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THE EFFECT OF INOCULUM LEVELS AND SUPPLEMENTATION OF LOCALLY PRODUCED CASING SOIL ON THE GROWTH AND PRODUCTION OF THE AGARICUS BISPORUS

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Abstract

This study aimed to enhance the quality of casing soil produced by the Al-Wadaq Trading Company, reduce the production costs of *Agaricus bisporus*, and improve energy efficiency in fruiting body production. The study involved two factors: preparation of organic and mineral supplements (C1, C2, C3) and use of different spawn inoculum levels (1%, 1.5%, 2%). The results showed that the shortest duration for complete mycelium growth and the addition of the casing layer was achieved with a 2% inoculum level, taking 18.3 and 18.2 days, respectively. The earliest fruiting body harvest occurred at 23.3 and 23.5 days with the 2% inoculum treatment combined with C2 and C3 casings, compared to 30 days for the C1 casing and 1% inoculum. The fastest batch production time of 41 days was observed with C2 and C3, and 2% spawn, whereas C1 and 1% spawn required 65 days. The highest yield was recorded with 2% and 1.5% inoculum levels combined with C3 and C2 casings, reaching 45.67, 45.17, 45.59, and 42.77 kg m^{-2}, respectively, with biological efficiency rates of 152.2% and 151.9%. Conversely, the lowest yield and efficiency were found with the 1% inoculum and C1 casing (20.79 kg/m² and 69.3%). The number of fruiting bodies and yields from the first three harvests were significantly higher with the 2% inoculum and C2 and C3 casings.

Keywords: Charcoal, Incineration, SMS, Casing, Spawn, Agaricus.

تأثير مستوى اللقاح ومكملات تربة التغطية المنتجة محلياً في نمو وإنتاج *Agaricus bisporus*

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الخلاصة

تهدف هذه الدراسة إلى تحسين جودة تربة التغطية التي تنتجها شركة الوَدْق، وتقليل تكاليف إنتاج فطر *Agaricus bisporus*، وتحسين كفاءة الطاقة في إنتاج الأجسام الثمرية. تضمنت الدراسة عاملين: تحضير المكملات العضوية والمعدنية (C1، C2، C3) واستخدام مستويات مختلفة من اللقاح (1%، 1.5%، 2%). أظهرت النتائج أن أقصر مدة لنمو الميسيليوم الكامل وإضافة طبقة التغطية تم تحقيقها باستخدام مستوى لقاح بنسبة 2%، حيث استغرقت 18.3 و18.2 يومًا على التوالي. وتم حصاد الأجسام الثمرية في أقرب وقت عند 23.3 و23.5 يومًا باستخدام معاملة لقاح بنسبة 2% مع التغطية C2 وC3، مقارنةً بـ 30 يومًا للتغطية C1 ولقاح بنسبة 1%. لوحظ أقصر مدة إنتاج دفعة كاملة بلغت 41 يومًا مع التغطية C2 و C3ولقاح بنسبة 2%، بينما استغرقت التغطية C1 ولقاح بنسبة 1% 65 يومًا. تم تسجيل أعلى إنتاجية مع مستويات لقاح بنسبة 2% و1.5% مع التغطية C3 وC2، حيث بلغت 45.67، 45.17، 45.59، و42.77 كغم م$^{-2}$ على التوالي، مع معدلات كفاءة حيوية بلغت 152.2% و151.9%. وعلى العكس، كانت أقل إنتاجية وكفاءة مع مستوى لقاح بنسبة 1% والتغطية C1 20.79 كغم/م² و69.3%. كان عدد الأجسام الثمرية والإنتاج من أول ثلاثة قطفات أعلى بشكل ملحوظ مع مستوى لقاح بنسبة 2% والتغطية C2 وC3.

كلمات مفتاحية: تغطية، فحم حيوي، ترميد، لقاح، فطر غذائي.

Introduction

The management of moisture is a critical factor in edible mushroom farms to ensure its availability in the casing soil, close to the mushroom mycelium. In this context, the quality of the casing layers greatly influences the quantity and attributes of the product. The casing layers and the size of the inoculum significantly affect production quantity and biological efficiency. Experiments on the cultivation of *A. bisporus* mushrooms point to the importance of their fruiting bodies containing approximately 90% water. This is provided primarily through the top cover layer. Mycelium growth is positively affected by high levels of carbon dioxide, which is also generated and supplied by soil cover (4 and 11). Coating materials should have high water-holding capacity and percentage of good air to facilitate air exchange and porosity (6). Practical coatings that have been developed to enhance soil cover include those having % 28 oak sawdust,

29% millet, 4% alfalfa, 4% soybean, 9% wheat bran, and 10% $CaCO_3$. These compounds shorten mushroom development periods and increase substrate colonization rates, and are essential for the mushroom production growth cycle. They have been found to reduce growth times and increase harvest rates (1). The casing layer helps initiate reproductive growth following full colonization and influences environmental modification for quality. As such, supplements may be incorporated into the casing layer to enhance its properties, such as biochar and ash (8 and 12). The correct use of biochar and ash along with casing application has been shown to provide significant yield benefits and decrease the primordia period leading to increased biological efficiency (BE) of oyster mushrooms, and also increase protein content in the fruiting body (9).

