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RESEARCH PAPER

Solid state fermentation of *Jatropha curcas* kernel cake with cocktail of fungi

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Abstract

The efficacy of four different fungi (*Aspergillus niger, Penicillium sp., Trichoderma harzanium and Trichoderma longibrachiatum*) in different combinations on the biodegradation of Jatropha curcas kernel cake was evaluated in a completely randomized design model. The inoculated substrates were incubated for 10 days at ambient temperature. The treatments include A (Control without fungi inoculation), B (50% *Trichoderma harzanium + 50% penicillium specie), C (33% Trichoderma harzanium* + 33% *Trichoderma longibrachiatum + 33% Aspergillus niger)* while Treatment D (*50% Trichoderma harzanium + 50% Aspergillus niger*). The results revealed highest protein content in treatment A followed closely by treatments B, C and D in that order. Contrarily, the ether extract content was significantly higher in Treatment C and poorest in treatment A. The ash content was 7.00% (A), 5.30 % (B), 7.7% (C) and 7.10% (D). The highest anti-nutrient contents were recorded for the Control treatment (A). The cyanide content was lowest in Treatment D while treatment C had the lowest saponin content. The incubation of the substrate

with *Trichoderma harzanium* and *Aspergillus niger* markedly reduced the tannin and phytate contents. It could be concluded that solid state fermentation of *Jatropha curcas kernel* cake with cocktail of fungi is a promising method of reducing the crude fibre content and the anti-nutrient factors of the cake thereby making available renewable feedstuff for livestock animals

Key words: *Jatropha curcas* kernel cake, cocktail of fungi, physico-chemical properties, anti-nutritional factors, biodegradation.

Introduction

The world population is increasing and there is an urgent need to increase animal production in order to meet the increasing demand of animal protein. However, the consumption of conventional feedstuffs like soybean, maize sorghum etc by human beings is undermining their availability to animals. Hence, the world is becoming increasingly aware of the looming food scarcity, the possibility of raising animal on unconventional but easily sourced and available feedstuffs in the tropics and subtropics deserves more attention (Belewu *et al*., 2009).

In Nigeria, the unbearable scarcity of animal feed therefore motivated nutritionists to find for alternative source of protein for livestock animals from waste agricultural residues and lesser known seed like *Jatropha curcas* seed. The Jatropha specie is an underutilized, small shrub or tree which does not require much care and with a lifespan of between 30 and 50 years. The plant is a goldmine hence it deserves more attention for the merits it possesses. The seed from the plant can be used as seed cake after extracting the oil either mechanically or chemically. The cake was found to contain a crude protein content of between 57 and 64% with 90% true protein. With the exception of lysine, the amino acid is higher than FAO preference protein required for animal wellbeing and growth. However, *Jatropha curcas* contains some toxins and anti-nutrients (Cyanide, saponin, tannin, phytate, etc). Various methods (physical, mechanical and chemical) of detoxification are well documented in literature (Aderibigbe *et al.,* 1997 Makkar and Becker, 1999). However, the

biological method (Cocktail of fungi) of detoxification is still at its infancy except for the reports of some workers (Peace and Aladesanmi, 2008, Belewu *et al* 2010[a,b c]) who used different fungI for the fermentation of Jatropha cake. These authors noted increased crude protein content and reduction of the fibre content of the inoculated samples while some anti-nutritional factors were removed / reduced in the inoculated samples.

Fermentation has always been an important part of our lives and it has some benefits exclusive to food. It can produce vital nutrients or eliminate antinutrients. Fermentation uses up food energy and can make conditions unsuitable for undesirable microbes. Belewu et al. (2010) reported on the effect of fungi treated Jatropha curcas kernel cake with encouraging results. Belewu and Sam (2010) noted that *Aspergillus niger* inoculated *Jatropha curcas* kernel cake gave a crude protein content of 65.75% which was similar to 63.06% found in *Trichoderma longibrachiatum* treated *Jatropha curcas* kernel cake. Belewu *et al*. (2010) opined that goat fed diet containing 50% Soybean meal plus 50% *Rhizopus oligosporus* treated *Jatropha curcas* kernel cake under confinement consumed adequate dry matter and other nutrients. Hence, the thrust of this study was to evaluate the efficacy of cocktail of fungi (*Trichoderma harzanium, Penicillum spp, Trichoderma longibrachiatum, Aspergillus niger)* on the biodegradation of *Jatropha curcas* kernel cake.

Materials and methods

Collection of Jatropha curcas seeds

The *Jatropha curcas* seed used for this study were harvested from *Jatropha* plantation, University of Ilorin, Nigeria.

Preparation of Jatropha curcas kernel cake

The seeds were dehulled in order to obtain the kernel while the extraneous materials were removed. The kernels were milled and defatted using both the mechanical and solvent extraction methods (cool extraction using petroleum ether: 40-60^{oc} for 30 minutes).

The petroleum ether was decanted and the remaining cake was strained through muslin cloth. The cake was later autoclaved at 121^{oc} for 30 minutes so as to get rid of any microbes.

