NIGERIAN AGRICULTURAL JOURNAL
ISSN: 0300-368X
Volume 51 Number 1, April 2020 Pg. 1-4
Available online at: http://www.ajol.info/index.php/naj

EFFICACY OF Aspergillus niger ISOLATED FROM TWO SOURCES IN THE BIODEGRADATION OF MAIZE CROP RESIDUES

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Abstract
Fungi generally grow on carbon-rich organic substrates; a few others including *Aspergillus niger* are more ubiquitous, as they are often found in usual environments such as damp walls. The present study assessed the performances of *Aspergillus niger* isolates obtained from two different environments, namely a dry mushroom conk (A. niger MC) and maize grains (A. niger MG), on the efficacy of biodegradation of maize crop residues. Treatments consist of the inoculation of 10g each of maize stover, maize husk or maize cob in a replicated randomized complete block design with suspensions of both isolates in a solid state fermentation study. The proximate composition, acid detergent fiber (ADF) and digestible dry matter (DDM) were investigated. Maize stover had the best proximate composition with 3.7% protein, 4.3 % ash and 92.9 % dry matter while cobs had the poorest with 1.9 %, 1.3 % and 87.2% respectively. There were significant different ($p<0.05$) levels of feed improvement following fermentation with both isolates. *A. niger* MC fermentation of stover gave the highest reduction in ADF from 52.2 % to 30.7%, while the least was observed with husk from 40.7% to 36.0 %. The highest increase in DDM was observed in stover which increased from 48.3% to 52.3% and 64.8 % following *A. niger* MG and *A. niger* MC fermentations respectively. Results show that *A. niger* mediated fermentation improved the digestibility parameters of the maize crop residues and *Aspergillus niger* isolated from dry mushroom was more effective compared to maize isolate.

Keywords: Aspergillus niger, Crop residues, Maize grains, Mushroom, and Solid state fermentation

Introduction

The vast volume of crop residues being generated through agricultural farming activities is grossly underutilized (Balarabe and Ibuowo, 2019). This is evident in developing countries like Nigeria where agriculture remains the primary occupation of the citizens. It is estimated that the total quantity of crop residues produced globally is 2.8×10^3Tg/year for cereal crops and 3.8×10^3 Tg/year for 27 of the most common food crops (Lal, 2005). More recently, the total quantity of cereal straws generated in 2010 was estimated to be about 82mt for all West African countries (FAO, 2014). The fuel value of the total annual residue produced is estimated at 1.13×10^{15} kcal (about 7.5 billion barrels) of diesel globally.

Farmers in resource-poor African and Asian countries would however, for social and economic reasons, continue to rely on the use of crop residues and agro-industrial by-products for livestock production. Agricultural by-products have high levels of the three major polymers in the plant cell wall vis-à-vis; cellulose, hemicelluloses and lignin that limit both intake and digestibility in feeds in both ruminant and non-ruminant animals (Ugwuanyi, 2016; Tagliapetra *et al.,* 2013 cited in Maggiolino *et al.,* 2019). Cellulose is metabolized by rumen microorganisms but not metabolized by non-ruminants. The lignin is a complex polymer which cements cellulose and hemicellulose to provide rigidity to plants, thus it is neither fermentable nor digestible in animal stomachs (Ugwuanyi, 2016).

Biological methods include: application of exogenous enzymes and fermentation processes. Cellulase and xylanase complexes in the diet of both ruminants and non-ruminants have aided the mobilization of energy resources from feed (Kiarie *et al.,* 2007; Kerr and Shurson, 2013). Several studies have shown that biological processing decreases the acid detergent fibre (ADF) content of crop residues thus improving feed quality through improved digestibility (Vansaun and Herdt, 2013). However, these commercial enzymes are expensive and not readily affordable to farmers in developing countries. Biological treatment of crop residues with microorganisms is a simple and relatively affordable way to improve feeding values. *Aspergillus niger* had been used (Vries and Visser, 2001; Ahmed El-Imam *et al.,* 2019) to produce exogenous enzymes for improvement of fibrous livestock feeds. It has great advantages (Villas, *et al.,* 2002) over the traditional physical methods such as grinding or chemical methods; treatment with caustic soda being cheaper than commercial enzymes.

