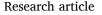
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# Enhancing yields of *Pleurotus ostreatus* and *Lentinula edodes* mushrooms using water hyacinth (*Eichhornia crassipes* [Mart.] Solms) supplemented with locally available feedstock as substrate

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# ABSTRACT

This study assessed the performance of Pleurotus ostreatus and Lentinula edodes mushrooms on a variety of substrate combinations. Water hyacinth, rice husk, and cow dung were employed as substrates. Mushroom growth performance, yield, proximate composition, and mineral content were among the variables evaluated. The results indicate significant differences (p < 0.05) in spawn run duration, first harvest duration, total yield, and biological efficiency among the substrate combinations for the mushroom species. The substrate combination of 80% water hyacinth and 20% cow dung consistently exceeded the performances of others, demonstrating higher total yield (863.00 and 799.81 g/bag) and biological efficiency (88.51% and 82.03%) for P. ostreatus and L. edodes mushrooms, respectively. Proximate analysis results also demonstrated that this substrate combination produced mushrooms with higher protein (14.72 and 12.04%) and carbohydrate (55.11 and 58.05%) contents for P. ostreatus and L. edodes, respectively. P, K, Mg, Na, Ca, Fe, Zn, and Cd levels in P. ostreatus samples ranged from 1700 to 2700, 28100 to 39500, 1600 to 7800, 291.55 to 400.23, 310.37 to 372.70, 26.42 to 45.47, 61.87 to 70.40, and 1.13-1.25 mg/ kg on average, respectively. The levels for P, K, Mg, Na, Ca, Fe, Zn, and Cd ranged from 19700 to 22700, 22500 to 25000, 2100 to 2500, 250.96 to 300.90, 284.66 to 296.19, 24.04 to 29.49, 74.03 to 83.98, and 1.31-1.45 mg/kg for L. edodes samples. The evaluated mushrooms grown on the various substrate combinations contain higher major and minor minerals needed in the human diet than toxic elements. This indicated that the evaluated edible mushrooms had high important mineral levels and could be considered a good source of vital elements. They are also very good at balancing nutrient supply scarcity, which is common in developing countries like Ethiopia. However, according to the World Health Organization's permissible limits for human intake, adequate attention and control of daily dietary intake is necessary for specific elements.

#### 1. Introduction

Water hyacinth (*Eichhornia crassipes* (Mart.) Solms.) has become a global concern due to its rapid proliferation, primarily attributed to human activities [1–4]. This invasive aquatic weed threatens freshwater ecosystems worldwide [5]. The introduction of water hyacinth to Africa dates to the late 19<sup>th</sup> century, first appearing in Egypt around 1882 and later in the White Nile in 1958. Its presence

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has expanded throughout the Nile River and East African regions, particularly infesting Lake Tana, Ethiopia's largest lake, since 2011.

The infestation of Lake Tana by water hyacinth has escalated dramatically, from covering a modest 100 ha in 2011 [6] to a staggering 50,000 ha by 2014 [1]. This unchecked growth threatens native flora and water quality while also adversely impacting crop production through the discharge of harvested biomass into surrounding areas [7–10]. Moreover, it impedes fishing activities and disrupts boat transportation on the lake [8]. Efforts have been made by different stakeholders to combat this infestation, using mechanical, biological, and chemical approaches. Between 2000 and 2013, approximately \$100,000 was allocated to this endeavor [11]. Manual removal efforts engaged around 800,000 labor hours, and over \$1 million was spent on harvester machines between 2012 and 2018 [8,10]. Despite these substantial activities and investments, the weed's expansion remains uncontrolled. This invasive weed problem could be solved by combining it with other commonly used agricultural wastes such as cow dung, rice straw, wheat straw, sawdust, and other substrates for mushroom cultivation. Eradicating this unpleasant weed by utilizing enormous quantities accessible for free is an environmentally friendly solution, motivating research into its application in mushroom cultivation. As a result, there is a demanding need for more research into the subject in order to validate discrepancies.

From 1990 to 2020 (over 30 years), mushroom production increased 13.8 times to 42.8 million tons globally [12]. Shiitake mushrooms (L. edodes) are the most abundant, followed by oyster mushrooms (Pleurotus spp.) [13]. China produces about 93% of the world's mushrooms including these species. L. edodes is widely regarded as an edible and medicinal mushroom, with a distinct fungal aroma and the ability to cure a variety of ailments such as cancer, diabetes, hypotension, inflammation, nociceptiveness, and hypocholesterolemia [14–16]. The China Edible Fungi Association reports that shiitake mushroom production reached 7.67 million tons in 2015, accounting for over 20% of total edible mushroom production in China, as cited in Ref. [17]. Ovster mushroom (*Pleurotus* spp.) is also one of the most common edible mushroom species cultivated industrially, accounting for about 16.3% of the total production [18]. This species of mushroom is the most frequently farmed in a country like Malaysia, accounting for more than 90% of total production with a capacity of over 65,000 tons in 2020, as cited in Ref. [19]. Among the Pleurotus spp., oyster mushrooms (also known as white rot fungus, abalone, or tree oyster mushroom) are a popular mushroom with several benefits, including pharmacological properties [19]. In recent years, the oyster mushroom farming sector has increased dramatically. It rose from \$2.79 billion in 2023 to \$2.94 billion in 2024, with a compound annual growth rate of 5.2% [20]. Mushroom cultivation, nowadays, is being promoted and practiced in Ethiopia for both personal consumption and income generation. The oyster (P. ostreatus) and shiitake (L. edodes) mushrooms are among the most produced mushrooms in the country. Different authors are trying to optimize various potential locally available substrates to establish sustainable mushroom cultivation in the country [21-27]. Water hyacinth as a mushroom substrate in Ethiopia is initiated for the first time by supplementing it with wheat and teff straws for ovster mushroom cultivation [28]. Nonetheless, the study has not explored supplementing with water hyacinth and other locally available biomasses. Furthermore, the authors did not investigate the suitability of water hyacinth for other mushrooms like shiitake.

