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# COMPARISON OF THE PHYSICAL AND CHEMICAL CHANGES IN LOCAL ORGANIC WASTE AFTER CULTIVATION OF THE GANODERMA LUCIDUM MUSHROOM AND COMPOSTING BY COMMON METHODS

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Abstract: A study was carried out using local organic waste to develop Ganoderma lucidum mushroom, assessing the physical and chemical changes occurring on the organic waste after the end of the mushroom cycle, and comparing it with the changes that occur to the organic waste after the composting process. The results showed that the highest production was achieved at a rate of 66.25 g / kg with the substrates B and decreased significantly (P > 0.05), and the lowest production was 18.03 g / kg with the substrates C (80% reeds straw + 20% wheat bran). The comparison results of the change in the physical and chemical properties of organic wastes after cultivation and after the composting showed an increased rate of loss significantly in the dry matter after the composting process, and achieved at a rate of 39, 43.2 and 44.7% with palm trunk sawdust, reeds straw, cogon straw respectively. Whereas, the percentage of loss after the end of the yield cycle reached (28.71, 23.5 and 23.45%) with the substrate of palm trunk sawdust, reed straw, and cogon straw, respectively. Conversely, the comparison results showed that the percentage of decrease in the carbon to nitrogen ratio increased significantly in the palm trunk sawdust after cultivation, and was achieved at a rate of 30.1%, while it reached a rate of 21.63% after the com-posting.

Keywords: Substrate, Spaw, Straw, Palm Trunk, Moisture Content.

#### 1. Introduction

The composting process is one of the biological treatment methods that help to get rid and use of the agricultural waste through turn it into biologically active material. This mechanism relies on reducing the ratio between carbon and nitrogen to limits that allow it to be used as organic fertilizer that added to the soil for improving its chemical and physical properties. The composting process for agricultural waste is accompanied by releasing energy and carbon dioxide,

thereby reducing the dry weight of the used organic waste of up to 50%. Many thermophilic and mesophilic microorganisms such as bacteria, fungi, and actinomycetes contribute to changes in agricultural waste during

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the decomposition process [1]. The group of Basidiomycetes fungi is one of the finest and most complex fungi groups, which has the ability to use cellulose and lignin compounds as a source of energy through its ability to secrete large quantities of external enzymes [2]. Cultivation mushrooms can be biotechnology for recycling lignocellulosic waste and maybe the only process that combines healthy food production and perfect waste disposal [3], which is one of the most profitable agricultural businesses [4]. Gamoderma Luciduim belongs to the group of nutritional Basidiomycetes fungi, used mostly in alternative and complementary medicine especially in Asian countries thousands of years ago [5], it has been widely used as herbal therapy for a variety of diseases ranging from bronchitis, liver inflammation, high blood pressure, and as a therapy for cancer [6].

These therapeutic properties for the fungus are due to it contains many bioactive compounds such as polysaccharides and un-saturated organic compounds [7]. Gamoderma Luciduim is cultivated in substrates consisting of a wide range of the lignocellulosic material [8], where the hard stems of trees, sawdust and many agricultural wastes have complex compositions of cellulose and lignin were used [9]. Gamoderma Luciduim cultivation is biotechnology that perfectly uses the complex organic compounds lignocellulosic. Large amounts of waste accumulate in the environment that may be used to produce energy by burning it, which leads to increased air pollution [10]. The residues of date palms Phoenix dactylifera, reeds Phragmites communis, and cogon Imperata cylindrica represent a large amount of biomass as lignocellulosic materials. This biomass is mostly carbohydrates including cellulose and hemicellulose combined with lignin [11].

In Iraq, the number of palm trees decreased due to wars and carelessness, from 21 million to 8-million in 2001, leaving large quantities of palm trunk [10]. On the other hand, weeds of reeds and cogon are spread in all regions of Iraq, especially in the marshes, irrigation and drainage canals that considered one of the weeds that is difficult to control. As well as, being very dangerous because it is perennials and resistant to salinity and it spawning by spawn run and by seeds [12]. Some studies have pointed out that the use of palm, reeds and cogon residues in preparing the substrates of some edible mushrooms such as white and oyster mushrooms [13]. Furthermore, the temperature is one of the most important factors in the mushroom mycelium growth, where the Gamoderma Luciduim grows within a temperature range of 15 - 40 °C, which is the best temperature range for the mycelium growth ranges between 30 - 35 °C [14]. As the common fungi were grown in the area, such as white mushrooms agaricus bisporus grow at a temperature range of 15-25 °C, while some lines of oyster mushrooms can grow up to 30 °C and are called Pleurotus. pulmonarius [15].

