OPEN ACCESS Freely available online

Journal of Nutrition & Food Sciences

Research Article

Comparison of Low-Cost Substrates and Spawn Levels for Oyster Mushroom (*Pleurotus ostreatus*) Cultivation in Afghanistan

Mohammad Hussain Falahzadah[1*], Abdul Saleem Jamily[1], Sayeed Qadir Danishiar[2], Aziz Ahmad Osmani[2], Gul Mohammad Ajir[2]

[1]Department of Plant Protection, Kabul University, Kabul, Afghanistan; [2]Department of Biotechnology and Seed Production, Kabul University, Kabul, Afghanistan

ABSTRACT

There is a significant interest in the cultivation of oyster mushrooms (*Pleurotus ostreatus*) as nutritious food, with the rise in its consumption. Thus, there is a need to compare different low-cost available substrates for its productivity in Afghanistan. In the present study, two substrates wheat straw and tree leaves along with their combination (1:1) as a single substrate were tested based on 10, 20 and 30 grams of spawn (wheat seeds mycelium growth). And the growth rate of fruiting bodies (fresh weight) was used for statistical analysis. When the wheat straw with 30 gr of wheat seeds spawn was used, resulting in the highest yield (348 gr/Kg substrate). While the growth of the mushroom showed better results (273 gr/Kg substrate) on mixed substrates with 30 gr of spawn as compared with the third substrate which consist of tree leaves+30 gr of wheat seeds growth spawn (111 gr/Kg substrate) indicating the lowest yield for *P. ostreatus*. This study concluded that the wheat straw substrate had affected significantly positive on fresh weight, and its yield was higher when the maximum dose of spawn was applied. To the best of our knowledge, this is the first study that evaluated local substrates for cultivation of the mushroom in Afghanistan.

Keywords: Cultivation substrates; Wheat straw; Tree leaves spawn levels; *Pleurotus ostreatus*

INTRODUCTION

Mushrooms are a nutritious food product, significantly valued as the healthiest food all over the globe, and are highly favorable in Asian countries for their taste. Besides the collection of wild-type mushrooms usually from mountain regions, the cultivation forms are growing in a widespread manner. Mushroom forms and commercially imported types are following dynamic graphs in various places of the world. A report just in 2019 indicated global mushroom production got over 11.9 million metric tons, with the most production in china about 8.9 million tons followed by Japan (0.47) and the United State of America (0.38) million tons. According to mushroom dry weight, its digestible proteins range is (10%-40%), carbohydrates (4%-21%) and fiber (3%-35%). Therefore, it is higher than those vegetables and is a super-quality food. Mushroom's high potassium-to-sodium ratio greatly helps patients with hypertension and heart diseases, and is an excellent affordable food source to decrease malnutrition in poor countries because of its flavor, nutritious value and high yield productivity [1]. Mushrooms belong heterotrophic and need external substrate for their vegetative mycelium growth and fruiting bodies.

Therefore, it is saprophytic fungi or decomposers. Nearly, all lignocellulosic plant substances including forestry, cereals, pulses, and horticultural wastes are used for mushroom production in the forms. *Pleurotus* species require a short time lifecycle in comparison to other mushrooms and can be normally cultivated under "axenic conditions" on non-degraded sterile agricultural substrates. Its fruiting bodies are directly produced of lignocellulosic materials which composes of cellulose, hemicellulose, and lignin. Oyster mushrooms (*P. ostreatus*) have been cultivated on different substrates based on availability and productivity including leaves, roots, straw, seed, and stems of different tree species, wheat, corn, cereal grains, and other agricultural materials. Oyster mushrooms require optimal temperature ranging from 20°C-30°C and relative best

Correspondence to: Mohammad Hussain Falahzadah, Department of Plant Protection, Kabul University, Kabul, Afghanistan, Tel: 251979874445; E-mail: abr2030@yahoo.com

Received: 03-April-2023, Manuscript No. JNFS-23-24009; **Editor assigned:** 06-April-2023, PreQC No. JNFS-23-24009 (PQ); **Reviewed:** 20-April-2023, QC No. JNFS-23-24009; **Revised:** 28-April-2023, Manuscript No. JNFS-23-24009 (R); **Published:** 26-May-2023, DOI: 10.35248/2155-9600.22.13.017