Given the limited success of current management strategies, there is an urgent need to explore alternative approaches to manage water hyacinth. One promising solution is the utilization of water hyacinth as a substrate for mushroom cultivation. This approach



Fig. 1. Substrate: (a) freshwater hyacinth, (b) chopped and dried water hyacinth, (c) dried rice husk, and (d) flattened and dried cow dung.

presents both environmental and socio-economic benefits. Therefore, the primary aim of this research is to investigate the feasibility of utilizing water hyacinth blended with locally sourced potential substrates as a sustainable and cost-effective medium for cultivating *P. ostreatus* and *L. edodes* mushrooms. The specific objectives are to (i) evaluate the spawn run and first harvest durations; (ii) evaluate overall yield and biological efficiency; and (iii) investigate the proximate composition and mineral contents of *P. ostreatus* and *L. edodes* mushrooms grown on various substrate combinations. By evaluating the growth performance and yield of these mushroom species on the novel substrate blend, this study seeks to offer an environmentally friendly approach to both managing water hyacinth abundance in Lake Tana and providing a viable alternative substrate for mushroom cultivation. This technology could be highly useful in the food and agriculture sectors, where accurate identification of foods and nutrients is essential to maintain food safety, quality, and sustainable practices.

#### 2. Materials and methods

#### 2.1. Cultures, substrates collection and preparation

The study was conducted at the facility of the Forest Products Innovation Center of Excellence (FPICE), Ethiopian Forestry Development (EFD), in Addis Ababa. *P. ostreatus* and *L. edodes* mushroom cultures were obtained from the Biology Department of Addis Ababa University and FPICE, respectively. These fungal cultures were consistently maintained on malt extract agar slants at a temperature of 4 °C and subcultured every 30 days.

Substrates (Fig. 1) were collected from various locations in the country. Fresh water hyacinth (Fig. 1a) was harvested from Lake Tana by carefully removing its roots and thoroughly washing the remaining biomass. Rice husks (Fig. 1c) were obtained from local farmers' rice fields in the Fogera district of the Amhara Regional State. Cow dung (Fig. 1d) was collected from the Holeta Agricultural Research Center of the Ethiopian Institute of Agricultural Research. The substrates were promptly transported to FPICE and air-dried. The dried water hyacinth biomass (Fig. 1b) was manually chopped into approximately 3 cm pieces. The substrates were stored in clean bags until they were ready for use.

#### 2.2. Mushroom cultivation and data collection

Sorghum grains underwent cleaning, overnight soaking in water, and softening through a 20-min boiling process. After draining excess water, the grains were allowed to cool at room temperature. Calcium carbonate (CaCO<sub>3</sub>) was added at a concentration of 2% (w/w basis) [29]. The grains were thoroughly mixed before being filled into glass bottles (500 mL), filling them up to about two-thirds of their capacity and sealed with cotton plugs. These bottles were subsequently autoclaved for 2 hrs. After cooling, each bottle was inoculated with 10 discs, each measuring 10 mm in diameter, of actively growing mycelium cultures that were cultivated on malt extract agar. All the bottles were incubated in a dark room at a constant temperature of 25 °C until full mycelial colonization was achieved, which took 16 days for *P. ostreatus* and 19 days for *L. edodes*.

It has been common practice to evaluate locally available substrates to find out the suitable combination for higher mushroom yields [27,30]. To examine the substrate effects, a completely randomized design (CRD) was employed. Likewise, different substrate combinations involving water hyacinth (WH), rice husk (RH), and cow dung (CD) on a dry weight basis were prepared (Table 1). The different proportions of CD and RH supplements to the main substrate (WH) were set to find the best combination for the mushrooms cultivation. WH and RH were soaked separately in water overnight, and excess water was removed by placing them on a clean inclined cement floor. The CD was moistened separately, and the three substrates were thoroughly mixed. Additionally, 2% CaCO<sub>3</sub> was mixed with each prepared substrate mix to buffer the pH of the substrate [31]. Finally, 1.5 kg of wet substrate was filled into each polypropylene container and autoclaved.

After cooling, each bag was inoculated with 2% (w/w wet basis) of the prepared spawn [32]. The mouth of each bag was loosely tied and then sealed with clean cotton for aeration. The bags were placed on a shelf in a dark room for spawn running at a temperature of approximately 25–30 °C and a relative humidity ( $R_H$ ) level of 70–80%. Before being moved to the fructification room, which had been disinfected with 70% alcohol applied using cotton, the bags were protected against damage by covering the shelf with iron wire mesh. The *L. edodes* shiitake mushroom bags underwent a 24-hrs shock treatment in a 4 °C refrigerator before being moved to the fructification room. To increase the room's  $R_H$  level and lower the temperature, both the floor and bags were sprayed with tap water three times daily. Mature fruit bodies were harvested when they were ready during the three consecutive flushing periods. Spawn run

#### Table 1

Optimization of WH for the cultivation of *P. ostreatus* and *L. edodes* mushrooms with combinations of CD and RH in different proportions.

Substrate code	Mixing proportions			
SC1	100% WH			
SC2	80% WH and 20% CD			
SC3	80% WH and 20% RH			
SC4	80% WH, 10% CD and 10% RH			
SC5	70% WH, 20% CD and 10%RH			
SC6	70% WH, 10% CD, and 20% RH			

(SR) and first fruit body harvest (FH) days for each bag were meticulously recorded. The total yields (TY) for the three flushing periods were also calculated, and biological efficiencies were determined using the following equation (1):

$$BE = \left(\frac{W_{FM}}{W_{DS}}\right) * 100 \tag{1}$$

where BE is biological efficiency in percentage, W<sub>FM</sub> is fresh mushroom weight in gram (g), and W<sub>DS</sub> is dry substrate weight in gram (g).

To determine the proximate compositions and mineral contents, the fruit bodies were dried in an oven (40 °C) until constant weight and then finely powdered to a 1-mm sieve size. The powdered samples were stored in clean polyethylene bags until analysis. The crude fat, crude fiber, and total carbohydrate contents were determined using the Association of Official Analytical Chemists procedures (AOAC), 1995. The protein content of samples was estimated by the macro-Kjeldahl method and corrected by using the N × 6.25 factor [33]. The total ash content was measured by incineration at 600  $\pm$  15 °C [34,35]. The mineral contents (including phosphorous (P), potassium (K), magnesium (Mg), sodium (Na), calcium (Ca), iron (Fe), zinc (Zn), and cadmium (Cd)) were determined using Atomic Absorption Spectrometer methods [36].

#### 2.3. Statistical analysis

Six different substrate combinations were used to cultivate two mushroom species, with each combination tested in three replicates, making a total of 18 bags for each type of mushroom. The inoculated bags were randomly arranged for both the spawn runs and fruit body production. Proximate analyses and mineral content tests were carried out on the fruit bodies, each in triplicate. Data on mushroom yield (including spawn run, first harvest, yield, and biological efficiency), as well as proximate and mineral contents, were subjected to statistical analysis using IBM SPSS version 24. The mean values of all the variables were calculated, and analysis of variance (ANOVA) was performed with the F-test. The significance of the differences among treatment means was determined using the least significant difference (LSD) test at a 5% probability level.

