Vol.2, No.3, Page 215- 222, 2024 https://doi.org/10.59628/jast.v2i3.767 ISSN 2958-9568 JAST

SUN PUBLISHER

مجلة جامعة صنعاء للعلوم التطبيقية والتكنولوجيا
Sana'a University Journal of Applied Sciences and Technology
https://journals.su.edu.ye/index.php/jast/

Effect of agricultural substrates mixtures on yield and components in oyster mushroom (*Pleurotus ostreatus*)

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Abstract

Mushroom cultivation is virtually non-existent in Yemen, despite it becoming increasingly common as an effective biotechnological practice for recycling agricultural by-products into valuable human food. Most farmers dispose of their agricultural waste, while it could be utilized beneficially as substrates for mushroom production.This study was conducted to investigate the effect of available agricultural waste mixtures on the growth of fungus *Pleurotus ostreatus*, yield and their components when used as substrates. Various agricultural waste and their mixtures were used, including banana, sorghum, barley, (banana + sorghum in a 1:1 ratio), (banana + barley in a 1:1 ratio), (sorghum + barley in a 1:1 ratio) and (banana + sorghum + barley in a 1:1:1 ratio), as substrates for improving fungus *Pleurotus ostreatus* growth. The experiment was carried out using a completely randomized design. The substrates were sterilized after preparation and packed into heat-resistant polypropylene bags. They were then inoculated with spawn and incubated. The results indicated that the shortest incubation period was observed (21.67 days) when using barley straw as a substrate. Additionally, the shortest duration for incubation and the formation of pinheads was achieved with the mixture of sorghum and barley, with durations of (25.67 and 10 days, respectively). The results showed that the highest number of clusters was obtained when using the substrate composed of a mixture of sorghum + barley, reaching 3.33 clusters. The maximum average number of fruits was recorded for the substrate mixtures when using the composed mixture of banana and barley, with a count of 7.00 fruit/cluster. Furthermore, the highest total productivity was obtained from the substrate composed of a mixture of (sorghum + barley), amounting to (810 g) with a biological efficiency of 91.2%. The fruiting bodies were analyzed for protein content, and the highest protein percentage (32.09%) was recorded when using the substrate mixture of (banana + sorghum + barley). Based on the research findings, we can conclude that agricultural waste mixtures can lead to increased productivity of oyster mushrooms and utilize the abundance of agricultural waste for mushroom production, enhancing their properties by incorporating them with barley residues. This, in turn, provides an alternative protein source.

Article Info

Keywords:
Mushroom, Mixtures, Yield, Efficiency, Substrates.

Article History:
Received: 30-November-2023,
Revised: 6-December-2023,
Accepted: 31-January-2024,
Available online: 30 June 2024.

1. INTRODUCTION

Providing protein-rich food is considered one of the most important challenges facing countries worldwide in light of the recent global food crisis that started in 2007/2008. The search for alternatives to consuming meat has become highly important, with increased consumer awareness of the risks associated with regular consumption of meat, such as high cholesterol levels and the spread of certain diseases affecting animals. Crow's mushroom is considered a suitable alternative to animal proteins [1]. Edible mushroom cultivation is a biotechnological strategy that involves reusing lignocellulosic organic waste. It may be the only existing procedure that brings together the production of food that's high in protein with the decrease of environmental pollution [2]. China is the world's largest producer of mushrooms, accounting for approximately 65% of the global production of mushrooms and

