



Upcycling of Oyster Mushroom Spent Through Reuse as Substrate in Sequential Production Cycles of Mushroom



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AGRO-INDUSTRIAL by-products are potential environmental pollutants. Achieving sustainability leads us to think for handling them in an environmentally friendly way. The present framework was designed to investigate the potential of subsequent utilization of mushroom spent substrate (MSS) of oyster mushroom (*Pleurotus ostreatus* var. *columbinus*) cultivation. Two consecutive trials (2018 and 2019) were conducted at Mushroom Laboratory, Sohag University, Egypt. Fresh rice straw (RS) was used along with mushroom spent substrate (MSS) as control treatments and compared with another three different mixtures of RS:MSS as follows: 1:2, 1:1, and 2:1. The agronomic parameters of the new growing cycle of oyster mushroom, as well as the chemical compounds of substrates were investigated. Data revealed that pure (RS) substrate gave the greatest fruiting bodies yield and number/500g substrate, average fruiting bodies weight, and both of the cap diameter and stem length. In contrast, sole (MSS) gave the least values in both trials. Employing RS and MSS substrate mixture at 2:1 ratio exceeded the other two formulations concerning the above-mentioned parameters. Relative to RS, the analyses of substrates nutritive compounds in MSS showed a significantly higher percentage of N, P, K, total carbohydrates and total ash after cultivation, but had a lower percent in total carbon, hemicellulose, cellulose, and lignin. In conclusion, MSS alone can't be considered a potent substrate for further mushroom production. However, it may be used as a filler material in combination with RS but at low ratio.

Keywords: Lignocellulosic materials, Mushroom cultivation wastes, Mushroom industry. *Pleurotus* yield, Sustainability.

Introduction

Mushrooms are great food for humans having valuable nutritional composition but low energy content (Gyenge et al., 2016). Mushroom production is an environmental-friendly technology; nevertheless, large volumes of solid waste are generated after harvesting, called mushroom spent substrate, (MSS) (Pecchia et al., 2014) or mushroom compost (MC). It also called mushroom bran or mushroom residue in China which is one among the mushroom biggest producers in the world (Chang, 2006). Sample et al. (2001) and Medina et al. (2012) reported that about 5 kg of waste substrates are produced from the production of 1 kg of mushrooms which may adversely affect the environment if they not

appropriately controlled. For the progress of the mushroom industry, it is necessary to manage these by-products sustainably.

Fortunately, MSS still has some nutrients available for growing mushrooms; however, most growers prefer to replace the substrate and start a new crop. (Beyer, 2016). It is considered a valuable source of major and minor nutrients as well as organic matter and interestingly, there are many appropriate uses for MSS. For example but not exclusively, MSS is an ideal soil organic fertilizer with a high N source for agricultural plants and could be used as a starting substrate for plants grown under the hydroponic system or potted plants to replace peat moss (Oei, 2007). Whereas, handle MSS for applying one of the

previously mentioned purposes is costly and needs some amendments and preparations to improve its using quality.

The present framework was designed to test the potential reuse of oyster mushroom (*P. ostreatus* var. *columbinus*) spent substrate for the production of a subsequent mushroom crop by determining:

- The agronomic performance and yield of new growing cycle of *P. ostreatus* var. *columbinus*
- The nutritive compounds of raw rice straw material (RS) and its spent (MSS).

Materials and Methods

This experiment was performed at Mushroom Laboratory, Horticulture Department, Faculty of Agriculture, Sohag University, Sohag, Egypt in two consecutive winter seasons in 2018 and 2019. Possibility of subsequent utilization of mushroom spent as substrate (MSS) was tested solely and in combinations with fresh rice straw (RS) in different ratios for growing further cycle of oyster mushroom *Pleurotus ostreatus* var. *columbinus*. MSS was obtained from rice straw left after cropping in the previous experiment. The mushroom strain was purchased as ready spawn grown on sorghum seeds from the Agricultural Research Centre, Giza, Egypt.

Treatments and Experimental Design:

A Simple experiment was designed of 2 sole substrates (RS) and sole (MSS) (control treatments and 3 different combinations of RS and MSS as follows: 1:2, 1:1, 2:1 (w/w) arranged in a completely randomized block design (RCBD) with 3 replicates.

General procedure

Preparation of the substrates for cultivation

After finishing the mushroom growing cycle, spent substrates were collected and left in the open air for 15 days to get rid of excessive humidity before reusing. The substrate was then pasteurized at 80°C for 2 hours and drained, resulting in the moisture content of approximately 70 % (Kumari and Achal, 2008).

