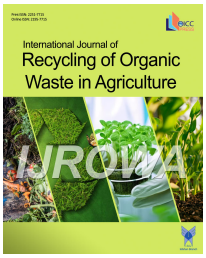

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Evaluating the performance of tea leaf waste as a substrate supplement for cultivating *Pleurotus ostreatus* L. on groundnut shell

Tedros Gebrezgiabhier Gebreyesus*, Kamal C. Semwal

Department of Biology, Mai-Nefhi College of Science, Eritrea Institute of Technology, Asmara, Eritrea.

***Corresponding author:** tedyhan.18@gmail.com

Original Research

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Abstract:

Purpose: There is no recycling plan for a tea leftover waste which is available in very high quantities in Eritrea. In view of the growing importance of environmental sanitation, food security and mushroom recipe in Eritrea this study was conducted to evaluate tea leaves waste as substrate mixed with ground nut shell for Oyster mushroom (*Pleurotus ostreatus* L.) cultivation.

Method: Different substrate mixtures were prepared and filled in polythene bags and were autoclaved (121 °C and 15 Lbs) for 2 hrs. Substrate bags were inoculated with Oyster spawn and incubated in a cultivation hat with controlled conditions. Growth parameters were recoded for each bag of each treatment and the data thus obtained were analysed statistically using R-software (R-4.3.3) at two significant levels ($P < 0.05$ and $P < 0.01$) and Chi-square test.

Results: In all replications 40% tea leaf waste supplied with 58% ground nut shell showed high performance in terms of mycelium colonization in bags, growth time, yield (189.9 ± 5.0 g) and biological efficiency ($47.5 \pm 1.3\%$) close to the control group (200 ± 0.39 g, BE $= 49 \pm 0.1\%$). At p-value ($p < 0.01$), the assessed treatments showed a significant difference with P-value 4.74E-200. All of the growth parameters were statistically different, with p-value $= 0.00$ at p-value ($p < 0.01$).

Conclusion: Therefore, tea leaf waste with aforesaid percentage of groundnut shell can be used as alternative substrate, which will reduce the cost of cultivation of this prized mushroom and the technology can be transferred to the local farmers.

Keywords: Food security; Growth parameters; Performance; Substrate; Significant

1. Introduction

Mushroom is fleshly, spore-bearing, fruiting macro fungi with epigynous sporocarp. It is one of the macro sized decomposers which recycle nutrients through decomposition in the natural ecosystems. Mushroom cultivation can be defined as the practice of obtaining fruit bodies artificially by repeating the growing stages. Mushrooms have been consumed since antiquity in prehistoric societies and been cultivated for their culinary and medicinal values. Nowadays about 14,000 mushroom species are documented with around 2,300 useful mushroom species where about 20 of them are cultivated on an industrial scale. Various wild and cultivated species of *Agaricus*, *Pleurotus*, *Lentinus*, *Volvariella* and *Calocybe* are some of the choice mushrooms for culinary purposes around the globe (Kumar et al., 2018; Mridu and Atri, 2017). Due to its exceptional nutritional and therapeutic qualities, oyster mushrooms are the second most widely farmed mushrooms in the world, behind *Agaricus bisporus*. The polysaccharide β-glucan, which has the highest antioxidant action, is abundant in *P. ostreatus* fruiting bodies (Hasan and Abdulhadi, 2022; Ejigu et al., 2022). Elsakhawy et al. (2022) declares that the genus *Pleurotus* grows in a wide range of temperatures ($10 - 30$ °C) and pH ($6 - 8$) and that it inhabits and colonizes a variety of agro-climatic settings. Oyster mushrooms are a diverse group of saprotrophic fungi, and different species of this mushroom cultivated globally. In different countries several kinds of substrate have been used to enhance the production of Oyster mushrooms. Though cereal

straw and sawdust are among the most common substrates used worldwide for its growth, oyster mushrooms may also be cultivated on a variety of different substrates because they contain a broad spectrum of oxidative and hydrolytic enzymes that allow them to break down complicated lignocellulosic biomass without the need for precomposting (Kamthan and Tiwari, 2017). According to studies done by Atila (2017), Oyster mushroom can degrade and grow on a variety of substrates. Oyster mushroom has maximum number of commercially cultivated species (varying in shape, color, texture and aroma), longer shelf life, highest productivity, high tolerance for variations in environmental shocks, higher growth rate, short cropping days and resistance to diseases and pests (Anjana and Savita, 2017). In the present study we have tried to investigate different treatments with tea leaf waste to estimate yields of this mushroom in controlled conditions. Ground nut shell is one of the crops cultivated on fields by the farmers in Eritrea for food purpose and the shell is mostly disposed uselessly while rarely used as fodder.

