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Der Pharmacia Lettre, 2016, 8 (8):85-88
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Nutritional attributes and cytotoxicity of the single cell protein enriched sugarcane bagasse

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ABSTRACT

Nutritional attributes of the fungal treated sugarcane bagasse which include the crude ash, crude fiber, crude fat, moisture, total carbohydrates and total energy value of the fungal treated sugarcane bagasse were probed in the study. This in in line with the previous study of Valentino et al 2016 wherein the crude protein content of sugarcane bagasse were enhanced by the same species of endophytic fungi after 20 days of solid state fermentation. Varying effect of the endophytes was noted on the moisture, crude ash, crude fiber and crude fat of the fermented sugarcane bagasse. Among which the following showed the highest increment; Fusarium sp. 2 and Penicillium citrinum-treated sugarcane bagasse of 9.10% for the moisture content; for the % ash, Aspergillus niger- treated sugarcane bagasse with 7.71%; Aspergillus flavus- treated sugarcane bagasse of 1.3% for the crude fat while Monascusruber-treated sugarcane bagasse for the crude fiber with 56.03%. Meanwhile, decrease in total carbohydrates and total energy value were recorded. For the cytotoxicity assay, non cytotoxic effect of the single cell protein enriched sugarcane bagasse was observed.

Key words: ash, crude fat, crude fiber, cytotoxity, single cell protein, sugar cane bagasse

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INTRODUCTION

Single cell protein is a microbial biomass from fermentation and other bioconversion processes which turned agro-industrial wastes into products with added nutritional values and is highly regarded as a promising source of protein [1]. It may also contains nucleic acids, carbohydrate cell wall material, lipids, minerals, vitamins, fats, carbohydrates, ash, water and other elements such as phosphorus and potassium. Moreover, the nature and quality of the substrates, carbon source, the microorganisms used and the method of harvesting drying can greatly affect its nutritive composition [2-5].

Single Cell Protein (SCP) from yeast and fungi has up to 50-55% protein, more lysine less amount of methionine and cysteine; it also contains high protein-carbohydrate ratio. Additionally, it has a good balance of amino acids and a high B-complex vitamins and more suitable as poultry feed. Single cell protein basically comprises proteins, fats, carbohydrates, ash, fiber, water and other elements such as phosphorus and potassium [2, 3, 4]. SCP if proven to be safe and non toxic will provide both the nutritional component in a food system and also perform a number of other functions [6].

Sugarcane bagasse is a by product of sugarcane industry and can be utilized as carbon source and substrate for single cell protein production. It is rich in lignocellulosic biomass, which is mainly composed of cellulose, hemicellulose and lignin. In contrary, it is low in protein and ash content, thus SCP can enhance its nutritive composition [7, 8, 9] Consequently, Valentino et al [10] reported the use of untreated sugarcane bagasse in the production of single cell protein of the fungal endophytes. The present study is a continuation of the previous report which aimed to evaluate the nutritive attributes and the toxicity of the fermented sugarcane bagasse.

MATERIALS AND METHODS

Methodology was adapted from the works of Valentino et al.[11], Valentino et al.[12], Paynor el at.[13], Ganado et al. [14], with some modifications. Additionally, this study is a continuation of the study of Valentino et al. [10].

Preparation of substrates:
One hundred (100) grams of sugarcane bagasse were added with 200ml of distilled water to obtain a moisture content of 70% and was sterilized at 15 psi at 121° C for one hour.

Solid state fermentation:
Spores of seven day old fungal endophytes were counted and adjusted to 5.0×10^6 cells per ml. Twenty (20) ml of the adjusted spore suspension of different endophytic fungi were aseptically inoculated to the sterile sugarcane bagasse were allowed to acclimatize in the substrate for 20 days at room temperature.

Harvesting and drying:
The fermented substrates were sterilized at 15 psi for one hour. Then, the fermented substrates were air dried for seven days. Dried substrates were pulverized using mortar and pestle and were sent to Philippine, Rice Research Institute for analysis.

Brine Shrimp cytotoxicity test:
Brine shrimp cytotoxity test was performed following the works of Valentino et al. [13] and Paynor et al. [12].

RESULTS AND DISCUSSION

Nutritional attributes of the single cell protein enriched sugarcane bagasse
In the previous study of Valentino et al 2016, nine species of endophytic fungi (*Aspergillus niger, Monascus ruber, Cladosporium cladosporioides, Fusarium* sp. 2, *Fusarium* sp. 1, *Penicillium citrinum, Fusarium semitectum, Aspergillus ochraceus,* and *Aspergillus flavus*) elevated the crude protein content of sugarcane bagasse fter 20 days of solid state fermentation. Hence, the study was designed to evaluate its effect on the nutritional attributes of the fermented substrate using the same procedure and the same fungal endophytes.