Solid state fermentation which occurs in the absence of free-flowing water is cheaper to operate, with low energy demand and less complex machinery and high products yield when compared with submerged fermentation process (Pandey, 2002). The potential for adoption of solid state fermentation technology in the biological treatment of crop residues would be enhanced by the ease of its adaptability to the prevailing socio-economic environment of the rural livestock farmers. The present study examines the efficacy of isolates of *Aspergillus niger* obtained from two sources, maize grains and a non conventional source-dried conk of a polyporous mushroom, in degrading maize stover, maize husk or maize cob by measuring the changes in the ADF component and estimating the changes in the digestible dry matter.

Materials and methods

Site and Conditions of the Study

The study was conducted at the Department of Microbiology and the University Central Research Laboratory, University of Ilorin, Ilorin Nigeria. Maize crop residues were sundried, milled and preserved in clean, air-tight plastic containers. Potato Dextrose Agar (Hi-Media, India) was prepared following the manufacturer's guidelines. A basal medium was prepared from $NaNO_3$ (2g), K_2HPO_4 (1g), $MgSO_4.7H_2O$ (0.3g), KCl (0.3g) and $FeSO_4.7H_2O$ (0.01g) made up to one liter with distilled water following Ahmed El-Imam *et al.,* (2019). This medium was used to wet the substrates.

Preparation of Spore Suspension

Two isolates of *Aspergillus niger* were used in this study. One isolate, *A. niger* MC was isolated from a dried-out conk of a polyporebasidiomycetes fungus following Ahmed El-Imam *et al.,* (2019) while the other, *A. niger* MG was from maize grains that were surface-sterilized in 0.4 % sodium hypochlorite. Seven-day old slants of each isolate were washed with sterile 0.1% Tween-80 solution and a sterile swab-stick to collect spores into sterile bottles. This suspension was appropriately diluted and then counted using a Neubauer haemocytometer.

Proximate Analysis

Proximate composition and Acid Detergent Fiber analyses were carried out following the AOAC (1990) and Goering and van Soest (1970) methods respectively. Digestible dry matter was estimated following Van Soest, (2003).

Biodegradation of Maize Crop Residues

Ten gram (10g) samples of maize stover, maize husk and maize cob each were separately moistened with basal medium to moisture contents of 79.8%,78.4% and 74.6% respectively and autoclaved at 121°C and 15psi for 15minutes following Sulaiman *et al.,* (2014) and Ahmed El-Imam *et al.,* (2019). Moisture lost due to evaporation was replaced with sterile distilled water. The samples were then inoculated to a final concentration of 1×10^6spores of *Aspergillus niger* per gram dry weight and incubated at 25°C for 21days. The bottles were gently agitated manually daily, with lids slightly loosened to ensure gaseous exchange. Lost moisture was replaced daily by addition of water to ensure stability in the moisture content. The treatments were terminated by sterilizing at 12°C for 20minutes.

Statistical Analyses

Data were analyzed by the GLM procedure (SAS, 1998) for a Randomized Complete Block Design (RCBD) experiment, and treatment means separated at $p<0.05$ by LSD.

Results and Discussion

Data on the proximate composition of the maize crop residues used in the present study are shown in Table 1.

Table 1: Chemical Composition of Maize Crop Residues

Residue	DM	Crude Fiber	Crude Protein	Ether Extract	Ash
Maize Stover	92.91	42.41	3.71	0.61	4.30
Maize Husk	94.30	31.23	3.50	0.86	3.31
Maize Cob	87.20	20.21	1.90	0.31	1.30

The highest content of crude fiber was recorded for maize stover while maize cob had the lowest value. The 42.4% observed was higher than 25.1 % and 32.3 % previously reported in corn stover by Islamiyati *et al.,* (2015) and Amuda *et al.,* (2017). All the three maize crop residues were nutritionally deficient as indicated by the low crude protein and ether extract contents. The relatively high crude fiber, low crude protein and low ether extracts contents suggest that these residues cannot adequately support livestock production as sole diets. A threshold value of 7-8% crude protein would be required in the diets of ruminants for sustained activities of the rumen microbial population.

