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MYCELIA AND FRUITING BODY PERFORMANCE OF PHILIPPINE WILD STRAINS OF *Ganoderma lucidum* AND *Schizophyllum commune* ON RICE BRAN ENRICHED RICE STRAW BASED SUBSTRATES

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ABSTRACT

The present study evaluated the influence of rice bran supplementation of rice straw based substrates on mycelial growth and fruiting body production of wild strains of *Ganoderma lucidum* and *Schizophyllum commune*. The formulated substrates were supplemented with different levels of rice bran (0%, 5%, 10%, 15% and 20%). Rice bran supplementation stimulated the mycelial growth of *S. commune* as shown by shorter incubation period in rice bran enriched substrates. The yield and biological efficiency significantly increased with an increase in the levels of rice bran. However, the duration primordial initiation was not affected by the supplementation. In*G. lucidum*, rice bran supplemented substrate registered significantly longer incubation period and duration of initiation of primordia. Moreover, substrate supplemented with 5% rice bran produced the highest yield and highest biological efficiency.

Keywords: *G. lucidum*, *S. commune*, rice bran, supplementation, wild strain

INTRODUCTION

Mushrooms are non-timber forest products that are recently becoming popular in the Philippines because they can be tapped as important sources of nutritious food and can be used in the development of nutraceutical and pharmacological products. They commonly grow on lignocellulosic substrates in tropical and temperate countries. The tropical climatic conditions of the country and the availability of abundant supply ofagro-industrial wastes make mushroom production a lucrative agribusiness venture. To date, several exotic species of mushrooms are commercially cultivated in different parts of the country. In our effort to utilize and harness the potential of wild mushroom genetic resources, our research group collected wild species of *Ganoderma lucidum* and *Schizophyllum commune* from Central Luzon region. These mushrooms are wood rotting basidiomycetous macrofungi that are usually found growing on dead logsduring rainy season. They have been reported to exhibit many biological activities such asanti-bacterial **[1, 2]**, antioxidant **[3, 4]**, anti-tumor **[5, 6]** and cholesterol lowering properties **[7, 8]**. Due to their potential in the nutraceuticaland pharmaceutical industry, Kalaw et al. **[9]** optimized the culture conditions for thesecondarymycelial growth of these mushroom species.

In the Philippines, rice straw is one of the most abundant agricultural biomass with an estimated annual production of 15.3 million tons a year **[10]**. In view of this, the Center for Tropical Mushroom Research and Development of Central Luzon State University developed production technology for growing mushrooms using rice straw based formulations.Supplementation of the substrate with nitrogenousorganic materials such as rice bran, wheat bran, sugarcanemolasses, maize powder and other materials is a common practice in mushroom production to increase the productivity, yield and biological efficiency. Among the organic additives, rice bran is the most popular and readily available. Herein, the influence of rice bran supplementation on mycelial growth and fruiting body production of *G. lucidum* and *S. commune* was evaluated.

MATERIAL AND METHODS

Source of Culture

Pure cultures of wild strains of *Ganodermalucidum* and *Schizophyllum commune* were obtained from the culture collections of the Center for Tropical Mushroom Research and Development, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines. The cultures were revived using potato dextrose agar.

Preparation of Grain Spawn

Unmilled rice were used in the preparation of grain spawn. One kilogram was boiled in two liters of water until tender. The moisture content of the grains was adjusted to 65%. Forty grams was placed in 4 x 8 inches heat resistant polypropylene bags and then autoclaved at 15 lbs/in^2, 121^{o}C for 30 minutes. The grain spawn were allowed to cool and then separately inoculated with 10 mm mycelial disc of *G.lucidum* and *S. commune*. The inoculated bags were incubated at room temperature until completely ramified with mycelia.

Substrate Preparation

The rice straw based substrate formulation was prepared based on the procedures described by Reyes et al. **[11]** as follows. The rice straw were soaked in water for three days, then hauled from the soaking tank and transferred to the composting area. The pile of rice straw was covered with plastic to stimulate the growth of natural decomposers that will initially decompose the substrate. The rice straw were composted for five days with turnings every two days. On the last day of composting, the rice straw were aerated by turning the pile using spading fork. The composted rice straw were chopped into approximately 2 to 3 cm long. Seven parts of the composted rice straw were mixed with 3 parts compstedsaw dust v/v. The moisture content of the substrates was maintained at 65%. The formulated substrates weresupplemented with different levels of rice bran (0%, 5%, 10%, 15% and 20%). Five hundred grams of formulated substrates was bagged and compacted in 6 inch x12 inch heat resistant polypropylene plastic bags. The bags were plugged with cotton and sterilized in an autoclave at a temperature of 121 ^{o}C, pressure of 15 psi for a period of one hour. The bags were cooled for 24 hours and inoculated with 40 grams of grain spawn of the mushroom. The inoculated bags were incubated at room temperature. The number of days of incubation and primordial initiation, weight of fruiting bodies were recorded and the biological efficiency was calculated.

