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Evaluating the Potential of two Invasive Plant Species as Sustainable Substrates for Oyster Mushroom (*Pleurotus* spp.) Cultivation in Nepal

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ABSTRACT

Oyster mushroom (*Pleurotus* spp.) is one of the most commonly cultivated mushrooms in Nepal due to its adaptability to a wide range of substrates. This study evaluates the potential of invasive plant species, *Lantana camara* and *Parthenium hysterophorus*, as substrates for oyster mushrooms (*Pleurotus* sp.) cultivation compared to traditional substrates such as rice straw and sawdust. The results show significant variations in mushroom weight, height, and cap width across substrates. *Lantana camara* substrate yielded a mean mushroom weight of 238.1 ± 21.1 g, height of 3.5 ± 0.8 cm, and cap width of 2.9 ± 0.7 cm, while *Parthenium hysterophorus* yielded 176.2 ± 37.6 g, 2.6 ± 1.2 cm, and 1.7 ± 0.6 cm, respectively on 2 kg of substrates. Traditional substrates performed better, with rice straw producing a mean weight of 529.2 ± 39.8 g, height of 2.6 ± 0.9 cm, and cap width of 2.1 ± 0.7 cm, while sawdust produced the highest mean weight (558.4 ± 301.4 g), tallest mushrooms (7.1 ± 1.6 cm), and largest cap width (5.7 ± 0.5 cm). One-way ANOVA confirmed a significant effect of substrate on mushroom weight ($F = 8.183$, p = =0.002 (round up), height ($F = 16.82$, $p < 0.001$), and cap width ($F = 40.47$, $p < 0.001$). Pairwise comparisons showed that sawdust consistently supported the tallest mushrooms and largest cap width, while rice straw also performed significantly better than *Lantana camara* and *Parthenium hysterophorus* in mushroom weight. Principal component analysis (PCA) revealed that PC1 (79.7% variance) was driven by mushroom height and cap width, while PC2 (18.6% variance) was influenced by mushroom weight. This study demonstrates that invasive species like *Lantana camara* and *Parthenium hysterophorus* produce some amount of oyster mushroom, however, traditional substrates remain superior as compared to these IAPS.

Keywords: Invasive alien plant species management, Alternative substrate, IAPS repurposing

INTRODUCTION

Oyster mushroom (*Pleurotus ostreatus*) is an edible mushroom commonly grown at altitudes ranging from 1000 to 2500m, with temperatures ranging from 18°C to 30°C. The cultivation of this mushroom has become an integral part of Nepal's agricultural economy (Shrestha and Dhakal, 2014). The commercial cultivation of oyster mushrooms in Nepal began in the early 1990s, driven by increasing awareness of its nutritional value and market potential (Raut, 2019). Oyster mushrooms are rich in protein (approximately 25-30% of dry weight), essential amino acids, vitamins (such as B-complex and vitamin D), minerals (including potassium, phosphorus, and iron), and dietary fiber, while being low in fat (Effiong, 2024). These nutritional properties make it a popular choice among health-conscious consumers. The market for oyster mushrooms in Nepal is growing steadily, catering to urban households, hotels, and restaurants (Raut, 2019). Although precise data on its economic contribution is limited, oyster mushroom farming has become a viable income source for smallholder farmers, contributing to rural livelihoods and agricultural diversification. Its ability to grow on locally available substrates like rice straw and sawdust makes it a sustainable option for promoting food security and rural economic development in Nepal (Pokhrel 2016).

Oyster mushrooms (*Pleurotus ostreatus*) can be cultivated on a variety of substrates, including agricultural residues, forest by-products, and invasive plant materials (Girmay et al., 2016; Pokhrel 2016). The choice of substrate plays a crucial role in the growth and yield of oyster mushrooms, as well as their nutritional composition. Ideal substrates should be rich in lignin, cellulose, and hemicellulose, which serve as energy sources for the mushroom's growth (Hoa 2015). A balanced carbon-to-nitrogen (C:N) ratio in the substrate, typically around 25:1 to 30:1, is critical for optimal mycelial development and fruiting body production (Temble 2018).

