elements [18]. Thus, these wastes can be inoculated with mushroom spawn to produce high-quality fruiting bodies.

Previously, limited studies have assessed the feasibility of wetland plant wastes for mushroom cultivation. Considering the environmental concern associated with using biomass, the efficient management of wetland plant biomass is necessary to reduce its environmental impacts. Keeping this in mind, the proposed study focused on utilizing the biomass of three wetland plants for the sustainable cultivation of oyster (*Pleurotus ostreatus* var. *florida*) mushrooms. These species were chosen for their high abundance in Haridwar wetlands. Further, the formulated mushroom substrates and harvested

fruiting bodies of *P. florida* were characterized in terms of physicochemical, proximate, and biochemical properties.

2. Materials and Methods

2.1. Collection of Wetland Plant Biomass and Mushroom Spawn

For the present study, fresh biomass of selected wetland plants such as lake sedge (CL: *Carex lacustris* Willd. 1805), water hyacinth (EC: *Eichhornia crassipes* Mart. Solms), and sacred lotus (NL: *Nelumbo nucifera* Gaertn) was collected from the local water bodies of Haridwar district, Uttarakhand, India (29.8876° N and 78.0195° E). Similarly, wheat straw (*Triticum aestivum* L.) was obtained from the local agricultural fields of Haridwar city. All wastes were collected in 10 kg capacity packaging-grade plastic bags and immediately transported to the laboratory for further processing. The collected wastes were sun-dried for one week and chopped into small pieces (2–3 cm) using a mechanical cutter. The freshly prepared grain spawn of oyster (*Pleurotus ostreatus* var. *florida*) mushroom was procured from Prakriti Mushroom and Spawn Lab located in Kaulagarh, Dehradun, Uttarakhand, India. One of the study's authors supervised the preparation of grain spawn at the aforementioned locations to ensure an adequate process. The grain spawn was stored at 4 °C until its final mixing with the substrate.

2.2. Experimental Design and Conditions

A total of seven treatments (having different ratios of selected wastes), such as control (100% WS.), CL50 (50% WH + 50% CL), CL100 (100% CL), EC50 (50% WH + 50% EC), EC100 (100% EC), NL50 (50% WH + 50% NL), and NL100 (100% NL), were prepared as substrates for the cultivation of *P. florida* mushrooms (Figure 1). The mixtures' proportions were chosen based on a previous pilot study performed (data not shown). All experiments were performed using five identical replicates. For this, the proportional weight of selected wastes was placed individually in plastic tubs of 25 L capacity (pre-sterilized using 10% formalin solution and sun-dried) for all treatments [19]. The wastes were moistened and chemically sterilized by dipping in the tubs containing 20 L tap water mixed with 20 mL formalin solution, 2 g carbendazim, and 50 g calcium carbonate for a period of 12 h [19]. Subsequently, the substrate was removed and placed on a plastic rack for rinsing the excess water and achieving optimum moisture content ($\approx 65\%$) [19]. Further, the substrate was placed in an aluminum container and then pasteurized at 60 °C for 4 h using an autoclave (7421PAD Equitron, Medica Instruments, Mumbai, India) [20].

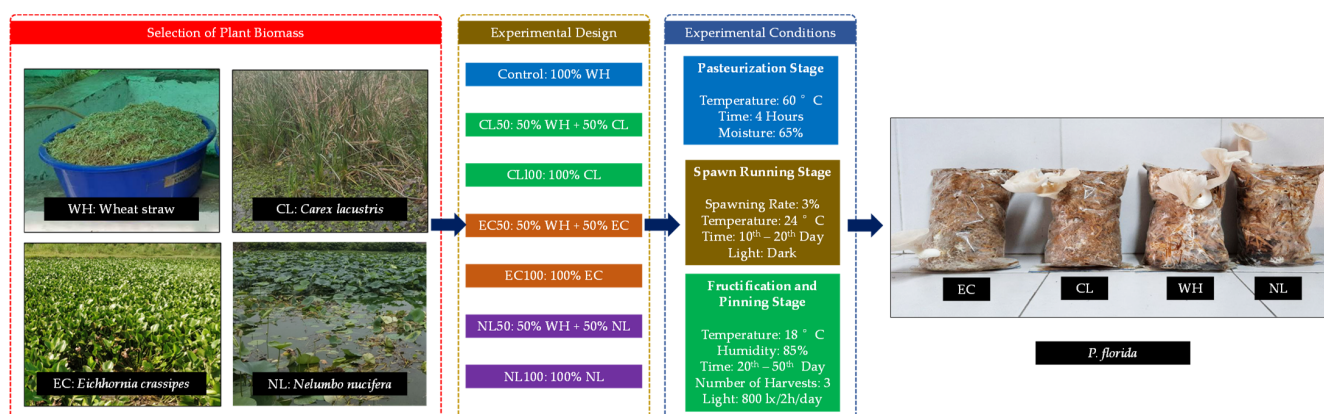


Figure 1. Study layout for plant biomass selection, experimental design, and conditions for cultivating *P. florida*.