Mushroom cultivation has been started in Eritrea with small unions under the Extension unit of the Ministry of Agriculture with conventional substrates. Few agricultural wastes (Corn cobs, banana leaves, and maize stem) are used as substrates for mushroom growing. Mushroom cultivation is a best alternative technology which recycles agro-industrial wastes in to protein rich stuff (Kamthan and Tiwari, 2017; Kinge, 2016). The habit of mushroom consumption is very poor in Eritrea compared to other neighbouring countries. This might be the result of poor knowledge on the nutritional and medicinal values of mushroom which requires media coverage to popularize mushroom consumption and cultivation among the populace. Mushrooms have long been prized as greatly tasty nutritional foods by many prehistoric and modern societies throughout the globe considering their medicinal role (Raymond et al., 2013; Upadhyaya et al., 2017). *Pleurotus ostreatus* has encouraging anti-cancer activity, immunomodulating effects, and antiviral, antibiotic and anti-inflammatory activities (Tupamahu and Budiarso, 2017).

Currently local growers are mainly providing fresh mushrooms to supermarkets, restaurants and hotels located in the same region of production mainly in the capital Asmara. However mushroom cultivation can be a sustainable and environmentally sound socio-economic activity in Eritrea as the agricultural wastes which can be used as substrates for mushroom cultivation are conventionally available. Therefore, this study is initiated to use readily available waste materials to reduce the cost of mushroom cultivation. In addition, tea leaf waste as an alternative substrate is costless; thereby avoiding expenses for the scarce wheat bran for oyster mushroom cultivation.

2. Materials and methods

Study area

Part of this study was conducted in the Mushroom Extension unit of Ministry of Agriculture, while major cultivation activities were done in Mai Nefhi college of Science, Eritrea Institute of Technology (15°14′35″N 38°46′30″E), Gala Nefhi, Maekel region, Eritrea.

Stock pure culture

Fruiting body of *Pleurotus ostreatus* L., was obtained from the Mushroom Extension unit in the Ministry of Agriculture, Eritrea. To obtain pure culture, a small piece of tissue was resected from the fruiting body of mushroom, and placed on the sterilized PDA medium under aseptic condition and kept for $7-9$ days in an incubator under 24 °C for sufficient mycelial growth. Further it was maintained by transferring to fresh potato dextrose agar media as previous studies by Das and Mukherjee (2007). In addition to this, PDA supplied with 40%, 60% of tea leaf and tea leaf alone was tested for oyster tissue culture. Oyster mushroom strain was selected for this study compared to other cultivable mushrooms (like, Agaricus and Shiitake) due to its versatile attributes such as; broad substrate adaptability, rapid growth, disease resistance, short cropping cycle, high yield, and simple cultivation technique.

Substrate collection

A black powdered tea leaf waste used as a substrate in this study was collected from student cafeterias in Eritrea Institute of Technology from March-April 2023. Every day, a tea is boiled in three large steam cookers which hold 600 litters of water in total; once it get consumed we used to collect the tealeaf residue from the bottom of each dish and sunbath it on a mat for about 5 days to remove the water from it. Ground nut shell was collected from Keren city and its surrounding areas. Wheat bran, lime and gypsum were purchased from market in Asmara, the capital city of Eritrea.

Spawn production

Wheat grain is used by extension unit of Ministry of agriculture in Eritrea for mushroom spawn production. For this study, spawning process was conducted in the extension unit. 30 kg of wheat was washed and dead floating grains was removed then soaked overnight in 15 L, 2% lime water and was rinsed three times with water according to the extension unit procedures. Then the wheat grain was boiled in hot water for $10-15$ minutes and cooled. The excess water was drained off and 20% wheat bran, 1% gypsum was added. The ingredients were thoroughly mixed, and filled equally in to 500 mL glass bottle at the rate of 350 g and was sterilized at 121 °C, 15 Ibs for 2 hours in an autoclave like previous studies by Upadhyaya et al. (2017) (Yamauchi et al., 2019). After cooling, each bottle was inoculated with 7 days old oyster mycelium culture which grown on PDA and incubated for 20 days at 25 °C until the substrate fully colonized with mycelia. Oyster mycelium invaded the whole wheat in the bottles with in 20 days of incubation and no contamination was encountered overall.

Substrate preparation

During substrate preparation, the collected ground nut shell was thrashed into small pieces using a stick and soaked in 2% lime water for 2 days. Moisture content with-in the substrate mixture was maintained around 60% which is determined by the ‘rule of thumb’ method where a handful of

substrate was squeezed to evaluate the substrate's moisture level (Alemu, 2013; Kinge, 2016). According to this principle, when the substrate is squeezed on the hand, a few drops of water should emerge between the fingers rather than the substrate being excessively wet or dry. Then substrate was supplemented with different ratios of supplements as indicated in Table 1 below. Supplementing the substrate improves the substrate power and mushroom harvest (Baysal et al., 2003). Lime and gypsum raises pH of the substrate thereby suppressing growth of competitors and additionally serve as sources of calcium and sulfur ions which are necessary for hyphae growth regulation, protein synthesis and cell differentiation. Moreover these supplements are crucial for keeping moisture in the substrate during the cultivation period.