Citation: Falahzadah MH, Jamily AS, Danishiar SQ, Osmani AA, Ajir GM (2023) Comparison of Low-Cost Substrates and Spawn Levels for Oyster Mushroom (*Pleurotus ostreatus*) Cultivation in Afghanistan. J Nutr Food Sci. 13:017.

performance humidity from 55%-70%. It is well documented that a mixture composed of various organic compounds is highly productive than a single compound of organic, and it is due to different nutritious elements in the mixture composition. The nutritious contents of mushrooms with essential minerals significantly increased the pharmaceutical value and their application for different purposes. Such essential elements like Se, Li, Zn, and Cu are useful for normal human body function. Traditional collection and cultivation of mushrooms in a polluted environment increase its risks due to toxic metals and leralloids (e.g. As, Ag, Cd, Hg, Ni, and Pb) for health [2]. The substrates used for oyster mushroom production are different in composition for elements and chemicals which may be a major factor in the growth and value contents of a mushroom. This study aimed to identify the best yield performance substrate out of a) Wheat straw, b) Tree leaves composed and c) a combination of wheat straw and tree leaves (1:1). Spawn level (10, 20 30 gr wheat seeds mycelium) for oyster mushroom (*Pleurotus ostreatus*) cultivation. The analysis was conducted based on its high productivity and economic efficiency for mushroom cultivation in Afghanistan as the majority of people's livelihoods belong to agricultural products.

MATERIALS AND METHODS

Site of experiment

This research work (spawn and substrate preparation) was conducted in the laboratory of the biotechnology and seed production department, Faculty of Agriculture, Kabul University. The cultivation of oyster mushrooms (*Pleurotus ostreatus*) was performed under controlled conditions (25°C-27°C) in the cultivation room of the department from June to September in 2021.

Treatment

The easiest and most locally available and low-cost substrates were selected for the cultivation of oyster mushrooms. The experiments were conducted in CRD-factorial design with three treatments; Wheat Straw (WS), deciduous Tree Leaves Composed (TLC) and Wheat Straw (WS) mixed with deciduous Tree Leaves composed (TL) at a rate of (1:1).

Spawn preparation

The mother spawns production and the wheat grain spawn produced according to. Mushroom mycelium culture and grain spawn preparation were carried out under laboratory conditions. The Mycelium of the mushroom (*Pleurotus ostreatus*) was obtained *via* single tissue isolation in our laboratory in previous studies used in this research. Potato Dextrose Agar (PDA) was used for fungal growth and the isolates were incubated at 25°C for 7 days at room temperature [3]. Then, the sub-culture was carried out under laminar flow and kept for 5 days to get the fast-grown mycelium for inoculation on wheat seeds.

Initially, wheat grains were boiled in water for 15 minutes to reach the softening stage. Then, the plastic bags of the wheat grains were autoclaved at 121°C for 20 minutes. Potato Dextrose Agar (PDA) containing the fresh mycelium was mixed with the autoclaved wheat seeds as a substrate for mycelium growth. For homogenous colonization of spawn on grains, the mycelium gently perturb the substrate well and was kept for 7 days at room temperature. For each substrate, three levels of 10, 20 and 30 grams of mycelium were used

Substrate preparation and cultivation

In this research, a perfect homogenized (1 cm-3 cm pieces) of wheat straw was collected from the Kabul city market. Then the substrate was pasteurized in a beaker by boiling in tap water at 100°C for one and a half hour. The pasteurized wheat straw is well-drained (exposed to free weather) to excess the extra water. The composed of tree leaves prepared under anaerobic conditions (leaves placed 1m deep underground with black-plastic cover on them) for three months from April to June 2021 [4]. The leave-composed substrate was prepared for sterilization in an autoclave plastic bag, and sterilized at 15 psi, 121°C for 60 minutes. One Kg of each Wet-Sterilized Substrate (WS, TLC, WS+TLC at 1:1 ratio) was separately packed into polypropylene bags (20 cm × 30 cm) in sizes in a clean room under aseptic condition. The three levels 10, 20, and 30 grams of grain spawn were added as each level of grain spawn in triplicates (1 replication=3 bags) and were prepared for inoculation on each substrate [5]. Grain spawn was spread on every layer of the substrate at two surfaces 10 cm and 20 cm height from the bottom of the bags and slightly pressed to be compacted. Small holes were made on the lateral and top sides of the bags for mushroom flash and aeration.