Nearly all research confirms that casing layers play a key role in the growth of edible mushrooms. It is considered one of the important growth factors and a source of variation in production, quality, and the consistency of mushroom yield (5). The feasibility of using supplements to enhance the substrate or casing layer and their role in mushroom production is significant. It not only involves the selection of raw materials for serving the basis of activity but also includes the correct determination of all procedures involved at each production stage. This is crucial for reapplying or recycling the waste generated during the activity back into the same company, as confirmed by (10). In applying this concept in mushroom production, the main focus will be on spent mushroom substrates (SMS) and their reuse (11). However, some research has already developed methods to use SMS as a supplementary material for new substrate formulations and as a casing layer for mushroom cultivation (7 and 8). As indicated by (11), biochar improves water retention, enhances nutrient absorption, and boosts biological activity, creating a sustainable and resilient environment for supporting mushroom production. The process of converting waste through pyrolysis into biochar and ash can be selected as supplements in the casing layer, offering high quality (2 and 18). It was concluded that the addition of biochar to the casing layer results in increased yield and biological efficiency (11).

A study was conducted to convert mushroom farm waste into biochar products at pyrolysis temperatures of 400, 600, and 800°C. The results showed that the porosity properties of the products significantly increased with the rise in pyrolysis temperature, reaching around 312.5 $m^2\ g^{-1}$. Many oxygen-containing functional groups were identified on the surface of the biochar products (4). A study (1) noted that adding biochar and ash to the casing layer in the production of *A. bisporus* mushrooms revealed significant increases of 4.8% and 12% in the yield of fruiting bodies and their protein content, respectively, compared to treatment without biochar and ash. Water-loving polymers have great potential in restoring and rehabilitating the casing soil as they act as gels that increase water absorption and retention in the environment. Managed correctly, hydrogel polymers (superabsorbent polymers) can retain about 95% of absorbed water. These hydrogels include polyacrylamide and superabsorbent polymers (3). A study was conducted on the use of gel recovered from waste diapers, which aligns with recent research developments in the production of hydrogels from waste materials (18).

Adding different quantities of spawn to agricultural substrates has a significant effect, with increased spawn quantities leading to faster mycelium growth and substrate colonization, thereby shortening the time required for full colonization before casing begins. This was confirmed by (7) who found that inoculating at a rate of 3% of the substrate reduced the days needed to complete mycelium colonization compared to a 1% spawn level. Additionally, it was found to have a significant impact on traits contributing to mushroom yield to some extent, making it a promising method for reducing production costs while also increasing yield and fruit body quality. The spawn level depends on the type of substrate and cost and can be expressed in units of volume per unit area (liters m^{-2}) in the case of shelf cultivation or weight-to-weight of the substrate in the case of bag cultivation. The average spawn rate ranged between 2-3%. A low spawn rate is about 1-2% of the dry weight, while a high rate is about 3% of the dry weight of the substrate. Higher spawn levels are better as they help lower costs and increase total yield. Numerous initial growth points are enabled in the substrate, resulting in efficient and fast colonization through minimal energy consumption from the substrate. This leads to a more effective use of nutrients for fruiting and an increased number of harvests (17).

In view of the considerable amount of energy contained in the substrate available for conversion into fruiting bodies this study aimed to improve the performance of the casing soil produced by Al-Wadaq Trading Company. These soils were supplemented with various additives to achieve concentration levels similar or very close the values for typical imported soil specifications. Moreover, it sought to establish the level of spawn and casings level for effective production as well as raising their quality.

Materials and Methods

The experiments were conducted from January 8, 2024 in the laboratory and mushroom production center of the College of Agriculture, Anbar University. They encompassed growth substrate preparation, casing layer fortification with supplements, and the production of the *Agaricus bisporus* A15 strain spawn. The study investigated the effects of two factors: the first being the spawn level (1%, 1.5% and 2%), and secondly, the casing layers fortified with two types of mushroom production supplements provided by the Al-Wadaq Trading Company. Three tons of wheat straw and two tons of poultry manure were gathered from local farms. Gypsum ($CaSO_4$) and calcium carbonate ($CaCO_3$) were acquired from agricultural supply centers. Additionally, 1.5 tons of casing layer material in 20 kg bags (72% moisture content) were procured from Al-Wadaq Trading Company and stored for later use.

