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Variation of Substrates and Steaming Time on Mushroom Production

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Abstract

One of the most important aspects of mushroom cultivation is substrate disinfection. Steaming is usually adopted to kill harmful microorganisms that cause contamination on the substrates. This study was designed to assess the most cost-effective steaming duration for the production of mushrooms. A factorial experiment was used and arranged in Completely Randomized Design (CRD). The study was laid out to test the following treatments, which were replicated four (4) times. For Factor A (Main plot) A1 – 4 hours, A2 – 6 hours, A3 – 8 hours and A4 – 10 hours. Factor B (Subplot) B1 – 100% sawdust, B2 – 100% rice straw, B3 – 75% rice straw + 25% sawdust, B4- 50% rice straw + 50% sawdust and B5 - 25% rice straw + 75% sawdust. The steam or sterilization method with a longer period was found to be more efficient or productive since it had higher yield and higher biological efficiency than the short duration of sterilization time. The use of rice straw can be used as an alternative to sawdust as substrates for mushroom production. The 75% rice straw could be combined with sawdust at 25% on white oyster mushroom production to save money, time, and effort. The use of rice straw could reduce the use of sawdust and increase the productivity of white oyster mushrooms.

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Introduction

Mushrooms are an edible fungus and a good source of delicious food. It is full of nutrients. It contains high-quality proteins, essential amino acids, fats, vitamins, carbohydrates and fibers, and some have medicinal value. The unique taste and nutritional properties make mushrooms an almost perfect food. However, growing mushrooms in the Philippines is still considered one of the not-so-popular entrepreneurship, perhaps due to the insufficiency of planting materials and the limited knowledge about its culture.

The mushroom industry in the Philippines could be improved and sustained through the support of the research and development entities to recognize the previous efforts provided by researchers on the importance of mushroom production in the country. Mushroom cultivation could be remarkably profitable.

Considering the advantages of mushroom production, it could be a potential invention in the rice-based farming system. Production materials are mainly agricultural waste like rice straw, which contributes the biggest portion of waste in rice areas. Mushrooms can be grown the whole year-round. It is an income-generating activity that can be done using low-cost materials and agricultural waste. Thereby, it is a prospective endeavor to provide additional income to rice farmers aside from its contribution to human nutrition.

Mushrooms are easy to produce. It can be grown in a little space, requires low capital, and without great effort. However, growing *Pleurotus* mushroom is associated with some problems like contamination and slow growth of mycelia. To avoid some problems like this, there is a need to determine the varying levels of substrates and proper sterilization time in order to attain an optimum high level of recovery in the production of mushrooms.

Generally, the study aimed to determine the variation of substrates and steaming time of steaming on mushroom production. Specifically, the study aimed to: (a) determine the best time of sterilization and variation of substrates on mushroom production; (b) evaluate the percentage contamination, and (c) determine the mycelial growth and yield components of mushroom using the variation of substrates and steaming time.

Materials and methods

Collection of substrates and other materials used

Substrates like rice straw were collected in a rice field/farm, while sawdust was also collected at the nearby furniture shops.

The consumable materials used are the following: spawn, measuring devices; sprayer, drum, polypropylene bag, polyvinyl chloride (PVC), rubber band, denatured alcohol, alcohol, 70% solution, and scratch paper.

Experimental design and treatment

A factorial experiment was used and arranged in Completely Randomized Design (CRD). The study was laid out to test the following treatments, which were replicated four (4) times. For Factor A (Main plot) A1 – 4 hours, A2 – 6 hours, A3 – 8 hours and A4 – 10 hours.

For Factor B (Subplot) B1 – 100% sawdust, B2 – 100% rice straw, B3 – 75% rice straw + 25% sawdust, B4- 50% rice straw + 50% sawdust and B5 - 25% rice straw + 75% sawdust.

Preparation of substrates and soaking

The substrates (rice straw) were chopped into small pieces using the shredding machine to obtain uniform size. After shredding, the substrates were placed in a plastic drum and soaked overnight to remove the dirt and soften the rice straw and wash it two (2) times to remove the foul odor.

Mixing and bagging

The substrates were mixed based on the treatment formulation. The mixture was packed in a 6 x 12 polypropylene bag weighing 750 grams each.