The substrate significantly affects both the yield and the nutritional content of oyster mushrooms (Temble 2018). Variations in the chemical composition of the substrate, such as the levels of nitrogen, phosphorus, and potassium, can alter the protein content, mineral profile, and other nutritional attributes of the harvested mushrooms (Effiong, 2024). For example, substrates high in nitrogen tend to produce mushrooms with a higher protein content (Diamantopoulou et al., 2023).

In Nepal, common substrates used for oyster mushroom cultivation include rice straw, wheat straw, maize stalks, sawdust, and banana leaves (Pokhrel, 2016; Raut, 2019). Recently, there has been interest in exploring invasive plant species like *Lantana camara* and *Parthenium hysterophorus* as alternative substrates (Mintesnot et al., 2014). These invasive species, which are abundant and problematic in Nepal (Shrestha 2016), not only offer a sustainable substrate option for mushroom production. If we can find a way to utilize them, it also contributes to ecological management. The choice of substrate in Nepal largely depends on its local availability, cost, and ease of processing, making oyster mushroom cultivation a flexible and sustainable agricultural practice (Pokhrel 2016; Thakur et al., 2024).

Nepal has around 30 invasive alien plant species (IAPS) that pose significant ecological, economic, and social challenges (Shah et al., 2020). These species invade natural ecosystems, agricultural lands, and urban areas, displacing native flora, altering habitats, and threatening biodiversity (Shrestha & Shrestha, 2021). Their impacts are severe, including reduced agricultural productivity, degradation of rangelands, disruption of water systems, and increased vulnerability of ecosystems to climate change. Invasive plants also hinder forest regeneration, affect the livelihood of local communities, and contribute to the loss of ecosystem services (Shrestha, 2019; Shrestha & Shrestha, 2021). Some of the most notorious invasive species in Nepal include *Lantana camara*, *Parthenium hysterophorus*, *Eichhornia crassipes* (water hyacinth), *Chromolaena odorata*, *Mikania micrantha* (mile-a-minute), *Ageratum conyzoides*, and *Alternanthera philoxeroides* (alligator weed) (Shrestha 2016). Management of IAPS in Nepal has become a pressing concern due to their rapid spread and extensive damage to ecosystems (Shrestha, 2019). Strategies like ecological restoration, sustainable utilization, and raising awareness are being explored to mitigate their impacts and maintain biodiversity (Shrestha, 2019; Bhatt et al., 2021; Shrestha & Shrestha, 2021)

Lantana camara and *Parthenium hysterophorus* are two of the most problematic invasive alien plant species (IAPS) in Nepal, significantly impacting ecosystems and livelihoods (Shrestha et al., 2017). We choose them in this study because of their proliferation and easy availability around the road sides and forests. *Lantana camara* is Native to Central and South America, this species has invaded forests, rangelands, and agricultural areas across Nepal (Bhatt et al., 2021). It forms dense thickets, outcompetes native vegetation, and hinders forest regeneration. It negatively affects biodiversity and is toxic to livestock (Bhatt et al., 2021). *Parthenium hysterophorus* is commonly known as 'Congress grass', this species is native to tropical America (Shrestha et al., 2015). It thrives in disturbed areas, agricultural fields, and along roadsides. Its rapid growth displaces native species, reduces crop yields, and causes allergic reactions and dermatitis in humans (Shrestha et al., 2015). Both species are listed as noxious weeds in Nepal, with their spread attributed to disturbed habitats and limited management efforts (Siwakoti et al., 2016). Their control has become a priority for ecosystem restoration and biodiversity conservation.

Several management strategies have been explored globally to mitigate the impacts of invasive alien plant species (IAPS) (Dhungana et al., 2024). These include mechanical removal, biological control, chemical methods, habitat restoration, and sustainable utilization of invasive plant biomass (Shrestha, 2019; Dhungana et al., 2024). Among these strategies, utilizing invasive plant biomass for productive purposes, such as bioenergy, composting, paper production, and mushroom cultivation, has gained attention as an innovative and sustainable approach (Feng et al., 2021). This dual-purpose strategy not only helps reduce invasive species proliferation but also creates economic and environmental benefits.

