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Evaluation of different lignocellulosic-wastes and their combinations on growth and yield of Oyster mushroom (*Pleurotus ostreatus*)

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Abstract

Lignocellulose wastes are generated in huge amounts by various sectors like agriculture, forestry, and industry but only a small portion of these wastes are utilized and a major portion is left unused. In this study, seven different lignocellulosic wastes and their combinations in different percentages were determined for the growth and yield of *Pleurotus ostreatus*. The maximum growth and yield of *P. ostreatus* were observed on a substrate made of rice straw, with a total yield of 399.70 gm per kg of substrate. The least growth and yield were recorded on a substrate made of wood flakes and sugarcane bagasse (80% + 20%), with a total yield of 13.54 gm per kg of substrate. Rice straw showed the highest biological efficiency (B.E) of 39.40, whereas wood flakes and sugarcane bagasse (80% + 20%) had the lowest B.E. of 1.35. Other substrates had a moderate effect, and citronella bagasse (*Cymbopogon nardus*), which was used as a substrate for the first time, gave a biological efficiency of 39.39 gm per kg substrate. The results showed a significant effect of substrates on mean yield and biological efficiency. Our study revealed that lignocellulosic waste can be profitably utilized for mushroom cultivation and could be one of the most economical and eco-friendly techniques.

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INTRODUCTION

Lignocellulosic wastes constitute a major portion of plant biomass and are generated in huge amounts annually in various sectors like agriculture, forestry and the food industry. These wastes consist of rich organic compounds and are worthy of being recovered and transformed[1]. Despite their usability, only a small fraction of the total waste is utilized and a major portion is left unused. Some of them are disposed in open dumps or burnt, resulting in emission of black carbon causing serious environmental pollution. Therefore, the utilization of these lignocellulosic wastes into profitable products has become one of the major objectives at present. Mushroom cultivation using lignocellulosic wastes could be one of the most economical and eco-friendly techniques for the conversion of these wastes into profitable products. Mushrooms are an excellent source of protein that can be a substitute for meat for vegetarians. Mushrooms contain about 85%–95% water, 3% protein, 4% carbohydrate, 0.1% fat, 1% minerals and vitamins[2].

Amongst various mushrooms, *Pleurotus* spp. (Oyster mushroom) can be cultivated on a wide range of lignocellulosic substrates. Therefore, cultivation of this mushroom species needs to be popularized so that unused lignocellulosic waste can be properly utilized for mushroom production. Oyster mushrooms are widely consumed worldwide and are regarded as a nutritious food option due to both its nutritional and medicinal properties. Over the past few decades, there has been a global trend toward the cultivation of significantly greater numbers of oyster mushrooms[3,4]. After button mushrooms, oyster mushrooms are the most common type of mushroom consumed[5]. The cultivation process of oyster mushrooms is cost-effective because of its easy cultivation techniques using substrates that are locally available[6,7]. Various agro-wastes have been utilized to cultivate the edible mushroom out of which paddy straw and wheat straw are the most common. The other substrates include sawdust, sugarcane, corn cob, corn stalks, leaves, and the pseudo stem of banana[8–10].

This study aims to investigate the yield and biological efficiency of the selected substrates and their combination on the productivity of *Pleurotus ostreatus* (Jacq.) P. Kumm through the usage of various locally accessible and unused lignocellulosic waste for its cultivation.

MATERIALS AND METHODS

Collection of substrates

Seven agricultural and plant-based lignocellulosic wastes were collected from agro-based and paper and pulp industries in Guwahati, Assam, India. The seven individual substrates and their combinations were T1 = rice straw, T2 = sugarcane bagasse, T3 = wood chips, T4 = wood flakes, T5 = citronella bagasse [*Cymbopogon nardus* (L.) Rendle], T6 = sawdust, T7 = leaf litter [*Monoon longifolium* (Sonn.) B.Xue & R.M.K. Saunders], and the combinations includes: T8 = rice straw + sugarcane bagasse (50% + 50%), T9 = Rice straw + wood chips (60% + 40%), T10 = wood flakes + sawdust (50% + 50%), T11 = wood flakes + sugarcane bagasse (80% + 20%), T12 = wood flakes + sugarcane bagasse + woodchips (35% + 35% + 30%), T13 = rice straw + wood flakes + sawdust (50% + 40% + 10%) and T14 = citronella bagasse + sugarcane bagasse + wood flakes + wood chips (25% each).

