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EVALUATION OF SUBSTRATE AND CONSORTIA OF SUBSTRATE ALTERNATIVES FOR OPTIMAL CULTIVATION OF OYSTER MUSHROOM (*PLEUROTUS SAPIDUS*)

Subhajit Khound[1], Dilip Kumar Sarmah[1], Jangila Hazowary[2]*, Apurba Das[1] and Ardita Biswas[3]

[1]Department of Plant Pathology, College of Agriculture, Assam Agricultural University, Jorhat (Assam), India.
[2]Department of Plant Pathology, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar (Uttarakhand), India.
[3]Department of Plant Breeding and Genetics, Assam Agricultural University, Jorhat (Assam), India.
*Corresponding author E-mail : jangilahajowary@gmail.com

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ABSTRACT

In this study, efforts were taken to assess the suitability of six locally available substrates including rice straw, mustard stalk and siliqua, sugarcane bagasse, water hyacinth, banana pseudostem and sawdust and also consortia of substrates for growing *Pleurotus sapidus*. Six different substrates were collected from ICR (Instructional-cum-Research) Farm, Assam Agricultural University, Jorhat campus and nearby locality of Jorhat district. The maximum cap size and stalk girth were observed in rice straw substrate and the longest stalk length was obtained from the mushroom beds with banana pseudostem as substrate. The highest yield was achieved using a substrate combination of mustard stalk and siliqua (1317.50 g/kg of substrate). To evaluate the growth parameters and yield attributes, eight substrate combinations were tested, with rice straw substrate as control. The highest yield was obtained in a mixed combination of substrates with rice straw 50% and Mustard stalk and siliqua 50% (1432.5 g/kg of substrate) followed by the rice straw (1290 g/kg of substrate). The composite substrate of rice straw 50% and mustard stalk and siliqua 50% demonstrated the highest yield among all the substrate combinations. This study suggests that mustard stalk and siliqua substrates can serve as superior alternatives to rice straw for mushroom cultivation, both when used individually and in combination. In the absence of these substrates, options such as water hyacinth and sugarcane bagasses may be considered for successful mushroom cultivation.

Key words : Oyster mushroom, *Pleurotus sapidus*, Protein, Phenol, Substrate, Spawn.

Introduction

The issue of global food security and malnutrition is an urgent matter that requires immediate attention. With the world's population expected to reach 9.1 billion by 2050 and the rapid expansion of urban areas, there is mounting pressure on food production and availability (World Population Review, 2018). Moreover, unsustainable practices are causing environmental degradation and desertification, which threatens arable land. The 2018 Global Hunger Index has highlighted the persistence of severe hunger-related issues in Asian and African countries, particularly India (Global Hunger Index, 2018). Given the current challenges in ensuring food security, it is essential to focus on alternative food sources to prevent malnutrition. Mushrooms are a great supplementary resource to cereals and have been used as an edible forest product for centuries. They were even considered the "food of gods" by the Romans and a delicacy by the Pharaohs (Arora and Shepard, 2008; Daba *et al*., 2008).

Mushrooms are a type of macro-fungi that belong to the kingdom fungi. They have a heterotrophic mode of nutrition and produce spores. Mushrooms belong to two groups, Basidiomycetes and Ascomycetes and have a two-phase growth cycle. The first phase is the vegetative phase, where the fungus grows as mycelia. The second phase is the reproductive phase, where fruit bodies are produced (Kumar *et al*., 2015). The spores of the fungus are found in a distinctive structure known as basidium (in Basidiomycetes) or the ascus (in Ascomycetes). Typically, mushrooms are grown above ground either on soil or on an artificial medium employed as a substrate (Thakur, 2020). It is estimated that there are more than 1.5 million fungal species worldwide. However, only 16,000 of these species are known as mushrooms, 700 are known as medicinal mushrooms and only 35 species are commercially exploited for human consumption (Varghese and Amritkumar, 2020). The oyster mushroom is an important member of the Pleurotaceae family and its cultivation has increased greatly around the world (Mshandete, 2011; Randive, 2012). Mushrooms are becoming an essential part of human diets due to their medicinal properties and nutritional values. Rich in vitamins, protein and minerals, they play a crucial nutritional role particularly due to their high protein content, dietary fibres and essential minerals (Chang and Miles, 2004; Khan *et al*., 2009; Khan*et al*., 2021). Mushrooms represent a nutritious food source with the potential to address malnutrition challenges in developing countries. They have low starch and fat content, making them suitable for patients with diabetes and heart diseases (Solovyev *et al*., 2018). Oyster mushrooms are a good source of protein (20-35%) have high water content (70-90%), low fat (0.6-3%) and contain 7.5-16.5% fibre (Jamil *et al*., 2019).