One of the promising dimensions explored is the use of invasive plant biomass as a substrate for mushroom cultivation (Mintesnot et al., 2014). For instance, invasive plants such as *Lantana camara*, *Parthenium hysterophorus*, and *Chromolaena odorata* have been studied for their potential in mushroom production (Feng et al., 2021; Lorenzo & Morais, 2023). Research has demonstrated that oyster mushrooms (*Pleurotus* spp.) can effectively degrade the lignocellulosic materials present in these plants, producing good yield and quality mushrooms. Studies such as those by Kundu et al., (2022) and Gamage et al., (2023) have highlighted the successful incorporation of *Lantana camara* and *Parthenium hysterophorus* into substrates for cultivating *Pleurotus ostreatus*, yielding comparable results to traditional substrates like rice straw or wheat straw.

The use of invasive plant biomass in mushroom cultivation not only addresses the issue of invasive species management but also provides an alternative substrate for mushroom growers, reducing reliance on conventional agricultural residues (Mintesnot et al., 2014, Feng et al., 2021). Furthermore, this approach aligns with circular bioeconomy principles by converting problematic biomass into valuable agricultural products (Sadh et al., 2023). It also offers additional ecological benefits, such as lowering the invasive species' impact on native ecosystems (Lorenzo & Morais, 2023). As *Lantana camara* and *Parthenium hysterophorus* are two of the most problematic invasive alien plant species (IAPS) in Nepal affecting ecosystems and livelihoods (Shrestha et al., 2017) and are widely available around the roadsides (*P. hysterophorus*) and forest (*L. camara*), we consider them in our study. Thus this study aims to evaluate the effect of these two invasive species as compared to the widely used sawdust and rice straw substrate on the oyster mushroom production. We hypothesized that using substrate from invasive plants specially *Lantana camara* and *Parthenium hysterophorus* could produce comparable yield as compared to widely used substrate saw dust and rice straw.

MATERIALS AND METHODS

Study site

The research was conducted in a controlled dark room environment in Tilottama-1, Rupandehi district of Nepal during May to July 2023 for the determination of effect of different substrates on the yield performance of oyster mushroom (*Pleurotus*).

Experimental Design

The experiment was based on a Single factor Completely Randomized Design (CRD) with 5 replications for each substrate treatment. A total of 20 polypropylene bags were used to evaluate the effect of substrates. Four different treatments, each weighing 2 kg, were applied and labeled as T1, T2, T3, T4 and T5. The treatments consisted of rice straw, sawdust, branches of *Lantana camara*, and above-ground parts of *Parthenium hysterophorus*, respectively. The sawdust was taken from a nearby saw mill processing species such as *Tectona grandis, Syzygium cumini, Haldina cordifolia, Terminalia elliptica, Dalbergia sissoo, and Shorea robusta*. However, the exact composition and proportions were unknown, which remains a limitation of our study.. The experiment was conducted at room temperatures ranging from 32°C to 34°C and 80-90% of relative humidity.

Spawn Source, Substrate Collection and Cultivation Process

Spawn was purchased from the Kalimati tarkari bajar, while the substrates were sourced locally. Rice straw was collected from nearby farms, sawdust from a local furniture shop, *Lantana camara* branches from Banbatika Forest, and *Parthenium hysterophorus* from nearby areas.

The substrates; rice straw, *Lantana camara* branches, and above ground parts of *Parthenium hysterophorus* were chopped into small pieces (3–4 cm), except sawdust. The substrates were soaked in tap water overnight, then drained to remove excess water. After that, different substrates were steam sterilized individually at 121°C for one hour using a stainless steel steamer.

The sterilized substrates were spread on a polyethylene sheet with swab dipped in 70% ethyl alcohol. Each substrate was then packed into polypropylene bags, with each bag containing 2 kg of substrate. Spawn was inoculated asceptically by thoroughly sprinkling at the corners every 3 inches by making 2-3 tiers of substrates filled in polypropylene bags. The bags were incubated in a well-ventilated dark room. Fans were used to maintain room temperature for about 2 hours every morning and evening, maintaining an internal temperature of 33.34°C in the morning and 35.7°C in the evening.

