**“Supplementing of Agro-Wastes with Wheat Bran to Improve Oyster Mushroom Growth, Yield and Protein Content”**

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***ABSTRACT***

Oyster **mushroom is the most important edible species of mushroom, having tremendous nutritional value with excellent flavor and taste. Due to their nutritional and medicinal importance, mushroom production has gained increasing interest. However, various constraints are reported for the low productivity of mushrooms, among which the use of the appropriate kind of Agro-wastes and the right amount of supplementation are the most influential ones. Therefore, a laboratory experiment was conducted at the Teda campus, University of Gondar at 2021. The objective of this study was to contribute to the improvement of oyster mushroom production by identifying Agro-wastes and supplements. The** experiment **comprised** of **factorial combination of four agro-wastes (wheat straw, water hyacinth leaves, tea waste, and sawdust) with two levels of wheat bran supplementation (0 and 15%) in a completely randomized design with three replications.** Except for weight of the fruiting bodies at second flush per bag, **Agro-wastes** and wheat bran supplementation had a significant (p<0.05) effect on the whole studied parameters**. Significantly faster days to mycelium completion rate (21 days) and maturity (4.2 days) were obtained from wheat straw substrate supplemented with 15% wheat bran.** Significantly highest total **weight of fruiting body per bag (1203.6g), was also obtained from wheat straw substrate supplemented with 15% wheat bran. On the other hand, the highest crude protein 30.3% was obtained from sawdust substrate supplemented with 15% wheat bran. The interaction effect of wheat straw substrate with 15% wheat bran supplementation and water hyacinth substrate with 15% wheat bran supplementation increased the total weight of fruiting bodies per bag by 127.1 and 115.9% compared to tea waste without supplementation, respectively. Therefore, use of wheat straw substrate along with 15% wheat bran supplementation could be advised for maximum yield and quality oyster mushroom production.**

*Key words*- **nutrition, mushroom fruiting body*, Pleurotus ostreatus*, substrate, water hyacinth leaves**

**Introduction**

Mushroom is a macro-fungus with a distinctive fruiting body which can be either epigeous (growing on or close to the ground) or hypogeous (growing underground). It has a unique texture, good aroma, taste and flavor that differs mushroom from other food crops (Fekadu, 2014). Commonly they are cultivated on any type of lignocellulosic material that supports growth, development, and fruiting (Zhang et al., 2014). The Agro-wastes on which mushrooms grow should supply specific nutrients required for cultivation (Chang and Miles, 2004). Almost all available lignocellulosic substances, inclusive of numerous wastes from agriculture, textile, and wood industries, can be used for growing mushrooms (Jeznabadi et al., 2016; Sanjay and Menuka, 2020). Growth and yield of oyster mushroom mainly depends on the chemical and nutritional content of Agro-wastes used (Miah et al., 2017). Addition of nutritional supplements to an agro-wastes is commonly employed to increase mushroom production (Ogundele et al., 2014).

Agro-wastes formulations with material based on wheat straw supplemented with wheat bran have shown good responses for oyster mushrooms, with supplemented mixes producing mushrooms with higher protein content (Iqbal et al., 2016). Yield and weight of mushroom fruiting bodies varied due to differences in chemical composition and the C/N ratio of Agro-wastes (Carrasco et al., 2018).

Mushroom cultivation is an emerging agriculture technology for sub-Saharan countries; its cultivation lies on utilization of plant derived agro-industrial waste, which causes environmental problems. The natural abilities of mushroom to digest and utilize almost all agricultural and agro-industrial residues is being considered as a profitable and bio-rationale technology for converting waste into nutritionally rich food (Antunes **et al**., **2020**). *Pleurotus ostreatus* is well-known for its nutritional value, medicinal effects, and biodegradability; it is also high in protein, vitamins, calcium, and various minerals such as copper, potassium, iron, and phosphorus; source of minerals, vitamins, proteins, and amino acids (Çağlarırmak, 2007).

Research findings on the effect of substrate and wheat bran supplementation on growth, yield and nutritional quality of oyster mushroom has been lacking in Ethiopia, particularly in central Gondar zone. Even if there is a great demand by different hotels due to its great nutritional value and customer need. Hence currently, there is a need for information that would contribute to identify the best and economically feasible cultural practice as substrate and wheat bran supplementation may be one agronomic tool to favor or increase yield and quality of fruiting body of oyster mushroom. Therefore, **the objective of this study was to contribute to the improvement of oyster mushroom production by identifying Agro-wastes and supplements.**

**Materials and Methods**

Four agro-wastes in mixes with wheat bran (15% of the total weight of the Agro-wastes) and without wheat bran were used. The experiment was arranged in a completely randomized design with 3 replications and 24 plots. The fungal strain *Pleurotus ostreatus* was obtained from the Mycology Laboratory, Department of Biology, Addis Ababa University, Addis Ababa, Ethiopia.