Substrate sterilization and spawn inoculation

The prepared substrate mixtures were filled equally into plastic bags at the rate of 500 g of substrate per bag and sterilized for 2 hrs at 121 °C, 15 lbs using an autoclave. After 4 hrs the sterilized bags were removed from the autoclave and kept for 20 hrs on shelves to be cooled. Each treatment involved 20 replicates. Cooled substrate bags were inoculated with the spawn (one glass bottle per 5 bags) in the inoculation box and mixed thoroughly to facilitate rapid and uniform mycelia growth. The mouth of the bags was closed using a plastic closure ring cup and incubated in the dark incubation room at $25+1$ °C with $65-70\%$ relative humidity for 40 days until mycelial colonization was completed. Holes were made over the polythene bags for aeration parallel to the running mycelium down the substrate. Rate of mycelial colonization and contamination per treatment were recorded every week.

Cultivation conditions

During the incubation stage the temperature was maintained at $25+1$ °C with $65-70\%$ relative humidity and with no light. After completion of incubation period the fruiting stage temperature was adjusted to 18 °C – 23 °C and $80-90\%$ Relative humidity with sufficient light and ventilation similar to previous study by Yamauchi et al. (2019). In this study, the temperature and relative humidity were measured using a room thermometer and a RH-controlling device, respectively. Moisture level was maintained by spraying water in the form of fine mist using a sprayer and covering the floor of the cultivation house with wet socks. Moreover water filled buckets were placed in the cultivation house. An adequate aeration was provided by opening the cultivation room's windows every two hours during the day. Bags with complete mycelium colonization were opened by removing the plastic closure cups and rings. Furthermore the fluorescent bulb was switched on during the fruiting days; because light is one of the stimulant factors for primordial initiation and fruit growth. Primordial growth was initiated $5-8$ days after stimulation (lower temperature, higher humidity level and illumination) depending on type of treatment. Cultivation house and its surrounding were disinfected by spraying diluted formalin once a week to avoid growth of contaminants.

Harvesting of mushroom

Maturity of a fruit body was determined by its curled margin of the cap with development of gills and harvested by twisting to uproot from the base as described by Bhattacharjya et al. (2014). Time required for maturity was observed to be different on different treatments. Freshly harvested mushrooms for each bag were weighted using a scale during harvesting time. Yield per bag and per treatment was assessed and biological efficiency was calculated with a formula below:

$$\text{BE} = \frac{\text{Fresh weight of the mushroom}}{\text{Dry weight of the substrate}} \times 100$$

Statistical analysis

All growth parameters per bag and treatment were recorded and analysed using R-software (version 4.3.3) to check the significant difference between growth parameters at two significant levels ($P < 0.05$ and $P < 0.01$) and to construct box plots showing significant differences between treatments. Moreover a chi-square test was used to select the best fitting treatment for the control group.

3. Result and discussion

Culture on PDA media

White dense mycelium of *P. ostreatus* was observed to colonize the whole potato dextrose media within $7-9$ incubation days at 24 °C while light dense mycelia was shown in the PDA media supplemented with 40% tea waste. PDA media supplied with 60% tea leaf displayed small patches of weak zonal mycelial growth (Fig. 1 (a)). Previously Mahadevan and Shanmugasundaram (2018), found *P. sapidus*

Table 1. Substrate mixture composition of the evaluated treatments.

Treatments (T)	Ratio of ground nut shell	Ratio of wheat bran	Ratio of tea leaf waste	Ratio of lime	Ratio of gypsum	Replicates
T1	80%	18%	0%	1%	1%	20
T2	58%	0%	40%	1%	1%	20
T3	38%	0%	60%	1%	1%	20
T4	0%	0%	100%	0%	0%	20
T**	**80% Sawdust**	**18% Wheatbran**	**0%**	**1%**	**1%**	**20**

*- Control group.

Figure 1. (a) Oyster culture plate with PDA and PDA supplied with tea leaf waste, (b) mycelial colonization of substrate bags, (c) Primordial growth after bag opening, (d) mushroom fruiting.

showing efficient mycelial growth on PDA, Malt Extract Agar (MEA) and Yeast Malt Agar (YMA) Medium compared to other evaluated media while other studies indicates that *P. ostreatus* showed best growth performance on a synthetic Kirk and Tein medium (Ali et al., 2012), PDA, PDA supplemented with yeast extract and Sabouraud Dextrose Agar (SDA) medium (Fletcher et al., 2019). Similarly *P. ostreatus* was observed to aggressively colonize the entire PDA medium in this study which attributes to the growth performance of oyster mushroom and the suitability of PDA as growth medium.