Oyster mushrooms are particularly suitable for cultivation in India due to their ease of cultivation, low-cost technology and ability to use various agro-waste in mushroom beds. They can be grown in both plains and hilly areas and have a high bio-efficiency (60-100%) due to favourable climatic conditions. They thrive in environments with relative humidity of 70-80%, temperature of 20-30°C and substrate pH of 6-8 (Alananbeh *et al*., 2014; Singh *et al*., 2013). The type of substrate used significantly affects both the yield and quality of oyster mushroom cultivation. A substrate in mushroom cultivation refers to a type of lignocellulose material that supports the growth, development and fruiting of mushrooms. As different materials are available in diverse locations, many edible mushroom species can adapt to and thrive on a variety of substrates (Chukwurah *et al*., 2012). However, the nutrient composition of the substrate is a limiting factor for the saprobiotic colonization of cultivated mushrooms. The cultivation of oyster mushrooms is beneficial not only for producing human food, but also for reducing agricultural waste. Agricultural wastes can serve as a substrate for cultivation of *Pleurotus* genus, offering a sustainable alternative to prevent air pollution caused by the burning of waste (Tshinyangu and Hennebert, 1995).

In India, paddy or wheat straw is the most commonly used substrate for the cultivation of oyster mushrooms. In Assam, paddy straw is widely available and mainly used for mushroom cultivation. However, during the summer season, the price of paddy straw increases significantly, leading to higher production costs. Therefore, to reduce the cost of cultivation other suitable agricultural wastes can be utilized by mixing them with paddy straw as the viable alternatives. Other suitable agricultural wastes which are rich in lignocelluloses can be used as a substrate such as sugarcane bagasse, mustard siliqua, mustard stalks, aquatic weeds like water hyacinth, banana pseudostem, sawdust, etc. which generally remain unutilized (Sharma *et al*., 2013). This study aims to assess the best possible mixture combinations to reduce the cost of cultivation, particularly during the period when the cost of rice straw becomes very high

Materials and Methods

The research was conducted in the Mushroom Production Facility Centre within the Department of Plant Pathology, Assam Agricultural University, Jorhat-13, Assam. during December 2021 to July 2022. Six locally available substrates, including rice straw, mustard stalk and siliqua, sugarcane bagasse, water hyacinth, banana pseudostem and sawdust were thoroughly assessed for their suitability in growing *Pleurotus sapidus*. These substrates were obtained from the ICR (Instructional-cum-Research) Farm, Assam Agricultural University, Jorhat campus, as well as the surrounding areas of the Jorhat district. The culture of the mushroom was maintained in Potato dextrose agar (PDA) media which was prepared by following the procedure outlined by Ricker and Ricker (1936). The rice grain was used for the preparation of spawn. The rice grain was thoroughly cleaned to eliminate any damage or infestation causedby pests.

To prepare the spawn, rice grains were soaked in water for 12 hours, followed by boiling, shade-drying, and mixing with 20 grams of $CaCO_3$ and 20 grams of dextrose per kilogram of grains to adjust the pH. The resulting mixture was placed in flasks and subjected to autoclaving for 1.5 hours at 15 lb/sq. inch pressure. For preparation of substrate for oyster mushroom cultivation, the substrates were cut into 3-4 cm long pieces and soaked overnight in clean tap water. The water was drained, and the substrates were chemically sterilized. The treatment consisted of 70 ml formalin and 7.5 g fungicide in 100 litres of water. The Compositions of mixed substrates used for Oyster mushroom cultivation

(*Pleurotus sapidus*) were Rice straw 50% + Sugarcane bagasse 50%, Rice straw 60% + Sugarcane bagasse 40%, Rice straw 50% + Mustard stalk & siliqua 50%, Rice straw 60% + Mustard stalk & siliqua 40%, Rice straw 50% + Water hyacinth 50%, Rice straw 60% + Water hyacinth 40%, Rice straw 50% + Banana Pseudostem 50%, Rice straw 60% + Banana Pseudostem 40%, Rice Straw (Control). For each bed, one kilogram of a substrate (dry weight basis) was used in a polythene bag measuring 60 cm × 40 cm. Before spawning, 25 to 30 holes (5 mm in diameter) were punched in polythene bags. Each bed was seeded with 100 g of spawn. The sterilized substrate was pressed into the bottom of a polythene bag to a depth of 8-10 cm. In the first layer of the substrate, a thin layer of 25 g spawn was sprinkled evenly. This procedure was repeated until the bag was filled with five layers of substrate planked by four layers of spawn and the polythene bag was tied and tagged. The bags were kept in a spawn running room for 15-18 days after 15-18 days of mycelial colonization, the substrates within the bags were enveloped by a densely proliferated, cottony growth of the mushroom fungus. The bags were then opened and transferred to the cropping room. The polythene bags were then removed from the beds by inverting them. The open beds were randomly arranged on three-tier shelves. Water was sprayed to 2-3 times a day. Two mushroom flushes were harvested and the average yield was recorded from each bed.