Preparation of the Casing Soil: To produce a diverse mixture of raw materials, various organic and mineral components in carefully measured proportions were gathered. Among these was a unique blend that included 10% reed plant roots, still clinging to the soil from a drainage ditch in the fields of the college. These reeds had thrived there for more than 50 years. A digging machine was used to reach down about 2-3 meters, extracting the materials, which were then dried and ground for use.

Also incorporated were 10% palm tree roots, taken from the trunks of palm trees that had been standing for over 50 years before being cut down. These decomposed, dead trunks were extracted with a digger from a depth of 1.5-2.0 m. Afterward, the

material was dried and ground to prepare for use. The mixture also included 5% ground limestone, 10% ground natural charcoal, 5% calcium sulfate ($CaSO_4$), 5% calcium carbonate, 10% local peat, and 8% vermicompost, all obtained from local commercial markets. Additionally, 15% spent mushroom substrate (SMS) was used, obtained from the mushroom production field at the College of Agriculture. The mixture also included 10% oven stone, replaced after being used in a bakery oven for no more than 10 years, and 4% crushed date pits, which were the byproducts from date syrup (cooked date juice) processing factories. Moreover, 3% polymer was utilized, extracted from used baby diapers after cleaning, drying, cutting, and disassembling them to obtain the polymer granules, sodium polyacrylate, known for its ability to retain water up to ten times its weight. Additionally, 5% raw phosphate rock ($Ca_5(PO_4)_3(F,Cl,OH)$) was used, sourced from the factory of the General Company for Phosphates in Al-Qaim.

The aforementioned materials were each individually passed through a 2 mm sieve. The components were then thoroughly mixed and divided into two parts: the first part being the mixture of natural materials, and the second moistened with distilled water to the point of wetness, and then ashed at 320°C for 3 hours, resulting in what was termed thermally ashed mixture. The casing layer produced by the Al-Wadaq Trading Company, referred to as C1, was supplemented with 5% each of the raw mixture and the thermally ashed mixture to create the C2 casing layer. Additionally, the commercial casing layer was fortified with 10% of the raw mixture and 5% of the thermally ashed mixture to create the C3 casing layer. These casing layers were used to determine their role in the production process of edible mushrooms and were applied to cover the production substrate according to (10).

Preparation of the Growing Substrate (Composting, Fermentation, and Pasteurization): Five tons of growing substrate were prepared by mixing thoroughly-washed wheat straw residues with poultry manure at a ratio of 2:1. To this mixture, 2% gypsum ($CaSO_4$) and 2% calcium carbonate ($CaCO_3$) were added. The materials were formed into a pile measuring 6 meters in length, 3 meters in width, and 1.6 meters in height. The components were well mixed and moistened under a shelter designed for this purpose, with a layer of polyethylene (plastic) placed underneath and over the pile. The fermentation process lasted for 24 days, with the pile being turned and moistened every 4 days, starting 7 days after fermentation began (12). After fermentation, the substrate components were transferred to the pasteurization unit, equipped as part of the edible mushroom production project, for sterilization.

The process involved raising the moist heat temperature with steam to 63±3°C for 24 hours. On the second day, the temperature was lowered by 2°C every 8 hours, with gradual reduction continuing until the seventh day, when it was brought down to 48°C. The pasteurization chamber was then fully ventilated until the temperature reached between 25-30°C, making the substrate ready for inoculation (12). The production room was sterilized in two stages. On the first day, 100% concentrated Setol was sprayed on all surfaces in the room and left for 24 hours. On the second day, formalin was used at a 38% concentration (11). The substrate was then distributed on 18 shelves, each resembling a bed and measuring 3 meters long, 1 meter wide, and 0.2 meters high) arranged inside the production hall.

Preparation and Production of A. bisporus Spawn, Inoculation, and Development: The spawn was prepared in the laboratory using a strain of the white mushroom *Agaricus bisporus* A15, obtained from the Al-Wadaq Trading Company. This strain is of French origin and was released to the market in 2022. It is characterized by its high ability to produce caps of uniform size, sensitivity to humidity levels below 70%, and vulnerability to sudden temperature changes. The strain yields fruiting bodies in the range of 25–40 kg m^{-2}. For spawn production, both solid PDA (potato dextrose agar) and liquid PD (potato dextrose) media were prepared. Pieces of the medium containing the *A. bisporus* mycelium were transferred into glass bottles filled with wheat grains. The incubation and shaking process lasted 16 days until the spawn was ready for use (18).

