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Improving the production and quality of white button mushroom (*Agaricus bisporus*) by adding biochar and ash to the casing layer

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ABSTRACT

This investigation was implied to assess the effect of adding different rates of biochar and ash (0, 5, 10, 20 g L $^{-1}$ casing layer) at the casing layer on production and quality indicator (total yield, biological efficiency, earliness of pinhead and fruit body appear, dry matter and protein content of fruit body) of common mushroom (*Agaricus bisporus*). The experiment was considered as a double factorial in a complete randomized design. Each treatment replicates three-time. There were significant variances between the four rates of biochar and ash. The highest total yield (2030.7 g 10 Kg^{-1} compost) was obtained using biochar 20 g L $^{-1}$ casing layer that was high rate compared with the 2009.5 g 10 Kg^{-1} compost for the control treatment also lowest total weight in this parameter (1951.1g 10 Kg $^{-1}$ compost) was obtained from biochar 5 g L $^{-1}$. The best protein values (26.871%) were observed in a biochar 20 g L^{-1} casing layer compared with the lowest rate 17.7115% which was the control, and the second value (25.085%) was observed in an Ash 20 g L^{-1} casing layer compared with the 18.920% for the control which was the lowest value. These studies will help to determine suitable casing layer conditions and the appropriate alternative casing materials for the cultivation of *A. bisporus.*

تحسين الاصفات الكمية و النوعية للفطر الزراعي الأبيض (*Agaricus bisporus*) بأضافة الفحم الحيوي والرماد إلى طبقة التغطية.

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الخلاصة

نفذ هذا البحث لتقييم تأثير معدلات الإضافة المختلفة للفحم الحيوي والرماد (0 ، 5 ، 10 ، 20 غم لتر $^{-1}$) الى طبقة التغطية على مؤشرات الإنتاج والجودة (الحاصل الكلي ، الكفاءة الحيوية ، التبكير في تكوين رؤوس الدبابيس والاجسام الثمرية، محتوى المادة الجافة والمحتوى البروتيني) للفطرالزراعي *Agaricus bisporus*. صممت التجربة ثنائية العامل ضمن التصميم العشوائي الكامل (CRD) كررت كل معاملة ثلاث مرات (كل مكرر يحتوي على وسط بوزن 10 كغم). وجد ان هناك اختلافات بين معاملات معدلات الاضافة الأربعة للفحم الحيوي والرماد. تم الحصول على أعلى حاصل كلي (2030.7 غم 10 كغم وسط$^{-1}$) باستخدام الفحم الحيوي20 غم لتر $^{-1}$ لطبقة التغطية مقارنة بمعاملة القياس التي بلغ الحاصل الكلي فيها 2009.5 غم 10 كغم وسط$^{-1}$ وأقل حاصل كلي (1951.1 غم 10 كغم وسط$^{-1}$) وجد عند اضافة الفحم الحيوي 5 غم لتر $^{-1}$ لطبقة التغطية. وسجل أفضل قيمة للمحتوى البروتيني (26.871٪) عند اضافة الفحم حيوي 20 غم لتر$^{-1}$ تلتها القيمة 25.085٪ عند اضافة الرماد 20 غم لتر$^{-1}$. ومن خلال هذه الدراسة نستفاد من تحديد ظروف طبقة التغطية المناسبة المخلفات البديلة المناسبة لزراعة الفطر *Agaricus bisporus*.

الكلمات المفتاحية: حموضة الوسط، الفطر الابيض، مواد طبقة التغطية، ، تحسين الانتاج، تحويل المخلفات.