Observation and data collection

The fruiting bodies of the mushroom were harvested at the appropriate time from the first flush by twisting the stipe before they began to curl upward. Ten numbers of fruiting bodies were collected randomly and brought to the laboratory to determine the cap size, stalk length and stalk girth of fruiting bodies. The yield attributing characters *viz*., time taken for spawn run (days), primordia formation in days and total duration of the experiment, were recorded along with the total yield (g/kg of substrate).

Crude protein content was also assessed by initially analysing the nitrogen content of the mushroom sample as per Crisan and Sands(1978). Using the modified Kjeldahl method described by Jackson *et al*. (1973), nitrogen content (%) in the mushroom sample was estimated. Total phenol was estimated by the Folin-ciocalteu method given by Bray and Thorpe (1954). The Ca and Mg in the digested filtrate were estimated by the Versenate titration method Richard (1954). Using an Atomic Absorption Spectrophotometer Lindsay and Norvell (1978) Zinc and Iron content in Mushroom samples were determined.

Data analysis

To conduct a statistical analysis of the experiment, the Fisher method of analysis of variance was utilized. The significance of variance was determined by comparing computed and tabulated "F" values at a 5 per cent probability level. The percentage values were transformed using the angular transformation method as per Gomez and Gomez (1984). The treatments were also compared using the critical difference (CD) formula.

$$C.D = S.Ed \times t_{0.05} \text{ or } t_{0.01} \text{ for error degrees of freedom.}$$

Where, S.Ed is the standard error of the difference of mean.

The standard error of the differences was calculated by using the following formula

$$S.\ Ed(\pm) = \sqrt{\frac{2 \times \text{Error Mean Sqaure}}{\text{Number of replication}}}$$

The treatments' significance and non-significance at the 5% probability level were calculated by multiplying the S.Ed by the appropriate tabulated value for error degrees of freedom.

Results and Discussion

Influence of different substrates and mixture of substrates on growth parameters and yield attributing characters of *Pleurotus sapidus*

The study revealed that the cap size of the *Pleurotus sapidus* mushroom cultivated on the rice straw substrate, employed as the control for the experiment, reached a maximum of 7.85 cm, followed by water hyacinth at 6.48 cm. The lowest cap size (4.05 cm) was observed in the bags with the sawdust as substrate. The longest stalk length (5.50 cm) of the fruiting body of the mushroom was observed on banana pseudostem substrate whereas the stalk length in the control bedwas 4.23 cm. The shortest stalk length (2.09 cm) was observed on the bed withwater hyacinth as substrate (Table 1). The average stalk girth of the fruiting body was found to be the highest (1.27 cm) on rice straw (control) andthe minimum stalk girth was observed on the bed with sawdust (0.85 cm) as substrate (Fig. 1). Similar results were noted by Dubey *et al*. (2019), who measured the cap diameter of the fruiting body as 5.13 cm on the mushroom bed with rice straw as substrate and the stipe length of the mushroom grown in substrate banana pseudostem was found to be 4.50 cm to 5.25 cm. Hoa and Wang (2015) also found similar results for the trait stalk girth, which is 1.02 cm for *Pleurotus ostreatus* in rice straw substrate. Singh *et al*. (2019) studied the effect of different substrates on

Fig. 1 : Growth of Mushroom in different substrates A. Rice straw (Control), B. Sugarcane bagasse, C. Mustard stalk & siliqua, D. Water hyacinth, E. Banana pseudostem, F. Saw dust and mixed substrates T_1: Rice straw 50% +Sugarcane bagasse 50%, T_2: Rice straw 60% + Sugarcane bagasse 40%, T_3: Rice straw 50% + Mustard stalk & siliqua 50%, T_4: Rice straw 60% + Mustard stalk & siliqua 40%, T_5: Rice straw 50% + Water hyacinth 50%, T_6: Rice straw 60% + Water hyacinth 40%, T_7: Rice straw 50% + Banana Pseudostem 50%, T_8: Rice straw 60% + Banana Pseudostem 40%.

