

Toxicity and Antimetabolic Properties of Biologically Treated Neem Seed (*Azadiracta indica*) Kernel Cake

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Abstract Seeds from Neem plants were ground and defatted to obtain the meal, the neem seed cake (NSC). The seed cake was fermented with two different fungi *Aspergillus niger* and *Mucor mucedo*. Proximate compositions as well as antinutrient factor of the treated cakes were analyzed. The results obtained reveals that the defatted Neem seed cake treated with *A. niger* contains protein of 47.70% which is higher than the seed cake treated with *M. mucedo* and the untreated sample. The untreated sample which is the control contains more dry matter and crude fiber of 80.67% and 0.51% respectively. It was also observed that the sample treated with *A. niger* contains more phytate and tannin of 7.95% and 1.30% respectively. Though the treatment has increased the crude protein content of Neem seed cake, but the biological treatment may not be suitable for the detoxification of neem seed cake as the antinutrient factors (oxalate, phytate, tannin and cyanide) determined were significantly higher in the treated than the untreated seed cake.

Keywords Neem Seed Cake, *Aspergillus Niger*, *Mucor Mucedo*, Antinutrients

1. Introduction

Several attempts have been made to find solutions to nutritional problems in Africa in relation to protein intake. Interest in newer sources of protein has grown due to protein shortage in developing countries, especially Nigeria. As part of the quest for newer sources, some lesser-known oil seeds have been evaluated for their nutritional qualities in India[1-5]. In Nigeria, locally available but unusual proteins containing foodstuffs have been evaluated for their nutritional potentials, aiming at reducing dependence and competition between livestock and man for the consumable sources[6-8].

Neem (*Azadirachta indica*), is an evergreen tree which is endemic to the Indian subcontinent and has been introduced to many other areas in the tropics[9]. The tree is known to cure more than one hundred diseases. Neem seed cake (NSC) is a by-product showing great potential for livestock feeding[10,11], NSC meal is a protein rich source (CP: 34-38%), the protein in the cake is relatively balanced in its amino acid and mineral profiles[12,13].

Despite the high nutritional value of NSC especially for livestock, its bitter taste and foul odour limits its use as a food source,[14]. The bitter taste of neem seed is as a result

of the presence of sulphur containing compounds, while the tignic acid is the main compound believed to be responsible for its odour,[15,16]. Consequently, the cake has been treated by various methods, including water washing[11], urea treatment[10] and solvent treatment[17-19]. Similarly, Regional Research Laboratory, Hyderabad in India has developed a simple extraction process of removing toxic compounds. When Udayashekara employed this process on neem seed, the seed was devoid of the bitter taste and its usual foul smell while retaining its nutritional value[14]. In another work Usman et al has attempted the extraction of protein from the neem seed[20]. This work thus aims at studying the effects of biological treatment (*Aspergillus niger* and *Mucor mucedo*) to inactivate the antinutritional factors in Neem Seed Cake.

2. Experimental

2.1. Preparation of Neem Seed Cake

The seeds were obtained from ripe fruits harvested from different locations in Ilorin, Nigeria. The exocarp and pulp of the riped fruit was removed and the endocarp was oven dried at 60°C. The dried endocarp was cracked to obtain the seed. The seeds were milled using magic blender (SHB – 515 model made by Sorex Company Limited, Seoul Japan) to obtain the seed cake. Standard Official and Tentative Method of Association of Official Analytical

Chemists procedure was used to defat the seed cake[21].

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The defatted seed cake was dried and kept for analysis. The cake was autoclaved using an autoclaving machine set at a temperature of 121°C for 30 minutes in the laboratory of the Department of Biochemistry University of Ilorin, Ilorin.

2.2. Preparation and sub-culturing of the Fungi

The fungi (*Mucor mucedo* and *Aspergillus niger*) were obtained from the laboratory of the Department of Microbiology, University of Ilorin, Ilorin.