Preparation of substrate

Each bag contained 1 kg of the collected materials as substrates. Firstly, the collected materials were cut into small pieces of 2–3 cm and thoroughly washed with normal water followed by surface sterilization with hot water treatment. After rinsing, the substrates were separately packed in polypropylene bags of 45 cm × 30 cm, autoclaved and then allowed to cool.

Preparation of mushroom beds and spawning

The spawn of *Pleurotus ostreatus* was collected from Assam Agriculture University of Kahikuchi campus, Guwahati, India. After cooling, the substrates were mixed with gram flour (8 g/kg substrate) and stacked in three layers in a separate clean polypropylene bag. Between each stacking layer, spawning was done on the entire surface of the beds. A number of holes (measuring ca. 2 mm in diam.) were created to maintain an aerobic condition.

Incubation

The bags were incubated by hanging them in a closed room with ventilation kept open throughout along with an exhaust fan, at a temperature ranging from 25–28.5 °C. Water was sprinkled regularly to maintain moisture.

Harvesting and determination of yield

After complete colonization, longitudinal slits were made to facilitate the proper development of fruiting bodies. Harvesting of the fruiting bodies was done on the fourth day after the appearance of pinheads. The mycelial growth, complete colonization, primordial initiation, and yield in terms of biological efficiency were recorded. Biological Efficiency (B.E) was calculated as the percentage of yield of fresh mushrooms in relation to the dry weight of the substrate as given by Chang & Miles[11].

Biological efficiency (B.E.) in % = yield of fresh mushroom (in gm)/ total weight of the dry substrate (i.e., 1,000 gm) × 100

Statistical analysis

Statistical analyses were performed for the comparison of treatment means of the first mycelial growth, complete mycelial colonization, pin-head initiation, the time required for first harvesting, yield and biological efficiency. The Shapiro-Wilk Normality Test was pre-performed to check for the goodness of fit normality of the data. Accordingly, after the log transformation of the original data, it eventually follows the assumption of normality. After that, the transformed data are implemented in a Completely Randomized Design with fourteen different substrates with three replications each and the analysis of variance (one-way ANOVA) along with a multiple comparison test viz. Least Significant Difference for comparison of the pairs of treatments using the RStudio version 1.2.1335. The primary software packages used in the analyses are agricolae, DescTools, ggplot2, tidyverse and dplyr.

RESULTS

In this study, *Pleurotus ostreatus* growth and yield were determined using seven lignocellulosic wastes and their combinations in varied proportions. The result showed that first mycelial growth in different substrates and their combinations ranged from 1.00–2.67 d (Table 1). The lowest day of first mycelial growth was observed on T5 and T14 substrates. Among all the substrates, T6 showed a significantly higher time for the appearance of first mycelial growth. Further, the time required for the appearance of the first mycelial growth was similar in T2, T3, T7 and T11. Similar trends were also observed in the other substrates as well. The study indicated that there was a significant effect of substrates on mean first mycelial growth (p-value = 0.04). Analysis using the Least Significant Difference (LSD) showed that the T6 substrate took a longer mean time for first mycelial growth (2.67 d).

From Table 1, it was observed that the completion of mycelial running in different substrates and their combinations ranged from 14.00–28.67 d. The lowest days of completion of mycelial running were observed on the T8 substrate i.e., 28.67 d. Again, among all substrates, T10 showed a significantly higher mean first completion of mycelial running (28.67 d) and this observation is also supported by the LSD analysis. The T1, T2, T4, T5, T9 and T14 substrates were not significantly different from each other and similar result was observed for the remaining substrates as well. There was a significant effect of substrates on mean complete mycelial running (p-value ≤ 2e-16).

