Changes of some chemical elements of agro-waste after shiitake cultivation

Mohammed Barakat Abdullah[1*], Idham Ali Abed[2] and Jamal Salih Alkobaisy[3]
[1] Department of care talent sports, Ministry of youth and sport, Fallujah, Anbar 31002. Iraq
[2]College of Agriculture, University of Anbar, Ramadi, Anbar 31001, Iraq.
[3]College of Agriculture, University of Anbar, Fallujah, Anbar 31002, Iraq
[*]Email: muhammadbrakat46@gmail.com

Abstract.

Lentinula edodes (Berck.) pegler is one of Basidiomycetes which is type of white-rot fungi that have the most efficient lignin, cellulose and hemicellulose degraders in natural like hemicellulases, cellulases, and lignin-modifying enzymes, as well as the elements were released from substrates. The aim of the study to test the changes of elements in six treatments of local agro-waste after shiitake mushroom cultivation which are : T1(sawdust 73%, Sesbania sesban20%), T2(Sawdust73%, Trifolium20%), T3(Phragmites australis vegetative73%, Sesbania sesban20%),T4 (Phragmites australis vegetative73%, Trifolium20%), T5 (Phragmites australis fruiting), T6 (Phragmites australis vegetative73%, Trifolium20%), T6(73%, Sesbania sesban20%) and 2% $CaCO_3$, 5% molasses factories residuals for all treatment. Spawn types used were wheat grains C_1, date seeds C_2, sorghum grains C_3. The results show that carbon percentage and C:N ratio were decreased after harvest from (48.79-52.72)% to (48.77-52)% and (74.41-115.54) to (56.21-81.37) and the best type of spawn was C_1 and C_3 respectively, while nitrogen percentage were increased from (0.4566-0.6691)% before inoculation to (0.639- 0.868) % after harvest and C_3 was the highest percentage , also phosphorous and potassium were increased after harvest (42.41-158.83) to (54.5-166.92) mg g^{-1} and (40.21-43.16) to (46.25-56.75) mg g^{-1} with type of spawn C_3 and C_2 respectively.

Keywords *Lentinula edodes*, agro-waste, phosphorous, potassium, shiitake residual

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Introduction

Lentinula edodes (Berck.) pegler, known as shiitake mushroom, is one of the most commonly produced mushrooms in the world. There has been a significant increase in shiitake production over the years, in 2012 the productivity of shiitake was 4.5 million tons (1) where shiitake consider as natural source of protein with high level of sugar and polyunsaturated fatty acid and its flavor taste (2), medical properties as antiviral (3), antibacterial (4),antifungal (5,6) cholesterol-lowering (7). Shiitake mushroom is Basidiomycetes, which is type of white-rot fungi that have the most efficient lignin, cellulose and hemicellulose degraders in natural like hemicellulases, cellulases, and lignin-modifying enzymes (7,8), different agricultural residues may be used as substrates for mushroom cultivation (9), in Iraq there is a

lot of agro-waste that is not used or burned, such as Sesbania sesban, Phragmites australis, Trifolium, as a source of cellulose, lignin and supplements (as nitrogen and sugar). Phragmites australis in Iraq is about (17300 ha) in 2000 as reported by (10), Phragmites content of cellulose ranged between 33-59% and lignin 22-33% (11). At the end of shiitake harvest, the growing substrates are considered spent and referred as (spent mushroom substrate) SMS. About 5 Kg of waste substrate produced by each 1 Kg of mushroom (12), the chemical composition of substrate changed continuously, and this changes in digestibility of mushroom growing substrate make recycling of these possible. Recycling spent mushroom substrate for different uses such as soil fertilizer, animal feed and grow plant. The aim of this study is determine the changes of element in local agro-waste after shiitake cultivation.