Once the spawn run was complete followed by proper colonization and initiation of the fruiting body, each bag was cut longitudinally into eight sections by a sterile blade to create openings for mushroom growth. The bags were shifted from dark room and kept moist by regularly spraying water. Mushrooms were harvested by carefully cutting the larger fruiting bodies at their base while allowing smaller ones to continue growing. Three harvests were performed.

The data regarding to the fresh weight of harvested mushroom (g), diameter of pileus (cm) and stalk and mushroom height (cm) were recorded. For the diameter of the pilus and mushroom height, three highest grown mushroom were sampled and their mean data for each replication were included in analysis.

Data collection and analysis

Data was recorded for stipe length (cm), pileus diameter (cm), and weight of mushroom (gm) from each substrate. The mean of the three tallest mushrooms per replication was used for pilus diameter and height analysis. ANOVA has been used to test among treatments (substrates) and means were separated using least significant difference (LSD) at 5% level of significance. The differences in each sample was compared by using multiple comparison t-tests with adjusted p-values using the Bonferroni method. Data were first entered on excel 2013 and further analysis was done by using R-studio (R-core team, 2024).

RESULTS

The results show the variation in weight of mushrooms, mushroom height, and cap width for each substrate. In *Lantana camara*, the mean mushroom weight was 238.1 ± 21.1 g, with a mean height of 3.5 ± 0.8 cm and a cap width of 2.9 ± 0.7 cm. For *Parthenium hysterophorus*, the mean weight was 176.2 ± 37.6 g, the height was 2.6 ± 1.2 cm, and the cap width was 1.7 ± 0.6 cm. Rice straw supported a higher mean weight of 529.2 ± 39.8 g, with a mean mushroom height of 2.6 ± 0.9 cm and a cap width of 2.1 ± 0.7 cm (Table 1). Lastly, saw dust yielded the highest mean weight of 558.4 ± 301.4 g, with a height of 7.1 ± 1.6 cm and a cap width of 5.7 ± 0.5 cm (Table 1).

One way ANOVA shows that substrate plays a significant role in the total production of oyster mushrooms along with their height and cap weight (Table 2). The analysis showed a statistically significant effect of substrate on weight of mushroom produced ($F = 8.183$, $p = 0.002$ (round up). Similarly, mushroom height showed a highly significant effect ($F = 16.82$, $p < 0.001$), and cap width also exhibited a highly significant difference ($F = 40.47$, $p < 0.001$) across the substrates (Table 2).

Table 1. Mean (± standard deviation) values for the weight of mushrooms, mushroom height, and cap width across different substrates.

Substrate	Weight of mushroom (g) (mean ± sd)	Mushroom height (cm) (mean ± sd)	Cap width (cm) (mean ± sd)
Lantana camara	238.1 ± 21.1	3.5 ± 0.8	2.9 ± 0.7
Parthenium hysterophorus	176.2 ± 37.6	2.6 ± 1.2	1.7 ± 0.6
Rice straw	529.2 ± 39.8	2.6 ± 0.9	2.1 ± 0.7
Saw dust	558.4 ± 301.4	7.1 ± 1.6	5.7 ± 0.5

Table 2. One way ANOVA tests for the effect of different substrates on oyster mushroom production.

	Df	Sum Sq	Mean Sq	F value	P value	
Weight of mushroom produced.	3	578469	192823	8.183	0.00158	**
Mushroom height	3	69.48	23.16	16.82	<0.001	***
Cap width	3	48.05	16.015	40.47	<0.001	***
Level of significance, ns: >0.05, *: <0.05, **:<0.01, ***:<0.001						

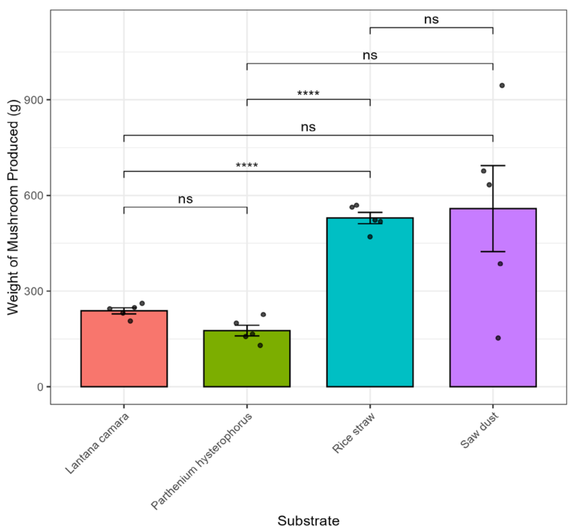

Figure 1. Comparison of mushroom production (g) across different substrates. The bar heights represent the mean weight of mushrooms produced, with error bars showing standard errors. Jittered points indicate individual replicate data. Statistical significance between substrates is denoted above the bars (adjusted p-values using the Bonferroni method).