Spawning and spawn run

The spawn was infested at the rate of 5% of the wet weight of the substrate. The substrate was then packed into clear polythene bags of (500 g per each) and transferred into the incubation

room at 24–27°C under dark conditions for spawn running. When mycelia completely colonized, small holes were manually made in the bags for the exhaust of gases. Bags were hanged using thread to initiate the primordial formation and facilitate spraying with water every day as needed. The relative humidity was maintained at 85-95% with the help of a humidifier. The temperature was adjusted to 18°C until the end of fruiting stage (8-10) weeks.

Data recorded

Agronomic parameters

Mushroom fruiting bodies produced from the different substrates were evaluated for their number/500g substrate and yield (g/ 500g substrate) across all flushes. Ten fruiting bodies from each flush were selected to measure average fruiting bodies weight (g). Additionally, the average cap diameter (cm), cap thickness (cm), and stem diameter (cm) were measured using a scale caliper. Further, stem length was also determined

Substrates nutritive compounds

The raw material of rice straw (RS) and its spent (MSS) were analyzed for their chemical composition. Three replicates of each were prepared for analysis by drying in oven 70-80°C and then grinding into a fine powder to be ready for all chemical analysis.

- *Elements (%)*: total N, P, K, organic carbon, and total ash were determined as percentages (%) following the methods of Metcalfe (1987) and George et al. (2013).
- *Total carbohydrates (g/100g)*: total sugars (g/100g) were determined following the method of Kostas et al. (2016).
- *Lignin, Cellulose, and Hemicellulose (%)*: percentages (%) of lignin, cellulose, and hemicellulose were determined following the method described by Ayeni et al. (2015).

Statistical analysis

All data were statistically analyzed using SAS version 17.0 software package. The breakdown of the total variance was conducted in the relevance of the two-way analysis of variance (ANOVA) according to Gomez and Gomez (1984). Variance of the treatments effect was partitioned into single degree of freedom orthogonal contrasts. Treatment means were presented along with their standard error.

Results

Agronomic parameters

Fruiting bodies yield

Oyster mushroom (*Pleurotus ostreatus* var. *columbinus*) fruiting bodies yield means are presented in Table 1 along with their standard errors and a set of orthogonal contrasts. Obviously, rice straw based substrate (RS) elevated the fruiting bodies yield in contrast with the spent substrate (MSS). On the other hand, the three mixtures of RS and MSS substrates varied significantly and the mushroom yield was increased when grown on RS and MSS substrate utilized at a ratio of 2:1 substrate, followed by RS and MSS 1:1 substrate. Clearly, the sole rice straw substrate gave the highest yield (180.0, 169.8 g/500g substrate) in the first and second trials respectively, followed by RS and MSS 2:1 substrate (110.2 and 103.6 g/500g substrate) in the first and second trials respectively whereas, the least fruiting bodies yield (48.76 g/500 g) was obtained when *Pleurotus* grown on sole spent mushroom substrate in 1st and 2nd trials. Positive and significantly high correlation coefficients were found between fruiting bodies yield and each of fruiting bodies number, weight, cap diameter, and stem length.

Fruiting bodies number

According to the results presented in Table 2, rice straw based substrate (RS) 100% yielded higher number of oyster mushroom fruiting bodies as opposed to the spent substrate 100% (MSS) in 1st and 2nd trials. The three different RS and MSS substrate combinations also showed significant variation concerning mushroom fruiting bodies number. Noticeably a significant increment occurred when the fruiting bodies were produced from RS and MSS 2:1 substrate mixt in both trials. On the other hand, RS and MSS 1:2 substrate combination yielded a lower number of fruiting bodies than RS and MSS 1:2 substrate combination in the 1st trial. However, no significant difference was detected between the two mentioned combinations in the 2nd trials.

Average fruiting bodies weight (g)

Apparently, as shown in Table 3, *Pleurotus* grown on sole rice straw substrate (RS) significantly produced heavier fruiting bodies compared to the spent substrate (MSS). Differently, *Pleurotus*

fruiting bodies weight was similar when grew using the substrate mixed at various ratios of RS and MSS; 1:1, 1:2, and 2:1 in the two trials. Using pure RS substrate in the cultivation of *Pleurotus* markedly gave the heaviest fruiting bodies weight among all treatments (9.50 and 7.873g in the first and second trials respectively). Using pure MSS substrate gave the least weight of fruiting bodies (7.00 and 5.90 g in the 1st and 2nd trials respectively).

