

Research Article

Assessment of Pollution and Detoxification Potentials of *Moringa oleifera* and *Vernonia amygdalina* leafs on Fumonisin B₁ in Maize and Rice

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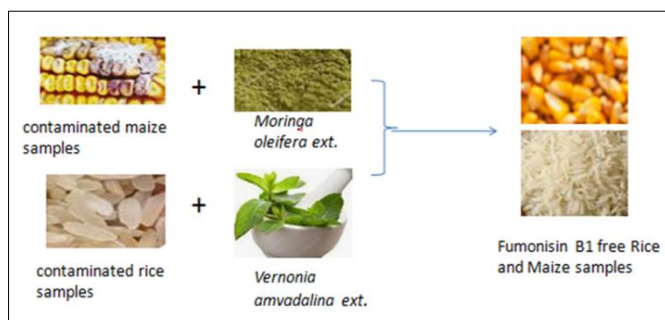


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Abstract – Several disadvantages have been identified with the already established Fumonisin B₁ remediation techniques, and these disadvantages, emphasizes the need to identify highly effective, low cost alternatives that are easy to deploy in the remediation of fumonisin B₁. This research aims to assess the contamination and investigates the efficiency of ethanolic extracts of *Moringa oleifera* and *Vernonia amygdalina* leafs on the detoxification of fumonisin B₁ in stored rice and maize obtained at keffi, Nasarawa state, Nigeria. Phytochemical constituents in both plant extracts were analysed using standard techniques. The phytochemical levels in *Moringa oleifera* extract were statistically ($p \leq 0.05$) higher than in *Vernonia amygdalina* extract. HPLC-Uv/Vis spectroscopy technique was employed in the determination of the residual levels of fumonisin B₁ in treated and untreated samples. Spectroscopic results confirmed the presence of fumonisin B₁ in all samples with residual levels of 101.77 ± 3.56 and 199.04 ± 7.55 $\mu\text{g/kg}$ in stored/untreated rice and maize samples respectively. Detoxification using $1 - 5 \text{ mg/cm}^3$ of *Moringa oleifera* leaf extract ranged from $56.28 (44.49 \pm 3.59 \text{ } \mu\text{g/kg})$ to 65.73% ($34.88 \pm 1.89 \text{ } \mu\text{g/kg}$) in rice and $86.86 (26.16 \pm 2.61 \text{ } \mu\text{g/kg})$ to 89.41% ($21.07 \pm 3.55 \text{ } \mu\text{g/kg}$) in maize while, detoxification using $1 - 5 \text{ mg/cm}^3$ of *Vernonia amygdalina* leaf extract were ranged from $34.80 (21.47 \pm 1.76 \text{ } \mu\text{g/kg})$ to 56.67% ($14.27 \pm 1.09 \text{ } \mu\text{g/kg}$) in rice and $38.87 (23.98 \pm 0.95 \text{ } \mu\text{g/kg})$ to 58.45% ($16.30 \pm 3.18 \text{ } \mu\text{g/kg}$) in maize respectively. Detoxification efficiency increased with increasing concentration of the leaf extracts used in the treatment and was in the order *Moringa oleifera* > *Vernonia amygdalina*.

Keywords- Mycotoxins, Fumonisin B₁ (FB₁), Phytoremediation

Graphical Abstract-



1. Introduction

Fumonisin B₁ is the most toxic member of the fomonisin homologue and is produced by certain *Fusarium spp* Majorly

F. verticillioides and *F. proliferatum* [1]. In a living system, fumonisin B₁ disrupts cell functions through its ability to alter the cell membrane and lipoprotein structure [2]. Fumonisin B₁ has been associated with variety of adverse health effects (mycotoxicoes) like liver cancer (hepatocarcinoma), stimulation and suppression of the immune system, neural-tube defects, inhibition of enzymes, esophageal cancer, weight loss, nephrotoxicity and foetal defects especially in spinal column and brain [3, 4, 5, 6, 7, 8, 9, 10]. It is for these reasons that fumonisin B₁ is classified as a group 2B carcinogen by The International Agency for Research on Cancer [11].