Due to the medicinal value of the fungus and its ability to decompose complex compounds, in addition to its suitability for growth within the environmental conditions of the area (compared to common fungi). Moreover, the possibility of using agricultural waste in production of mushrooms and converting this waste from materials with low nutritive value to high nutritive value, at the same time reducing the environmental impact resulting from improper disposal for this waste. Finally, this study conducted an attempt to develop the organic cultivation technique by developing mushrooms on local organic wastes in polypropylene bags and testing the role of the fungus in de-compose the used organic waste and comparing it with the results of decomposition the materials by composting.

#### 2. Materials and Methods

The study was conducted at the laboratories of the Desert Studies Center and College of Agriculture, University of Anbar. The mushroom spawn was obtained from the America Company Everything Mushrooms, which specializes in the production of food and medicinal mushrooms spawn loaded on small wooden pieces Plug Spawn. In the Gamoderma Luciduim production experiment was used oak and wheat bran as a comparison substrate and three types of local organic waste, which were also used in the composting experiment as shown in table (1). The stems of oak were sawed with sawmill, the palm trunk and reeds and cogon were crushed by a mill of the College of Agriculture, the materials were spread under the sun for a week with flipping until completely dried. Prepare the fungal mother spawn: The mother spawn of the fungal G. lucidum was prepared on wheat seeds that obtained from the local market, after cleaning them from broken seeds and impurities, they were washed and placed in a metal bowl, then submerged in an equal amount of water. Then heated till boiling and left for 15 minutes, after which the water was filtered by placing it on a mat for 7-8 hours until the seeds surface dry and the humidity becomes about 52%, which should not exceed 55% because it creates ideal conditions for the bacterial fermentation. Then the seeds were put on a clean piece of polyethylene and added calcium carbonate (CaCO3) at 0.5% dry weight (to maintain the pH) and calcium sulfate (CaSO4) at 2% (to absorb excess moisture). The seeds were distributed on 250 ml glass bottles at 50 g seeds/ bottle, the bottles were closed with a cotton pad and sterilized by autoclave for 20 minutes at 121 °C and a pressure of 1.5 atmospheres.

0		-	-		1 0 1	
Seq.	Material	Source	<b>N%</b>	C%	C:N	
	name					
1	Oak	Forests of the Sitk region - Sulay-	0.39	53.1	136.15:1	
		maniyah				
2	Palm trunk	Orchards of the banks of the Eu-	0.51	49	96.07:1	
		phrates River – Al Rumadi				
3	Reeds	Orchards of the banks of the Eu-	0.44	50	113.63:1	
		phrates River – Al Rumadi				
4	Cogon	Orchards of the banks of the Eu-	0.44	51.88	117.9:1	
		phrates River – Al Rumadi				
5	Wheat bran	Local market	2.36	52	22.03:1	
5	Wilcat Diali	Local market	2.00	52	22.00.	

Table 1. the organic materials used in the mushroom production experiment and the composting experiment.



## Picture 1. Prepare the mother spawn.

Fungal spawn production: Barley grains were used as a source for the fungal spawn production, where the procedures for the production of the mother spawn were adopted to prepare the fungal spawn, barley seeds are packed in a 150g heat resistant polypropylene bag, and closed with a non-absorbent cotton pad, sterilized by autoclave for 20 minutes at 121 °C and pressure 1.5 atmospheres. The substrates are inoculated under sterile conditions by adding 5 seeds to each bag of the mother spawn and then placed in the incubator at a temperature of  $30 \pm 1$  °C, mycelium mushroom has covered the used substrates completely for 14 days [16], as shown in picture (2).



picture 2. fungal spawn production

Preparation of the substrates: After preparing the sawdust and local waste, the treatments were prepared according to the percentages shown in table (2).

Treatment	Sawdust	Palm trunk	Reeds	Cogon	Wheat	Weight of the	N%	С%	C:N
	%	sawdust %	straw	straw	bran %	dry treatment			
			%	%		kg			
А	80	-	-	-	20	3	0.78	52.88	67.79:1
В	-	80	-	-	20	3	0.87	49.6	57.01:1
С	-	-	80	-	20	3	0.82	50.4	61.46:1
D	-	-	-	80	20	3	0.82	51.9	63.29:1
Е	20	60	-	-	20	3	0.84	50.42	60.02:1
F	20	-	60	-	20	3	0.8	51.02	63.77:1
G	20	-	-	60	20	3	0.8	52.14	65.17:1

Table 2. Components and materials percentage for the substrates used in the experiment.