Cap diameter and cap thickness (cm)

In trial 1 and trial 2, cap diameter of *Pleurotus* grown on sole RS and MSS substrates varied significantly; RS 100% based substrate produced greater cap diameter (6.60 and 6.60 cm) than MSS 100% (4.63 and 4.70 cm). The three different substrate combinations of RS and MSS exhibited no significant variations in both trials. However, comparing RS and MSS 1:2 mixture versus RS and MSS 1:1 mixture; cap diameter in the latter mixture had a slight significant variance in the second trial only. (Table 4) On the other hand, cap thickness of *Pleurotus* fruiting bodies was similar whether grown on sole RS and MSS substrates or their mixtures (Table 5).

Stem diameter and length (cm)

Data collected and presented in Table 6 showed that stem diameter of oyster mushroom fruiting bodies was not significantly varied weather cultivated on sole RS, sole MSS or their combinations in different ratios in the two trials. On the other hand, substrates had a significant effect concerning *Pleurotus* stem length (Table 7). Obviously, stem length of fruiting bodies obtained from MSS (1.40 and 1.40 cm) was shorter than RS (2.90 and 2.96 cm) in the 1st and 2nd trials respectively. RS and MSS 1:2 along with sole MSS mixture produced the least stem length values (1.46 and 1.50 cm) in the 1st and 2nd trials respectively.

Substrates nutritive compounds

Data in Table 8 presented the chemical analysis of fresh rice straw substrate (RS) and the mushroom spent substrate (MSS). The analyses included the macro elements N, P, K, and the total ash as well as total carbohydrates and organic carbon. Percentages of cellulose, hemicellulose, and lignin were also estimated. Results showed that,

after the mushroom cultivation, valued alterations were observed on chemical and lignocellulosic content of spent substrates (MSS). Levels of N, P, and K in (MSS) were higher than that of (RS) consequently, total ash content of MSS increased from 14.42% in RS to 39.27% in SMS. Similarly, a significant increment in total carbohydrates from 14.60% in RS to 38.90% in SMS has occurred.

Adversely, C content was decreased from 85.58% to 61.39% throughout the growth of mushroom fungi. Lignin is the most abundant fiber content in the substrate. Cellulose, hemicellulose, and Lignin concentrations decreased by the growth of *Pleurotus ostreatus var. columbinus* however; the degradation seemed higher in lignin than those in cellulose and hemicellulose.

TABLE 1. Fruiting bodies yield of oyster mushroom produced on rice straw 100%, spent substrate 100% and, three of their different mixtures.

Treatments	Fruiting bodies yield (g/500g substrate)	
	Trial 1	Trial 2
1-Rice straw100%	180.0±4.50	169.8±7.59
2-Spent 100%	48.76±1.66	48.76±1.37
3-Rice + Spent 1:1	84.30±3.70	88.36±8.00
4-Rice + Spent 1:2	74.16±3.01	77.86±3.01
5-Rice + Spent 2:1	110.2±3.70	103.6±6.51
C.V.%	3.53%	4.26%
Source of variation	Mean Square	
T1 vs T2	25833.2**	21985.7**
T5 vs T3&T4	1917.86**	844.60**
T3 vs T4	154.026**	165.370*
T3&T4&T5 vs T1&T2	2219.10**	1345.6**

*, ** are significant at 0.05 probability level, respectively.

TABLE 2 . Fruiting bodies number of oyster mushroom produced on rice straw 100%, spent substrate 100% and, three of their different mixtures.

Treatments	Fruiting bodies number	
	Trial 1	Trial 2
1-Rice straw100%	19.66±1.26	20.50±1.50
2-Spent 100%	7.16±0.76	8.33±0.29
3-Rice + Spent 1:1	12.33±0.76	11.66±1.53
4-Rice + Spent 1:2	10.53±0.50	10.23±0.40
5-Rice + Spent 2:1	17.33±1.04	14.00±1.80
C.V. %	6.87%	10.62%
Source of variation	Mean Square	
T1 vs T2	234.375**	222.041**
T5 vs T3&T4	69.6200**	18.6055*
T3 vs T4	4.860000*	3.0811 ns
T3&T4&T5 vs T1&T2	0.00100 ns	21.609**

*, ** are significant at 0.05 probability level, respectively.