Sources of Inoculum

The fungi used (*Trichoderma harzanium, Trichoderma longibrachiatum, Penicillium spp and Aspergillus niger)* were obtained from Institute of Agricultural Research and Training, Oyo State , Nigeria in McCartney bottle.

Preparation / Sub- culturing of the Fungi

The fungi were sub-cultured on potato dextrose agar (PDA) by transferring the mycelium aseptically from the cultures to freshly prepared PDA containing in Petri-dishes. The PDA was amended with Streptomycin to suppress any bacterial growth and later autoclaved to sterilize it. The petri-dishes were incubated at ambient temperature for some days to stimulate the growth of the fungi.

Inoculation of Jatropha curcas kernel cake

There were three different treatments based on the various species of fungi used and a control treatment which was not inoculated but received same sterilization as others. The inoculated Treatments were inoculated with 50% *Trichoderma harzanium and 50% Penicillum spp (treatment B), 33.3% Trichoderma longibrachiatum* plus 33.3% *Trichoderma harzanium* plus 33.3% *Aspergillus niger* (treatment C) , 50% *Trichoderma harzanium* plus 50% *Aspergillus niger* (treatment D) All the Treatments were inoculated at ambient temperature in the Laboratory. The substrates were enveloped with the various fungi in about 10 days. The fungal growth was terminated by oven drying the spent substrates in an air draught oven at 70^{OC} for 24 hours.

Chemical analysis

The proximate composition and the Cyanide content of the Control and the Experimental treatments were determined by using the methods of A.O.A.C (1990). Saponin content was evaluated by the method of Edeoga *et al*. (2005). Tannin was determined by the method of Josyln (1970) while phytate was determined using the method Wheeler and Ferrel (1971).

Statistical analysis

All collected data were subjected to analysis of variance (ANOVA) of a completely randomized design model (Steel and Torrie, 1980) while treatment means were separated using Duncan (1955) Multiple Range Test.

Results and discussion

The proximate composition of the fungi fermented and unfermented *Jatropha curcas* kernel cake was presented in table 1. The dry matter content which ranged between 79.20 and 85.20% was not significantly different ($p>0.05$) among the treated and untreated samples. The highest crude protein content was noticed in the control sample (A) followed closely by B while samples C and D are similar but significantly different from other samples. The crude fibre content followed similar trend as the crude protein content. The least crude fibre recorded for samples C and D could be due to the ability of the cocktail of the fungi in degrading the fibre content. It is presume that both solubilization and degradation of the cake could have been done by the cocktail of the fungi. This shows that the fungi could have worked synergistically due to various enzymes secreted (catalase, cellulose, Dextrose, Glucose oxidase, Beta glucanase , Hemicellulase, xylanase, amylase, invartase, protease, lactase, etc) that degrade complex polysaccharides (Felse and Panda 1999). The lower crude fibre content reported here is supported by other results (Belewu and Belewu and Belewu, 2005. Belewu et *al*., 2006, Belewu and Sam, 2010) .

The ash content did not follow any particular trend; however samples C and D were numerically higher than the control sample (A). The slightly higher content agreed with the results of Belewu (2003) who used corn cob and sawdust treated with *Pleurotus pulmonarium.*

An interesting consequence of the cocktail of fungi used was the appreciable reduction in the antinutrient contents of samples B to D (Table 2). The lowest cyanide, tannin and phytate contents were noted in sample D (*Tricoderma harzanium* plus *Aspergillus niger*). Another point of note in this study was the least saponin content found in Sample B (*Trichoderma harzanium plus Penicillium spp*).

Table 1. Proximate composition of Fermented and Unfermented *Jatropha curcas* Kernel cake (%).

Treatments	Dry matter	Crude protein	Crude fibre	Ether extract	Ash
A	85.20	45.94[a]	29.6[a]	29.20[b]	7.00[ab]
B	80.70	40.69[b]	24.90[a]	32.30[ab]	5.30[b]
C	79.20	36.46[c]	9.10[b]	36.30[a]	7.71[a]
D	82.50	36.75[c]	16.00[b]	35.30[ab]	7.10[ab]
±SEM	11.61NS	0.92*	5.83*	2.98*	0.54*

Means along the same column with similar superscripts are not significantly different from each other (p>0.05)

Table 2. Some antinutrient contents of Fermented and Unfermented *Jatropha curcas* kernel cake.

Treatments	Cyanide (mg/kg)	Saponin (%)	Tannin (%)	Phytate(mg/kg)
A	208.00[a]	3.40[a]	0.48[a]	0.376[ab]
B	188.00[a]	1.90[b]	0.32[b]	0.316[b]
C	125.00[a]	1.70[b]	0.28[b]	0.188[b]
D	104.00[b]	2.20[b]	0.22[bc]	0.157[b]
±SEM	41.70*	0.25*	0.03*	0.074*

Means along the same column with similar superscripts are not significantly different from each other (p>0.05)

It could be concluded from this study that incubation of *Jatropha curcas* kernel cake with cocktail of fungi is promising as it reduced the anti-nutrient contents of the cake significantly so as to make a renewable feedstuff for livestock animals.

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