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Effect of agricultural substrates and spawn rates on growth and production of oyster mushroom (*Pleurotus ostreatus*)

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Abstract The results of cultivating *Pleurotus ostreatus* at different spawn rates and using various agricultural substrates indicated that the fastest incubation occurred on barley substrate at a spawn rate of 4% , while the sorghum substrate significantly outperformed in the shortest period of formation of the pinhead and the number of clusters at a spawn rate of 4%, while the maximum number of fruiting bodies was recorded in the barley substrate at a spawn rate of 4%. The highest total yield was obtained in the barley substrate with a spawn ratio of 4 %, which amounted to (743 g), with a biological efficiency of (99.3%).

Keywords: Agricultural waste, Yield, Cultivation, Incubation period

Introduction

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 (Fakoya *et al*., 2020). Mushroom production has increased dramatically over the previous decade, with an approximate worldwide annual production of over 9 million tons. *Pleurotus* species, one of the most produced mushroom species, is responsible for more than 19% of the worldwide mushroom production (Moshtaghian *et al*., 2022).

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 after Agaricus mushrooms. It is also an easy and low-cost mushroom to cultivate (Adjapong *et al*., 2015). Mushrooms are eukaryotic macrofungi with fleshy, spore-bearing fruiting bodies that grow above the ground or on plant-based sources of food (Pradeep *et al*., 2018).There are about 5,000 different types of mushrooms that

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can be used for food and medicine, many of the edible mushrooms are members of the Basidiomycotina and Ascomycotina families (Mishra *et al*., 2015). 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 (less than 0.3%) (Akter *et al*., 2019). Important nutrients (riboflavin, niacin, vitamin D, potassium) are also supplied (Arruda *et al*., 2023). Mushrooms are an excellent source of nonstarchy carbs and vitamins. They are high in protein and are used as a meat substitute in vegetarian diets. Furthermore, mushrooms are high in dietary fiber and due to their chemical structure, have immunostimulatory and anticancer potential. Mushrooms' other biological effects include anti-diabetic, and antioxidant (Muswati *et al*., 2021). Mushroom growing can help to reduce poverty vulnerability and boost livelihoods by producing a high-yielding, nutritious source of food as well as a consistent source of income. Mushroom farming is a practical and appealing pastime for both rural farmers and semi-urban residents. Farming on a relatively small scale does not involve big sums of money (Bose, 2016). Mushroom cultivation is regarded as an agricultural waste clean-up and a profitable crop for farmers (Gowda *et al*., 2021). As a mushroom substrate, most organic materials containing cellulose, hemicelluloses, and lignin, such as rice straw and wheat straw, banana leaves, sawdust, cottonseed hulls, corncob, sugarcane bagasse, and so on, can be used (Dubey *et al*., 2019).

The cultivation technology of this mushroom has not received any attention in Yemen and the majority of the Yemeni population suffers from malnutrition, especially with the rise in protein sources such as meat, chicken, fish, and milk. To some extent, mushrooms can be part of the solution to this nutritional suffering. The present study aimed to assess the impact of agricultural residues available in Yemen using different spawn rates on the growth and production of oyster mushrooms. State the objectives of the work.

Materials and methods

The experiment was conducted during the years 2021-2022 in the Horticulture Laboratory of the Department of Horticulture at the College of Agriculture / Sana'a University / Yemen. The Oyster mushroom (*Pleurotus ostreatus*) spawn was obtained from the Agricultural Research Center in the Arab Republic of Egypt, and then it was multiplied for preservation purposes for further study.

Preparation of spawn

The sorghum grains were soaked in water for 16 hours, after which the excess water was drained, then 3% calcium carbonate and 2% calcium sulfate were added and mixed well. The grains were packed in polypropylene bags which were then connected with cotton wool, sealed tightly with a rubber band, and sterilized at 121°C for 1 hour. When cooled, the grains were inoculated from the mother spawn and incubated at 25°C for two weeks.

Substrates and spawn rates

Various agricultural wastes were collected from various locations in Yemeni cities. A factorial experiment was conducted using designing the completely random block of three different substrates, with three different spawn rates and three replicates. Treatmente were Banana waste + 2% of spawn, Banana waste + 3% of spawn, Banana waste + 4% of spawn, Sorghum waste + 2% of spawn, Sorghum waste + 3% of spawn, Sorghum waste + 4% of spawn, Barley waste + 2% of spawn, Barley waste + 3% of spawn and Barley waste + 4% of spawn.