Spawning

Spawn is a mushroom's vegetative seed that a mushroom grower should sow or inoculate on a substrate medium during cultivation time. Spawn making is putting mycelium of desired mushroom in a suitable sterilized substrate under aseptic conditions (Aditya et al., 2024). There are two forms of mushroom spawn; called as the mother spawn and the planting spawn, where the former is refers to the first generation spawn prepared in glass bottles using a pure plate or tube culture, while the latter is the spawn which is actually sewed on substrate bags during mushroom cultivation. Mother spawn is necessary for propagation and maintenance of a renewed spawn or for producing planting spawn to cultivate mushrooms. Mother spawn is prepared through inoculating a pure culture to a sterilized substrate (Sharma et al., 2014). There are four types of mother spawn called grain spawn, sawdust spawn, liquid spawn, and stick spawn (Zhang et al., 2019). Different growth medium can be used to grow spawn including grains like *Sorghum bicolar* (guinea corn), *Triticum aestivum* (wheat) *Pennisetum tyhiodes* (millet) to produce grain spawn, sawdust to grow sawdust spawn or liquid medium to produce liquid spawn (Bilal et al., 2014). In this study we used collected sawdust spawn from the Mushroom extension unit and propagated it on wheat grain. The inoculated wheat grain substrate was incubated in a dark room at room temperature for 20 days. Although previous studies showed that grain spawn has higher chance of contamination, mycelium was observed to percolate and colonize the whole substrate successfully in the incubation days without contamination. The quality and amount of spawn is one of the most decisive factors for successful mushroom cultivation. The stock culture selected should be acceptable in terms of yield, flavor, texture, fruiting time, and so on (Mamiro and Royse, 2008).

Mycelial growth and substrate colonization

Due to its higher capacity of producing extracellular enzymes in its hyphae that break down cellulose, hemicellulose, and lignin. Oyster mushroom is considered as a potent saprophytic decomposer (Akcay et al., 2023). Sterilization of substrate bags avoids contaminants with in the substrate which competes with the mushroom mycelia for nutrients and retards mushroom mycelial spreading as stated by Kurtzman (2010). Mixing of the spawn inoculum with the substrate thoroughly and creating tiny holes on bags using a sharp tip of a sterile tooth sticks (to avoid wide pore formation) after mycelial colonializes about half of the substrate enhances the rate of mycelial colonization during incubation time. Inserting the tooth stick deeper can create wider holes on the bag which might cause either entrance of competitors through it or loss of substrate moisture thereby retarding mycelium growth. The effect of aeration through the tiny holes on enhancement of mycelial invasion of the substrate was promising; where accumulation of CO_2 within the substrate might get reduced thereby improving mycelium extension (Bellettini et al., 2019). Mycelium running (MI) in the spawning bags took an average of 36.20 to 47.76 days to finish, with statistically significant variations between treatments ($p < 0.01$). In this study mycelial colonization rate was faster in T1 (36.20 ± 1.0 days) followed

by T2 (39.94 ± 1.0 days) and quite slower in treatment T3 (47.76 ± 0.7 days) while no mycelial growth displayed on T4 supporting the findings of Ahmed et al. (2023) where 75% tea leaf waste was evaluated and showed no mushroom mycelium growth at all. Leaves of tea plants often contain more nitrogen than many other plant materials, which lowers the carbon-to-nitrogen (C/N) ratio of this waste and making it to frequently used in composting mixtures to speed up decomposition because microorganisms can readily break down any organic substrate if it is composed of lower C/N ratio. So an increase in tea leaf waste weakens the C/N ratio of the substrate and increases substrate compaction thereby reducing space for penetration of mushroom hyphae (Fig. 1 (b)) which indirectly cause asphyxiation of the mycelium (Chukowry et al., 2009). In addition, a compacted substrate can hold high moisture which motivates growth of other competitors which competes for the limited nutrients with the growing mushroom mycelium thereby deterring spread and growth of oyster hyphae like on treatment 3 while T4 do not show any mycelial growth which seems to be similar to previous findings by Yang et al. (2016) and supporting the suggestion by Ahmed et al. (2023) where substrates with higher C/N ratio are more favorable for mycelial growth. Contamination rate appeared to increase with increasing proportion of tea leaf supplementation to the substrate which was found to be deviated from the findings by Chukowry et al. (2009).

Primordial formation and growth

Primordia are tiny nascent emerging structures which seems as precursors to mature mushroom arising from the dense mycelium network in the substrate. Primordia were observed to appear fast on treatment 1 (4.47 ± 0.6 days) followed by T2 (7.78 ± 0.4 days) after bag opening while T3 (7.65 ± 0.47 days) showed poor primordial growth. Treatment 4 displayed no primordial growth at all. This was may be due to the antifungal capability of the tea leaf which slows down the growth of fungal mycelia and lower C/N ratio which is similar to what was reported before by Yang et al. (2016), Naraian et al. (2016) and Chukowry et al. (2009). So, tea leaf should be mixed with carbon-rich substrates for effective mushroom cultivation. The number of pinhead or primordial emergence was observed to determine the number and size of fruiting bodies formed later. The higher the pinhead formation the higher will be the number of fruits formed and the smaller the size of fruiting bodies and Vise versa. The time required for primordial initiation and number of primordial growth/bag was found to be affected by fluctuation of the environmental triggers/shocks (Fig. 1 (c)) indicating the necessity of maintaining the required level of fruiting conditions (Temperature, moisture and light intensity) (Chen et al., 2022). An increase in temperature coupled with decreasing humidity level were observed to severely reduce growth and number of pinhead formation.