***Innoculant Preparation***

The pure culture of *P*. *ostreatus* was transferred to Potato Dextrose Agar prepared in the laboratory of the Department of Plant Science, University of Gondar. The culture media was prepared using the mixture of 20g potato infusion, 20 g glucose (dextrose), 15g agar and 0.2g chloramphenicol in 1L of distilled water then autoclaved at 121°C for 15 min. Potato infusion was prepared by boiling 200g sliced and peeled potato by 1 liter distilled water for 30 minute, then filter it through cheesecloth to save the effluent. The medium was poured into Petri-dishes and allowed to cool under aseptic conditions in a laminar flow chamber. The cooled, solidified, medium was inoculated with a 1 × 1 cm agar block of the fungal strain and incubated at 28°C. Growth of the culture and presence of contamination were visually inspected at a 3-day interval.

***Spawn Preparation***

Spawn of *P*. *ostreatus* was produced on yellow-colored sorghum (*Sorghum bicolor* L) grain. The grain was initially soaked overnight in tap water. The grain were washed and drained to remove dead, and floating, seed with water and lightly boiled for 15 min to kill the seed.

After draining excess water the grain was thoroughly mixed with 2% (w/w) gypsum and 4% (w/w) calcium carbonate, then poured into glass bottles to two-third of capacity and plugged with cotton. Calcium sulfate and calcium carbonate were used to maintain pH close to neutrality and reduce grain adhesion (Smith and Love, 1995). Cotton plugged bottles were sterilized at 121°C for 1 h. The bottles were shaken to break clumps of grains and cooled overnight. After cooling for a day, bottles were inoculated with a pure stock culture of 15 day-old agar with mycelium (1 × 1 cm). The culture and grain were thoroughly mixed to uniformly distribute the mycelium and incubated at 25°C. After all grains were fully covered with mycelium, the bottles were used as spawn or planting material.

***Substrate Preparation, Planting and Harvesting***

Wheat straw and water hyacinth leaves were cut into 1-3 cm long pieces. They were weighed and soaked in a sufficient amount of water (10 liter of water per kg of straw) overnight. The tea waste and saw dust were weighed after soaking in water overnight then all Agro-wastess, and the required amount of wheat bran, were boiled in water for 30 min for sterilization. After excess water was drained aseptically, the Agro-wastess were mixed with 1% calcium carbonate, and for treatments with supplements, mixed with wheat bran (15% of total weight of Agro-wastes).

Sterilized Agro-wastess were transferred to transparent polyethylene cultivation bags for observation of mycelial growth and presence of contamination. Agro-wastes moisture content was determined by the oven-dry method. Weight of a Agro-wastes sample of approximately 200 g, was dried in convection drying oven at 105 to 110°C for 24 h, then weighed and moisture loss determined by subtracting the oven-dry weight from the moist weight. Transparent polyethylene bags were filled with 1.7 kg of Agro-wastes and 170 g spawn (10% of the total weight of Agro-wastes). Spawn was planted using layers between 5 cm depth until the polyethylene bag weighed 1.87 kg. Pinholes were made with sterilized needle through bags (1/100 cm2) or (10-14) per bag for drainage and aeration. Inoculated bags were tightly tied with string and kept in a spawn running room at 23-25°C in the dark until primordia formed.

After primordial formation, large holes were made in the polythene bags to allow development of fruiting bodies. The bags were arranged in a simple randomization under a constructed fruiting house. Bags were sprayed with tap water 3 times a day until all flushes of fruiting bodies were harvested.

At the end of harvests growth parameters (mycelia completion period, pin head formation duration, fruiting body maturity, stipe length and cap diameter) were recorded; yield parameters (total weight of fruiting bodies at first, second, third flush per bag and total weight of fruiting bodies during the season per bag) were weighed for evaluation and biological efficiency calculated by the formula described by Pokhrel and Ohga, (2007).

The data were subjected to analysis of variance (ANOVA) using SAS (ver. 9.4, SAS Institute, Cary, NC), general linear model procedures. If the interaction was significant it was used to explain results. If the interaction was not significant means were separated by least significant difference (LSD).