Acquisition of Spawn and Sterilization Time

The spawn was acquired at the Southern Cagayan Research Center of the Department of Agriculture Minanga, Iguig, Cagayan, a day before planting. The fruit bags were steamed/sterilized in a steel drum based on the treatment procedure and it was cooling down for 4 hours.

Inoculation of Fruiting Bags

The fruiting bags were inoculated by introducing an optimum amount of grain spawn surrounded with an alcohol lamp to avoid contamination. Inoculated fruiting bags were placed in a dry, cool place for three (3) weeks or when the fruiting bags were fully ramified.

Hanging of Fruiting Bags and Watering

Watering of fruiting bags was arranged based on treatment combinations/layout. Watering was done early in the morning and late in the afternoon, specifically when the weather was too hot, which may damage the crop. Watering was done by using a hand sprayer or when the mushroom house reached 25°C.

Harvesting

Harvesting was done as the fruit reached the desirable or marketable size through hand picking.

Data Gathered

Weekly Mycelial Growth (cm): This was done by measuring the growth of mycelia one week after the inoculation up until fully ramified.

Percentage contamination

This can be obtained using the formula:

$$PC\ (\%) = \frac{total\ number\ of\ conatmination}{total\ number\ of\ fruiting\ bags} \times 100$$

Cumulative number of pinheads

This was done by counting the number of pinheads produced in every fruiting bag during harvesting. The total number of pinheads was divided by the number of samples harvested.

Yield (kg)

This was done by adding the total produced per treatment up to the *termination of the study*.

Statistical analysis

The data were analyzed using STAR, version 2.0.1 2014. Biometrics and Breeding Informatics, PBGB Division, International Rice Research Institute, Los Baños, Laguna following procedures for analysis of variance (ANOVA) for Complete Randomized Design (CRD) to test the significant differences among treatments. The Least Significance Difference (LSD) test was also used to analyze the mean comparisons.

Results and discussion

Weekly mycelial growth (cm)

The weekly mycelial growth of oyster mushrooms as affected by the number of hours of sterilization was presented in Table 1. Results show the increment from the first week up to the third week that A2 (6 hours), A3 (8 hours) and A4 (10 hours) were the fastest in terms of mycelial growth. Only A1 (4 hours) was considered the slowest from the start of the observation period. Results further showed highly significant differences were observed among the treatments tested.

Table 1. Weekly growth of mycelia as affected by the number of steaming time.

Treatment	Week		
	1	2	3
A1	41.14b	86.08c	142.91c
A2	48.67a	128.54ab	141.45c
A3	48.37a	133.55a	151.22b
A4	48.91a	128.65ab	160.28a
Result	**	**	*
CV%	3.58	5.32	8.02

ns = not significant
* = significant @ 1%
** = highly significant @ 5%

These results can be inferred that sterilization time really affects the growth of mycelia on mushrooms. Rapid growth and development are desirable for a profitable business; however, multiple factors, including ambient environmental conditions, substrate, physical and chemical properties, and the presence of competitive and pathogenic organisms, and antigrowth substances, will influence the rate of growth. These results conform to the study of Shah *et al.*, 2004; Kalita, 2015; the oyster mushroom takes up to three weeks for a full spawn run in its optimal environment.

Table 2. Weekly growth of mycelia using different substrates on mushroom production.

Treatment	Week		
	1	2	3
B1	31.6^{c}	94.86	127.97
B2	42.64^{b}	127.41	142.84
B3	51.06^{ab}	136.36	157.4
B4	53.76a	135.17	155.86
B5	54.8a	131.7	160.79
Result	**	**	**
CV%	3.11	6.19	4.82

ns = not significant

* = significant @ 1%

** = highly significant @ 5%.

In terms of the effect of substrates on mycelial growth, fruiting bags consistently increased their ramifications up to the third week of observation (Table 2). Results revealed highly significant differences from week 1 and week 2 except for week 3, which had a significant result. It can be gleaned that B3, B4 and B5 did not differ significantly but were significantly different from B1 and B2. This means that substrates at different levels will affect the growth of mycelia. The appreciable days to complete the mycelium running of oyster mushrooms on different substrates might be due to variation in their chemical composition and C: N ratio, as reported by Bhatti *et al.* (1987).