After cooling, 2 kg substrate was filled in the breathable polypropylene bags (3 kg capacity), and 3% w/w grain spawn was aseptically mixed [21]. The spawned bags were then placed on a cultivation rack inside the mushroom cultivation room of the Agro-ecology and Pollution Research Laboratory, Department of Zoology and Environmental Science,

Gurukula Kangri (deemed to be a university), Haridwar, India. Spawn running was allowed at a room temperature of 24 °C in dark conditions. The room was opened for 2 h (daily) for efficient fresh air exchange (FAE). Once spawn running was completed, the room conditions were adjusted to 18 °C temperature, 85% relative humidity, and 800 lx of light intensity (2 h/day), respectively [22]. The substrate bags were pierced using sterile needles to make holes for efficient pin-head formation. The fruiting bodies of *P. florida* were carefully harvested (cut with a knife) once they reached marketable size and color. In this experiment, the *P. florida* crop was harvested in three subsequent flushes.

2.3. Analytical Methods

The mushroom substrates prepared from selected wastes were analyzed for selected physicochemical and proximate parameters before and after the cultivation of *P. florida*. Specifically, standard methods of chemical analyses were adopted as prescribed by AOAC [23] and used by Kumar et al. [24]. The pH and electrical conductivity (EC: dS/m) were measured using the pre-calibrated meter (ESICO 1611, Parwanoo, India). The contents of carbon and nitrogen in the substrate were determined with the methods of Walkley and Black [25] and Kjeldahl [26]. The proximate parameters such as ash, cellulose, hemicellulose, and Klason-lignin contents in the mushroom substrate were assessed using techniques previously reported by Gessner [27]. The harvested *P. florida* mushroom bodies were characterized for selected parameters such as moisture (%), protein (%), lipids (%), carbohydrates (%), ash (%), ascorbic acid (µg/100 g), carotenoids (µg/100 g), and total phenol (mg/100 g) contents. The moisture content of *P. florida* mushrooms was estimated using the oven-drying method by placing the fresh fruiting bodies at 60 °C until a constant weight was achieved. Similarly, the protein content was determined by the Kjeldahl method [28]. Total lipids and carbohydrate contents were determined using modified methods as previously adopted by Alam et al. [29]. Ash, ascorbic acid, carotenoids, and total phenol contents were analyzed following the methodology described by Kumar et al. [30]. Briefly, ash content was determined using a muffle furnace at 550 °C for 1 h. The ascorbic acid content was ascertained (absorbance peak 245 nm) using a 5% metaphosphoric acid solution as an extraction reagent. Similarly, carotenoids were determined by extracting them with petroleum ether and measuring the absorbance at 450 nm. Total phenol content was determined using the acetone extraction method (adding sodium carbonate and Folin–Ciocalteu reagent) and spectrophotometric analysis at 725 nm (Cary 60, UV-Vis, Agilent, Santa Clara, CA, USA).

2.4. Data Analysis

In this study, the obtained data were tested using analysis of variance (ANOVA) and Tukey's multiple range test based on Prob. (p) < F value of 0.05. The changes in the mushroom substrate properties were presented using the percent reduction efficiency (BE) tool [24]. The following Equation (1) was used for RE (%) calculation:

$$\text{Reduction Efficiency} = [(IV - FV)/IV] \times 100 \quad (1)$$

where IV and FV are the initial and final (after cultivation) parameter values of the *P. florida* mushroom substrate. Moreover, the biological efficiency (BE: %) of selected waste substrates for *P. florida* production was also calculated in order to understand their cultivation suitability [31]. The following model (Equation (2)) was used for the calculation of BE:

$$\text{Biological Efficiency (\%)} = [(FM/DW)] \times 100 \quad (2)$$

where FM refers to the total fresh weight of mushrooms (g) harvested from mushroom bag treatment having DW, i.e., initial dry weight of substrate (g), respectively. The interactive effects of different waste materials and their properties on growth, yield, and biochemical constituents of harvested *P. florida* mushrooms were studied using Pearson correlation analysis. This tool measures the strength of the linear relationship between variables with −1 and +1 representing the respective values of total negative and positive correlations and

a neutral (0) value corresponding to no correlation. Further, a hierarchical cluster analysis tool tested the similarities among different treatment groups and different properties of *P. florida* mushroom. The latter consists of agglomerative nesting that uses single-element clusters for each group to develop similarity. As a result, a combined tree is developed, and a heatmap showing similarities between participating groups is obtained.

2.5. Software and Tools

The obtained data were analyzed and processed using Microsoft Excel 2021 (Microsoft, Redmond, WA, USA) and OriginPro 2022b (OriginLab, Northampton, MA, USA) software packages.