**Results**

Mycelium completion rate, pin head formation and maturity were affected by agro-wastes, wheat bran supplement and their interaction. Treatment combination affected responses. When the agro-waste was wheat straw supplemented with 15% wheat bran, days required for mycelium to fully colonize the growing bag; to pin head formation and to mature was least.

Total fruiting body weight at first flush per bag, total fruiting body weight at second flush per bag, total fruiting body weight for the season and biological efficiency were affected by agro-wastes, wheat bran supplement and their interaction. When the agro-wastes was wheat straw supplemented with 15% wheat bran, the highest yield were harvested at 1st (Figure 1) and 3rd flush (Figure 2). Total fruiting body weight for the season (Table 3) and biological efficiency (Figure 3) were also highest.

Total fruiting body weight at second flush per bag was affected by the main effects (Agro-wastes and wheat bran supplement) whereas it is not affected by their interaction. When the Agro-wastes was wheat straw, total fruiting body weight at second flush per bag was highest (Figure 2). Total fruiting body weight at second flush per bag was also recorded highest yield when 15% wheat bran supplement was used

Yield of the first harvest were higher compared with the 2nd and 3rd harvests from the same Agro-wastes treatment combination. Using wheat straw agro-wastes with 15% wheat bran supplement increased total fruiting body weight at first flush per bag by 91.1% compared to tea waste without supplement. Use of wheat straw agro-wastes with 15% wheat bran supplement increased total fruiting body weight at third flush per bag per bag by 116.8% compared to sawdust Agro-wastes without supplement; both tea waste (TW)+10% wheat straw Agro-wastes with, or without, supplement did not yield after the second harvest.

Crude protein was affected by agro-wastes, wheat bran supplement and their interaction. Treatment combination affected responses. When the agro-waste was saw dust supplemented with 15% wheat bran, crude protein content was higher (Figure 2).

**Discussion**

Oyster mushroom manufacturing from agricultural waste has a number of advantages for the country, including lower pollution, lower costs, a shorter time commitment, and the provision of nutritionally rich food. The type, and amount of Agro-wastes, supplements, and their interaction with mushroom strains influence mushroom productivity (Sing et al., 2017).

The chemical and nutritional content of the Agro-wastess influences the growth and yield of mushrooms (Patil et al., 2010; Badu et al., 2011). The Agro-wastes on which mushrooms are grown should supply specific nutrients required for cultivation, and the main nutritional sources include cellulose, hemicelluloses, and lignin. For quality production of mushrooms, the Agro-wastes should possess an optimum C/N ratio (Chang and Miles, 2004). Supplementing the Agro-wastes with nitrogen-rich substances increases the overall productivity of the mushroom (Shashirekha et al., 2005). Nitrogen is an essential element for cellular functions, growth, and various metabolic activities, particularly protein and enzyme synthesis. The high nitrogen content of supplements could be a key factor for growth and fruit body formation. The Agro-wastes should also possess good aeration and water holding capacity (Mane et al., 2007). Appropriate preparation of the Agro-wastes is important for the production of a maximized mushroom yield. To reduce the risk of Agro-wastes contamination and pathogenic infections, nitrogenous supplements should be applied with care (Kazemi-jeznabadi et al., 2016).

 Different researchers reported that Agro-wastess play a role in the nutritional composition of Oyster mushrooms, and previous studies found differences in the growth and yield of oyster mushrooms cultivated using different Agro-wastess (Bhattacharjya et al., 2015; Sing et al., 2017). The fastest mycelium growth and pin head formation could be attained due to the natural characteristics of lignin and cellulose of the growing media, which hastened mycelium extension and shortened the days required for attaining mycelium completion of the Agro-wastes (Kimenju et al., 2009).

Various additives are recommended after sterilization for enhancement of mushroom yield (Naraian et al., 2009). According to (Pardo-Giménez et al., 2018) wheat and rice brans, chicken manure, cottonseed meal, urea, superphosphate, ammonium sulphate, and grape pomace, are ingredients to supplement Agro-wastess in mushroom cultivation.

**Conclusion**

On the basis of results of the present study, it could be recommended that agro-waste containing wheat straw supplemented with 15 % wheat bran could help to obtain higher yield and quality oyster mushroom. On the other hand, the study also showed that water hyacinth can also be used as Growing media for oyster mushroom production, which results in relatively higher yields next to wheat straw substrate. Hence, it is advisable to use water hyacinth leaves as a substrate to harvest reasonable yield with a less production cost. At the same time, it might be possible to minimize the infestation of Lake Tana by using this invasive weed as a substrate for mushroom production.

