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Agro-industrial waste based substrate for production of two major cultivated oyster mushrooms in sub-Himalayan West Bengal

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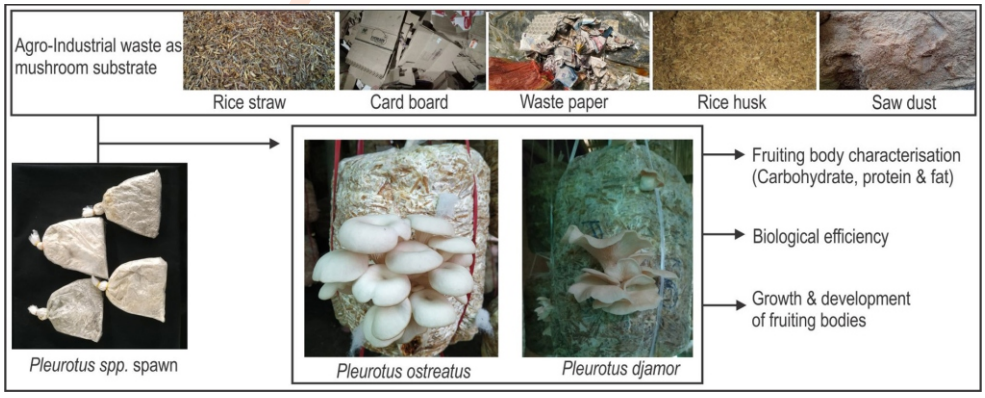
Abstract

Aim: To compare the outcomes of various agro-industrial wastes on the growth, yield and nutritional contents of two major cultivated oyster mushrooms, *Pleurotus ostreatus* and *Pleurotus djamor*.

Methodology: Locally available agro-industrial wastes were utilized as substrate in five formulations including rice straw alone and in combination with card board (7: 3), waste paper (7: 3), rice husk (17:3) and jack fruit saw dust (7:3). After sterilization substrates were inoculated with mature spawn and placed in cropping room.

Results: Total yield, biological efficiency and nutritional contents were found better in the fruiting bodies of *Pleurotus ostreatus* and *Pleurotus djamor* cultivated on rice straw as the sole substrate than the mixed substrate formulations. Total colonization period (22.6 days for *P. ostreatus* and 20.2 days for *P. djamor*) was quite less for both the oyster mushroom cultivated in rice husk mixed substrate among mixed substrate formulations. Among mixed substrate formulations, highest total yield was obtained from waste paper mixed substrate for *Pleurotus ostreatus* (713.8 g) and card board mixed substrate for *Pleurotus djamor* (737.7 g). The highest carbohydrate content in *Pleurotus ostreatus* and *Pleurotus djamor* were found in card board mixed substrate. Total protein content was high in fruiting bodies cultivated on saw dust mixed substrate for both the oyster mushrooms.



Interpretation: Card board, waste paper, rice husk and saw dust mixed substrates showed relatively high fruiting body yield and nutritional values. It can be concluded that these agro-industrial waste appears to be the promising ingredients in combination with rice straw for cultivation of oyster mushroom.

Key words: Agro-Industrial waste, Nutrition, Oyster mushrooms, *Pleurotus*, Rice straw

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Introduction

Mushroom is a fleshy, spore-bearing sporocarp of a eukaryotic macro-fungus that usually grows above the surface of the soil or on some of its natural food sources. It plays a crucial role in forest ecosystem as it has a distinctive characteristic to biodegrade wood, leaves and other organic matters (Zhang *et al.,* 2014; Waktola and Temesgen, 2018). Edible mushrooms have gained worldwide popularity due to their medicinal as well as nutritional utility since ancient Greek and Roman civilization (Arora and Shepard, 2008; Gan *et al.,* 2013). Considering a huge diversity of mushroom, only 7000 species have been secured varying degree of edibility (Kaliyaperumal *et al.,* 2018). About 35 mushroom species are utilized in commercial cultivation scale but only 20 species are cultivated at industrial scale (Sánchez, 2004). Mushroom cultivation is purely dependent on the utilization of agro-based solid waste. Integration of edible mushrooms in food chain and its cultivation has captured an important position in agricultural field due to its potential for converting waste into wealth and being considered as a valuable and environmentally friendly technology (Grimm *et al.,* 2021).