Table 3 shows the interaction effect between the steaming time and varying levels of substrates on mushroom production. Highly significant results were observed among the treatments tested. This can be inferred that steaming time and varying levels of substrates will affect mycelial growth.

The substrate sawdust resulted in the lowest mycelium running rate might be due to the presence of different kinds of polyphenolic substances in them, as suggested by Wang (1982) and the low content of cellulose (Gohl, 1993).

Percentage contamination

The percentage contamination of oyster mushrooms as affected by steaming time is reflected in Table 4. Results show that A1 (4 hours) steaming time obtained the most number of contamination with 9.33%, followed by A2 (6 hours) and A3 (8 hours) with a contamination percentage of 4.8% and 4.33%, respectively. The lowest was obtained in T4 (10 hours) with 3.4%. Analysis of variance shows highly significant differences were observed among treatments tested. This means that a shorter duration of steaming will produce more contaminations.

Table 5 also indicates the data on percentage contamination using varying levels of substrates on mushroom production. Results show that contamination will range from 4-6%. Despite numerical differences, statistical analysis shows no significant results were observed among the treatments tested.

Table 3. Interaction effect of the growth of mycelia on the number of hours of steaming and varying levels of substrates on mushroom production from Week 1 – Week 3.

Treatment	W1	W2	W3
A_1B_1	31.27	72.59	89.56
A_1B_2	41.42	125.95	135.84
A_1B_3	42.23	131.0	168.5
A_1B_4	45.35	117.95	154.49
A_1B_5	45.44	100.9	166.16
A_2B_1	32.67	83.84	129.00
A_2B_2	41.74	132.47	137.12
A_2B_3	54.03	140.09	139.8
A_2B_4	56.98	143.01	147.73
A_2B_5	57.95	143.29	153.72
A_3B_1	31.6	110.84	140.15
A_3B_2	43.71	132.54	142.61
A_3B_3	53.55	140.63	156.47
A_3B_4	55.5	141.25	156.45
A_3B_5	57.49	142.46	160.48
A_4B_1	41.25	112.22	153.17
A_4B_2	43.67	118.69	155.81
A_4B_3	51.06	137.05	164.84
A_4B_4	57.23	138.46	164.78
A_4B_5	58.3	140.14	162.79
Result	**	**	**

ns = not significant

* = significant @ 1%

** = highly significant @ 5%.

Moreover, no interaction effect was observed among the two factors tested (Table 6). In mushroom cultivation, substrate contamination is economically important as yield can be reduced. The most common contaminants observed were species of Aspergillus, Penicillium, Rhizopus, and Trichoderma. Similar contaminants were reported by others (Mazumder *et al.*, 2001; Ramhakana *et al.*, 2002). Trichoderma species are one of the organisms that, when present, have the potential to inhibit the growth of *Pleurotus* mushrooms; therefore, it is important that pasteurization methods are used to eliminate the contaminants.

Cumulative number of pinheads

The mean number of pinheads is presented in Table 4. It can be observed in the figure that A3 (8 hours) obtained the most number of pinheads with 45.66, followed by A4 (10 hours) and A2 (6 hours) having a means of 40.42 and 39.33 in the same order.

Table 4. Summary of statistical analysis on the yield parameters of different steaming time on mushroom production.

Treatment	Number of Pinheads	Percentage contamination	Yield (grams)
A1	27.6	9.37a	311.38
A2	39.33	4.8b	443.01
A3	45.66	4.33b	458.87
A4	40.42	3.4b	504.22
Result	*	**	**
CV%	13.73	3.02	3.93

ns = not significant

* = significant @ 1%

** = highly significant @ 5%

The least was observed in A1 (4 hours) with a mean of 27.6. Analysis of variance shows significant differences among treatments tested. On comparison among means, when A2 and A4 compared with each other, no significant difference existed; but when A3 compared to each other significant difference was observed. These results show that proper sterilization of substrates is important for the effective and smooth cultivation of mushrooms.

As shown in Table 5, the number of pinheads as affected by varying levels of substrates reveals that B3 (50% rice straw + 50% sawdust) obtained the most number of mushroom pinheads with 46.33, and the least was observed in B2 (100% sawdust) with a mean of 26.07. Analysis of variance shows highly significant differences among treatments tested. In the study of Quimio (1976) and Shah *et al*. (2004), fruiting body formation took between 3-6 weeks after pinhead formation and this agrees with the findings of this study. Onuha (2007) also worked on mushroom cultivation on different substrates and observed that the mushrooms grown on sawdust produced the least number of fruiting bodies.

