



Research Article

Use of effective microorganisms on enhancing the mycelial growth of *Pleurotus florida* on unsterilized rice straw

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Abstract: The use of unsterilized rice straw with (T₁) or without (T₂) effective microorganisms (EM-I), sterilized pure rice straw (T₃) and (T₄) Control which used the standard protocol for mushroom production developed by the Center for Tropical Mushroom Research and Development (CTMRD), were evaluated as substrates for edible mushroom production and lignin degradation using the *Pleurotus florida* fungal species. The treatments were laid-out in a Completely Randomized Design with three replications, composing of 20 fruiting bags as the sampling units. Each fruiting bag containing 750 grams substrate was inoculated with 20 grams mushroom grain spawn. The mycelia growth, pinhead formation and yield of harvested mushroom were the parameters studied in the production of edible mushroom *P. florida*. The proximate (organic matter, Ash, and Crude Protein) and cell wall contents (Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Hemicellulose, Cellulose, and Lignin) of pure rice straw and the mushroom spent beddings after harvesting the mushroom were also evaluated. Results showed that the sterilized rice straw without Effective Microorganisms substrate (T₃) and substrate containing standard CTMRD (T₄) showed significantly higher yield and biological efficiency with fast mycelial colonization of the bag compared to the unsterilized rice straw with or without EM. Producing mushroom without sterilization was made possible in the study. Significant reduction in lignin content was observed when *P. florida* was grown in unsterilized rice straw with EM. Fungal treatment of unsterilized rice straw with or without Effective Microorganisms and pure sterilized rice straw significantly increased the crude protein, crude ash, and available cellulose contents of the substrates. Fungal treatment reduced the Neutral Detergent Fiber, lignin and hemicellulose contents of the substrates.

INTRODUCTION

The sterilization and pasteurization of substrate for mushroom production is performed primarily in order to prevent the presence of contaminants like bacteria and other fungi that tend to compete for the available nutrients in an unsterilized substrate. Pressurized steam pasteurization is used in order to rid of the harmful contaminants that can hamper the growth of the mycelia and basidiocarp production of the desired mushroom. For some practical reasons, some mushroom producers used natural sources such as limestone and pulse powder to successfully dispense with the pasteurization or sterilization steps, [1] On the other hand, the use of Effective Micro-organisms as potential biological agent for pre-treatment of lignocellulosic materials or as an

alternative to expensive steam sterilization was studied, hence this research is in line with the quest for practical and more economic production of high value *Pleurotus florida* mushroom production using rice straw as biomass.

Various varieties of mushroom are found in the Philippines, these includes the tropical white rot fungi and most of these are well-known edible basidiomycetes cultivated by mushroom farmers and enthusiasts alike using organic agricultural wastes available to the locality. Mushroom farmers use sawdust and rice straw to cultivate edible mushrooms in order to augment food production and generate additional income for the family while the fibrous mushroom substrate produced from mushroom cultivation is usually discarded.

Faced with the urge to find more practical methods to increase the digestibility of forage, the potential for utilizing

indigenous edible white rot fungi is apparent. The excellent properties of fungi can be exploited for the benefit of the livestock animals. The goal of this study is to improve the lignin degradability, chemical composition, and digestibility of rice straw treated with edible white rot fungi *Pleurotus florida*, [2].

The Effective Microorganisms-I is a microbial inoculant and a performance enhancer composed of mixed microorganisms such as lactic acid bacteria, actinomycetes, yeast, photosynthetic and propionic bacteria. Effective microorganism-I is produced by EM Research Philippines, Inc, Alabang Muntinlupa City, Philippines. The main mechanism or action of EM-I on pathogenic microorganisms and on the substrate is not yet fully understood but this had been used as deodorizer and is known to dominate some pathogenic or unwanted microbial contaminants.

At present the effect of EM-I on the nutrient composition of lignocellulosic substrates has not been studied and its effect on the growth of fungal mycelia has only been confined on pre-treated sterilized substrates. The main aim of this research is to study the influence of using EM-I for enhancing the mycelial growth performance of indigenous white rot fungal species that are well known to produce edible basidiocarps, on unsterilized / unpasteurized substrates.