A paste of Potato Dextrose Agar (PDA) was prepared by weighing 10 g of PDA in 250 ml of distilled water; it was then autoclaved (Autoclave machine model YSQ-LS-/00SII) at 121°C at 15 lbs per square inch for 15 minutes. It was allowed to cool to about 45°C and streptomycin injection was added. The mixture was then poured into a sterile plate and allowed to set (solidify), after setting, the required organism was inoculated into the plate and incubated at room temperature for 72 hours. A pointed needle was used to pick the organism from the stock and stab the center of the plate.

2.3. Inoculation and Incubation of Neem Seed cake

The autoclaved cake was divided into three portions, a portion was inoculated with *Aspergillus niger* (treatment A) another *Mucor mucedo* (treatment B) while the third was left untreated (Control experiment). All the treatments were inoculated at ambient temperature in the laboratory. The fungal growth was terminated after 10 days by oven drying the spent substrates in an air draught oven at 70°C for 24 hours in the laboratory of the Department of Animal Production, Faculty of Agriculture, University of Ilorin.

2.4. Analyses

The proximate compositions of the fermented samples were analyzed using the method of AOAC[21]. All data were analyzed using ANOVA of a completely randomized design model.

3. Results and Discussions

Neem seed cake can be a source of protein for animal feed if the cake is properly treated to remove the antinutritional principles in the seed. This work attempted the improvement of the nutritional quality of the neem seed cake using biological treatment method. Table 1 present the proximate composition of fungi treated and untreated neem seed cake.

The results presented in Table 1 shows the effect of the biological treatment on the dry matter, ether extract, crude fibre, ash content and crude protein contents of neem seed cake. The results clearly indicate that the treatment reduces the dry matter, crude fibre and ash content of the neem seed cake, while the ether extract was drastically increased. There was on the other hand an increase in the crude protein of the *A. niger* but a decrease in the *M. mucedo* treated cakes, Table 1. These observations are similar to those of

Belewu & Sam and Belewu M. A. who also treated *Jatropha curcas* kernel for nutritional improvement biologically[22-23].

Table 1. Proximate Composition of Fungi Treated and Untreated Neem Seed Cake

| Parameters | Control | <i>Aspergillus niger</i> treated sample | <i>Mucor mucedo</i> treated sample | ±SEM |
|---------------|---------|---|------------------------------------|-------|
| Dry matter | 80.67 | 40.00 | 47.00 | 37.39 |
| Ether extract | 3.50 | 52.50 | 26.50 | 9.04 |
| Crude fibre | 0.51 | 0.11 | 0.14 | 0.055 |
| Ash content | 14.00 | 8.00 | 10.50 | 12.56 |
| Crude protein | 41.30 | 47.70 | 24.10 | 40.22 |

Key: + means of three rdeterminations; SEM = Standard error of the mean

Table 2. Antinutritional factors of fungi treated and untreated Neem seed cake*

| Parameters | Control | <i>Aspergillus niger</i> treated sample | <i>Mucor mucedo</i> treated sample | ±SEM |
|------------|---------|---|------------------------------------|-------|
| Oxalate | 0.54 | 1.08 | 1.62 | 3.635 |
| Phytate | 6.41 | 7.95 | 6.92 | 3.636 |
| Tannin | 1.10 | 1.30 | 1.20 | 3.635 |
| Cyanide | 0.675 | 0.675 | 0.675 | 3.635 |

Key: + means of three determinations; SEM = Standard error of the mean

Table 2 presents the antinutrient factors in the treated and untreated neem seed cake. The oxalate, phytate, tannin and cyanide were determined. From the results obtained it is clearly shown that the biological treatment may not be suitable for the detoxification of neem seed cake as the factors determined were significantly higher in the treated than the untreated seed cake, Table 2.

4. Conclusions

Various methods has been described for the treatment of neem seed cake so that it could be useful in animal feeds, this attempt at using biological treatment for the detoxification of neem seed cake was not successful as the antinutritional factors in the treated cakes were significantly higher than the untreated cake. Thus it can be concluded that *A. niger* and *M. mucedo* may not be suitable for the treatment of neem seed, however treatment with other types of fungi may give positive results or other treatments can be improved upon.

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