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Effect of Different Substrates and Supplement with Three Types of Spawn on Letinula Edodes Parameters for First Production in Iraq

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Abstract. Shiitake mushroom (Lentinula edodes) can have an important role specially in agriculture where land is limited. This study was the first step to improve mushroom cultivation in Iraq by using locally available lignocellulosic materials as substrates, supplements and types of spawn. Effect of substrates, supplements and spawn on mycelium and browning period, time of three flush, weight of three flush, biological efficiency (BE), total yield, diameter of cap and length of stalk. Used substrates were sawdust and Phragmites australis, supplements were Sesbania sesban, Trifolium and molasses factories residuals. The treatment were 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 used were wheat grains C_1, date seeds C_2, white corn grains C_3. Results show that faster mycelium covering substrate was at T1 (23.167 days), T6 gave the first complete browning (55.417 days), the days of three flushes were (68.91, 88.42 and 107.83 days) for T5, T6 and T6 respectively. T6 was superior by showing highest values of weight of three flush (73.93, 76.13 and 49.06 g for the 1st, 2nd, and 3rd respectively), total yield and BE (199.14 g and 49.784 % respectively), and diameter of cap and length of stalk (6.7 and 6.0 cm respectively). Hence, T6 which content of Phragmites australis in fruiting stage, Trifolium and molasses was the best substrate used in this study, while C2 (date grains) was best type of spawn.

Keywords. Shiitake cultivation, Lentinula edodes, Phragmites australis, Date grains.

1. 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]. There are two methods to produce shiitake mushroom, one of them is log cultivation placed on forest floor which made of dead trees cut to 12 inches in length and 2.5 to 7 inches as diameter [6], this method is too expensive and it takes long time to harvest, the 2nd method by using bags cultivation or sawdust indoor method and this is the most main method for mushrooms cultivation which recently applied word wide (75%), this method is applying by using residual agro-wastes and make its sawdust in small pieces. 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 [3,4], different agricultural residues may be used as substrates for mushroom cultivation [8], 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 [9] Phragmites content of cellulose ranged between 33-59% and lignin 22-33% [10]. The aim of this study is to produce shiitake mushroom for the first-time in Iraq using agricultural residuals with different types of spawn.

2. Methods and Materials

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 [11]. 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.

2.1. 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 80℃ for 2 h after that then the water drained until 55-60% moisture [12]. After that sterilized at 121c̊ 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, white corn grains C_3) for each treatment in four replicates, which make it 72 treatments. Bags were incubated in dark at 25±2 c̊, 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 c̊ , 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. The yield and various quality parameters for mushrooms were recorded regularly.

2.2. Evaluation of The Cultivation Parameters

Spawn run time (days), time to complete browning (days),time to first harvest (days), weight ~~for~~ of three flushes (g), yield (g/kg),biological efficiency (%).Yields were obtained from three flushes and expressed as grams of fresh mushrooms harvested at maturity per gram of wet substrates (w/w), diameter of cap (cm), length of stalk (cm), biological efficiency was calculated as a percentage ratio of the fresh weight of harvest per gram of dry substrates[13].

Table 1. Formula of treatment and carbon, nitrogen ratio.

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
C%	52.75	51.86	50.93	51.30	49.64	49.95
N%	0.46	0.50	0.56	0.59	0.66	0.68

2.3. 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%.

3. Results

3.1. Time From Inoculation to Complete Mycelium and Browning

Presented results in table (2) showing that there are a significant differences in time of mycelia fully covered the bags, the shorter period from inoculation was 23.167 days at T1 (sawdust + Sesbania sesban+ molasses), the type of spawn was C2 (date seeds) 24.833 days, the shorter treatment between T& C was 22.5 days at T2C3 and T1C2 with decrease percent of 8% from longer period 30.5 days at T3C2. significant different was found in time to full browning between used substrates, the shorter period from inoculation (55.417 days) was shown by T6 (Phragmites australis fruiting stag +Trifolium+ molasses), C2 was the best type of spawn by showing shorter time (58.33 day) to browning, the best combination between T&C was T6C2 which gave 54.5 day to browning with decrease percent of 12% compared to longer period (66.5 day) at T2C3.