The results of the pairwise comparisons for mushroom weight across different substrates indicate there was no significant difference between *Lantana camara* and *Parthenium hysterophorus* (p = 0.102), or between *Lantana camara* and Saw dust (p = 0.456). However, a highly significant difference was observed between *Lantana camara* and Rice straw (p<0.001), with Rice straw resulting in significantly higher mushroom weights. Similarly, a significant difference was found between *Parthenium hysterophorus* and Rice straw (p<0.001), where Rice straw produced significantly more mushrooms. There was no significant difference between *Parthenium hysterophorus* and Saw dust (p = 0.276), nor between Rice straw and Sawdust (p = 1.00), indicating that Sawdust did not significantly affect mushroom weight compared to the other substrates.

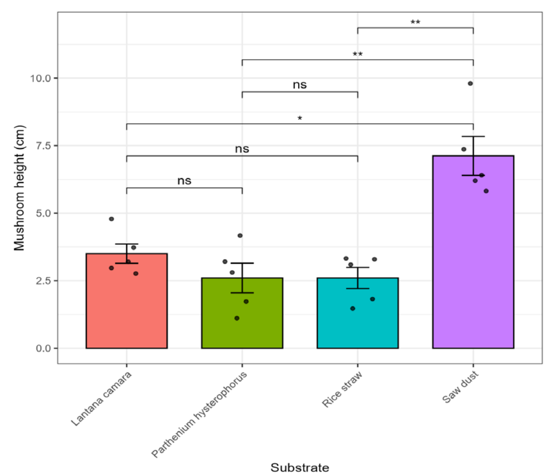

Figure 2. Comparison of mushroom height across different substrates. The bar heights represent the mean height, with error bars showing standard errors. Jittered points indicate individual replicate data. Statistical significance between substrates is denoted above the bars (adjusted p-values using the Bonferroni method).

There was no significant difference in height between *Lantana camara* and *Parthenium hysterophorus* (p = 1.00), or between *Lantana camara* and Rice straw (p = 0.774). However, a significant difference was observed between

Lantana camara and Saw dust, with Saw dust resulting in significantly taller mushrooms (p = 0.024). Similarly, *Parthenium hysterophorus* and Rice straw showed no significant difference (p = 1.00), but a significant increase in height was found with Saw dust compared to *Parthenium hysterophorus* (p = 0.006). A similar significant effect was observed between Rice straw and Saw dust, with Saw dust again promoting significantly taller mushrooms (p = 0.006). These results suggest that Saw dust consistently led to taller mushrooms compared to the other substrates, while no significant differences were found between the other pairings.

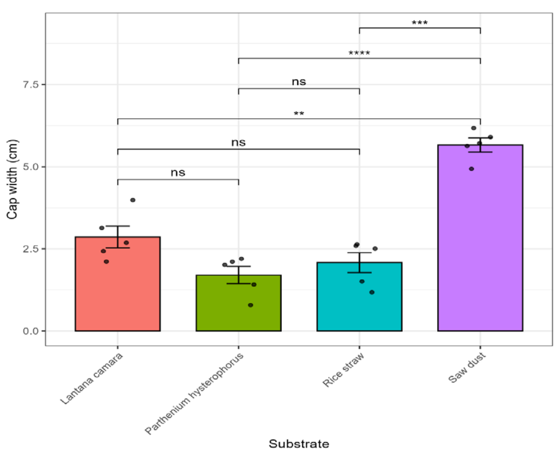

Figure 3. Comparison of mushroom cap width across different substrates. The bar heights represent the mean width, with error bars showing standard errors. Jittered points indicate individual replicate data. Statistical significance between substrates is denoted above the bars (adjusted p-values using the Bonferroni method).