Preparation of substrates, inoculation, and incubation

The waste material was completely dried under sunlight. After that, the various agricultural wastes were cut and soaked in water for 14 hours until their moisture content ranged between 70-80%, and wheat bran and agricultural gypsum (calcium sulfate) (5% each) were added. The mixture is then packed into polypropylene bags and sterilized in an autoclave at a temperature of 121°C for a full hour. They were refrigerated for 1 day, inoculated by different spawn rates inside a sterile space (inoculation chamber), and closed with sterile cotton and rubber band. The incubation process is carried out by transferring the inoculated bags to a dark incubation room at a temperature of 22-26°C until the fungus grows inside the bag. This can be identified by the appearance of a distinctive white color (mycelia growth), and the incubation period varies depending on the substrates and seeding rate used.

Formation of fruiting bodies

After completion of the incubation process, the environmental changes encouraged the formation of fruiting bodies. The growth chamber temperature is maintained between 16-25°C. When the substrate is fully saturated with fungal growth, the pinheads and fruiting bodies begin to grow.The bags were

exposed to light by creating side openings in them and utilizing indirect natural light, In cases where natural light was not available, artificial lighting was used for 4-6 hours daily. The humidity in the growth chamber was maintained between 80-90% by sprinkling water on the floor. The moisture requirements of the bags were met by spraying water on them twice daily using a sprinkler. The place was also ventilated twice a day.

Harvesting

The mushroom fruiting bodies were harvested upon reaching maturity, at which fruiting bodies start to curl up, and the edges of the fruit turn light brown. The fruiting bodies were picked up in several flushes. Data for the following traits were collected as the number of days required to complete mycelial growth after spawning (incubation period), the time required for pinhead formation, the number of clusters, the number of fruiting bodies, and total yield (g) after the cropping period.

Biological efficiency (BE%)

The biological efficiency is used to indicate a mushroom's ability to consume the organic matter utilized in agriculture for fruiting body production. Biological efficiency was calculated by the following equation reported by Stamets (1993) as follows :

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

The collected data was statistically analyzed using the GenStat 12th version. The means were compared using the least significant (LSD) at the probability level of (0.05).

Results

Incubation period, initiation of pinhead formation

The results showed significant differences in the effect of agricultural substrates on the average incubation period, the shortest incubation period (30 days) was observed when using the barley substrate and the longest incubation period in the banana substrate was (36. 56 days) which was not morally different from the incubation period in the sorghum substrate (Table 1).

It is evident that there are significant differences in the effect of spawn rates on the average incubation period, with the spawn rate (4%) excelling in reducing the incubation period, which was (32 days). On the other hand, the longest incubation period of (35.67 days) was observed at a spawn rate of 2%.

The results demonstrated significant differences in the average incubation period due to the interaction between different agricultural substrates and spawn rates, the shortest incubation period of (24.33 days) was observed when using the barley substrate with a spawn rate of 4%. At a spawn rate of 2%, the longest incubation duration (39.00 days) was recorded in the sorghum substrate.

Table 1. Effect of substrates and spawn rates on average incubation period(day)

Spawn rates	***Substrates***			**Average (B)***
	banana	sorghum	barley	
2 %	35.33^{ab}	39.00^{a}	32.67^{b}	35.67^{a}
3 %	36.33^{ab}	36.00^{ab}	33.00^{b}	35.11^{ab}
4 %	38.00^{ab}	33.67^{ab}	24.33^{c}	32.00^{b}
Average (A)*	36.56^{a}	36.22^{a}	30.00^{b}	

(A)* Substrates, (B)* Spawn rates

Table 2. Effect of substrates and spawn rates on average initiation of pinhead formation (day)

Spawn rates	***Substrates***			**Average (B)***
	banana	sorghum	barley	
2 %	21.00^{abc}	21.33^{abc}	26.00^{a}	22.78^{a}
3 %	16.00^{bcd}	15.00^{cd}	22.00^{ab}	17.67^{b}
4 %	22.00^{ab}	12.67^{d}	23.33^{ab}	19.33^{ab}
Average (A)*	19.67^{b}	16.33^{b}	23.78^{a}	

(A)* Substrates, (B)* Spawn rates

Table 3. Effect of substrates and spawn rates on average number of clusters

Spawn rates	***Substrates***			**Average (B)***
	banana	sorghum	barley	
2 %	1.00^{c}	2.00^{abc}	1.33^{c}	1.44^{b}
3 %	1.67^{bc}	2.67^{ab}	2.00^{abc}	2.11^{a}
4 %	1.67^{bc}	3.00^{a}	2.67^{ab}	2.44^{a}
Average (A)*	1.44^{b}	2.65^{a}	2.00^{ab}	

