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The Potentiality of different agro-wastes combinations for growth of *Volvariella* and *Pleurotus* sp.

Dibyarupa Pal[1], Sujan Maity[2] & Sudipto Sarkar[3]

1. Department of Biosciences, JIS University, 81, Nilgunj Rd, Agarpara, Kolkata, West Bengal 700109, India, Corresponding author's email: dibyarupa.pal@gmail.com
2. Department of Biosciences, JIS University, 81, Nilgunj Rd, Agarpara, Kolkata, West Bengal 700109, India
3. Department of Agricultural Engineering, Assam University, Silchar, 788011, India

Abstract:

India being agrarian based economy has huge turnover of agricultural waste which needs suitable management system for their dispersal. These are ligno-cellulosic wastes which are burnt openly creating threat for environment and human health. Mushroom production utilizing the agro-waste as substrates offers a 'golden opportunity' to turn waste into wealth with minimum investment. Mushroom harbours both nutritional and medicinal properties beneficial for humans with diabetes and provides essential amino acids to eradicate malnutrition. Indian climatic conditions prefer cultivation of different edible mushrooms throughout the year. In return the economy of the rural people is uplifted and food security is also ensured. Post-harvest gives us spent mushroom substrate which can be re-utilized in agriculture as compost, as livestock feed or generation of unconventional fuel. Valorisation of mushroom products is achieved by fortifying different food items with mushroom powder and used as nutraceuticals also. In this study Volvariella sp. and Pleurotus sp., was grown utilizing ligno-cellulosic substrates like paddy straw, areca palm leaves, sugarcane bagasse and saw dust. To determine feasibility of such substrates different parameters like mushroom yield, cap diameter and stipe length was observed. Though paddy straw was the preferred substrate for both mushroom species but areca palm leaves and sugarcane bagasse also showed huge potential. Further investigations are needed for efficient utilization of these unconventional substrates by certain pre-treatment procedures. This study aims to find cheap, alternative eco-friendly ligno-cellulosic substrates which farmers can access easily for cultivating mushroom.

Keywords: *Mushroom, Lignocellulosic Waste, Volvariella Sp., Pleurotus Sp., Substrates.*

Introduction:

The demand for continuing food supply has culminated in the increase in the cultivation of agricultural crops globally. In India in the year 2019-2020, approximately 500 million tonnes (MT) of agricultural wastes are generated, where total food grains account for 284.95 MT. Major crops like rice (116.425 MT), wheat (98.38 MT), maize (26.26 MT) pulses (22.95 MT), sugarcane (377.77 MT), cotton (33.09 MT) are cultivated across

the states (MoA and FW, 2019-2020). Simultaneously reports of clearing the land post-harvest by stubble burning in unmanaged paddy and wheat fields of Punjab (20.17 MT) and Haryana (7.93 MT) is also on the rise (IARI, 2012). To curb such open burning of crop residue, waste recycle and crop diversifications are encouraged where mushroom cultivation holds huge and untapped potential. Mushroom cultivation can act as agricultural waste clean-up process and mitigate this serious problem of crop residue management.

With increasing demand for food and biofuel, cultivation of primary agricultural crops has accelerated globally by 54% between the years 2000-2023 [8]. To ensure nutritious and healthy food to the growing population and also simultaneously maintain environment sustainability, FAO has projected on Sustainable Development Goals (SDGs) to reduce waste generation [8]. In this aspect promoting mushroom cultivation serves both purposes of nutritional security as well as reducing the growing agricultural waste on the farmland. Lignocellulosic components are the main constituents and mushrooms are excellent biodegraders of theses substrates, by releasing enzymes like endoglucanases, laccases and phenoloxidases which break them into simple sugars [19;17]. This replenishes the soil with nitrogen, phosphorus, potassium and provides nutrition for growth of mushroom. Earlier report showed various substrates like rice straw, wheat straw, corn cob, saw dust, sugarcane bagasse, banana leaves, waste paper used successfully for growth of different mushrooms [6;19;3;13]. Though growth rate and yield is different for different substrates, the ratio of Carbon:Nitrogen in the substrate is significant as it determines the protein content and flavour of mushroom [16;20;17;14].

Among the commonly cultivated edible mushrooms in India, Oyster mushroom (*Pleurotus* spp.) and Paddy straw mushroom (*Volvariella* sp.) are of great demand after *Agaricus* sp. in the state of Bengal and Orissa. The cultivation process of *P. ostreatus* is very easy, less costly and shorter growth time compared to other edible mushrooms [1;3]. It is highly adapted to various lignocellulosic substrates with optimum growth temperature and relative humidity as 20-30°C and 55-70% respectively [6]. *V. volvacea*, commonly paddy straw mushroom can grows outdoors as well as indoors without any effort and has a short life cycle of 14 days [2]. The species favours 30–35 °C temperature for the development of mycelia stage, and 28–30 °C for fruiting body stage.

