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Optimizing tea waste as a sustainable substrate for oyster mushroom (*Pleurotus ostreatus*) cultivation: a comprehensive study on biological efficiency and nutritional aspect

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Introduction: In Bangladesh, rice straw (RS) and sawdust (SD) substrates have traditionally been used in the production of oyster mushrooms (*Pleurotus ostreatus*). However, the rising costs of these substrates have led many to look for alternatives.

Objectives: The present study thus focuses on the potential of waste tea leaves (WTL) for mushroom farming.

Methods: We prepared various substrate mixtures by combining WTL with SD and RS, subsequently evaluating mushroom yield and various quality parameters such as amino acid concentration, mineral content, and biological efficiency.

Results and discussion: Our investigation revealed that WTL alone is not a suitable substrate for mushroom (*Pleurotus ostreatus*) growth. However, when combined with SD at a 50% ratio, it significantly boosts mushroom yield and biological efficiency (BE). Conversely, a reduction in yield was noted when WTL was mixed with RS in all tested treatments, although BE surpassed 50%. In summary, incorporating WTL into both substrates proves economically viable from the BE standpoint. According to PCA analysis, the minerals and amino acid content varied based on the different substrate formulations involving WTL blending with both SD and RS at different ratios. Remarkably, mushroom fruiting bodies exhibited lower levels of Na and Fe despite these elements being present in higher concentrations in the growing substrates, suggesting the inability of *P. ostreatus* to bioaccumulate Na and Fe. Conversely, we observed higher bioaccumulation of Zn and P, even exceeding substrate levels. Importantly, our findings showed that mushrooms cultivated on WTL-based formulations consistently contained elevated Zn levels irrespective of substrate types, indicating that WTL enriched Zn in mushrooms. Additionally, the Fe level increased specifically in RS + WTL-based formulations. All essential and non-essential amino acids were detected, with the highest concentration of histidine, isoleucine, and methionine found in the WTL + SD formulation. Non-essential amino acids (NEAA) like alanine and glutamic acid were more prominent in formulations combining WTL with RS. This study represents the first documented exploration of the impact of WTL on the accumulation of intracellular metabolites including minerals and amino acids, in *P. ostreatus*.

KEYWORDS

tea waste, oyster mushroom, yield, biological efficiency, mineral, amino acid, PCA

1 Introduction

Oyster mushrooms, known scientifically as *Pleurotus* spp. are well recognized as primary decomposers, and their ability to thrive in fruiting bodies on a diverse range of substrates. Mushroom production is one of the most effective biotechnological approaches for recycling lignocellulosic organic waste. It can secrete an array of lignocellulosic enzymes for degrading environmental pollutants (Kabel et al., 2017; Carrasco et al., 2018).

In addition to converting environmental pollutants into protein-rich human food, mushrooms also produce notable nutraceutical compounds like biologically active proteins, antioxidants, terpenoids, and sterol-which makes mushrooms a great choice for human health (Bell et al., 2022; Jana and Acharya, 2022; Kour et al., 2022). Due to the saprophytic nature of mushrooms, they derive their nourishment from the growing substrate, which has an impact on the chemical, functional, and sensory properties of mushroom fruit bodies (Saba et al., 2016). Additionally, mushrooms can accumulate various metals from their substrates, including essential nutrients like Cu, Fe, Mn, and Zn (Damodaran, 2014; Lalotra et al., 2016; Seyfferth et al., 2016; Demková et al., 2021).

In Bangladesh, Oyster mushrooms (*Pleurotus ostreatus*) are primarily produced on sawdust and rice straw. Currently, commercial mushroom farmers are finding it more difficult and expensive to obtain due to the rising demand in the livestock sector and mushroom farming. In light of this problem, finding substitute substrates or reducing the use of sawdust (SD) and rice straw (RS) for mushroom growing are now beginning to receive much-deserved attention.

Like other nations, tea stands out as one of the most widely consumed beverages in Bangladesh. The consumer markets for tea in the country are currently expanding, showcasing a variety of tea products. Among these, black tea holds greater popularity in local tea stalls compared to other types like oolong, green, and white tea. However, a significant challenge arises in the form of tea waste generated after extracting water-soluble components from tea leaves. The growing amount of tea waste not only poses a threat to the environment but also requires considerable labor and space for proper disposal. Consequently, finding effective solutions to manage this issue has become an urgent priority. Various measures have been proposed to address the challenge of tea waste, including utilizing it as livestock feed or converting it into fertilizer. However, implementing these solutions on a practical scale to meet the demands of tea processing businesses has proven to be a challenging task. As a result, it is crucial to critically assess and explore viable options to tackle this issue and ensure sustainable practices within the tea industry.

Interestingly, tea waste is characterized by a composition of cellulose (28–30%), hemicellulose (10–20%), and lignin (28–30%) (Sharma et al., 2015; Mahmoud et al., 2020). In contrast, a commonly used mushroom growing substrate such as rice straw and sawdust typically consists of cellulose (32–39%), hemicellulose (23–24%), and Lignin 18–36%, for rice straw (El-Tayeb et al., 2012; Limayem and

Ricke, 2012) and cellulose (40–55%), Hemicellulose (24–40%), and Lignin (18–25%) for hardwood sawdust (Sun and Cheng, 2002).