INTRODUCTION

Agaricus bisporus, commonly known as button mushroom or white mushroom, is one of the world's most frequently farmed edible mushroom species, particularly in Europe, North America, China, and Australasia (Atila *et al*., 2017; Kabel *et al*., 2017; Sonnenberg *et al*., 2017). *A. bisporus* had highly nutritious qualities with a rich source of delicious food. *A. bisporus* white strain were custom as antifungal activity, antibacterial, anti-cancer, and antioxidant, reduction of blood pressure and cholesterol, liver protective, antifibrotic, anti-inflationary, anti-diabetic. Depending on harvest and growth, *A. bisporus* samples have a high moisture content (Harikrishnan *et al*., 2021; Dheyab *et al*., 2020). It has both medicinal and nutritional characteristics. Due to their excellent digestibility, *A. bisporus* was thought to be a viable alternative for meat protein. In addition to being a great source of protein, mushrooms are also a great source of vitamin D, and minerals, and are low in calories, fat, cholesterol, gluten-free, and sodium (Netam *et al*., 2018). Two different media are needed for the cultivation of mushrooms (*A. bisporus*) to grow fruiting bodies, one of them is called the casing layer, the casing layer is the material utilized to cover the mushroom compost to induce the transition from asexual to reproductive growth after complete colonization (Pardo-Giménez *et al*., 2017; Grimm *et al*., 2018). This casing layer had the effect to promotes an ecological change and improving the quality of the compost also had an impact on the management of cultivation conditions, meaning induction of fruiting not only depended on the genetic capacity of the mycelium (Alkaisi, 2019) rather depended on physical, chemical and microbiological factors as pH, particle size and electrical conductivity. The casing layer has an alkaline pH of 7-8 this rate has important to grow this mushroom and another hand help control the presence of competitors such as Trichoderma, which grows better at acidic, sometime could adding some supplement to improve character casing layer, also could adding biochar and ash.

Biochar is one of the compounds that has benefits for enhancing the physical and chemical characteristics of the casing layer (Tangmankongworakoon, 2019), High mineral content of biochar which can remove heavy metals using intricate processes such as mineral precipitation, cation exchange, complexion of surface functional groups, and heavy metal interaction with electrons (W. Zhang *et al*., 2020; Chang *et al*., 2020). In conclusion, mineral-rich biochar is more cost-effective than other materials and may be useful for eliminating a range of heavy metals from water (G. Zhang *et al*., 2021). While adding ash to the casing layer changed how different casing materials affected the mixture's bulk density and water-holding capacity. Additionally, the use of ash in the casing materials can improve yield for white button mushrooms, such as stalk length and diameter, pileus thickness (K. Kumar *et al*., 2020). The aim of this study was to use biochar and ash as environmentally safe additions, to improve the yield quantity and quality of white button mushrooms.

MATERIAL AND METHODS

The study project was conducted in the mushroom house, Department of Horticulture, Agriculture College University of Sulaimani, Kurdistan -Iraq during the October to January year 2021-2022. The experiment included a study of two factors, the first factor included biochar and the second factor included ash added at four levels (0, 5, 10 and 20 g L^{-1} casing layer) to the casing layer (peat moss 50% (imported a company /hobby): and sand 50% after mixing, 10% calcium carbonate was added. This compost was usage before added to bag was measured some chemical analysis as explained (table 1). Ash was prepared from Oakwood (*Quercus infectoria*) Dutch oven was used, where sawdust was placed under a temperature of 400 ^{o}C for 4 hours, at the beginning cleaned the oven very well after putting this wood burned without usage any other matter to burned until all wood was become grey or ash. Also, Biochar was prepared from white poplar (*Populus alba*) in a small piece and was used as a source of biochar for this purpose microwave and pot clay was used under maximum temperature for 20-25 min, after putting this small pieces in pot clay and put cover pot also surrounded this cover pot by clay to prevent the entry of oxygen inside the pot, until all wood was became as ash or changed color to black. (Adibah *et al*., 2020). Before added casing layer to compost was measured some physical and chemical analysis as (table 2). Regarding analysis compost as determinate moisture content was taken 100 g of compost after that dried in an electric oven at a temperature 60±5 °C until the weight is stable. Determinate ash by usage dry weight in muffle for 4 hours in 550 °C, The dry matter (DM) was analyzed according to the methods of (Kim *et al*., 2011). Measured organic matter and organic carbon by depended of rate ash and calculated equation as follow: Organic matter =1-ash percentage , Organic carbon=organic matter *0.58 (Chu *et al*., 2012). Total nitrogen was determined by Kjeldhal method, and percentage protein was calculated as mentioned in equation: Percentage of protein= percentage of nitrogen * 4.38 (Pardo, de Juan, *et al*., 2010)**,** (Romanjek Fajdetić *et al*., 2019). C/N ratio was determinate by carbon on nitrogen. The casing layer analysed for chemical and physical properties, pH was measured by pH meter (Kerketta *et al*., 2019), electrical conductivity EC was measured by EC meter (R. Zhang *et al*., 2002), total dissolved solids (TDS) which could measure by using EC*F (640) (Kim *et al*., 2011), porosity (Verruijt, 2010) and water-holding capacity (WHC) (Vengadaramana *et al*., 2012),

Table (1): some of chemical analyzed for compost

Parameters	Compost
Moisture %	55
Ash %	39
Organic matter %	61
Nitrogen %	2.51
Carbon %	36
C/N ratio	14.4

Total weight of mushrooms

Total weight of white button mushroom was harvesting out once a week, total yield (g 10 kg $^{-1}$ compost) was calculated by summing up the picks.