85% of oyster mushrooms. An oyster mushroom (*Pleurotus ostreatus*) is among the world's second-largest commercially grown and important edible fungus [3]. Based on statistics from the Food and Agriculture Organization [4], global agricultural mushroom production reached 10,242,541 tons in 2017. Fresh mushrooms are about 85% water and 3.2% protein. Dried mushrooms, on the other hand, have a low water content, a high percentage of protein (34 to 44%), and a low-fat content [5]. Important nutrients (niacin, vitamin D, potassium) are also supplied [6]. Oyster mushroom (*Pleurotus ostreatus*), is distinguished by its content of carbohydrates, vitamins, and minerals. It also contains a high proportion of fiber [7]. Research indicates that consuming oyster mushrooms may reduce the risk of various diseases, such as heart disease, hepatitis B, high cholesterol levels, digestive system cancers, as well as high blood pressure, and diabetes. Incorporating mushrooms into daily meal planning can help mitigate the risk of these diseases [8–10]. Mushroom cultivation contributes to reducing poverty rates and achieving livelihoods by producing a high-yielding food source and providing a steady source of income. It does not require large amounts of money to be cultivated on a relatively small scale [11]. Furthermore, mushroom cultivation helps in the disposal of agricultural waste and protect the environment [12]. The determining factor for using any substrate in mushroom cultivation is the amount of organic matter it contains [13]. Mixing different agricultural waste materials is a contributing factor in increasing productivity and can be utilized to reduce production costs. Creating a blend composed of multiple waste sources can improve structural properties, such as moisture retention capacity, and enhance the agricultural medium's structure and porosity, which increases environmental permeability and ventilation [14]. Sorghum ranks first among cereal crops in Yemen, representing approximately 59% of the total cultivated area. The total cultivated area of sorghum in Yemen is approximately 313,916 hectares, with a total production of 230,766 tons. Our country is characterized by an abundance of agricultural waste resulting from banana cultivation. The total cultivated area for bananas in Yemen is approximately 9,431 hectares, with a total production of 116,300 tons [15]. Despite the importance of using barley straw to achieve higher productivity, researchers have found that the highest fresh weight of mushrooms was achieved on a barley straw substrate [16]. However, barley is considered one of the least cultivated cereal crops in terms of both area and production in Yemen. The total cultivated area for barley is approximately 27,344 hectares, with total production from this area reaching 26,933 tons [15]. Thus, barley residues are relatively low, and they are mostly used for animal feed. This prompted the research objective of utilizing agricultural waste from sorghum and banana and studying the extent to which their nutritional value can be enhanced for mushroom cultivation through the formulation of a blend consisting of sorghum and banana residues, supplemented with barley as an enhancer for these substrates, and examining their impact on oyster mushroom productivity.

Table 1. Percentage of organic carbon and nitrogen in agricultural substrates.

Substrates	C %	N %	C/N ratio
Banana	**42.69**	**1.00**	**42.69**
Sorghum	**40.71**	**0.88**	**46.26**
Barley	**35.40**	**0.87**	**40.68**
Banana+ Sorghum	**38.73**	**0.84**	**46.11**
Banana+ Barley	**44.53**	**0.82**	**54.30**
Sorghum+ Barley	**36.40**	**0.81**	**44.94**
Banana+ Sorghum +Barley	**45.53**	**0.71**	**64.13**

2. MATERIALS AND METHOD

Part of this study was conducted at the Orchards and Tissue Culture Laboratory in the Department of Horticulture and its Technologies at the Faculty of Agriculture, Food, and Environment at Sana'a University. The other part was carried out in a private laboratory in Sana'a city, which was equipped for cultivating and producing mushrooms. It was conducted during the period from July to November of 2022.

2.1. Fungal Strain

The oyster mushroom (*Pleurotus ostreatus*) strain was obtained from the Agricultural Research Center in Cairo, Arab Republic of Egypt.

2.2. Agricultural substrates

The agricultural substrates used were obtained from various locations and were used either individually or in the form of mixtures as follows:

1. Banana waste, sourced from Al Hudaydah Governorate.
2. Sorghum waste, sourced from Sana'a Governorate.
3. Barley waste, sourced from Yareem Directorate in Ibb Governorate.
4. A mixture of banana waste and Sorghum (1:1) (w:w).
5. A mixture of banana waste and barley (1:1) (w:w).
6. A mixture of Sorghum waste and barley (1:1) (w:w).
7. A mixture of banana waste, Sorghum, and barley (1:1:1) (w:w:w).