the yield performance of oyster mushrooms (*Pleurotus sajorcaju*) and observed the highest stipe length (4.86 cm) and cap diameter (5.14 cm) in rice straw substrate. Johnnie *et al*. (2023) also found that the rice straw substrate produced the highest fresh and dry weight fruiting bodies of oyster mushroom, followed by sugar cane bagasse, wood shaving and coconut coir. The total number of days required for a full spawn run in the mushroom beds with rice straw, mustard stalk and siliqua as the substrate was found to be thelowest of 19 days among other substrates. However, a complete spawn run in sawdust substrate required an average of 23.50 days. Compared to other substrates in the experiment, rice straw demonstrated a shorter time from their emergence to harvest (47.75 days) as compared to the other substrates while the sawdust recorded the longest duration of (54 days) (Table 1). Kumar *et al.* (2019) found that the time taken for spawn run and primordia formation for rice straw substrate, with an average of 17.20 and 21.10 days, respectively. The maximum time taken for spawn run and primordia formation was observed in the substrate sawdust, with an average of 21.00 and 25.10 days, respectively. The study also revealed that the average time taken for primordia formation was minimum for mushroom beds with rice straw as substrate (22.00 days) and maximum for the mushroom beds with sawdust as substrate (29.00 days). From a yield perspective, the average yield of the mushroom bed cultivated on mustard stalk and siliqua was found to be superior (1317.50 g/kg of the substrate) compared to the control (1290.00 g/kg of the substrate) (Table 1) and this difference was also found to be statistically insignificant. The lowest yield was obtained from the beds with the water hyacinth (260.00 g/kg of the substrate) and sawdust (481.25 g/kg of the substrate) as substrates. Raghav *et al*. (2016) investigated the various substrates for *Pleurotus florida* and the highest yield was observed in the mustard stalk and siliqua substrate. (1750.00g/ bag) followed by rice straw (1090.00g/ bag), which aligns with our present findings. Nasir *et al*. (2021) found lower production in *Pleurotus sapidus* when cultivated in a sawdust substrate. Similarly, Jamil *et al*. (2019) reported that *P. sapidus* exhibited superior production capabilities yielding the maximum compared to other *Pleurotus* species such as *P. eryngii* and *P. sajor-caju*.

The fruiting body obtained from the *Pleurotus sapidus* mushroom bed cultivated on T_7 (Rice straw 50% + Banana Pseudostem 50%) had the largest average cap size of 8.25 cm, followed by T_8 (Rice straw 60% + Banana Pseudostem 40%) of average cap size of 8.20 cm although both treatments were at par statistically. In T_2 (Rice straw 60% + Sugarcane bagasse 40%), a minimum cap size of 4.19 cm was noted. Similar findings were obtained by Muswati *et al*. (2021), who evaluated the effect of different substrate combinations on the growth parameters of *Pleurotus ostreatus* and recorded the largest cap diameter cotton waste followed by (baobab fruit shell + cotton husks, 1:1 combination). It was revealed from Table 2 that the average stalk length of a single fruiting body of *Pleurotus sapidus* cultivated on T_2 (Rice straw 60% + Sugarcane bagasse 40%) was found to be higher (4.355 cm) compared to control T_9 (Rice Straw) of 4.23 cm followed by T_1 (Rice straw 50% +Sugarcane bagasse 50%) of 4.27 cm. The shortest average stalk length was observed in the treatment in T_6 (Rice straw 60% + Water hyacinth 40%), which was 3.33 cm. Singh *et al*. (2017) reported similar findings for the trait of stalk girth in *Pleurotus* sp. on mixed substrate, where the treatment (rice straw + corn cob) yielded a higher value. The fruiting bodies recovered from the mushroom bed in T_1 (Rice straw 50% +Sugarcane bagasse 50%) exhibited the largest average stalk girth (0.968 cm), whereas T_5 (Rice straw 50% + Water hyacinth 50%) showed the minimum stalk girth of 0.68 cm. Similar findings were observed by Neupane *et al*. (2018), who recorded a cap size of 8.11 cm in the oyster mushroom (*Pleurotus florida*) and also recorded a stalk length of 6.07 cm when cultivated in the mixture of 50% rice straw and wheat straw. The minimum days required for the spawn run were recorded in T_5 (Rice straw 50% + Water hyacinth 50%) with 16.5 days, which was found superior to all the other treatments. The maximum number of days for spawn run was found in the treatment T_2 (Rice straw 60% + Sugarcane bagasse 40%) and T_9 (Rice Straw) of 19 days. The minimum number of days required for primordial production was found lowest in T_9 (Rice straw) at 22 days and highest in T_8 (Rice straw 60% + Banana pseudostem 40%) at 27.5 days. Kumar *et al*. (2019) experimented on the yield parameters of Oyster mushrooms (*Hypsizygus ulmarius*) and they found that the average minimum spawn run days for the treatments of (Paddy straw + Water hyacinth) and (Paddy straw + Banana pseudostem) were 16.7 and 17.30 days, respectively. They also observed that the treatment of (Paddy straw + Water hyacinth) required a minimum of 20.70 days, on average, for the primordial formation.