Biological efficiency

Percentage of Biological efficiency calculated by (100*Fresh weight of mushroom (kg). Dry weight of compost $^{-1}$ (kg)) (Kumar *et al*., 2021).

Time required for mushroom pinheads

Period required pinheads is calculated by the number of days, which is calculated days from carrying out the casing process until the beginning of the appearance of the pinheads.

Time required for mushroom fruit bodies

Period required to form fruit bodies in *A. bisporus* is calculated by the number of days from carrying out the casing process until the beginning of the appearance of the fruit bodies, they become ready for harvesting.

Table (2): Some Physical and chemical properties of casing layer

N. of treatment	Treatments of key (biochar, ash g L^{-1})	pH	EC (ms cm^{-1})	TDS (mg L^{-1})	Porosity (%)	WHC (%)
T1	(0, 0	7.34	0.3	192	40	59.32
T2	(5, 0)	7.25	0.3	192	37.5	52.9
T3	(10, 0)	7.35	0.2	128	40	51.44
T4	(20, 0)	7.34	0.2	128	42	56.32
T5	(0, 5)	7.37	0.3	192	38	56.7
T6	(5, 5)	7.27	0.2	128	40	49.84
T7	(10, 5)	7.3	0.2	128	35	54.86
T8	(20, 5)	7.3	0.2	128	39	50.04
T9	(0, 10)	7.25	0.3	192	41	51.48
T10	(5, 10)	7.36	0.3	192	40	54.1
T11	(10, 10)	7.42	0.2	128	35	52.26
T12	(20, 10)	7.35	0.3	192	40	54.28
T13	(0, 20)	7.43	0.3	192	35.5	53.82
T14	(5, 20)	7.45	0.3	192	40	52
T15	(10, 20)	7.44	0.3	192	44	51.28
T16	(20, 20)	7.44	0.3	192	40	52.48

EC = electrical conductivity TDS = total dissolved solids WHC = water holding capacity

Mushroom dry matter

The percentage of dry matter from the fresh fruit body, was taken 100 g then it was cut into small pieces after that dried in an electric oven at 60±5 °C until the weight is stable, was calculated as mentioned in the equation (100*Dry weight (g)/ Wet weight (g)) (Abd Allah *et al*., 2020).

The protein of mushrooms

The protein was taken from dry weight the samples were crushed after drying with an electric grinder and kept until use in airtight plastic containers to be ready for estimation total nitrogen was determined by Kjeldhal method, and percentage protein was calculated as mentioned in equation (Percentage of protein= percentage of nitrogen * 4.38) (Romanjek Fajdetić *et al*., 2019).

Statistical analysis

The factorial experiment was designed according to a completely randomized design (CRD) and was analyzed using Fisher test (analysis of variance), and the mean of the treatments were tested according to the least significant difference (LSD) test at the 5% probability level.

The results were extracted statistically using the statistical by GENSTAT software (12th ed). (Alqaisi, 2022).

RESULT AND DISCUSSION

Table 3. illustrated the effect of biochar, ash and interaction between both factors were added to the casing layer on those times needed to appear pinheads on the casing layer of white button mushroom by (day), Data pertaining to the time taken for initiation of pinheads, The pin head of *A. bisporus* on casing materials were initiated ranged between 14.33 to 17.33 days, effect Biochar was added to casing layer had no significant differences on this time, also affect ash for the period of pinhead initiation was not found a significant difference, influenced interaction to period were no differences.

Table 3. The effect of Biochar, ash and their interactions on first appearance of pinheads in *Agaricus bisporus* (day).