Table 1. Effect of substrates on the mycelial growth of *Pleurotus ostreatus*.

Substrates	First mycelial growth in the substrate (d)	Time required for completion of mycelial running (d)
T1 = Rice straw	1.67abc	16.33de
T2 = Sugarcane bagasse	2.33ab	15.67ef
T3 = Wood chips	2.33ab	26.00b
T4 = Wood flakes	1.67abc	17.33de
T5 = Citronella bagasse	1.00c	16.33de
T6 = Sawdust	2.67a	20.00c
T7 = Leaf litter	2.33ab	24.67b
T8 = Rice straw + sugarcane bagasse (50% each)	1.33bc	14.00f
T9 = Rice straw + wood chips (60% + 40%)	1.33bc	17.33de
T10 = Wood flakes + sawdust (50% each)	1.67abc	28.67a
T11 = Wood flakes + sugarcane bagasse (80% + 20%)	2.33ab	18.00cd
T12 = Wood flakes + sugarcane + wood chips (35% + 35% + 30%)	1.33bc	15.33ef
T13 = Rice straw + wood flakes + sawdust (50% + 40% + 10%)	1.33bc	18.33cd
T14 = Citronella bagasse + sugarcane bagasse + wood flakes + wood chips (25% each)	1.00c	17.00de
Significance	*	***
CV (%)	82.42	1.50

Treatments followed with the same letter are not significantly different by LSD (Least Significance Difference) test at a 5% level of significance.

First pinhead initiation in different substrates and their combinations ranged from 18.67–34.00 d (Table 2). The lowest days of the first pinhead initiation were observed on T2 and T8 substrates. Substrates T3, T7, and T10 showed considerably longer mean initial pinhead initiation times than the other substrates. The remaining substrates were not significantly different from each other in terms of first pinhead initiation. However, there was significant effect of the substrates on the first pinhead initiation (p-value ≤ 2e-16). Similar to previous studies, LSD analysis showed that the T10 substrate took the longest duration for first pinhead initiation among all substrates (34.00 d).

The first harvest in different substrates ranged from 21.67–37.00 d (Table 2). T2 and T8 substrates needed the least time (21.67 d) for the first harvest out of all the substrates. However, T3, T7, and T10 substrates required much more time than other substrates. Similar to the previous finding, T10 had a mean first harvest of 37.00 d, which was longer than the other substrates (p-value = 2e-16). After the pin head initiation, harvesting was done within a week (Fig. 1). A total of four harvests were made depending upon the yield on different substrates. The results showed that the first harvest in different substrates and their combinations ranged from 13.50–222.43 gm (Table 3).

T4 and T11 substrates had the lowest first harvest yield of 13.50 gm, whereas the T5 substrate had the highest mean yield. T3 and T10 substrates yielded similarly in the first harvest. Other substrates had similar first-harvest yields. The study indicated that there was a significant effect of substrates on the mean yield of the first harvest (p-value ≤ 2e-16). Least Significant Difference (LSD), analysis showed that T5 substrates produced the highest mean yield of the first harvest (i.e., 222.43 gm).

The yield of the second harvest ranged from 5.63–203.97 gm (Table 2). The lowest yield of the second harvest was observed on T2 and T4 substrates i.e., 5.63 and 8.55 gm, respectively.

Table 2. Effect of different substrates on first pin-head initiation and the time required for the first harvest.

Substrates	Time required for first pin- head initiation (d)	Time required for first harvesting (d)
T1 = Rice straw	23.33b	26.33b
T2 = Sugarcane bagasse	18.67d	21.67d
T3 = Wood chips	33.33a	36.33a
T4 = Wood flakes	22.67bc	24.67bc
T5 = Citronella bagasse	23.33b	26.33b
T6 = Sawdust	20.00d	23.00d
T7 = Leaf litter	33.00a	36.00a
T8 = Rice straw + sugarcane bagasse (50% each)	18.67d	21.67d
T9 = Rice straw + wood chips (60% + 40%)	22.00bc	25.00bc
T10 = Wood flakes + sawdust (50% each)	34.00a	37.00a
T11 = Wood flakes + sugarcane bagasse (80% + 20%)	21.67c	24.67c
T12 = Wood flakes+ sugarcane + wood chips (35% + 35% + 30%)	20.00d	23.00d
T13 = Rice straw + wood flakes + sawdust (50% + 40% + 10%)	22.67bc	25.67bc
T14 = Citronella bagasse + sugarcane bagasse + wood flakes + wood chips (25% each)	22.00bc	25.00bc
Significance	***	***
CV (%)	3.98	3.53