MATERIALS AND METHODS

The research was conducted at the National Water Buffalo Gene Pool Farm of the Philippine Carabao Center. Two empty refrigerated vans were used as the facility for growing the mushroom. Each van was equipped with three layers of wooden shelves, which served as the beddings of the fruiting bags. In addition, the vans were provided with screen doors and to monitor the temperature and relative humidity inside the mushroom house, a thermometer, relative humidity equipment and a ventilator exhaust fan were installed.

The experimental treatments that were used in this study for the production of edible mushroom using rice straw as the basal substrate were as follows:

- Treatment 1= Unsterilized rice straw without EM
- Treatment 2= Unsterilized rice straw with EM
- Treatment 3= Sterilized rice straw without EM
- Treatment 4= Standard CTMRD technology (control)

Treatments and preparation of the substrates

Treatment 1: The harvested rice straws were chopped at approximately 2-3 cm in length using a mechanical forage chopper. The chopped rice straw was soaked and moistened in tap water for 24 hours inside a plastic drum with a capacity of 200 li. The water in the soaked rice straw was completely drained off. Exactly 750g of rice straw was placed in a heat resistant polypropylene bag. A PVC pipe measuring one-fourth inch in diameter and one inch in length was prepared and served as a neck for the bag. The opening which serves as the mouth of the fruiting bag was plugged with cotton ball.

Treatment 2: As in T₁, the rice straws were chopped at approximately 2-3 cm in length. The straw was soaked in 33% EM solution at a ratio of 3 lit EM to 2 kg chopped rice straw for 24 hours and drained afterwards. Exactly 750g of rice straw was placed in heat resistant polypropylene bag, the previously cut PVC pipes served as a neck for the bag and the opening were plugged with cotton.

Treatment 3: Rice straws were chopped at approximately 2-3 cm in length and was soaked in water for 24 hours and drained afterwards. Exactly 750g of rice straw were placed in heat resistant polypropylene bags, the previously cut PVC pipes served as a neck for the bag and the opening were plugged with cotton. This was sterilized at the CTMRD for 6-8 hours inside the boiler.

Treatment 4 (CTMRD technology/control): Rice straw was soaked in water for 24 hour and drained afterwards. The substrates used consisted of mixtures of 70% rice straw and 30% saw dust. The same quantity of 750g substrate was placed in heat resistant polypropylene bags, the previously cut PVC pipes served as a neck for the bag and the opening were plugged with cotton. This was pasteurized for 6-8 hours.

Source of spawn

Mushroom *Pleurotus florida* spawn used in this study was obtained from small scale supplier that is accredited by the CTMRD.

Source and activation of the effective microorganism

The effective microorganisms that was used for this study was acquired free from the existing EM stock solution used at the Philippine Carabao Center (PCC) Gene Pool farm. In preparation or activation of the effective microorganism stock solution, the following standard formulation used by the Gene Pool farm was followed; 18 Lt water was poured in a plastic container and this was mixed with 1 Lt molasses and 1 Lt of EM stock solution. The container containing the mixtures was tightly covered and stands for 48 hours to complete the activation. The activated EM was then diluted with tapped water at 1: parts by volume and used in soaking the rice straw, T₂.

Inoculation and cultivation of the prepared fruiting bags

Each prepared fruiting bag was inoculated with approximately 20g of grain spawn. Each bag after inoculation was plugged with cotton and stored at 28-30°C in the prepared incubation room, while humidity was maintained at 80-85% by means of sprinkling of water inside the room until the mycelia have ramified the whole bag. Each bag fully ramified by fungal mycelia was transferred to the specially prepared flushing room. The opened bags from the treatments were sprayed regularly with tap water.

The bags were allowed to produce fruit-bodies. The fruit-bodies were harvested, weighed, and noted for calculation of the biological efficiency of the substrate. Contaminated fruiting bags were immediately removed and destroyed to avoid further contamination of the remaining treatments.