Sawdust and other waste were soaked in water for 12 hours to reach moisture up to 60 -70%, the remaining water was eliminated by placing it on a mat, after that, wheat bran was added and then calcium carbonate and calcium sulfate were added by 1, 3%, respectively of the dry weight of the treatment [14]. The treatments were mixed homogeneously, where the substrates are then packed in sterile plastic bags with 6 bags by equal weight for each treatment. Finally, the bags were closed with a plastic ring and with a cotton pad, then were transported to the autoclave and sterilized for 20 minutes at 121 °C and pressure 1.5 atmospheres as shown in picture 3.



## Picture 3. Preparation of the substrates.

Inoculating the substrates: After the bags were left for 24 hours till cooled, the fungal inoculation process with the produced spawn was done inside the laminar flow chamber. The fungal spawn was added by 2% based on the dry weight of the substrate [17]. Furthermore, three bags of each treatment were inoculated with fungal spawn produced on barley grains and the other three with fungal spawn produced on palm trunk sawdust, bags were then shaken after inoculation to mix the spawn with the substrate.

Incubated the substrates and harvest: The substrates were packed in bags after the inoculation process was transferred to the incubation and production room. The substrates were incubated without exposure to light at a temperature of  $30 \pm 1$  °C and relative humidity 75 - 85% without ventilation in order to raise the carbon dioxide concentration inside the incubation room. The process continued until the mycelium growth in most substrates completed [17], as soon as the mycelium invaded most of the substrate, then the cotton pads were lifted and the bags were opened, the incubation process continued at a temperature of  $30 \pm 1$  °C with controlling the other environmental conditions in order to stimulate fruiting. Substrates were exposed to 150-200 lux of light with a capacity of 13-watt LED lamp. The relative humidity of the incubation room was raised to 90 - 95% and the oxygen concentration increased through the operating the air pullers and left the doors open for 30 minutes three times per day [17], with frequent sprinkler irrigation to maintain high relative humidity [9]. The fruiting bodies were formed within different periods, depending on the type of the substrate; these bodies were harvest cycle [18].

Composting and production of organic fertilizer: Equal volumes were weighed separately from each of the palm trunk sawdust, reeds straw, and cogon straw, and after being moistened for three days. The remaining water was removed, and then the nitrogen was added by 1% as urea fertilizer (46% N) and phosphorus by 0.5% as Di-ammonium phosphate fertilizer 50%, based on the dry weight of each type of organic waste [19]. Furthermore, the waste was inoculated with soil extract collected from different areas of a cultivated field with a depth of 0-15 cm [20]. The wastes are individually composting in pans of dimensions (1 x 1 x 1.2 m), formed from a metal clip under a roof to protect them from sunlight and rain. Moreover, a copper tube of 70 cm length is then passed from the middle of the upper surface of each stack to its center through which the electronic thermometer sensor is placed to track thermal changes of the stack. The stack was stirred and moistened according to the changes in the temperature in each stack, where the composting continued for 90 days as shown in picture 4.



#### Picture 4. Composting the organic waste.

Estimate the percentage of loss in the dry matter of organic waste: The substrates were dried after the composting and production stage in an electric oven at a temperature of 60 °C for two days. The percentage of loss in the dry matter was calculated according to the following equation:

Loss in dry matter % = [substrate dry weight (before inoculation, composting) - substrate dry weight (after production, composting) / substrate dry weight (before inoculation, composting) ×100] [21].

Estimate the percentage of carbon in organic waste before and after cultivation and composting: Carbon was estimated using the dry heat method described by [22]. The specified models for measuring this percentage were dried in an electric oven at 60 °C for two days, after that, the burning process was done by an electric oven at a temperature of 600 - 500 °C for three hours in ceramic utensils prepared for this purpose until the ash became white. Then the formed ash was weighed and calculate the percentage of volatile solids % during the burning. The percentage of carbon was calculated according to the following equation:

% C = (% VS) / 1.8

% VS = 100 - % Ash

Estimate the percentage of nitrogen in organic waste before and after cultivation and composting: The nitrogen was estimated using the Kjeldahl method described by [23], which included three steps that included digestion, distillation, and titration, and then the nitrogen amount was calculated according to the following equation:

N % =  $[(ml of HCl \times 0.00014 \times 50) \times 100 gm] / 10.$ 

Estimate the decrement percentage in carbon content: The percentage of carbon content decrease is calculated according to the following equation:

Decrease in carbon content% = [carbon content of substrates (before inoculation, composting) - carbon content of substrates (after harvesting, composting) / carbon content of substrates (before inoculation, composting) ×100].