3. Results and Discussion

3.1. Changes in Substrate Properties before and after *P. florida* Cultivation

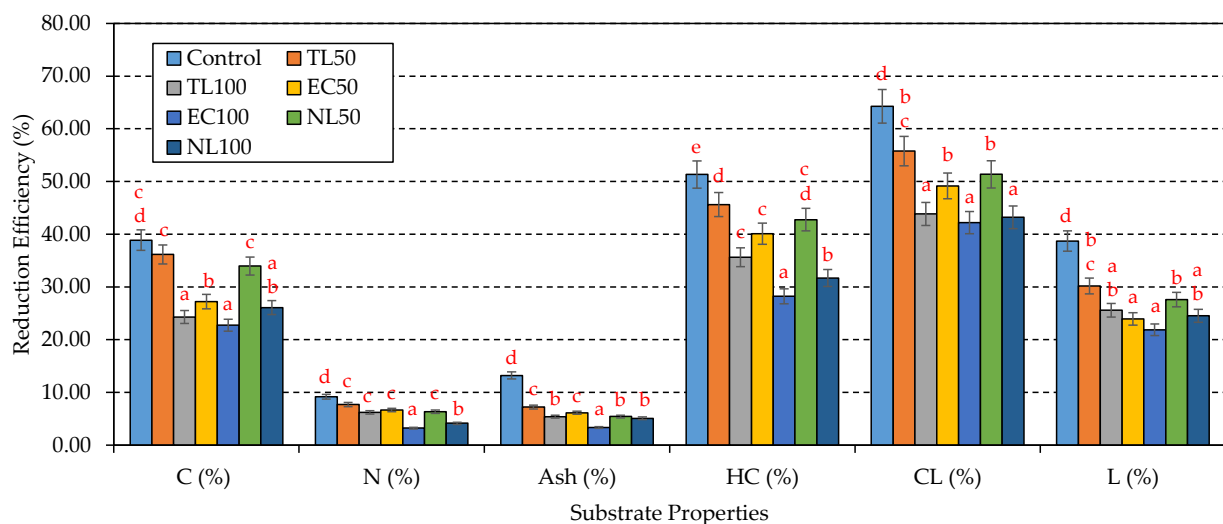
The mushroom substrate prepared from different combinations of wetland plant biomass and wheat straw was analyzed for selected properties to evaluate its efficiency in *P. florida* cultivation. The changes in the physicochemical and proximate characteristics of the substrate before and after *P. florida* mushroom cultivation are presented in Table 1. The results showed that the initial characteristics of the mushroom substrate were significantly ($p < 0.05$) reduced after the termination of experiments. Specifically, it was observed that the pH and EC of the mushroom substrates in all treatments were maximally reduced in the control and CL50 treatment, while the minimum value was observed in the EC100 treatment. Similarly, the contents of C and N were also significantly ($p < 0.05$) reduced as a result of mycelial action on the substrate and utilizing them as a primary energy source. Other proximate parameters of the substrate also declined significantly ($p < 0.05$). Based on the reduction efficiency index, the highest reductions in C (38.88%), N (9.16%), ash (13.21%), hemicellulose (51.32%), cellulose (64.27%), and lignin (38.70%) were observed in control treatment followed by CL50, NL50, EC50, CL100, NL100, and EC100, respectively (Figure 2). Overall, a 50% blend of wetland plant biomass with 50% wheat straw waste showed better reduction than their absolute treatments (CL100, EC100, and NL100). Mycelia produces acidic enzymes that help in the breakdown of β -galactoside bonds inside cellulose thereby reducing the pH of substrate rapidly in the colonization stage; however, it becomes stationary during the fructification stage [32]. It is reported that mushrooms require greater contents of C to grow and reproduce efficiently than N, which might be the reason behind their rapid reduction [33]. In this study, the C/N ratio ranged from 23.45 to 36.72, which is the ideal range as previous studies have shown that a substrate having a C/N ratio of 7–40 is considered optimum for mushroom cultivation [34].

Previously, limited studies have assessed the feasibility of wetland plant wastes for mushroom cultivation. Hultberg et al. [9] utilized the residues of the common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) plant for *P. ostreatus* (M2140) mushroom cultivation. They reported that wetlands produced significant biomass in two subsequent years (2016–2017) having a C/N ratio of 24 ± 5 . A significant reduction in common-reed-based mushroom substrate was reported, producing high-quality fruiting bodies of *P. ostreatus*. Similarly, Mukhopadhyay et al. [10] also evaluated the potential of water hyacinth (*E. crassipes*) biomass in combination with wheat straw for the cultivation of selected *Pleurotus* spp. They found that water hyacinth biomass had sufficient energy sources and nutrient loads to support *Pleurotus* spp. growth.