Treatments followed with the same letter are not significantly different by LSD (Least Significance Difference) test at a 5% level of significance.

Table 3. Effect of different substrates and substrate combinations on yield of *Pleurotus ostreatus*.

Substrates	Weight of the fruiting bodies (in gm)				Net weight (in gm)
	1st harvest	2nd harvest	3rd harvest	4th harvest	
T1 = Rice straw	131.67c	163.33b	90.00b	14.70b	399.70a
T2 = Sugarcane bagasse	14.86ij	8.55i	–	–	23.41j
T3 = Wood chips	63.17e	22.84g	–	–	86.01g
T4 = Wood flakes	13.50j	5.63i	3.33d	–	22.45j
T5 = Citronella bagasse	222.43a	123.73c	47.75c	–	393.90b
T6 = Sawdust	161.56b	15.20h	–	–	176.76d
T7 = Leaf litter	23.33h	13.05h	–	–	36.38i
T8 = Rice straw + sugarcane bagasse (50% each)	50.73f	77.51d	–	–	128.24f
T9 = Rice straw + wood chips (60% + 40%)	17.56i	–	–	–	17.56k
T10 = Wood flakes + sawdust (50% each)	65.81e	71.74e	–	–	137.55a
T11 = Wood flakes + sugarcane bagasse (80% + 20%)	13.54j	–	–	–	13.54l
T12 = Wood flakes + sugarcane + wood chips (35% + 35% + 30%)	53.23f	25.12g	–	–	78.35h
T13 = Rice straw + wood flakes + sawdust (50% + 40% + 10%)	83.55d	203.97a	104.97a	–	392.49b
T14 = Citronella bagasse + sugarcane bagasse + wood flakes + wood chips (25% each)	32.34g	66.09f	106.80a	66.67a	271.90c
Significance	***	***	***	***	***
CV (%)	3.36	3.30	5.58	5.66	1.16

Treatments followed with the same letter are not significantly different by LSD (Least Significance Difference) test at a 5% level of significance.

Fig. 1 Growth of *Pleurotus ostreatus* on different substrates (a) T1 = rice straw, (b) T2 = sugarcane bagasse, (c) T3 = wood chips, (d) T4 = wood flakes, (e) T5 = citronella bagasse (*Cymbopogon nardus*), (f) T6 = sawdust, (g) T7 = leaf litter (*Monoon longifolium*), (h) T8 = rice straw + sugarcane bagasse (50% + 50%), (i) T9 = rice straw + wood chips (60% + 40%), (j) T10 = wood flakes + sawdust (50% + 50%), (k) T11 = wood flakes + sugarcane bagasse (80% + 20%), (l) T12 = wood flakes + sugarcane bagasse + woodchips (35% + 35% + 30%), (m–n) T13 = rice straw + wood flakes + sawdust (50% + 40% + 10%), and T14 = Citronella bagasse + sugarcane bagasse + wood flakes + wood chips (25% each).

Among the substrates, T13 a showed significantly higher mean yield in the second harvest (203.97 gm). Similar to the previous observations, there was a significant effect of substrates on the mean yield of the second harvest (p-value = 1.01e-0.5).

The yield of the third harvest ranged from 3.33–106.80 gm (Table 3). The lowest yield in the third harvest was observed on the T4 substrate i.e., 3.33 gm, while the highest yield in the third harvest was recorded in T13 and T14 substrates. The results revealed a significant effect of substrates on the mean yield of the third harvest (p-value = 6.88e-11). However, LSD analysis showed that the T14 substrate gave the highest mean yield in the third harvest (106.80 g).