Although knowledge of fumonisin B₁ contamination in food dates back six decades, several researches have indicated a worldwide increase in its incidence in cereals 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

mycotoxins exposure [12], mainly due to their cultivation in large quantities [13] and poor post-harvest handling techniques [14].

Since the discovery of mycotoxins and their toxic effects even at minute concentrations, food safety has been modified to involve determination of these toxins. Fumonisin B₁ as one of the toxins of concern and accounts for 70 – 80 % of the total fumonisin content in cereals [15, 16]. FB₁ is a common contaminant of maize and other cereal crops [17] existing at concentrations as high as 8150 µg/kg in Nigeria [18]. However, very little data has been generated about fumonisin B₁ and its remediation in North central region of Nigeria and Nasarawa state to be precise.

The knowledge of the detoxification of fumonisin B₁ through physical techniques like washing/Cleaning, mechanical sorting, separation (e.g. filtering), heat treatment (e.g. boiling), ultrasonic treatment, and irradiation; chemical techniques which involves the use of alkaline compound/hydrated oxides like sodium hydroxide and calcium hydroxide, ammonia and oxidants like peroxide and chlorine [19]; and biological techniques have associated with certain limitations bothering on the introduction of chemical impurities in food matrices, selectivity, nutrient loss, poor removal and high cost [20, 21, 22, 23, 24]. There is therefore need to identify novel broad spectrum, cost effective, easy to deploy, highly effective remediation technique with high acceptability in other to minimize contamination, improving quality and minimize nutrient loss in cereals.

Plants stand out as the major producers of promising bioactive substances with identified antimicrobial and antimycogenic abilities and the advantage of being more effective, less toxic, safe and economically viable. Furthermore, the additional advantage of their low-cost of extraction, fewer side effects, easy accessibility, abundant nature and wide distribution geographically, makes it easy to research their mycotoxigenic efficacy [25, 26, 27, 28, 29]. *Moringa oleifera* is one of the 13 species of moringa genus, evenly distributed across all ecological zones of Nigeria and well adapted to the climatic conditions in central Nigeria [30, 31]. There are also close to 1000 species of *Vernonia* genus and among them *Vernonia amygdalina* is well adapted and vastly cultivated across all the ecological zones in Nigeria.

1.1 Objective of the study

The general objective/aim of this research was to evaluate the detoxification potentials of *Moringa oleifera* and *Vernonia amygdalina* on fumonisin B₁ residues in stored rice and maize obtained at Keffi, Nasarawa state, Nigeria.

The specific objectives are to;

- Isolate fumonisin B₁ residues in the food samples (rice and maize).
- Extract phytochemicals from the leaves of *Moringa oleifera* and *Vernonia amygdalina* plants.
- Clean up the phytochemical extract by using dispersive Solid Phase Extraction (dsPE).

- Qualitatively and quantitatively determine the phytochemicals (phenols/phenolic compounds, flavonoids, terpenoids, tannins, saponins, steroids, anthraquinone, alkaloids, glycosides and cardiac glycosides) of the plants extracts.
- Carry out treatment processes on the fumonisin B₁ extracts using the phytochemical extracts at different concentrations from leaves of both plants (*Moringa oleifera* and *Vernonia amygdalina*).
- Determine residue concentration of fumonisin B₁ in the stored and treated samples using HPLC- UV/Vis spectroscopy.
- Compare the detoxification percentages of the extracts in both samples.

1.2 Organization of the article

In this article, Section 1 contains the introduction of the fumonisin B₁. It highlights its toxicity/health effects, prevalence and predominance in cereals and cereal based products, the various detoxification techniques and establishes plant as potential detoxifiers. Section 2 contain the related works on FB₁ determination and detoxification, Section 3 contain the methodology used for the research, Section 4 contains the results and its implications and, section 5 summarizes the conclusion and recommendation.