Table 1. Changes in properties (mean \pm SD of five replicates) of wetland plant biomass-based substrates before and after *P. florida* cultivation.

| Treatment | | Mushroom Substrate Properties | | | | | | | | |
|-----------|--------|-------------------------------|-------------------|--------------------|-------------------|-------|-------------------|--------------------|--------------------|-------------------|
| | | pH | EC (dS/m) | C (%) | N (%) | C/N | Ash (%) | H.C.L. (%) | CL (%) | L (%) |
| Control | Before | 7.01 \pm 0.01 a | 3.01 \pm 0.03 a | 48.10 \pm 1.90 a | 1.31 \pm 0.01 a | 36.72 | 2.12 \pm 0.03 a | 24.20 \pm 0.56 a | 45.37 \pm 2.74 a | 9.07 \pm 0.03 a |
| | After | 5.10 \pm 0.03 b | 2.07 \pm 0.05 b | 29.40 \pm 2.02 b | 1.19 \pm 0.03 b | 24.71 | 1.84 \pm 0.04 b | 11.78 \pm 1.78 b | 16.21 \pm 3.06 b | 5.56 \pm 0.09 b |
| CL50 | Before | 6.96 \pm 0.03 a | 2.79 \pm 0.06 a | 47.23 \pm 0.95 a | 1.30 \pm 0.02 a | 36.33 | 2.08 \pm 0.02 a | 22.99 \pm 1.05 a | 43.69 \pm 1.59 a | 8.74 \pm 0.12 a |
| | After | 5.13 \pm 0.02 b | 2.09 \pm 0.10 b | 30.15 \pm 1.64 b | 1.20 \pm 0.01 b | 25.13 | 1.93 \pm 0.03 b | 12.50 \pm 2.66 b | 19.32 \pm 2.20 b | 6.10 \pm 0.07 b |
| CL100 | Before | 6.90 \pm 0.03 a | 2.56 \pm 0.05 a | 46.35 \pm 1.31 a | 1.29 \pm 0.02 a | 35.93 | 2.04 \pm 0.01 a | 21.78 \pm 0.97 a | 42.01 \pm 1.11 a | 8.40 \pm 0.05 a |
| | After | 5.87 \pm 0.04 b | 2.12 \pm 0.09 b | 35.09 \pm 1.05 b | 1.21 \pm 0.01 b | 29.00 | 1.93 \pm 0.02 b | 14.02 \pm 1.32 b | 23.58 \pm 3.01 b | 6.25 \pm 0.19 b |
| EC50 | Before | 6.76 \pm 0.01 a | 2.91 \pm 0.08 a | 38.59 \pm 2.10 a | 1.28 \pm 0.01 a | 30.27 | 1.96 \pm 0.04 a | 21.62 \pm 0.63 a | 38.97 \pm 2.48 a | 8.99 \pm 0.08 a |
| | After | 5.49 \pm 0.02 b | 2.25 \pm 0.03 b | 28.09 \pm 1.47 b | 1.19 \pm 0.02 b | 23.61 | 1.84 \pm 0.03 b | 12.95 \pm 1.70 b | 19.81 \pm 0.97 b | 6.84 \pm 0.10 b |
| EC100 | Before | 6.50 \pm 0.03 a | 2.81 \pm 0.04 a | 29.08 \pm 0.89 a | 1.24 \pm 0.02 a | 23.45 | 1.80 \pm 0.02 a | 19.03 \pm 1.07 a | 32.57 \pm 1.76 a | 8.91 \pm 0.04 a |
| | After | 5.75 \pm 0.05 b | 2.34 \pm 0.07 b | 22.47 \pm 0.52 b | 1.20 \pm 0.01 b | 18.73 | 1.74 \pm 0.02 b | 13.66 \pm 0.81 b | 18.82 \pm 2.55 b | 6.96 \pm 0.11 b |
| NL50 | Before | 6.92 \pm 0.02 a | 2.87 \pm 0.10 a | 41.02 \pm 1.73 a | 1.26 \pm 0.01 a | 32.56 | 1.85 \pm 0.01 a | 23.75 \pm 1.25 a | 41.25 \pm 2.02 a | 8.80 \pm 0.09 a |
| | After | 5.12 \pm 0.01 b | 2.18 \pm 0.05 b | 27.09 \pm 0.86 b | 1.18 \pm 0.03 b | 22.96 | 1.75 \pm 0.02 b | 13.59 \pm 2.04 b | 20.06 \pm 1.17 b | 6.37 \pm 0.03 b |
| NL100 | Before | 6.82 \pm 0.04 a | 2.73 \pm 0.04 a | 33.94 \pm 1.24 a | 1.21 \pm 0.02 a | 28.05 | 1.58 \pm 0.03 a | 23.29 \pm 1.42 a | 37.13 \pm 2.35 a | 8.52 \pm 0.10 a |
| | After | 5.78 \pm 0.03 b | 2.22 \pm 0.06 b | 25.09 \pm 0.92 b | 1.16 \pm 0.02 b | 21.63 | 1.50 \pm 0.01 b | 15.91 \pm 0.63 b | 21.09 \pm 1.60 b | 6.43 \pm 0.04 b |

The same letters (a,b) indicate no significant difference in substrate properties before and after *P. florida* cultivation; CL: *Carex lacustris*; EC: *Eichhornia crassipes*; NL: *Nelumbo nucifera*; EC: electrical conductivity; C: carbon; N: nitrogen; C/N: carbon to nitrogen ratio; HCL: hemicellulose; CL: cellulose; L: lignin.