Biochar / **Ash**	**Time required for mushroom pinheads (day)**				**Ash Mean**
	0	5	10	20	
0	16.00	16.00	16.33	16.00	16.08
5	15.00	15.33	15.67	17.00	15.75
10	14.33	14.67	17.00	16.33	15.58
20	17.33	16.33	17.00	16.00	16.67
Biochar Mean	15.67	15.58	16.50	16.33	
L.S.D	**Ash**			**Biochar**	**Interaction**
	n.s			n.s	n.s
CV	8.4				

Table 4. revealed the effect of Biochar, ash and the interaction between both factors were added to the casing layer at those times needed formation sporophores after casing layer of white button mushroom by (day), effect Biochar was added to casing layer had no significant differences on this time, also affect ash for this period required to formation fruit body was not found a significant difference, however, factor interaction had significant differences on a number of days which needed to formation sporophores, the highest value 23.33 days was recorded in this treatment added 10 g L^{-1} casing layer of Biochar with 10g L^{-1} casing layer of ash, when added 0g L^{-1} casing layer of Biochar with 20g L^{-1} casing layer of ash while the lowest value was 20.00 days,

Table 4. The effect of Biochar, ash and their interactions on period required to form fruit bodies in *Agaricus bisporus* (day).

Biochar / **Ash**	**Time required for mushroom fruit bodies (day)**				**Ash Mean**
	0	5	10	20	
0	23.00	20.33	22.67	20.67	21.67
5	20.67	20.33	22.33	23.00	21.58
10	21.00	22.67	23.33	21.33	22.08
20	20.00	22.67	21.00	21.67	21.33
Biochar Mean	21.17	21.50	22.33	21.67	
L.S.D	**Ash**			**Biochar**	**Interaction**
	n.s			n.s	2.147
CV	6.0				

Table 5. illustrated, the effect of Biochar, ash and the interaction between both factors added to the casing layer on the total weight of the fruit body of white button mushroom, biochar factor had significant differences in total weight in production, the highest value was recorded 2030.7 g 10 kg $^{-1}$ compost this value from added 20g L^{-1} casing layer of Biochar was came, while the lowest value 1951.1 g 10 kg $^{-1}$ compost was observed from added 5g L^{-1} casing layer of Biochar. But effect factor ash to total weight were no significant differences. Also in the same table, the effect interaction to total weight were no significant differences in this study.

Table 5. The effect of Biochar, ash and their interactions on the total weight of fruit bodies in *Agaricus bisporus* (g. 10 kg $^{-1}$ compost).

Biochar / **Ash**	**Total weight (g 10 kg $^{-1}$ compost)**				**Ash Mean**
	0	5	10	20	
0	2024.2	1931.1	1925.2	2058.4	1984.9
5	2031.6	1981.3	1978.8	2045.0	2009.2
10	1991.0	1961.0	1958.5	2024.7	1983.8
20	1991.1	1931.1	1958.5	1994.8	1968.9
Biochar Mean	2009.5	1951.1	1955.4	2030.7	
L.S.D	**Ash**			**Biochar**	**Interaction**
	n.s			45.79	n.s
CV	2.8				

Table 6. shows the effect of each factor as Biochar, ash to casing layer and interaction between factors on the percentage biological efficiency of white button mushroom (*Agaricus bisporus*), Biochar factor was significant differences in biological efficiency, the highest value with this

factor was recorded 50.77% during was added 20g L^{-1} casing layer of Biochar while the lowest value in this treatment was 48.78% observed when added 10g L^{-1} casing layer of Biochar to casing layer, Data presented in the same table shows effect ash factor had no significant differences on percentage biological efficiency, for the interaction between ash and Biochar shows had no significant differences.

Table 6. The effect of Biochar, ash and their interactions on the percentage of biological efficiency in *Agaricus bisporus* (%).

Biochar / **Ash**	**Biological efficiency (%)**				**Ash Mean**
	0	**5**	**10**	**20**	
0	50.60	48.28	48.15	51.46	49.62
5	50.79	49.53	49.47	51.13	50.23
10	49.77	49.03	48.96	50.62	49.60
20	49.78	48.28	48.96	49.87	49.22
Biochar Mean	50.24	48.78	48.89	50.77	
L.S.D	**Ash**			**Biochar**	**Interaction**
	n.s			1.145	n.s
CV	2.8				

Table 7. shows the effect of Biochar, ash and the interaction between both factors added to the casing layer on the Percentage of dry matter in the fruit body of white button mushroom by (%), effect of Biochar added to the casing layer had no significant differences on the Percentage of dry matter in fruit body, also influenced both factor ash and interaction to dry matter were no significant differences in this study.