Materials and methods

This study was carried out at the Dep. of Soil & Water Resources Sciences / College of Agricultural- University of Anbar, Mycelium of shiitake mushroom *L. edodes* was obtained from the UK (13). The shiitake mycelia was produced on potato dextrose agar (PDA) and incubated at 25±2 for two weeks and then used, spawn was prepared on wheat grain, prepared mixture of white corn grain and date seeds inoculated with actively growing shiitake mycelia and incubated at 25 ± 2 °C for mycelial growth until the mycelium fully covered the grains.

Preparing substrates

Sesbania sesban, Phragmites australis, Trifolium collected from nature, cut to small pieces and dried, sawdust and molasses factories residuals then mixed with each one as show in table (1), all substrates weight 400 g in bags and pasteurized in hot water at 80̊C for 2 h after that then the water drained until 55-60% moisture (14). After that sterilized at 121č at 1.5 br for 40 min, the process was repeated for 40 min again. After bags cooled down translocated to the laboratory and spawned with three types of spawn (wheat grains C_1, date seeds C_2, sorghum C_3) for each treatment in four replicates, which make it 72 treatments. Bags were incubated in dark at 25±2 č, 80-85% Rh until bags fully covered with mycelium and dark brown-colored crust. After that stage the bags exposed to low temperature stimulus, as well as vibration stimulus, then opened and transported to fruiting room at 16 č , 90-95% Rh, for 12hr lighting per day and fresh air every 2 h until the first flush, after that the substrates returned to vegetative room for 10 days, bags then soaked in water to recover the moisture moved to fruiting room, this process repeated until the third flush.

Substrate analyzed

All substrate were oven-dried at 60̊C for 48h and ground to less than 1 mm. carbon, nitrogen, phosphorus and potassium content was assessed according to (15).. The carbon /nitrogen (C:N) ratio of each substrate was then calculated.

Statistical analysis

The experiments were set up in a two way design to test four replicates of six treatment of substrates and supplements mixed and three types of spawn. The data obtained from the experiments were subjected to variance and means analyses using GINSTAT 12.0 for windows statistical computer program at a significant level LSD 5%.

Table 1. compositional contents of substrate in the study

Treatments	T_1	T_2	T_3	T_4	T_5	T_6
Sawdust	73	73	-	-	-	-

Phragmites australis vegetative	-	-	73	73	-	-
Phragmites australis fruiting	-	-	-	-	73	73
Trifolium	-	20	-	20	-	20
Sesbania sesban	20	-	20	-	20	-
molasses factories residuals	5	5	5	5	5	5
$CaCo_3$	2	2	2	2	2	2

Table 2. the chemical composition of treatments before inoculated

treatments	C %	N %	C:N %	K mg g^{-1}	P mg g^{-1}
T1	52.72	0.4566	115.454	40.91	42.41
T2	52.29	0.5016	104.239	41.41	51.33
T3	50.93	0.5475	93.037	43.16	66.16
T4	51.11	0.5891	86.761	43.08	75.66
T5	49.80	0.6566	75.836	41.33	141.91
T6	49.79	0.6691	74.413	41.75	158.83

Results and discussion

Change in Carbon

Table (2) shows the percentage of carbon after harvest, there are significant differences ($p<.001^{**}$) between the treatments. The lowest carbon content was 48.77% for the T_5 treatment, which included (Phragmites australis fruiting + Sesbania sesban + molasses factories residuals) with a difference Significant amounted to 3.23% for the highest carbon content of treatment T_1(sawdust+ Sesbania sesban + molasses factories residuals), as the table indicates that the lowest carbon content of 50.11% was achieved with the treatments that inoculated with the source of the spawn C_1 (wheat), as for the relation between the treatments and the source of The spawn, There were significant differences for the intervention ($p = 0.401$), and the lowest interference amounted to 48.39% for the treatments T_6C_3(Phragmites australis fruiting + Sesbania sesban + molasses factories residuals+ sorghum), with a significant difference of 3.73% for the lowest T_1C_2(sawdust + Sesbania sesban + molasses factories residuals+ date seeds). This is consistent with what (16) found when he was found that the percentage of carbon decreased after the crop cycle was completed for all

media, as well as (17) that the percentage of carbon decreased after the end of the mushroom cycle from (45.88-47.13) to (45.08-46.89).) %. This decrease because the mycelium product many of enzymes to degradation substrate (7).