Fruiting Body production

The fully ramified fruiting bags were transferred to the growing house. To allow the growth of the fruiting bodies, one end of the bag was cut using a scissor. The open end of the bags was sprayed with clean water three times a day. The fruiting bodies were harvested and weighed.

Statistical Analysis

The experiment was laid out following the completely randomized design. All the data collected were analyzed using SAS version 9.1. Means were compared using least significant difference at 5% level of significance.

RESULTS AND DISCUSSION

Incubation period

Incubation period was recorded as the number of days from inoculation of the substrate to complete mycelial ramification of the fruiting bags. The rate of mycelial colonization of the substrate is dependent on the nutruents present in thethe substrates and prevailing environmental conditions **[12]**. The incubation period of *G. lucidum* and *S. commune* on rice straw supplemented with different amounts of rice bran is presented in Table 1. In *S. commune*, the longest incubation period was recorded in substrate formulation without rice bran (control) with a mean of 18 days while the shortest incubation period was noted in substrate enriched with 20% rice bran with a mean of 12 days. However, in *G. lucidum,*the longest incubation period was observed in substrate enriched with 20% rice bran with a mean of 34.67 days. On the other hand, the shortest incubation period was noted in substrate without rice bran (control) with a mean of 20.60%. Statistical analysis revealed significantinfluence of rice bran supplementation on the incubation periodof the two mushroom species.Themycelial growth response to different concentration of rice bran varies according to the mushroom species. Rice bran stimulated the rapid mycelial proliferation of*S. commune* while it slowed down the mycelial colonization of *G. lucidum*. This result indicates that the effect of addition of rice bran supplementation is species dependent.For instance, Moonmoon et al. **[13]** reported that highest mycelium growth of *L. edodes* was attained when 20% rice bran was added to sawdust fruiting substrate. Pokrel et al. **[14]** also observed faster mycelial growth of *Pleurotussajorcaju* when cultured on maize stalks with rice bran. They explained further that that rice bran significantly supported the mycelial growth in all substrates used in the study.On the contrary, Rossi et al. **[15]** disclosed that myceliationrate of *Lentinulaedodes* decreased significantly with increasing proportion of rice bran. They explained that the addition of this supplementpromoted a gradual decline in C: N ratio of the substrate which suggests that large amount of rice bran inhibits the growth of *L. edodes*. Moreover, Donini et al. **[16]** reported that besides affecting the formation of fruit bodies, the excess of nitrogen may have affected the degradation of lignin which may prevent the mycelium from developing.

Table 1. Mycelia and fruiting body performance of *S. commune* on rice straw based substrate supplemented with varying levels of rice bran					
Species	**Substrate Formulations**	**Incubation Period (days)**	**Days to Primordial Initiation**	**Yield /bag (grams)**	**Biological Efficiency (%)**
S. commune	0% (Control)	18.00^{a}	10.00^{a}	14.22^{e}	5.69^{e}
	5% rice bran	12.56^{b}	10.00^{a}	19.11^{d}	7.65^{d}
	10% rice bran	12.44^{b}	10.00^{a}	25.11^{c}	10.00^{c}
	15% rice bran	12.33^{b}	9.44^{a}	28.11^{b}	11.24^{b}
	20% rice bran	12.00^{b}	10.00^{a}	38.00^{a}	15.20^{a}
G. lucidum	0% (Control)	20.60^{d}	30.85^{d}	37.56^{bc}	6.26
	5% rice bran	21.00^{d}	31.08^{d}	54.00^{a}	9.03^{a}
	10% rice bran	24.25^{c}	35.08^{c}	45.27^{b}	7.55^{b}
	15% rice bran	28.45^{b}	38.44^{b}	29.46^{cd}	4.89^{cd}
	20% rice bran	34.67^{a}	43.67^{a}	28.32^{d}	4.72^{d}
Means with the same superscript are not significantly different from each other.					

Days to Primordia Initiation

The duration of primordial initiation is the number of days from inoculation of the substrate to the appearance of fruiting initials or primordial. The appearance of these structures indicates the start of fruiting body development. The number of days to primordial production is presented in Table 1. *S. commune* recorded shorter duration to primordial initiation in substrate supplemented with 15% rice bran with 9.44 days while longer duration was recorded in the rest of the treatments with an identical mean of 10 days.Analysis of variance revealed no significant influence of rice bran supplementation on the duration of primordia initiation. This finding implies that the addition of rice bran to the substrate did not influence the formation of primordial.This is in congruent with the observation of Abere and Stanley **[17]** who reported no significant difference in the time of primordial appearance of Pleurotus *tuber-regium* cultivated on corn cob supplemented with rice bran.