(A)* Substrates, (B)* Spawn rates

Table 4. Effect of substrates and spawn rates on average number of fruits

Spawn rates	*Substrates*			Average (B)*
	banana	sorghum	barley	
2 %	2.81[b]	8.66[a]	8.33[a]	6.60[a]
3 %	3.55[b]	6.55[a]	7.56[a]	5.89[a]
4 %	3.67[b]	8.03[a]	8.39[a]	6.69[a]
Average (A)*	3.34[b]	7.75[a]	8.09[a]	

(A)* Substrates, (B)* Spawn rates

Table 5. Effect of substrates and spawn rates on the total yield (g)

Spawn rates	*Substrates*			Average (B)*
	banana	sorghum	barley	
2 %	131[d]	432[bc]	318[cd]	294[b]
3 %	298[cd]	397[bcd]	519[abc]	405[ab]
4 %	275[cd]	634[ab]	743[a]	551[a]
Average (A)*	235[b]	488[a]	527[a]	

(A)* Substrates, (B)* spawn rates**.**

Table 6. Effect of substrates and spawn rates on the biological efficiency (BE%)

Spawn rates	*Substrates*			Average (B)*
	banana	sorghum	barley	
2 %	57.9[c]	77.1[abc]	86.2[ab]	73.7[a]
3 %	74.2[bc]	74.2[bc]	93.0[ab]	80.5[a]
4 %	74.0[bc]	79.9[ab]	99.3[a]	84.4[a]
Average (A)*	68.7[c]	77.1[bc]	92.8[a]	

(A)* Substrates, (B)* Spawn rates.

Concerning the impact of agricultural substrates on the average pin head formation period, the results indicated that there were computational differences that fell short of morale when using the sorghum substrate and the banana substrate (Table 2). It was the shortest time to form a pinhead (16.33 days) in the sorghum substrate. The longest pinhead formation period was observed in the barley substrate, which was 23.78 days.

Regarding the effect of different spawning ratios on the average initiation of pinhead formation, it was observed that the shortest duration was observed at

a 3% spawning ratio (17.67 days), while the longest duration was recorded at a 2% spawning ratio (22.78 days).

There are also significant differed in the impact of the interaction between different substrates and spawn rates on the average pinhead formation period. The smallest period (12.67 days) was observed while using sorghum substrate and a 4% spawning rate, whereas the greatest period (26 days) was observed when using barley substrate and a 2% spawning rate.

Number of clusters, Number of fruits

The results indicated significant differed in the effect of the substrates used on the average number of clusters (Table 3). The highest number of clusters (2.65) was observed in the sorghum substrate, while the lowest number (1.44) was recorded in the banana substrate. As for the effect of different spawn ratios on the number of clusters, the highest number (2.44) was achieved at a 4% spawn ratio, and there was no significant difference between this ratio and the 3% spawn ratio, where the number of clusters (2.11).

The significant differences were found in the interaction effect between different environments and spawn ratios on the average number of clusters. The highest number (3.00) was observed when using the sorghum substrate and a 4% spawn ratio, while the lowest number (1) was recorded in the banana substrate with a 2% spawn ratio.

Result demonstrated significant differences in the impact of the substrates on the average number of fruits (Table 4). The highest number of fruits (8.09 fruit) was observed in the barley substrate, whereas the lowest number of fruits was recorded in the banana substrate (3.34 fruit).As for the effect of different spawn ratios on the average number of fruits, the results indicated that there were arithmetic differences that did not reach the significant level of the three spawn rates, which amounted to (6.60, 5.89, 6.69 fruits), respectively. The effect of the interaction between the substrates and spawning rates on the average number of fruits showed significant differences, as the highest number of fruits (8.39) which was observed when using the barley substrate with a spawning rate of 4%, while the lowest number (2.81) was observed when using the banana substrate with a spawning rate of 2%.

Total yield, biological efficiency

Regarding total yield and biological efficiency, It was significantly differed in the effect of different substrates on total yield and biological efficiency (Tables 5 and 6). The results showed that the highest total yield was observed in the barley substrate, reaching (527 g) with a biological efficiency

of 92.8%. On the other hand, the lowest productivity (235 g) with a biological efficiency of 68.7% was recorded in the banana substrate.

The results also revealed significant differences in the effect of spawn rates on total yield and biological efficiency. The highest total yield (551 g) with a biological efficiency of 84.4% was achieved at a spawn rate of 4%. Conversely, the lowest total yield (294 g) with a biological efficiency of 73.7% was obtained at a spawn rate of 2%.

In terms of the interaction effect of different substrates and spawn rates on average total yield and biological efficiency, the results showed that the barley substrate with a spawn rate of 4% produced the highest total yield (743 g) with a biological efficiency of 99.3%. In comparison, utilizing the banana substrate with a spawn rate of 2% resulted in the lowest total yield (131 g) with a biological efficiency of 57.9%.