Fruiting and effects of growth conditions

T1 (37.41 ± 2.4) and T2 (41.28 ± 4.1) showed higher number of fruiting bodies while T3 (27.12 ± 1.6) produced fewer fruit bodies (Table 2). Fruiting body size and number were found to be inversely correlated; the larger the number of fruiting bodies in a bag, the smaller the majority of the fruiting bodies; conversely, the smaller the number of fruiting bodies in a bag, the larger the fruiting body size. This inverse relationship is attributed to the distribution of available nutrients; the more fruiting bodies there are, the less resources will be available for each fruiting body, which will limit a fruit's size and growth. In contrast, the bags with fewer fruits had larger fruiting bodies and a broader cap width due to the adequate nutrient supply relative to the fruit count. In addition to this, type of substrate used has also a significant effect on the growth parameters of oyster mushrooms (Hoa et al., 2015; Zhang et al., 2023). In this study the groundnut is used as a main carbon source while tea leaf waste was evaluated as a nitrogen source. Due to its crucial role in the synthesis of proteins, amino acids, and nucleic acids, nitrogen is a necessary element for life. Type and amount of supplements added to a substrate was found to have influence on number and size of fruiting body supporting previous findings by Naraian et al. (2016). Increasing the amount of tea leaf waste was observed to negatively affect the performance of the substrate to support growth of fruiting bodies both in number and yield by promoting the growth of contaminating competitors thereby deterring effective mycelium percolation through the substrate which seems related to previous study by Ogundele et al. (2017). According to Sassine et al. (2021) the weight of *P. florida* fruiting bodies was found to decrease with an increase in additives. Nitrogen concentrations also have a substantial impact on how white-rot basidiomycetes regulate their cellulase enzymes. The growth rate may be limited

Table 2. Comparative results of the growth parameters of oyster mushroom in all evaluated treatment.

Substrate (X)	Treatment (T)	Mean time for mycelial colonization (day)	Mean time from stimulation to Primordial initiation (day)	Mean of Primordia/ bag	Mean of fruiting body/bag	Mean time from primordia initiation to harvest (day)	Mean of total cropping time (day)	Mean of Yield (g)/bag	Mean of Biological efficiency (%)	No. of contamination encountered
Ground nut shell	T1	36.20 ± 1.0	4.47 ± 0.6	97.41 ± 5.3	37.41 ± 2.4	6.76 ± 0.4	47.41 ± 1.2	216.48 ± 6.9	54.13 ± 1.7	3
	T2	39.94 ± 1.0	7.78 ± 0.4	95.00 ± 13.5	41.28 ± 4.1	7.89 ± 0.25	55.61 ± 1.1	189.9 ± 5.0	47.5 ± 1.3	2
	T3	47.76 ± 0.7	7.65 ± 0.47	82.41 ± 9.7	27.12 ± 1.6	9.76 ± 0.44	65.18 ± 0.9	90.12 ± 9.5	22.53 ± 2.4	3
	T4	0	0	0	0	0	0	0	0	14
Control group	T**	40 ± 0.5	8 ± 0.13	82 ± 00	32 ± 01	8 ± 0.01	56 ± 0.19	200 ± 0.39	49 ± 0.1	3

**-control parameters from extension unit, ± -standard deviation.

by both an excess of nitrogen and a deficiency of nitrogen in the substrate. In growth media, nitrogen sources are typically provided in the right proportions to maintain a good nitrogen balance because of its impact on expression of genes and the activities of enzymes, particularly those of cellulases and xylanases (Zhang et al., 2023). The effect of optimal Nitrogen content on substrate was also found to boost biological efficiency of *Agaricus bisporus* according to Pardo-Gimenez et al. (2017) but Osunde et al. (2019) found a contradicting idea that higher amount of nitrogen to Corncob (carbon source) increases productivity of *Pleurotus pulmonarius*.

The only two Pleurotus strains with fully sequenced genomes to date are PC9 and PC15. In light of this, it was discovered that these strains had more genes encoding carbohydrate-active enzymes (CAZymes), which enabled them to degrade cellulose and hemicellulose aggressively (Wang et al., 2018; Yang et al., 2023). Despite not being thermophilic, *Pleuratus ostreatus* has been shown to contain some isenzymes that are thermostable (about 50 °C) (Fernández-Fueyo et al., 2014). At genetic level PoWC, the Fbh1 gene and the Pofst gene are found responsible for structural changes, respiratory requirements along with several other biosynthesis genes (PAL, PoLAC gene) and respiratory enzymes (G6PD, PEP, DAHP synthase, PFK) regulating the entire fruiting body development process during the primordial, fruiting body and spore development process of *Pleurotus ostreatus* (Barh et al., 2019; Yoneyama et al., 2021).