**Figure 2.** Reduction efficiency (%) of mushroom substrate properties before and after *P. florida* cultivation (The same letters (a–e) indicate no significant difference between treatment group values; TL: *Typha latifolia*; EC: *Eichhornia crassipes*; NL: *Nelumbo nucifera*; EC: electrical conductivity; C: carbon; N: nitrogen; HCL: hemicellulose; CL: cellulose; L: lignin).

3.2. Effect of Wetland Plant Biomass on Growth and Yield of *P. florida*

The fastest colonization of *P. florida* for substrates was detected with the control and CL50 as 12.0 ± 1.0 and 13.0 ± 1.0 days, respectively (Table 2). The growth of *P. florida* on plant extracts cultivated on Petri dishes at a 25 °C incubation showed fast colonization, thus, corroborating with the current field findings [35]. Moreover, the high cellulose content in these substrates promotes the mycelial colonization of *Pleurotus* spp., as previously outlined by Sassine et al. [36]. In the first flush, the highest yield was observed with the control followed by CL50 with 115.46 ± 4.08 and 95.10 ± 3.57 g/kg, respectively ($p < 0.05$). A similar trend was observed in the second and third flushes, with 93.55 ± 2.77 and 86.81 ± 4.23 g/kg, and 45.05 ± 1.50 and 42.18 ± 2.74 g/kg, respectively. Consequently, the total yield was also the highest in those two treatments, with 254.06 ± 6.82 and 224.17 ± 8.10 g/kg, respectively. The lowest yields and BEs were observed when *P. florida* mushrooms were grown on substrates fully based on wetland biomass. This could be explained by the fact that these biomasses had a lower C/N ratio than other treatments; mycelial growth and development

are normally associated with a high C/N ratio [31]. Lower cellulose content in such substrates may have contributed to the delay of substrate colonization and the reduction in yield and BE; this component acts as the main source of energy for the mycelium [37]. It is worth noting that mushroom yields decrease as the growing cycle proceeds; i.e., subsequent flushes outline decreased yields [22]. The BE is an important parameter for mushroom substrate selection. Although no substrate reached 80% BE—the economically feasible BE for mushroom cultivation [38]—the control and CL50 treatments (72.59 ± 0.80 and $64.05 \pm 1.47\%$, respectively) showed significantly higher ($p < 0.05$) BEs compared to the remaining substrates, as depicted in Table 2. Figure 3a outlines a strong correlation between wheat straw on the one hand and yield and BE on the other hand, which explains the highest yield and BE observed with the control. A previous study outlined the use of the wetland common reed (*Phragmites australis*) in the production of *P. ostreatus* [9]. The results delineated a 140% BE, which is 2.2–3.7 times higher than the values obtained with wetland plant biomass in this study. Incorporating other plant biomass, e.g., alfalfa pulp, also resulted in 166.3% BE [39]. These authors referred to the increase in BE in the protein content of the used substrate. This corroborates the strong correlation between these two parameters, as shown by Pearson's correlation (Figure 3a).

Table 2. Effect of wetland plant biomass on growth and yield properties (mean \pm SD of five replicates) of *P. florida*.

| Treatment | Colonization (Days) | Yield (g/kg Fresh Substrate) | | | | Biological Efficiency (%) | Pileus Diameter (cm) | Stipe Length (cm) |
|-----------|---------------------|------------------------------|--------------------|--------------------|----------------------|---------------------------|----------------------|-------------------|
| | | First Flush | Second Flush | Third Flush | Total | | | |
| Control | 12.00 \pm 1.00 a | 115.46 \pm 4.08 e | 93.55 \pm 2.77 e | 45.05 \pm 1.50 d | 254.06 \pm 6.82 f | 72.59 \pm 0.80 e | 7.30 \pm 0.04 f | 6.39 \pm 0.07 e |
| CL50 | 13.00 \pm 1.00 a | 95.10 \pm 3.57 d | 86.81 \pm 4.23 e | 42.18 \pm 2.74 d | 224.17 \pm 8.10 e | 64.05 \pm 1.47 d | 6.79 \pm 0.07 e | 5.75 \pm 0.05 d |
| CL100 | 18.00 \pm 2.00 c | 70.22 \pm 5.16 a | 56.63 \pm 2.06 b | 30.08 \pm 0.98 a | 156.96 \pm 5.17 ab | 44.85 \pm 1.82 b | 4.66 \pm 0.03 b | 4.22 \pm 0.18 b |
| EC50 | 15.00 \pm 1.00 b | 77.36 \pm 2.09 b | 64.09 \pm 3.42 c | 34.10 \pm 1.26 b | 175.45 \pm 7.32 c | 50.13 \pm 3.25 c | 5.30 \pm 0.10 c | 4.42 \pm 0.09 b |
| EC100 | 19.00 \pm 2.00 c | 61.09 \pm 4.82 a | 46.32 \pm 1.85 a | 24.67 \pm 2.30 a | 132.09 \pm 9.51 a | 37.74 \pm 0.78 a | 4.20 \pm 0.18 a | 3.46 \pm 0.13 a |
| NL50 | 14.00 \pm 1.00 b | 82.24 \pm 2.70 c | 70.40 \pm 2.57 d | 39.30 \pm 1.10 c | 191.99 \pm 4.08 d | 54.85 \pm 1.35 c | 5.82 \pm 0.05 d | 4.75 \pm 0.06 c |
| NL100 | 14.00 \pm 1.00 b | 67.02 \pm 3.56 a | 51.06 \pm 3.14 a | 26.50 \pm 3.12 a | 144.58 \pm 8.66 a | 41.31 \pm 2.92 b | 4.38 \pm 0.12 a | 3.64 \pm 0.20 a |