Analysis of Mushroom Spent beddings and *P. florida* fruiting bodies

Sample was brought to University of the Philippines Los Baños for the proximate analysis to determine the nutritional content. Percentage hemicelluloses, cellulose, lignin, Neutral Detergent Fiber and Acid Detergent Fiber of the mushroom spent and percentage crude fibre, protein fat and ash of the mushroom spent and *P. florida* were determined based from the standard procedure of chemical analysis.

Data gathered

The following parameters were properly gathered during the duration or conduct of the experiment:

- 1.Duration for full mycelia colonization of the bag this corresponds to the number of days after inoculation until the bag is fully ramified.
- 2.Duration for pinhead formation this corresponds to the number of days when the formation of pinhead was observed after the bag is fully ramified.
- 3. Duration for the maturation of fruit body this is the time when the fruiting body is ready for harvest after the pinhead formation.
- 4.Percent contamination- the presence of unwanted growth of other species of fungi or bacteria in the fruiting bags other than the *Pleurotus florida*.

The percent contamination was computed using the following formula:

% Contamination= $\left[\frac{\text{No.of contaminated bag per treatment}}{\text{Total number of bags per treatment}}\right]\times 100$

- 5. Biological efficiency/ yield performance

The Biological Efficiency (BE) was computed on a dry weight basis using the following formula of Ahmed et al., (2009).

% BE = $\left[\frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}}\right]\times 100$

- 6.Nutrient composition of rice straw before and after the mushroom harvest was analyzed in terms of proximate constituents using the procedure of AOAC, 1991[3] and the fiber contents (ADF, NDF, cellulose, hemicellulose and Lignin) by Van Soest, 1963 [4])

- a. % Organic matter (OM) = % DM of sample - % Ash
- b. % Ash content = The residue after subjecting the substrates inside the muffle furnace set at 600 °C for about 4- 6 hours;
- c. % Crude protein (CP) – This is determined by the formula: %Crude Protein = %N x 6.25
- d. % Neutral detergent Fiber (NDF)
- e. % Lignin (72% H₂SO₄)
- f. % Acid Detergent Fiber
- g. % Hemicelluloses (HCL) = %NDF - % ADF
- h. % Cellulose (CL) = %ADF - % Lignin
- i. % Dry matter = 100 - % Moisture content

Experimental design

The experiment was laid out following completely randomized design with three replications. The means was compared using Duncan’s Multiple Range Test at 5% level of significance.

RESULTS AND DISCUSSIONS

Mushroom production

During the course of the research, the white rot fungi *Pleurotus florida* showed noticeable growth on the unsterilized substrate with or without EM. However, the growth of the fungi was observed to be favourable on the sterilized rice straw substrates. Based on the observed data presented in Table 1 showed the mean number of days of total mycelia colonization, pin head formation and duration for the maturation of the fruit- body of *P. florida* as influenced by different preparation of the rice straw as substrates.

Table1. Mean number of days of total mycellial colonization, pinhead formation and duration for the maturation of the fruit-body of *Pleurotus florida* as influenced by different preparation of the rice straw substrates.

Treatment	Full mycelia colonization of the bag after inoculation	Pinhead formation after the bag was fully ramified	Maturation of fruit-body after pinhead formation
Unsterilized Rice straw W/o EM	39.03 ^a	8.08 ^b	3.99
Unsterilized Rice straw w/ EM	38.08 ^a	13.56 ^a	4.48
Sterilized rice straw w/0 EM	22.91 ^b	8.00 ^b	3.65
Sterilized rice straws	23.22 ^b	7.98 ^b	3.76

a, b = significant at 5%, SAS, 2002

Statistical analysis showed the significant effect of treatments on the full mycelial colonization of *Pleurotus florida* at 5% level of significance, analysis of variance in Completely Randomized Design, (SAS, 2002) [5]. Treatment means

showed that unsterilized rice straw substrates without EM (T1) had the highest number of mycelia growth, followed by unsterilized substrate with EM (T2), sterilized without EM (T3) and sterilized rice straws as component substrate of the

control. Significant mean comparison showed that unsterilized rice straws both with EM (T2) and without EM (T1) had significantly higher mycelia growth compared to sterilized substrates of rice straws T3 and T4. Result showed that sterilized rice straw in T3 and control (T4) are equally significant but significantly lower output than the T1 and T2. The difference could be attributed to factors such as contaminations from the environment during the mycelia stage.

