Mycological and Aflatoxin B1, B2, G1 and G2 Analysis and Its Effect On Storage Practices for Herbal Medicines in Yobe State Northeast Nigeria

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ABSTRACT

There is increased reliance on traditional herbal medicines by several millions of people worldwide, especially in West Africa and Nigeria in particular. This is due to escalating cost of good quality drugs and consequent proliferation of faked cheaper drugs. However, non standardization of production and handling methods have resulted in herbal medicines with varying quality and safety indices, thus resulting in possible public health concerns. This work investigated mycological and aflatoxin B1, B2, G1 and G2 analysis and its effect on storage practices for herbal medicines in Yobe State Northeast Nigeria**.** A total of five (5) samples was obtained from renowned herbal medicine practitioners in Damaturu Yobe state in Nigeria, fresh and stored for 35, 70, 105 and 105 days, based on their medicinal uses, were analyzed to determine the microbial load by the plate count method and aflatoxin contamination levels using thin layer chromatography with aflatoxin standards. At least seven (7) fungi genera (*Aspergillus, Penicillium, Fusarium*) were isolated. Aflatoxin B1, B2, G1 and G2 were detected in varying concentrations in the samples analyzed. Some of these herbal concoctions were found to contain unacceptably high bioload, according to WHO standards. Microbial contamination and the presence of aflatoxins in herbal medicines appear to be an endemic problem in Nigeria, as observed in this work, probably due to poor observation of basic hygiene during preparations and poor storage conditions. The findings in this work may serve in developing and instituting public health standards for the production and safety of herbal remedies in Damaturu, Yobe state Nigeria.

Keyword: *Herbs, Fungi, chromatography, Aflatoxin, Contamination*

INTRODUCTION

Humans have used extracts derived from medicinal plants in folk medicine systems for thousands of years (Ong and Bodela, 2005) and these traditions continue to the present day. According to the World Health Organization (WHO, 1997), a medicinal plant is defined as any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis" (WHO 1997). Many licensed drugs were originally derived wholly or partially from extracts of medicinal plants that had long histories of folk usage (Bent 2008). An herb is a plant or part of a plant valued for its medicinal, aromatic, or savory qualities (Kunle et al 2012). Herbs can be viewed as biosynthetic chemical laboratories, producing a number of chemical compounds. Herbal remedies or medicines consist of portions of plants or unpurified plant extracts containing several constituents, which often work together synergistically (Kunle *et al.*, 2012). Herbal medicine or herbalism is the use of herbs or herbal products for their therapeutic or medicinal value. They may come from any part of the plant but are most commonly made from leaves, roots, bark seeds, and flowers. They are eaten, swallowed, drunk, inhaled, or applied topically to the skin. Herbal products often contain a variety of naturally-occurring biochemical from plants, many of which contribute to the plant's medicinal benefits. Chemicals known to have medicinal benefits are referred to as "active ingredients" or "active principles" and their presence depends on a number of factors including the plant species, the time and season of harvest, the type of soil, the way the herb is prepared, etc. (Kunle *et al.*, 2012). The use of plants for medicinal purposes for the treatment, cure and prevention of diseases is one of the earliest known medical practices in History. At present, a significant amount of medicinal plant commercialization is carried out in drugstores and natural product stores, where vegetable preparations are marketed under industrialized labeling (Junior, 2005).

In 1995, the global market for medicinal herbs was approximately USD 17 billion, but a recent market analysis suggests that by 2023 this will increase to approximately USD 111 billion (compound annual growth rate of 7% to 8%). Market drivers for this increased demand in developed countries include the expense of insurance-based medical care, a rising desire of people in industrialized nations to take charge of their own health, and an increasing elderly population (GHMMRR, 2018).

Some well-known systems of using herbal medicine are Ayurveda from India and Traditional Chinese Medicine (TCM), although the latter includes a variety of products of animal and fungal origin. In developed countries, herbal medicines are also consumed. The United States Food and Drug Administration (US-FDA) estimated a decade ago that more than 20,000 different plant species were used in herbal remedies each year, and that one out of five US citizens used herbal medicines regularly (Bent, 2008). In the European Union (EU), France, and Germany are the main markets for herbal remedies (GHRMMRR, 2018).