The same letters (a–f) indicate no significant difference among different substrate groups; CL: *Carex lacustris*; EC: *Eichhornia crassipes*; NL: *Nelumbo nucifera*; EC: electrical conductivity; C: carbon; N: nitrogen; HCL: hemicellulose; CL: cellulose; L: lignin.

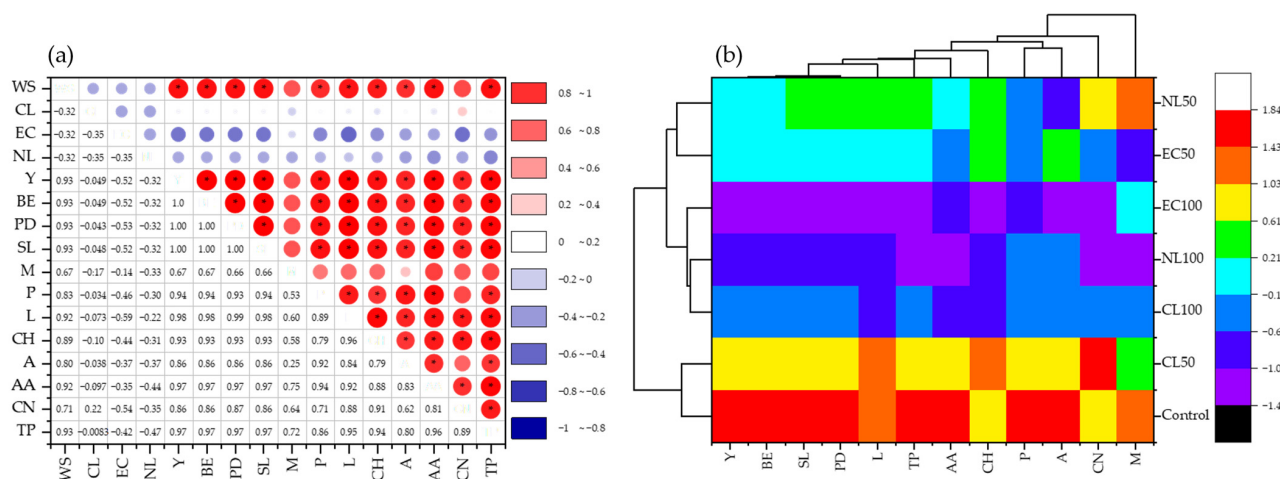


Figure 3. (a) Pearson correlation and (b) hierarchical cluster matrices showing the effect of different biomass treatments on growth, yield, and biochemical constituents of *P. florida* (TL: *Typha latifolia*; EC: *Eichhornia crassipes*; NL: *Nelumbo nucifera*; WS: wheat straw; Y: yield; BE: biological efficiency; PD: pileus diameter; SL: stipe length; M: moisture; P: protein; L: lipid; CH: carbohydrates; A: ash; AA: ascorbic acid; CN: carotenoids; TP: total phenols).

Commercially, mushrooms with large pilei and short stipes are the most preferred by consumers. The largest pileus (PD) and longest stipe (SL) were observed with the control and CL50 (7.30 ± 0.04 and 6.79 ± 0.07 cm, and 6.39 ± 0.07 and 5.75 ± 0.05 cm, respectively).

EC100 and NL100 had the shortest and most economically preferred stipes (3.46 ± 0.13 and 3.64 ± 0.20 cm, respectively). Therefore, CL50 showed the most economically feasible mushrooms in terms of pileus diameter, while EC100 and NL100 were the most suitable in terms of stipe length. A previous study indicated the close interrelationship between shorter oyster mushroom stipes and the inoculation of plant biomass residues in the growing substrate [37]. Strong correlations were observed between PD on the one hand, and yield and BE on the other hand (Figure 3a), which is obvious as caps generally weigh more than stipes. Increased PD and SL simulate increased protein, lipid, and carbohydrate contents in the substrate; those contents need further investigation. Similar findings were reported on *P. ostreatus* where PD and SL were closely related to substrate composition, especially in terms of protein and carbohydrates [37]. Hierarchical cluster analysis (HCA) is a descriptive classification that helps identify high similarities/dissimilarities between parameters or groups of parameters [24]. Herein, the similarities between groups of treatments were depicted in a heat-map-based cluster diagram (Figure 3b) in terms of the production and composition parameters of *P. florida* mushrooms. The minimum and maximum distances were -1.42 and 0.67 , respectively, based on the nearest neighboring method of Euclidian clustering. The highest similarity was observed between the control and CL50, and dissimilarity between their grouping on the one hand and the remaining treatment group on the other hand. Although HCA effectively outlined the interactive effect of the composition parameters, no clear understanding of the effect of the production parameters of *P. florida* treatments was obtained.