Change in Nitrogen percentage

Table (4) shows the percentage of nitrogen after harvest, as it shows significant differences (p<.001**) in the average percentage of nitrogen in substrate after the completion of the cultivation cycle of mushrooms, It was found that the highest percentage of nitrogen in it was 0.868% for the T_6, with a significant difference of 0.23% for the lowest treatment of T_1. The table also shows that the highest percentage of nitrogen was achieved with the treatments that inoculated with the type of spawn C_3 (sorghum). As for the interaction between the treatments and the type of spawn, there were significant differences between the interactions, and the best interaction was 0.888% for the combination T_5C_3 (Phragmites australis fruiting + Trifolium + molasses factories residuals+ sorghum), with a significant difference of 0.26% for the lowest interaction with T_1C_2. This is agree with the findings of (16) when it was found that the nitrogen percentage increased after the end of the crop cycle for shiitake mushrooms and for all media, also agrees with what (17) found that the percentage of nitrogen increased from (1.08-1.51) to (1.36-2.05)% after the end of the harvesting process. Nitrogen media is higher than other media, as the percentage of nitrogen in the T_6 mixture before inoculated was 0.6691%, which is the highest among the treatments.

Table 3. Effect of treatments and type of spawn on carbon percentage in substrate after harvest (%)

Spawn	Treatments						
	T_1	T_2	T_3	T_4	T_5	T_6	
C_1	51.92	51.20	49.76	50.60	48.40	48.78	50.111
C_2	52.12	51.98	50.03	50.63	48.91	48.89	50.426
C_3	51.95	51.73	50.09	49.98	49.01	48.71	50.244
	52.0	51.64	49.96	50.40	48.77	48.80	means
LSD 5%	TC=0.6765		C = 0.2762		T= 0.3906**		

Table 4. Effect of treatments and type of spawn on Nitrogen percentage in substrate after harvest (%)

	Treatments						
Spawn	T_1	T_2	T_3	T_4	T_5	T_6	
C_1	0.645	0.713	0.743	0.778	0.848	0.855	0.763
C_2	0.628	0.685	0.748	0.800	0.863	0.868	0.765
C_3	0.645	0.710	0.753	0.805	0.888	0.883	0.780
	0.639	0.703	0.748	0.794	0.866	0.868	means
LSD 5%	TC= 0.01381		C = 0.00564		T= 0.00797		

Change in C:N ratio

Table (5) shows the C:N ratio after harvest, as it shows significant differences (p<.001**) in the

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average of C:N ratio in substrate after the completion of the cultivation cycle of mushrooms, It was found that the lowest ratio was 56.21 for the T_6, with a significant difference of 25.16 for the lowest treatment of T_1. The table also shows that the lowest ratio was 65.42 achieved with the treatments that inoculated with the type of spawn C_3 (sorghum). As for the interaction between the treatments and the type of spawn, there were significant differences between the interactions, and the lowest interaction was 55.20 for the treatment T_6C_3, with a significant difference of 25.34 for the highest interaction with T_1C_2. This agree with (16, 17) they found that C:N ratio was decreased after harvest. The C:N ratio changed from before inoculation (74.413-115.454) to (56.21- 81.37) after harvest.

Table 5. Effect of treatments and type of spawn on C:N ratio in substrate after harvest.