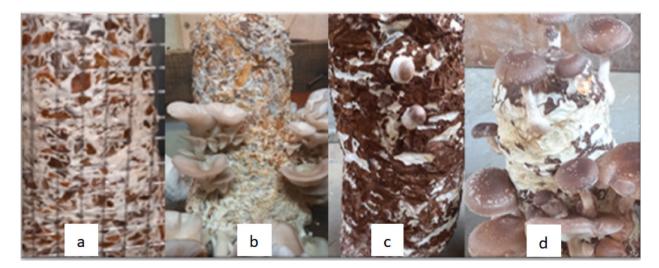
# 3. Results and discussion

Fig. 2 shows the *P. ostreatus* (Fig. 2a and b) and *L. edodes* (Fig. 2c and d) fruit bodies cultivated on WH mixed with RH and CD substrates. The next sections discuss mushroom cultivation parameters, as well as the relative composition and mineral content of *P. ostreatus* (section 3.1) and *L. edodes* (section 3.2) cultivated on different substrate combinations.

# 3.1. Cultivation of P. ostreatus

#### 3.1.1. Spawn run and first harvest durations

Spawn run duration varied significantly among the substrate combinations (p < 0.05) (Fig. 3). SC1 appears to have the longest mean spawn run duration (22.33 days), followed by SC3 and SC6. SC5, on the other hand, exhibited the shortest mean spawn duration (14.67 days). This indicates notable differences in mycelial colonization rates, with SC5 potentially promoting faster mycelial growth. First harvest duration also differed significantly (p < 0.05) among the substrates, ranging from approximately 20 days for SC5 to 29 days for SC1. SC5 demonstrated the shortest time to first harvest, suggesting quicker fruiting initiation and maturation compared to



**Fig. 2**. *P. ostreatus* and *L. edodes* fruit bodies cultivated on WH mixed with RH and CD substrates. (a) Fully colonized with substrate with *P. ostreatus*; (b) fruit bodies of *P. ostreatus*; (c) fully colonized substrate with *L. edodes*; (d) mature fruit bodies of *L. edodes*.

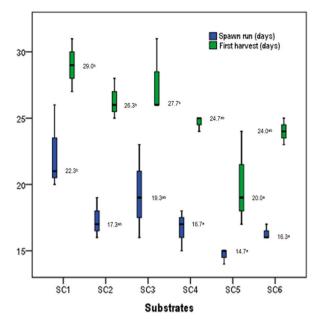


Fig. 3. Spawn run and first fruit body harvest days for P. ostreatus on different substrate combinations.

SC1. Subedi et al. [37] reported an increasing spawn run duration with increasing WH proportion with RS with the highest 25.0 days in a 2:1 (WH and RS) proportion.

#### 3.1.2. Total yield and biological efficiency

In terms of total yield, substrate combination SC2 produced significantly more mushrooms (863.00 g/bag) than all other substrates (Fig. 4). Conversely, SC1 yielded significantly fewer mushrooms than all other substrates. SC5 and SC6, essentially, fall in between SC1 and SC2 in terms of mushroom yield. They are statistically different (p = 0.02) from SC1 but not as productive as SC2. SC3 and SC4 were moderately productive compared to SC1 and SC2. They are statistically different from both SC1 and SC2 but not significantly different from each other. SC2 resulted in significantly higher (p < 0.05) biological efficiency (88.51%) in converting substrate into

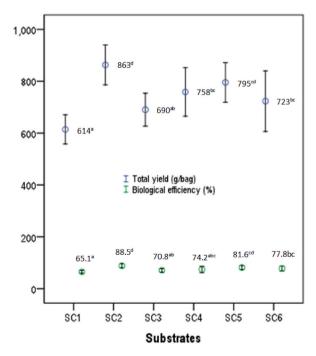


Fig. 4. Total yield and biological efficiency of P. ostreatus cultivated using various substrates combinations.

mushroom biomass compared to all other substrates. On the contrary, SC1 showed the lowest biological efficiency (65.12%) in converting substrate into mushroom biomass compared to all other substrates. Like total yield, SC5 and SC6 have higher biological efficiencies compared to SC1 but lower than SC2. Substrate combinations, SC3 and SC4, with biological efficiencies falling between SC1 and SC2, are statistically different from both SC1 and SC2 but not significantly different from each other.

In essence, SC2 consistently exceeds other substrates in both total yield and biological efficiency, while SC1 consistently shows the lowest values. SC5 and SC6 are intermediate, falling between SC1 and SC2, and SC3 and SC4 are also intermediate but not significantly different from each other. Higher biological efficiency (90%) for *P. ostreatus* grown on a composted water hyacinth was reported by Ref. [38]. About 83% biological efficiency was reported by cultivating *P. ostreatus* on a 1:1 ratio of water hyacinth to wheat straw in Ethiopia, which is relatively lower than our findings when WH mixed with RH and CD [28].

#### 3.1.3. Proximate composition

Significant (p < 0.05) differences existed in total ash content among the substrate combinations (p < 0.05) (Table 2). SC1 displayed the highest total ash content (19.96%), while SC6 showed the lowest (11.55%). A gradual decline in total ash content was observed from SC1 to SC6, with significant differences between each combination. Previous research found ash content ranging from 6.0% to 13.7% for *P. ostreatus* [39]. The ash content of mushrooms ranged between 5.4% and 27.6% as indicated in their study, which is consistent with our findings. The ash content directly reflects the presence of several minerals that play important roles in immunological modulation, homeostasis maintenance, illness prevention, and metabolic process maintenance [40].

Significant (p < 0.05) variations were observed in crude protein content among the substrate combinations (p < 0.05). SC2 exhibited the highest crude protein content (14.72%), while SC5 showed the lowest (8.58%). The protein content of *P. ostreatus* in this study is close to the protein amounts of 10.09–19.14% [41], 10.99–20.81% [29], and 14.64–22.74% [42]. A surpassed crude protein content compared to all substrate combinations for *P. ostreatus* was also reported by Ref. [43]. However, the protein content obtained in this study is substantially higher than 2.11–3.99% that reported by Onyeka et al. [44], who investigated the effect of substrate composition on the growth, yield, and nutritional composition of *P. ostreatus* grown domestically. These results demonstrate that *P. ostreatus* growth, repair, and maintenance.