With respect to pinhead formation after ramification, data showed that T2 with applied EM has produced higher pinhead compared to T1, T3 and Control. The difference between T2 and T1, T3 and Control was significant while T1, T3 and

Control has insignificant differences at 5% level of significance. The high pin head formation of T2 could be attributed to the EM solution that was applied to the substrates prior to incubation. EM solution contains molasses sugar, nitrogen source and other nutrients that were available during the cell growth. The approximate composition of molasses is: 17- 25% of water, 30-40% of sucrose, 4 - 9% of glucose, 4 -12% of fructose, 2 - 5% of starch, 7 - 15% ash, 2.5 -4.5%nitrogen compounds, 0.5 - 4.5% of protein and 1.5 - 6% non-nitrogenous acids with varying amounts of vitamins, [6]. Result of the study showed that the nutrients in EM have contributed nutrients to the fungus assimilation and enhanced the mycelial growth.

Table2. Total yield, biological efficiency and contamination rate of *Pleurotus*

Treatments	Total yield (g)	Biological efficiency (%)	Contamination rate (%)
W/o EM unsterilized (T1)	88.09 ^{bc}	11.65 ^{bc}	36.67 ^a
With EM unsterilized (T2)	78.27 ^c	10.44 ^c	33.33 ^a
W/o EM sterilized (T3)	113.58 ^a	15.15 ^a	8.33 ^b
CTMRD technology (T4)	106.46 ^{ab}	14.19 ^{ab}	5.00 ^b

Table 2 showed the total yield of the mushroom on different treatments. Treatment 3 showed the highest yield of 113.58 g, followed by Control with 106.46 g and T1 with 88.09 g and T2 with 78.27 grams. Analysis of the variance showed significant differences among treatments at 5% level of significance. Comparison of means showed T3 and T4 as significantly different with T3 significantly higher than T1 and T2 respectively. Treatment 1 and treatment 2 on the other hand has insignificant difference with respect to the biological efficiency, the results followed the trend on harvested yield per treatment. The higher yield in T3, using sterilized substrates result from biological efficiency of 15.15% followed by the Control at 14.19% followed by T1 at 11.65%, and Treatment 1 with 10.44% biological efficiency.

The significant effect of treatment could be attributed by the level of contaminations. Calculated contamination rates showed that contamination rates had significantly influenced efficiency of the treatments. Result showed that Control treatment (T₄) has the least contamination, followed by T₃, T₂ and T₁. With respect to treatment with EM, results showed that EM had reduced the contamination in unsterilized substrate, but the difference was low to effect significance compared to T1. Observation such as rapid growth of mycelia, pinhead formation and fruit-body formation did not result into higher yield and efficiency at the later stage. EM was not able to control contamination as compared to control treatment. Control treatment was the standard process for spawning *P. florida*. Result of the study did not concur with the previous study [7], contradictory results obtained in the performance of the mycelia on the substrates. This might be caused by the uncontrolled conditions took place environmental, temperature, relative humidity and air ventilation, and the presence of many contaminants in the mushroom house. Duration for the maturation of the fruit-body all the preparation of the substrates showed insignificant difference. Cultivation of oyster mushroom on lignocellulosic products has variable

levels of biological efficiencies. These differences are mainly related to the supplement added to the substrate, fungal species used and spawn rate [8]. In the present study, the inclusion of EM solution as biological agent on rice straw substrate did not bring significant effects on the mycelial colonization, pinhead formation and the yield of the fungi. Some commonly used substrate soybean straw 11.58% [9], paddy straw 83.43% [8]. In a previous study [10] recommended the use of fertilizer such as urea and dihydrogen along with the paddy straw for the cultivation of *Pleurotus florida*.