Presented in Table 1 are the dnutritional attributes of the single cell protein enriched sugarcane bagasse. Both *Fusarium* sp. 2- treated sugarcane bagasse and *Penicillum citrinum*- treated sugarcane bagasse obtained the highest moisture content of 9.10%. Meanwhile, *Monascus ruber*- treated sugarcane bagasse with 7.70% had the lowest moisture percentage followed by the uninoculated sugarcane bagasse with 8.20%. All except *Monascus ruber* were statistically higher than the untreated sugarcane bagasse. This is in accordance with several studies wherein increase in moisture content of the substrate due to enzymatic and metabolic activities of the microorganisms which releases water, thus the increase in moisture content [15, 16, 17].

Aspergillus niger increased the % ash from 5.22% to 7.71%, followed by *Fusarium semitectum* and *Aspergillus ochraceous* treated sugarcane bagasse with 6.15% and 6.13%, respectively. Thus, the aforementioned endophytic fungi contribute to the increase of ash percentage of the sugarcane bagasse. Increment in the ash content due to microbial activity resulted decrease in the dry matter content and increase in mineral content [18, 19, 20]. *Aspergillus niger* based treatment of residue has led to increase in ash percentage absorbed material coefficient of compound digestion and raw protein content but it led to a decrease in raw fibers [21].

For the evaluation of crude fat percentage, *Aspergillus flavus*- treated sugarcane bagasse had the highest crude fat of 1.3% followed by *Penicillum citrinum* and *Fusarium semitectum* –treated sugarcane bagasse both with 1.2% crude fat. Meanwhile *Aspergillus ochraceus* –treated sugarcane bagasse obtained the least crude fat of 0.5% followed by uninoculated sugarcane bagasse of 0.6%. Statistically, *Aspergillus ochraceus, Penecillium citrinum and Fusarium semitectum* were significantly higher than the uninoculated sugarcane bagasse thus enhancing the crude fat content of sugarcane bagasse. The increase in fat content of the fungal fermented sample could be due to the possibility of the fermenting fungi in transforming the carbohydrate content to fat and synthesize microbial oil during the process of fermentation [22, 23]. Several studies obtained the same results with regards to the increase in crude fat of the fermented substrate [25, 26,27, 28]. Furthermore, *Aspergillus* strains have been utilized in the production of enzymes and organic acids [29, 30, 31].

For crude fiber percentage, *Monascus ruber*- treated sugarcane bagasse recorded the highest crude fiber with 56.03% followed by *Fusarium* sp 1- treated sugarcane bagasse with 43.47%. The least crude fiber content was

observed in *Fusarium* sp 2–treated sugarcane bagasse with 22.63%. Hence the ability of *Aspergillus niger* and *Monascus ruber* to enhance the crude fiber while *Fusarium* sp2 exhibited a degradative effect on sugarcane bagasse.

Total carbohydrates and total energy value were decreased which can be due to the enhancement on the nutritional attributes of the sugarcane bagasse by the fungal endophytes. Total carbohydrates were reduced from 59.87 to 54.12.In addition, among the nine endophytes tested, *Cladosporium cladosporioides* recorded the highest total carbohydrates of 56.94 followed by *Monascus ruber* of 56.14 while the least of 52.16 was recorded by *Aspergillus niger*. Whereas the energy value of untreated sugarcane bagasse of 310 was lowered to 290.94 by *Aspergillus niger*. Decrease in total carbohydrates and total energy values can lead to a better digestibility and growth of the animals, if proven safe and appropriate as animal feeds.

Table 1. Mean percentage (%) of proximate composition of enriched sugarcane bagasse