Table 2. Effect of different agro-waste in time of mycelia growth and browning period.

	Time run mycelia days				Time run browning days			
TREATMENTS	**C_1**	**C_2**	**C_3**		**C_1**	**C_2**	**C_3**	
T_1	23.500	22.500	23.500	23.167	61.500	61.750	61.250	61.500
T_2	24.250	24.500	22.500	23.750	63.500	62.500	66.500	64.167
T_3	27.250	27.750	30.500	28.500	57.250	57.250	56.250	56.917
T_4	27.500	23.750	27.500	26.250	57.500	57.500	58.250	57.750
T_5	27.500	25.250	26.500	26.417	58.500	56.500	55.250	56.750
T_6	25.500	25.250	25.250	25.333	55.250	54.500	56.500	55.417
	25.917	24.833	25.958	Means	58.917	58.333	59.000	Means
LSD 5%	TC = 0.777	C= 0.317	T= 0.449		TC= 0.771	C= 0.315	T= 0.445	

3.2. Effect of Treatment and Spawn on Time of Flushes

Table (3) show significant different ($p<0.001$) in the time of three flushes, time from inoculation to the first flush was 68.91 day at T_5 and the shorter time for spawn was 71.92 for C_2, the shorter treatment between T&C was T_5C_2 (68.500 day) which decreased around 14% from longer treatment T_2C_3. The time for second flush was 88.42 day for T_6, the shorter time for spawn type was 91.71 day at C_2, the relationship between T&C was T_5C_3 87.25 day with significant decrease reached 14.25 from long treatment T_2C_3. The time for third flush was 107.833 days for T_6, the shorter time for spawn type was C_2 111.71 day, the relationship between T&C was T_6C_3 106.50 days with significant decrease 15% from long treatment T_2C_3.

Table 3. Effect of substrates with supplement and types of spawn on time of three flushes.

	Time to First flush (days)				**Time to Second flush (days)**				**Time to Third flush(days)**			
Treat.	C_1	C_2	C_3		C_1	C_2	C_3		C_1	C_2	C_3	
T_1	78.50	75.50	75.50	76.50	97.75	95.50	95.5	96.25	117.50	116.5	118.5	117.50
T_2	72.75	76.50	82.50	77.25	93.75	96.50	101.5	97.25	114.25	116.5	121.5	117.42
T_3	72.25	71.50	70.50	71.41	91.75	91.25	90.5	91.17	111.5	110.75	111.75	111.33
T_4	71.00	70.50	73.25	71.58	90.75	90.75	92.5	91.33	110.75	110.5	112.5	111.25
T_5	70.75	68.50	67.50	68.91	90.75	87.75	87.25	88.58	110.50	107.5	107.0	108.33
T_6	70.00	69.00	70.25	69.75	88.50	88.50	88.25	88.42	108.50	108.5	106.5	107.83
	72.54	71.92	73.25	Means	92.21	91.71	92.58	Means	112.17	111.71	112.96	Means
LSD 5%	TC = 0.9402 T= 0.5428		C= 0.3838		TC= 0.895 T= 0.517		C= 0.363		TC = 0.863 C= 0.352		T = 0.498	

3.3. Weight of Three Flushes

Table (4) show significant different ($p<0.001$) in weight of three flushes, weight for first flush was 73.935 g appeared in T6 and the high weight for spawn was 70.315g for C_2. Best interaction between T&C was at T_6C_1 which showed 90.823 day with increase percent of 47.67% compared to the lowest (T_1C_1.) The weight for second flush was 76.138 g shown by T_6 , while spawn type had the highest weight at C_2 and gave 59.565 g. for the interaction between T and C; 84.750 g was the highest value shown by T_6C_3 with significant increase reached 49.83% from the lowest treatment T_1C_1. Third flush obtained at T6 was the highest (49.065 g), the highest weight obtained from used spawn type was 54.618 g at C_2, T and C had the best interaction at T_6C_1 by giving value reached (51.180 g) which increased significantly by 30.41% from lowest value shown by treatment T_1C_1.

