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# Occurrence of Aflatoxins and Fumonisins Contamination in Herbal Medicinal Products Sold in Nairobi, Kenya

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**Abstract**
Aflatoxins and fumonisins are referred to as Mycotoxins. They are secondary metabolites of some moulds which are highly toxic, mutagenic or teratogenic compounds. These substances are not formed by all mould species but are characteristic of their producers. The aim of this study was to determine the occurrence of Aflatoxin and Fumonisins contamination in herbal medicinal products sold in Nairobi Kenya. The study was undertaken in Nairobi, the capital and largest city in Kenya. Nairobi has several herbal clinics, especially in densely populated areas. This study employed an exploratory as well as laboratory-based experimental design to sample 138 herbal medicinal products. The sample were in different preparations, which included liquids, powders, capsules, creams/lotions, and syrups. Screening of the presence of aflatoxins and fumonisins were done using Envirologix Quick Tox™ Kit following the manufacturer's instructions. Fumonisins and aflatoxins concentration in parts per billion (ppb) was analyzed statistically using Pearson Chi square test at 95% confidence interval. Contaminations were presented in form of frequencies and percentages. Aflatoxins were detected in 74(53.6%) samples while fumonisins were detected in 75(54.3%). Four (11.8%) samples from herbal clinics and 3(4.1%) from street vendors in form of powders and liquids had aflatoxins levels above 4.0ppb. Nine (26.5%) samples from herbal clinics, 10(13.5%) from streets vendors/hawkers and two (10.5%) from the supermarkets in form of powders and liquids had fumonisins levels above 4.0ppb. There was no significant association ($\chi^2$ test; $p>0.05$) between detection of fungi from an herbal product and the occurrence of mycotoxins. *Aspergillus flavus* and *A. parasiticus* isolated in this study were associated with occurrence of aflatoxins while the *Fusarium* isolated were responsible for the presence of fumonisins among the herbal products. We conclude that herbal products investigated were contaminated with fumonisins and aflatoxins in varying degrees. Some were contaminated beyond the accepted limits. There were many genera of molds isolated in this study, which are associated with mycotoxins production, but the current study only focused on aflatoxins and fumonisins and therefore other mycotoxins should be investigated so as to ensure overall fungi toxins safety among the herbal products.
**Key words:** aflatoxin, fumonisins, contamination, herbal medicinal products, Aspergillus, Fusarium, Nairobi.

## 1. Introduction

Aflatoxins and fumonisins are referred as Mycotoxins. They are secondary metabolites of some moulds which are highly toxic, mutagenic or teratogenic compounds. These substances are not formed by all mould species but are characteristic of their producers. The occurrence of toxigenic mycoflora and mycotoxins in medicinal plants and herbal products has been reported by Alwakeel, (2009). *Aspergillus*, *Penicillium* and *Fusarium* are the major genera reported to produce mycotoxins (Rodriguez-Amaya and Sabino, 2002). Several environmental factors are reported to influence mycotoxin production, but temperature and humidity are considered to be the most critical (Simsek *et al.*, 2002).

Aflatoxins are secondary metabolites produced by various species of the genus *Aspergillus* which includes *A. flavus* and *A. parasiticus*. Sometimes the fungus is damaged by adverse conditions especially during processing of the products. However aflatoxins are not affected by processing because they are heat stable. The stability of aflatoxins during food processing is affected by many factors, including: the moisture of the product, the toxin concentration and its location and the presence of additives. These factors should therefore be considered with respect to each type of processing (e.g. milling, roasting, canning, oil extraction) when estimating the fate of the mycotoxin (Ezekwesili-Ofili *et al.*, 2014).

 

The prevalence of *Fusarium* and the subsequent production of fumonisins are enhanced by warm climates and under drought conditions, factors that cannot be controlled, although growers and consumers should be made aware that high concentrations of fumonisins are to be expected under such conditions (Dragan *et al.,* 2001). Insect damage also affects the accumulation of fumonisins, and both the prevalence and degree of insect damage are significantly correlated with the concentrations of the toxins (Korir *et al.,* 2017). The safe moisture content for natural product is 14–15%. Although mycotoxin contamination of foods and feed has been a worldwide problem as demonstrated by an estimate from the Food and Agriculture Organization (FAO) of the United Nations, recent focus has however been on contamination of medicinal herbal products (Simsek *et al.,* 2002). In the present study; we evaluated selected herbal medicinal products (HMPs) from Nairobi, Kenya, for the presence of aflatoxins and fumonisins contaminations and compared them with European pharmacopeia specifications.

## 2. Materials and methods

### 2.1 Study site and design

The study was undertaken in Nairobi, the capital and largest city in Kenya. Nairobi has several herbal clinics, especially in densely populated areas. However HMPs are also sold in nutrition stores, pharmacies/chemists, supermarkets, local retailers, and hawkers, among other outlets. This study employed an exploratory as well as laboratory-based experimental design.

### 2.2 Sample collection

We collected HMPs from different herbal vendors across Nairobi City in Kenya. The study sample included 138 different HMPs in different preparations, which included liquids, powders, capsules, creams/lotions, and syrups.