Knowledge gaps that motivate to work on this problem

Lignocellulosic components are the main ingredients that the mushroom degrade in the agricultural waste (Table 1). Various agricultural wastes are available which can act as suitable substrates but certain components might need pre-treatment processes like lignin [4;12]. Limited information on a uniform standardization process is available to degrade such recalcitrant components in biodegradable stage. In our study we have undertaken different agro-wastes as well as their combinations to find the suitable substrate composition preferred by *Volvariella* sp., and *Pleurotus* sp.

Table 1: Lignocellulosic composition of various types of agricultural wastes used by *Pleurotus* sp. and *Volvariella* sp.

Substrate	Composition	References
Paddy	Cellulose 41%, Hemicellulose 22%, Lignin14%	[7]
Sugarcane bagasse	Cellulose 35-40%, Hemicellulose 20-25%, Lignin 18-24%	[7]
Areca Husk	Cellulose 41-42%, Hemicellulose 17-18%, Lignin 43-44%	[18]
Saw Dust	Cellulose 47.82%, Hemicellulose 16.63%, Lignin 33.29%	[3]

Methodology

Preparation of Substrate

All kind of straw and other agro-wastes were obtained from farmers of local market of East Midnapore, chopped in small pieces of 5-8 cm (Figure. 1) and soaked in water for 10-12 hours using bavistin.

Figure 1: Different Substrates utilized for growth of *Volvariella* and *Pleurotus* sp.

Then pasteurized by boiling for 2 hours in wide containers (Figure. 2). All the substrates were cut into 1.5-2 inches in length and kept in a cool place to semi-dry overnight.

Figure 2: Sterilization of substrates and preparation of spawning bags

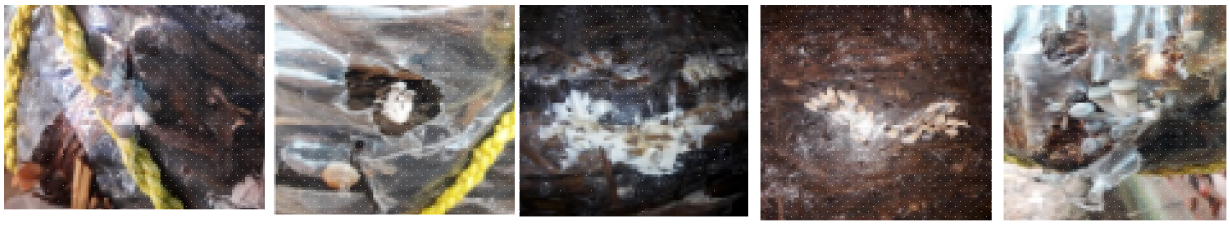
Preparation of spawning bags

Freshly prepared wheat grain spawn (25-30 days old) was procured from local farms of East Midnapore. For mushroom growth, 60 x 30 cm polybags were taken with one end tied and respective substrates were laid in the different bags to a height of 5 cm. 50-60 gm of spawn was sprinkled on the substrates in outwardly fashion. The process was repeated to get 4 layers of substrates and 3 layers of spawn. Then the mouth of the polybags were tied tightly with cotton plugs. Several holes are made aseptically outside the polybags in random fashion. All polybags are hung in a semi-lit room at 24-28°C for 18-30 days (Figure 2). Water was sprinkled intermittently in the polybags to prevent dehydration of the substrate and spawn.

Harvesting

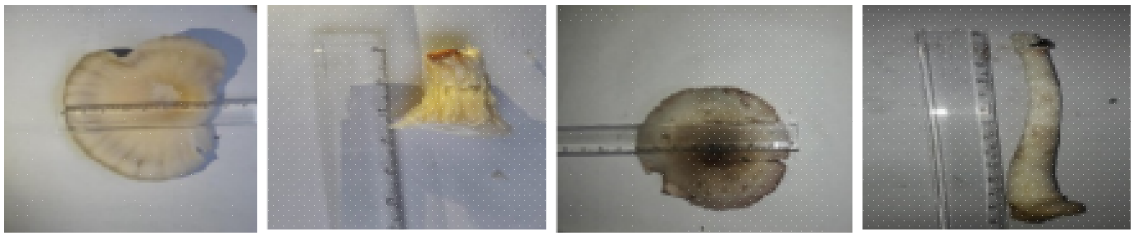
Both *Pleurotus* sp. and *Volvariella* sp. mushrooms were harvested before their volva broke. In the current study mushrooms were harvested at different times due to different substrates used. For three flushes the harvest was obtained and the total crop cycle was completed within 4-5 weeks' time (Figure 3).

a) Mycelia growth on different Substrates by *Pleurotus* sp. and *Volvariella* sp.



b) Pinhead formation on different Substrates by *Pleurotus* sp. and *Volvariella* sp.

c) Maturation stages on different Substrates by *Pleurotus* sp. and *Volvariella* sp.



d) Measurement of cap heads and stipe of *Pleurotus* sp. and *Volvariella* sp.