The minimum total duration of the experiment was observed by T_5 (Rice straw 50% + Water hyacinth 50%) *i.e.*, 46.75 days which shows superiority among all the other treatments. T_4 (Rice straw 60% + Mustard stalk & siliqua 40%) and T_8 (Rice straw 60% + Banana Pseudostem 40%) were significantly found *at par* of 51 days for the maximum duration. Similarly, Bilal and Asgher (2016) also investigated the total duration of the experiment which was found to be 48.90 days for the treatment (Paddy straw + Banana pseudostem). It was observed that the average yield of the mushroom bags cultivated on T_3 (Rice straw 50% + Mustard stalk & siliqua 50%) was found to be a maximum (1432.50 g/ kg of substrate) followed by T_9 (Rice Straw) of 1290 g/ kg of substrate. The lowest yield was observed in T4 (Rice straw 60% + Mustard stalk & siliqua 40%) of 247.50 g/ kg of substrate. Similar results were obtained by Devi *et al*. (2015), who investigated the yield parameters in substrate mixture and found a better yield in rice straw followed by the treatment of Paddy straw and Banana leaf with a substrate combination.

Effect of different substrates and substrate mixture on total protein and phenol content of *Pleurotus sapidus*

The total protein content of *P. sapidus* grown in banana pseudostem as substrate was found to be higher (26.02%) compared to the control (25.26%) followed by the substrate water hyacinth (25.48%) (Fig. 2). Mushrooms cultivated on sawdust exhibited the lowest total protein content (22.74%). The total phenol content of *P. sapidus* grown on rice straw (control) substrate was found to be the highest (10.55mg catechol /g dry wt). Whereas, the lowest amount of total phenol was observed from the mushroom grown on sawdust (8.213 mg catechol /g dry wt) as substrate. Similar varied results were also observed by Rashid *et al*. (2016), who evaluated the protein content of *Pleurotus florida* in various sawdust substrate Tirkey *et al*. (2017) found varied amounts of total protein content of the oyster mushroom

Table 1 : Influence of different substrates on growth parameters and yield attributing characters of *Pleurotus sapidus*.

Treatments	Cap size (cm)	Stalk length (cm)	Stalk girth (cm)	Spawn run (days)	Primordia formation in days	Total duration of experiment (days)	Yield (g/kg of substrate)
T_1: Rice straw (control)	7.85[a]	4.23[bc]	1.27[a]	19.00[c]	22.00[d]	47.75[c]	1290.00[a]
T_2: Sugarcane bagasse	5.93[bc]	4.47[b]	1.25[a]	21.75[b]	26.00[b]	52.50[a]	728.75[b]
T_3: Mustard stalk & siliqua	5.61[c]	3.81[cd]	1.14[a]	19.00[c]	26.50[b]	50.00[b]	1317.50[a]
T_4: Water hyacinth	6.48[b]	2.09[e]	0.90[b]	20.50[bc]	22.50[d]	48.50[bc]	260.00[c]
T_5: Banana pseudostem	5.59[c]	5.50[a]	1.08[ab]	19.50[c]	24.00[c]	49.75[bc]	700.00[b]
T_6: Saw dust	4.05[d]	3.30[d]	0.85[b]	23.50[a]	29.00[a]	54.00[a]	481.25[bc]
S.Ed (±)	**0.407**	**0.283**	**0.109**	**0.737**	**0.505**	**0.977**	**120.022**
CD (P=0.05)	**0.867**	**0.603**	**0.234**	**1.570**	**1.077**	**2.083**	**255.647**