The oyster mushroom holds second position after button mushroom in mushroom cultivation in India (Sharma *et al.,* 2017). It consists of several species including *Pleurotus ostreatus*, *P. pulmonarius*, *P. sajor-caju, P. sapidus, P. cystidiosus*, *P. flabellatus*, *P. citrinopileatus*, etc. It is cultivated in the subtropical and temperate zones of the world. Oyster mushroom is traditionally harvested from forest during March to September. However, it can be grown in a greenhouse or in plastic tunnels throughout the year (Kibar and Peksen, 2008). *Pleurotus* mushroom is considered as a healthy as it is a rich source protein, fiber, amino acids, vitamins and minerals (Feeney *et al.,* 2014). *Pleurotus* mushroom possess diverse medicinal values, like antimicrobial, antiviral, antitumor, anti- HIV, antineoplastic, antimutagenic, antidiabetic, antilipidemic, antioxidant, anti-inflammatory, hepatoprotective, immunomodulatory properties (Patel *et al.,* 2012).

Substrate composition has a remarkable impact on the growth and nutritional constituents of mushrooms. Several agro-waste including rice straw, wheat straw, corn straw, thatch grass, banana leaves, palm leaves, soybean straw, etc., have been utilized in the production of mushrooms (Bellettini *et al.,* 2019). However, rapid growth of mycelia in mushroom cultivation requires ideal substrate that should have appropriate levels of nitrogen and carbon contents, as well as cellulose and lignin because of its effective lignin degrading traits (Das and Mukherjee, 2007). India is the leading rice producing country in the world and within Indian state West Bengal is at the top. Huge amount of rice straw and rice husk are produced every year in India as agricultural wastes. Paper is nearly 100% cellulosic in nature. At international level, about 40- 65% of paper is wasted and discharged in the environment causing pollution. Waste paper refers to newspaper, printing paper, magazine paper, brown paper, kite paper, etc., that is discarded from residential places, offices, markets and shopping malls (Ukaogo *et al.,* 2020). Card board is a quite thick sheet of paper mainly utilized for packing purpose. Card boards are highly rich in lignocellulosic substances. In India, several million tones of dry recyclables waste are generated every year in which major part is paper and paperboard. Sub-Himalayan West Bengal has congenial climatic conditions, abundance of man power, and availability of agro-waste resources that is appropriate for cultivation of oyster mushrooms, however, due to lack of nutritional awareness and cultivation technology, mushroom production in this region is limited to winter months, and only mushroom cultivation units are present ranging from moderate to large scale production. In view of the above, the present study was conducted to utilize various agro-industrial wastes in mushroom cultivation and to evaluate the nutritional values as well as yield performance of two oyster mushroom species, *Pleurotus ostreatus* and *Pleurotus djamor*.

Materials and Methods

Study area and mushroom strain: The study was conducted in mushroom cultivation House, Coochbehar, West Bengal, India. The strains utilized in the present experiment were obtained from Directorate of Mushroom Research (Solan, Himachal Pradesh, India). *Pleurotus ostreatus* (Code- DMRP 115) and *Pleurotus djamor* (Code- DMRP 205) were utilized in the present experiment. Strains were maintained in the potato dextrose agar media.

Spawn production: Wheat grains were taken for spawn production. They were boiled for 30 min and then washed in fresh flowing water. Extra water present in the grains was drained off and spread over a blotting paper and air dried. Thereafter, calcium carbonate and calcium sulphate were thoroughly mixed with grains. Approximately, 200 g of grains (wet weight) were taken in each plastic bag and autoclaved at 121ºC for 120 min. After cooling, each sterile plastic bag was inoculated with fungal inoculum and kept at 27ºC for 15 days in a BOD incubator. After 15 days of incubation when the grains were fully invaded by mycelia, the spawn was ready for use.

Collection and preparation of substrate: Rice straw, card board, jackfruit saw dust, domestic waste paper (newspaper, printing waste paper of office and home), rice husk were collected from local area. All the substrates were chopped into 2cm long pieces, except for sawdust and rice husk. The substrates were submerged in water for about 16-18 hrs. Thereafter, all the substrates, except for card board and waste paper, were boiled for 60 min and cooled. For card board and waste paper sterilization, fresh water was heated at 60ºC and both the substrates were placed in hot water for 30 min. Finally, once pasteurized, substrates were stocked in sterile place for few hours to remove excess water from the substrates to attain 60-70% moisture level.