Crude fat variability, ranging from 1.42% to 1.76%, was observed across all the substrate combinations, with no significant differences. The fat level found in the present study agrees with the range 0.15–1.83% reported for *P. ostreatus* grown on different substrates [44]. *P. ostreatus* has a fat content ranging from 0.2 g to 8 g per 100 g of dried fruit bodies, according to Ref. [39]. Other studies have also reported that the fat content of *P. ostreatus* ranges between 0.5% and 1.3% [39] and stated that this mushroom species contained less fat than other common mushrooms (1.0-9.5%). A higher crude fat content of 5% was also reported for the mushroom [45]. In this work, *P. ostreatus* cultivated on several substrate combinations had very low-fat levels when compared to carbohydrate and protein levels. Previous studies have also found that *P. ostreatus* mushrooms had lower fat content than carbohydrate and protein [39,46]. Mushroom fat is primarily composed of unsaturated fatty acids. Oleic acid is the predominant monounsaturated fatty acid, while linoleic acid is the primary polyunsaturated fatty acid in *P. ostreatus* [39,47].

Crude fiber contents significantly varied among the substrate combinations (p < 0.05). SC5 (16.49%) exhibited the highest crude fiber content, while SC1 (12.96%) showed the lowest. These findings deviated slightly from previous observations [48], who reported lower crude fiber content when cultivating *P. ostreatus* on rubber wood sawdust. The fiber content of the cultivated mushroom found in this study is higher than 9.89–11.18% reported by Ref. [41], but it is in good accord with the reported value of 11.01–29.00% [49]. The substrate combinations also showed significant differences in total carbohydrate content (p < 0.05). SC2 exhibited the highest total carbohydrate content of 55.11%, compared to SC4, which exhibited the lowest content (50.44%). Lower carbohydrate compositions were reported elsewhere compared to this work [45,48]. Carbohydrates are mostly found in *P. ostreatus* as polysaccharides, which include compounds such as monosaccharides and their derivatives; oligosaccharides are generally referred to as soluble sugars [50]. The amount of dietary fibre in 100 g of edible parts varied from 4.1 g in *P. ostreatus* mushroom as reported in Ref. [47].

#### 3.1.4. Mineral content

Minerals identified in cultivated *P. ostreatus* include P, K, Mg, Na, Ca, Fe, Zn, and Cd, as presented in Table 3. Minerals generally in the diet are essential for proper metabolic function in the body. Nerve impulse transmission, bone development, and water and salt

Proximate composition of <i>P. ostreatus</i> cultivated on various substrate combinations.							
Substrate combinations	Proximate composition						
	Total ash (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Total carbohydrate (%)		
SC1	$19.96\pm2.97^a$	$13.41\pm2.28^{ab}$	$1.75\pm0.21^{\text{a}}$	$12.96\pm1.59^a$	$54.79\pm2.95^a$		
SC2	$16.79\pm0.94^{\rm a}$	$14.72\pm3.10^{\rm a}$	$1.76\pm0.25^{\rm a}$	$13.05\pm1.59^{\rm a}$	$55.11\pm2.96^{\rm a}$		
SC3	$12.51\pm3.32^{\rm b}$	$14.54\pm3.05^a$	$1.74\pm0.24^{\text{a}}$	$12.89 \pm 1.57^{\rm a}$	$54.45\pm2.93^a$		
SC4	$12.11\pm1.30^{\rm b}$	$10.87\pm1.08^{\rm ab}$	$1.57\pm0.03^{\rm a}$	$14.23\pm1.65^{\rm a}$	$50.44\pm2.66a^{\rm b}$		
SC5	$11.67\pm0.87^{\rm b}$	$8.58 \pm 1.95^{\rm b}$	$1.43\pm0.15^{\rm a}$	$16.49\pm2.23^{\rm a}$	$45.86\pm2.46^b$		
SC6	$11.55\pm0.86^{\rm b}$	$9.18\pm3.09^{\rm b}$	$1.42\pm0.11^{\rm a}$	$16.32\pm2.21^{\rm a}$	$45.37\pm2.43^{b}$		

 Table 2

 Proximate composition of P. ostreatus cultivated on various substrate combination

The results represent the mean  $\pm$  standard deviation (SD) of triplicate measurements of dry matter in fruit bodies. When the means share the same alphabet within each column, there are no significant differences.

balance are among others [51]. Nutritionally, *P. ostreatus* was found to contain significant amounts of essential minerals and very low heave metal content (Cd). The mineral uptake by fungi and subsequent accumulation in their fruit bodies are influenced by substrate composition (Table 3). The macroelements (such as P, K, Na, Ca, and Mg), which occur in relatively large amounts, are one type of essential element. In our study, we observed variations in the P content of cultivated *P. ostreatus* fruit bodies across different substrate combinations (Table 2). While substrates SC1 to SC4 exhibited similar P content, they differed significantly from substrates SC5 and SC6. The highest P content 0.27% (2700 mg/kg) was recorded in SC2 and SC3, whereas the lowest 0.17% (1700 mg/kg) was found in SC5 and SC6. P levels ranging from 710 mg/kg to 2820 mg/kg were reported in Ethiopia [35], which is consistent with the findings of this study. Notably, our values were also substantially lower than those reported elsewhere [52], who documented a P content of 1.49% (14,900 mg/kg) in *P. ostreatus* mushrooms. Other reports indicate that the phosphorus concentration of mushrooms ranges from 640 to 4490 mg/kg dry weight [53]. Compared to vegetables, mushrooms revealed to be rich suppliers of a variety of minerals, including K and P.

In terms of K content, SC4 displayed the highest value of about 3.95% (39,500 mg/kg), significantly surpassing all other substrates, while SC6 exhibited the lowest content of 2.81% (28,100 mg/kg). The K levels found in the current investigation were consistent with those reported by other studies: 3660–42,400 mg/kg [35], 590.30–36,340.00 mg/kg [54], and 5950–29,230 mg/kg [55]. Lower K levels of 2652.66–19,918.66 mg/kg [56] and 0.98% (9800 mg/kg) [53] have also been reported in literature. A K level of 3.5% (35000 mg/kg) in *P. ostreatus* fruit bodies grown on a 100% water hyacinth substrate was reported [57]. The WHO/FAO specified K values ranging from 190 to 5020 mg/kg [58]. This indicates that the investigated mushroom samples contained higher amounts of K. This shows that mushrooms would be beneficial for lowering blood pressure, reducing appetite loss, and maintaining bone health [59]. However, because the amounts found here exceeded the FAO/WHO prescribed values of 190 to 5020 mg/kg, daily dietary K intake must be carefully monitored [56].