Data collection

The spawn-inoculated plastic bags were incubated in a dark cultivation room under the controlled condition temperature of 25°C-27°C, relative humidity of 85% with a light intensity of 300 lux-500 lux. The cultivation of mushroom plastic bags was carried out for 3 months in the cultivation room [6]. Within the three months of incubation, fruiting bodies (fresh weight) were harvested three times (one flash per week) and were considered as biological efficiency for statistical analysis.

RESULTS

Analysis of variance

Analysis of variance was done for the growth rate and yield of oyster mushroom. Effect of the studied treatments (wheat straw, a mixture of wheat straw with tree leaves and tree leaves only), level of spawn in gram (30 grams, 20g rams and 30 grams) and the result index in harvesting times (first, second and third harvest) are shown in Table 1. The ANOVA table shows that the studied substrates for *Pleurotus ostreatus* are significantly different ($p<0.05$) [7]. In the same way, the levels of studied spawn show significant effects on fresh weight of oyster mushroom, this indicates the dose spawn (30, 20 and 10 grams) could bring efficient positive effects on fresh weight index of mushroom at

significant level ($p<0.05$). In addition to the two mentioned treatments, the yield of fresh mushroom in consideration of each substrate and the level of spawn used were significantly different at the levels of 0.001, 0.004 and 0.002, as the results clearly indicate in Table 1. The results obtained in the table confirm that three types of low cost and accessible substrates in a low-income country like Afghanistan can be implemented to increase employment rate and local economics effectively. Each of these selected substrates can be used anywhere in Afghanistan to grow the well-known oyster mushroom *P. ostreatus*.

Table 1: Factorial-ANOVA table for fresh mushroom weight on three different substrates in regard to spawn levels and harvest times.

Source	df	SS	MS	F	Sig.
Substrates	2	54080.68	27040.34	326.027	0
Harvests	2	46970.08	23485.04	283.161	0
Spawn level	2	7900.896	3950.448	47.631	0
Substrates × Harvests	4	5150.112	1287.528	15.524	0
Substrates × Spawn level	4	1807.646	451.912	5.449	0.001
Harvests × Spawn level	4	1425.649	356.412	4.297	0.004
Substrates × Harvests × Spawn level	8	2402.624	300.328	3.621	0.002
CV	14.38				

Morphology and biomass of fruiting bodies

The effects of the substrates tested in this study show that there are no significant differences in the morphology of Mushroom regardless of the substrates used [8]. Each bag of mushroom contains 1 Kg of the substrate, on wheat straw substrate all the parts of the cap of the fruiting bodies are white, but the color of fruiting bodies caps changed a little to white-yellowish color in the mixed substrate (wheat straw+tree leaves compost) and the tree leaves compost can be seen in Figure 1.

The amount of spawn produced through wheat seeds mycelium in this experiment was three levels higher as mentioned [9]. At 30 gr of spawn, the first harvest mean was 154.00 gr ± 10.6 gr as compared to 134.7 gr ± 13.87 gr and 113.33 gr ± 12.5 gr fresh weight Mushroom through the use of 20 grams and 10 grams of spawn respectively (Figure 1).

Figure 1: Morphological characteristics of oyster mushroom cultivated on different substrates and three different level of spawn.

Effect of the substrates on Mushroom (*P. ostreatus*)

To find the best substrate for the cultivation of oyster mushroom, the cheapest and locally available substrates were

used separately and in combinations [10]. Figure 2 shows wheat straw has a significant and positive effect on fresh weight of Mushroom, followed by the mixed substrate (wheat straw mixed with tree leaves compost at 1:1 ratio) and tree leaves substrate respectively. That result is true in consideration of 30 grams of spawn for producing of the highest fresh weight, followed by 20 and 10 grams of spawn.