Preparation of cultivation bag (Inoculation): Cultivation bags were prepared using polypropylene bags (12×18 inch) with each combination of substrates (Table 1) and inoculated with previously prepared spawn at 100 g in 1 kg substrate (dry weight basis) in each cultivation bag. The bags were tightly closed with pin holes on the surface for aeration and placed in cropping room.

The cropping room had an average temperature and limited light. The temperature (26º to 30ºC) and humidity (85-90%) of cropping room was maintained by spraying water at a regular interval.

Harvesting and evaluation of yield: Mature fruiting bodies were harvested without harming the substrate. The yield performance was noted with respect to time periods required for completion of spawn running, period taken for first appearance of pin head formation, period taken for first flush, number of flushes and total yield from each treatment formulation. Fully mature fruiting bodies were weighed to determine the biological efficiency. The percent biological efficiency was calculated.

Quality of fruiting body: Harvested fruiting bodies were sun dried and ground into powder and kept for further analysis. Total carbohydrate, total protein, lipid, energy value and moisture content were evaluated. Moisture content, total fat and total protein content were estimated following the standard protocols (Ahmed *et al*., 2009; Folch *et al.,* 1957; Lowry *et al.,* 1951). Total carbohydrate was estimated by the method of Yemm and Willis (1954). Energy value (kcal 100 g^{-1}) was calculated using the equation of Nielsen (2017).

Chemical toxicity test of fresh fruiting body: Juice from fresh fruiting bodies was pressed out on a waste newspaper and after drying a drop of hydrochloric acid was placed on it. Appearance of a blue spot revealed the presence of toxins (Svrcek, 1998).

Data collection and statistical analysis: The cultivation experiment was done in a completely randomized design with five replications (n=5) for each of the five treatment formulation and nutritional analysis of each treatment formulation was done with three replications (n=3). Data were analyzed by statistical tool SPSS version 22 and graphically represented using Microsoft excel version 2007. One-way ANOVA and then post hoc Tukey's B test ($p < 0.05$) were conducted for the statistical analysis of data.

Results and Discussion

The period (days) taken for completion of mycelial growth differed significantly ($p<0.05$) among the treatments. In this study (Table 2), the fastest mycelial colonization period (18.2±0.83 days) of *Pleurotus ostreatus* was observed in *Pleurotus ostreatus* treatment 1 and the maximum number of days (27.4±1.14) were required by *Peurotus ostreatus* treatment 5 to complete the spawn run. From the mixed substrate groups, *Pleurotus ostreatus* treatment 4 showed minimum time period (22.6±0.54 days) for completion of spawn run. Colonization of substrate in all treatment formulations of *Pleurotus ostreatus* was completed between 18.2± 0.83 to 27.4±1.14 days. In case of *Pleurotus djamor* (Table 2), the fastest completion of mycelial run (15.4±1.34 days) was noted on *Pleurotus djamor* treatment 1 and the maximum number of days (29.8±0.83) were required by *Pleurotus djamor* treatment 5. From the mixed substrate formulations, *Pleurotus djamor* treatment 4 took less time (20.2±1.48 days) to colonize the substrate completely. Colonization of substrate by *Pleurotus djamor* was accomplished within 15.4±1.34 to 29.8±0.83 days among all treatment formulations. Shah *et al.* (2004) reported that *Pleurotus* species cultivated on wheat straw and in a mixture of wheat straw and saw dust as substrates, required 2-3 weeks for mycelial expansion whereas present finding showed 3-4 weeks period for mycelial colonization, which is close to their results. Rice husk as a substrate shows quite fast mycelial run of *P. ostreatus* as reported by Frimpong-Manso *et al.* (2011).

Results of the present study are analogous to their results. The mean time for pin-head formation among treatments varied significantly ($p<0.05$). It was observed that the time taken (Table 2) for the first appearance of pin-head from *Pleurotus ostreatus* was 22.6±0.54 days (*Pleurotus ostreatus* treatment 1) and the maximum time taken for pin-head formation was 32.6±1.51 days by *Pleurotus ostreatus* treatment 2. First pin-head appearance by *Pleurotus djamor* (Table 2) was found on 21.6±1.51 days (*Pleurotus djamor* treatment 1), while the maximum time (33.4±1.34 days) was required by *Pleurotus djamor* treatment 5 for pin-head generation. The results of the present study are in accordance with the findings of Dissasa *et al.* (2022) who reported that pin-head formation of *Pleurotus* species cultivated on coffee waste varied between 20 to 28 days. Time taken for first flush (harvesting) of oyster mushroom differed significantly ($p<0.05$) among treatments (Table 2). Considering the minimum time required for maturity of fruiting bodies, *Pleurotus ostreatus* treatment 1 (26.8±0.44 days) appeared to be