The inoculation of the growing substrate was carried out in the production room by evenly distributing the spawn among the layers of the substrate at rates of 1%, 1.5%, and 2%, depending on the treatment. Each spawn level was applied to nine shelves, and the spawn was thoroughly mixed into the substrate, with special attention given to the corners. Optimal growth conditions were maintained using equipment in the production hall that maintained the temperature at around 24°C and humidity of approximately 85%. Humidity was provided through mist distributors suspended from the ceiling of the cultivation room. After the mycelium appeared and spread throughout the substrate according to the spawn treatments, the substrate was covered with the casing layers C1, C2, and C3, corresponding to each spawn treatment, with three replicates per treatment (3 shelves). A 5-cm-thick layer was added to each treatment, and the substrate was incubated again at 25°C without ventilation to increase CO_2 levels to 5000 mg L^{-1} to encourage the mycelium to emerge on the casing layer (12).

After the mycelium has grown and spread throughout the growth medium and covering layer, the surface of the medium was scarified to a depth of 6-8 cm using a tool designed by the researcher. This tool consisted of nails arranged in a single row resembling a comb, attached to a handle at the top. The aim of this scarification process was to ensure even growth, disrupt the dominance of the fungal mycelium, and encourage the development of fruiting bodies. After scarifying, the casing layer was carefully leveled to return it to its natural state, setting the stage for optimal fruiting body production. The carbon dioxide level was lowered to between 500-800 mg L^{-1} by improving ventilation, and the temperature adjusted to around 17°C. Afterward, the humidity was increased to 95%. Soon after, tiny "pinheads" appeared on the casing layers, signaling the onset of fruiting body formation. The temperature was then fine-tuned to 18±1°C, allowing the process to continue until the fruiting bodies fully matured. Once ready, the mushrooms were harvested, cleaned, and collected in plastic containers for necessary measurements and testing. After the harvest, the surface of the casing soil was leveled again to ensure ongoing mushroom production.

The harvests were gathered based on the specific treatments, carefully considering factors such as the time taken to begin casing, the time to start harvesting, the yield of each harvest, the number of fruiting bodies per square meter, and the overall yield from all harvests. The biological efficiency (BE) of the substrate in producing fruiting bodies was calculated using the formula:

$$\text{BE } \% = \frac{\text{Fresh weight of fruiting bodies (kg)}}{\text{Dry weight of substrate (kg)}} \; x \; 100$$

The dry weight of the substrate was estimated at 30 kg m^{-2} (20).

Statistical analysis: The data collected was analyzed using a factorial experiment set within a randomized complete block design (RCBD). To understand the differences between the means, the least significant difference (LSD) method at a 5% significance level was used. This statistical analysis was performed using GenStat software.

Results and Discussion

The Effect of Spawn Density and Casing Layer on Growth Duration, Casing, Harvesting Time (Days), Mushroom Yield, and Biological Efficiency: The results indicated that the duration for complete mycelium growth and the time to start casing was 18.3, 18.2, and 20 days for the 2% spawn density treatment with casing layers C2, C3, and C1, respectively (Table 1). For the 1.5% spawn density, the times were 20.2 and 23 days for casing layers C1, C3, and C2, respectively. For the 1% spawn density, the times were 24 and 25 days with casing layers C3, C2, and C1, respectively.

The results also indicate that the earliest average harvesting times for the fruiting bodies was achieved at 23.3 and 23.5 days for the 2% spawn density treatment with casing layers C2 and C3, compared to 27 days with casing layer C1. The 1.5% spawn density harvest times were 26.5, 26.3, and 30 days for casing layers C3, C2, and C1, respectively. This trend continued, with shorter harvesting times for the second, third, fourth, and fifth harvests with the 2% spawn density and casing layers C2 and C3, resulting in the shortest overall cycle time of approximately 41 days. In contrast, the longest cycle times were 62, 60, and 65 days for casing layer C1 with spawn densities of 1%, 1.5%, and 2%, respectively. The shortest time for the five harvests after casing completion was around 23 days with the 2% spawn density and casing layers C2 and C3. The longest times were around 40, 42, and 47 days with casing layer C1 and spawn densities of 1%, 1.5%, and 2%, respectively.

Table 1 further shows that the highest total yield averages were achieved with 2% and 1.5% spawn densities and casing layers C3 and C2 at 45.67, 45.17, and 45.59, 42.77 kg m^{-2}, respectively. The lowest average yield was 20.79 kg m^{-2} with the 1% spawn density and casing layer C1.

The highest average biological efficiency was between 152.2% and 151.9%, achieved with the 2% and 1.5% spawn densities and the C3 casing layers. In contrast, the lowest average biological efficiency was 69.3% with the 1% spawn density and C1 casing layer.

Table 1: Effect of spawn density and casing layer on growth duration, harvest time, average yield, and biological efficiency.