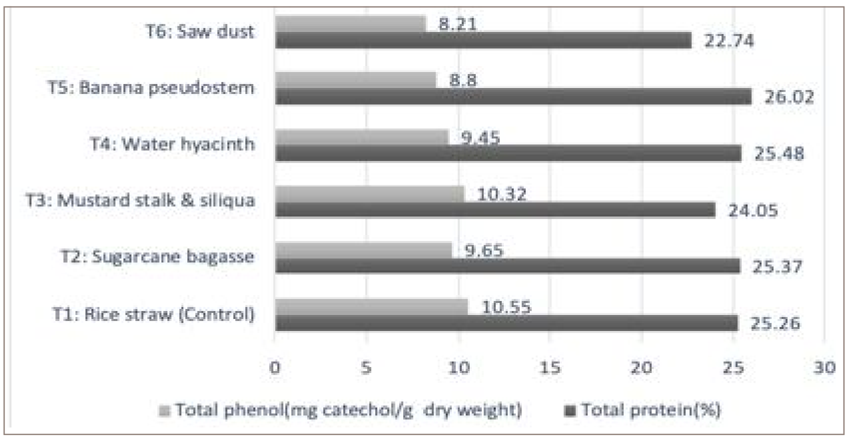

Fig. 2 : Effect of different substrates on total protein and total phenol content of *Pleurotus sapidus*.

(*Pleurotus florida)* grown on different substrate and they found 27.56% total protein from the mushroom grown on banana pseudostem substrate. Regarding the total phenol content, Mansuri *et al.* (2017) also found similar observations in *Pleurotus sapidus* (9.64 mg catechol /g dry wt).

The protein content of *P. Sapidus* grown on the substrate combination of T_7 (Rice straw 50% + Banana Pseudostem 50%) was found to be the best (25.89%) followed by T_8 (Rice straw 60% + Banana Pseudostem 40%) (25.29%). The lowest protein percentage (24.17%) was found in T_3 (Rice straw 50% + Mustard stalk & siliqua 50%) (Fig. 3). The total phenol content was maximum (11.70 mg catechol /g dry wt) from the mushroom grown on the substrate combination of T_6 (Rice straw 60% + Water hyacinth 40%) followed by T_2 (Rice straw 60% + Sugarcane bagasse 40%) with 10.75 mg catechol /g dry wt (Fig. 3). The minimum amount of phenol content was observed from T_7 (Rice straw 50% + Banana Pseudostem 50%) (9.68 mg catechol/g dry wt). Yang *et al.* (2002) experimented with shiitake and oyster mushrooms to analyze the total phenol content and observed varying amounts of total phenol (6 to 15 mg catechol/g dry wt) of mushroom, which varied depending on the species and substrate used. These findings were similar to our present study. A similar observation was also found by Bandopadhyay *et al*. (2013), where they found varied amounts of total protein content (16% to 25%) from the oyster mushroom grown on various substrates.

Effect of different substrates and substrate mixtures on total Ca, Mg, Zn and Fe content of *Pleurotus sapidus*

The calcium content of the fruiting bodies was higher in sugarcane bagasse substrate (475.07 mg/100g dry weight) followed by Mustard stalk & siliqua (406.04 mg/100g dry wt) and control *i.e.* rice straw substrate (320.84 mg/100g dry weight) (Table 3). The Magnesium content of the fruiting bodies cultivated in mustard siliqua & stalk was 125.06 mg/100g dry weight) followed by Sugarcane bagasse (105.93 mg/100 g dry wt) and control (95.12 mg/100 g dry wt). The Zinc content of mustard stalk & siliqua substrate was found significantly higher (3.91 mg/100 g dry wt) than control (3.82 mg/100g dry wt), which was followed by Sugarcane bagasse (3.53 mg/100 g dry wt). The total Iron content of the mushroom cultivated on mustard stalk & siliqua substrate was found higher

Table 2 : Influence of mixed substrates on growth parameters and yield attributing characters of *Pleurotus sapidus.*