Regarding Mg content, SC5 and SC6 showed similar values of 0.78% (7800 mg/kg), which were significantly higher than those of other substrates (ranging from 0.16% (1600 mg/kg) to 0.22% (2200 mg/kg)). The levels of Mg obtained in this study were also higher than 570-2120 mg/kg [35] and 16.00–30.38 mg/kg [56], which were reported in Ethiopia. Other studies have reported values of 180-1930 mg/kg in Turkey [55] and 210.10–400.70 mg/kg in India [54]. In contrast to our findings, a much lower Mg content of 0.018% (180 mg/kg) in the mushrooms was also reported elsewhere [60]. The nutritional composition of mushrooms varies according to species, age of fruiting bodies, meteorological circumstances, medicines, and substrate [61]. Mg levels in some mushroom samples evaluated in this study exceeded the WHO/FAO permissible limit value of 45-4520 mg/kg [58], and daily dietary Mg intake must be carefully monitored. Consuming mushrooms as a dietary source for people gives essential nutrients for bones and teeth [62].

The Na content was the highest in SC2 (400.23 mg/kg), with no significant difference compared to SC1, SC3, and SC4. SC6 exhibited the lowest Na content (291.55 mg/kg). Compared to our results, much lower Na content, 132.1 mg/kg, was reported [48] for *P. ostreatus* mushrooms. The permissible limit of Na in food is 300 to 1340 mg/kg according to WHO/FAO [58], which agrees with the values obtained in this study. The maximum daily intake of 2 g/day is recommended by WHO [63]. Ca content varied across substrates, with SC4 having the highest value (372.70 mg/kg) and SC3 the lowest (310.37 mg/kg). Calcium consumption is necessary for maintaining calcium balance and skeletal integrity. The permissible limit for Ca level in foods according to WHO/FAO is 8810 mg/kg [58]. The Ca contents in the mushroom samples were within the WHO safe limits for human consumption and did not pose any health risks [56]. This study results are consistent with a range of 290–6450 mg/kg [35] and 40–5720 mg/kg reported for different mushrooms grown in Ethiopia and Turkey, respectively. However, the values in this study were higher than reports made in Bangladesh [64].

Micro or minor elements are minerals that occur in trace levels and are required in a few milligrams or less each day including Fe and Zn. In this study, Fe content was the highest in SC4 (45.47 mg/kg) and the lowest in SC6 (26.42 mg/kg), differing significantly across substrates. The levels of Fe found in *P. ostreatus* mushrooms cultivated on various substrate combinations were within the WHO acceptable limits [65]. Consumption of these mushrooms could thus serve as an excellent source of Fe supplementation, particularly in low-income countries where iron deficiency (e.g., anemia) is a severe health issue. Tsegay et al. [56] reported levels of Fe for *P. ostreatus* mushroom cultivated on cotton waste (34.13 mg/kg), wheat straw (48.96 mg/kg) and wood waste 87.50 mg/kg. A much higher Fe content (212.12 mg/kg) in *P. ostreatus* was reported in Ethiopia [66]. Zn content was the highest in SC4 (70.40 mg/kg), followed by SC2 (65.85 mg/kg), while SC6 had a significantly lower content (61.87 mg/kg). A Zn content of 87.07 mg/kg in *P. ostreatus* 

Table 3
Mineral contents of P. ostreatus cultivated on various substrate combinations.

Substrate	Mineral content							
	P (%)	K (%)	Mg (%)	Na (mg/kg)	Ca (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Cd (mg/kg)
SC1	$0.25\pm0.02^a$	$3.66\pm0.17^{b}$	$0.18\pm0.03^a$	$395.25 \pm 28.21^{\text{a}}$	$312.26\pm2.44^{\text{c}}$	$37.16 \pm \mathbf{1.92^{b}}$	$65.46 \pm 3.69^{ab}$	$1.14\pm0.07^{a}$
SC2	$0.27\pm0.03^{a}$	$3.68\pm0.12^{\rm b}$	$0.16\pm0.02^{a}$	$400.23 \pm 28.38^{a}$	$314.14\pm2.46^{c}$	$\textbf{37.47} \pm \textbf{1.85}^{b}$	$65.85\pm3.71^{ab}$	$1.15\pm0.09^{a}$
SC3	$0.27\pm0.02^{a}$	$3.64\pm0.11^{\rm b}$	$0.17\pm0.02^{a}$	$390.27 \pm 28.04^{a}$	$310.37 \pm 2.43^{\rm c}$	$37.02 \pm \mathbf{1.84^{b}}$	$65.06\pm3.66^{ab}$	$1.13\pm0.08^{\rm a}$
SC4	$0.24\pm0.05^a$	$3.95\pm0.21^{a}$	$0.22\pm0.06^{a}$	${\bf 389.04 \pm 18.12^{a}}$	$372.70 \pm 8.21^{a}$	$\textbf{45.47} \pm \textbf{3.15}^{a}$	$\textbf{70.40} \pm \textbf{1.40}^{a}$	$1.18\pm0.07^{\rm a}$
SC5	$0.17\pm0.01^{\rm b}$	$2.84\pm0.14^{c}$	$0.78 \pm 1.02^{a}$	$299.48 \pm 19.00^{\rm b}$	$338.63\pm4.88^{\mathrm{b}}$	$26.71\pm2.97^{\rm c}$	$62.54\pm5.50a^b$	$1.25\pm0.09^{a}$
SC6	$0.17\pm0.02^{b}$	$\textbf{2.81} \pm \textbf{0.11}^{c}$	$\textbf{0.78} \pm \textbf{1.01}^{a}$	$291.55 \pm 18.79^{b}$	$334.95 \pm 4.83^{b}$	$\textbf{26.42} \pm \textbf{2.94}^{c}$	$61.87\pm5.44^{c}$	$1.24\pm0.09^{a}$

The results represent the mean  $\pm$  standard deviation (SD) of triplicate measurements of dry matter in fruit bodies. When the means share the same alphabet within each row, there are no significant differences. It's important to note that nickel (Ni) was not determined in any of the oyster fruit bodies tested.

was reported [66]. The permissible limit for Zn level in foods based on WHO data is 60 mg/kg [65]. Thus, consumption of the *P. ostreatus* mushroom cultivated on WH supplemented with other substrates needs due attention and control of the daily dietary intake of Zn. The recommended daily mineral intakes are 1,000, 400, 3,500, 2,400, 15, and 18 mg of Ca, Mg, K, Na, Zn, and Fe, respectively [67].