Figure 3: Different stages of growth of *Pleurotus* sp. and *Volvariella* sp. on varied substrates and their combinations.

Mushroom growth Limiting Factors and Cost effective solutions

Mushroom cultivation can be highly productive, but several factors can limit growth and yield. Identifying these constraints and implementing cost-effective solutions can improve production efficiency and performance of the mushroom:

Temperature: The temperature range for *Pleurotus* sp. is 22-25°C, elevated temperature can hamper mycelium growth and pinhead formation [10]. Cost effective way to control the temperature is by three layer process where the cultivation room has to be modified in a way that the walls contain concrete, thermocol and narrow leaves of cat's tail plant. Also adding false ceiling can aid in the process of cooling.

Humidity: The humidity ranges from 60-90 % depending on the temperature [10]. To control humidity sometimes wet cotton sacs can be hanged and the floor is covered with wet white sand. Too high or too low humidity slow growth or cause crop failure. Low humidity leads to drying; excess humidity promotes mold growth.

Indirect Sunlight: Mushroom cultivation requires indirect sunlight for their growth. If required greenhouse nets can be used to cover the open windows.

O_2 and CO_2 Balance: Mushroom has two growth phases, before and after discharge of the fruiting body. In the first phase (mycelium colonization phase), higher CO_2 levels required and in the later fruiting stage,

excess CO_2 leads to poor mushroom development. For this purpose the air circulation has to be adjusted for fresh air exchange, like position of the window and door in the cultivation room.

Table 2. Different growth stages of *Pleurotus* sp. cultivated on varied substrates

Mold control: 10% formalin water can be sprayed on regular basis and inside the room before hanging the cylinders to control green and black mold growth. For excessive unwanted mold growth, scissors are used to cut and Calcium Hydroxide $Ca(OH)_2$ can be sprayed on that area.

To evaluate Biological efficiency

To assess the growth performance of mushrooms on various substrates, both yield and biological efficiency were measured. The biological yield (g) was recorded by weighing the entire cluster of fruiting bodies, including the base of the stalks, while the economic yield (g) was determined by weighing only the harvested fruiting bodies after trimming the stalk bases. Biological efficiency (BE %) was then calculated using the following formula:

BE = (weight of harvest / weight of dry substrate) x 100 %

Results and Discussions

The effect of different substrates on growth and yield of *Volvariella and Pleurotus* was studied from a period of November-March, 2025. The observations were recorded for mycelium growth, pinhead formation and maturation stages for respective mushroom varieties. Paddy and all the substrate combinations yielded mushroom though colonization but the maturation stages varied. All the experimental data was measured in triplicate and the means are presented in the tables below.

Substrates	Observation (days)		
	Mycelium growth	Pin head formation	Maturation stage
Paddy (P)	8	10	13
Paddy:Sugarcane (PS) (1:1)	10	13	17
Paddy:Areca (PA) (1:1)	9	11	15
Paddy:Saw dust (PS) (1:1)	11	13	16
Mixed substrate (MS) (1:1:1:1)	11	14	18

Table 3. Growth performance and Biological Efficiency of *Pleurotus* sp. cultivated on varied substrates

Substrates	Cap Diameter (cm)	Stipe Height (cm)	Weight (g)	Biological Efficiency (g)
Paddy (P)	7.46	4.36	9.56	27
Paddy:Sugarcane (PS) (1:1)	5.70	2.76	8.33	24

Paddy:Areca (PA) (1:1)	4.90	3.16	5.10	15
Paddy:Saw dust (PSA) (1:1)	4.20	3.0	6.32	18
Mixed substrate (MS) (1:1:1:1)	5.63	3.76	7.20	21

Table 4. Different growth stages of *Volvariella* sp. cultivated on varied substrates

Substrates	Observation (in days)		
	Mycelium growth	Pin head formation	Maturation stage
Paddy (P)	7	10	12
Paddy:Sugarcane (PS) (1:1)	9	12	14
Paddy:Areca (PA) (1:1)	8	11	13
Paddy:Saw dust (PSA) (1:1)	11	14	17
Mixed substrate (MS) (1:1:1:1)	11	15	18

Table 5. Growth performance and Biological Efficiency of *Volvariella* sp. cultivated on varied substrates