Numerous studies have delved into incorporating tea waste with various substrates like sugarcane bagasse and tea leaves (Chukowry et al., 2009) cottonseed hull and tea waste (Yang et al., 2016), tea waste combined with bran (Gökhan et al., 2018). Notably, In Bangladesh, cottonseed hull, or sugarcane bagasse are not utilized for commercial mushroom growing as these are not available at farmers' level. Therefore, it is important to consider tea waste as an alternative to sawdust and rice straw in mushroom production, either independently or in combination. Furthermore, the absence of published research on the influence of tea waste combined with sawdust and rice straw on the yield and quality of oyster mushrooms underscores the need for further investigation. In addition, it is crucial to develop and optimize a detailed substrate formula for practical large-scale production.

Therefore, this study aims to assess the commercial feasibility of using tea waste either alone or combined with RS and SD as an alternative substrate for oyster mushroom production. Additionally, our study investigates how these substrates influence essential mushroom attributes such as major mineral content, heavy metal status, and amino acid composition. Our data establishes a foundation for the utilization of tea waste for commercial oyster mushroom cultivation in tropical countries. This innovative approach has the potential to convert tea waste into valuable, marketable, edible biomass and offer a cost-effective substrate option for mushroom growers.

2 Materials and methods

2.1 Materials

Rice straw and Sawdust were sourced from local farmers and sawmills, while waste black tea leaves were collected from the nearby tea stalls. At the tea stall, black tea leaves underwent boiling with water to extract the water-soluble compound. This extract was later mixed with sugar and milk, forming the beverage served to consumers. The remaining insoluble tea leaves were discarded as waste tea leaves. The mushroom inoculum (*Pleurotus ostreatus*) was obtained from the National Mushroom Development Institute in Savar, Dhaka.

2.2 Treatment

Different ratios of waste tea leaves (WTL), sawdust (SD), and rice straw (RS) (detailed in Table 1) were used as substrate for mushroom cultivation. It is noteworthy that in Bangladesh, the exclusive use of SD as a substrate at the farmers' level is not practiced due to its low yield potential, rendering it non-profitable from a business standpoint. Thus, in the experiment, one control group used SD supplemented with wheat bran, while another control group used RS without any supplement.

TABLE 1 Different substrate combinations used as a treatment.

Treatment	Composition
Control	90% Sawdust + 10% wheat bran
T _{1.1}	75% of SD + 25% of WTL
T _{1.2}	50% of SD + 50% of WTL
T _{1.3}	25% of SD + 75% of WTL
Control	100% Rice straw
T _{2.1}	75% of RS + 25% of WTL
T _{2.2}	50% of RS + 50% of WTL
T _{2.3}	25% of RS + 75% of WTL

Intriguingly, T_{1.3} (75% WTL + 25% SD) and T_{2.3} (75% WTL + 25% RS) showed weak mycelial growth that eventually ceased, rendering these bags unfitting for colonization. Consequently, these packets were discarded.

2.3 Packet preparation, mushroom cultivation, and data collection

2.3.1 Sawdust packet preparation and incubation

The preparation of sawdust packets was initiated by combining SD and WTL in a mixing machine. We gradually added tap water to adjust the moisture to 65–70%. Subsequently, 500 g of this moistened substrate was placed in a polypropylene bag, which was then autoclaved at 121°C for 1 h. After cooling, each bag was spawned with fungal seed and incubated at 25 ± 2°C with a humidity level of 50–60%. The packets were allowed to become densely colonized with mycelium before transferring to the culture room to induce fructification.

2.3.2 Rice straw packet preparation and incubation

Before use, dried RS was chopped into small pieces (3–5 cm in length) using a chaff cutter. These pieces of RS were completely moistened by soaking them in tap water for 24 h. Both RS and WTL were pasteurized in a clean steel drum. To briefly describe the pasteurization process, the water was heated at 60°C initially. Then the substrate was added and maintained in warm water for 30 min. Following pasteurization, both substrates were wrapped in a sterile plastic sheet (2 m × 4 m) and placed on a steep concrete surface to drain excess water, reaching a moisture level of 65–75%. Layer spawning was employed to mix fungal seed with the RS and WTL before tightly sealing in polypropylene bags (35 × 17 cm²). These bags were incubated at a temperature of 25 ± 2°C, and humidity 50–60%. Once the mycelium fully colonized and attained a dense, and white appearance, the plastic bags were transferred to the cropping room to initiate the formation of fruiting bodies.

2.3.3 Mushroom cultivation

After transferring fully colonized bags to the culture house, a few holes are punctured on the sides to facilitate the emergence of fruiting bodies. Within the cropping room, environmental conditions are carefully maintained to stimulate the formation of fruit bodies. The temperature was maintained within a range of 25 to 30°C (Agustianto et al., 2021), the humidity was 80–90% by spraying a fine mist of water three times a day (Girmay et al., 2016), and the illumination level was

controlled within the ranged between 150 and 200 lux (Wu et al., 2019).

2.3.4 Data collection for yield performance

The growth and development of mushrooms were monitored daily. The time (numbers of days) required from inoculation to completion of mycelium running and time elapsed between opening the plastic bags to pinhead formation were recorded. Mushrooms were harvested when the mushroom cap surface was flat to slightly up-rolled at the cap margins. For the collection of data for yield parameters and biological efficiency, the harvested fruiting bodies in each bag were then counted and weighed. A total of two flushes were recorded. Accordingly, biological yield (g) was determined by weighing the entire cluster of fruiting bodies without removing the base of stalks. Finally, the biological efficiency (%) was calculated as follows: $BE = (wf/wd) \times 100$, where BE represents biological efficiency, wf is the weight of the freshly harvested fruiting body, and wd is the weight of dry matter of the substrate.

