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Proximate composition profiling of the rice straw enriched with mycoprotein of fungal endophytes

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ABSTRACT

The present study focused on the proximate composition profiling of the mycoprotein enriched rice straw by endophytic fungi which include the moisture, crude fat, crude ash, crude fiber, total carbohydrates and total energy value of the fermented rice straw. Additionally, cytotoxicity of the fermented rice straw was also undertaken. Fusarium sp.1 – treated rice straw had the highest moisture content with 8.09%, whereas, the highest value of 20.53% for % ash was recorded in Aspergillus flavus – treated rice straw. For the crude fiber content, Fusarium sp.2 – treated rice straw increased the crude fiber content of the rice straw from 27.84% to 32.63%. Meanwhile, crude fat content (2.21% to 0.58% by Penicillium citrinum, total carbohydrates (40.91% to 35.40% with Fusarium sp 1) and total energy value (203.82% to 172.15% by Cladosporium cladosporioides) were reduced significantly upon solid state fermentation of the rice straw. Moreover, cytotoxicity test disclosed the non – toxicity of the mycoprotein enriched rice straw in brine shrimp. Thus along with their single cell protein potentials is the capability of the endophytic fungi in enhancing the proximate composition of the rice straw.

Key words: cytotoxicity, mycoprotein, proximate composition, rice straw, single cell protein

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INTRODUCTION

Endophytes are microorganism that inhabits the healthy tissue of plants without causing damage to the plants [1, 2]. Additionally, their ability to produce various enzymes and their industrial and pharmaceutical potentials has been widely explored [3, 4, 5, 6, 7]. They are capable of utilizing starch and lipids as energy sources releasing amylase, proteinase and oxidases which actively degrade the components of the substrates [8].

Several studies has been conducted demonstrating the microbial ability to upgrade low protein organic material to high protein food, thus microbial proteins was termed as single cell protein. Consequently, proteins from fungi are known as mycoprotein. Fungal organisms specifically the filamentous ones, utilize starch as the substrate for bioconversion processes and SCP production [9, 10]. Several investigations were carried out using various forms of organic waste such as cellulose hemicelluloses, hydrocarbon and different types of agricultural waste cellulose and hemicelluloses waste as a suitable substrate for increasing SCP production [11, 12, 13, 14].

In a study of Valentino et al. [15], nine species of fungi were identified as endophytes of bamboo including *Aspergillus niger, Aspergillus flavus, Aspegillus ochraceus, Penecillium citrinum, Cladosporium cladosporioides, Monascus ruber, Fusarium semitectum , Fusarium sp1* and *Fusarium sp2*. Similarly, several studies revealed their ability in enriching the crude protein content of various subtrates (corn cob, rice bran sugar cane bagasse) thus their potentials in single cell protein production [15, 16, 17, 18, 19]. However, other nutritional attributes in relation to mycoprotein production in rice straw (crude ash, crude fat, crude fiber, moisture, total carbohydrates and total energy value) has yet to probe. Hence, the study was conducted.

MATERIALS AND METHODS

Methodology was adapted from the works of Valentino et al. [18], Valentino et al.[19], Paynor el at.[16] (2016), Ganado et al. [17] with some modifications. Additionally, this study is a continuation of the study of Valentino et al. [15].

Preparation of substrates
One hundred (100) grams of rice straw were placed in fermentation bottles separately and 150 ml of distilled water were added and sterilized.

Solid state fermentation
Spores of fungal endophytes were adjusted to 5.0×10^6 cells per ml and 20 ml of which were aseptically transferred to the sterile sugarcane bagasse. Solid state fermentation was carried for 20 days at room temperature. After which, substrates were air dried and pulverized.

Proximate analysis
Dried samples were pulverized using mortar and pestle. After which, samples were sent to Lipa Quality Control Center, Bocaue Bulacan, Philippines for proximate composition analysis.

Brine Shrimp cytotoxicity test
Adapted from the works of Valentino et al. (2016), Paynor et al. (2016).

RESULTS AND DISCUSSION

The study is a continuation of the study of Valentino et al. [19], wherein the single cell protein capability of *Aspergillus niger, Aspegillus flavus, Aspergillus ochraceus, Fusarium* sp.1, *Fusarium* sp.2, *Fusarium semitectum, Monascus ruber, Penicillium citrinum* and *Cladosporium cladosporoides* using rice straw as substrate was probed.
Proximate composition of the fungal enriched rice straw including moisture, ash, crude fiber, crude fat, total carbohydrates and total energy value were evaluated in the present study (Table 2). Results showed significant increase in moisture, crude ash and crude fiber. Meanwhile, the crude fat, total carbohydrates and total energy values were reduced.

Moisture content was reduced from 8.00% of the untreated rice straw to 5.89% by *Aspergillus ochraceus*. Meanwhile, *Fusarium* sp1 obtained 8.09% moisture which is statistically comparable with the untreated substrate. For percentage ash content of 20.59% and 20.53% was obtained in *Aspergillus niger* and *Aspergillus flavus* – treated rice straw correspondingly while the uninoculated rice straw had the lowest ash content of 15.69%. These findings agreed with those of Bakshi et al.[20] and Oboh & Akindahunsi [21], suggesting that microbial fermentation increases the ash content of cassava peel which could be useful in animal feeds. In addition, high ash content is a reflection of the mineral contents preserved in the food materials [22]. For the crude fiber content *Fusarium* sp.2– treated rice straw recorded the highest mean value of 32.63% followed by *Fusarium semitectum* and *Cladosporium cladosporoides* – treated rice straw with 31.87% and 30.84% respectively. On the other hand, *Aspergillus niger* – treated rice straw had the lowest crude fiber of 26.90% and *Aspergillus flavus* – treated rice straw with 26.94%. Mycelial growth contributed to the increased in crude fiber while the reduction in crude fiber can be due to the enzymatic activities by the fungus [23]. This also coincides of Adenipekun & Okunlade [24] wherein the crude fiber was decreased significantly by the microorganisms compared to untreated substrates. Low fiber content can would result to an improved digestibility of animals [25]. Finally, crude fat content was reduced from 2.21% of the uninoculated rice straw to 0.32% by *Fusarium sp 1*– treated rice straw.