	Measurements			
Substrates	Cap Diameter (cm)	Stipe Height (cm)	Weight (g)	Biological Efficiency (g)
Paddy (P)	11.40	13.07	13.76	39
Paddy:Sugarcane (PS) (1:1)	7.97	11.37	11.26	33
Paddy:Areca (PA) (1:1)	9.7	9.03	8.53	24
Paddy:Saw dust (PSA) (1:1)	7.23	7.0	7.56	21
Mixed substrate (MS) (1:1:1:1)	10.12	12.0	9.8	24

Mycelium growth was first observed on paddy substrate after 8 and 9 days for *Pleurotus* and *Volariella* sp. compared to the mixed combinations (Table 2 and 4). Thus paddy was the preferred substrate to initialize mycelium growth but among the substrate combinations, Paddy and Areca (PA) showed mycelium growth on 9th and 8th day respectively by *Pleurotus* and *Volvariella* sp. Mixed combination of Paddy and Sugarcane (PS) was the next ideal substrate. Similar result was obtained by De *et al*., 2023 [5] where rice straw and sugarcane bagasse (3:2) was the ideal ration for growth of oyster mushroom.

Pinhead stage formation was early in case of rice straw substrate (10 days) compared to mixed substrates. The reason might be the high lignin component and crystalline fraction of cellulose which are recalcitrant to the mushroom enzymes as evident form the lignocellulosic fractions exhibited in Table 1. Biological efficiency (g) was determined by weighing the whole fruiting bodies against the substrate required for their growth. Paddy straw substrate showed better yield and biological efficiency than other combinations (Table 3 and 5). Throughout the experiment saw dust yielded low biological efficiency for *Volvariella* sp. whereas Areca leaves gave low performance for *Pleurotus* sp., but again proper pre-treatment processes can initially help in breakdown of the recalcitrant portions of agri-waste which can then be properly utilized to its full potential.

The statistical analysis of the growth stages of *Pleurotus* mushrooms provides vital insights into their development across different substrates. ANOVA p-values are all significantly low (< 0.05), indicating that there are statistically significant differences in growth stages across different substrates. The mean duration for Mycelium Growth was recorded at 9.92 days, with highest coefficient of variation at 15.77%, indicating that mycelium growth had the greatest relative variability among the three stages. Further, external factors, like substrate composition, moisture content and environmental conditions, might play a significant role in influencing mycelium growth phase. Pinhead Formation and Mature Stage had a mean duration of 12.25 days and 15.92 days respectively, demonstrating moderate variability, suggesting that once the mycelium establishes itself, the subsequent developmental stages progress with more consistency. All p-values are extremely low (< 0.05), indicating significant differences in Cap Diameter, Stipe Height and Weight across different substrates. The presence of highly significant differences suggests that some substrates provide superior nutrients and environmental conditions for mushroom cap expansion compared to others. Comparing the substrate performance, *Pleurotus* grown on paddy substrate produced the largest and heaviest mushrooms followed by PS, MS, PSA and PA. The combination of Paddy and areca in equivalent ratio led to the smallest mushrooms in terms of cap diameter, height and weight.

Similarly for *Volvariella* mushroom development the p-value of 0.00027 indicates a statistically significant difference in the duration of mycelial colonization across substrates. This signifies that certain substrates provide more favourable conditions and easy breakdown for rapid mycelial spread. Factors such as lignocellulosic fraction, moisture retention and aeration likely contribute to these differences. Pinhead formation represents the transition from vegetative mycelium to the reproductive fruiting body. Faster pinhead formation generally leads to improved productivity and shorter cultivation cycles, making substrate selection a key consideration for mushroom growers. A delayed mature stage may indicate suboptimal substrate conditions, whereas an accelerated maturity phase suggests favourable substrate properties that enhance overall production efficiency. Comparing the substrate performance, *Volvariella* grown on paddy substrate produced the best mushrooms along with early mycelium growth, pin head formation and fruiting body development. The substrate preference is followed by PS, MS, PA and PSA. In corroboration, paddy straw was stated as the best substrate for *Volvariella* by other investigator also [11;15].

Conclusion and future research directions

Mushroom cultivation is the most prominent and suitable technology for generation of wealth and health out of agro-wastes which are bounteously available on earth. Though paddy is the ideal substrate for mushroom cultivation but after reducing the amount of indigestible material in the ligno-biomass, others substrates can also be equally feasible for cultivation. Future studies should explore specific substrate compositions and their nutrient profiles to determine their direct impact on growth variability. Additionally, research on environmental conditions such as temperature, humidity, and aeration would provide deeper insights into optimizing mushroom yield and quality. By leveraging the findings from this study, cultivators can improve mushroom quality, yield and overall economic returns.

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