2.4 Minerals and toxic metals analysis

2.4.1 Sample preparation

The obtained mushroom samples and the substrates were oven-dried at 60 ± 2°C until a constant weight was achieved. The dried samples were ground into a fine powder using a mortar & pestle. All mushroom samples were mineralized prior to analysis. In this regard, 0.5 g of powdered samples were placed into the Teflon vessels with 5 mL 65% Nitric Acid (Analar Grade, Merck, Germany) and 2 mL 30% Hydrogen peroxide (Merck, Germany). The digestion was completed in Microwave Digester (Model: Ethos One, Milestone, United States) at 180°C for 45 min (Lao et al., 2023). After the completion of digestion, the digests were transferred into 50 mL volumetric flasks and made up the volume by Class I (18 MΩ) deionized water. All reagents used for heavy metal analysis were of Analar grade.

2.4.2 Analytical methods

Analyses of mineral elements (Na, Mg, P, Zn, Fe, Cu) were performed using Flame Atomic Absorption Spectrophotometry (FAAS) (Brzezicha et al., 2019). The toxic heavy metals (Pb, and As) were performed using Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS) (Dowlati et al., 2021). Both flame and electrothermal modes were operated by the Shimadzu Instrument, Model AA-7000 (Japan origin). The analytical conditions of the AAS instrument such as detection limits, wavelength (nm), cathode lamp current (mA), slit width (0.2–0.7), and flame (air-acetylene mixture) were adjusted based on the heavy metals according to available literatures. The standard recovery percentages of the analytes ranged to be from 95 to 105%.

2.5 Amino acids analysis

The mushroom samples were subjected to amino acid analysis using an Automatic Amino Acid Analyzer (Model: S4300, Sykam GmbH, Germany). The analysis followed chromatographic techniques

according to Ferré et al. (2019), Isildak et al. (2023). In brief, 0.20 g of powdered mushroom samples underwent initial acidification with 25 mL of 7N HCl, ensuring thorough homogenization, followed by filtration to remove acid-insoluble components. Subsequently, this mixture underwent a 24-h hydrolysis period within a hydrolyzer. After complete hydrolysis, any excess HCl was neutralized using a 7.5 N NaOH solution, with confirmation of the neutralization point using a pH meter (Model: Sension TM 156, HACH, United States). The final sample volume was adjusted to 250 mL in a calibrated volumetric flask containing a specialized buffer solution designed for sample dilution, maintaining a pH level of 3.4. Following this, the sample underwent a secondary filtration step using a microfilter (0.45 mm) to eliminate any unwanted foreign particles. Subsequently, a mixture comprising 100 µL of the sample solution and 900 µL of the sample dilution buffer (pH 3.4) was placed into a sample vial, resulting in a total sample volume of 1 mL (representing a 10-fold sample dilution). This diluted sample was then sequentially analyzed for amino acid content. Additionally, a standard solution of amino acids was simultaneously analyzed to ensure dependable sample quantification, quality control, and data comparisons.

2.6 Statistical analysis

One-way analysis of variance (ANOVA) was used to analyze significant differences and mean values at the 5% level of significance. One-way ANOVA was performed using SPSS software version 20 (SPSS, Inc., Chicago, Illinois, United States). DMRT test for the separation of means was applied for $p < 0.05$ when the ANOVA was significant ($p < 0.05$). Additionally, a principal component analysis (PCA) was also carried out using R-program.

3 Results

3.1 Substrate qualities

Regarding substrate qualities, we observed distinct concentration patterns of macro-nutrients such as Magnesium (Mg), Sodium (Na), and phosphorus (P). Specifically, in the SD substrate, the pattern is $Mg > Na > P$, while in the WTL and RS substrates, it followed $Na > Mg > P$. According to our analysis, trace elements including Fe, Zn, and Cu were detected in the substrate, which is significant differences, showing a clear trend of $Fe > Zn > Cu$. Furthermore, WTL content has a lower C/N ratio than the other two substrates as detailed in Table 2.

3.2 Time elapsed for mycelium running and pin-head formation

The total colonization time and the duration from bag opening to primordia formation of oyster mushroom cultivation on different substrates are illustrated in Figures 1A,B. Notably, when the WTL ratio was set at 75% and combined with both SD and RS substrates, a cessation of mycelium growth was observed in all samples. This suggests that WTL alone is not conducive to the growth of *Pleurotus Ostreatus*. Furthermore, no discernible changes were found in the number of days needed for mycelium completion (MCD) among the various substrate formulae. The time needed for completing spawn running ranged from 27 to 30 days for SD + WTL-based substrate formulations including control and 30 to 31 days for RS + WTL-based substrate formulations including control. This finding suggests that the presence of tea waste did not have a notable impact on either slowing down or speeding up mycelial growth, as the control also showed a similar timeframe. Figures 1A,B explain the time taken for the appearance of pinhead following the opening of fully colonized spawn packets. The time for pinhead initiation (TPI) varied significantly ($p < 0.05$) among the treatment groups. In the case of SD + WTL preparation, T1.2 (50% WTL + 50% SD) showed the shortest TPI ranging between 6 to 8 days. To the contrary, in RS + WTL, the lowest TPI (ranging from 4 to 5 days) was observed in control (100% RS).

3.3 Yield and biological efficiency

The yield from two flushes was carefully recorded and analyzed as presented in Table 3. The yield significantly ($p < 0.05$) varied among the different WTL ratios, regardless of whether SD or RS was used as a substrate. Specifically, when 50% WTL was added to SD (T1.2), a substantial increase in yield was observed, totaling 189 g fresh mushroom per packet, with a corresponding biological efficiency (BE) of 79%. In contrast, the control group of SD exhibited the lowest yield, producing 130 g per packet and a BE of 62%. Unlikely, the RS control group showed the highest yield 357.4 g/packet, along with a remarkable BE of 89%. The lowest yield, on the other hand, was observed in 50% of RS + 50% of WTL, which also displayed a lower BE of 64%.