Aflatoxins

Mycotoxins are natural contaminants in raw materials, food and feeds (Boscon and Mollea, 2012). Mycotoxigenic molds are widespread throughout the world; thus, mycotoxins, including aflatoxin, contaminate a variety of crops and foods worldwide, especially those grown in tropical regions. As mentioned earlier,

the level of aflatoxin contamination is determined by a myriad of factors, including crop types and environmental factors (Reddy *et al.,* 2010). Although aflatoxin's occurrence is widespread and affects many food crops, certain crops are more susceptible than others. Aflatoxins are toxic metabolites produced by different species of toxigenic fungi, called mycotoxins.

Diseases caused by Aflatoxin.

Humans may be exposed to aflatoxins through the consumption of aflatoxin-contaminated foods or the ingestion of foods produced by animals previously exposed to aflatoxins (Leong et al., 2012). Chronic dietary exposure to aflatoxins poses severe health complications in humans and animals (Williams *et al.,* 2004). Aflatoxin B1, due to its toxic, mutagenic, immunotoxic, teratogenic, and carcinogenic effect on humans and animals, is classified as a group1 carcinogen in the International Agency for Research on Cancer (IARC) classification of carcinogenic substances (Ostry *et al.,* 2017). As potent carcinogen, aflatoxin B1 may affect organs like the liver and kidneys (Alvarez *et al.,* 2020; Li *et al.,* 2018).

Puttashwamy and kotteshwar (2015) also reported incidence of Aflatoxin and other mycotoxin in India according to A total of 302 isolates of 42 fungal species belonging to 17 genera were found in association with test of the samples. More than 61% of *A. flavus* isolates tested positive for production of AB_1 and highest yield recorded was 5008.20 ppb from the isolates of *T. cordifolia.* Among the six highly contaminated samples three samples tested positive for AB1. Highest AB_1 was recorded from T. *cordifolia* (104.19 µg/kg), followed by *A. calamus* (13.73 µg/kg) and *M. fragrans* (12.02 µg/kg). In 2017 research conducted in china, five types of commonly used herbal medicines including *Lilii Bulbus, Hordei Fructus Germinatus, Nelumbinis Semen, Polygalae Radix* and *Bombyx Batryticatus* were selected as the subjects of study, optimized high-performance liquid chromatography with fluorescence detection (HPLC- FLD) method was validated for the quantitative analysis of four aflatoxins (AFG_2, AFG_1, AFB_2 and AFB_1) (Yujiao *et al*., 2017). The incidence of toxigenic fungi producing aflatoxins, ochratoxin A, and fumonisin on medicinal herbs was also reported from Argentina (Rizzo et al. 2004). An investigation from South Africa showed the presence of fumonisin B_1 in dietary and medicinal wild plants (Sewram et al. 2006). Two analytical methods, trifluoroacetic acid and Kobra cell derivatization methods, were compared; The latter was selected based on high linearity and sensitivity. The limits of detection of AFs using the Kobra cell method were 0.07 – 0.32 ng/g. Recoveries of AFs using various matrixes such as solid, semi-solid, liquid samples and CRM were 81.81-119.87%. The Z-score and linearities of calibration curves were 0.53 and 0.9996e0.9999, respectively.

Bugno *et al*. (2006) had reported the occurrence of Aflatoxins-, ochra- toxin A-, and citrinin-producing Aspergillus and Pencillium in medicinal herbs in Brazil. Visenuo and Alko (2015) examined Sixty-three samples for fungal contamintion and fungal load determined using standard microbiological method in India. Aflatoxin and citrinin were detected using thin layer chromatography and high-performance chromatography technique. Fifty-eight out of the 63 samples were found contaminated, while five were free from fungal con- tamination. Analysis revealed that 47 % of the samples had a fungal load above 1 9 103 cfu/g which is the permissible limit set by World Health Organization According to a report published by the

International Agency for Research on Cancer (IARC),

EXPERIMENTALS

STUDY AREA

Damaturu, the Yobe state capital, North east geopolitical Zones of Nigeria was used to carry out the study.

MOISTURE CONTENT ANALYSIS OF HERBS

Moisture content was measured prior to rinsing in distilled water. For moisture content, prepared samples were dried at 60°C for 24h or until their weight remain constant and the difference in weight was be calculated. Each sample was analyzed in triplicate (Amal 2011).

MYCOLOGICAL ANALYSIS

Ten grams of sample was added to 90 ml portion of sterile saline solution (0.35%) in 500 ml Erlenmeyer flask and wias homogenized thoroughly on an electric shaker at constant speed for 15 min. Tenfold serial dilutions was then be prepared according to (Aziz and Youssef, 1991). One milliliter portion of suitable dilutions was used to inoculate Petri dishes containing 15 ml dextrose agar fortified by 0.5 mg chloromphnicol/ml medium plates was incubated at 28℃ for 7 to 15 days and the growth of molds was examined. Fungi was isolated and identified according to Amal (2011).

