

Research Article

Detoxification Efficiency of Ethanolic Leaf Extracts of *Moringa oleifera* and *Vernonia amygdalina* on Aflatoxin B₁ in Rice and Maize

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Abstract - The prevalence of aflatoxin B₁ in stored agricultural produce and their subsequent effect on food safety has stepped up researches to identify novel phytoremediation techniques. This research evaluates the potency of phytochemical remediation on aflatoxin B₁ (AFB₁) in stored rice and maize using ethanolic leaf extracts of *Moringa oleifera* and *Vernonia amygdalina*. HPLC-UV/Vis spectroscopy was employed in the determination of the detoxification levels of the AFB₁ in the samples. Phytochemical constituents in the plant extracts were quantified using standard methods. Phytochemical concentrations ranged from 27.83 ± 1.53 – 184.73 ± 0.06 mg/g and 23.00 ± 1.00 – 171.67 ± 3.06 mg/g in the *Moringa oleifera* and *Vernonia amygdalina* extracts respectively. The levels of phytochemicals in *Moringa oleifera* extract were statistically ($p \leq 0.05$) higher than in *Vernonia amygdalina* extract. AFB₁ was present in all the samples with residual concentrations of 89.77 ± 10.56 and 94.83 ± 2.63 µg/kg in the stored rice and maize samples respectively. Detoxification using 1 – 5 mg/cm³ of *Vernonia amygdalina* leaf extract ranged from 25.25 (67.10 ± 2.18 µg/kg) to 75.33 % (22.15 ± 1.41 µg/kg) in rice and 29.71 (66.66 ± 12.39 µg/kg) to 69.69 % (28.74 ± 0.67 µg/kg) in maize while, detoxification using 1 – 5 mg/cm³ of *Moringa oleifera* leaf extract were ranged from 23.19 (68.95 ± 12.60 µg/kg) to 84.23 % (14.16 ± 2.03 µg/kg) in rice and 70.99 (27.51 ± 8.76 µg/kg) to 80.21 % (18.77 ± 2.57 µg/kg) in maize respectively. Detoxification efficiency of the leaf extracts of *Vernonia amygdalina* and *Moringa oleifera* on AFB₁ increased with increasing concentrations of the extracts. The efficiency of detoxification was in the order *Moringa oleifera* > *Vernonia amygdalina*.

Keywords – Mycotoxin, Phytochemicals, Aflatoxin B₁ (AFB₁)

1. Introduction

Mycotoxins are toxic micromolecules and secondary metabolite compounds that are naturally produced in the hyphae structures of certain moulds (filamentous fungi) during growth [1], [2]. Production of mycotoxin by fungi occurs when they grow as parasites or as saprophytes on a host plant metabolizing many organic substances such as proteins, lipids, soluble and insoluble sugars and others [3], [4], [5], followed by a secondary growth which leads to sporulation and production of secondary metabolites (mycotoxins).

According to WHO (2018), over 500 mycotoxins have been identified. Most researches and international regulations have however focused on the group of the most toxic and prevalent mycotoxins which include aflatoxins (AFs), fumonisins (FUMs), patulin (PAT), ochratoxin A (OTA), zearalenone (ZEN) and the trichothecenes group (deoxynivalenol (DON), nivalenol (NIV), HT-2 and T-2 toxins). The discovery of aflatoxin stemmed from the sudden death of over 100,000

young turkeys of 1960 in an English poultry farm. This caught the attention of scientists and resulted into a scientific research that attributed these turkey deaths to feeds contaminated with a toxin (mycotoxin) produced by the fungus *Aspergillus flavus*, hence, the name “aflatoxin”.