Diseussion

Studies have shown that lowest incubation period in *Pleurotus cornucopia* was 23 days when using a 4% spawn rate and the longest incubation period was 30 days at 2% spawn and both were in maize straw. These results reported by (Kumar *et al*., 2021) are almost identical to the current study, while the finding of the study is not entirely consistent with the results obtained by (Hossain, 2017), who indicated that the time required to complete the incubation period in *Pleurotus sajor-caju* varies according to the substrates used, ranging from 17-26 days. He explained that the waste of banana, paddy straw, and wheat straw took 21.5, 21, and 22 days respectively, while the longest incubation period was recorded in the substrate of maize waste (26 days), this is consistent with the the study, which showed a longer incubation period in the sorghum substrate at a 2% spawn rate, with only a difference in the number of days.

He also stated that pinhead formation took 24.20 days at a 2% spawn rate in banana substrate, whereas it took 25 and 30 days in wheat and maize straw, respectively.

The findings were consistent with those published by (Tesfaw *et al*., 2015) about the period of pinhead formation, which ranged from 26 to 31 days. when they used different substrates to cultivate Pleurotus ostreatus among these substrates (barley, wheat straw, and others).

On the other hand, (Pala *et al*., 2012) observed that when cultivating *Pleurotus sajor-caju* using various substrates such as wheat straw, rice straw, chinar leaves, and apple leaves, the longest incubation and pinhead formation periods were observed in apple leaves and chinar leaves. In contrast, the shortest incubation and pinhead formation periods were observed in rice straw,

with durations of 17-19 and 21-23 days, respectively. This was followed by wheat straw with an incubation of 22-24 days and a pinhead formation period of 28-30 days.

Similar results were observed regarding the number of clusters in experiment by (Bhatti *et al*., 2007) on *Pleurotus ostreatus* (*Jacq. Ex. Fr.*) using wheat straw as a substrate, the number of clusters reached 3.00 at a spawn rate of 4%, while the number of clusters was equal at spawn rates of 2% and 3%, both yielding a count of (2.00).

However, the results did not align regarding the number of fruits. The highest number of fruits (4.22) was achieved at a spawn rate of 4%, whereas the numbers were 3.07 and 4.13 fruits at spawn rates of 2% and 3%, respectively, were recorded in the banana substrate with a spawning rate of 2%.

The results reported by (Pal *et al*., 2017) in their experiment on *Pleurotus pulmonarius (Fr.) Quel*, using different spawn rates (%0.5, 1, 2, 4, 6, 8), showed that the highest yield of the mushroom was 168.7 g / 200 g dry substrate, with a biological efficiency of 84.33%, achieved at a spawn rate of 8%. The yield at a spawn rate of 4% was (157.3 g) with a biological efficiency of 78.65%. At a spawn rate of 2%, the yield reached (151.7 g) with a biological efficiency of 75.83%.

In the case of wheat straw, the yield was 84.3 g / 200 g dry substrate, with a biological efficiency of 42.15%. These results are inconsistent with the findings of the current study.

Furthermore, the results reported by Iqbal *et al*. (2016) in their experiment on *Pleurotus Florida*, using a spawn rate of 2.5%, indicated that the total yield reached (453.3 g) when using wheat straw, (320 g) when using maize, and (388.33 g) when using sorghum. It approximately aligns with the current research findings, particularly regarding the substrates of sorghum and barley at a spawn rate of 2%.

The study results do not align with those obtained by (Sharma *et al*., 2013), as the total yield of *Pleurotus ostreatus* fungus was recorded when using rice straw, which reached (381.85 g) with a biological efficiency of 95.46%. Meanwhile, the yield when using a mixture of rice straw and wheat straw was (309.99 g) with a biological efficiency of 77.32%, both at a spawn rate of (2.5%).

According to (Pala *et al.*, 2012), when cultivating the *Pleurotus sajor-caju* mushroom on different substrates, the highest total yield was reported with the use of paddy straw, reaching (747.1 g). In contrast, the yield was (623 g) when wheat straw was used.

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.

I It is concluded that barley waste is found to be one of the best substrates used in this research, especially when using a spawn ratio of 4%. These factors had a major impact on the features that contribute to mushroom productivity.It can be inferred that the substrate of sorghum ifound to be s a suitable substrate for the growth of mushrooms after barley and that banana wastes can be used in banana growing areas to produce mushrooms to provide a source of protein for the residents of these areas.

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Conflicts of interest

The authors declare no conflict of interest.

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