The organic carbon ratios of agricultural substrates were determined according to **George et al.** [17] and the nitrogen content was determined using the Kjeldahl apparatus, according to **A.O.A.C** [18].At the Laboratory of Soil and Water Department, Faculty of Agriculture, University of Sana'a.

2.3. Spawn Preparation

The sorghum grains were cleaned to remove any impurities and washed several times. Then, they were soaked in water overnight. Additionally, the soaked grains were briefly boiled and then washed again. Afterward, the grains are filtered to remove the excess water and spread out on a clean surface. Next, 3% calcium carbonate and 2% calcium sulfate were added to the grains and mixed thoroughly. The grains were then packed into polypropylene bags, which were sealed with sterilized cotton and tightly closed with a rubber band. The bags were sterilized at a temperature of 121°c for one hour. Afterward, the cooled bags were inoculated and incubated at a temperature of 25°c for two weeks until the mycelium fully colonized the sorghum grains.

2.4. Preparation of Agricultural Substrates

The agricultural waste was completely dried under sunlight to remove any excess moisture. To enhance the surface area for mycelium growth, the waste materials were cut into small pieces, approximately 3-5 cm in size. Then, they were soaked in water overnight, followed by thorough drainage to remove excess water. The hand-press method was employed to determine the moisture content. When the waste material was squeezed between the fingers, only a minimal amount of water was released, as described by **Varghese and Amritkumar** [19]. Afterward, wheat bran (to increase productivity) and agricultural gypsum (calcium sulfate to adjust the pH) were added to the substrates at a rate of 5% each, and they were mixed thoroughly. The mixture was then packed into polypropylene bags. The prepared plastic bags were sterilized at a temperature of 121°C for one hour, as recommended by **Ogundele et al.** [20]. After sterilization, the bags were cooled for one day. Subsequently, they were inoculated with *P. ostreatus* spawn, which had been prepared beforehand. The inoculation was carried out in a sterile environment (inoculation chamber). Finally, the bags were sealed with sterilized cotton and a rubber band.

2.5. Incubation

The inoculated bags were incubated in a dark incubation room at a temperature of approximately 22-26°C until the fungus fully colonized the substrate. This can be identified by the distinct white coloration (fungal growth) The incubation period varies depending on the type of waste material used and the spawn rate, and the incubation period for each agricultural substrate was recorded.

2.6. Fruits Formation Stimulation

After the incubation process is completed and the substrate is fully colonized by the mycelium, the environmental conditions need to be optimized for fruiting. Side openings have been made in the bags to initiate service operations that will assist in the initial formation and development of pinheads into fruits.

2.6.1. Ventilation

Adequate ventilation is essential to reduce CO_2 concentration and increase O_2 concentration, which promotes fruiting. Ventilation is achieved by opening the windows for 1-2 hours daily and occasionally opening the door.

2.6.2. Lighting

The bags are exposed to light, utilizing indirect natural light. In cases where natural light is insufficient, artificial lighting is used for 4-6 hours daily.

2.6.3. Temperature

The growth room temperature is maintained between 16-25°C.

2.6.4. Irrigation and Relative Humidity

The humidity level in the growth room was maintained between 80-90% by spraying water on the floor and in the air. The moisture requirements for the bags were met by spraying them twice daily using a mist sprayer, ensuring that the surface was moist but not dry. During this process, the relative humidity and room temperature were monitored and maintained using a hygrometer. The aforementioned procedures continued twice daily until the end of the cycle. As time progresses, fruits begin as branched growths resembling pinhead, slightly swollen and dark-colored. These serve as the initial stages of fruiting bodies. They continue to develop and branch out until they form clustered fruits, where the fruits are not individual but rather interconnected with each other, resembling a hand palm.