TREATMENTS	Moisture	Ash	Crude Fat	Crude Fiber	Carbohydrates	Energy
Uninoculated sugarcane Bagasse	8.2^{b}	5.22^{a}	0.6^{a}	36.13^{b}	59.87^{a}	310^{a}
A. niger -treated sugarcane bagasse	8.9^{cde}	7.71^{c}	07^{a}	39.95^{c}	52.16^{e}	290.94^{f}
M. ruber -treated sugarcane bagasse	7.7^{a}	5.73^{ab}	0.7^{a}	56.03^{e}	56.14^{bc}	303.48^{c}
C. cladosporioides-treated sugarcane bagasse	9.0^{cde}	5.90^{ab}	0.7^{a}	35.21^{b}	56.94^{bc}	306.08^{b}
Fusarium sp. 2 -treated sugarcane bagasse	9.1^{e}	5.44^{ab}	0.7^{a}	22.63^{a}	55.08^{d}	300.86^{d}
Fusarium sp.1 -treated sugarcane bagasse	9.0^{cde}	5.85^{ab}	0.7^{a}	43.47^{d}	54.89^{e}	298.48^{de}
P. citrinum-treated sugarcane bagasse	9.1^{de}	5.76^{a}	1.2^{b}	35.16^{b}	54.12^{e}	299.51^{de}
F. semitectum -treated sugarcane bagasse	8.8^{cd}	6.15^{b}	1.2^{b}	34.37^{b}	54.82^{e}	299.25^{de}
A. ochraceus -treated sugarcane bagasse	8.7^{c}	6.13^{b}	1.3^{b}	34.05^{b}	54.78^{e}	300.82^{d}
A. flavus-treated sugarcane bagasse	8.4^{b}	5.55^{ab}	0.5^{a}	36.73^{b}	55.02^{d}	304.50^{c}

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

Cytotoxicity of the single cell protein enriched sugarcane bagasse

Cytotoxicity of protein -enriched sugarcane bagasse revealed their non-cytotoxic effect at all incubation periods.

Table 2 revealed that there is 0% mortality rate among the treatments at 6 hrs incubation excluding *Penicillium citrinum* with the highest percentage mortality of 6.67% followed by *Clasdosporium cladosporioides*-treated sugarcane bagasse and *Fusarium semitectum* -treated sugarcane bagasse both had 3.33%. At 12 hrs of incubation, *Penicillium citrinum*-treated sugarcane bagasse had the highest percentage mortality of 6.67% followed by *Monascus ruber*-treated sugarcane bagasse, *Clasdosporium cladosporioides*-treated sugarcane bagasse, *Fusarium* sp.1 -treated sugarcane bagasse and *Fusarium semitectum* -treated sugarcane bagasse had the same mortality rate of 3.33%. At 18 hrs of incubation, there is 0% mortality rate among all the treatments except for *Fusarium semitectum* -treated sugarcane bagasse which obtained a percentage mortality of 3.70%. At 24 hrs of incubation, *Penicillium citrinum*-treated sugarcane bagasse had the highest percentage mortality of 4.76% followed by *Monascus ruber* -treated sugarcane bagasse, *Fusarium semitectum* -treated sugarcane bagasse and *Aspergillus flavus*-treated sugarcane bagasse with 4.16%. Statistical analysis revealed no significant difference among the treatment means, hence, signifies the non cytotoxicity of the fungal enriched sugarcane bagasse.

Table 2. Mean percentage of brine shrimp mortality

Treatment	6hrs	12hrs	18hrs	24hrs
Yeast	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
A. niger -treated sugarcane bagasse	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
M. ruber -treated sugarcane bagasse	0.00^{a}	3.33^{a}	0.00^{a}	4.16^{a}
C. cladosporioides-treated sugarcane bagasse	3.33^{ab}	3.33^{a}	0.00^{a}	0.00^{a}
Fusarium sp. 2 -treated sugarcane bagasse	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Fusarium sp.1 -treated sugarcane bagasse	0.00^{a}	3.33^{a}	0.00^{a}	0.00^{a}
P. citrinum-treated sugarcane bagasse	6.67^{b}	6.67^{a}	0.00^{a}	4.76^{a}
F. semitectum -treated sugarcane bagasse	3.33^{ab}	3.33^{a}	3.70^{a}	4.16^{a}
A. ochraceus -treated sugarcane bagasse	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
A. flavus-treated sugarcane bagasse	0.00^{a}	0.00^{a}	0.00^{a}	4.16^{a}

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

CONCLUSION

Inoculation of nine endophytic fungi into sugarcane bagasse had resulted to varying influence of the endophytic fungi in the nutritional attributes of the substrate. Moisture content was elevated by all except *M. ruber* and *A. flavus.* In addition, *A. niger, F. semitectum* and *A. ochraceus* enhanced the ash content of the sugarcane bagasse and *P. citrnum, F. semitectum* and *A. ochraceus* increased the crude fat. Similarly, *A. niger, M. ruber* and *Fusarium* sp 1 elevated the crude fiber of the sugarcane bagasse. Meanwhile, reduction in total carbohydrates and total energy values were recorded. Thus, the ability of the endophytic fungi in enhancing the nutritional attributes of the substrate

which were also found to have a non- cytotoxic effect. However, further studies most be carried out prior to the utilization of the product as animal supplement.

Acknowledgement
To God Be The Highest Glory! Thy Will Be Done.

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