2. Related Works

Fumonisin residues have been observed to be the most prevalent in cereals and cereal based products [32]. They are produced by *Fusarium* spps such as *F. moniliforme*, *F. verticillioides*, *F. proliferatum*, *F. nygamai* and sometimes by *Aspergillus* spps like *Aspergillus nigr* that live as saprophytes in soil and plants [33, 34, 35]. FB₁ is also one of the most virulent as it is responsible for a number of health effects [32, 36].

In Nigeria, there are limited articles with up-to-date information on fumonisin B₁ determination and detoxification in food. In a 2015 research conducted by [37], results obtained indicated a high incidence of fumonisin B₁ with residual levels between 0.9 - 59.6 µg/kg and 10.0 - 3644.0 µg/kg in rice and maize respectively. Fumonsin B₁ was the predominant toxin detected in terms of frequency (78.6 % of samples) and quantity (concentration range 70 – 1780 µg/kg) in 15 samples of post harvested maize in south-western Nigeria [38]. In a study by [39], 363 samples of cereals comprising of maize (136), sorghum (110), millet (87) and soaked corn starch (30) collected randomly from selected markets in four agro-ecological zones in Nigeria, a high incidence of fumonisin (FB₁ + FB₂ + FB₃) was reported with total fumonisin observed to be as high as 32–8508 µg/kg (65 %), 45–180 µg/kg (8 %), 74–22,064 µg/kg (14 %), and 125–3557 µg/kg (93 %) respectively.

Physical detoxification processes of fumonisin showed that a temperature as high as 200 °C is required to detoxify between 87–100 % of FB₁ [40] and such temperatures are higher than food and feed processing temperatures. Physical processes

such as sorting and separation: which is achieved by sieving, gravity separation, aspiration, photoelectric separation and image processing have been known to generate at least 63 % removal of fumonisin [41]. Three independent studies reported 73 - 74 % of FB₁ was removed by washing grains with water [41, 42, 43]. The use of thermal processes generated various detoxification of FB₁ in maize; 15 % detoxification at a oven temperature of 100 °C for 2 h, 98 % detoxification at 220 °C for 30 min, 70 % at 200 °C for 20 min by baking and an average loss of 67 % was achieved by frying at 190 – 210 °C for 5 to 10 min [44]. Detoxifications involving high temperatures are limited by certain drawbacks like loss of nutrient [45], high processing costs [46] and loss of texture and so on.

Detoxification of FB₁ using chemical agent treatments leads to the formation of harmful and mildly toxic results and changes the physical and chemical composition of the produce such as changes in colour, changes in starch structure, lipid oxidation, and protein denaturation [47, 48]. In an oxidative degradation study of FB₁, there was a significant decreased following treatment with O₃ for 15 sec [48]. A study to determine the effectiveness of ammonia in FB₁ detoxification generated 30 - 45 % detoxification of FB₁ contaminated corn [49]. 74.1 % conversion of FB₁ to aminopentol was achieved through alkaline hydrolysis of contaminated ground corn when 0.1 M solution of calcium hydroxide was used to treat the maize for 24 hours and at room temperature [50].

In an innovative mycotoxin detoxification study by [51], there was a complete removal of fumonisins with Aloe vera (*Bryophyllum pinnatum* Leaf) -derived nanocarbon particles from spoiled pap.

3. Materials and Methods

3.1 Sample Collection

Shelled maize (800 g) and parboiled/dehusked rice (800 g) were collected from four designate farmers in four villages within the study area. Each sample (200 g) was collected after 6 months of storage and combined to obtain a representative sample. Samples were then transported using labelled sterile paper bags and stored 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 [52, 53]. Plants were collected by hand and transported using pyrex glass jars (uncorked).

3.2 Sample Pre - Treatment

The rice and maize samples (800 g) were grounded to fine powder using a food grade high speed blender and passed through a sieve (particle $\leq$ 1 mm). They were then mixed thoroughly to ensure the matrices were homogeneous.

Leaves were stripped from stalk/stems, washed under running 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 and passed through a sieve (particle $\leq$ 1 mm) to ensure a high surface area for the extractions.

3.3 Phytochemical Extraction

Phytochemical extractions were carried out using cold maceration extraction as described by [53, 54, 55]. 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.