T1 and T14 substrates yielded 14.70 and 66.67 gm in the fourth harvest (Table 3). The yield of the total harvest in different substrates and their combinations ranged from 13.54–399.70 gm. T11 substrate had the lowest harvest yield of 13.54 gm. T1 and T5 substrates had higher average harvest yields, but T1 had the highest overall yield (399.70 gm).

The effect of different substrates and their combination on the yield of *Pleurotus ostreatus* was determined in terms of biological efficiency. From Table 4, it was observed that biological efficiency ranged from 1.35%–39.40%. The lowest biological efficiency was observed in T11 and the highest was that on T1, respectively. The higher the total yield, the higher the biological efficiency. It was observed that there was a significant effect of substrates on the mean biological efficiency of substrates and their combinations (p-value ≤ 2e-16). Using Least Significant Difference (LSD) analysis, the additional study revealed that T1 was connected to the highest mean biological efficiency (39.40%). In the present study, the highest biological efficiency of *P. ostreatus* was observed on Straw (39.40%) followed by Citronella (39.39%) and the T13 substrate (rice straw 50%, wood flakes 40%, and sawdust 10%) (39.25%). The lowest B.E. of 1.35% was observed on wood flakes (80%) plus Sugarcane bagasse (20%) substrate combination.

DISCUSSION

The choice of substrate significantly influenced the yield of *Pleurotus ostreatus*. In our study, most of the substrates used for

Table 4. Yield of *Pleurotus ostreatus* in terms of biological efficiency.

Substrates	Biological efficiency (%)
T1 = Rice straw	39.40a
T2 = Sugarcane bagasse	2.34j
T3 = Wood chips	8.60g
T4 = Wood flakes	2.25j
T5 = Citronella bagasse	39.39b
T6 = Sawdust	17.68d
T7 = Leaf litter	3.64i
T8 = Rice straw + sugarcane bagasse (50% each)	12.82f
T9 = Rice straw + wood chips (60%+40%)	1.76k
T10 = Wood flakes + sawdust (50% each)	13.76a
T11 = Wood flakes + sugarcane bagasse (80% + 20%)	1.35l
T12 = Wood flakes + sugarcane + wood chips (35% + 35% + 30%)	7.83h
T13 = Rice straw + wood flakes + sawdust (50% + 40% + 10%)	39.25b
T14 = Citronella bagasse + sugarcane bagasse + wood flakes + wood chips (25% each)	27.19c
Significance	***
CV (%)	1.16

Treatments followed with the same letter are not significantly different by LSD (Least Significance Difference) test at a 5% level of significance.

the cultivation were lignocellulosic wastes, and similar work was also carried out by Zadrazil[12] where several unused agro-wastes in the form of straws, leaves, stems, roots, etc. were selected for the cultivation of mushroom. Our finding showed that although T5 and T14 substrates required the fewest days for the first mycelial growth, the T8 substrate required the least days for the first mycelial running over the substrate. The substrate combination (T10), which was a combination of wood flakes and sawdust in equal amounts, dried out after the initial flushing since it had a lower water retention capacity and moisture content[13]. Similarly, supplementation of mushroom beds with gram powder provided a better yield of mushrooms as earlier reported by Bano et al.[8]. It was observed that the total yield of Oyster mushrooms on lemon grass (*Cymbopogon citratus*) after three flushes was 264.80 gm on 1 kg of substrate[14]. The yield of fruiting bodies on T5 substrate, or Citronella bagasse (*Cymbopogon nardus*) was 393.90 gm on 1 kg of the substrate after three flushes, which is significantly higher than the yield on lemon grass reported by Mumtaz et al.[14]. The biological efficiency of mushrooms varied significantly in different substrates and their combinations. In many instances, the production of mushrooms was found to be low as the substrates accounted for various changes like temperature, the activity of microbes, and aeration that affected the mushroom production.

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Conflict of interest

The authors declare that they have no conflict of interest.

Dates

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