3.3. Effect of Wetland Plant Biomass on Nutrient and Biochemical Composition of *P. florida*

The results of the proximate and biochemical constituents of *P. florida* grown on different wetland plant biomass spp. are presented in Table 3. Moisture, protein, lipid, carbohydrates, ash, ascorbic acid, carotenoids, and total phenol levels were significantly ($p < 0.05$) the highest in control and CL50 treatments (86.30 ± 2.10 and $85.66 \pm 1.98\%$; 25.14 ± 0.22 and $24.42 \pm 0.24\%$; 2.36 ± 0.05 and $2.35 \pm 0.05\%$; 48.70 ± 0.40 and $49.02 \pm 0.21\%$; 7.52 ± 0.04 and $7.48 \pm 0.05\%$; 0.49 ± 0.01 and 0.47 ± 0.02 $\mu\text{g}/100$ g; 0.23 ± 0.01 and 0.24 $\mu\text{g}/100$ g; 6.12 ± 0.03 and 6.10 ± 0.04 $\mu\text{g}/100$ g, respectively) compared to the remaining treatments. Increased moisture content in mushrooms can hasten the deterioration process during the postharvest period leading to enzymatic browning [40]. Herein, the incorporation of wetland plant biomass decreased the moisture content in harvested *P. florida* mushrooms. Oyster mushrooms grown on alfalfa pulp showed slightly lower moisture content (83.13%) [39]. These authors also outlined a lower protein content in harvested mushrooms than those grown on wetland plant biomass in this study (lower by 2.54–4.82%). The observation of Figure 3a depicts a correlation between moisture content in *P. florida* mushroom and WS. This finding corroborates with Abou Fayssal et al. [37], who found a direct influence of WS in *P. ostreatus* growing substrate on the moisture content of harvested mushrooms. Moreover, lower protein and carbohydrate contents in mushrooms grown on substrates fully based on wetland biomass can be related to the lower C/N ratio of such substrates, as observed in Table 1. Furthermore, the lower lignin content found in mushrooms grown on these substrates compared to the control explains the fact that *P. florida* had a lower capability to degrade this component from the substrate and translocate it into the fruiting body. This finding corroborates a similar hypothesis lately raised on *P. ostreatus* [22].

Our findings outlined a strong positive correlation between protein content in the harvested mushrooms and WS in the growing substrate. This contradicts the findings of Piskov et al. [41] and Gao et al. [42] on wheat and rice straws in *P. ostreatus* and *L. edodes* cultivation, respectively. Moreover, the protein content was significantly correlated with increased yield, BE, PD, and SL (Figure 3a). Mushrooms are generally low in fat/lipid; the incorporation of different wetland plant biomass reduced this content, especially with CL100, EC100, and NL100 ($p < 0.05$). Therefore, mushrooms harvested from these treatments are healthier than those from the control and can help in the remediation

of cardiovascular problems [37]. CL50 increased the carbohydrate content in harvested mushrooms, which can present a promising additive to carbohydrate-rich diets. Wetland plant biomass-based substrates resulted in mushrooms with lower ash content. Overall, harvested mushrooms in this study were not very rich in ash; it may be returned to their freshness as, during storage or processing, mushrooms dry, and their ash content tends to increase accordingly [43]. Promising ascorbic acid contents (also known as vitamin C) were found in *P. florida* mushrooms grown on wetland plant biomass spp. This vitamin is essential for humans as a primary antioxidant found in plasma and cells [44]. CL50 mushrooms had a higher carotenoid content than the control ones; carotenoids play a crucial role in the non-enzymatic ROS defense mechanisms [45]. When wetland plant biomass was added to the growing substrate, good total phenol values were noted in harvested mushrooms. Phenolic compounds hold interesting redox properties, which are responsible for antioxidant activity [46].

Table 3. Effect of wetland plant biomass on proximate and biochemical constituents (mean \pm SD of five replicates) of *P. florida*.