Cd content was comparable across all substrates, ranging from 1.13 to 1.25 mg/kg, with no statistical differences. These values were lower than those reported for the mushrooms cultivated on wheat straw (1.94 mg/kg), cotton waste (2.08 mg/kg), and wood waste (2.52 mg/kg) [56]. Woldegiorgis et al. [35] reported a value of 0.00–4.08 mg/kg Cd levels for Ethiopian mushrooms. Other studies elsewhere have reported Cd levels ranging from <0.03–19.00 mg/kg [55]. The Cd level obtained for *P. ostreatus* in this study is consistent with literature values, but at lower concentrations. Further, the values for Cd in the *P. ostreatus* mushrooms cultivated on the various substrate combinations were below the permissible limits recommended for food by WHO, 2 mg/kg [68]. Acceptable weekly intakes of Cd for adults are 0.42–0.49 mg as cited in Ref. [35], which corresponds to 0.06 mg of Cd on a daily basis. Cadmium accumulates mostly in the kidneys, spleen, and liver, with a significant increase in blood serum levels after mushroom ingestion [69]. Thus, consuming contaminated mushrooms may provide a health risk to consumers, particularly during the rainy season when intake is high.

#### 3.2. Cultivation of L. edodes

#### 3.2.1. Spawn run and first harvest days

Spawn run duration varied significantly among the substrate combinations (p < 0.05) (Fig. 5). SC5 had the shortest spawn run duration (28.67 days), while SC1 exhibited the longest duration (43 days). This indicates variations in the rate of mycelial colonization among the substrates, with SC5 offering more favorable conditions for rapid mycelial growth compared to SC1. The spawn run durations of the mushroom ranged from 43.0 to 61.8 days, and in line with our report, when cultivated on different sawdust substrates [70]. Time to first harvest also showed significant differences across the substrates (p < 0.05). SC5 had the shortest time to first harvest, 40.67 days, whereas SC1 took the longest at 63.33 days. This suggests differences in fruiting initiation and maturation rates, with SC5 exhibiting quicker fruiting compared to SC1. Longer first harvest durations of 55.2–70.00 days were reported elsewhere on different sawdust substrates [70].

#### 3.2.2. Total yield and biological efficiency

Total yield varied significantly among the substrates (p < 0.05) (Fig. 6). SC2 yielded the highest yield of 799.81 g/bag, followed by SC5 (775.59 g/bag), while SC6 yielded the lowest (646.95 g/bag). This disparity highlights differences in substrate composition and its impact on mushroom productivity. Biological efficiency also differed significantly among the substrates (p < 0.05) (Fig. 6). SC2 showed the highest efficiency of 82.03%, followed by SC1 (79.55). Conversely, SC6 exhibited the lowest biological efficiency (60.00%). Lower biological efficiencies were reported by Ranjbar et al. [71] (69.88%) from wheat straw supplemented with wheat bran and by Ref. [4]. On the other hand, higher biological efficiency (93.65%) was reported for shiitake mushroom from wheat stalk and wheat bran mix [72]. Similar biological efficiency was reported by Alberti et al. [73] from eucalyptus sawdust and chips and by Desisa et al. [27] from sugarcane bagasse and chicken manure mix.

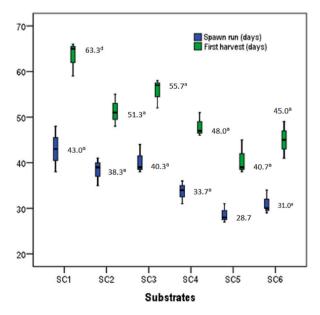
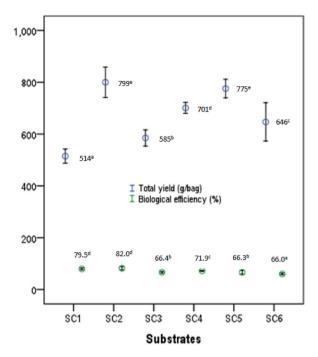


Fig. 5. Spawn run and first harvest days for *L. edodes* from the inoculation time. Mean numbers of days for spawn run and first harvest; identical letters indicate no significant differences.



**Fig. 6.** Displays the yield and biological efficiency of shiitake mushrooms cultivated using various substrate combinations. Groups with the same letter and color in their alphabet notations do not exhibit statistically significant differences. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

#### 3.2.3. Proximate composition

Table 4 provides analysis of the proximate composition and mineral content of *L. edodes* fruit bodies harvested from substrate combinations (SC1 to SC6). Total ash content, indicative of mineral content, exhibited variation from 10.48% (SC6) to 12.24% (SC1). This indicates that there are no statistically significant differences between substrate combinations (p < 0.05). Comparable ash content (10.39%) was reported after cultivating the mushroom on eucalyptus sawdust [73]. But lower ash contents were reported for *L. edodes* from *Quercus acutissima* and supplemented sugar cane bagasse, respectively, [74,75]. Significant disparities in crude protein content were evident among the substrate combinations (p < 0.05). Notably, SC4 displayed the highest crude protein content (16.19%), while SC5 and SC6 exhibited the lowest (13.92%). The crude protein content of *L. edodes* mushrooms obtained in this study agrees with the one reported in Ref. [76], with protein values ranging from 13.4 to 17.5%. Similar crude protein content of 14.5% was also reported [74], whereas a much higher content was reported for the mushroom [73] after growing the mushroom on eucalyptus sawdust.

In terms of crude fat, substrates SC2 and SC3 had the highest (1.73%), while SC4 exhibited the lowest (1.33%). The crude fat content of *L. edodes* mushrooms obtained in this study is slightly lower than the reports by Ref. [76], with crude fat values ranging from 4.9 to 8.0%. Higher crude fat content, 2.13%, was also reported [73] from eucalyptus sawdust. Additionally, crude fiber content ranged from 13.65% (SC6) to 15.57% (SC4), with SC4 showing the highest fiber content (p < 0.05). The highest crude fiber content of 7.69% was found in the fruit bodies of *L. edodes* cultivated on the differently optimized sawdust substrate [77]. The crude fibre content of *L. edodes* mushrooms obtained in this study is slightly lower than that reported by Ref. [76], which ranged from 7.3 to 8.0%. Significant discrepancies were observed in total carbohydrate content across the substrate combinations (p < 0.05). While carbohydrate contents of 49.25% and 52.62% were reported for two different varieties of *L. edodes* [77]. Other studies found higher carbohydrate values

 Table 4

 Proximate compositions of L. edodes cultivated on various substrate combinations.