Table 5. Summary of statistical analysis on the yield parameters of variation of substrates on mushroom production.

Treatment	Number of Pinheads	Percentage contamination	Yield (grams)
B1	26.07	6.08	439.08
B2	36.59	5.17	441.76
B3	46.33	4.92	466.09
B4	45.05	5.58	455.33
B5	37.2	6.0	394.71
Result	**	ns	**
CV%	14.64	2.21	3.82

ns = not significant

* = significant @ 1%

** = highly significant @ 5%.

No significant interaction was also noted among fruiting bags when exposed to the two factors tested (Table 6).

Yield (grams)

Table 4 shows the average yield of mushrooms in grams as affected by the steaming time. Disparities among means show highly significant differences in terms of the yield of mushrooms. Mushrooms grown on steamed substrates had a significantly higher yield than those grown on substrates dipped in boiling water or unheated substrates.

Table 5 shows the yield of mushrooms as affected by the use of different levels of substrates. Results reveal that B3 (75% rice straw + 25% sawdust) out-yielded other treatments with a mean yield of 466.09 grams per fruiting bag produced, followed by B4 (50% rice straw and 50% sawdust) and B2 (100% rice straw) with a yield of 455.33 grams and 441.76 grams, respectively.

The lowest yield was obtained from B1 (100% sawdust) with a mean of 439.08 grams. Comparison among means shows that when B3 and B4 were compared with each other, no significant difference was observed, but not with B1 and B2 where a significant difference exists.

This result could be attributed to the main function of rice straw which provides a reservoir of cellulose, hemicelluloses and lignin which is utilized during the growth and fructification (Yildiz *et al*., 2002). The highest yield on rice straw appeared to be due to comparatively better availability of nitrogen, carbon and minerals from this substrate (Shah *et al*., 2004).

Table 6. Summary of statistical analysis on the varying levels of substrates and steaming time on mushroom production.

Treatment	Number of Pinheads	Percentage contamination	Yield (grams)
A_1B_1	24.84	12.00	258.09
A_1B_2	25.15	8.33	312.18
A_1B_3	26.15	8.00	337.67
A_1B_4	31.09	10.00	336.82
A_1B_5	30.68	10.00	312.14
A_2B_1	23.69	4.33	372.07
A_2B_2	39.44	5.00	479.48
A_2B_3	42. 25	4.33	470.89
A_2B_4	48.82	4.67	480.81
A_2B_5	35.80	5.67	438.50
A_3B_1	26.55	4.67	444.46
A_3B_2	42.62	4.00	480.37
A_3B_3	53.14	4.00	478.84
A_3B_4	69.17	4.33	486.15
A_3B_5	40.15	4.67	404.46
A_4B_1	29.19	3.67	481.71
A_4B_2	39.16	3.33	508.37
A_4B_3	43.8	3.33	576.94
A_4B_4	41.18	3.33	530.33
A_4B_5	42.16	3.33	423.77
Result	**	ns	**

ns = not significant

* = significant @ 1%

** = highly significant @ 5%

Analysis of variance reveals a highly significant interaction effect was noted among the two factors involved in the experiment (Table 6). Siwulski also supported the results that pure sawdust was not too effective for mushroom growth but that if different supplements were mixed, nutrients provided by them had significant effects on mushroom yield.

Conclusions and Recommendations

Based on the above findings, the following conclusions were drawn: Proper sterilization of substrates is important for the effective and smooth cultivation of mushrooms. The steam or sterilization method with a longer period was found to be more efficient or productive since it had higher yield and higher biological efficiency than short-duration sterilization time. The use of rice straw can be used as an alternative to sawdust as substrates for mushroom production. The 75% rice straw could be combined with sawdust at 25% on white oyster mushroom production to save money, time, and effort. The use of rice straw could reduce the use of sawdust and increase the productivity of white oyster mushrooms. Further studies can be done thoroughly, modification of environment making suitable temperature and humidity for the better growth and development of oyster mushroom.

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