Biological efficiency (BE) serves as a valuable metric for assessing the efficiency of substrate conversion in the mushroom cultivation process. This metric is computed by dividing the fresh weight of harvested mushrooms by the dry weight of the cultivation substrate. A higher BE score indicates a greater likelihood of

TABLE 2 Minerals content and C/N ratio of the substrates.

Treatments	Macronutrient (mg/100 g dm)			Micronutrients (mg/100 g dm)			Toxic Minerals (mg/100 g dm)		C/N ratio
	Na	Mg	P	Zn	Fe	Cu	Pb	As	
Sawdust	2951.64 ^b ± 0.7	3177.56 ^a ± 0.5	1.78 ^c ± 0.12	1.58 ^c ± 0.4	94.71 ^a ± 0.5	1.01 ^c ± 0.5	0.012 ^b ± 2.9	0.03 ^b ± 3.5	244 ^a
Waste Tea Leaves	2903.53 ^b ± 1.2	1550.47 ^b ± 1.1	2.98 ^b ± 0.1	6.58 ^b ± 1.1	65.72 ^b ± 1.1	2.55 ^a ± 0.6	0.026 ^a ± 3.7	0.014 ^a ± 4.2	25.2 ^c
Rice Straw	4862.34 ^a ± 4.1	1453.60 ^c ± 2.1	3.89 ^a ± 0.09	7.62 ^a ± 2	23.84 ^c ± 2	1.24 ^b ± 1.1	0.026 ^a ± 2.8	BDL	63 ^b

BDL, Below detectable level.

Values are the means of three replicates. Means followed by different letters in each column are significantly different ($p < 0.05$) according to Duncan's Multiple Range Test (DMRT).

efficient substrate utilization for mushroom growth. To assess the economic viability of a particular substrate formulation for mushroom farming, it is important to note that the BE value ideally surpasses 50% as suggested by Wakchaure (2011). Moreover, it's important to acknowledge that BE is influenced by various factors, including mushroom species, substrate composition, and environmental conditions, as highlighted by Girmay et al. (2016), Sath et al. (2018). In our study, it was observed that the BE value exceeded 50% in all tested treatments. This implies that using WTL in the tested ratio is viable from a business standpoint, regardless of the presence of both RS and SD.

3.4 Minerals composition of mushroom fruiting bodies

Table 4 presents the mineral profile, based on dry weight, encompassing both macronutrients Sodium (Na), Magnesium (Mg), and Phosphorous (P) as well as micronutrients Zinc (Zn), Iron (Fe), and Copper (Cu) in the fruiting bodies of oyster mushrooms cultivated using different substrate formulation. The concentrations of macronutrients in the mushroom fruiting bodies varied significantly across different substrate formulations. For sodium (Na) the range was 680.95 mg/100 g to 828.24 mg/100 g, magnesium (Mg) ranged from 1594.3 mg/100gm to 2104.1 mg/100gm, while phosphorous (P) concentration ranged from 1.29 mg/100gm to 2.39 mg/100gm. Notably, formulation 75% SD+ 25% WLT tended to have the highest concentration of all studied macronutrients, except for P, which was highest in the control group. Likewise, the fruiting bodies in 75% RS+ 25% WTL displayed the highest Na (4249.43 mg/100 g) and Mg (2196.29 mg/kg), while the control (100% RS) had the lowest concentration of Na (1196.33 mg/100 g) and Mg (2120.09 mg/100 g). Contrarily, P concentration was highest (2.77 mg/100 g) in control and lowest (1.83 mg/100 g) in 50% RS+ 50% WTL.

Our analysis of trace elements, including Fe, Zn, and Cu in mushroom fruiting bodies exhibited significant differences, with a general trend of Cu < Fe and < Zn. Our observation aligns with the findings of Falandysz and Borovička (2013) who also asserted higher Zn concentration compared to Cu in mushrooms. In formulations based on SD+ WTL, the highest Zn concentration (14.61 mg/100 g) was found in 75% SD + 25% WTL, while Fe (5.56 mg/100 g) and Cu (1.58 mg/100 g) concentration were maximum in control. Conversely, Control had the lowest Zn (8.82 mg/100 g) concentration, while 50% SD+ 50% WTL had the lowest Fe (2.89 mg/100 g) and Cu (1.09 mg/100 g) concentration. In formulation using RS+ WTL, 50% RS +50% WTL exhibited the highest Zn (11.54 mg/100 g), Fe (5.30 mg/100 g), and Cu (1.50 mg/100 g) concentration, while the control had the lowest Zn (5.41 mg/100 g), Fe (1.77 mg/100 g), and Cu (0.72 mg/100 g) concentration. These findings indicate that the incorporation of WTL effectively enriched the microelement content of mushroom fruiting bodies when combined with RS. In contrast, the SD+ WTL formulation yielded opposite outcomes except for Zn, where the results differed.