Reduction in total carbohydrates and total energy values was also observed in all endophytic fungi treated rice straw (Table 2). Total carbohydrates of rice straw decreased from 40.91% to 35.40% by *Fusarium semitectum*. Similarly, total energy values were lowered from 203.82% to 172.15% by *Cladosporium cladosporoides*. Apparently, decrease in the carbohydrate content is due to the increase in crude protein, crude ash and its varying effect of the endophytic fungi to the proximate composition of the substrate. This also conform with Oboh et al. [21], and Abdel- Azim [26] that fungal treatment of rice straw produce sufficient amount of cellulolytic enzymes namely exo, endogluconases and ß- glycosidase and the decrease of energy content.

For the cytotoxicity assay (Table 3), 10.00% mortality rate was recorded at 6hrs of incubation in *Aspergillus ochraceus*, *Penecillium citrinum*, and *Cladosporium cladosporoides*- treated rice straw. At 12 hrs of incubation, yeast, *Monascus ruber* and *Cladosporium cladosporoides*– treated rice straw had the highest percentage of mortality of 16.67%. At 18 hrs of incubation, *Fusarium* sp.2 *and M. ruber* recorded 9.52% mortality rate while *Cladosporium cladosporoides* obtained the highest mortality rate of 12.50%.

Table 1. Mean percentage (%) of proximate composition of enriched rice straw

TREATMENTS	%Moisture	%Ash	% Crude Fiber	%Crude Fat
Control (Uninoculated rice straw)	8.00[h]	15.69[a]	27.94[a]	2.21[b]
A. niger – treated rice straw	5.64[a]	20.59[f]	26.90[a]	1.07[a]
A. flavus – treated rice straw	6.32[d]	20.53[f]	26.94[a]	0.58[a]
A. ochraceus – treated rice straw	5.89[b]	19.70[e]	30.31[b]	0.73[a]
Fusarium sp.1 – treated rice straw	8.09[h]	19.01[d]	30.74[bc]	0.32[a]
Fusarium sp.2 – treated rice straw	6.16[c]	18.69[c]	32.63[d]	1.06[a]
F. semitectum – treated rice straw	7.21[e]	18.59[c]	31.87[cd]	0.91[a]
M. ruber – treated rice straw	7.41[f]	18.32[b]	27.55[a]	0.92[a]
P. citrinum – treated rice straw	7.22[e]	19.24[d]	30.24[b]	0.58[a]
C. cladosporoides – treated rice straw	7.69[g]	19.10[d]	30.84[bc]	0.78[a]

[*] Treatment means with the same letter are not significantly different

Table 2. Total carbohydrates and total energy value of the untreated and treated rice straw

TREATMENTS	Carbohydrates	Energy
Control (Uninoculated rice straw)	40.91[b]	203.82[e]
A. niger – treated rice straw	39.57[b]	192.40[d]
A. flavus – treated rice straw	39.29[b]	187.74[cd]
A. ochraceus – treated rice straw	36.87[a]	181.75[bc]
Fusarium sp.1 – treated rice straw	35.40[a]	172.95[a]
Fusarium sp.2 – treated rice straw	36.01[a]	176.68[ab]
F. semitectum – treated rice straw	35.55[a]	172.77[a]
M. ruber – treated rice straw	39.91[b]	191.06[d]
P. citrinum – treated rice straw	36.48[a]	177.63[ab]
C. cladosporoides – treated rice straw	35.85[a]	172.15[a]

** Treatment means with the same letter are not significantly different*

Table 3. Percentage of brine shrimp mortality rate (%).

Treatment	6hours	12hours	18hours	24hours
Control/ Yeast	0.00[a]	0.00[a]	26.67[bc]	30.00[ab]
A. niger- treated rice straw	0.00[a]	0.00[a]	3.33[a]	3.33[a]
A. flavus- treated rice straw	0.00[a]	16.67[a]	20.00[abc]	26.67[ab]
A. ochraceus- treated rice straw	10.00[a]	13.33[a]	20.00[abc]	23.33[ab]
Fusarium sp.1- treated rice straw	3.33[a]	3.33[a]	10.00[ab]	16.67[ab]
Fusarium sp.2- treated rice straw	6.67[a]	6.67[a]	10.00[ab]	16.67[ab]
F. semitectum- treated rice straw	0.00[a]	6.67[a]	6.67[ab]	10.00[ab]
M. ruber- treated rice straw	6.67[a]	16.67[a]	33.33[c]	33.33[b]
P. citrinum- treated rice straw	10.00[a]	10.00[a]	13.33[abc]	13.33[ab]
C. cladosporoides- treated rice straw	10.00[a]	16.67[a]	23.33[abc]	30.00[ab]

** Treatment means with the same letter are not significantly different*

CONCLUSION

The results of the present study probed the ability the varying effect of the endophytic fungi in the composite composition of the rice straw in line with their single cell protein potentials. Moisture, crude ash and crude fiber content were increased significantly while crude fat, total carbohydrates and total energy values were reduced. Cytotoxicity test disclosed the non- cytotoxicity of mycoprotein enriched rice straw.

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