Aflatoxin is the name ascribed to the most virulent collection of specialized metabolites produced by *Aspergillus spp.* There are presently over 20 types of aflatoxins but, the major ones are classified as the B-series (aflatoxins B₁ and B₂), M-series (aflatoxin M₁ and M₂) and the G-series (aflatoxins G₁ and G₂) [6]. They are structurally related toxic, teratogenic, mutagenic, immunotoxic, and carcinogenic mycotoxins that contaminate vast amount of food and agricultural produce, affecting the health of human, animals and the environment. Aflatoxin has been reported in food produce like cereals (maize, rice, wheat and sorghum), nuts (almond, coconut, pistachio and walnut), oil plants (cotton seed, peanut, soybean and sunflower) and spices (ginger, turmeric and peppers) [7]. Contamination of food produce with aflatoxins occurs in the

field before harvest and concentration is observed to increase during storage after harvest.

Since the discovery of aflatoxin B₁ and its toxic effects even at minute concentrations, it was classified as a group 1 carcinogen by the International Agency for Research on Cancer (IARC) [8]. Some of the toxic effects of aflatoxin B₁ include its effects on organs like the liver and kidneys [9], [10]. It is also reported to suppress humans' immune systems, rendering them vulnerable to infectious diseases like HIV and AIDS [11]. This has made the determination of the toxins a necessity in food safety.

For over six decades, a worldwide increase in the prevalence of aflatoxin B₁ infections has been observed in produce like maize, rice, sorghum, beans and their products, as well as by-products. These produce are consumed daily in significant quantities in different localities and are the most sensitive crops for aflatoxin B₁ exposure [12], mainly due to their cultivation in large quantities [13], poor postharvest handling techniques [14], and favourable climatic conditions.

Moringa oleifera and *Vernonia amygdalina* are widely popular, nutrient-rich plants with medicinal properties that have been used traditionally for several purposes including curing wounds and various fungal diseases [15], [16]. Both plant species contain about 110 secondary metabolites (phytochemicals) for defence against insect, pathogens and adverse climatic conditions. These metabolites are promising chemopreventive/detoxifying agents and they include alkaloids, saponins, tannins, steroids, phenolic acids/compounds, phytosterols, carotenoids, tocopherols, aromatic acids, essential oils, chlorophylls, protease inhibitors, organic acids, glucosinolates, flavonoids, and terpenoids. [17], [18], [19], [20], [21].

Phytochemicals confer certain biological activities such as antimicrobial, antiviral, antigenotoxic, antimutagenic, antiproliferative, antitoxic, antifertility, anti-convulsant, anticancer, antitrypanosomal, antileishmanial, antihyperglycemic, antihyperlipidemic, hypocholesterolemic, anti-inflammation, antihypertension, antispasmodic, antioxidant, and most importantly antifungal properties, resulting in cytotoxicity, disruption of plasma membrane permeability and function, inhibition of cytoplasmic, and mitochondrial enzymes which are involved in the synthesis of the components of the cell wall and alters cellular homeostasis and redox balance in fungi [22], [23], [24], [25], [26], [27], [28], [29], [30], [31], [32], [33].

This research aims to evaluate the efficacy of phytochemical remediation on aflatoxin B₁ (AFB₁) in stored rice and maize obtained at Keffi, Nasarawa state, Nigeria.

2. Materials and Methods

2.1 Sample Collection

Samples of shelled maize (800 g) and parboiled/dehusked rice (800 g) were collected from four designate farmers termed F₁, F₂, F₃ and F₄ from four villages within the study area. Each sample (200 g) was collected after 6 months of storage. Samples were combined (to obtain a representative sample) and placed in labelled sterile paper bags before they were transported to the laboratory. Samples were then stored between at 6 °C in a refrigerator until the next day before evaluative processes were carried out.

Plants under consideration (*Moringa oleifera* and *Vernonia amygdalina*) were collected from the wild. Plants collected from the wild are found to contain little or no pesticides [35]. Plants were collected by hand and transported using pyrex glass jars (uncorked).

2.2 Sample Pre - Treatment

The rice sample (800 g) was grounded to a fine powder using a food grade high speed blender. The rice particles were passed through a food grade sieve (particle ≤ 1 mm) and then mixed thoroughly to ensure that the matrix was homogeneous.