Estimate the increasing percentage in nitrogen content: The increasing percentage in nitrogen content of the organic wastes before and after cultivation and composting duration were calculated according to the following equation:

Increasing in nitrogen content% = [(nitrogen content of substrates (after harvesting, composting) - nitrogen content of substrates (before inoculation, composting)) / (nitrogen content of substrates (before inoculation, composting)] x 100.

Estimate the decrement percentage in the carbon to nitrogen ratio: The percentage in the decrease of carbon to nitrogen ratio was calculated according to the following equation:

Decrease in the carbon to nitrogen ratio % = [(carbon to nitrogen ratio for substrates (before inoculation, composting) - the carbon to nitrogen ratio for substrates (after harvesting, composting)) / (the carbon to nitrogen ratio for substrates (before inoculation, composting)] x 100.

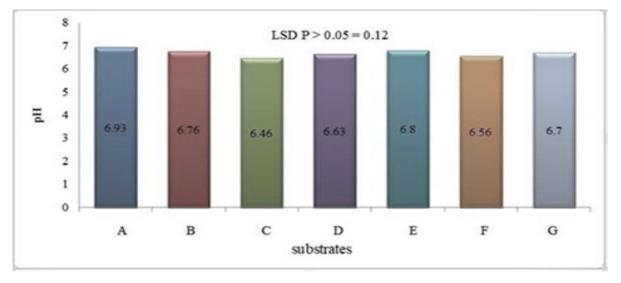
Statistical Analyses: Data were collected and statistical analyses according to the complete random design with respect to the characteristics of the culture media and the production of Fungus. The characteristics of organic wastes after Fungus cultivation and after bunching were compared with the adoption of a standard error test

# 3. Results and Discussion

The pH of the Substrates Ready for Inoculation: The results showed that there were significant differences (P>0.05) in pH values between the different substrates ready for inoculation. Figure (1) shows that the highest pH value was achieved at a rate of 6.93 with a substrate A, followed by the substrate E with a rate of 6.8, while the pH decreased with the rest of the substrates, and reached the lowest value at a rate of 6.46 with the substrate C. In comparison with [24] results, the reaction degrees were high, as they stated that the reaction degree of the substrate of oak sawdust and wheat bran reached 5.75, while the results were consistent with [25] results, where they indicated that the reaction degree of the substrate of sawdust and wheat bran ranges between 6.8 - 7.0.

The substrate reaction degree after the inoculation process changes and begins to decrease with time as a result of the mycelium growth. Furthermore, the decomposition process, the formation of organic acids and secondary metabolites products, especially, increasing the concentration of carbon dioxide that dissolves with moisture inside the bags to form carbonic acid which works to reduce the reaction degree of the substrate until it reaches the appropriate number for the formation of primitive fruiting bodies [26]. Therefore, calcium carbonate and calcium sulfate are added when preparing the substrate to stabilize the reaction degree at a range between 5.5 - 6.5 [7].

The moisture content of the substrates ready for inoculation: Figure (2) shows that the highest moisture content of the substrates was achieved with the substrate B at a rate of 67.36%, followed by a non-significant difference with the substrates E and A at a rate of (67.33 and 67.26%). The moisture content decreased significantly and achieved at a rate of 59.06, 58.03, 58, and 57.16% in media G, F, D, and C respectively.



# Figure 1. pH of the substrates ready for inoculation.

Total production: Figure (3) and Figure (4) showed that the best production of fruiting bodies was achieved at a rate of 66.25 g/kg dry-substrate with substrate B for two harvest cycles. While the production decreased significantly (P>0.05) to (65, 59.43, 22.46, 21.86, 18.9 and 18.03 g /kg) substrate with the substrates A, E, G, D and C by a decreasing percentage of 1.88, 10.29, 66.09, 67, 7147, and 72.78%, respectively.