3.4 Extraction of Fumonisin

The ground and homogenized sample (1.0 g) was weighed into a stoppered 250 cm³ conical flask. The sample was then extracted with acetonitrile/water/formic acid (20/79.9/0.1, 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 at 4 °C for 15 minutes at 4000 rpm. The supernatant was filtered through a developed set up of filter paper and celite. The resulting filtrate was stored in an amber autosampler vial at below 4 °C for further analysis.

3.5 Quantitative Analysis of Phytochemicals

UV/Vis Spectrophotometer was used in the determination of phytochemical concentrations. All analysis was carried out in triplicate.

3.6 Phytochemical Treatment of Extract

To 5.0 cm³ of the fumonisin extract in a test tubes, 1.0 mg/cm³ of *Vernonia amygdalina* leaf extract /ethanol was added. The resulting mixture was vigorously shaken for 30 seconds in a 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.

3.7 Clean-up of the fumonisin 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.

3.8 HPLC Quantification of Fumonisin B₁

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 202 nm for FB₁ 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).

3.9 Statistical Analysis

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

3.10 Determination of Percentage Detoxification

The percentage degradation of fumonisin B₁ was calculated using the following formula:

$$\text{Percentage degradation} = (1 - C_b/C_a) \times 100 \% \quad (1)$$

Where,

C_a is the concentration of fumonisin B₁ in the control/stored sample

C_b is the concentration of fumonisin B₁ in the sample after detoxification and [56].

4. Results and Discussions

4.1 Results

Table 1: Residual concentrations (µg/kg) of fumonisin B₁ in stored rice and maize samples

Samples	Residual concebtration (µg/kg)
Rice	101.77 ± 3.56 ^b
Maize	199.04 ± 7.55 ^a

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

Table 2: Residual concentrations (µg/kg) and detoxification levels (%) of fumonisin B₁ in rice and maize samples treated with *Moringa oleifera* extract

Extract concentration (mg/cm ³)	Rice	Detoxification	Maize	Detoxification
1	44.49 ± 3.59 ^{bc}	56.28	26.16 ± 2.61 ^a	86.86
2	42.58 ± 3.01 ^{abc}	58.16	25.89 ± 4.25 ^a	86.99
3	38.64 ± 8.03 ^{ab}	62.03	23.94 ± 4.27 ^a	87.97
4	38.66 ± 6.43 ^{ab}	62.01	23.13 ± 11.00 ^a	88.38
5	34.88 ± 1.89 ^a	65.73	21.07 ± 3.55 ^a	89.41

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

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

Extract concentration (mg/cm ³)	Rice	Detoxification	Maize	Detoxification
1.0	73.78 ± 5.22 ^c	27.50	63.75 ± 3.34 ^c	67.97
2.0	60.85 ± 4.38 ^d	40.21	49.06 ± 10.76 ^b	75.35
3.0	52.72 ± 1.99 ^e	48.20	44.00 ± 3.28 ^b	77.89
4.0	36.82 ± 1.35 ^b	63.82	45.97 ± 6.97 ^b	76.90
5.0	24.00 ± 4.79 ^a	76.42	39.46 ± 3.17 ^{ab}	80.17

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

4.2 Discussions

Qualitative investigation of some phytochemicals in hexane/methanol extracts of *Moringa oleifera* and *Vernonia amygdalina* leafs indicated the presence of major phytochemical constituents to include phenols, tannins, flavonoids, saponins and steroids in both leaf extracts with an exception of anthraquinone in *Vernonia amygdalina*.

Quantitative analysis of the plant extracts show 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 average concentrations of flavonoids, alkaloids, saponins, terpenoids, steroids, glycosides and cardiac glycosides were significantly higher ($p \leq 0.05$) in *Moringa oleifera* than *Vernonia amygdalina*. However, phenols/phenolic compounds were observed to be significantly higher in *Vernonia amygdalina* compared to *Moringa oleifera*. Total tannins were observed not to be statistically ($p < 0.05$) the same in both extracts. The mean for *Moringa oleifera* was M = 84.58, SD = 51.34 and *Vernonia amygdalina* was M = 59.06, SD = 43.92. Independent sample *t*-test revealed a significant difference between the two groups, $t_{(58)} = 1.194$, $p = 0.044$. The effect size for the difference was large $d = 0.61$. Thus, there is a

significant difference between the means of the phytochemicals in the samples.