No significant difference was found between *Lantana camara* and *Parthenium hysterophorus* (p = 0.156), or between *Lantana camara* and Rice straw (p = 0.72). However, a significant difference was observed between Lantana camara and Saw dust (p<0.001), with Saw dust producing significantly larger cap widths. Similarly, no significant difference was found between *Parthenium hysterophorus* and Rice straw (p = 0.37). A highly significant difference was noted between *Parthenium hysterophorus* and Saw dust (p<0.001), where Sawdust again produced significantly larger cap widths. Finally, Rice straw and Saw dust also exhibited a significant difference (p <0.001), with Saw dust yielding a larger cap width. These results highlight Saw dust as the substrate with the largest cap width, while the other substrates did not show significant differences in their effects.

The principal component analysis (PCA) revealed that the first principal component (PC1) explains 79.7% of the variance in the data, primarily driven by mushroom height and cap width. The second component (PC2) explains 18.6% of the variance and is influenced by the weight of the mushroom produced, with some negative contributions from mushroom height and cap width. Based on the PCA result, there is wide variation in the mushroom performance while the mushroom properties in the rice straw are significantly different in *Lantana camara* and *Parthenium hysterophorus*. The performance of the mushroom shows wider variability along both domension1 and 2 axis for the saw dust. However for the other substrate there is variation along the dim 1. The demarcation between the mushroom performance on different substrate is performed by dim 2 (Figure 4). On the mushroom performance, it is seen that mushroom height and cap width are correlated, however the weight of the mushroom produced is clearly demarcated with them along dim 2.

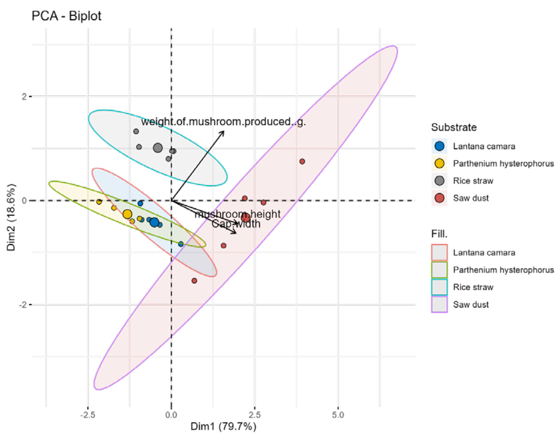

Figure 4. Principal Component Analysis (PCA) of mushroom growth in different substrates. The plot shows the distribution of samples based on the first two principal components (PC1 and PC2), which explain 79.7% and 18.6% of the total variance, respectively. Each point represents a sample, colored by substrate type and shaped by replication. The ellipses represent the 95% confidence intervals for each substrate group.

DISCUSSION

This study shows that although some amount (nearly half than widely used substrate) of the oyster mushroom could be produced on substrate of invasive species like *Lantana camara* and *Parthenium hysterophorous*, they are significantly less than widely used substrate like rice straw. The saw dust yields more average yield than rice straw, however yield is much higher in some replication and low on the some replication and analysis does not signify any significant higher in the saw dust. This wide range of the mushroom production in the saw dust may be due to variation in substrate nutrient content such as carbon to nitrogen ratio, cellulose and lignin (Mortada et al., 2020). As the saw dust is borrowed from a furniture factory, the source of the saw dust was unknown and during the experiment, the substrate was thought to be of same quality in all the replication. This remains one of the areas for research on how the different types of the saw woods dust effects on the oyster mushroom production.