To obtain concrete and reasonable results on effects of substrate and supplementation on yield and nutritional compositions of mushroom, it would be valuable to repeat the study under different substrate formulation and seasons to arrive at a sound conclusion. Also, evaluation of mushroom production with different kind of supplementation practice deserve further investigation. Moreover, it is recommended that a further study be carried out to investigate differences in sensory characteristics of the oyster mushroom that are grown on the selected substrates.

**Data Availability**

The data used to support the findings of this study are included within the article.

**Disclosure**

The authors confirm that the content of the manuscript has not been published or submitted for publication elsewhere.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

 **Authors’ Contributions**

AW is the first author of the research article, whereas the coauthors have contributed equally to the literature collection, data collection and analysis, manuscript documentation, and its revision. All the authors have read and approved the final manuscript.

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**List of Tables and Figures**

Table 1. Treatment combination used in the study.

|  |  |
| --- | --- |
| Agro-wastes | Rate of wheat bran supplement (%) |
| Water hyacinth (WH) | 0 |
| Wheat straw (WS) | 0 |
| Sawdust (SD) | 0 |
| Tea waste (TW) + 10% wheat straw | 0 |
| Water hyacinth (WH) | 15 |
| Wheat straw (WS) | 15 |
| Sawdust (SD) | 15 |
| Tea waste (TW) + 10% wheat straw | 15 |

Table 2 Interaction effect of Agro-wastes and wheat bran supplementation on morphological and growth parameter of oyster mushroom.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Wheat bran supplement | Agro-wastesa | Mycelium completion rate (days) | Pin head formation (days) | Maturity (days) |
| Supplemented | WS | 21.00eb | 5.00fb | 4.16gb |
| Not supplemented | 25.00bc | 6.00d | 5.66d |
| Supplemented | WH | 22.33d | 5.50e | 4.76f |
| Not supplemented | 24.66bc | 6.46c | 6.00c |
| Supplemented | SD | 24.00c | 5.85d | 5.25e |
| Not supplemented | 24.66bc | 6.63c | 6.33b |
| Supplemented | TW | 25.66ab | 7.00b | 6.41b |
| Not supplemented | 26.50a | 7.45a | 7.00a |

a WS = Wheat straw; WH = Water hyacinth leaves; SD = Saw dust; TW = Tea waste+10% wheat straw.

b Data analyzed with least squares means. Values in a column followed by the same letter are not significantly different, P < 0.05, Tukey’s test.

Table 3: Interactioneffect of Agro-wastes and wheat bran supplement on total weight of fruiting body.

|  |  |  |
| --- | --- | --- |
| Wheat bran supplement | Agro-wastesa | Total fruiting body weight of the season(g) |
| supplemented | WS | 1203.60ab |
| Not supplemented | 931.67d |
| supplemented | WH | 1144.33b |
| Not supplemented | 890.67d |
| supplemented | SD | 1046.33c |
| Not supplemented | 773.67e |
| supplemented | TW | 694.33f |
| Not supplemented | 530.10g |

a WS = Wheat straw; WH = Water hyacinth leaves; SD= Saw dust; TW = Tea waste (TW) + 10% wheat straw.

b Data analyzed with least squares means. Values in a column followed by the same letter are not significantly different, P < 0.05, Tukey’s test.

Figure 1. Interaction effect of Agro-wastes and wheat bran supplement on fruiting body weight at first flush per bag (WF1). Bars with different letters for treatments are significantly different at p ≤ 0.05. Supplemented (S) not supplemented (NS)

**Figure 2.** Interaction effect of Agro-wastes and wheat bran supplement on fruiting body weight at third flush per bag (WF3). Bars with different letters for treatments are significantly different at p ≤ 0.05. Supplemented (S) not supplemented (NS)

**Figure 3.** Interaction effect of agro-wastes and wheat bran supplement on biological efficiency. Bars with different letters for treatments are significantly different at p ≤ 0.05. Supplemented (S) not supplemented (NS).

**Figure 4**. Main effect of Agro-wastes, or wheat bran supplement, on fruiting body weight at second flush per bag (WF2), respectively. Bars with different letters for treatments are significantly different at p≤0.05. Supplemented (S) not supplemented (NS)

**Figure 5.** Interaction effect of agro-wastes and wheat bran supplement on biological efficiency. Bars with different letters for treatments are significantly different at p ≤ 0.05. Supplemented (S) not supplemented (NS).