Table 7. The effect of Biochar, ash and their interactions on the percentage of dry matter in the fruit bodies in *Agaricus bisporus* (%).

Biochar / **Ash**	**Percentage of dry matter in fruit body (%)**				**Ash Mean**
	0	**5**	**10**	**20**	
0	9.94	10.14	11.14	9.79	10.25
5	9.81	10.52	10.03	10.74	10.27
10	10.95	9.45	11.03	11.23	10.67
20	10.10	11.21	11.22	10.66	10.80
Biochar Mean	10.20	10.33	10.86	10.60	
L.S.D	**Ash**			**Biochar**	**Interaction**
	n.s			n.s	n.s
CV	10.4				

Table 8. shows the effect of factor Biochar, ash and the interaction of those factors on the percentage protein of in the fruiting bodies (%), during added Biochar factor was significant differences in the percentage protein of in fruit body mushroom meaning protein gradually increased, the highest value of protein was recorded 26.871% in this treatment was added 20g L^{-1} casing layer of Biochar while the lowest value in this treatment was 17.711% observed when no biochar was added to casing layer, Data presented in the same table shows effect ash factor significant differences were observed on the protein content of fruit bodies, protein content was reached 25.085 % it is the maximum value during added 20g L^{-1} casing layer of ash and the minimum value by this factor was 18.920% observed when added any amount of ash. Regarding the effective interaction between ash and Biochar, the highest value of 28.606 % was recorded in this treatment added 20g L^{-1} of Biochar with 20g L^{-1} casing layer of ash, while the lowest value was 14.951% in this interaction when treatment was controlled, that is meaning interaction between Biochar and ash was a significant effect and by added rate, each factor increased protein content.

Table 8. The effect of Biochar, ash and their interactions on the percentage of protein content of fruit body in *Agaricus bisporus*) (%).

Biochar / Ash	Protein content (%)				Ash Mean
	0	**5**	**10**	**20**	
0	14.951	17.080	19.074	24.573	18.920
5	16.123	19.762	23.852	26.387	21.531
10	17.993	20.843	25.147	27.919	22.976
20	21.778	22.488	27.468	28.606	25.085
Biochar Mean	17.711	20.043	23.885	26.871	
L.S.D	**Ash**			**Biochar**	**Interaction**
	0.1111			0.1111	0.2222
CV	0.6				

The results obtained clearly demonstrate that it is possible to use biochar and ash to produce *A. bisporus*, using biochar and ash was evaluated in this study and resulted in significant increases in quality and quantity yield, High level of biochar had a significant effect on total weight (2030.4 g 10 kg $^{-1}$ compost) when it was used alone (20g L^{-1} casing layer of Biochar) compared to values obtained (2009.5 g 10 kg $^{-1}$ compost) were added low level of biochar (5 g L^{-1} casing layer of Biochar) as explained in table 5) because biochar was improved more physical and chemical characters to casing layer (Table 2) (Tangmankongworakoon, 2019). Also, biochar had an effect on the percentage of biological efficiency in this investigation and recorded values were started at (50.24% to 50.77%, table 6) sequentially, these results were in agreement with (Al qaisi ., 2015). Regarding the percentage of protein in fruit bodies both factor biochar and ash had a positive impact on the rate of protein content in fruit bodies compared to the control treatment which had a low rate of protein, the values were recorded (26.871%, 25.085% and 28.606%, table 8.) sequentially, it was an agreement with the result (Pardo, Emilio Pardo, *et al*., 2010). Because the elemental composition of biochar generally includes carbon, nitrogen, and hydrogen and, to a lesser extent, K, Ca, Na and Mg Biochar is a polar or non-polar material with high specific surface area, a strong affinity for inorganic ions including phosphate, nitrate, and heavy metal ions. Different studies have reported on biochar production from a variety of edible mushrooms and their spent substrates (Kumar *et al*., 2021).

CONCLUSION

The practice of added casing layer is an important technique for improving yield in the industrial production of *A. bisporus*. Waste conversion such as biochar and ash can be selected as quality supplements. These wastes were suggested to enhance properties of the physical and chemical of casing layer thus had effect for the development and growth of the fruiting bodies. Concluded that the biochar added to casing layer when applied properly has significant benefits, including an increase in yield and biological efficiency and the most important one is reduction in fruit formation cycle when combined with ash. On the other hand, the application of biochar, ash and interaction added increase protein fruit body content.

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