Treatments	Cap size (cm)	Stalk length (cm)	Stalk girth (cm)	Spawn run (days)	Primordia formation in days	Total duration of experiment (days)	Yield (g/kg of substrate)
T_1: Rice straw 50% +Sugarcane bagasse 50%	4.94^{d}	4.27^{ab}	0.96^{a}	17.50^{d}	23.50^{cd}	47.00^{d}	737.50^{d}
T_2: Rice straw 60% + Sugarcane bagasse 40%	4.19^{d}	4.35^{a}	0.76^{bc}	19.00^{a}	24.50^{c}	49.75^{b}	900.00^{c}
T_3: Rice straw 50% + Mustard stalk & siliqua 50%	6.21^{b}	3.85^{bcd}	0.92^{a}	18.00^{c}	24.00^{cd}	48.50^{c}	1432.50^{a}
T_4: Rice straw 60% + Mustard stalk & siliqua 40%	5.91^{c}	4.08^{abc}	0.90^{a}	17.50^{d}	23.50^{cd}	51.00^{a}	247.50^{b}
T_5: Rice straw 50% + Water hyacinth 50%	4.92^{d}	3.53^{de}	0.68^{c}	16.50^{f}	23.00^{cd}	46.75^{d}	967.50^{c}
T_6: Rice straw 60% + Water hyacinth 40%	4.95^{d}	3.33^{e}	0.75^{bc}	17.00^{e}	24.00^{bc}	48.75^{bc}	901.25^{c}
T_7: Rice straw 50% + Banana Pseudostem 50%	8.25^{a}	4.00^{abcd}	0.85^{ab}	17.50^{e}	25.50^{ab}	49.00^{bc}	637.50^{de}
T_8: Rice straw 60% + Banana Pseudostem 40%	8.20^{a}	3.72^{cde}	0.85^{ab}	18.50^{b}	27.50^{a}	51.00^{a}	550.00^{e}
T_9: Rice Straw	7.85^{a}	4.23^{ab}	1.27^{a}	19.00^{a}	22.00^{cd}	47.75^{d}	1290.00^{b}
S.Ed (±)	**0.422**	**0.293**	**0.030**	**0.206**	**0.545**	**0.496**	**77.231**
CD (P=0.05)	**0.871**	**0.604**	**0.063**	**0.425**	**1.123**	**1.023**	**159.097**

(53.75 mg/100g dry wt) compared to the control (51.93 mg/100 g dry weight). Muthangya *et al.* (2013) also estimated Fe and Zn of *Pleurotus* sp. and observed similar results 56.24 mg/100g and 3.59 mg/100g, respectively in the sugarcane bagasse substrate. Wang *et al.* (2015) found 521.28 mg/100g dry wt of Ca in Sugarcane bagasse. Our results followed asimilar trend to the obtained findings. Similarly, Paul *et al.* (2015) evaluated the Fe content of *Pleurotus florida* in different sawdust substrates and found that the Fe content varied from 40 to 44 mg/100g with different substrates. Salami *et al.* (2017) also evaluated the nutrient and mineral content of *Pleurotus florida* cultivated in four lignocellulosic substrates and recorded the calcium contents in the range of 342 to 410 mg/100g.

The fruiting bodies grown in T_1 (Rice straw 50% + sugar cane bagasse 50%) had the highest calcium content at 397.875 mg/100g dry wt, followed by T_2 (Rice straw 60% + Sugarcane bagasse 40%) at 374.93 mg/100g dry wt and T_3 (Rice straw 50% + Mustard stalk & siliqua 50%) at 362.68 mg/100g dry wt. The lowest calcium content was found in T_5 (Rice straw 50% + Water hyacinth 50%) at 291.31 mg/100g dry wt (Table 4). The fruiting bodies grown in T_3 (Rice straw 50% + Mustard stalk & siliqua 50%) had the highest magnesium content at 110.18 mg/100g dry wt, followed by T_4 (Rice straw 60% + Mustard stalk & siliqua 40%) at 107.25 mg/100g dry wt. The lowest magnesium content was found in T5 (Rice straw 50% + Water hyacinth 50%) at 88.75 mg/ 100g dry wt. The highest zinc content was found in T_9 (Rice Straw) at 3.82 mg/100g dry wt, followed by T_3 (Rice straw 50% + Mustard stalk & siliqua 50%) at 3.81 mg/100g dry wt. The lowest zinc content was found in T_5 (Rice straw 50% + Water hyacinth 50%) at 3.29 mg/ 100g dry wt. The mushroom cultivated in T_1 (Rice straw

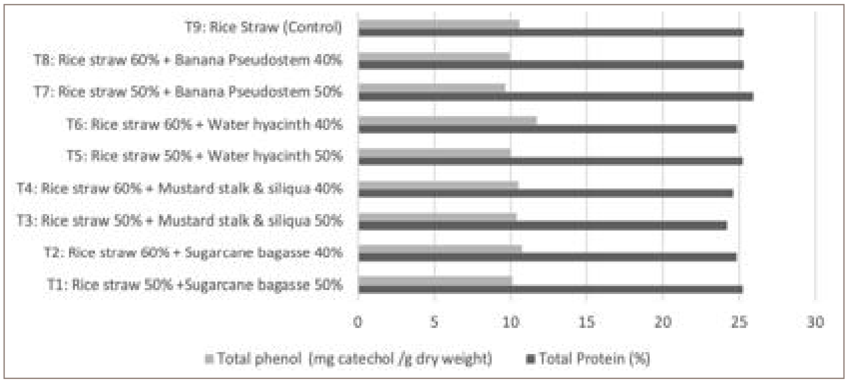

Fig. 3 : Effect of mixed substrates on total protein and total phenol content of *Pleurotus sapidus*.