First treatment (Wheat straw) with 30 grams of spawn resulted the highest fresh weight in the first harvest (154 grams), in comparison to the second harvest (111 grams) and third harvest (71 grams). To consider total fresh weight of 336 gr per 1 Kg of substrate (wheat straw) with the highest yields, ranked at first harvestmen, followed by the result obtained from 20 grams of spawn on wheat straw substrate 135, 104, and 48 grams/Kg respectively in the first, second and third harvests whit the total of fresh weight 287 grams/Kg of substrate [11]. The results obtained from 10 grams of spawn on wheat straw were 114, 81 and 49 grams at first, second and third harvests respectively. According to Table 1, the difference in the effect of the level of spawn used is significant ($p<0.05$). In the first harvest of 30 grams of spawn show increased in yield compared to 20 grams of spawn and 10 gr of spawn by 14% and 48% respectively. In the second harvest, the yield (fresh weight) collected from 30 grams of Spawn compared to 20 grams and 10 grams showed a higher yield of about 6.7% and 37%. Finally, in the third harvest through 30 grams of spawn, the yield increased by 48% and 47% compared to 20 grams and 10 grams [12].

The mushroom collected from mixed substrate of wheat straw and tree leaves (1:1 ratio) using 30 grams of spawn in the first harvest was 130 grams, in the second harvest it was 92 grams and in the third harvest it was 35 grams, where the total fresh weight of mushroom was 237 grams/1 kg of the substrate. In the same manner, the results obtained from 20 grams of spawn in 1Kg of this mixed substrate, a fresh weight of 90, 78 and 48 grams were harvested from the first, second and third harvest times respectively with the total of 216 grams of fresh weight in 1 kg of the substrate. In the last stage, the weights of 67, 45 and 28 grams' mushroom produced in the first, second, and third harvesting time, which total weight is 140 grams by addition of 10 gr of spawn in the substrate. So, the effects of the level of spawn on the yields of mushroom in the mixed substrate is significant ($p<0.05$) [13]. In the first harvest, 30 grams of spawn has caused an increase of 22% to 64% in the fresh weight of mushroom compared to 20 grams and 10 grams respectively. Likewise, in the second harvest, the difference of 30 grams of spawn compared to 20 grams is shown 17.9%, but significantly compared to 10 grams of spawn, it shows an upward increase of 104%.

The results obtained from the tree leaves substrates (100%) with 30 grams' spawn in the first, second and third harvests were 59, 33 and 18 grams respectively, and the total fresh weight was 130 grams. In 20 and 10 grams of spawn used in the first harvest (48 gr and 43 gr) and in the second harvest (37 gr and 33 gr) and in the third harvest (11 gr, 30 gr) of fresh weights of Mushroom were obtained, it shows the lowest yield levels in the Figure 2. As the total fresh weight of 97 and 86 grams/1 Kg of tree leaves substrate were harvested from 20 grams to 10 grams of applied spawn respectively. The production rate of oyster mushroom is also significantly depending on level of spawn [14]. The level of 30 grams of spawn has increased the fresh weight of mushroom by 22% compared to 20 grams of spawn as shown in Figure 2.

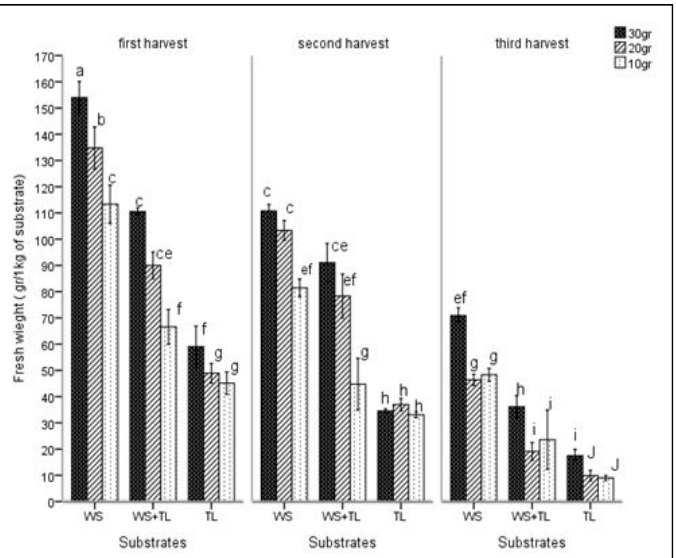

Figure 2: Effects of different substrates and the level of spawn on the production of oyster mushroom (*P. ostreatus*) in the first, second and third harvests.