TABLE 3. Average fruit weight of oyster mushroom produced on rice straw 100%, spent substrate 100%, and three of their different mixtures.

Treatments	Average fruiting body weight (g)	
	Trial 1	Trial 2
1-Rice straw100%	9.50±0.50	8.73±0.25
2-Spent 100%	7.00±0.87	5.90±0.36
3-Rice + Spent 1:1	7.06±0.51	7.46±0.47
4-Rice + Spent 1:2	7.26±0.38	7.53±0.06
5-Rice + Spent 2:1	7.00±0.50	7.56±0.38
C.V. %	7.89%	4.85%
Source of variation	Mean Square	
T1 vs T2	9.3750**	12.041**
T5 vs T3&T4	0.0555 ns	0.0088 ns
T3 vs T4	0.0600 ns	0.0066 ns
T3&T4&T5 vs T1&T2	4.6694**	0.1521 ns

*, ** are significant at 0.05 probability level, respectively.

TABLE 4. Cap diameter of oyster mushroom produced on rice straw 100%, spent substrate 100%, and three of their different mixtures.

Treatments	Cap diameter (cm)	
	Trial 1	Trial 2
1-Rice straw100%	6.60±0.36	6.60±0.36
2-Spent 100%	4.63±0.35	4.70±0.20
3-Rice + Spent 1:1	5.16±0.21	5.50±0.30
4-Rice + Spent 1:2	4.90±0.10	4.80±0.10
5-Rice + Spent 2:1	5.00±0.20	4.76±0.31
C.V. %	4.50%	5.67%
Source of variation	Mean Square	
T1 vs T2	5.8016**	5.4150**
T5 vs T3&T4	0.0022 ns	0.2938 ns
T3 vs T4	0.1066 ns	0.7350*
T3&T4&T5 vs T1&T2	1.2721**	1.4187**

*, ** are significant at 0.05 probability level, respectively.

TABLE 5. Cap thickness (cm) of oyster mushroom produced on rice straw 100%, spent substrate 100%, and three of their different mixtures.

Treatments	Cap thickness (cm)	
	Trial 1	Trial 2
1-Rice straw100%	0.98±0.02	0.98±0.02
2-Spent 100%	0.93±0.06	0.96±0.12
3-Rice + Spent 1:1	0.90±0.10	0.92±0.06
4-Rice + Spent 1:2	0.90±0.10	0.93±0.06
5-Rice + Spent 2:1	0.96±0.06	0.96±0.12
C.V. %	7.73%	9.14%
Source of variation	Mean Square	
T1 vs T2	0.0032 ns	0.000 ns
T5 vs T3&T4	0.0088 ns	0.002 ns
T3 vs T4	0.0000 ns	0.000 ns
T3&T4&T5 vs T1&T2	0.0000 ns	0.003 ns

*, ** are significant at 0.05 probability level, respectively.

TABLE 6. Stem diameter of oyster mushroom produced on rice straw 100%, spent substrate 100%, and three of their different mixtures.

Treatments	Stem diameter (cm)	
	Trial 1	Trial 2
1-Rice straw100%	1.04±0.14	1.02±0.06
2-Spent 100%	1.03±0.16	1.02±0.07
3-Rice + Spent 1:1	0.95±0.05	0.94±0.06
4-Rice + Spent 1:2	0.95±0.04	0.96±0.04
5-Rice + Spent 2:1	0.98±0.10	0.98±0.02
C.V. %	9.50%	5.83%
Source of variation	Mean Square	
T1 vs T2	0.000 ns	0.000 ns
T5 vs T3&T4	0.001 ns	0.001 ns
T3 vs T4	0.000 ns	0.001 ns
T3&T4&T5 vs T1&T2	0.018 ns	0.013 ns

*, ** are significant at 0.05 probability level, respectively.

TABLE 7. Stem length of oyster mushroom produced on rice straw 100%, spent substrate 100%, and three of their different mixtures.

Treatments	Stem length (cm)	
	Trial 1	Trial 2
1-Rice straw100%	2.90±0.10	2.96± 0.05
2-Spent 100%	1.40±0.20	1.40± 0.10
3-Rice + Spent 1:1	1.87±0.16	1.86± 0.05
4-Rice + Spent 1:2	1.46 ±0.21	1.50± 0.10
5-Rice + Spent 2:1	1.90±0.10	1.94±0.04
C.V. %	8.99%	3.32%
Source of variation	Mean Square	
T1 vs T2	3.375**	3.681**
T5 vs T3&T4	0.105 ns	0.135**
T3 vs T4	0.248*	0.201**
T3&T4&T5 vs T1&T2	0.585**	0.615**

*, ** are significant at 0.05 probability level, respectively.