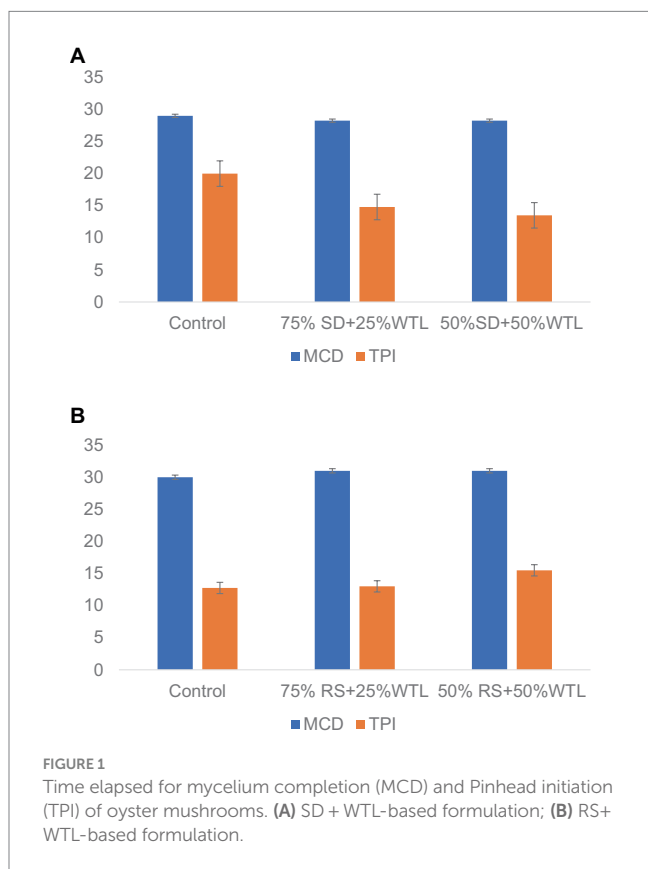


TABLE 3 Yield performance of waste tea leaves with different combinations of substrate.

Treatment	Biological Yield (g)			Biological efficiency (%)
	1st Flush	2nd Flush	Total	
Sawdust based substrate				
Control (SD)	79.75 ^c ± 0.95	50.25 ^c ± 2.21	130.00 ^c ± 1.41	65
75% of SD + 25% of WTL	91.50 ^b ± 1.29	63.80 ^b ± 2.69	155.30 ^b ± 2.05	62
50% of SD + 50% of WTL	109.30 ^a ± 0.95	80.25 ^a ± 2.62	189.50 ^a ± 3.31	79
Rice Straw Based Substrate				
Control (RS)	195.64 ^a ± 0.95	161.76 ^a ± 1.20	357.40 ^a ± 2.04	89
75% of RS + 25% of WTL	166.36 ^b ± 2.56	141.82 ^b ± 2.64	308.18 ^b ± 0.19	68
50% of RS + 50% of WTL	156.27 ^c ± 1.56	101.81 ^c ± 2.76	258.08 ^c ± 2.96	64

Values are the means of three replicates. Means followed by different letters in each column are significantly different (p < 0.05) according to Duncan's Multiple Range Test (DMRT).

TABLE 4 Effect of WTL on minerals content of oyster mushroom.

Treatment	Macronutrient (mg/100 g dm)			Micronutrient (mg/100 g dm)			Toxic mineral (mg/100 g dm)	
	Na	Mg	P	Zn	Fe	Cu	Pb	As
<i>Fruiting bodies grown in sawdust-based formula</i>								
Control	680.99 ^a ± 2.06	1594.28 ^f ± 0.85	2.384 ^b ± 0.95	8.82 ^d ± 0.88	5.56 ^a ± 0.07	1.58 ^a ± 1.01	0.0013 ^d ± 0.006	BDL
75% SD + 25% WTL	828.21 ^b ± 0.95	2104.15 ^d ± 1.20	1.25 ^d ± 0.88	14.61 ^a ± 1.01	5.38 ^{ab} ± 1.10	1.21 ^{cd} ± 0.92	0.010 ^e ± 0.015	BDL
50% SD + 50% WTL	803.84 ^b ± 1.53	1781.37 ^e ± 1.90	1.29 ^d ± 0.97	11.32 ^b ± 0.81	2.89 ^d ± 0.94	1.09 ^d ± 0.99	0.010 ^e ± 0.006	BDL
<i>Fruiting bodies grown in rice straw-based formula</i>								
Control	1196.33 ^a ± 2.51	2120.09 ^e ± 1.0	2.77 ^a ± 0.03	5.41 ^c ± 2.02	1.78 ^a ± 1.01	0.72 ^e ± 0.03	0.0091 ^c ± 0.05	BDL
75% RS + 25% WTL	4249.43 ^d ± 1.05	2739.88 ^a ± 2.90	1.97 ^c ± 0.03	9.98 ^c ± 2.00	3.84 ^c ± 1.01	1.33 ^{bc} ± 0.02	0.015 ^b ± 0.095	BDL
50% RS + 50% WTL	3740.82 ^c ± 0.96	2196.29 ^b ± 1.06	1.83 ^c ± 0.02	11.54 ^b ± 2.10	5.30 ^b ± 0.98	1.50 ^{ab} ± 0.05	0.028 ^a ± 0.05	BDL

Values are the means of three replicates. Means followed by different letters in each column are significantly different ($p < 0.05$) according to Duncan's Multiple Range Test (DMRT). Sodium (Na), Magnesium (Mg), Phosphorous (P), Zinc (Zn), Iron (Fe), Copper (Cu), Lead (Pb), and Arsenic (As).

In our study, the levels of lead and Arsenic in the mushroom samples were found to be very low, indicating that these mushrooms are safe for consumption.