Table 1: Substrate composition of treatment formulations used for the cultivation of oyster mushroom

Treatment groups	Composition of substrate (1 kg substrate in each bag)
Pleurotus ostreatus treatment 1	Rice straw (100%)
Pleurotus ostreatus treatment 2	Rice straw (70%) + card board (30%)
Pleurotus ostreatus treatment 3	Rice straw (70%) + waste paper (30%)
Pleurotus ostreatus treatment 4	Rice straw (85%) + rice husk (15%)
Pleurotus ostreatus treatment 5	Rice straw (70%) + saw dust (30%)
Pleurotus djamor treatment 1	Rice straw (100%)
Pleurotus djamor treatment 2	Rice straw (70%) + card board (30%)
Pleurotus djamor treatment 3	Rice straw (70%) + waste paper (30%)
Pleurotus djamor treatment 4	Rice straw (85%) + rice husk (15%)
Pleurotus djamor treatment 5	Rice straw (70%) + saw dust (30%)

Table 2: Influence of different substrates for production of oyster mushroom *Pleurotus ostreatus* and *Pleurotus djamor*

Treatment	Spawn run (Days)	Initiation of pin head formation (Days)	Time taken for first flush (Days)	Time taken between first flush to second flush (Days)	Total yield* (g)
Pleurotus ostreatus treatment 1	18.2±0.83^{a}	22.6±0.54^{a}	26.8±0.44^{a}	14.2±1.09^{a}	837.0±28.35^{b}
Pleurotus ostreatus treatment 2	25.4±0.89^{c}	32.6±1.51^{d}	37.8±1.09^{d}	15.2±0.44^{ab}	696.5±97.26^{a}
Pleurotus ostreatus treatment 3	23.6±0.89^{b}	29.4±1.14^{bc}	34.6±1.14^{c}	16.6±1.14^{b}	713.8±50.26^{a}
Pleurotus ostreatus treatment 4	22.6±0.54^{b}	27.6±0.89^{b}	32.2±0.83^{b}	16.4±0.54^{b}	643.8±30.66^{a}
Pleurotus ostreatus treatment 5	27.4±1.14^{d}	31.2±1.30^{cd}	36.6±1.14^{d}	19.4±0.54^{c}	706.9±26.55^{a}
Pleurotus djamor treatment 1	15.4±1.34^{A}	21.6±1.51^{A}	25.8±1.30^{A}	12.0±1.41^{A}	818.8±23.79^{B}
Pleurotus djamor treatment 2	21.0±0.70^{B}	30.8±1.30^{C}	35.2±1.64^{C}	15.8±0.83^{B}	737.7±54.91^{AB}
Pleurotus djamor treatment 3	20.8±1.09^{B}	28.8±1.30^{C}	33.2±1.64^{BC}	15.2±0.44^{B}	713.0±78.88^{A}
Pleurotus djamor treatment 4	20.2±1.48^{B}	26.4±1.14^{B}	31.4±0.89^{B}	16.6±1.34^{B}	661.0±69.78^{A}
Pleurotus djamor treatment 5	29.8±0.83^{C}	33.4±1.34^{D}	38.2±1.78^{D}	18.8±0.83^{C}	725.3±17.02^{AB}

All values are mean± Standard Deviation (SD); Different letters along the column indicate significant differences of the mean ($p<0.05$) according to Tukey's B test (n=5). *Total yield from two flushes

Table 3: Effect of different substrate formulation on nutritional composition of *Pleurotus ostreatus* and *Pleurotus djamor*