TABLE 8. Correlation coefficients between fruiting bodies yield and four traits of its components in oyster mushroom produced on raw rice straw, spent substrate and three of their different mixtures.

Trait ⁽¹⁾	Correlation coefficient (r)	Trait	Correlation coefficient (r)
Fruiting bodies number	0.979** ⁽²⁾	Cap diameter	0.919*
Fruiting bodies weight	0.963**	Stem length	0.980**

⁽¹⁾ n=5; ⁽²⁾ * and ** refer to a significance at 0.05 or 0.01 probability level.

TABLE 9. Some chemical components alteration in mushroom spent (MSS) in contrast with rice substrate (RS)

Samples	Total N (%)	Total P (%)	Total K (%)
RS	0.51±0.06	0.138±0.02	0.12±0.01
MSS	0.77±0.05	0.278±0.10	2.19±0.21
Samples	Total Carbohydrates (g/100g)	Total O.C (%)	Total Ash (%)
RS	14.60±3.42	85.58±0.31	14.42±0.31
MSS	38.09±2.28	61.39±5.03	39.27±0.95
Samples	Hemi Cellulose (%)	Cellulose (%)	Lignin (%)
RS	18.04±0.69	21.95±0.27	33.14±1.76
MSS	15.77±1.06	14.07±1.44	22.69±1.48

Discussion

The present investigation provides useful information toward establishing an effectual upgrading for the mushroom spent substrate (MSS). Our assessment outcome affirms the feasibility of producing oyster mushroom (*Pleurotus ostreatus* var. *columbinus*) fruiting bodies crop utilizing spent mixed with a fresh (raw) lignocellulosic substrate of rice straw (RS). The mixture ratio, however, is crucial. The lower ratio of the spent is in favor of increased fruiting bodies number and yield.

In a previous study, Ashrafi *et al.* (2014) used different proportions of MSS with fresh sawdust for the cultivation of two *Pleurotus* species viz., *Pleurotus ostreatus* and *Pleurotus florida*. They found a decrease in fruiting bodies yield by increasing MSS portion in the mixture and also indicated that MSS alone produced a low relative yield of oyster mushroom. Furthermore, Siddhant-Singh (2009) used mushroom spent substrate as ingredient in cultivation of three *Pleurotus* spp. (*P. sajor-caju*, *P. florida*, and *P. flabellatus*). They recorded the highest yields in the sets supplemented with 25% of recycled MSS. They confirmed that sole MSS can't be used as a potential substrate for mushroom production. Similarly, Pecchia (2014) recycled white *Agaricus bisporus* wastes in conjunction with fresh substrates in different quantities and reported a reduction in total fruiting bodies yield. They found that yield loss was not much different when using either 50% or 75% of MSS as compared to control (fresh substrate). Our results, in general, are in line with those previous studies that used different substrates and *Pleurotus* mushroom species.

In the current research study, the fruiting bodies yield of oyster mushroom (*P. ostreatus* var. *columbinus*) grown on sole mushroom spent was 27.09% and 28.72% (in both trials) of the yield produced on sole rice straw. Growing the mushroom on the mixed substrate at 1:1 ratio gave 46.83% and 52.04% (in the first and second trials, respectively) of the yield produced

on sole fresh rice straw. Utilizing a substrate mixture of 1:2 (fresh rice straw: spent rice straw) gave 41.2% and 45.85% (in the first and second trials, respectively). An appreciable elevation (61.22% and 61.01%, in the first and second trials, respectively) occurred when employing a mixture of 2:1 (fresh rice straw: spent rice straw). Chemical properties of the substrate had a great influence on *Pleurotus* spp. growth rate and the quality of yield produced (Ozcelik and Peksen, 2007; Peksen and Yakupoglu, 2009 and Fanadzo *et al.*, 2010). Interestingly, the fruiting bodies number, weight, and diameter, and stem length strongly associated with fruiting bodies yield. This suggested that these component traits were influential in determining of the fruiting bodies yield.