3.4. Total Yield and Biological Efficiency BE

Result of total yield and biological efficiency (Table 5) demonstrated significant differences between applied treatments ($P<0.001$), the highest yield and BE% were found in T6 and type of spawn was C2, T and C interacted significantly and was superior at T6C1 that gave 212.60 g and 53.149 % with increased percent of 113.77% and 28.441% for yield and BE respectively from the lowest treatment T1C1.

Table 4. Effect of different substrates and supplements and type of spawn on weight of three flushes.

	Weight of first flush g				**Weight of second flush g**				**Weight of third flush g**			
Treat.	C_1	C_2	C_3		C_1	C_2	C_3		C_1	C_2	C_3	
T_1	43.150	60.560	65.778	56.496	34.915	46.675	53.538	45.043	20.767	35.447	44.037	33.418
T_2	65.748	75.705	70.650	70.701	54.410	50.490	52.578	52.493	35.282	35.475	35.635	35.464
T_3	54.465	59.105	53.173	55.581	65.368	55.832	47.977	56.393	49.802	48.492	30.185	42.827
T_4	51.135	77.653	60.643	63.143	48.013	70.980	49.765	56.253	45.167	50.883	38.837	44.963
T_5	61.885	67.632	60.850	63.456	55.935	60.347	50.960	55.748	46.305	55.270	44.797	48.791
T_6	90.823	81.235	49.748	73.935	70.595	73.067	84.750	76.138	51.180	45.618	47.875	49.065
	61.201	70.315	60.140	mean	54.873	59.565	56.595	mean	41.417	45.618	40.228	mean
	TC= 1.0349		T= 0.5975	C= 0.4225	TC= 0.9156		T= 0.5286	C= 0.3738	TC=0.1146		T=0.1620	C = 0.2806

Table 5. Effect of different substrates and supplements and type of spawn in total yield and BE.

	Total yield g				**BE%**			
TREAT.	**C_1**	**C_2**	**C_3**		**C_1**	**C_2**	**C_3**	
T_1	98.83	142.68	163.35	134.96	24.708	35.671	40.838	33.739
T_2	155.44	155.44	158.66	158.66	38.860	40.417	40.417	39.664
T_3	158.66	158.66	131.34	154.80	39.664	39.664	32.834	38.700
T_4	144.31	199.52	149.25	164.36	36.079	49.879	37.311	41.090
T_5	164.12	183.25	156.61	167.99	41.031	45.812	39.152	41.999
T_6	212.60	202.44	182.37	199.14	53.149	50.611	45.593	49.784
	157.49	175.50	156.96	MEAN	39.373	43.875	39.241	MEAN
LSD 5%	TC= 1.496	C=0.611	T= 0.864		TC = 0.3741	C= 0.1527	T= 0.2160	

3.5. Diameter of Cap and Length of Stalk cm

Obtained results of cap diameter and stalk length (table 6) showing significant different between the treatments ($P<0.001$), the highest diameter and length was found in T6 and type of spawn was C2, the relationship between T&C the highest was T6C2 7.950 cm and 7.575 cm with increase 2.45% and 3.925 % for diameter and length, respectively from the lowest treatment T1C1 and T1C3.

Table 6. Effect of substrate and type of spawn on cap and stalk.

	Diameter of cap cm				**Length of stalk cm**			
Treatment	**C1**	**C2**	**C3**		**C1**	**C2**	**C3**	
T1	5.500	6.450	6.225	6.058	3.900	4.550	3.650	4.033
T2	6.375	6.450	6.775	6.533	4.075	4.900	4.475	4.483
T3	7.150	7.350	6.825	7.108	5.225	5.625	5.200	5.350
T4	6.750	7.150	7.075	6.992	5.150	6.325	6.450	5.975
T5	7.150	7.600	7.575	7.442	6.775	7.025	7.150	6.983
T6	7.500	7.950	7.650	7.700	7.275	7.575	7.200	7.350
	6.737	7.158	7.021	means	5.400	6.000	5.688	means
LSD 5%	TC= 0.2406	C= 0.982	T= 0.1389		TC = 0.2514	C= 0.1062	T= 0.1451	

4. Discussion

Mentioned results were agreed with [5], findings where they reported that period of spawn run time of shiitake mushroom was 24-29 day, while [14], confirmed that time was 38.83 -59.0 days on different growing substrates, this may be due to the concentration of nitrogen in the substrates, Nitrogen supplementation play a significant regulatory role, the faster mycelia were found in low nitrogen [15], and that what we found in T1& T2 which had the faster growth and lowest nitrogen concentration as presented in table (1).