EXTRACTION OF SAMPLE

Ground herbal medicines was individually prepared for ELISA methods. Samples were prepared for ELISA analysis as follows; 5 g of the sample was mixed with 25 ml of a mixture of methanol and water (60:40, vol/vol) and 1 g of NaCl, extracted and shaked for 15 min. After centrifugation, the supernatant was filtered through Whatman no. 1 filter paper, diluted with PBST to minimize matrix influence, and immediately applied to ELISA.

SCREENING OF AFLATOXIN B1 USING ELISA

Two hundred (200 µl) of the conjugate solution was placed in each mixing well,100 µl sample extract was added to each dilution well containing the 200 µl of the conjugate solution, a dry multi-channel pipet was used to thoroughly mixed the liquids in the well, one hundred (100 µl) of the solution was pipetted and transferred to each corresponding antibody coated well and the content of the mixing well was incubated at room temperature for fifteen (15) minutes, after incubation the content of the antibody wells was shaked out into a beaker and discarded, the antibody coated wells was then filled using distilled water and washed five times. The antibody coated well was taped to dry with adsorbent paper until the remaining water was removed. One hundred (100 µl) of the substrate solution was then added into the antibody coated well and incubated at room temperature for 5 minutes, one hundred (100 µl) of stop solution was added into the antibody coated well, the change in color from blue to yellow indicate the presence of AFM1. The content was mixed by sliding back and forth on the flat surface, the bottom of the well was then wiped with a dry cloth. The absorbance was read within 20 minutes of the addition of the stop solution using Elisa at 450 nM filter and 630 nM differential filter, the result was obtained and calculated in form of concentration using the ELISA stat fax micro well reader or equivalent (Romer labs 2015). Scheme 3.1 present a block diagram giving a summary of ELISA procedure**.**

RESULTS AND DISCUSSION

Fresh samples of *Acacia sirberana*, *Tamarindus indica*, *Parkia biglobosa*, *Acacia nilotica* and *Cassia tora* had moisture content of 73.5, 25.5,

24.4, 28.5 and 27% respectively while that of the stored samples decreased to 9.2 and 8.5, 3.9 and 3.1, 4.0 and 3.2, 5.2 and 4.6, 5.3 and 4.7 for *Acacia sirberana, Tamarindus indica, Parkia biglobosa, Acacia nilotica and Cassia* tora respectively after 105 and 140 days of storage (Table 1). Moisture content of more than 12% may contribute significantly to the spoilage of any food commodity among other factors such as temperature and relative humidity (Christensen and Kaufmann, 1965). From Table 1, it was noticed that the moisture condition that will promote the growth of aflatoxigenic fungi has been achieved between the first 35 days of storage. This could be responsible for the establishment of these spoilage fungi in the storedherbs sasmple. Moisture is required by the fungi for metabolism and activation of enzymes. (Jonathan *et al, 2010*) .Fresh samples and 35 day old samples of Acacia sirberana and Acacia nilotica have higher moisture contents than other stored herbs samples .(Table 1). The total fungal counts were increasing as the storage time increases in both samples. *Pleurotus ostreatus* increased from 3.0 × 101 (cfu/g) (fresh samples) to 1.6 × 103 (cfu/g) (140day old samples) while *P. pulmonarius* ranged from 2.5 × 102 (cfu/g) in fresh samples to 1.8 × 103 (cfu/g) in 140-day old samples).

Table 1 Moisture content and total fungal counts for *Acacia sirberana, Tamarindus indica, Parkia biglobosa, Acacia nilotica* and *Cassia tora* samples

Period of storage /sample	Fresh	35	70	105	140
Acacia sirberana	73.5	14.50	10.5	9.2	8.5
Count (cfu/g)	3.10×10^1	6.30×10^5	1.60×10^3	1.3×10^2	1.0×10^1
Tamarindus indica	25.5	12.2	5.2	3.9	3.1
Count (cfu/g)	4.1×10^1	1.15×10^5	1.0×10^2	1.0×10^2	1.0×10^2
Parkia biglobosa	24.4	11.50	4.9	4.0	3.2
	$4.0\ 10^1$	1.35×10^3	1.0×10^2	1.0×10^2	1.0×10^2
Acacia nilotica	28.5	12.60	6.5	5.2	4.6
Count (cfu/g)	4.2×10^3	1.20×10^4	1.0×10^2	1.0×10^2	1.0×10^2
Cassia tora	27	12.10	6.2	5.3	4.7
Count (cfu/g)	3.8×10^2	1.13×10^4	1.0×10^2	1.0×10^2	1.0×10^2