Treatments	Protein (g 100 g^{-1})	Carbohydrate (g 100 g^{-1})	Fat (g 100 g^{-1})	Energy (kcal 100 g^{-1})
Pleurotus ostreatus treatment 1	26.8±0.80^{b}	38.5±1.04^{b}	3.4±0.20^{bc}	292.3±8.28^{c}
Pleurotus ostreatus treatment 2	24.6±0.40^{a}	39.3±0.65^{b}	3.0±0.11^{ab}	283.3±2.61^{c}
Pleurotus ostreatus treatment 3	23.8±0.80^{a}	34.2±0.80^{a}	2.7±0.17^{a}	256.7±3.55^{a}
Pleurotus ostreatus treatment 4	25.0±0.55^{a}	36.0±0.85^{a}	2.7±0.11^{a}	269.1±4.34^{b}
Pleurotus ostreatus treatment 5	27.2±0.41^{b}	35.3±1.05^{a}	3.7±0.20^{c}	283.7±2.25^{c}
Pleurotus djamor treatment 1	29.4±0.90^{C}	40.5±0.65^{C}	2.5±0.36^{AB}	302.2±8.11^{B}
Pleurotus djamor treatment 2	27.4±0.45^{AB}	40.2±0.37^{C}	2.3±0.37^{AB}	291.5±2.36^{B}
Pleurotus djamor treatment 3	26.3±0.45^{A}	36.8±0.30^{A}	1.9±0.25^{AB}	270.0±0.68^{A}
Pleurotus djamor treatment 4	26.5±0.50^{A}	35.8±0.50^{A}	1.8±0.30^{A}	265.8±2.70^{A}
Pleurotus djamor treatment 5	28.6±0.30^{BC}	39.0±0.56^{B}	2.7±0.30^{B}	295.4±4.85^{B}

the best substrate and among the mixed substrates, *Pleurotus ostreatus* treatment 4 (32.2±0.83 days) was found to be the best substrate. In case of *Pleurotus djamor* (Table 2), the first harvest was done from *Pleurotus djamor* treatment 1 (25.8±1.30 days). Among the mixed substrates, *Pleurotus djamor* treatment 4 took minimum time (31.4±0.89 days) for the first harvest.

In the present study, the outcome showed that fruiting body formation took 4 to 6 weeks after inoculation which is in accordance with the results reported by Girmay *et al.* (2016), where 27- 40 days were required for maturation of *Pleurotus ostreatus* fruiting bodies cultivated on waste paper as substrate. From Table 2, it was observed that there were only 2 flushes among all the treatments. However, Islam *et al.* (2017) reported three consecutive flushes from the oyster mushroom cultivation unit. Time taken between two harvest differed significantly ($p<0.05$) between the treatments. Least number of days taken in between two flushes was 14.2±1.09 days in case of *Pleurotus ostreatus* and 12.0±1.41 days in case of *Pleurotus djamor*. Maximum time was required in saw dust mixed substrate for both the oyster mushrooms. Our findings are in accordance with the results of Yang *et al.* (2016) who reported the time period between the first and second flushes of oyster mushroom cultivated on tea waste between 9 to 12 days.

Yield and biological efficiency varied significantly ($p<0.05$) among the treatment formulations. Cultivation of Pleurotus ostreatus on different substrate showed maximum yield from *Pleurotus ostreatus* treatment 1 (837.0±28.35 g), followed by *Pleurotus ostreatus* treatment 3 (713.8±50.26 g). Cultivation of *Pleurotus djamor* on different substrate indicated highest yield from *Pleurotus djamor* treatment 1 (818.8±23.79 g), followed by *Pleurotus djamor* treatment 2 (737.7±54.91 g). As shown in Fig. 1 (B), the maximum biological efficiency among mixed substrate was recorded from waste paper mixed substrate (71.3±5%) for *Pleurotus ostreatus,* while the maximum biological efficiency among mixed substrate was documented from card board mixed substrate (73.7±5%) for *Pleurotus djamor*. Least biological efficiencies (64.3±3% and 66.0±6%) were recorded from rice husk mixed substrate for *Pleurotus ostreatus* and *Pleurotus djamor*, respectively. In the present study, it can be concluded that variation was observed in the yield of oyster mushroom on