50% + Sugarcane bagasse 50%) had the highest total iron content at 54.21 mg/100g dry wt, followed by T_3 (Rice straw 50% + Mustard stalk & siliqua 50%) at 53.56 (Rice straw 50% + Banana Pseudostem 50%) mg/100g dry wt. The lowest iron content was recorded in T_7 at 49.50 mg/100g dry wt. Ahmed *et al.* (2009) also found that the calcium content in *Pleurotus* sp cultivated in wheat straw + paddy straw as substrate was 304 mg/100g and 310 mg/100g, while grown in

Table 3 : Effect of different substrates on total Ca, Mg, Zn and Fe content of *Pleurotus sapidus.*

Treatments	Calcium (mg/100g dry weight)	Magnesium (mg/100g dry weight)	Zinc (mg/100g dry weight)	Iron (mg/100g dry weight)
T_1: Rice Straw (Control)	320.84^{d}	95.12^{c}	3.82^{a}	51.93^{c}
T_2: Sugarcane bagasse	475.07^{a}	105.93^{b}	3.53^{b}	52.57^{b}
T_3: Mustard stalk & siliqua	406.04^{b}	125.06^{a}	3.91^{a}	53.75^{a}
T_4: Water hyacinth	265.65^{f}	82.12^{e}	2.86^{d}	49.56^{d}
T_5: Banana pseudostem	308.15^{e}	85.25^{d}	2.90^{d}	47.64^{e}
T_6: Saw dust	360.96^{c}	61.97^{f}	3.36^{c}	52.12^{bc}
SEd (±)	**0.765**	**1.349**	**0.061**	**0.258**
CD (P=0.05)	**1.631**	**2.874**	**0.132**	**0.551**

Table 4 : Effect of different mixed substrates on total Ca, Mg, Zn and Fe content of *Pleurotus sapidus.*

Treatments	Calcium (mg/100g dry weight)	Magnesium (mg/100g dry weight)	Zinc (mg/100g dry weight)	Iron (mg/100g dry weight)
T_1: Rice straw 50% +Sugarcane bagasse 50%	397.87^{a}	101.18^{c}	3.63^{abc}	54.21^{a}
T_2: Rice straw 60% + Sugarcane bagasse 40%	374.93^{b}	98.50^{d}	3.70^{ab}	53.75^{ab}
T_3:Rice straw 50% + Mustard stalk & siliqua 50%	362.68^{c}	110.50^{a}	3.81^{a}	53.56^{ab}
T_4: Rice straw 60% + Mustard stalk & siliqua 40%	355.06^{d}	107.25^{b}	3.37^{d}	52.75^{bc}
T_5: Rice straw 50% + Water hyacinth 50%	291.31^{h}	88.75^{g}	3.29^{d}	50.72^{de}
T_6: Rice straw 60% + Water hyacinth 40%	297.00^{g}	90.62^{ef}	3.575^{bc}	51.75^{cd}
T_7: Rice straw 50% + Banana Pseudostem 50%	313.87^{f}	90.00^{f}	3.360^{d}	49.50^{e}
T_8: Rice straw 60% + Banana Pseudostem 40%	321.43^{e}	91.18^{e}	3.450^{cd}	51.25^{d}
T_9: Rice Straw (Control)	321.87^{e}	95.12^{e}	3.820^{a}	51.93^{bc}
SEd (±)	**0.472**	**0.402**	**0.093**	**0.618**
CD (P=0.05)	**0.973**	**0.829**	**0.193**	**1.274**

soybean straw + paddy straw as substrate.

This experiment revealed that the highest yield was achieved when utilizing mustard stalk and siliqua as substrates, followed by the rice straw substrate. However, the combination of 50 per cent rice straw and 50 per cent mustard stalk & siliqua exhibited the best results among all substrate combinations, with respect to yield. It can be inferred that mustard stalk and siliqua might be apromising alternative to rice straw for mushroom cultivation. Furthermore, these substrates can be effectively utilized in combination with other substrate to achieve better yield. In the absence of mustard stalk and siliqua substrates, sugarcane bagasse emerges as a viable option for mushroom cultivation.

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