Treatments		Casing	Harvest Time (Days)					Growth Duration	Yield	BE
I	C		1	2	3	4	5		(kg m^{-2})	%
1%	C1	25.0	36.0	42.0	50.0	59.0	65.1	40.1	20.79	69.3
	C2	24.0	31.3	37.7	44.0	51.2	58.6	34.6	34.65	115.5
	C3	24.0	31.3	37.6	44.0	51.1	58.4	34.4	39.97	133.2
1.5%	C1	23.0	30.0	38.0	45.0	52.0	60.0	47.0	25.64	85.5
	C2	20.2	26.3	31.4	36.6	42.4	48.7	28.5	42.77	142.5
	C3	20.2	26.5	31.7	37.0	42.2	48.3	28.1	45.59	151.9
2%	C1	20.0	27.0	35.0	43.0	52.0	62.0	42.0	27.97	93.2
	C2	18.3	23.5	27.7	31.9	36.2	41.5	23.2	45.17	150.1
	C3	18.2	23.3	27.4	31.6	35.8	41.2	23.0	45.67	152.2

Increasing spawn levels results in higher mushroom production and reduces the time required for their growth. The higher mushroom yield is attributed to the more efficient use of substrate nutrients, which is linked to more primary growth points and faster substrate colonization, as well as the nutritional value of the spawn grains themselves. However, adding more spawn requires additional nutrients, and there are practical limits to the amount of spawn that can be added while continuing to achieve increased yields. Studies (8, 10 and 11) noted a 100% increase in mushroom yield when soybean and cottonseed were added to the casing layer. Most of the increase in mushroom production came from treatments where the casing layer was fortified with supplements, especially within the first 23 days of harvesting. This period roughly corresponds to the first two or three mushroom harvests. After that, production quickly declined to the same level as the no-supplement substrate.

Natural materials of mineral origin, such as soil, gravel, and calcium carbonate in various forms, as well as spent *A. bisporus* farm waste, are typically mixed with mineral materials or any substance that can be considered a substitute for peat to be used in the mushroom casing layer. These materials should have performance characteristics at least equal to peat in quality (15 and 16). Therefore, the casing layer must possess certain properties that define its environmental function to complete the production process. It helps stimulate the formation of fruiting bodies by providing moisture to the spawn and supporting good mycelium growth. It also facilitates gas exchange and the transfer of dissolved nutrients from the substrate to the mushroom (8, 12 and 15). The casing layer performs several functions, including providing shelter for mature mushrooms from water, support during harvesting, facilitating nutrient transfer, and offering a well-aerated environment for the mycelium. In commercial mushroom farming, selecting the appropriate casing layer is a crucial practice (3). Various casing materials have been used in mushroom cultivation, including peat moss, loamy soil, spent mushroom substrate, coconut coir, and farmyard manure. The casing layer has been supplemented with different materials to influence the growth of the spawn, yield, and biological efficiency. Some of the materials used include soybean meal, cornmeal, wheat bran, cottonseed meal, pea powder, lentil powder, rice bran, clay soil, and spent mushroom substrate. These materials have been employed by many researchers to enhance mushroom yield and biological efficiency (3, 14, 15 and 17).

The Effect of Spawn Levels and Casing Layer Type on the Average Number of Fruiting Bodies and Yield of Edible Mushrooms in the First Harvest: Table 2 demonstrates that the average number of fruiting bodies produced in the first harvest was influenced by varying spawn levels. The 2% spawn level resulted in the highest average number of fruiting bodies at 163.86 bodies m^{-2}, compared to 143.03 and 121.93 bodies m^{-2} for the 1.5% and 1% spawn levels, respectively. There was no significant difference between the C3 and C2 casing layers, which had 149.4 and 148.1 bodies m^{-2}, respectively. However, both layers significantly outperformed the control treatment C1 at 130.9 bodies m^{-2}.

The interaction between spawn levels and casing layers showed a significant effect, with the highest averages of 173.5 and 167.7 bodies m^{-2} for the interaction of the 2% spawn level with casing layers C2 and C3, respectively. The lowest average was 112.4 bodies m^{-2} for the interaction of casing layer C1 with the 1% spawn level.

According to the results, the first harvest's average mushroom yield for the 2% spawn treatment was 9.55 kg m^{-2}, significantly greater than the lowest average of 7.79 kg m^{-2} for the 1% spawn level. Furthermore, casing layer C3 showed a notable advantage over C2, with an average yield of 9.03 kg m^{-2} as opposed to 8.58 kg m^{-2}. With a yield of 7.17 kg m^{-2}, the control treatment C1 was surpassed by both casing layers. An average yield of 9.65 kg m^{-2} was obtained for both casing layers C3 and C2 when they interacted with the 2% spawn level, but the lowest average yield was 6.57 kg m^{-2} when casing layer C1 interacted with the1% spawn level.