4.2.1 Fumonisin B₁ levels in stored samples

The mean residual concentrations (**Table 1**) in stored samples of rice and maize produce revealed the presence of fumonisin B₁ in all the investigated samples, with mean concentrations of 101.77 ± 3.56 and 199.04 ± 7.55 $\mu\text{g/kg}$ in rice and maize respectively. The relatively high concentration of fumonisin B₁ in the stored grains may be attributed to the predominance of *Fusarium spp*s during grain cultivation and storage [56, 57] which is favoured by conditions such as high relative humidity, favourable water activity, temperatures of 30 – 35 °C [56, 58, 59], and geographical factors like high rainfall and topography [58, 59].

The obtained residual levels were higher than the 2 $\mu\text{g/kg}$ FAO/WHO safety limit for human consumption but lower than the FDA and EU safety limits.

4.2.2 Detoxification of Fumonisin B₁ with Leaf Extracts

4.2.2.1 Use of *Moringa oleifera* leaf extract

A reduction in residual concentrations of fumonisin B₁ was observed in rice as concentration of *Moringa oleifera* leaf extract used for the treatment increased from 1 – 5 mg/cm^3 (**Table 2**). The trend, however, was different in maize; as detoxification levels remained high and were not significantly different ($p \leq 0.05$) with increasing concentration of the extract. The highest detoxification levels obtained were 65.73 % (34.88 ± 1.89 $\mu\text{g/kg}$) and 89.41 % (21.07 ± 3.55 $\mu\text{g/kg}$) while the lowest were 56.28 % (44.49 ± 3.59 $\mu\text{g/kg}$) and 86.86 % (26.16 ± 2.61 $\mu\text{g/kg}$) for the rice and maize samples respectively.

4.2.2.2 Use of *Vernonia amygdalina* leaf extract

Detoxification levels of fumonisin B₁ in rice and maize decreased as concentration of *Vernonia amygdalina* extract increased from 1 – 5 mg/cm^3 (**Table 3**). A significant decrease in residual levels with stepwise increase in *Vernonia amygdalina* leaf extract used for the treatment was obtained in the rice sample while, a significant decrease in residual levels between 1 – 2 mg/cm^3 which remained statistically the same from 2 – 4 mg/cm^3 and later decreases further between 4 – 5 mg/cm^3 was obtained in the maize sample. The highest detoxification levels obtained were 76.42 % (24.00 ± 4.79 $\mu\text{g/kg}$) and 80.17 % (39.46 ± 3.17 $\mu\text{g/kg}$) while the lowest were 27.50 % (73.78 ± 5.22 $\mu\text{g/kg}$) and 67.97 % (63.75 ± 3.34 $\mu\text{g/kg}$) for the rice and maize samples respectively.

4.2.3 Comparative Variations in Fumonisin B₁ Detoxification Levels

4.2.3.1 Variations in fumonisin B₁ detoxification in rice

A comparative variation in the detoxification results (**Figure 1**) indicates an increase in percentage detoxification of fumonisin B₁ in rice with as extract concentration increased (1

– 5 mg/cm^3). The highest detoxification levels obtained were 65.73 and 76.42 % while the lowest were 56.28 and 27.50 % after treatment with *Moringa oleifera* and *Vernonia amygdalina* extracts respectively.