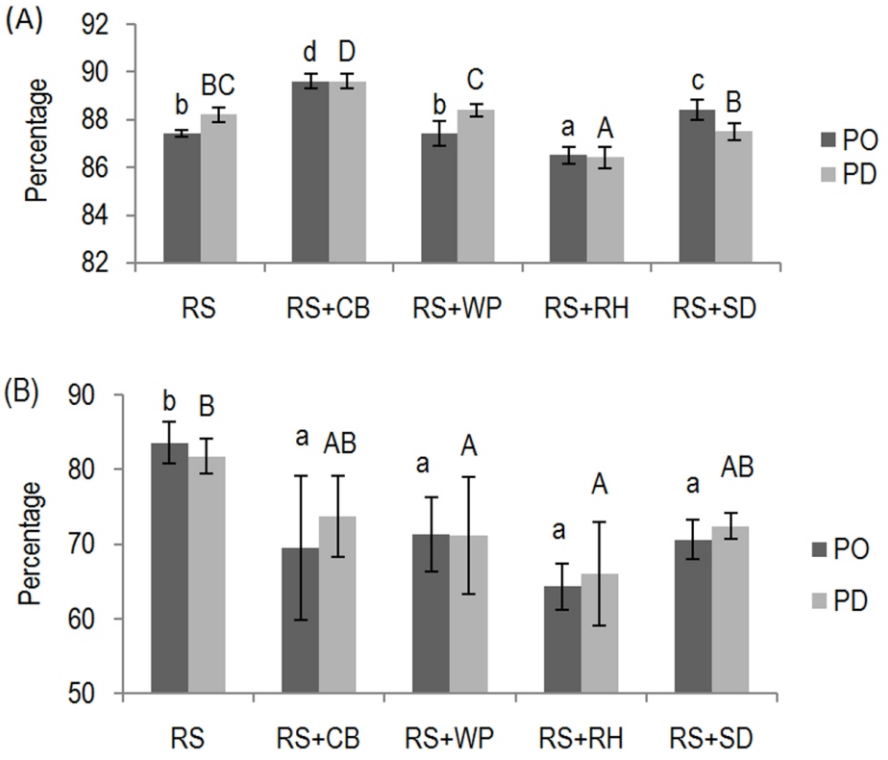

Fig 1: (A) Moisture content *Pleurotus ostreatus* and *Pleurotus djamor*; (B) Biological efficiency of *Pleurotus ostreatus* and *Pleurotus djamor*. RS-rice straw, CB- card board, WP- waste paper, RH- rice husk and SD- saw dust.

various substrates utilized. Among all the five treatment formulations, the maximum yield was obtained from rice straw as the only substrate for both the oyster mushrooms, where the lowest yield was recorded from rice husk mixed substrate. Biological efficiency was high in both *Pleurotus ostreatus* and *Pleurotus djamor* mushroom cultivated on rice straw; however, variation in the biological efficiency among all five treatment formulations was noticed. Variations in biological efficiency were reported by Liang *et al.* (2009) in different lignocellulosic wastes used for *Pleurotus* spp. cultivation. The results of the present study are in agreement with the biological efficiency of oyster mushroom cultivated on card board (Afify *et al.,* 2022). Yang *et al.* (2013) reported 44.3 to 125.6% biological efficiency for oyster mushroom cultivated on rice and wheat straw as basal substrate supplemented with cotton seed hull. Hoa *et al.* (2015) suggested that variability of biological efficiency is due to physical and chemical compositions of the substrates including cellulose/ lignin ratio, pH, carbon/ nitrogen ratio and mineral contents.

According to Beje *et al.* (2013), substrate that provide more than 40% biological efficiency can be recommended for the cultivation of oyster mushroom cultivation. So it can be concluded from the present investigation that all experimental treatment formulations are efficient for cultivation of oyster mushroom. The quality of mushroom depends on the nutritional constituents of fruiting bodies. The nutritional constituents tested in this study varied significantly ($p<0.05$) among different treatment formulations. The moisture content in *Pleurotus ostreatus* fruiting bodies ranged from 86.5±0.35-89.6±0.32% whereas in *Pleurotus djamor*, the moisture content varied between 86.4±0.43-89.6±0.30% (Fig. 1a). Regarding total carbohydrate content of *Pleurotus ostreatus*, the highest carbohydrate content (39.3±0.65 g 100 g^{-1}) was detected in fruiting bodies grown on *Pleurotus ostreatus* treatment 2. Fruiting bodies from *Pleurotus ostreatus* treatment 1 showed statistically identical carbohydrate content with *Pleurotus ostreatus* treatment 2. In contrast to total carbohydrate content of *Pleurotus djamor, Pleurotus djamor* treatment 1 showed the maximum carbohydrate content (40.5±0.65 g 100 g^{-1}). Among mixed substrate *Pleurotus djamor* treatment 2 (40.2±0.37 g 100 g^{-1}) revealed maximum carbohydrate content followed by *Pleurotus djamor* treatment 5 (39.0±0.56 g 100 g^{-1}). The maximum protein content was noted in *Pleurotus ostreatus* treatment 5 (27.2±0.41 g 100 g^{-1}), while the lowest protein content was observed in *Pleurotus ostreatus* treatment 3 (23.8±0.80 g 100 g^{-1}). Similarly, the maximum protein content was recorded from *Pleurotus djamor* treatment 1 (29.4±0.90 g 100 g^{-1}). Saw dust mixed substrate (*Pleurotus djamor* treatment 5) exhibited maximum protein content (28.6±0.30 g 100 g^{-1}) among mixed substrate formulations while *Pleurotus djamor* treatment 3 (26.3±0.45 g 100 g^{-1}) showed minimum protein content. The maximum fat content of fruiting bodies of *Pleurotus ostreatus* was recorded (3.7±0.20 g 100 g^{-1})