Table 2: Effect of spawn levels and casing layer type on the average number of fruiting bodies and edible mushroom yield in the first harvest.

Spawn %	Mean number of fruiting bodies				Mean yield kg m^{-2}			
	Casing			Mean spawn	Casing			Mean spawn
	C1	C2	C3		C1	C2	C3	
1.0	112.0	123.4	130.5	121.93	6.57	7.62	8.37	7.48
1.5	130.5	148.4	150.2	143.03	6.99	8.75	9.07	8.27
2.0	150.4	173.5	167.7	163.86	7.96	9.65	9.65	9.09
Mean casing	130.9	148.4	149.4		7.17	8.58	9.03	
LSD 0.05	I=5.373, C=4.387, IC=7.59				I=0.176 C=0.144, IC= 0.249			

Number of Fruiting Bodies and Yield of Edible Mushrooms in the Second Harvest: The results indicate that the average yield of fruiting bodies in the second harvest was influenced by different spawn concentrations (Table 3). The 2% spawn level produced the highest average number of fruiting bodies, reaching 178.9 bodies m^{-2}, compared to 151.76 and 130.13 bodies m^{-2} for the 1.5% and 1% spawn levels, respectively. There was also a significant difference between casing layers C3 and C2 with averages of 162.3 and 155.5 bodies m^{-2}, respectively, both outperforming the C1 average of 143.06 bodies m^{-2}. The interaction between spawn levels and casing layers showed a significant effect, with the highest averages of 184.8 and 186.9 bodies m^{-2} for the 2% spawn level interaction with casing layers C2 and C3, respectively, compared to the lowest average of 130.1 bodies m^{-2} for C2 and the 1% spawn level.

For the second harvest yield the results also confirmed the superiority of the 2% spawn level, with a yield of 11.45 kg m^{-2}, compared to the lowest average of 8.55 kg m^{-2} for the 1% spawn level. Although there was no significant advantage of casing

layer C3 over C2, with averages of 10.47 and 10.89 kg m^{-2}, respectively, both significantly outperformed the 8.82 kg m^{-2} yield for treatment C1. The interaction of casing layers C4 and C2 with the 2% spawn level achieved the highest average yield of 12.2 and 12.27 kg m^{-2}, respectively. In comparison, the lowest was 7.66 kg m^{-2} for the interaction of casing layer C1 with the 1% spawn level.

Table 3: Effect of spawn levels and casing layer type on the average number of fruiting bodies and edible mushroom yield in the second harvest.

Spawn %	Mean number of fruiting bodies				Mean yield kg m^{-2}			
	Casing			Mean spawn	Casing			Mean spawn
	C1	C2	C3		C1	C2	C3	
1.0	124.0	130.1	136.3	130.13	7.66	8.76	9.25	8.55
1.5	140.2	151.6	163.5	151.76	8.91	10.37	11.20	10.16
2.0	165.0	184.8	186.9	178.90	9.89	12.27	12.20	11.45
Mean casing	143.0	155.5	162.3		8.82	10.47	10.89	
LSD 0.05	I=5.373, C=4.387, IC=7.59				I=0.176 C=0.144, IC= 0.249			

Number of Fruiting Bodies and Yield of Edible Mushrooms in the Third Harvest: The results revealed that the average number of fruiting bodies harvested in the third harvest varied according to different spawn concentrations (Table 4). The 2% spawn level produced the highest average number of fruiting bodies, reaching 201.5 bodies m^{-2}, compared to 174 and 131.66 bodies m^{-2} for the 1.5% and 1% spawn levels, respectively. There was also a significant difference between casing layers C3 and C2, with averages of 195.7 and 181.6 bodies m^{-2}, respectively. Both casing layers significantly outperformed the average of 130 bodies m^{-2} for the C1 treatment. The interaction between the two factors, spawn levels and casing layers, indicated a significant effect. The maximum average for the interaction was with the 2% spawn level and casing layers C3 and C2 at 231.5 and 222.6 bodies m^{-2}, respectively. The lowest average was in the combination of casing layer C2 and 1% spawn level at 105.4 bodies m^{-2}.

The results for average yield during the third harvest confirm a high superiority of the spawn level of 2%, obtaining 11.75 kg m^{-2} against the lowest average for the 1% spawn level of 8.26 kg m^{-2}. Casing layer C3, with its average yield of 11.89 kg m^{-2}, edged C2's 11.11 kg m^{-2}, with both exceeding the 7.49 kg m^{-2} yield of C1. The average highest yields were recorded from the interactions of casing layers C3 and C2 with the 2% spawn level at 13.5 and 13.13 kg m^{-2}, respectively. The lowest average yield was for the interaction of casing layer C1 and the 1% spawn level.