In another hand particle size of substrate plays an important role in growth of mycelium. Phragmites australis in this study was larger than sawdust, so the mycelia will take additional time to move to another piece in Phragmites australis and that clearly shown in figure (1.A), (12,14) reported that used corncob 50% with sawdust and wheat bran was best browning color and that related to corncob effect on substrate's physical properties, such as air permeability and water holding capacity. Result of flushes time agree with [16], which reported that the time from inoculation to first flush was 58-76 days, while [8], reported that time for first flush was 81-117 day, [17] reported (50-65 , 70-138 and 113-160) days for first, second and third flush respectively.

Nitrogen plays limited role in shiitake mushroom cultivation, T1,T2 was the lowest concentration of nitrogen, which could explain the reduced productivity of the three flushes, conjunction to their relatively high lignin content, and shiitake known have demonstrated higher ligninolytic enzyme activities in nitrogen sufficient condition [18]. Obtained results were in agreement with [19], who reported that high weight was 72.5, 104.7 and 69.2 g for the first, second and third flush for substrates chickpea straw, sunflower head residue and chickpea straw respectively, which were higher from [20], that reported values of that weight of 37.69, 60.04 and 30.92 g for the first, second and third flush respectively. While lowest weight (of three flush was 293, 268 and 297g respectively) recorded by [21]. Nitrogen plays limited role in shiitake mushroom cultivation, T_1 and T_2 were lower in nitrogen concentration, which could explain the reduced productivity of the three flushes, and that was agreed with [22], who reported that low nitrogen in substrate affect the yield, when they add (1%) whey the yield increased. The total productivity and BE% results were highest from [23,24], findings where they reported that the total yield was 118.5 g. Kg (w\w) and 113-194 g respectively, but lowest from [2], who reported that total yield for three flushes was 233g. BE result agreed with [25]they reported that BE% was 25.12-54.17 %.

The yield and BE correlation with the increase in weigh of flush. The diameter and length result was highest from [26], they report that the diameter was 5.85- 7.09 cm and the length of stalk was 4.05-4.98 cm for different substrates, while, [27], they report that the diameter of cap was 7.37- 9.70 cm and the length of stalk was 6.2 cm when grown on wheat straw with different strains. There is many studies on showed that the formula sometimes effect on the shape of fruit body [28,29].

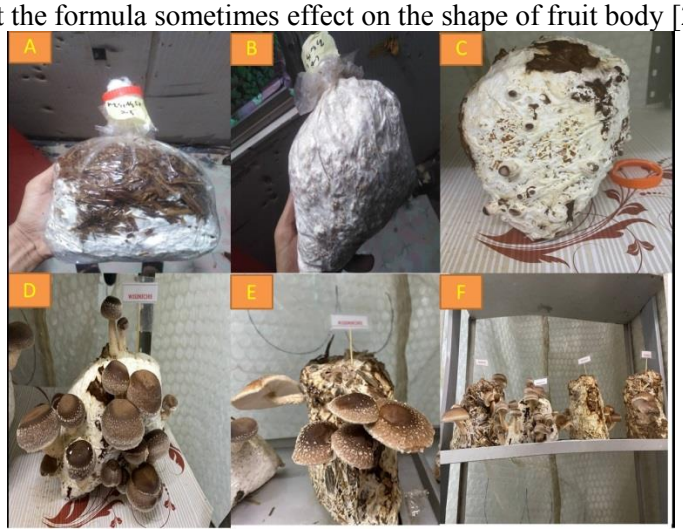

Figure 1. A: mycelium growth in substrate Phragmites australis, B: complete mycelium in sawdust, C starting fruit, D: complete fruiting in sawdust, E: complete fruiting in Phragmites australis, F: Phragmites australis substrate.

Conclusion

In this study T_6 was best treatment for cultivation of shiitake mushroom, which content high ratio of nitrogen with enough ratio of sugar from molasses residuals, best type of spawn was C_2 which is date grains that make a spores in substrate and make suitable gas exchangeable.

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