Three mushroom cultivation substrates (wheat straw, mixed substrate (wheat straw mixed with tree leaf compost at ratio of 1:1) and tree leaves compost) were tested at three levels 10, 20 and 30 grams of spawn.

Economic analysis of the substrates

Based on the economic analysis, the ratio of benefit and cost of the used substrates was taken into account. The ratio of income to the cost of the substrates were calculated for every 10 kg of the three cultivation substrates. It is defined as the ratio for the benefit cost ratio from the sale of mushroom to the total cost.

In Mushroom farm, when the ratio of income and cost is=1, the project is accepted, and if it is higher than 1, from the economic aspect and accessibility of the local substrate produced, it indicates a higher level of sustainability of the project. In the first treatment (wheat straw), the benefit-cost ratio is 5.22, and is 4.22 times more than the cost of substrate (Table 2). Similarly, the mixed substrate outcome for fresh mushroom is 3.82 times compared to the input (cost of substrate). The third substrate (Tree leaves) with a very small difference (0.38), recorded the lowest benefit overall the substrate [15]. The difference in benefit-ratio of wheat straw and tree leaves is 2.84 (315%), which shows a significant difference as compared with the wheat straw substrate. Based on the data recorded, it can be concluded that wheat straw is one of the cheapest and accessible substrate that has the highest income overall and is considered a lucrative job in Afghanistan. Benefit cost ratio calculated as fallow:

T1-Wheat straw=1044/200=5.22.

T2-Wheat straw tree leaves compost=819/170=4.82.

T3-Tree leaves compost=333/140=2.38.

Table 2: The ratio of gross income from the sale of oyster mushroom (*Pleurotus ostreatus*) to the total cost in Afghanistan currency.

Treatment	Cost of 10 kg substrate	Yield/kg substrate	Income/10 kg substrate	Gross margin	Benefit-cost ratio
WS	200 AFN	348 gr	1044 AFN	844 AFN	5.22
WS+TL	170 AFN	273 gr	819 AFN	649 AFN	4.82
TL	140 AFN	111 gr	333 AFN	193 AFN	2.38

Statistical analysis

Analysis of Variance (ANOVA) performed through statistics 10 and IBM SPSS statistics 21 to test the treatment means for all groups including substrates and spawn levels and their interactions [16]. SPSS (v21) conducted for multiple comparison (LSD) and chart for substrates and spawn levels based on F-ratio and P-value (α=0.05, P<0.05).

DISCUSSION

Oyster mushrooms is the healthiest food worldwide and is highly favorable in Asia for its taste and nutritious values. Beside the collection of wild type mushrooms, cultivation forms are growing in a widespread manner in many countries across. According to the report by mushroom production in Afghanistan is at initiation stage in comparison to other commercially producer such as India, Japan, China and USA. To cultivate oyster mushroom (*P. ostreatus*) on easily available substrates (wheat straw and tree leaves) is a need for the family's income in the poor country like Afghanistan.

This study conducted to evaluate whether the three substrates used for cultivation of oyster mushroom, are different in chemical composition and properties, may cause effects on biomass of mushroom fruiting bodies, and to find whether the amount of spawn can be effectively grown for fresh weight of mushroom or can be different [17]. The results of this study are determinant in prospects of maximum yield efficiency that is right practical and can be commercially important for commercial producer.

As the properties of the selected substrates differ in various components, the yield collected and observed morphological features of the fruiting bodies have some deviation. In wheat straw the caps of the fruiting bodies are whitish in color, but in the substrates (wheat straw+tree leaves compost in (1:1) ratio and the tree leaves composed mostly the centers of fruiting bodies caps deviated to yellowish. This change might be created by leaves compost as the nutrient contents might be differ from wheat straw [18]. In regards to economic analysis, this alteration of color may decrease the market product values by consumers and have inappropriate effects on sales. In these experiments, significant differences were recorded between all substrates used for mushroom cultivation included wheat straw, combination of wheat straw and tree leaves compost (1:1) and tree leaves compost as pure substrate [19]. However, the fresh weight of P. ostreatus were significantly higher (p<0.01) when grown on straw of wheat. It is thus interpreted using this substrate potentially over those both tree leaves or combination substrates.