High dietary crude fiber as indicated in Table 1 would limit both feed intake and digestibility which are the two major problems militating against extensive use of crop residues for ruminant livestock production. Significant reduction in crude fiber contents of barley straw was reported (Hassan *et al.*, 2012) following fungal treatments. The extent of reduction in the indigestible fraction of crude fiber in maize stover, maize husk and maize cob was assessed through ADF and digestible dry matter measurements (DDM) (Table 2).

Table 2: Acid Detergent Fiber and Digestible Dry Matter Estimates (%) in Maize Crop Residues

A. niger Source/ Residue	Maize Stover	Maize Husk	Maize Cob
Acid Detergent Fiber			
Unfermented residue	52.17[a]	40.67[a]	37.33[a]
***A. niger* MG** Fermented residue	47.00[b]	37.67[b]	35.17[a]
***A. niger* MC** Fermented residue	30.67[c]	36.00[b]	29.33[b]
LSD ($p<0.05$)	3.06	2.18	2.74
Digestible Dry Matter			
Unfermented residue	48.27[c]	56.95[b]	60.19[b]
***A. niger* MG** Fermented residue	52.29[b]	59.56[a]	61.51[b]
***A. niger* MC** Fermented residue	64.81[a]	60.86[a]	66.05[a]
LSD ($p<0.05$)	2.67	2.15	2.93

Values are means(n=3); means along the column with different superscripts differ significantly ($p<0.05$)

The ADF represents the sum of cellulose and the indigestible lignin fraction of the feed and its level could predict feed digestibility (Vansaun and Herdt, 2013). Treatments with both *A. niger* isolates resulted in a significant decrease ($p<0.05$) in ADF level of all residues with a consequent increase ($p<0.05$) in the digestible dry matter (DDM) component, except in cobs where the decrease in ADF by the *A. niger* MG isolate was not significant. Similarly, the *A. niger* MC isolate had greater ($p<0.05$) effect on ADF levels in maize stover (30.7% vs. 47.0 %) and maize cob (29.3 % vs. 35.2 %) when compared with *A. niger* MG, while the decrease in maize husk was not significantly different. Biological treatment of the residues with both *A. niger* isolates resulted in significant decreases in ADF on stover and cob while the effect on maize husk was not significant. The 21.5 % reduction in ADF in stover by *A. niger* MC was considerably higher than about 5.2% and 3.7 % reduction, and a 3.4 % increase as reported by Yakin *et al*., (2017), Güngör *et al.,* (2019) and Altop *et al.,* (2019) following *A. niger* solid state fermentation of nettle, cottonseed meal and cocoa pod respectively. These two maize crop residues could thus be identified as suitable candidates for fungal fermentation for high-value animal feed production. The effects of *Aspergillus niger* treatments on estimated digestible dry matter of the three maize crop residues were significant ($p<0.05$). Digestible dry matter increased with a reduction in the indigestible feed components. *A. niger* MC isolate however significantly increased the DDM better than *A. niger* MG except in the husk where the increase was not significant. Similarly only *A. niger* MC significantly increased DDM in cob. The findings of this study further confirms the numerous reports of the ability of *A. niger* and other fungi to improve the proximate composition, decrease ADF in potential animal feed and improve the DDM content and overall nutritional profile (Belewu and Adeniyi, 2001; Sulaiman *et al.,* 2014; Yakin *et al.,* 2017; Ahmed El-Imam *et al.*, 2019). It also shows that the specific fungal isolate used also determines the degree of success that will be achieved.

Conclusion

There study shows a significant effect of the source of a fungus employed in a bio-modification such as in reducing ADF levels. The *Aspergillus niger* isolate recovered from a dried out mushroom conk was found to be superior to maize isolate in the reduction of the acid detergent fibre and improvement in DDM content of maize stover, husk and cob. The maize isolate also improved the ADF and DDM in all but the cob, although at a lower level than the conk isolate. This study thus shows that *A. niger* fermentation improves the digestibility of maize residues and should be a source of an isolate and also determines the extent of its effect on digestibility parameters.

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