Aspergillus niger and *Fusarium oxysporum*, were the most frequently encountered fungi among the seven fungal species isolated. These two fungal species were isolated from all the fresh and stored samples of Acacia sirberana, Tamarindus indica,Parkia biglobosa, Acacia nilotica and

Cassia tora. These were followed in order (P>0.05) by *A. tamarii*, *A. flavus*, *Penicillium chrysogenum*, *P. oxalicum* and *F. compaticum* (Table2). Some of the fungi reported in this study have been isolated by other authors from diverse food products like dried/stored yam and cassava chips (Gnonlonfin, *et al.,* 2008), stored bush mango seeds (Adebayo, *et al.,* 2006), dry roasted groundnut (Bankole and Mabekoje, 2004). *A. niger* had the highest percentage of occurrence. This fungus was recovered throughout the storage periods. The frequency of occurrence was 38.52, 30.48, 28.65, 26.22 and 22.84% in Acacia sirberana, Tamarindus indica,Parkia biglobosa, Acacia nilotica and Cassia tora, respectively. *Fusarium oxysporum* was also recovered throughout the storage periods with 20.63% 20.52, 20.40, 20.20 and 20.25% in Acacia sirberana, Tamarindus indica,Parkia biglobosa, Acacia nilotica and Cassia tora. *Aspergillus tamarii* was the third most frequently encountered fungus. The percentages of occurrence were 31.63 25.24, 24.50. 28.35 and 7.96 % in Acacia sirberana, Tamarindus indica,Parkia biglobosa, Acacia nilotica and Cassia tora, respectively. A*spergillus flavus* had 18.9 17.5, 15.8 14.5 and 13.88% for Acacia sirberana, Tamarindus indica,Parkia biglobosa, Acacia nilotica and Cassia tora respectively. The presence of *Aspergillus* spp. and *Fusarium* spp. were not unexpected in fresh and stored mushroom samples because they are known as major contaminants of the environments occurring as ubiquitous saprophytes, with great ability of their spores to survive and reproduce on many organic substrates (Mbata *et al*, 2008)

Table 2 Individual fungal counts from *Acacia sirberana, Tamarindus indica, Parkia biglobosa, Acacia nilotica* and *Cassia tora* samples when fresh and at various storage durations

FUNGAL SPECIES	Fresh (0 day) (cfu.g)	35 day (cfu.g)	70 days (cfu.g)	150 days (cfu.g)	140 days (cfu.g)
Acacia sirberana					
Aspergillus niger	2.0×10^{1c}	$2.5\times10^{3\,b}$	3.5×103 a,b	$4.0\times10^{3\,a}$	4.5×10^{3a}
Aspergillus tamarii	-	$1.5\times10^{3\,a}$	$2.0\times10^{3\,a}$	-	-
Aspergillus flavus	-	-	$2.0\times10^{3\,c}$	$5.0\times10^{3\,b}$	6.5×10^{3a}
Penicillium oxalicum	-	$5.0\times10^{2\,a}$	$1.0\times10^{3\,a}$	-	-
Penicillium chrysogenum	-	-	$1.0\times10^{3\,b}$	$2.5\times10^{3\,b}$	2.5×10^{3a}
Fusarium oxysporum	1.0×10^{2b}	1.5×103 a,b	2.0×103 a,b	$2.0\times10^{3\,a}$	$2.5\times10^{3\,a}$