Leaves were stripped from stalk/stems, washed under a flow of water and air-dried at room temperature for a few days until they were completely dried. The air-dried samples were grounded to fine particles using a food grade high speed blender. The resulting grounded particles were passed through a food grade sieve (particle ≤ 1 mm) to ensure a high surface area for the extractions.

2.3 Extraction Process for Aflatoxin

The ground and homogenized sample (1.0 g) was weighed into a stoppered 250 cm³ conical flask. The sample was then extracted with 10 cm³ acetonitrile/water/formic acid (80/19.9/0.1, in v/v/v) for 30 minutes on a shaker. MgSO₄ (4 g) and NaCl (1 g) were then added and shaken for 30 seconds in vortex and then centrifuged further for 15 minutes at 5 °C and 4000 rpm. The supernatant was filtered through a developed set up of filter paper and celite. The resulting filtrate was then stored in an amber autosampler vial at below 4 °C for further analysis.

2.4 Phytochemical Extraction

Phytochemical extractions were carried out using cold maceration extraction as described by [34], [35], [36] to obtain a robust extraction. The air-dried and powdered leaf material (250 g) from each plant were soaked separately (in stoppered containers) in hexane/methanol 1:1 mixture (1/4 w/v) for 48 hours at room temperature and vigorously shaking for 5 minutes, five times per day. The mixture was filtered through a whatman No. 1 filter paper and the filtrate was concentrated by evaporation at 45 °C using a rotatory evaporator (Buchi R-200) to obtain the crude extract that was subsequently dried in an oven at 40 °C. The extract was finally stored at 4 °C until further use.

2.5 Quantitative Analysis of Phytochemicals

Phytochemical concentrations were determined using UV/Vis Spectrophotometer and all analysis was carried out in triplicates.

2.6 Phytochemical Treatment of Extract

To 5.0 cm³ of the aflatoxin extract in a test tubes, 1.0 mg/cm³ of *Vernonia amygdalina* leaf extract was added. The resulting mixture was vigorously shaken for 30 seconds in vortex and allowed to remain for 72 hours at ambient temperature. The mixture was subjected to filtration through a developed set up of filter paper and celite (developed as filter paper-celite-filter paper set up). This process was carried out in triplicate and repeated for 2 – 5 mg/cm³ concentrations of the leaf extract. This process was repeated using ethanolic extract of *Moringa oleifera* leaf.

2.7 Clean-up of the Treated Aflatoxin Extract

An aliquot of 4.0 cm³ of the filtrate was diluted to 20.0 cm³ with phosphate-buffered saline (PBS) solution pH 7.4. After conditioning the immunoaffinity column (IAC) with 8.0 cm³ PBS, 20.0 cm³ of the diluted filtrate was passed through it at a flow-rate of 2.0 cm³/min. The column was washed with 50 % methanol (4.0 cm³) and dried by pressing air. In the next step, retained mycotoxin extracts were eluted at a flow rate of 0.5 cm³/min using 1.0 cm³ of methanol and 1.0 cm³ of distilled water and collected in a glass vial. 10.0 cm³ of eluate was used for HPLC analysis.

2.8 HPLC Quantification of Aflatoxin

High performance liquid chromatography analysis was performed on a Biobase machine with reversed phase column that consists of a microprocessor-controlled delivery pump and a fixed wavelength Biobase model UV-Vis spectrophotometer detection system. The mobile phase consisted of water, acetonitrile and methanol (60:20:20, v/v/v) which was filtered, degassed, and pumped at a flow rate of 1 cm³/min. The injection volume was 20 µL and the eluent was monitored using UV detection at 360 nm for aflatoxin B₁ under isocratic condition. Separation was achieved on a 4.6 mm×250 mm C₁₈ reversed phase column with 5 µm ultra-pure silica gel packing material (vertex). All the system was maintained at ambient temperature. Data collection and handling were carried out by Biobase software (N2000 Chromatographic Data System).

2.9 Statistical Analysis

The IBM SPSS statistical software version 27 and MExcel version 2010 were used to process all quantitative data obtained. Results are expressed as mean ± standard deviation (SD).