The main purpose of this study was to explore the effect of the different substrate on the yield of the oyster mushroom and suitability of substrate from the invasive species on the oyster mushroom growth. Based on the result of this study we argue that there is a tradeoff between the mushroom production and management of invasive species. One possibility for the management of the *Lantana camara* and *Parthenium hysterphorous* can be the uses of them on the oyster mushroom productions as they can yield nearly half of the mushroom as the widely used rice straw. Though the yield is low, it can be covered up economically as the rice straw costs much higher than the invasive species which is the waste and has no economic value (Mintesnot et al., 2014; Pandey et al., 2018). If some authority manages to give incentive on the uses of such invasive species on the oyster mushroom, it has a double benefit, one is the environmental sustainability and smooth ecosystem functioning and another is the economic profit. However, this research alone can't guarantee these benefit because the nutraceutical analysis of the mushroom grown in the invasive species and their comparison with the mushroom grown in the widely used substrates like rice straw and woods has not been done, so this study is not sure whether there is any alteration in the properties and their edible suitability. The study by Elkanah et al., (2022) has shown that the substrate significantly affects the nutritional quality of *Pleurotus ostreatus* mushrooms by increasing or decreasing nutrients like carbohydrates and vitamins in the fruiting body. However, this study does not address the alteration of nutrient production or the formation of new compounds by the substrates. Moreover this research is done in the three month time frame and to make the comparisons, their mushrooms were sown and harvested in the same amount of time. So this result does not provide insight on the total mushroom production till the end and also it does not study the capacity of the multiple harvesting of these substrates.

PCA results show that the performance of Oyster mushroom is similar in *Lantana camara* and *Parthenium hysterophorous*. The similarity in their ecological characteristics - such as nutrient availability and moisture retention - might explain why oyster mushrooms exhibit similar growth patterns on both of these invasive species. Invasive species often exhibit rapid growth and high biomass production, which could result in a substrate rich in nitrogen (Sharma and Raghubanshi 2009; Defar et al., 2024). Rice substrate was clearly separated from all other substrates, indicating that it supports mushroom growth under very different conditions. Rice straw, commonly used as a substrate, may have distinct physical and chemical properties - such as a different carbon-to-nitrogen ratio or moisture-holding capacity - that make it less conducive to mushroom growth compared to the other substrates. A study by Rahman et al., (2010) while studying nutrition value of different varieties of rice straw reported that the nutrient content in rice straw includes Dry Matter (DM) ranging from 92.21% to 93.05%, Organic Matter (OM) from 81.21% to 86.24%, Crude Protein (CP) from 3.49% to 5.10%, Acid Detergent Fibre (ADF) from 41.38% to 46.32%, and Neutral Detergent Fibre (NDF) from 72.16% to 77.57%. Additionally, the lignin content ranges from 4.3% to 6.97%, calcium (Ca) from 0.10% to 0.245%, and phosphorus (P) from 0.046% to 0.146%. The sawdust substrate, on the other hand, showed a larger ellipse with minimal overlap with the invasive species, suggesting a wide range of variation in mushroom growth. This could be due to the diverse nature of sawdust, which can vary based on the wood source and preparation. Sawdust contains a mix of cellulose and lignin, which decomposes at varying rates depending on environmental conditions, leading to a broader spectrum of growth conditions. As a result, oyster mushrooms growing on sawdust may experience more variability in growth patterns, reflected by the larger ellipse in the PCA plot.

Oyster mushrooms can grow on a wide variety of substrates. However, the yield and the quality of oyster mushrooms depend on the chemical and nutritional content of substrates (Sopanrao et al., 2010). The main nutrients are less nitrogen and more carbon so materials containing cellulose, hemicellulose and lignin (i.e., rice and wheat straw, cotton seed hulls, sawdust [SD], waste paper, leaves, and sugarcane residue) can be used as mushroom substrates (Hoa et al., 2015). *Lantana camara* has comparatively lower C/N ratio than other plants in forest because it has higher nitrogen and lower cellulose and lignin contents (Sharma and Raghubanshi 2009). This may be the reason for the lower oyster production in *Lantana camara* substrate. Similarly in *Parthenium hysterophorus* also, the carbon to nitrogen (C:N) ratio is low, with some examples ranging from 7.1 to 10:1 (Defar et al., 2024). The carbon to nitrogen (C:N) ratio of rice straw is around 80:1 (Goyal and Sindu, 2011), which is way more higher than in *Parthenium hysterophorus* and *Lantana camara*, this explains why there is higher oyster mushroom production in the rice straw in this study. The C:N ratio in the saw dust varies according the nature of wood and the plants, some study reported the lowest carbon to nitrogen (C:N) ratio of 15:1 (Abubakari et al., 2019) and some has reported up to 500:1 (Mortada et al., 2020), this explains the variability of mushroom yield in different replication of sawdust substrate.

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