Environmental conditions play a crucial role in determining whether or not mushrooms will form. Vegetative mycelia can produce fruit through a variety of environmental conditions, including temperature, humidity, CO_2 concentration, and lighting. The growth of mushrooms may be adversely affected if environmental conditions deviate from the optimal level (Yamauchi et al., 2019). For instance thermal deviation inside a cultivation room affects the fruiting body's quality and productivity immensely (Wang et al., 2018). While certain edible mushrooms, such as *Pleurotus giganteus*, may withstand high temperatures, the majority of sequenced wood-rotting mushrooms grow their fruiting bodies at relatively low temperatures. According to Barh et al. (2019) the mitochondrial genome of *Pleurotus ostreatus* also showed the presence of 73,242 bp, 26.4% GC content, 44 genes encoding 18 proteins and 26 RNA genes. This shows respiration rate can be higher in oyster mushroom than in animals including humans and plants; that's why it requires higher ventilation (sufficient O_2 for cellular respiration) during fruiting time. Ventilation capacity was also found to determine morphological and physiological features of a mushroom (Chen et al., 2022). Thong-un and Wongsaroj (2022) uses a programmable logic controller (PLC), and wireless module to control and monitor temperature, humidity and ventilation using water spray, cooling fan and come up with higher yield than that of farm crops produced in the traditional way. However, in this study it appears that inadequate ventilation at night tends to minimize the growth of cap size and stipe length, indicating that poor ventilation can retard mushroom development, producing poor quality mushrooms thereby affecting yield. Furthermore, previous studies confirmed that light is a stimulant for primordial growth and crucial during the fruiting stage; affecting both quality and quantity of harvest. Moreover mushroom fruits were found to respond differently (through cap color) to different light intensities (Red, Blue and Green). Similarly in this study the growth of primordia during the night time (in the absence of light source) was observed to decrease compared to the day-time when the light bulb is switched on for hours. The genome sequences can provide a solid foundation for mining genes of important agronomic traits, such as quality, resistance, and adaptability (Zou et al., 2018)..

Yield and Biological efficiency

Yield and Biological efficiency depends on the genetic nature of a mushroom strain, its enzymatic system to degrade the substrate, type of a substrate (Physical and chemical composition) and growth conditions. Maintaining the required temperature and humidity level according to the growth stages (incubation and fruiting) are crucial for optimal harvest. Moreover, the required amount of ventilation and light should be supplied during the fruiting stage to avoid carbon dioxide accumulation in the cultivation room and triggering of primordial or fruit formation respectively. In this study the mean weight of all the bags belonging to a single treatment was computed after recording the harvest of each bag from each treatment in each flash (Table 2). T1 (216.48 ± 6.9 g) was found to score the highest followed by T2 (189.9 ± 5.0 g), displaying a good outcome compared to the control (200 ± 0.39 g) while T3 (90.12 ± 9.5 g) displayed a lower result. In terms of biological efficiency T1 ($54.13 \pm 1.7\%$) showed highest biological efficiency closely followed by T2 ($47.5 \pm 1.3\%$) while T3 ($22.53 \pm 2.4\%$) showed the lowest record. Dissasa (2022) also found that *Pleurotus ostreatus* can grow on coffee wastes as substrate scoring cost-effective yield and biological efficiency indicating the suitability of using oyster mushroom to degrade agro-wastes and generate income.

According to previous studies the nutritional content of the substrate used for mushroom cultivation majorly determines the yield and biological. Girmay et al. (2016) investigated the performance of Oyster mushroom on four different waste substrates and found different ranges of yield and biological efficiency. In addition Akcay et al. (2023) similarly evaluated five waste substrates alone and seven different mixtures of these substrates to cultivate *P. ostreatus* and recorded a range of yield ($105.2 - 257.0$ g) and biological efficiency ($11.3 - 64.3\%$) for total of the three flushes. Furthermore; Hoa et al. (2015) cultivated oyster mushrooms on saw dust of acacia wood, sugarcane bagasse, corncob and different mixtures of these substrates and calculated the total yield and BE for six flushes. According to his findings corncob as a standalone substrate scores highest yield and BE (Table 3). Moreover an increase in corncob and sugarcane bagasse was found to reduce C/N ratio and enhance protein and mineral content of a fruiting body. Our study reveals that higher C/N ratio tends to enhance mycelium growth thereby facilitating mycelial colonization and reduc-

Table 3. Previous findings regarding performance of Oyster mushroom (*P. ostreatus*) on different agro-waste substrates in terms of yield and biological efficiency.