Table 4: Effect of spawn levels and casing layer type on the average number of fruiting bodies and edible mushroom yield in the third harvest.

Spawn %	Mean number of fruiting bodies				Mean yield kg m^{-2}			
	Casing			Mean spawn	Casing			Mean spawn
	C1	C2	C3		C1	C2	C3	
1.0	105.4	144.8	150.8	131.66	5.93	9.27	9.60	8.26
1.5	140.2	177.3	204.9	174.00	7.93	10.93	12.57	10.47
2.0	150.4	222.6	231.5	201.5	8.62	13.13	13.50	11.75
Mean casing	130.0	181.6	195.7		7.49	11.11	11.89	
LSD 0.05	I=8.0, C= 6.53, IC=11.32				I=0.45, C=0.37, IC=0.64			

Number of Fruiting Bodies and Yield of Edible Mushrooms in the Fourth Harvest: The results presented in Table 5 show that the average number of fruiting bodies in the fourth harvest decreased compared to previous harvests and were influenced by different spawn concentrations. The 1.5% spawn level resulted in the highest average number of fruiting bodies, achieving 133.2 bodies m^{-2}, compared to 83.36 bodies m^{-2} for the 1% spawn level. There was no significant difference between casing layers C3 and C2, with averages of 146 and 149.7 bodies m^{-2}, respectively, though both significantly outperformed the C1's 42.3 m^{-2} average. The interaction between spawn levels and casing layers showed a significant effect, with the highest averages of 170.8 and 168 bodies m^{-2} for the interaction of the 1.5% spawn level for casing layers C2 and C3, respectively. The lowest average was 20.4 bodies m^{-2} for the interaction of the C1 casing layer with the 1% spawn level.

The results also confirmed that the average yield in the fourth harvest decreased compared to previous harvests. The 1.5% spawn level demonstrated a statistically significant increase in yield, averaging 5.92 kg m^{-2}, in contrast to the lowest average yield of 4.13 kg m^{-2} observed at the 1% spawn level. Furthermore, casing layers C3 and C2 exhibited a significant superiority over C1, with average yields of 7.13 kg m^{-2}, 6.94 kg m^{-2}, and 1.11 kg m^{-2}, respectively. The interaction of casing layers C3 and C2 with the 1.5% spawn level resulted in the highest average yield of 8.12 kg m^{-2} for both, while the lowest average yield of 0.48 kg m^{-2} was recorded for the interaction of the C1 casing layer with the 1% spawn level.

Table 5: Effect of spawn levels and casing layer type on the average number of fruiting bodies and edible mushroom yield in the fourth harvest.

Spawn %	Mean number of fruiting bodies				Mean yield kg m^{-2}			
	Casing			Mean spawn	Casing			Mean spawn
	C1	C2	C3		C1	C2	C3	
1.0	20.4	113.5	123.3	83.36	0.48	5.74	6.16	4.13
1.5	64.8	170.8	168.0	133.2	1.52	8.12	8.12	5.92
2.0	52.0	153.8	157.8	121.2	1.32	6.98	7.12	5.14
Mean casing	42.3	146.0	149.7		1.11	6.94	7.13	
LSD 0.05	I=10.59, C=12.32, IC=13.22				I=0.45, C=0.53, IC=0.91			

Number of Fruiting Bodies and Yield of Edible Mushrooms in the Fifth Harvest: Table 6 confirms the significant decrease in averages and yields compared to previous harvests. The average number of fruiting bodies in the fifth harvest also varied with different spawn levels. The 1.5% spawn level had the highest average number of fruiting bodies, reaching 58.20 bodies per m^2, compared to the lowest at 36.20 bodies m^{-2} for the 2% spawn level. There was no significant difference between casing layers C3 and C2, with averages of 64.61 and 68.11 bodies m^{-2}, respectively; however, both significantly outperformed the C1's average of 6.96 bodies m^{-2}. The interaction between spawn levels and casing layers showed a significant effect, with the highest averages of 81.98 and 83.52 bodies m^{-2} for the 1.5% spawn level interaction with C2 and C3, respectively. The lowest average was 5.2 bodies m^{-2} for the C1 interaction at the 1% spawn level.

The results also indicated that the average yield in the fifth harvest was highest with the 1.5% spawn level achieving 3.17 kg m^{-2}, compared to the lowest average yield of

2.18 kg m-² with the 2% spawn level. There were no significant differences between casing layers C3 and C2, but both showed a significant increase compared to the C1 layer, with average yields of 3.84, 3.8, and 0.22 kg m^{-2}, respectively. The interaction of casing layers C3 and C2 with the 1.5% spawn level resulted in the highest average yields of 4.63 and 4.6 kg m^{-2}, respectively, while the lowest average yields were 0.15 and 0.18 kg m^{-2} for the interaction of the C1 casing layer with the 1% and 2% spawn levels, respectively.