3.5 Amino acid profile of mushroom fruiting bodies

The distribution of the amino acid (AA) concentration (in g/100gm of dry matter) in mushrooms cultivated using different substrate formulations is presented in Table 5. Significant differences were observed among the tested treatments for all measured AAs. In the case of SD and WTL formulation, concentration range of AA in were as follows: Histidine (2.01–2.40), Isoleucine (0.23–0.53), Leucine (0.67–0.93), Methionine (0.19–0.53), Phenylalanine (0.49–1.04), Valine (0.73–1.05), Lysine (0.81–1.04), Threonine (0.81–1.18), Alanine (0.81–1.56), Aspartic acid (1.79–2.76), Glutamic acid (3.81–4.48), Cystine (1.35–2.25), Glycine (0.65–1.23), Proline (0.60–1.01), Argine (0.99–1.44), Serine (0.98–1.45) and Tyrosine (0.44–0.95). For the RS and WTL preparation, the AA concentration ranges were as follows: Histidine (0.97–1.99), Isoleucine (0.34–0.62), Leucine (0.93–1.11), Methionine (0.20–0.33), Phenylalanine (0.36–1.11), Valine (0.77–1.05), Lysine (0.41–0.75), Threonine (0.66–0.89), Alanine (0.98–1.17), Aspartic acid (1.74–2.66), Glutamic acid (2.96–3.11), Cystine (1.07–1.30), Glycine (0.73–0.93), Proline (0.62–0.93), Argine (0.78–0.95), Serine (0.65–0.95) and Tyrosine (0.41–0.82). Interestingly, when incorporating WTL with SD, there was a notable increase of EAA such as Histidine, Isoleucine, and Methionine. Non-essential amino acids (NEAA) like Alanine and Glutamic acid also exhibited an increase in concentration when WTL was combined with RS.

3.6 Principal components analysis

To enhance visual interpretation and simplicity in understanding the data, a principal component analysis was conducted (Figure 2).

The first principal component (PC1) explained 42.3% of the variability, while the second principal component (PC2) contributed a modest 24% to the overall variation. These outcomes differed in magnitude and signified a stronger positive relationship with all the examined traits when integrating WTL in both RS and SD. The elongated arrows, representing 50% of the WTL ratio, signify the highest observed variabilities in mushroom qualities across both substrates.

4 Discussion

According to Lu et al. (2021), the substrate carbon-nitrogen ratio (C/N) significantly influences the mycelial growth and fruiting body development of edible fungi. In our study, a substantial variation in the C/N ratio among the materials was observed, with WTL exhibiting a low C/N ratio (25.2) compared to the other two substrates, SD (244) and RS (63). The control group using RS outperformed all tested treatments in our study, including formulations based on RS + WTL, SD control, and SD + WTL-based formulation, in terms of mycelium growth, biological yield, and biological efficiency. Noteworthy, no mushroom mycelium growth was observed in a 75% WTL combination, regardless of substrate, which aligns with the findings by Yang et al. (2016). This lack of growth may be attributed to the content less amount of cellulose, hemicelluloses, low C/N, and greater amount of lignin as described (Sharma et al., 2015; Mahmoud et al., 2020). It is essential to note that the variation in the yield of *Pleurotus* spp. was primarily driven by differences in the physical and chemical composition of substrate formulations, including cellulose, hemicellulose, lignin, mineral content, and C/N ration (Suwannarach et al., 2022). Furthermore, research by Yang (2000) and Mintesnot et al. (2014) suggested that substrates having greater C/N ratio are conducive to mycelial growth and less favorable for fruiting body formation. Additionally, a substrate with a high lignin concentration impedes mushroom growth by limiting enzyme accessibility (Kaya

TABLE 5 Effects of WTL on the amino acid concentration of oyster mushroom (mg/100g dm).

Treatment	Essential AA										Non-Essential AA						
	His	Ile	Leu	Met	Phe	Val	Lys	The	Ala	Asp	Glu	Cys	Gly	Pro	Arg	Ser	Tyr
<i>SD + WTL-based preparation</i>																	
Control	2.21 ^a ± 0.33	0.52 ^b ± 0.12	0.93 ^b ± 0.28	0.47 ^b ± 0.29	1.04 ^a ± 0.19	1.05 ^a ± 0.13	1.04 ^a ± 0.37	1.18 ^a ± 0.37	1.56 ^a ± 0.19	2.76 ^a ± 0.09	4.48 ^a ± 0.32	1.56 ^a ± 0.31	1.23 ^a ± 0.22	1.01 ^a ± 0.22	1.44 ^a ± 0.44	1.45 ^a ± 0.22	0.95 ^a ± 0.27
75% SD + 25% WTL	2.01 ^a ± 0.21	0.53 ^b ± 0.27	0.83 ^a ± 0.23	0.53 ^b ± 0.50	0.60 ^b ± 0.15	0.73 ^b ± 0.24	0.99 ^a ± 0.23	0.88 ^a ± 0.35	1.23 ^a ± 0.04	2.19 ^a ± 0.22	4.16 ^a ± 0.26	2.25 ^a ± 0.41	0.97 ^a ± 0.13	0.82 ^a ± 0.12	1.06 ^a ± 0.33	1.07 ^a ± 0.24	0.43 ^a ± 0.13
50% SD + 50% WTL	2.40 ^a ± 0.10	0.23 ^a ± 0.14	0.67 ^b ± 0.15	0.19 ^a ± 0.29	0.49 ^a ± 0.59	0.83 ^b ± 0.17	0.81 ^b ± 0.11	0.81 ^b ± 0.33	0.81 ^b ± 0.20	1.79 ^a ± 0.48	3.81 ^a ± 0.28	1.35 ^a ± 0.25	0.65 ^a ± 0.18	0.60 ^a ± 0.36	0.99 ^a ± 0.36	0.98 ^a ± 0.24	0.44 ^a ± 0.23
<i>RS + WTL-based preparation</i>																	
Control	0.97 ^a ± 0.27	0.34 ^a ± 0.28	1.11 ^a ± 0.27	0.33 ^a ± 0.42	1.11 ^a ± 0.38	1.05 ^a ± 0.15	0.75 ^a ± 0.21	0.89 ^a ± 0.21	0.99 ^a ± 0.30	2.66 ^a ± 0.30	2.96 ^a ± 0.17	1.30 ^a ± 0.17	0.93 ^a ± 0.33	0.93 ^a ± 0.18	0.95 ^a ± 0.19	0.94 ^a ± 0.39	0.82 ^b ± 0.08
75% RS + 25% WTL	1.99 ^a ± 0.29	0.52 ^b ± 0.16	0.97 ^b ± 0.24	0.38 ^a ± 0.04	0.51 ^b ± 0.38	0.78 ^b ± 0.24	0.47 ^a ± 0.08	0.75 ^a ± 0.35	0.98 ^a ± 0.19	1.95 ^a ± 0.29	2.42 ^a ± 0.24	1.29 ^a ± 0.39	0.79 ^a ± 0.19	0.73 ^a ± 0.40	0.78 ^a ± 0.24	0.95 ^a ± 0.32	0.42 ^a ± 0.22
50% RS + 50% WTL	1.82 ^a ± 0.24	0.62 ^a ± 0.23	0.93 ^b ± 0.41	0.20 ^a ± 0.09	0.36 ^b ± 0.30	0.77 ^b ± 0.20	0.41 ^a ± 0.40	0.66 ^a ± 0.17	1.17 ^a ± 0.33	1.74 ^a ± 0.25	3.11 ^a ± 0.14	1.07 ^a ± 0.25	0.73 ^a ± 0.19	0.62 ^a ± 0.23	0.81 ^a ± 0.18	0.65 ^a ± 0.29	0.41 ^a ± 0.22