In case of *G. lucidum*, substrate supplemented with 20% rice bran exhibited the longest period with a mean of 43.67 days. On the other hand, the shortest duration was noted in substrate without rice bran (control) with a mean of 30.85 days. Analysis of variance revealed significant influence of rice bran supplementation on the duration of primordialinitiation.The appearance of primordia is prolonged with an increase in the amount of rice bran.This result implies that the addition of rice bran can delay the appearance of primordial of *G. lucidum*.This finding does not conform with the observation ofPokhrel et al. **[14]** who reported fastest primordial initiation in maize stalk, banana leaves and pea residue with rice bran. Moreover, Alam et al. **[18]** disclosed shortest time for primoridial initiation in the 40% rice bran supplemented substrate while the longest time was recorded in the unsupplemented rice straw substrate.

Yield per bag

The yield is the most important parameter in mushroom production. The amount of fruiting bodies produced by *S. commune*and *G. lucidum*as influenced by rice bran supplementation is shown in Table 1. *S. commune* grown in substrate supplemented with 20% rice bran produced the highest yield with a mean of 38 grams per bag. In contrast, the lowest yield was obtained in substrate without rice bran (control) with a mean of 14.22 grams per bag. Analysis of variance revealed significant influence of rice bran supplementation of fruiting body production. Addition of rice bran to the substrate can produce significantly. The result of the presentstudy is in agreement with the findings of Adenipekun and Omolaso **[19]** who disclosed higher yield of*Pleurotuspulmonarius*cultivated in rice straw enriched with rice bran. Furthermore, Abere and Stanly **[17]** reported that substrates supplemented with rice bran produced better yield of *Pleurotus tuber-regium*.

Meanwhile, in *G. lucidum* the highest yield was obtained in substrates supplemented with 5% rice bran while the lowest yield was recorded in substrate supplemented with 20% rice bran. Analysis of variance revealed significant effect of rice bran supplementation on the yield. This result suggests that supplementation of rice bran can increase the mushroom yield. However, increasing the amount of rice bran beyond 5% did not significantly increase the yield. This finding does not conform to Rossi et al. **[15]** who obtained higher yield of *Lentinulaedodes* with the addition of 25% and 30% rice bran. The increase in the yield could be due to the nutrients present in rice bran.Faria et al. **[20]** reported that rice bran is rich in proteins, carbohydrates and minerals such as calcium, iron, sodium, potassium. Rossi et al. **[15]** disclosed that addition of rice bran to the substrate improved the nutrient content. Peng et al **[21]** found that the average yield of *Pleurotuseryngii* increased significantly with the increase of rice bran in the substrate from 0 to 9.20, 18.34, 28.32, 38.08 and 47.95%.. The increase in the yield could be attributed to the nutrients present in rice bran. Faria et al. **[20]** reported that of rice bran contain proteins, mineral and carbohydrates. Donini et al **[16]** noticed that substrates excessively enriched with rice bran, as well as the nature or some component in the soybean bran, influenced productivity and biological efficiency. In contrast, Stanley et al. **[22]** found that unsupplemented corn cob gave best yield than the rice bran supplemented substrate.

Biological Efficiency

Biological efficiency is the ability of the mushroom organism to convert the substrate into fruiting bodies. The biological efficiency of *S. commune* and *G. lucidumon* different substrate formulations is also presented in Table 1. In *S. commune,* the substrate with 20% rice bran yielded the highest biological efficiency with a mean of 15.20% while the control (without rice bran) exhibited the lowest biological efficiency with a mean of 5.69%.Similar to the yield, there was a significant increase in biological efficiency with increasing amount of rice bran. This observation is consistent with the report of Adenipekun and Omolaso **[19]** that the total yield and biological efficiency of*Pleurotuspulmonarius* was significantly influenced by the substrate and additives, with high percentage of rice bran concentration performing best in rice straw. Furthermore,Peng et al. **[21]** whoreported that the biological efficiency of *Pleurotuseryngii* increased significantly with supplementation of rice bran to the substrate. Meanwhile, in *G. lucidum,* the substrates with 5% rice bran exhibited the highest biological efficiency with a mean of 9.03% while the lowest biological efficiency was observed in substrate enriched with 20% rice bran. This result implies that the 5% rice bran is the best level of rice bran supplementation to meet the highest biological efficiency. The data obtained in the present study is in agreement with the finding of Adenipekun and Omoloso **[19]** who disclosed that addition of 10% wheatbran to banana leaves produced significantly higher biological efficiency than 20% and 30%. Moreover, in *Calocybeindica,* Alam et al. **[18]**observed that increasing the amount of supplements resulted in an increase in biological efficiency up to 30% and then the efficiency decreased again. They explained that the reduction in efficiency may be due to the compactness or poor aeration of the substrates, which results from insufficient utilization of nutrients.

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