Substrate	Proximate composition							
	Total ash (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Carbohydrate (%)			
SC1	$12.24\pm0.74^{\rm a}$	$11.93\pm2.51^{\rm b}$	$1.71\pm0.12^{\rm a}$	$13.92\pm1.37^{\rm a}$	$57.52\pm2.44^{\mathrm{b}}$			
SC2	$11.62\pm0.69^{\rm ab}$	$12.04\pm2.54^{\rm b}$	$1.73\pm0.11^{\rm a}$	$14.05\pm1.39^{\rm a}$	$58.05 \pm 2.47^{\rm ab}$			
SC3	$11.49\pm0.69^{ab}$	$11.96\pm2.52^{\rm b}$	$1.71\pm0.12^{\rm a}$	$13.95\pm1.37^{\rm a}$	$57.66\pm2.44^{\rm b}$			
SC4	$10.87\pm1.04^{\rm ab}$	$16.19\pm1.99^{\rm a}$	$1.33\pm0.19^{\rm b}$	$15.57\pm0.57^{\rm a}$	$60.99\pm4.18^{ab}$			
SC5	$10.63\pm1.02^{\rm b}$	$13.96\pm0.17^{ab}$	$1.62\pm0.12^{\rm a}$	$13.69\pm1.64^{\rm a}$	$64.58\pm4.37^{a}$			
SC6	$10.48\pm0.61^{b}$	$13.92\pm0.18^{ab}$	$1.63\pm0.13^{a}$	$13.65\pm1.62^{\rm a}$	$64.37 \pm \mathbf{4.35^a}$			

The results represent the mean  $\pm$  standard deviation (SD) of triplicate measurements of dry matter in fruit bodies. When the means share the same alphabet within each row, there are no significant differences.

ranging from 67.5 to 78.0% [76].

#### 3.2.4. Mineral content

Mineral composition analysis of *L. edodes* cultivated on the differently combined substrates was conducted. Findings indicated that substrate composition may have limited influence on the mineral composition of cultivated *L. edodes* (Table 5). While there were variations (p < 0.05) in mineral contents among the substrate combinations in Na and Fe, no statistically significant differences were observed for most minerals. SC4 consistently demonstrated elevated mineral levels across various elements, contrasting with SC6, which generally exhibited lower concentrations. P content ranged from 1.96% (19,600 mg/kg) to 2.27% (22,700 mg/kg) across the different substrate combinations. P levels in Ethiopian mushrooms were reported to range between 710 mg/kg and 2820 mg/kg [42], which is lower than this study's values for *L. edodes*, which were grown on different substrate combinations.

K content ranged from 2.25% (22,500 mg/kg) to 2.50% (25,000 mg/kg) for the various substrate combinations evaluated in this study. The K levels found in the current investigation were slightly higher than 14,300 mg/kg reported for *L. edodes*, but consistent with 3660 to 42,400 mg/kg reported for other mushroom species grown in Ethiopia [35]. Other studies reported K levels ranging from 5950 to 29,230 mg/kg for various mushroom species grown on different substrates elsewhere [55]. The WHO/FAO specified K values ranging from 190 to 5020 mg/kg [58]. This demonstrates that the *L. edodes* mushroom samples grown on the various substrate combinations under investigation had higher K levels than the allowed limit and should be closely monitored depending on daily dietary intake.

The Mg level of *L. edodes* mushroom samples grown on various substrate combinations ranged between 0.21% (2100 mg/kg) and 0.25% (2500 mg/kg). The Mg levels found in this study were slightly greater than 1650 mg/kg reported for *L. edodes* and 570-2120 mg/kg reported for other mushroom species grown in Ethiopia [42]. However, the Mg level in all the *L. edodes* mushroom samples evaluated in this study were within the WHO permitted limit value of 45-4520 mg/kg [58].

Na content, measured in mg/kg, ranged from 250.95 to 300.90. The Na levels found in this study were slightly lower than 990 mg/kg reported for *L. edodes* and 410-34,800 mg/kg reported for other mushroom species cultivated in Ethiopia [35]. Other studies have reported salt concentrations in mushrooms ranging from 30 to 4850 mg/kg [78], which is comparable with the results obtained in this study. The WHO permissible limit for Na in food ranged from 300 to 1340 mg/kg [58], which corresponds to the values obtained in this investigation for the *L. edodes* mushroom samples examined. Calcium content ranged from 284.66 mg/kg to 296.19 mg/kg. The WHO/FAO allows a maximum Ca content of 8810 mg/kg in meals [58]. Thus, the Ca levels in the *L. edodes* mushroom samples evaluated in this study were within the WHO's allowed range for human consumption and caused no health risk [56].

Fe content ranged from 24.04 mg/kg to 29.49 mg/kg. Another study found that the Fe content in *L. edodes* was 98.0 mg/kg, while other mushroom species growing in Ethiopia had Fe levels ranging from 32.5 to 6835.9 mg/kg [35]. Zn content ranged from 74.03 mg/kg to 83.97 mg/kg. Another study conducted in Ethiopia found that the Zn level in *L. edodes* was 80.9 mg/kg, while other mushroom species growing in Ethiopia had Zn levels ranging from 26.6 to 87.6 mg/kg [35]. The permissible limit for Zn in food is about 60 mg/kg, according to WHO data [65].

Cd content ranged from 1.30 mg/kg to 1.44 mg/kg. Mn content ranged from 13.88 mg/kg to 15.96 mg/kg. Much lower Cd content of 0.15 mg/kg, much higher Fe content of 60.51 mg/kg, lower Mn content of 6.34 mg/kg, and lower Zn content of 46.09 mg/kg were reported from cultivation of the mushroom on 100% dairy plant waste [79] compared to findings of this work. Cd level less than 0.03 mg/kg in *L. edodes* grown in Ethiopia was also reported by Ref. [35], while Cd levels ranged from 0.4 to 91.8 mg/kg in mushrooms grown in China [80]. The WHO limit for Cd levels in food is 2 mg/kg [68]. This indicates that the Cd levels in the studied mushrooms grown on the various substrate combinations were within WHO's permitted limits for food. This leads to the conclusion that consuming *L. edodes* mushrooms grown on the various substrate combinations may not pose a toxicological concern in terms of short-term impacts.