Values are the means of three replicates. Means followed by different letters in each column are significantly different ($p < 0.05$) according to Duncan's Multiple Range Test (DMRT). Essential Amino Acid (EAA), His = Histidine, Ile = Isoleucine, Leu = Leucine, Met = Methionine, Phe = Phenylalanine, Val = Valine, Lys = Lysine, Thr = Threonine, Non-Essential Amino Acid (NEAA) Ala = Alanine, Asp = Aspartic Acid, Glu = Glutamic acid, Cys = Cystine, Gly = Glycine, Pro = Proline, Arg = Arginine, Ser = Serine, Tyr = Tyrosine.

and Cam, 2022). Noteworthy, our study revealed a significant increase in yield and biological efficiency when combining WTL and SD, suggesting that WTL may aid in reducing the substrate's C/N ratio.

Mushrooms are important sources of minerals and play a vital role in the proper development of the human body. Thus, we evaluated whether the mineral contents of the substrate influenced the yield and quality of *Pleurotus ostreatus*. The analysis of minerals elements (Na, Mg, P, Zn, Fe, and Cu) in three substrates used in the study revealed macronutrient Na and Mg concentrations were high while P concentration was significantly lower (Table 2). Similar trends were also observed in mushroom fruiting bodies. Furthermore, our result established that mushrooms possess the ability to accumulate minerals in substantial amounts, often exceeding those present in the substrate which is supported by the line findings (Florczak et al., 2014; Mleczek et al., 2016). Surprisingly, the P concentration in the SD substrate was significantly lower than RS and WTL substrates. However, this difference was not reflected in the elemental composition of the fungal fruiting bodies, as a significantly higher p value was observed in the fruiting bodies produced on control SD.

Our result also showed that Na content in the substrate is higher than that found in the mushroom fruiting bodies. This suggested that mushrooms lack the capacity for bioaccumulation of Na. In contrast, multiple investigations have shown that mushrooms bioconcentrate Mg and P in their fruiting bodies (Frankowska et al., 2010; Kojta and Falandysz, 2016; Li et al., 2017; Malinowski et al., 2021; Hultberg et al., 2023). The biological conversion factor (BCF) of Mg ranged from 1.5 to 7.2, and P ranged from 10 to 50 times (Rudawska and Leski, 2005; Frankowska et al., 2010; Falandysz and Borovička, 2013). Our research finding confirmed that *Pleurotus ostreatus* exhibits the ability to bioconcentrate Mg and P but not Na. In RS+WTL formulas, micronutrients like Zn, Fe, and Cu concentration are significantly increased with the addition of tea leaves, while interestingly significantly decreased by the addition of WTL to SD except Zn. Consistent with other studies, we found that in mushroom fruiting bodies, the amount of Zn is higher than that of Cu irrespective of the substrate. Outstandingly, zinc (Zn) levels in fruiting bodies were significantly increased in both SD and RS substrates blended with WTL in all tested ratios. So, our study claimed, that the enrichment of Zn in mushrooms can be achieved by incorporating WTL as a supplement in the substrate. Zinc stands out as a crucial trace element essential for various aspects of human metabolism including transcription of DNA, translation of RNA, and cellular division (Brown et al., 2004). The introduction of Zn supplementation has shown substantial and positive effects on both growth and weight gain, as evidenced by the work of Rahman et al. (2016). Furthermore, it is noteworthy to note that the substrate's Fe content was significantly higher than that of mushroom fruiting bodies. Our findings contradict those made by Almeida et al. (2015), who claimed that *P. ostreatus* was capable of bioaccumulation of Fe. It is well known that Fe uptake by fungus is a complex process, and it depends on the speciation of Fe (II) and Fe (III) forms (Philpott, 2006). The present study reveals that the WTL had a remarkable impact on the mineral content in the fruiting bodies of the mushrooms. More importantly, our results for trace elements falling within acceptable nutritional levels for human consumption are shown in Table 6.