from *Pleurotus ostreatus* treatment 5, followed by *Pleurotus ostreatus* treatment 1 (3.4±0.20 g 100 g^{-1}).

The lowest fat content of fruiting bodies of *Pleurotus ostreatus* was recorded (2.7±0.11 g 100 g^{-1}) from *Pleurotus ostreatus* treatment 4 and statically identical result was obtained from *Pleurotus ostreatus* treatment 3. The maximum fat content of *Pleurotus djamor* was detected from *Pleurotus djamor* treatment 5 (2.7±0.30 g 100 g^{-1}), while the minimum fat content was from *Pleurotus djamor* treatment 4 (1.8±0.30 g 100 g^{-1}). *Pleurotus ostreatus* cultivated on rice straw (*Pleurotus ostreatus* treatment 1) showed the highest energy (292.3±8.28 kcal 100 g^{-1}) value while least energy content (256.7±3.55 kcal 100 g^{-1}) was identified from waste paper mixed substrate formulation (*Pleurotus ostreatus* treatment 3) (Table 3). The maximum energy content (302.2±8.11 kcal 100 g^{-1}) was detected from the fruiting bodies of *Peurotus djamor* cultivated on rice straw as substrate (*Pleurotus djamor* treatment 1), while the minimum energy content (265.8±2.70 kcal 100 g^{-1}) was obtained from the fruiting bodies cultivated on rice husk mixed substrate (Table 3).

In the current study, the proximate composition of nutritional constituents varied significantly ($p<0.05$) among treatment formulations in both oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus djamor*). Maftoun *et al.* (2015) reported that the nutritional constituents of oyster mushrooms are highly fluctuating according to mushroom species and the substrate used. Total carbohydrate content in the fruiting bodies of oyster mushrooms grown on different substrates were identical to those cultivated on cattail weed substrate (Naraian and Dixit, 2017). Mshandete and Cuff (2007) concluded that the protein content present in edible mushroom is dependent on species or strain and also depends on the substrate used. Crude protein contents of all experimental treatment formulations in the present study showed close similarity with previous work done by Elattar *et al.* (2019) where protein contents of oyster mushroom ranged from 19.21% to 29.76%. The moisture, protein and fat content in *P. ostreatus* and *P. djamor* fruiting bodies were similar to *Pleurotus florida* cultivated on cotton stalks and cotton seed hull (Sardar *et al.,* 2020). Moisture content of fruiting bodies of both *Pleurotus* species in the present experiment cultivated on rice husk mixed substrate showed less moisture content than other treatment formulations. The results of energy contents of *Pleurotus* species also showed similarity with the findings of Khan *et al.* (2008) who reported energy contents between 242.6 kcal 100 g^{-1} to 262.8 kcal 100 g^{-1}. *Pleurotus ostreatus* and *Pleurotus djamor* showed no blue spot on reacting with HCl, indicating the presence of non-toxic compounds on the fruiting bodies. Nutritional contents of the mushrooms grown on paddy straw were similar with that of mushrooms grown on agro-industrial waste based substrate in the present study. Thus the present study shows its significance of using agro-industrial waste.

Mushroom cultivation is one of the most efficient ways to recycle agro-industrial solid wastes. Mushrooms can provide protein to the poor people. Fruiting bodies from the mixed substrate formulations have shown moderate to high nutritional components (carbohydrates, protein and fat) and energy values. Therefore, it can be concluded that rather than using only rice straw as a substrate, card board, waste paper, saw dust and rice husk mixed substrates can be utilized for the cultivation of oyster mushroom in view of recycling of agro-industrial waste.

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