| Treatment | Proximate Constituents (%) | | | | | Biochemical Constituents | | |
|-----------|----------------------------|--------------------|--------------------|---------------------|--------------------|--|--|---|
| | Moisture | Protein | Lipid | Carbohydrates | Ash | Ascorbic Acid ($\mu\text{g}/100\text{ g}$) | Carotenoids ($\mu\text{g}/100\text{ g}$) | Total Phenol ($\text{mg}/100\text{ g}$) |
| Control | 86.30 \pm 2.10 ab | 25.14 \pm 0.22 c | 2.36 \pm 0.05 b | 48.70 \pm 0.40 cd | 7.52 \pm 0.04 b | 0.49 \pm 0.01 d | 0.23 \pm 0.01 b | 6.12 \pm 0.03 b |
| CL50 | 85.66 \pm 1.98 a | 24.42 \pm 0.24 c | 2.35 \pm 0.05 b | 49.02 \pm 0.21 d | 7.48 \pm 0.05 b | 0.47 \pm 0.02 cd | 0.24 \pm 0.01 b | 6.10 \pm 0.04 b |
| CL100 | 85.10 \pm 2.06 a | 23.15 \pm 0.17 b | 2.30 \pm 0.06 a | 46.20 \pm 0.19 b | 7.38 \pm 0.10 a | 0.43 \pm 0.02 b | 0.22 \pm 0.01 a | 6.05 \pm 0.01 a |
| EC50 | 84.90 \pm 1.55 a | 23.23 \pm 0.30 b | 2.32 \pm 0.04 ab | 47.81 \pm 0.28 c | 7.44 \pm 0.05 ab | 0.44 \pm 0.01 c | 0.22 \pm 0.02 a | 6.07 \pm 0.04 ab |
| EC100 | 85.53 \pm 2.91 a | 22.90 \pm 0.13 a | 2.28 \pm 0.06 a | 45.75 \pm 0.13 a | 7.35 \pm 0.07 a | 0.43 \pm 0.02 b | 0.21 \pm 0.01 a | 6.03 \pm 0.02 a |
| NL50 | 86.37 \pm 1.72 ab | 23.28 \pm 0.25 b | 2.33 \pm 0.07 ab | 47.94 \pm 0.36 c | 7.36 \pm 0.03 a | 0.45 \pm 0.01 c | 0.23 \pm 0.01 ab | 6.08 \pm 0.04 ab |
| NL100 | 84.48 \pm 1.05 a | 23.20 \pm 0.18 b | 2.30 \pm 0.04 a | 46.09 \pm 0.09 b | 7.39 \pm 0.06 a | 0.42 \pm 0.02 a | 0.21 \pm 0.01 a | 6.02 \pm 0.02 a |

The same letters (a–d) indicate no significant difference among different substrate groups; CL: *Carex lacustris*; EC: *Eichhornia crassipes*; NL: *Nelumbo nucifera*; EC: electrical conductivity.

4. Conclusions

The findings of this study concluded that the biomass of selected wetland plants (TL: *Typha latifolia*; EC: *Eichhornia crassipes*; NL: *Nelumbo nucifera*) could be efficiently utilized in combination with wheat straw waste for the sustainable cultivation of oyster (*Pleurotus ostreatus* var. *florida*) mushrooms. Overall, a 50% blend of wetland plant biomass and wheat straw (WS) gave the highest *P. florida* yield with enhanced proximate and biochemical constituents. However, the use of absolute (100%) wetland plant biomass gave a relatively lesser yield than treatments carried out in combination with WS. The highest yield, growth, proximate, and biochemical properties of *P. florida* were reported using WS (100%) treatment followed by 50% blends of CL, NL, and EC, respectively. Therefore, this study suggests that the biomass of selected aquatic plants harvested from wetlands can be used as renewable substrate resources for *P. florida* mushroom cultivation to reduce the environmental risk associated with their self-degradation. Further studies on using other aquatic plants to cultivate *P. florida* and other mushroom species are highly recommended.

Author Contributions: Conceptualization, P.K. (Pankaj Kumar, rs.pankajkumar@gkv.ac.in); Formal analysis, P.K. (Pankaj Kumar, rs.pankajkumar@gkv.ac.in) and P.K. (Pankaj Kumar, pankaj9991kumar@gmail.com); Funding acquisition, M.E. and I.Š.; Investigation, P.K. (Pankaj Kumar, pankaj9991kumar@gmail.com); Methodology, P.K. (Pankaj Kumar, rs.pankajkumar@gkv.ac.in); Project administration, E.M.E.; Software, P.K. (Pankaj Kumar, rs.pankajkumar@gkv.ac.in); Supervision, V.K.; Validation, M.E., S.E.-N., A.E.-D.O., E.M.E., A.B., S.A.F., B.A., R.K.B., B.M., V.K. and I.Š.; Visualization, P.K. (Pankaj Kumar, rs.pankajkumar@gkv.ac.in); Writing—original draft, P.K. (Pankaj Kumar, rs.pankajkumar@gkv.ac.in) and S.A.F.; Writing—review and editing, M.E., S.E.-N., A.E.-D.O., E.M.E., B.A., R.K.B., B.M., V.K. and I.Š. All authors have read and agreed to the published version of the manuscript.

Funding: The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University for funding this work through the Large Groups Project under grant number R.G.P. 2/138/43.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are grateful to their host institutes for providing the necessary facilities to conduct this study. This is joint work from the members of the Sustainable Agro-Environment International Research Group (SAEIRG). The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University for funding this work through the Large Groups Project under grant number R.G.P. 2/138/43.

Conflicts of Interest: The authors declare no conflict of interest.

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