Fusarium compaticum	-	5.0×10^{2}	-	-	-
Tamarindus indica					
Aspergillus niger	$5.0\times10^{1\,b}$	$1.5\times10^{3\,a}$	$2.5\times10^{3\,a}$	$3.7\times10^{3\,a}$	$4.5\times10^{3\,a}$
Aspergillus tamarii	$1.5\times10^{\,b}$	$2.5\times10^{3\,a}$	$3.0\times10^{3\,a}$	$2.5\times10^{3\,a}$	$2.5\times10^{3\,a}$
Aspergillus flavus	-	-	$1.5\times10^{3\,b}$	$4.5\times10^{3\,a}$	6.0×10^{3a}
Penicillium oxalicum	-	-	-	-	-
Penicillium chrysogenum	-	5.0×10^{2}	-	-	-
Fusarium oxysporum	-	$5.0\times10^{2\,a}$	$1.5\times10^{3\,a}$	$2.0\times10^{3\,a}$	2.5×10^{3a}
Fusarium compaticum	$1.0\times10^{2\,b}$	$1.5\times10^{3\,a}$	$2.0\times10^{3\,a}$	$2.5\times10^{3\,a}$	2.5×10^{3a}
Parkia biglobosa					
Aspergillus niger	3.0x	5.0×10^{2}	-	-	-
Aspergillus tamarii	-	$3.0\times10^{2\,a}$	$1.5\times10^{3\,a}$	2.0×10^{2a}	2.5×10^{3a}
Aspergillus flavus	$1.0\times10^{2\,b}$	$1.5\times10^{3\,a}$	$2.0\times10^{3\,a}$	$2.5\times10^{3\,a}$	2.5×10^{3a}
Penicillium oxalicum	-	-	-	-	-
Penicillium chrysogenum	-	5.0×10^{2}	-	-	- -

Fusarium oxysporum	$1.0\times10^{2\,b}$	$1.5\times10^{3\,a}$	$2.0\times10^{3\,a}$	$2.5\times10^{3\,a}$	2.5×10^{3a}
Fusarium compaticum	$1.0\times10^{2\,b}$	$1.5\times10^{3\,a}$	$2.0\times10^{3\,a}$	$2.5\times10^{3\,a}$	2.5×10^{3a}
Acacia nilotica					
Aspergillus niger	-	5.0×10^{2}	-	-	-
Aspergillus tamarii	-	$5.0\times10^{2\,a}$	$1.5\times10^{3\,a}$	$2.0\times10^{3\,a}$	2.5×10^{3a}
Aspergillus flavus	$1.0\times10^{2\,b}$	$1.5\times10^{3\,a}$	$2.0\times10^{3\,a}$	$2.5\times10^{3\,a}$	2.5×10^{3a}
Penicillium oxalicum	-	-	-	-	-
Penicillium chrysogenum		5.0×10^{2}	-	-	- -
Fusarium oxysporum	1.0×10^{2b}	$1.5\times10^{3\,a}$	$2.0\times10^{3\,a}$	$2.5\times10^{3\,a}$	2.5×10^{3a}
Fusarium compaticum	$1.0\times10^{2\,b}$	$1.5\times10^{3\,a}$	$2.0\times10^{3\,a}$	$2.5\times10^{3\,a}$	2.5×10^{3a}
Cassia tora					
Aspergillus niger	-	5.0×10^{2}	-	-	-
Aspergillus tamarii	-	$5.0\times10^{2\,a}$	$1.5\times10^{3\,a}$	$2.0\times10^{3\,a}$	2.5×10^{3a}
Aspergillus flavus	1.0×10^{2b}	$1.5\times10^{3\,a}$	$2.0\times10^{3\,a}$	$2.5\times10^{3\,a}$	2.5×10^{3a}
Penicillium oxalicum	-	-	-	-	-

Penicillium chrysogenum		5.0×10^{2}	-	-	- -
Fusarium oxysporum	$1.0\times10^{2\,b}$	$1.5\times10^{3\,a}$	$2.0\times10^{3\,a}$	$2.5\times10^{3\,a}$	2.5×10^{3a}
Fusarium compaticum	$1.0\times10^{2\,b}$	$1.5\times10^{3\,a}$	$2.0\times10^{3\,a}$	$2.5\times10^{3\,a}$	2.5×10^{3a}

Table 3 shows that the concentration of AFB_1, AFB_2, AFG_1 and AFG_2 *Acacia sirberana, Tamarindus indica, Parkia biglobosa, Acacia nilotica* and *Cassia tora* after 35 days of storage were 1.10, 0.22, 0.17 and 0.08µg/kg, 1.95, 1.06, 0.55 and 0.12, 1.06, 0.18, 0.15 and 0.08, 1.14, 0.23, 0.18 and 0.10 and 1.16, 0.21, 0.16 and 0.07 respectively. The increased fungal population in the herb's samples contributed to the increment in aflatoxins concentration with storage.

Food commodities having moisture content below 12% have long been reported to be safe for storage (Christensen & Kaufmann, 1965).