3. Results and Discussions

3.1 Phytochemical Analysis

Statistical analysis of the plant extracts (Table 1) shows that the highest phytochemical concentration in *Moringa oleifera* extract was total saponins content at 184.73 ± 0.06 mg/g while; the lowest constituent was total cardiac glycoside at 27.83 ± 1.53 mg/g. The highest phytochemical concentration in *Vernonia amygdalina* extract was total phenols/phenolic compounds content at 171.67 ± 3.06 mg/g while, the lowest constituent was total cardiac glycoside at 23.00 ± 1.00 mg/g. The levels of phytochemicals in *Moringa oleifera* extract were statistically ($p \leq 0.05$) higher than in *Vernonia amygdalina* extract.

Table 1: Phytochemical contents of ethanolic extracts for *Moringa oleifera* and *Vernonia amygdalina* leaf

Phytochemicals	Mean concentrations (mg/g) of phytochemicals in ethanolic extracts of plant leaves	
	<i>Moringa oleifera</i>	<i>Vernonia amygdalina</i>
	leaf extract	leaf extract
Flavonoids	116.67 ± 0.76 ^a	82.33 ± 1.25 ^b
Phenols/Phenolic compounds	152.17 ± 1.04 ^b	171.67 ± 3.06 ^a
Total Tannins	49.68 ± 2.84 ^a	47.84 ± 1.53 ^a
Alkaloids	67.67 ± 3.06 ^a	30.67 ± 2.52 ^b
Saponins	184.73 ± 0.06 ^a	58.99 ± 0.10 ^b
Anthraquinone	70.07 ± 1.45 ^a	–
Terpenoids/Triterpenes	66.10 ± 0.23 ^a	37.75 ± 1.39 ^b
Steroids	79.22 ± 0.84 ^a	40.89 ± 0.77 ^b
Glycosides	31.67 ± 0.76 ^a	27.37 ± 0.56 ^b
Cardiac glycosides	27.83 ± 1.53 ^a	23.00 ± 1.00 ^b

Values are mean ± standard deviation, n = 3. Values with the same superscript across the same row are not significantly different ($p \leq 0.05$).

3.2 AflatoxinB₁ in Stored Samples

Aflatoxin B₁ was present in the stored rice and maize samples at residual levels higher than the 20 µg/kg FDA acceptable limit for human consumption and the 2 µg/kg safety levels set by the European Union. Mean residual level of AFB₁ (Table 2) in maize was 94.83 ± 2.63 µg/kg and was observed to be statistically ($p \leq 0.05$) higher than the mean level in rice 89.77 ± 10.59 µg/kg.

Table 2: Residual concentrations (µg/kg) of aflatoxinB₁ in stored rice and maize samples

Samples	Residual concebtration (µg/kg)
Rice	89.77 ± 10.56 ^b
Maize	94.83 ± 2.63 ^a

Values are mean ± standard deviation, n=3. Values with different superscript down the column are significantly different ($p \leq 0.05$).

3.3 Detoxification of aflatoxin

The detoxification of aflatoxin B₁ using *Vernonia amygdalina* leaf extracts (Table 3) show a reduction in residual concentrations of aflatoxin B₁ in rice and maize as concentrations of *Vernonia amygdalina* extract increased from 1 – 5 mg/cm³. The highest detoxification levels obtained were 75.33 % (22.15 ± 1.41 µg/kg) and 69.69 % (28.74 ± 0.67 µg/kg) while the lowest were 25.25 % (67.10 ± 2.18 µg/kg) and 29.71 % (66.66 ± 12.39 µg/kg) for the rice and maize samples respectively.