	Treatments						
Spawn	T_1	T_2	T_3	T_4	T_5	T_1	
C_1	80.50	71.86	67.02	65.09	57.107	57.07	66.44
C_2	83.06	75.89	66.94	63.29	56.704	56.36	67.04
C_3	80.55	72.86	66.57	62.09	55.235	55.20	65.42
	81.37	73.54	66.84	63.49	56.35	56.21	means
LSD 5%	TC= 0.9803			C = 0.5660		T= 0.5051	

Change in phosphorous concentration

Table (6) shows phosphorous concentration after harvest, as it shows significant differences (p<.001**) in the average phosphorous concentration in substrate after the completion of the cultivation cycle of mushrooms, It was found that the highest concentration was 166.92 mg g^{-1} for the T_6, with a significant difference of 112.42 mg g^{-1} for the lowest treatment of T_1. The table also shows that the highest concentration was 104.38 mg g^{-1} achieved with the treatments that inoculated with the type of spawn C_3 (sorghum). As for the interaction between the treatments and the type of spawn, there were significant differences between the interactions, and the highest interaction was 116.75 mg g^{-1} for the treatment T_6C_3, with a significant difference of 116.75 mg g^{-1} for the highest interaction with T_1C_1(sawdust+ Sesbania sesban + molasses factories residuals+ wheat).

Table 6. Effect of treatments and type of spawn on phosphorous in substrate after harvest.

	Treatments						
Spawn	T_1	T_2	T_3	T_4	T_5	T_6	
C_1	52.75	60.50	71.75	86.00	140.75	164.75	96.08
C_2	54.00	62.00	83.25	86.00	150.50	166.50	100.38
C_3	56.75	68.25	80.75	88.75	162.25	169.50	104.38
	54.50	63.58	78.58	86.92	151.17	166.92	means
	LSD 5%	T= 1.207		C = 0.854		TC= 2.091	

Change in potassium concentration

Table (7) shows potassium concentration after harvest, as it shows significant differences (p<.001**) in the average phosphorous concentration in substrate after the completion of the cultivation cycle of mushrooms, It was found that the highest concentration was 56.75 mg g^{-1} for the T_6, with a significant difference of 10.5 mg g^{-1} for the lowest treatment of T_2 (sawdust+ Trifolium + molasses factories residuals). The table also shows that the highest concentration was 51.62 mg g^{-1} achieved with the treatments that inoculated with the type of spawn C_2 (date seeds). As for the interaction between the treatments and the type of spawn, there were significant differences between the interactions, and the highest interaction was 58.25 mg g^{-1} for the treatment T_6C_3, with a significant difference of 13.72 mg g^{-1} for the highest interaction with T_2C_1 (sawdust+ Trifolium + molasses factories residuals+ wheat). The reasons that make potassium and phosphorus increase was that maycelium of shiitake mushroom product many types of enzymes like (Cellulases, hemicellulases, Laccase, Lignin peroxidase, Manganes peroxidase) and work to degradation of substrates and release the elements.

Table 7. Effect of treatments and type of spawn on phosphorous in substrate after harvest.

	Treatments						
Spawn	T_1	T_2	T_3	T_4	T_5	T_6	
C_1	47.25	44.50	50.25	50.25	54.25	54.50	50.17
C_2	47.00	48.00	51.75	51.25	54.25	57.50	51.62
C_3	45.00	46.25	49.00	47.00	53.00	58.25	49.75
	46.42	46.25	50.33	49.50	53.83	56.75	means
LSD 5%	TC= 1.388		C = 0.567		T= 0.801		

Conclusion

The results show that shiitake mushroom can degradation substrates, and can release the elements from substrate, it was show that carbon percentage and C:N ratio decreased after harvest, while nitrogen, phosphorous and potassium increased after shiitake cycle. It can used this substrate to product another types of mushroom and soil fertilizer or grown plant with added soil.

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Authors contributions

JSA and IAA conceptualized and designed the study, MBA carried out the research work, acquired the data and wrote the manuscript. Finally all authors edited the manuscript and approved the final version for submission.

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Ethical issues: None

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