Substrate type	Dry weight of substrate per bag (g)	Number of flashes harvested	Yield (g)	Biological efficiency (%)	Reference
Wheat straw	573.70	2	174.25	35.88	(Girmay et al., 2016)
Paper waste	689.10	2	192.45	34.22	(Girmay et al., 2016)
Coffee ground	1000	3	174.4	43.6	(Akcay et al., 2023)
Wheat straw	1000	3	172.5	43.1	(Akcay et al., 2023)
Hazelnut husk	1000	3	157.5	39.4	(Akcay et al., 2023)
Wheat straw + Coffee ground	1000 (50:50)	3	155.3	11.3	(Akcay et al., 2023)
Hazelnut husk + Coffee ground	1000 (50:50)	3	105.2	26.3	(Akcay et al., 2023)
Wood dust (*Triplochiton scleroxylon*)	300	2	45.81	48.40	(Victor Familoni et al., 2018)
Wood dust (*Terminalia ivorensis*)	300	2	46.97	48.83	(Victor Familoni et al., 2018)
Saw dust (acacia wood)	1000	6	232.54	46.44	(Hoa et al., 2015)
Sugarcane bagasse	1000	6	257.70	65.65	(Hoa et al., 2015)
Corncob	1000	6	270.60	66.08	(Hoa et al., 2015)
Saw dust + Sugarcane bagasse	1000(50:50)	6	250.51	58.94	(Hoa et al., 2015)
Saw dust + Corncob	1000(50:50)	6	258.82	58.82	(Hoa et al., 2015)
Sisal leaf	450	1	12.03	8.95	(Raymond et al., 2013)
Sisal bole	450	1	98.93	33.69	(Raymond et al., 2013)
Sisal bole + Sisal bole	(50:50)	1	119.16	43.02	(Raymond et al., 2013)

ing mycelium colonization time while reduced C/N ratio favors growth of fruiting body which is in line with findings of Hoa et al. (2015) and Raymond et al. (2013). Substrate mixtures with higher C/N ratio reduces the yield and quality of fruiting bodies while extremely lower C/N ratio enhances contamination and render mycelium running through substrate. The records of yield and BE of this study on T1 and T2 are promsing compared to the previous findings stated on Table 3.

The three treatments showed significant differences on mycelium colonization and cropping time while considering amount of yield harvested and biological efficiency treatment one and treatment two showed significant differences when compared with treatment three. Treatments with overly compressed box plots either below or above the median line of the box indicate overlapping of the recorded data (Fig. 2). Treatment three displayed high significance in the four cultivation parameters (with delayed mycelial colonization and cropping time, lower yield and biological efficiency) compared with the two treatments due to the effect of higher nitrogen source (tea leaf waste) to carbon source (groundnut shell) ratio. The three evaluated treatments displayed significant differences with P-value 4.74E-200 at p-value ($p < 0.01$). In addition, all the growth parameters were found significantly different with p-value $= 0.00$ at p-value ($p < 0.01$) considering each growth parameter among the treatments. Chi-square analysis of the data reveals that T2 of ground nut shell ($X^2 = 8.419793$) was found to be the best fitted treatment than the control group (standard treatment) considering all the cultivation parameters together.

According to the correlation analysis done through R-software the amount of harvest/yield (Y) and its biological efficiency (BE) are inversely correlated with the time needed for the substrate bag to fully colonize with mycelium (Fig. 3). From previous studies; mycelium tends to colonize a substrate rapidly either if the amount of substrate material per bag is smaller or a substrate is not compressible (higher space between substrate material eg: straw substrate, grass substrate etc.). But this leads to insufficiency of substrate content to support higher fruiting body growth in each flash, thereby affecting yield and biological efficiency (Markson et al., 2012). In this study since the amount of a substrate is large (500 g/bag) and the nature of the substrate is prone to compaction; the rate of mycelium colonization was a bit slower while the amount of harvest and BE were satisfactory. As per a research work done by Valenzuela-Cobos et al. (2023) biological efficiency of a substrate is highly linked (displaying strong correlation) to the yield, similarly Fig. 3's results suggested that the number of fruiting bodies per bag (FB) exhibited positive correlations with both "Y" and "BE." This is because a higher number of fruiting bodies on a substrate bag increases the likelihood that fruits reaching maturity, which raises yield and biological efficiency in each flash.

This study is contributing on finding and usage of wastes to grow nutritionally rich oyster mushrooms thereby enhancing environental sanitation and food security. But this