Overall, the study findings indicate that water hyacinth biomass has the potential to be used as a sustainable source of mushroom cultivation when combined with other agricultural residual biomass resources. According to the study, water hyacinth can be safely combined with cow dung (up to a ratio of 4:1) as an alternate substrate for cultivating mushrooms while also recycling undesired weeds in an environmentally responsible manner and generating income. However, given the limitations of the current study, more research into combining water hyacinth and other agricultural residual waste biomass resources is needed to maximize optimal

Table 5			
Nutritional composition of L.	edodes cultivated or	n various substrate	combinations.

Substrate	Mineral content							
	P (%)	K (%)	Mg (%)	Na (mg/kg)	Ca (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Cd (mg/kg)
SC1	$2.04\pm0.41^{a}$	$\textbf{2.48} \pm \textbf{0.05}^{a}$	$0.22\pm0.03^{\text{a}}$	$296.13 \pm \mathbf{12.45^a}$	$288.93 \pm 17.05^{a}$	$29.22 \pm 6.37^{a}$	$83.22\pm5.74^{a}$	$1.33\pm0.13^{\text{a}}$
SC2	$2.27\pm0.47^{a}$	$2.50\pm0.17^{a}$	$0.23\pm0.03^{\text{a}}$	$298.83 \pm 12.56^{a}$	$291.56 \pm 17.21^{\rm a}$	$29.49 \pm \mathbf{6.43^a}$	$83.98\pm5.79^a$	$1.35\pm0.12^{\rm a}$
SC3	$2.06\pm0.34^a$	$2.25\pm0.15^a$	$0.21 \pm 0.03^a$	$296.82 \pm 12.48^{\mathrm{a}}$	$289.60 \pm 17.09^{a}$	$29.29\pm6.39^a$	$83.41\pm5.75^a$	$1.34\pm0.13^{\rm a}$
SC4	$1.97\pm0.13^{\rm a}$	$2.26\pm0.26^a$	$0.25\pm0.02^a$	$300.90\pm9.91^{a}$	$296.19 \pm 9.29^{\mathrm{a}}$	$26.36\pm2.52^a$	$74.80\pm3.19^{a}$	$1.45\pm0.17^{a}$
SC5	$2.02\pm0.22^{\rm a}$	$2.43\pm0.21^{a}$	$0.21 \pm 0.04^a$	$251.77 \pm 13.58^{\rm b}$	$285.60\pm9.11^{a}$	$24.12 \pm 5.98^{\mathrm{a}}$	$74.27 \pm 4.92^{\mathrm{a}}$	$1.31\pm0.07^{\rm a}$
Sc6	$1.98\pm0.25^{a}$	$\textbf{2.34} \pm \textbf{0.10}^{a}$	$0.21\pm0.03^a$	$250.96 \pm 13.54^{\rm b}$	$284.66\pm9.08^a$	$24.04\pm5.96^a$	$\textbf{74.03} \pm \textbf{4.90}^{a}$	$1.31\pm0.07^a$

The results represent the mean  $\pm$  standard deviation (SD) of triplicate measurements of dry matter in fruit bodies. When the means share the same alphabet within each row, there are no significant differences. It's important to note that nickel (Ni) was not determined in any of the shiitake fruit bodies tested.

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substrate combinations across a variety of substrate types. Future research should also focus on establishing and/or customizing the best mushroom cultivation practices in response to regional conditions and demands, as well as collaborating with potential users and stakeholders to reduce capital costs and, as a result, the need for financial support. Mushroom cultivation technologies can be developed on a variety of scales, and the size of a project effects economies of scale, which must be considered throughout the planning process.

Furthermore, in order to maximize resource utilization and improve mushroom production in low-income countries (like Ethiopia), basic information and experience must be collected, communicated, and database created, as well as industrial-scale sterilization and culture facilities installed and maintained. Mushroom cultivation is declining or stagnant despite its benefits and age. Awareness creation and education are a significant component that can help to improve these trends. Mushrooms contribute to the production of nutritious food ingredients and/or dietary supplements, and are a useful source of vitamin D for persons who have religious or philosophical dietary restrictions and preferences. However, in low-income countries where food production including the three major nutrients, particularly carbohydrates, is prioritized, increasing mushroom cultivation may be viewed as less urgent than producing other crops. In the future, habits of eating based only on the calorie content of high-carbohydrate foods may result in social problems such as obesity. The scientific community and policymakers must collaborate to reduce this detrimental impact by actively disseminating information about mushrooms' benefits. Mushrooms are high in dietary fiber, low calorie count, compounds with several health benefits, and vitamins, particularly vitamin D.

#### 4. Conclusions

To address water hyacinth (*Eichhornia crassipes*) infestations, a number of management strategies have been adopted to reduce the weed spread and biomass. The invasion of water hyacinth in Lake Tana, Ethiopia, has emerged as a significant ecological and economic concern. Despite considerable efforts and investments in conventional control methods, the spread of water hyacinth continues. This study demonstrates the potential of using water hyacinth as a sustainable substrate for mushroom cultivation, particularly oyster and shiitake mushrooms. Among the evaluated substrate combinations, water hyacinth (80%) and cow dung (20%) yielded the highest mushroom biomass and superior biological efficiency. This substrate combination also consistently exceeded other substrate combinations for both mushroom species in terms of crude protein and crude carbohydrate content, while maintaining comparable crude fibre, crude fat, ash, and mineral contents. The results suggest that water hyacinth, when mixed with agricultural bio-wastes, can effectively recycle unwanted weeds, mitigate infestations, generate income, and create employment opportunities. However, to further enhance the efficacy of water hyacinth utilization for mushroom cultivation, future research should focus on evaluating and optimizing various substrate formulations, exploring other mushroom species, and evaluating the economic feasibility of large-scale production. Collaboration among researchers, local communities, and government agencies is essential for implementing integrated management strategies and promoting community participation in water hyacinth management and mushroom cultivation.

#### CRediT authorship contribution statement

**Shasho Megersa:** Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Amsalu Tolessa:** Writing – review & editing, Resources.

# **Ethical approval**

Not required.

#### Ethics statement

Not applicable.

# Data availability statement

Data included in the article is referenced in the article.

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#### Declaration of competing interest

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