Table 3 Aflatoxins level in *Acacia sirberana, Tamarindus indica, Parkia biglobosa, Acacia nilotica* and *Cassia tora* samples when fresh and at different storage periods

Aflatoxins	Fresh (0 day) (µg/kg)	35 days (µg/kg)	70 days (µg/kg)	105 days (µg/kg)	140 days (µg/kg)
Acacia sirberana,					
B1	ND	1.10^{c}	1.28^{c}	2.79^{c}	3.48^{c}
B2	ND	0.22^{d}	0.29^{c}	1.55^{b}	2.26^{a}
G1	ND	0.17^{d}	0.25^{c}	0.78^{b}	1.23^{a}
G2	ND	0.08^{c}	$0.09^{b,\,c}$	0.12^{b}	0.22^{a}
Tamarindus indica					
B1	0.06^{d}	1.95^{c}	2.18^{c}	3.78^{b}	4.23^{a}
B2	ND	1.06^{d}	1.14^{c}	1.62^{b}	2.72^{a}
G1	ND	0.55^{d}	0.70^{c}	1.07^{b}	1.26^{a}
G2	ND	0.12^{d}	0.19^{c}	0.28^{b}	0.35^{a}
Parkia biglobosa,					
B1	ND	1.06^{c}	1.24^{c}	2.75^{c}	3.44^{c}
B2	ND	0.18^{d}	0.25^{c}	1.51^{b}	2.22^{a}
G1	ND	0.15^{d}	0.24^{c}	0.74^{b}	1.20^{a}

G2	ND	0.08[c]	0.09[b, c]	0.12[b]	0.22[a]
Acacia nilotica					
B1	ND	1.14[c]	1.32[c]	2.83[c]	3.52[c]
B2	ND	0.23[d]	0.28[c]	1.54[b]	2.23[a]
G1	ND	0.18[d]	0.22[c]	0.75[b]	1.20[a]
G2	ND	0.08[c]	0.10[b, c]	0.14[b]	0.24[a]
Cassia tora					
B1	ND	1.16[c]	1.30[c]	2.82[c]	3.50[c]
B2	ND	0.21[d]	0.24[c]	1.50[b]	2.20[a]
G1	ND	0.16[d]	0.24[c]	0.73[b]	1.22[a]
G2	ND	0.07[c]	0.09[b, c]	0.12[b]	0.24[a]

After 70th day of storage, the AFB_1 level in Acacia sirberana were 1.28µg/kg, AFB_2 0.29µg/kg, AFG_1 0.25µg/kg and AFG_2 0.09µg/kg, in Tamarindus indica AFB_1 level were 2.19µg/kg, AFB_2 1.14µg/kg, AFG_1 0.70µg/kg and AFG_2 0.19µg/kg, in Parkia biglobosa AFB_1 level were 1.24µg/kg, AFB_2 0.25µg/kg, AFG_1 0.24µg/kg and AFG_2 0.09µg/kg in Acacia nilotica AFB_1 level were 1.32µg/kg, AFB_2 0.28µg/kg, AFG_1 0.22µg/kg and AFG_2 0.10µg/kg while in Cassia tora AFB_1 level were 1.30µg/kg, AFB_2 0.24µg/kg, AFG_1 0.24µg/kg and AFG_2 0.09µg/kg. Considering the levels of all the aflatoxins with significant increase at this period, it could also be said that most fungal activities were occurring mainly at the exponential phase of growth. As earlier recounted, the stationary phase is a period of secondary metabolites production (Jonathan, *et al.*,2010) even though small amounts of mycotoxins may start appearing at the exponential growth phase due to the presence of non-dividing cells;

The results obtained with 105-days stored samples of *Acacia sirberana*, *Tamarindus indica*, *Parkia biglobosa*, *Acacia nilotica* and *Cassia tora* revealed further decreased in moisture content, increased individual fungal flora and more aflatoxins levels. The increase noticed with all the aflatoxins detected increased more at this period than what was initially obtained. The increase noticed with *A. flavus* was more prominent therefore; high levels of AFB_1 and AFB_2 would be expected.

With the 140-days stored samples, the values of AFB_1 and AFB_2 further increased (Table 3).What was observed at this period shows that lengthened storage time for food commodities could give room for fungal activities and risk of aflatoxins contamination in such foods. *A. flavus* has been reported to be a major fungus capable of producing AFB_1 and AFB_2 (Abba, *et al.*, 2009) and, occurred in higher percentage in the samples investigated in this study.

CONCLUSIONS

In conclusion, storage (especially in humid environment) may increase microbial population and creates an avenue for increasing aflatoxins production under other favorable conditions. Therefore, necessary precautions in preventing contamination of dried and stored herbs with must be ensured, in order to reduce the risk of aflatoxin and other mycotoxins that are deleterious to human health.

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