Table 6: Effect of spawn levels and casing layer type on the average number of fruiting bodies and edible mushroom yield in the fifth harvest.

Spawn %	Mean number of fruiting bodies				Mean yield kg m^{-2}			
	Casing			Mean spawn	Casing			Mean spawn
	C1	C2	C3		C1	C2	C3	
1.0	5.2	60.92	69.74	45.28	0.15	3.66	3.70	2.51
1.5	9.1	81.98	83.52	58.20	0.29	4.60	4.63	3.17
2.0	6.6	50.94	51.06	36.20	0.18	3.14	3.20	2.18
Mean casing	6.96	64.61	68.11		0.22	3.80	3.84	
LSD 0.05	I=3.23, C=NS, IC=4.57				I=0.185, C=NS, IC=0.261			

Discussion:

Converting mushroom farm waste and other organic and mineral materials into porous biochar products through pyrolysis alters some of their chemical and physical properties, including surface area, pore volume, and average pore size. This was confirmed by the findings of this study, which align with the results of (9 and 15) that biochar products exhibited a significant increase in surface area, reaching approximately 312.5 $m^2 g^{-1}$, and identified many oxygen-containing functional groups on the surface of the biochar products. (14) also confirmed that biochar produced through pyrolysis improves water retention capacity, enhances nutrient absorption, and boosts microbial activity in casing layers. Additionally, it acts as a carbon reservoir, contributing to long-term carbon sequestration and mitigating the effects of increased carbon dioxide concentrations. (5) emphasized the effect of biochar and ash cover on the production of the *A. bisporus* edible mushroom.

The findings of this study showed a significant increase of 4.8% in yield and 12% in protein content of productive body compared to treatments without biochar and ash application. The results also indicated that the cover was important to induce the transition from sexual to reproductive maturity after complete colonization and also played a role in improving environmental conditions and substrate quality. In addition, it influenced the management of agricultural conditions, indicating that productive physiology is driven not only by mushroom genetic abilities but also by physiological and microbiological factors. Therefore, adding certain additives, such as biochar and ash, to improve the casing properties can sometimes be beneficial, as also confirmed by (8, 13 and 18). The inclusion of ash in the casing layer modified its moisture retention properties and improved the edibility of mushrooms according to (11 and 15). Extensive research supports the conclusion that casing layers are a critical factor in mushroom cultivation. They are regarded as a key element influencing growth, as well as a significant source of variability in yield, quality, and consistency of mushroom production (9). The use of primary calcium sources such as $CaCO_3$ compounds,

gypsum, and phosphate rocks is also important in mushroom production as they significantly influence mushroom growth and metabolism. Moderate levels of calcium are beneficial for mushroom growth, as they help enhance the production of essential compounds like phenols, sugars, and minerals (2 and 12).

This study found that increasing the amount of spawn in the growing medium hastened the growth of mushroom mycelium. This meant the mushrooms were ready to harvest sooner, more frequently, and in less time, as also noted by (7) that using 3% instead of 1% spawn accelerated the spread of mushroom roots (mycelium) through the growing medium. This method significantly influenced the traits contributing to mushroom yield and was considered a promising approach for reducing production costs while increasing yield and fruit body quality. The higher the spawn level used, the better, as it is cost-effective, increases overall production, and provides abundant initial growth points within the substrate, leading to rapid and complete colonization with less energy consumption. This efficiency in nutrient use results in the production of more fruiting bodies and a higher number of harvests. Additionally, it improves substrate colonization in all corners, helping to ensure mushrooms grow faster than other competitors in the mushroom environment (16).

Discussions on the feasibility of using supplements to enhance substrates or casing layers and their role in mushroom production should go beyond considerations of the integrated economic concept of accurately selecting the raw materials forming the foundation of the activity. They should also include a proper determination of all procedures involved at each production stage, aiming to recycle the waste generated in the activity back into the producing company itself, as confirmed by (10). In applying this concept to mushroom production, spent mushroom substrates have been reused, and research has indeed developed methods to utilize them as a supplementary material for new substrate formulations and as casing layers in mushroom cultivation (5 and 10).

This research highlights the importance of better moisture management to ensure it remains available in the casing soil near the mushroom mycelium. In this context, polymers have a significant role in mushroom cultivation as gel-like materials that absorb water (14). Water-absorbing polymers have great potential in the restoration and rehabilitation of casing soil. Managed properly, hydrogels (superabsorbent polymers) retain about 95% of the absorbed water. The goal is to improve moisture management by increasing its efficiency and retention, as well as preventing some material loss from the substrate, which positively impacts edible mushroom farming.

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