2.7. Harvesting

The fruiting bodies (clusters) are harvested when they reach maturity, which is indicated by the cessation of their growth and the light brown (beige) coloration and downward curling of their edges. To harvest, the fruiting bodies are gently grasped at the base between the thumb and forefinger and twisted in a clockwise direction with a complete turn, while ensuring the removal of any remnants of the harvested cluster to encourage subsequent flushes. Multiple clusters are harvested from each bag in several flushes. At this stage, the sequence of tasks is as follows: ventilation, fruiting body harvesting, irrigation, humidity control, along with appropriate temperature and lighting.

2.8. Experimental design

A pilot study was conducted, consisting of seven agricultural substrates that were replicated three times using a complete randomized design.

2.9. Studied Traits

following data were recorded:

1. Average incubation period.
2. Average period of formation of pinheads.
3. Average number of fruiting clusters: Calculated as the number of clusters present per bag/substrate.
4. Average number of fruits per cluster.
5. Total yield (gram/kilogram substrate).
6. Estimation of the biological efficiency (BE%): Biological efficiency was calculated by the following equation reported by **Stamets** [21] as follows:

$$Biological\ efficiency(\%) = \frac{\text{Fresh weight of mushroom (g)}}{\text{Dry weight of substrate}} \times 100 \quad (1)$$

7. Estimating of crude protein content was performed using the Kjeldahl method and the utilization of the factor (N× 6.25) [22]. The analysis was conducted at the Central Laboratory of the Agricultural Research Center in Cairo.

2.10. Statistical analysis

The collected data were analyzed using the statistical software GenStat (Version 12) according to the adopted design. The means were compared using the least significant difference (LSD) test at a significance level of 0.05, as described by **Steel and Torrie** [23].

3. RESULTS AND DISCUSSION

Table 2 shows significant differences in the effect of agricultural substrates mixtures on the average incubation period. The shortest incubation period (21.67 days) was observed when using barley straw, while the longest incubation period (39.33 days) was found in the substrate mixture composed of banana waste and barley straw. The shortest incubation period for agricultural substrates mixtures was recorded in the mixture of sorghum and barley, which reached 25.67 days. Similarly, the table demonstrates significant differences in the effect of agricultural substrates mixtures on the average onset of pinhead formation. The shortest duration (6.67 days) was observed when using sorghum waste alone, while the longest duration (18 days) was observed when using banana waste. The shortest duration for pinhead formation among the substrate mixtures was recorded in the mixture of sorghum and barley, which reached 10 days. The previous results are nearly consistent with the findings of **Kumar et al.** [24], who demonstrated that the shortest incubation period was 23 days when using maize straw. According to **Hossein** [25], the required time to complete the incubation period varies depending on the waste materials used. The findings differed from Hussein's [25] study, as the incubation period for fungi when using only banana waste was 21.5 days. However, the results aligned with the same researcher's findings when using rice straw and wheat straw waste, where the shortest incubation period was achieved, reached 21 and 22 days, respectively. Researchers **Tesfaw et al.** [26] indicated that the pinhead formation period from the beginning of incubation until pinheads were formed ranged from 26 to 31 days when using various agricultural wastes for cultivating *Pleurotus ostreatus*, including barley waste, wheat straw, and others. On the other hand, **Pala et al.** [27] observed that when cultivating mushrooms using various agricultural residues such as rice straw, wheat straw, Chinar leaves, and apple leaves, the shortest incubation periods and pinhead formation were observed in rice straw, ranging between 17-19 and 21-23 days, respectively. This was followed by wheat straw, with an incubation period of 22-24 days. The results differed from those reported by **Dlamini et al.** [28], where the incubation period was 70 days in a substrate mixture consisting of maize straw and corn cobs. In contrast, the incubation period was 58 days in maize straw alone and 83 days in banana leaf residues. This variation could be attributed to the prevailing temperature conditions. These results closely aligned with those reported by **Sahoo et al.** [29] regarding the emergence period of pinheads, which reached 14.7 days when utilizing corn straw. The table 2 reveals that there are numerical differences in the impact of substrate mixtures on the average

Table 2. Effect of agricultural substrates mixtures on average incubation period(day), pinhead (day), number of clusters, number of fruits.