The analysis of cellulose, hemicellulose and lignin demonstrated a clear drop in MSS as compared to fresh RS. Thus the spent seemed to be exhausted concerning the contents of these carbon sources. The percent reductions were 35.9% for cellulose, 12.6% for hemicellulose, and 31.5% for lignin. Consequently, percent of organic carbon decreased and percent of ash rose. The percent of total nitrogen, phosphorus, and potassium were higher in the spent than in the raw rice straw. This may be attributed to the mycelia content of mushroom remaining in the spent. It would need a bio-decomposing to be available for the current growing mushroom (Mohamed *et al.*, 2016). Okhuoya *et al.* (2000) reported that a well-balanced carbon to nitrogen ratio enhances the growth and development of mushroom while an imbalance of C/N impedes their growth. Sarkar *et al.*, (2008) and Philippoussis *et al.*, (2000) also reported a positive correlation between the C/N ratio and *Pleurotus eryngii* mushroom yield. It is a rationale, therefore, to obtain lower yield using spent as contrasted to raw lignocellulosic straw. Furthermore, the need for nutrient elements enrichment to decompensate the depletion of these nutrients is not excluded (Soliman *et al.* 2011; Siqueira *et al.* 2012). It is believed that further enhancement in the fruiting bodies yield would be realized at lower ratios than 2:1 (RS:

spent). Easy visual estimation of trait like fruiting bodies number and cap diameter which strongly correlated with the crop yield would facilitate research in optimizing RS: spent ratio.

Conclusion

The present work emphatically indicated that oyster mushroom spent alone is not a potential substrate for mushroom production. However, it may be utilized mixed with raw straw at low portions which is suggested to be much lower than that used here. Potential spent upgrading to be used in sequential mushroom production cycles is awaiting further research.

Acknowledgment and funding statement

The authors would like to appreciate Prof. Ahmed Aziz, the president of Sohag University for the financial support provided to establish the Mushroom Production Unit and to conduct this research. We would like also to thank Dr. Mohamed Tharwat, lecturer at Agronomy Department, Fac. of Agri. Assuit Univ. for his cooperation in the statistical analysis.

Declaration of interest

The authors declare that they have no conflicts of interests with anybody.

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إعادة تدوير مخلفات زراعة عيش الغراب المحاري في دورات إنتاجية متتابعة

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يقودنا تحقيق الإستدامة إلي التفكير في إستغلال كافة المنتجات الثانوية الزراعية بطريقة صديقة للبيئة. تم تصميم الدراسة لبحث إمكانية إستخدام المخلفات الناتجة من زراعة عيش الغراب المحاري كبيئة لإنتاج محصول آخر من الفطر. وقد أجريت الدراسة مرتين متتابعتين في الوحدة الخاصة بزراعة عيش الغراب بكلية الزراعة جامعة سوهاج. تم إستخدام قش الأرز الطازج والمخلفات الناتجة من زراعة محصول الفطر السابق كمعاملات كنترول. كاتم عمل ثلاث خلطات من البيتين السابقين، وكانت النسب كالآتي: ٢:١ و ١:١ و ١:٢. تم أخذ القياسات المحصولية لثمار عيش الغراب الناتجة وكذلك تقدير التركيب الكيميائي لبيئة قش الأرز قبل وبعد الزراعة. أظهرت النتائج تفوقاً لبيئة قش الأرز المنفردة في كل من المحصول، عدد الثمار، متوسط وزن الثمرة، سمك القبة وطول الساق. في المقابل أعطت بيئة المتبقية المنفردة أقل القيم فيما يتعلق بالصفات السابق ذكرها. علاوة علي ذلك أثبتت المعاملة التي كانت نسبتها ١:٢ جزئين قش أرز إلي جزء واحد متبقية تفوقاً علي المعاملتين ٢:١ و ١:١. لم توجد أية فروق معنوية بين المعاملات فيما يخص قيم سمك القبة وقطر الساق. وأثبتت نتائج التحليل الكيميائي للبيئات المنفردة زيادة في تركيزات كل من النيتروجين، الفوسفور، البوتاسيوم، الكربوهيدرات الكلية، والرماد الكلي. بينما أظهرت نقصاً في الكربون الكلي ونسب الهيميسليلوز والسليولوز واللجنين. وقد انتهت الدراسة بالتوصية بعدم إستخدام البيئة الناتجة من زراعة عيش الغراب المحاري منفردة لإنتاج محصول لاحق. بينما قد يمكن إستخدامها فقط كمادة مالئة مخلوطة مع قش الأرز الطازج وبنسبة قليلة