The highest contamination rate was reported on unsterilized substrate without EM with 36.67%. The usual contaminants that appear are microscopic mites (unknown species) and those unwanted species of competitive fungi like *Trichoderma* that hamper the growth of the mycelia. The use of unsterilized rice straw substrate with or without EM really affects significantly the yield of the mushroom harvested specifically due to high contamination.

Nutrient composition of mushroom

Table 3 presents the protein, fat, fibre and ash contents of the mature fruiting bodies or spent beddings of *P. florida* mushroom cultivated on different preparation of rice straw as substrates. The *Pleurotus florida* cultivated on T3, sterilized without EM has the highest percentage of protein content, followed by the substrate generated by the control at , CTMRD technology and T1, the unsterilized rice straw without EM. The least protein content was reported on T2, where rice straw was unsterilized with EM. The difference was insignificant at 5% level of significance. The highest percentage of fiber and fat content of *P. florida* on different preparation of the substrates reported on unsterilized without EM 18.91% and 2.57%, least on CTMRD technology 8.06% and 2.08%. Results showed insignificant differences among treatments. The maximum ash content of *P. florida* was found on CTMRD technology at 6.65% and *P. florida* grown on

T2, unsterilized with EM substrate being the least. The high ash content was attributed to high yield of fungal biomass in

T3 and control. The per cent content of protein and fat were similar results reported [8].

Table 3. Proximate composition of *Pleurotus florida* mushroom on different preparation of rice straw substrates

Substrate	%Crude protein	%Fiber	%Fat	%Ash
Unsterilized rice straw w/o EM	22.24	8.84	2.56	4.64
Unsterilized rice straw with EM	18.52	8.54	2.28	4.63
Sterilized rice straw w/o EM	25.24	8.91	2.57	6.63
Sterilized rice straw	22.70	8.06	2.08	6.65

Nutrient composition of substrates

Table 4. Nutrient composition of rice straw and fungal treated spent rice straw

Component (%)	Initial rice straw content	Substrate1	Substrate 2	Substrate3	Substrate4
Dry matter	97.43	95.95	95.91	96.75	96.31
Organic matter	88.79	80.21	81.66	84.79	79.29
Crude fibre	28.72	11.31	12.89	21.12	12.53
Crude protein	5.81	6.82	8.08	5.39	8.40
Ash	17.29	31.48	28.5	23.91	34.04
NDF	75.62 ^a	54.37 ^c	46.42 ^d	58.46 ^b	56.04 ^{bc}
ADF	55.15 ^c	54.99 ^c	48.65 ^d	59.05 ^b	67.08 ^a
Hemicelluloses	20.46 ^a	0 ^b	0 ^b	0 ^b	0 ^b
Cellulose	49.37 ^c	49.73 ^c	44.21 ^d	54.40 ^b	58.44 ^a
Lignin	5.79 ^b	5.26 ^b	4.44 ^c	4.67 ^c	8.64 ^a

Substrate1= fungal treated w/o EM unsterilized
Substrate3=fungal treated w/o EM sterilized

substrate2=fungal treated with EM unsterilized
substrate4=fungal treated CTMRD technology

The nutrient composition of untreated rice straw and the treatment spent rice straw were presented in Table 4. The neutral detergent fibre content represented the hemicelluloses and cellulose of the biomass. Results showed the significant difference in NDF, decreasing in percentage of dry matter with treatment applications. Similarly, ADF of rice straw declined with treatments applications. The hemicelluloses in NDF were totally reduced in treatment compared to rice straw. Cellulose declined in percentage of dry matter with treatments. The results implied that mushroom *P. florida* has consumed all the hemicelluloses in rice straw than celluloses. The effect could be related to the reduced percentages of lignin in rice straw and cellulose that forms the major component of the lignocellulose complex. Hemicelluloses content on initial rice straw was 20.46%, while the other four substrates were zero. This means that the *P. florida* completely degraded the available hemicelluloses of the substrates after 60 days of the incubation period. Significant reduction in the lignin content was also observed in fungal treated rice straw substrates compared to the pure untreated rice straw. The lower lignin content in the substrate 2 was possibly attributed to the complementary effects of the