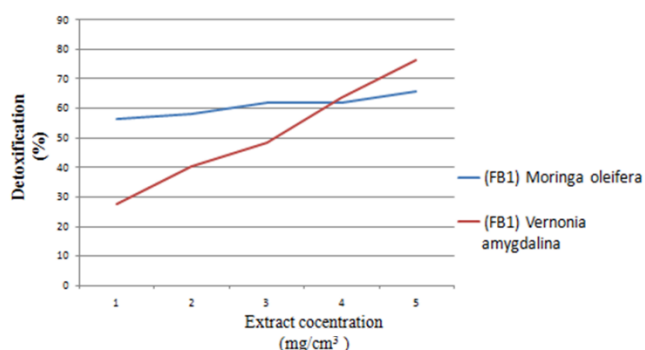


Figure 1: Comparative variations in the detoxification levels of FB₁ in rice samples

Initial detoxification levels and a higher percentage detoxification obtained from 1 – 3 mg/cm^3 of *Moringa oleifera* extract compared to *Vernonia amygdalina* extract suggests greater detoxification efficacy for *Moringa oleifera* extract. However, at higher concentration of the leaf extracts used for the treatment (5 mg/cm^3), a higher detoxification by *Vernonia amygdalina* was obtained.

4.2.3.2 Variations in fumonisin B₁ detoxification in maize

The variations in detoxification levels of fumonisin B₁ in the maize samples after treatment with the leaf extracts (**Figure 2**) show initial detoxification results at 1 mg/cm^3 extract concentration were 86.86 and 67.97 % for *Moringa oleifera* and *Vernonia amygdalina* extracts respectively. Detoxification by *Moringa oleifera* remained statistically high and the same as extract concentration increased while, detoxification by *Vernonia amygdalina* increased steadily as extract concentration increased from 1 – 5 mg/cm^3 . Highest detoxification levels obtained in the maize samples were 89.41 and 80.17 % for *Moringa oleifera* and *Vernonia amygdalina* extracts respectively. The detoxification levels after treatment in the maize samples varied in the order; *Moringa oleifera* > *Vernonia amygdalina*.

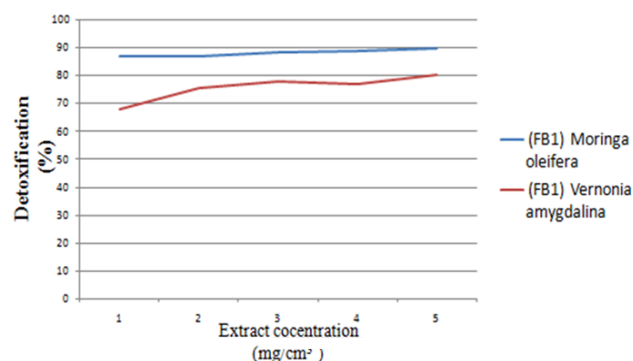


Figure 2: Comparative variations in the detoxification levels of FB₁ in maize samples.

5. Conclusion

The research identified fumonisin B₁ in all samples of the stored food produce at residual concentrations higher than the FAO/WHO acceptable limits for human consumption. These high residual levels indicate that the samples were potential sources of fumonisin B₁ and establish them as exposure point of the mycotoxin to humans. The use of both plant extracts as phytoremediation techniques demonstrated a higher reduction in the fumonisin B₁ residues in the food matrices.

The corresponding increase in detoxification levels of fumonisin B₁ as leaf extract concentration increased may correlate with the increased phytochemicals presence in the extract. The use of *Moringa oleifera* and *Vernonia amygdalina* leaf extracts as phytoremediation techniques proved to be effective green techniques in the preservation of maize and rice against fumonisin B₁. The overall application of this technique will help to enhance food safety.

The detoxification efficiency of these green techniques on other agricultural produce and other classes of mycotoxins should be investigated to encourage acceptability of green remedy. Also, the use of these techniques as proactive new technology in the possible prevention of mould growth and mycotoxins' production through field application of the extracts of these plants should be researched into, this could help minimize field production of the toxins during cultivation,

Author's statements

This work was a combined effort of all the authors with no conflict of interest.

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Authors' Contributions

Authors 1, 2 and 3 conceived the study. Author 1 researched the literature and prepared the manuscript. Authors 2 and 3 supervised the research and approved the final version of the manuscript while Author 4 provided external additions to the research.

Conflict Of Interest

The authors declared that they have no conflict of interest.

Data Availability

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

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