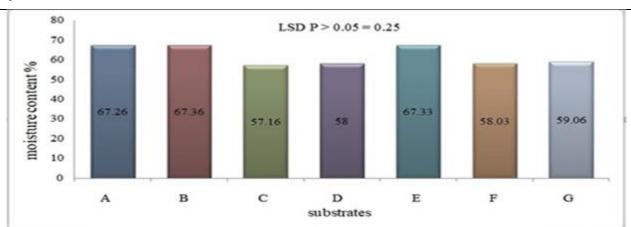
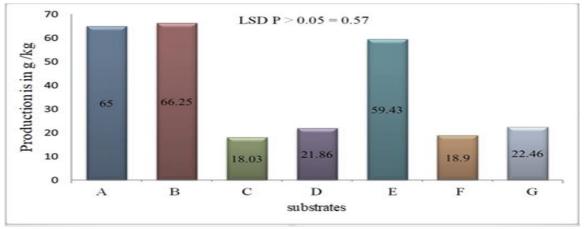


Figure 2. Moisture content of the substrates ready for inoculation





The total yield varies with different environmental conditions, additives materials to the substrate [27], and the components of the substrate itself (cellulose, hemicellulose, etc.). As well as, the pH of the substrate, the strength of the fungal spawn, the used generation of the spawn and line [28]. In addition, the sterilization method [29] and the spawn percentage [27], these are all factors that influence mushroom production. The palm trunk sawdust consists of 50.6% cellulose, 8.1% hemicellulose and 31.9% lignin and they are close to percentages in the solid wood sawdust [30]. Despite of the similarity of the two substrate components of the oak sawdust and the palm trunk sawdust, and the moisture content, the environmental conditions, the fungal spawn, as well as their ratio and the way of preparation, but the substrates of the palm trunk sawdust has significantly exceeded in the amount of the yield.

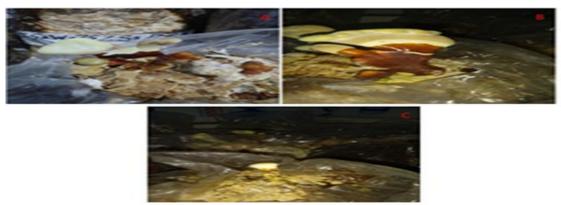


Figure 4. Production of fruiting bodies on the substrates (A, B and C)

This can be attributed to the influence of the significant difference in the reaction degree of the two substrates, where the reaction degree of the substrates has a direct effect on the fungus growth rate with the availability of other necessary nutritional requirements by regulating the absorption of ions. As well as, its effect on

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the enzymatic activity [31]. The results of the total yield on the reed and cogon substrates were not consistent with the results of [32] for the development of G. lucidum on weeds. They were stated that some types of weeds have higher productivity than those grown on sawdust, which may reach 30% and with a quality equal to the quality of the fungus produced on sawdust. Alternatively, [8] pointed out that the highest production of the juicy fruiting bodies for oyster mushrooms may be achieved on the reeds substrate at a rate of 97 g/kg in a dry substrate. It was followed by the cogon substrate at a rate of 89.2 g/kg superior over the wheat straw substrate, which is the comparison substrate that it was achieved at an average of 57.5 g/kg.

The reason for the total yield decreasing of the reed and cogon substrates can be attributed to the weakness of mycelium growth during the vegetative growth. In spite of the decrease in the total productivity of the reed and cogon substrates, but the researcher believes that these substrates can be considered an alternative for the mushroom cultivation substrates that environmentally desirable. The researcher also believes that if the soaking period of these two substrates is increased during the preparation of the substrate, their moisture content may increase to the optimum limits for the mycelium growth, which reflects positively on rapid growth and thus increase productivity, especially at the first harvesting.

Comparison of the change percentage of physical and chemical properties of organic waste after mushroom cultivation and after composting: It was observed from table (3) that the treatment of palm trunk sawdust, reed straw, cogon straw after cultivation has significantly exceeded in the percentage of dry matter loss at a rate of 28.71, 23.5 and 23.45%. Moreover, the decrease percentage of 26.38, 45.6 and 47.53% are less than the decrease percentage in the dry matter of the treatment of palm trunk sawdust, reed straw, and cogon straw after composting, which was achieved at a rate of 39, 43.2 and 44.7%, respectively. Furthermore, the treatment of palm trunk sawdust after composting was significantly exceeded in the decrease percentage of carbon by 10.2% compared to the treatment of palm trunk sawdust after cultivation, which was achieved at a rate of 4.8%. However, the treatment of palm trunk sawdust after cultivation was significantly superior in the decrease percentage of carbon to nitrogen ratio by 30.1.