P. florida in combination of effective micro-organisms in the degradation of the lignin content of the substrate. The effect could be attributed to the fungi ligninolytic enzymes and the complementary effect of the EM in the degradation of the hemicelluloses after the breakdown of the lignin by *P. florida*. The use of EM had reduced the time taken to breakdown of lignocellulose in agricultural residues [11]. Results showed that the highest lignin content among treatments was recorded in the Control, which contain sawdust and sterilized rice straw. Residues like sawdust with high lignin have slow decomposition rates while lignin in the rice straw was rapidly degraded in terms of reduced level of contamination of *P. florida* by opportunistic microbes from the environment [12]. Sterilization has enhanced the growth of the mushroom. Mushroom production results in increase in the following nutrients in spent rice straw like the crude protein content and ash content. The treatment effect like the mushroom hyphal growth which was related to high yield could be attributed to the increased crude protein and minerals or ash content in the spent biomass of the substrates in Control. The significant increase in the crude proteins of spent biomass could be potential nutrient for the ruminants, [13]. The same result was obtained using Fungal treated rice

straw [14]. There is significant difference in the cellulose content and tended to be lower in unsterilized straws than those in sterilized straws, [15].

Income from mushroom production and cost of producing a kilogram of mushroom

The benefit derived from mushroom production and the cost of producing a kilogram of mushroom is presented in Table 5. The highest benefit or net income was from Control, mushroom production using the CM Technology. Result

revealed a total benefit of Php 782.2 and least cost of production of 65.00. The application of EM with sterilization of rice straw revealed a net income of P223.90 and cost of production of P98.19. The EM thus contributed additional cost of producing a kilogram of mushroom and has not improved mushroom production as alternative to sterilization. On the other hand, the cost of producing a kilogram rice straw was lowest when the control substrate was used. The highest production cost was registered by substrate with EM with P98.19 to produce a kilogram of rice straw.

Table 5. Cost of producing a 100 kilogram of *Pleurotus florida* mushroom

Item	Unsterilized Rice straw/o EM	Unsterilized Rice straw w/ EM	Sterilized Rice straw w/o EM	Sterilized rice straw
A. Expenses, Supplies:				
• Cost of hauling & chopping rice straw	40	40	40	40
• Cost of saw dust				50
• Cost of EM		140		
• Cost of plastic bag	133	133	133	133
• Cost of PVC ¼ "	33.2	33.2	33.2	33.2
• Cost of cotton	150	150	150	150
Cost of water	50	50	50	50
Depreciation				
• Drum	2.5	2.5	2.5	2.5
• Weighing scale	5.0	5.0	5.0	5.0
• Chopper	80.0	80.0	80.0	80.0
• Mushroom housing	55.0	55.0	55.0	55.0
Labor:				
• Sterilization			150	
• Soaking/draining straw, MD	79	79	79	79
• Bagging	316	316	316	316
• Harvesting of mushroom	39.5	39.5	39.5	39.5
B. Total expenses, P	875.34	1015.34	1194.34	925.34
C. Mushroom produced from 100 kg substrate	11.84	10.41	15.16	14.23
D. Cost of mushroom, P	1,420.8	1249.2	1819.20	1707.5
E. Net Income, P	545.5	223.9	624.9	782.2
F. Cost of producing/kg mushroom, P	73.93	98.19	78.78	65.02

CONCLUSIONS

The results of the study led to the conclusion that unsterilized rice straw with EM was a poor substrate and treatment combination with EM for edible mushroom production reduced yield. Unsterilized rice straw with EM supported only the preliminary growth of the mycelia but the use of EM as alternative to sterilization did not support mushroom production in terms of yield and reduction in contamination. The proteinaceous, low lignin content of the spent rice straw from mushroom production has a potential feed quality for ruminants.

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RECOMMENDATIONS

- The results of the study suggest that the rice straw should be subjected to sterilization prior to use in growing edible mushroom *P. florida*.
- *In vivo* or *in vitro* study on the nutritive value of spent rice straw from mushroom production as feed source for buffaloes is recommended to determine animal acceptability.

COMPETING INTERESTS: The authors have declared that no competing interests exist.

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