Substrates	Incubation	pinhead	No.clusters	No.fruits
Banana	**38.67 a**	**18.00 a**	**2.67 a**	**3.33 b**
Sorghum	**37.67 a**	**6.67 d**	**3.00 a**	**7.33 ab**
Barley	**21.67 c**	**15.67 ab**	**3.17 a**	**10.17 a**
Banana+ Sorghum	**38.67 a**	**17.33 a**	**3.00 a**	**5.75 b**
Banana+ Barley	**39.33 a**	**16.33 a**	**2.67 a**	**7.00 ab**
Sorghum+ Barley	**25.67 bc**	**10.00 cd**	**3.33 a**	**6.50 ab**
Banana+ Sorghum +Barley	**30.00 b**	**12.67 bc**	**3.20 a**	**5.83 ab**
L.S.D 0.05	**6.359**	**3.388**	**0.695**	**4.358**

number of clusters, but they did not reach a level of significance. The highest number of clusters (3.33 clusters) was achieved when using a substrate composed of a mixture of sorghum and barley. On the other hand, the lowest number of clusters (2.67clusters) was observed in the following substrates: banana, and a mixture of banana and barley. The table 2 also shows significant differences in the effect of agricultural substrate mixtures on the average number of fruits. The highest average number of fruits (10.17 fruit/cluster) was achieved when using only barley residues, while the lowest number of fruits (3.33 fruit/cluster) was observed when using only banana residues. Additionally, the table reveals numerical differences that did not reach a level of significance regarding the effect of some agricultural residue mixtures on the average number of fruits. In the substrates mixture consisting of banana and barley in a ratio of 1:1, the average number of fruits reached 7.00 fruit/cluster and did not significantly differ from the other substrates where barley was present. the lowest average number of fruits was observed in the substrate mixture of banana and sorghum in a ratio of 1:1, with a value of 5.57 fruit/cluster. From these results, it is observed that barley plays a significant role in the substrate in which it is included in affecting the growth and productivity of fungi, although the impact of barley alone when used as a 100% substrate gave the highest number of fruits/clusters. However, barley production in Yemen is considered limited and confined to specific regions. On the other hand, bananas, when used as a 100% substrate, gave the lowest number of fruits (3.33 fruit/cluster) and did not differ significantly when compared to using sorghum as a 100% substrate. Similarly, when using a mixture of banana and sorghum in a ratio of 1:1, it resulted in the lowest average number of fruits/clusters.It is worth noting that banana production in Yemen is 116,300 tons, while the production of sorghum is 230,766 tons, both of which are higher compared to barley production in Yemen, which is 26,933 tons. Therefore, the residues resulting from banana and sorghum, which are abundant in their cultivation and production, can be used in mushroom cultivation and improving the agricultural substrate, whether for banana or sorghum, by adding barley to them and benefiting from these residues. There is an important factor that may contribute to a decrease in mushroom fruit production, especially in banana substrate, and that is the high nitrogen content, it was found to be high in banana substrate compared to other substrates. Nitrogen is a crucial factor in mushroom cultivation; however, high concentrations of nitrogen in the agricultural substrate can burden mushroom cultivation [30, 31]. These results are in line with previous researchers **Richard et al.** [32] and **Bahatti et al.** [33] who found that the average number of clusters did not show a significant difference when using banana residue substrate alone or wheat straw alone, as the number of clusters reached (3). These results also align with the findings of **Hoa et al.** [34] who found that the highest number of fruits was obtained when using a mixture of corn residues and sawdust, with a count of 8.07 fruit/cluster. Furthermore, the results are consistent with the findings of **Aguilar et al.** [35], who obtained the lowest value in the number of fruits/clusters when using banana residue alone. Our findings may align with **Fufa et al.** [36], who found that using mixtures yielded reasonable results in the average number of fruits/clusters. Specifically, in the substrate composed of a mixture of corncobs and bamboo in a ratio of 1:1, the average number of fruits/cluster reached 4.25, which is comparable to the result obtained in the substrate composed of a mixture of banana and sorghum, where it was 5.75 fruit/cluster.