As wheat straw with 30 grams of spawn used for cultivation, that recorded significant differences (p<0.01). As in the previous study also suggested the same result on *P. ostreatus*. Low dose of spawn seem has less effectivity for mushroom production, it is may be due to less source of mycelium in cultivation plastic bags of mushroom. In the case of harvesting time, this study observed that the highest weight of fresh mushroom collected from first harvest and the consequent decreased in the second and third harvest in wheat straw, tree leaves and combination substrate is in accordance to the findings by research team that reported the yield decreases by the harvesting times. To postulate it scientifically, that the nutrient compositions of the substrates could be used mostly at the initiation stage of mushroom flushes, which show high yield and fast growth, then this nutritious values of substrates decrease by the harvest times for mushroom growth, as mushroom fruiting bodies growth positively associated with the nutritious values of the substrates.

From the economic point of view, the ratio of input (cost of substrate) and output (benefit of mushroom) calculated in Table 2 were most effective. The benefit taken from wheat straw substrate is 5.22 times in compare to its cost, which can be considered a profitable option. And the leaves of the tree and the combination substrate recorded the lowest benefit-cost ratio. Since the compost of tree leaves recorded the lowest fresh weight. The yield of combination substrate (1:1) also affected in 50% by the tree leaves compost which had less than wheat straw benefits.

CONCLUSION

This study proved that fresh weight of mushroom fruiting bodies has significantly affected under the studied treatments. Among the substrates used for cultivation of *P. ostreatus*, wheat straw recorded the highest fresh weight of mushroom in the first, second and third harvest times over the tree leaves and combination substrates. The amount of spawn used in this experiments, 30 grams out of 10 and 20 grams had the potential influence on fresh weight which positively associated for high yield of oyster mushroom production in a low-income country like Afghanistan.

FUNDING

Food and Agriculture Organization (FAO) supported 5% for facility requirements for this research, but its support stopped due to political change in Afghanistan. We by our-self completed this research for the purpose of creating jobs for poor people of Afghanistan. We performed this study design, data collection, analysis and decision to publish the manuscript (we have no any funding recourse for this paper publication).

DECLARATION OF COMPETING INTEREST

The authors have no conflict of interest or personal influence on the works reported in this paper.

DATA AVAILABILITY

The data reported in this paper will be send by the request of the journal.

AUTHOR'S CONTRIBUTION

Sayeed Qadir Danishiar and Aziz Ahmad Osmani designed the research and performed all experiments; Sayeed Qadir Danishiar, Abdul Saleem Jamily and Mohamad Hussain Falahzada conducted data analysis; Sayeed Qadir Danishiar and Gul Mohammad Ajir wrote the manuscript. All authors reviewed and approved the final manuscript

ACKNOWLEDGEMENT

This work was conducted in the department of biotechnology and seed production faculty of agriculture Kabul university. And also we thank the reviewers and editors for their helpful comments on this paper.

ETHICAL APPROVAL

There is any human participants or animals applied in this research.

CONSENT TO PARTICIPATE

All authors participated for this research and have seen and approved this manuscript.

CONSENT FOR PUBLICATION

All the mentioned authors have seen and approved this article submission.