Table 3: Residual concentrations (µg/kg) and detoxification levels (%) of aflatoxin B₁ in rice and maize samples treated with *Vernonia amygdalina* leaf extract

Extract concentration (mg/cm ³)	Rice	Detoxification	Maize	Detoxification
1.0	67.10 ± 2.18 ^b	25.25	66.66 ± 12.39 ^b	29.71
2.0	61.90 ± 2.98 ^b	31.05	63.32 ± 7.66 ^b	33.23
3.0	27.61 ± 1.08 ^a	69.24	35.64 ± 2.16 ^a	62.42
4.0	25.08 ± 0.98 ^a	72.06	28.87 ± 2.08 ^a	69.56
5.0	22.15 ± 1.41 ^a	75.33	28.74 ± 0.67 ^a	69.69

Values are mean ± standard deviation, n=3. Means with same superscripts down the same column are not significantly different ($p \leq 0.05$).

The efficacy of *Moringa oleifera* leaf extract in the detoxification of aflatoxin (Table 4) also show a reduction in residual concentrations of aflatoxin B₁ in rice and maize as the concentration of *Moringa oleifera* extract increased from 1 – 5 mg/cm³. The highest detoxification levels obtained were 84.23 % (14.16 ± 2.03 µg/kg) and 80.21 % (18.77 ± 2.57 µg/kg) while the lowest were 23.19 % (68.95 ± 12.60 µg/kg) and 70.99 % (27.51 ± 8.76 µg/kg) for the rice and maize samples respectively.

Detoxification levels increased sharply as extract concentration increased from 1 – 2 mg/cm³ in rice and remained statistically ($p \leq 0.05$) high from 2 – 5 mg/cm³ of extract concentration. Detoxification in maize remained statistically ($p \leq 0.05$) the same from 1 – 5 mg/cm³ of the extract concentration.

Table 4: Residual concentrations (µg/kg) and detoxification levels (%) of aflatoxin B₁ in rice and maize samples treated with *Moringa oleifera* extract

Extract concentration (mg/cm ³)	Rice	Detoxification	Maize	Detoxification
1	68.95 ± 12.60 ^b	23.19	27.51 ± 8.76 ^a	70.99
2.0	20.11 ± 4.32 ^a	77.60	20.17 ± 5.04 ^a	78.73
3.0	18.32 ± 3.47 ^a	79.59	19.66 ± 0.83 ^a	79.27
4.0	16.27 ± 1.69 ^a	81.88	18.96 ± 1.51 ^a	80.01
5.0	14.16 ± 2.03 ^a	84.23	18.77 ± 2.57 ^a	80.21

Values are mean ± standard deviation, n=3. Means with the same superscripts down the same column are not significantly different ($p \leq 0.05$).

3.4 Comparative Variations in AFB₁ Detoxification Levels

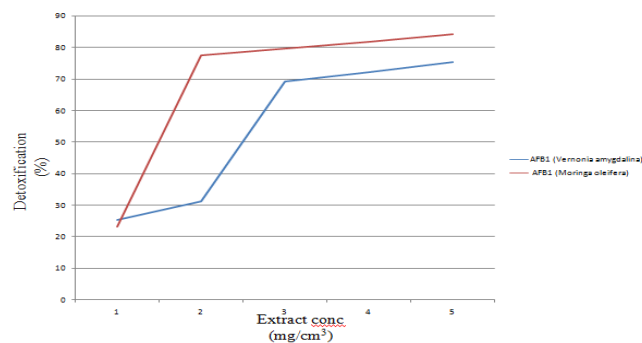


Figure 1: Comparative variations in the detoxification levels of AFB₁ in rice samples

The detoxification results in figure 1 also show an increase in percentage detoxification of aflatoxin B₁ in rice as the concentrations of *Vernonia amygdalina* and *Moringa oleifera* extract increased from 1 – 5 mg/cm³. The highest detoxification levels obtained were 75.33 and 84.23 % while the lowest were 25.25 and 23.19 % after treatment with *Vernonia amygdalina* and *Moringa oleifera* extracts respectively. These results indicated a higher detoxification of aflatoxin B₁ using *Moringa oleifera* than *Vernonia amygdalina* leaf extract.