Additionally, a decreasing percentage of 28.13% higher than the treatment of palm trunk sawdust after composting, which the decrease percentage of carbon to nitrogen ratio in it was 21.63%, due to the effect of the increase exceeded of the nitrogen percentage in the treatment of palm trunk sawdust after cultivation. This increase was achieved at a rate of 36.2%, with an increase percentage of 59.77% compared with the nitrogen percentage increase in the treatment of palm trunk sawdust after composting achieved by 14.56%. This can be attributed to the fungal biomass formed on the palm trunk sawdust as the mycelium growth of G. lucidum perfectly within a suitable environment leads to the formation of the highest biomass as the amino acid and protein percentage in its composition increases. table (3) also showed significant superiority of the reed straw treatment after composting in the decrement percentage for the carbon to nitrogen ratio at a rate of 28.16% compared to the treatment of reed straw after cultivation, which was achieved at a rate of 8.89%. These results were achieved due to the superiority of reed straw treatment after composting in the decrease percentage of carbon and the increasing percentage of nitrogen which achieved by 22.68 and 7.63%.

In addition to the decrease percentage of carbon and the increasing percentage of nitrogen that exceeds by 85.27 and 20.18% compared to the decrease percentage of carbon and the increasing percentage of nitrogen in the treatment of reed straw after cultivation, which was achieved at a rate of 3.34 and 6.09%. The results also showed that the treatment of cogon straw after composting was significantly superior in the decrease percentage of the carbon to nitrogen ratio, and it was achieved by 31.78% compared to the treatment of cogon straw after cultivation, which was achieved at a rate of 8.68%. These results were achieved due to the significant superiority of cogon straw treatment after composting in the decreased percentage of carbon that amounted to 27.52% and with a higher decreased percentage of 88.62%, compared to the decrease percentage of carbon for the cogon straw treatment after cultivation, which was achieved at a rate of 3.13%. In addition to the increase percentage of nitrogen which reached 6.25% with a higher increase percentage of 2.56% compared to the increase percentage of nitrogen in the cogon straw treatment after cultivation, which was achieved at a rate of 3.13%. In addition to the increase percentage of nitrogen in the cogon straw treatment after cultivation, which was achieved at a rate of 3.68% compared to the increase percentage of nitrogen which reached 6.25% with a higher increase percentage of 2.56% compared to the increase percentage of nitrogen in the cogon straw treatment after cultivation, which was achieved at a rate of 6.09% as shown in table (3 and 4).

	Loss in weight %		Decrease in carbon%		Increase in nitrogen %		Decrease in carbon to nitrogen ratio%	
	After cul- tivation	After com- posting	After cul- tivation	After com- posting	After cul- tivation	After com- posting	After cul- tivation	After com- posting
Palm trunk	28.71	39	4.8	10.2	36.2	14.56	30.1	21.63
sawdust	SE= 5.14		SE= 2.7		SE= 10.82		SE= 4.23	
Reed straw	23.5	43.2	3.34	22.68	6.09	7.63	8.89	28.16
	SE= 9.85		SE= 9.69		SE= 0.77		SE= 9.63	
Cogon	23.45	44.7	3.13	27.52	6.09	6.25	8.68	31.78
straw	SE= 10.62		SE= 12.19		SE= 0.08		SE= 11.55	

#### Table 3. Percentage of change in organic waste after cultivation and composting the mushroom %.

#### Table 4. Chemical characteristics of organic waste before and after cultivation and composting.

<b>:N</b> 45:1		
45:1		
72:1		
02:1		
Before composting		
:N		
43:1		
94:1		
57:1		

#### 4. Conclusion

In conclusion, the highest pH value of the substrates was achieved at a rate of 6.93 with the A substrate (80% oak sawdust + 20% wheat bran), and the highest moisture content of the substrates was achieved at a rate of 67.36% with the B substrate (80% palm trunk sawdust + 20% wheat bran). Contrarily, the comparison results revealed that after cultivation, the palm trunk sawdust's percentage of drop in the carbon-to-nitrogen ratio increased dramatically and was attained at a rate of 30.1%, whereas it reached a rate of 21.63% after composting.

#### Supplementary Materials:

No Supplementary Materials.

#### **Author Contributions:**

K. H. Ohmayed and H. M. Rashid; methodology, writing—original draft preparation, A. I. Mohammed and M. M. Sharqi; writing—review and editing, K. H. Ohmayed; paraphrasing. All authors have read and agreed to the published version of the manuscript.

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The study was conducted in accordance with the protocol authorized by the University of Anbar, Ethics Committee, Iraq.

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The authors declare no conflict of interest.

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