REFERENCES

1. Amirta R, Herawati E, Suwinarti W, Watanabe T. Two-steps utilization of shorea wood waste biomass for the production of oyster mushroom and biogas-a zero waste approach. Agric Agric Sci Procedia. 2016;9:202-208.
2. Ashraf J, Ali MA, Ahmad W, Ayyub CM, Shafi J. Effect of different substrate supplements on oyster mushroom (*Pleurotus* spp.) production. Food Sci Technol. 2013;1(3):44-51.
3. Assan N, Mpofu T. The influence of substrate on mushroom productivity. Sci J Crop Sci. 2014;3(7):86-91.
4. Bhattacharjya DK, Paul RK, Miah MN, Ahmed KU. Effect of different saw dust substrates on the growth and yield of oyster mushroom (*Pleurotus ostreatus*). IOSR J Agric Vet Sci. 2014;7(2): 38-46.
5. Buah JN, van der Puije GC, Bediako EA, Abole EA, Showemimo F. The growth and yield performance of oyster mushroom (*Pleurotus ostreatus*) on different substrates. Biotechnology. 2010;9(3):338-342.
6. Dubey D, Dhakal B, Dhami K, Sapkota P, Rana M, Poudel NS, et al. Comparative study on effect of different substrates on yield performance of oyster mushroom. Glob J Biol Agric Health Sci. 2019;7.
7. Fang W, Zhang X, Zhang P, Morera XC, van Lier JB, Spanjers H. Evaluation of white rot fungi pretreatment of mushroom residues for volatile fatty acid production by anaerobic fermentation: Feedstock applicability and fungal function. Bioresour Technol. 2020;297:122447.
8. Zahid A, Alharbi NK, Khan MI, Noreen A, Khan AA, Qamar S, et al. Thiourea enriched cotton waste enhances biomass and nutrition contents in (White oyster) and (Phoenix oyster) mushrooms. J King Saud Univ Sci. 2022;34(4):102054.
9. Girmay Z, Gorems W, Birhanu G, Zewdie S. Growth and yield performance of *Pleurotus ostreatus (Jacq. Fr.) Kumm (oyster mushroom)* on different substrates. Amb Express. 2016;6:1-7.
10. Hu W, Di Q, Liang T, Liu J, Zhang J. Effects of spent mushroom substrate biochar on growth of oyster mushroom (*Pleurotus ostreatus*). Environ Technol Innov. 2022;28:102729.
11. Iossi MR, Cobos JD, Alegria FJ, Zied DC. *Pleurotus* spp. cultivation on *Brachiaria* sp. straw treatment with alkaline water. Braz J Microbiol. 2018;49:64-67.
12. Jeznabadi EK, Jafarpour M, Eghbalsaied S. King oyster mushroom production using various sources of agricultural wastes in Iran. Intl J Recycl Org Waste Agric. 2016;5:17-24.
13. Liu SR, Zhang WR, Kuang YB. Production of stalk spawn of an edible mushroom (*Pleurotus ostreatus) in l*iquid culture as a suitable substitute for stick spawn in mushroom cultivation. Sci Hortic. 2018;240:572-577.
14. Maurya AK, John V, Murmu R, Simon S. Impact of different substrates for spawn production and production of milky mushroom (*Calocybe indica*). Int J Pharma Bio Sci. 2019;10(2):5-10.
15. Muswati C, Simango K, Tapfumaneyi L, Mutetwa M, Ngezimana W. The effects of different substrate combinations on growth and yield of oyster mushroom (*Pleurotus ostreatus*). Int J Agron. 2021;2021:1-0.
16. Nam WL, Phang XY, Su MH, Liew RK, Ma NL, Rosli MH, et al. Production of bio-fertilizer from microwave vacuum pyrolysis of palm kernel shell for cultivation of oyster mushroom (*Pleurotus ostreatus*). Sci Total Environ. 2018;624:9-16.
17. Pal J, Sharma R, Lal M, Suman BC. Effect of different spawn rates and substrate supplementation on yield of Indian Oyster mushroom, *Pleurotus pulmonarius* (Fr.) Quel. J Appl Nat Sci. 2017;9(3):1406-1410.
18. Xie C, Gong W, Yan L, Zhu Z, Hu Z, Peng Y. Biodegradation of ramie stalk by *Flammulina velutipes*: Mushroom production and substrate utilization. Amb Express. 2017;7(1):1-8.
19. Besufekad Y, Mekonnen A, Girma B, Daniel R, Tassema G, Melkamu J, Asefa M, Fikiru T, Denboba L. Selection of

appropriate substrate for production of oyster mushroom (*Pleurotus ostreatus*). J Yeast Fungal Res. 2020;11(1):15-25.