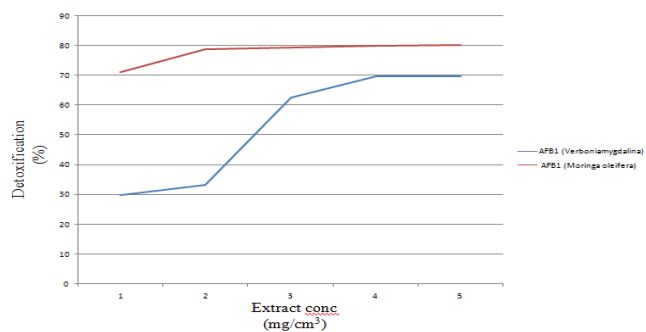


Figure 2: Comparative variations in the detoxification levels of AFB₁ in maize samples

The detoxification results in figure 2 also show an increase in percentage detoxification of aflatoxin B₁ in maize as the concentrations of *Vernonia amygdalina* and *Moringa oleifera* extract increased from 1 – 5 mg/cm³. The highest detoxification levels obtained were 69.69 and 80.21 % while the lowest were 29.71 and 70.99 % after treatment with *Vernonia amygdalina* and *Moringa oleifera* extracts respectively. These results indicated a higher detoxification of aflatoxin B₁ using *Moringa oleifera* than *Vernonia amygdalina* leaf extract.

3.5 Regression Models

The regression models in Tables 5 were developed from the detoxification results obtained after treatments with ethanolic extracts of *Vernonia amygdalina* and *Moringa oleifera* leaves

respectively. These models provide insights into the detoxification of the Aflatoxin B₁ in rice and maize hence, enabling the estimation of their respective residue concentrations after treatment with extracts of known concentrations.

Table 5: Regression models for residual aflatoxin B₁ levels in rice and maize treated with ethanolic extracts of *Vernonia amygdalina* and *Moringa oleifera* leaves

Samples	Treatment Extract	Models	R ² values
Rice	<i>Vernonia amygdalina</i>	$y = -0.066x + 5.711$	0.842
Maize	<i>Vernonia amygdalina</i>	$y = -0.077x + 6.476$	0.858
Rice	<i>Moringa oleifera</i>	$y = -0.052x + 4.446$	0.595
Maize	<i>Moringa oleifera</i>	$y = -0.346x + 10.27$	0.647

The generated R² values indicate the strength and accuracy of each model. All R² values between 0.5 – 1.0 summarizes a positive relationship for both variables (extract concentration and expected residual levels of aflatoxin B₁) in the models. The R² values generated were in the range of 0.595 - 0.858. These indicate that the developed models can estimate accurately ($p \leq 0.05$) the expected residual levels after treatment with a known extract concentration.

4. Conclusion

This study aimed to identify and investigate the efficacy of phytochemicals (from medicinal plants) in AFB₁ detoxification in stored rice and maize. This was achieved by quantifying the residual levels of AFB₁ in the samples after the treatment period.

The high residual levels of aflatoxin B₁ in the stored rice and maize samples indicated that the samples were potential exposure sources of aflatoxin B₁, which adversely affect food safety. The use of *Moringa oleifera* and *Vernonia amygdalina* leaf extracts as phytoremediation techniques proved to be effective green techniques in the detoxification of maize and rice against aflatoxin B₁, thereby enhancing food safety. Detoxification efficiency of ethanolic extract of *Moringa oleifera* leaf was greater than for *Vernonia amygdalina* leaf.

This work however couldn't take into account the nutritional implication of using green technique.

In other to encourage the use of this technique, detoxification potentials of both leaf extracts on other toxic mycotoxins and in multiple food matrices should be investigated.

Data Availability

The data supporting the conclusion of this research are presented in this article.

Conflict of Interest

The authors declared there is no conflict of interest to declare.

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Authors' Contributions

Author 1 carried out the research while authors 2 and 3 reviewed it and edited the manuscript.

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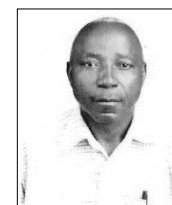
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