The Fig.1 illustrates significant differences in the effect of agricultural substrate mixtures on total yield and biological efficiency. The highest yield (810 g) was achieved when using the substrate composed of a mixture of sorghum and barley with a biological efficiency of 91.2%. On the other hand, the lowest yield (232 g) was observed when using only banana residues with a biological efficiency of 52.2%. Although the effects of agricultural residues varied in their impact on biological efficiency, it is noteworthy that the highest impact was observed in the substrate consisting of barley alone compared to substrates composed of banana residues alone or sorghum residues alone. However, it is possible to improve the poor substrates by adding them to the environments that had a high impact, such as adding barley to sorghum

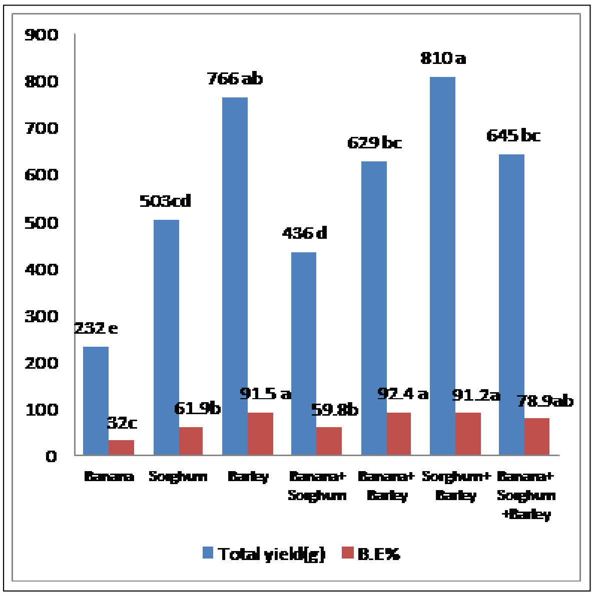

Figure 1. Effect of agricultural substrates mixtures on total yield(g), biological efficiency(BE%).

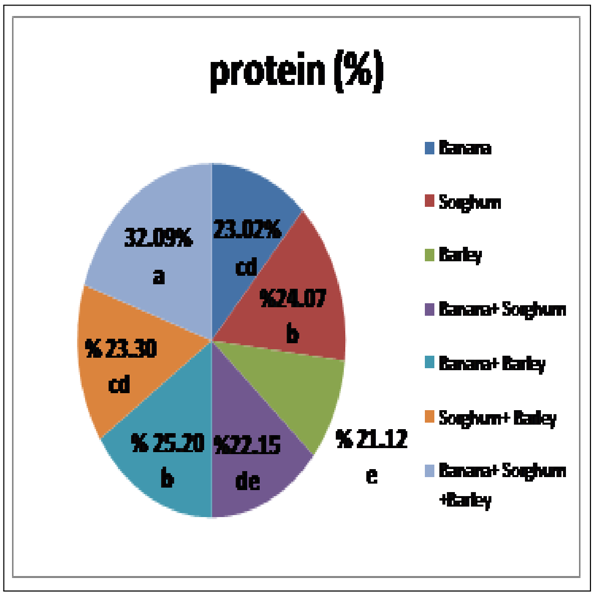

Figure 2. Effect of agricultural substrates mixtures on average protein content of fruits.