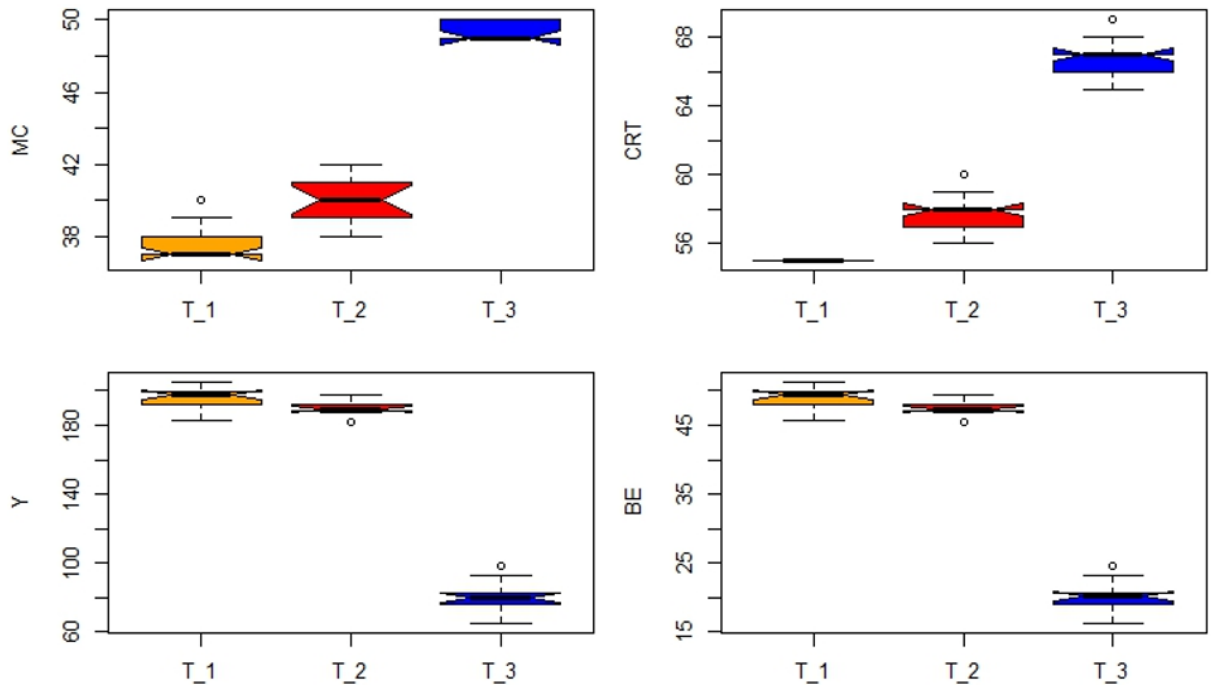

Figure 2. Significance between treatments; all treatments show significant differences in mycelial colonization (MC) and in cropping time (CRT); treatment three showed significant difference in amount of yield (Y) and in biological efficiency (BE) compared to the two treatments. NB: compressed boxes indicate overlapping of data.

finding is limited to two susbtrate and one mushroom strain; so a more comprehensive study is necessary to investigate the broad substrate and strain range. Moreover this work lacks focus on nutritional value of substrates, biochemical mechanisms behind the observed effects and potential presence of inhibitory compounds in the evaluated substrates, which needs further research.

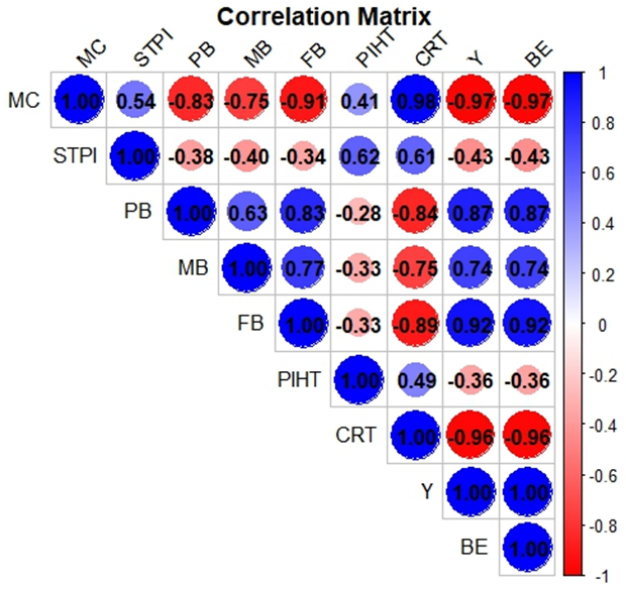

Figure 3. Sample correlation coefficient matrix between evaluated growth parameters of oyster mushroom.

4. Conclusion

Mushroom cultivation is the best alternative technology to convert agro-wastes into nutrient rich food stuffs in addition to its contribution on the environmental sanitation and amelioration. Tea leaf waste can be used as economic alternative substrate for oyster mushroom cultivation at specified (40%) ratio mixed with 58% ground nut shell which showed satisfactory biological efficiency (BE $= 47.5 \pm 1.3\%$) and yield per bag (189.9 ± 5.0 g) closely approaching to the control considered (BE $= 49 \pm 0.39\%$, 200 ± 0.39 g) while tea leaf waste alone cannot be used as a substrate for mushroom cultivation. With a P-value of 4.74E-200 at p-value (p ¡ 0.01), the three assessed treatments showed a significant difference. Furthermore, tea leaf waste also will lower the cost of fruiting bag preparation so it can be used as an alternative to conventional substrate i.e. wheat bran used in Eritrea.

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Authors contributions
The authors confirm the study conception and design: T.G.G, K.C.S; Substrate collection, cultivation, data curation, data analysis, manuscript preparation and editing –T.G.G. Supervision and editing, K.C.S. Both authors have checked and approved the final version of the manuscript.

Availability of data and materials
The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Conflict of interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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