When comparing mushrooms to fruits and vegetables, mushrooms emerge as superior sources of both essential amino acids (EAAs) and non-essential amino acids (NEAAs) as reported in numerous literature

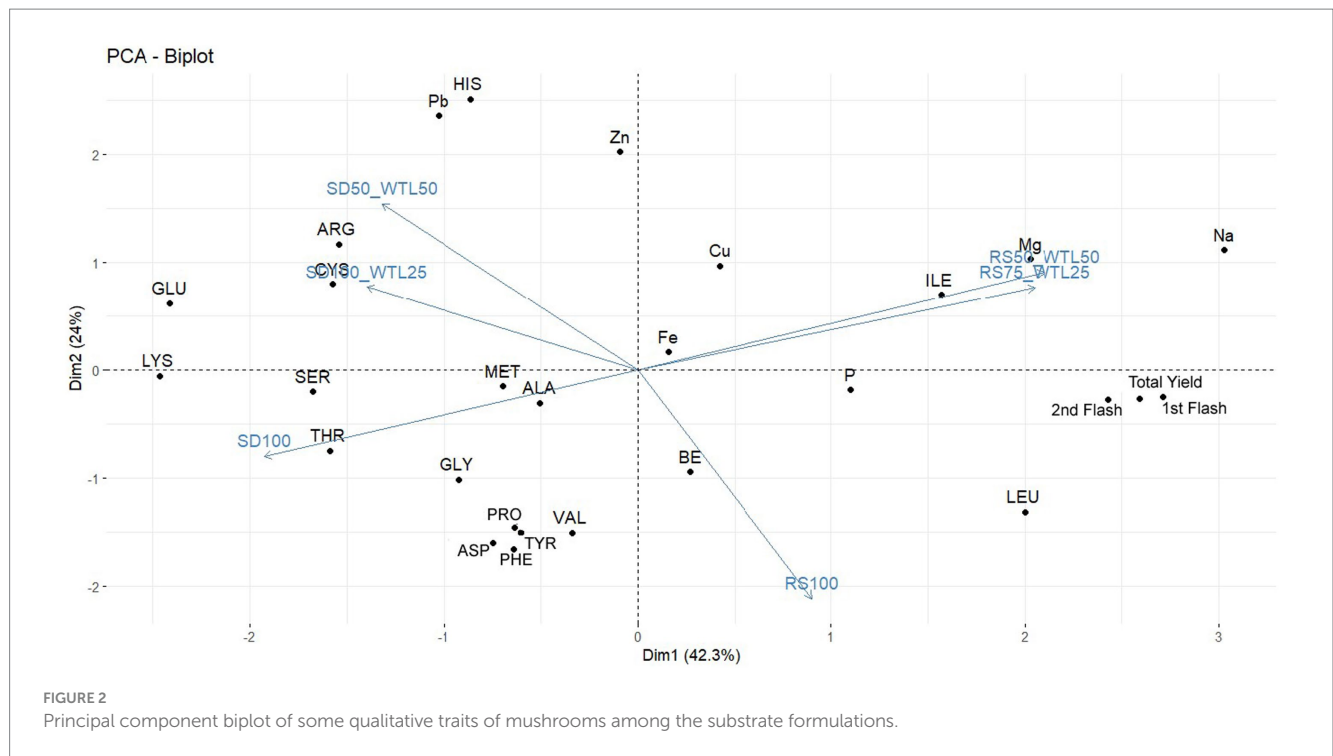


TABLE 6 Recommended daily intake (RDI) of trace elements.

Element	RDI	Reference
Cu	2 mg/day	World Health Organization (1996)
Fe	18 mg/day	World Health Organization (1996)
Zn	15 mg/day	World Health Organization (1996)

(Yang et al., 2001). The data of AA composition shows that mushrooms particularly rich in EAA like histidine and NEAA such as glutamic, aspartic acids, and cystine irrespective of the growing substrate. In contrast, methionine and isoleucine are presented in relatively lower quantities, categorizing them as limiting amino acids. The significant increase in glutamic and aspartic acid content in mushrooms is attributed to their roles as precursors for other amino acids such as serine, alanine, lysine, and proline, among others (Akindahunsi and Oyetayo, 2006). Markedly, the umami flavor associated with certain foods including mushrooms can be attributed to the presence of glutamic and aspartic acids. Furthermore, our study observed that the addition of WTL to substrate formulations resulted in a significant reduction in the concentration of both essential and non-essential amino acids, except for histidine in SD-WTL formulates and isoleucine, alanine in RS-WTL-based preparation. It is important to highlight that the amino acid concentration in WTL-based formulation exceeded the levels reported in the study involving *Pleurotus* spp. grown on perilla stalks (Li et al., 2017) and a substrate composed of wheat straw combined with grape marc (Tagkouli et al., 2020).

5 Limitations and future research

Our investigation explored the impact of WTL on the yield and qualities of oyster mushrooms (*Pleurotus ostreatus*). Nevertheless,

certain limitations should be acknowledged. Firstly, our study concentrated exclusively on fresh mushrooms of *Pleurotus ostreatus*. Secondly, we utilized black tea waste sourced from the local tea stall. Future research endeavors could extend to other oyster mushroom species and consider diverse types of tea waste including industrial sources. Furthermore, assessing the applicability of our findings in varied geographical contexts, such as Bangladesh or other countries, would be valuable for future studies.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

RA: Writing – original draft, Investigation. MN: Investigation, Writing – original draft. MI: Data curation, Formal analysis, Writing – review & editing. MR: Writing – review & editing. SY: Data curation, Writing – review & editing, Methodology. SR: Data curation, Writing – review & editing, Methodology. JK: Writing – review & editing, Conceptualization, Project administration, Supervision, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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