adding barley to banana, or adding banana together with sorghum and barley. The suitability of banana residues as a substrate for mushroom cultivation ranges from moderate to poor. This may be due to the fact that banana residues take a longer time to decompose, resulting in a decreased potential for biological efficiency and availability of nutrients in the agricultural medium [35]. These results align with the findings of **Hoa et al.** [34] who found that the highest productivity was achieved when using a mixture of corn residues and sawdust, with a yield of 258.82 g and a biological efficiency of 58.82%. These results are in line with the findings of **Sharma et al.** [37] when using substrate mixtures that yielded the highest average yield and biological efficiency. Specifically, when using a mixture of rice straw and wheat straw, the yield reached (309.99 g) with a biological efficiency of 77.32%. However, the results do not align with **Richard et al.** [32], who found that the average yield for banana leaf residues was (181 g/2 kg substrate), with a biological efficiency of 37%. The results are consistent with **Agba et al.** [38], who used mixtures composed of sawdust and dried plantain leaves in a ratio of (1:1). The total yield obtained was (104.92 g) with a biological efficiency of 34.97%. The results were close in proximity to **Onyeka et al.** [39], who used a mixture of sawdust and cassava peel residues, which yielded the highest productivity of (463 g/kg substrate) with a biological efficiency of 46.30%. Additionally, the results are similar to the researchers **Tesfay et al.** [40], who used a mixture of printing paper waste and corn stalk residues in a ratio of 1:1, resulting in a total yield of (620.04 g). The results of the study are consistent with what researchers **Kamthan et al.** [41] have demonstrated, that agricultural waste mixture lead to increased productivity, they obtained the highest total yield from a blend consisting of barley straw and sugar beet. The Fig.2 demonstrate significant differences in the impact of agricultural substrates on the average protein content of fruits. The highest protein percentage (32.09%) was achieved when using the agricultural substrate composed of a mixture of banana waste, sorghum, and barley together. In contrast, the lowest protein percentage (21.12%) was recorded when using barley straw alone. The results approached those reported by [34], where the protein content in the cultivated mushroom fruiting bodies on a corn substrate reached 29.70%. Meanwhile, the protein content reached 25.65% when a substrate mixture of corn and sawdust was used. Especially considering that sorghum and barley belong to the same family as maize. The results also approached those of **Hamed et al.** [42], who found that the protein content in oyster mushrooms reached 22.16% when cultivated on sorghum residue. They also found that mixing agricultural substrates enhances the nutritional value of the mushroom cultivation medium, which is reflected in the nutritional components of the mushrooms. The results obtained in this study regarding the protein content in mushrooms fall within the range (14.10 to 29.36%) reported by researchers **Ritota and Manzi** [43] for Pleurotus spp. species whose fruiting bodies were cultivated on different substrates. The results were consistent with those obtained by researchers **Pokhrel et al.** [44], who found that the protein content in mushrooms cultivated on a maize substrate was 25.89%. Additionally, researchers **Stanely and Odu** [45] reported that oyster mushrooms contain a protein content ranging from 25% to 30%. The study results aligned with the findings of **Abid et al.** [46], who affirmed that the increase in protein content is high due to the utilization of a blend of substrates, the maximum protein content (22.34%) was achieved when using a substrate mixture consisting of rice straw and sawdust in a 1:1 ratio. Furthermore, **Ogundele et al.** [20] confirmed that the protein content in *Pleurotus ostreatus* is elevated when utilizing a mixture of substrates. The variations in the previous results can be attributed to environmental and climatic conditions in Yemen, particularly in the city of Sana'a, where low relative humidity plays a significant role. Additionally, temperature plays a crucial role in the formation of pinheads. The differences in the chemical composition and C:N ratio of the different substrates used may also have a significant impact on the results. All of these factors can also affect the total yield and nutritional properties of the mushroom.

4. CONCLUSION

Based on the research results, we can conclude that despite the availability of large quantities of agricultural waste in the Yemeni environment, such as residues from sorghum and banana, which showed a low impact on mushroom productivity, their properties can be improved by integrating them with barley residues to enhance mushroom production. Improving individual income in both rural and urban areas can be achieved through small-scale mushroom production projects, which simultaneously provide an alternative source of protein. Utilizing banana waste in banana cultivation areas for mushroom cultivation, especially since banana cultivation areas are suitable for growing and producing oyster mushroom in climatic conditions, It is important to consider improving the banana waste substrate by mixing it with waste from other crops.

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