### 2.3 Determination of aflatoxins and fumonisins

Screening of the presence of aflatoxins and fumonisins were done using Envirologix Quick Tox™ Kit following the manufacturer's instructions. Briefly, the toxins were extracted from the sample with 70% methanol and shaken for 2 minutes and incubated for five minutes. Exactly 100 µl of the top layer were drawn and dispensed into a small vial and then mixed with 100µl buffer and mixed well before applying the strip and incubated for five minutes. The two lines in the rapid strip indicate a positive test while one line indicates a negative test. The strips were placed in a Quick Scan Machine-reader and the machine reads the concentrations of the toxins and displays the results on a computer screen in parts per billion (ppb). Two standards with 0.0025 ppb levels of aflatoxins and 0.004ppb of fumonisins were included in the analysis for quality control purposes.

### 2.4 Data analysis

We used Statistic Package for Social Scientist (SPSS) for statistical analysis (SPSS version 20) in this study. Fumonisins and aflatoxins concentration in parts per billion (ppb) was analysed statistically using Pearson Chi square test. Contamination was presented in form of frequencies and percentages. A significance level of 0.05 which is 95% confidence interval was used for all the tests.

## 3. Results

We collected and analysed 138 samples of HMPs. These samples included 106 powders (76.8%), 18 liquids (13.0%), 8 syrups (5.8%), 4 creams/lotions (2.9%), and 2 capsules (1.4%). Seventy-four of the samples (53.6%) came from street vendors/hawkers, 34 (24.6%) from herbal clinics, 19(13.8%) from supermarkets/shops, 7(5.1%) from manufacturers/wholesalers, and 2 each (1.4%) from chemists and health food stores, respectively.

### 3.1Determination of aflatoxins from the herbal products

The herbal products were contaminated with aflatoxin to varying degrees. They were detected in 74(53.6%) samples while in 64(46.4%) samples, their levels were below the limit of detection (Table 1). All the samples from the chemist had aflatoxin levels below the limit of detection (<LOD). Among the aflatoxins contaminated samples 67(48.6%) had aflatoxin contamination range from 0.001-4.00ppb. Only 7(5.1%) samples had aflatoxins levels above 4.0ppb. These samples were; 4(11.8%) from herbal clinics and 3(4.1%) from street vendors. The general safety limits for total mycotoxins in herbal products should not exceed 4 ppb which is equivalent to 4µg/g. Data analysis showed that there was significant association between samples from herbal clinics and street vendors with aflatoxins contaminations ($\chi^2$ test; p=0.001).

 

Table 2 shows that, 1(6.3%) liquid and six (5.8%) powders had aflatoxins levels above 4.0ppb. Seven (43.8%) liquids, 2(66.7%) creams/lotion, 48(45.2%) powders and 7(87.5%) syrups had aflatoxins levels below the limit of detection. Some samples had aflatoxins ranging from 0.001-4.00ppb. There was significant association between samples formulated in terms of powders and liquids with aflatoxins contaminations ($\chi^2$ test; p=0.001) as shown in Table 2.

**3.2 Relationship between fungi and aflatoxins**

Table 3 showed that, 44(59.5%) samples which were not contaminated with fungi were positive for aflatoxins. Thirty four (53.1%) fungal contaminated samples were also positive for aflatoxins. There was no significant association bewteen the presence of fungi and aflatoxins contamination among the herbal products ($\chi^2$ test; p=0.779).

**3.4 Determination of fumonisins from the herbal products**

Sixty-four (46.4%) samples had fumonisins levels below the limits of detection. All samples from health food stores, 16(47.1%) from herbal clinics, 3(42.9%) from manufacturers/wholesalers, 26(35.1%) from street vendors/hawkers and six (31.6%) from supermarkets had fumonisins ranging from 0.001-4.0ppb. Nine (26.5%) samples from herbal clinics, 10(13.5%) from streets vendors/hawkers and two (10.5%) from the supermarkets had fumonisins levels above 4.0ppb. Data analysis showed that there was significant associations among samples from herbal clinics, street vendors/hawkers and supermarkets/shops ($\chi^2$ test; p=0.001) with fumonisins contaminations (Table 4).

Table 5 shows that, 2(100.0%) capsules, 5(31.3%) liquids, 43(41.3%) powders and one cream/lotion, juice and syrups, respectively had fumonisins contamination ranging from 0.001-4.0ppb. Two (12.5%) liquids and 19(17.3%) powders had fumonisins levels above 4.0ppb. There was significant association between liquids and powders formulations with aflatoxins contaminations ($\chi^2$ test; p=0.001).

**3.5 Relationship between fungi and fumonisin**

Table 6 shows that, 36(47.4%) samples were not contaminated with fungi yet they were positive for fumonisins contamination. Thirty eight (62.3%) samples were contaminated with fungi and positive for fumonisins. There was no significant association between fungal contamination and the presence of fumonisins ($\chi^2$ test; p=0.081).

**4. Discussions**

Aflatoxin contaminations were determined among the sampled products in this study. The results showed that the herbal products were contaminated with aflatoxins to varying degrees. The general safety limits for total mycotoxins in herbal products should not exceed 4 ppb (µg/kg) according to European Pharmacopoeia (2007). Most (53.6%) samples were contaminated with aflatoxins although a few (5.16%) had aflatoxins levels beyond 4ppb hence were not safe for human consumptions. Four (11.8%) samples from herbal clinics and three (4.1%) from street vendors had aflatoxins levels above 4.0ppb. These samples from herbal clinics and street vendors/hawkers were contaminated with aflatoxins levels above the accepted limits. These contaminated samples were in form of powders and liquids formulations. Aflatoxins are secondary metabolites of some moulds, which include highly toxic, mutagenic or teratogenic compounds (Simsek *et al.*, 2002).

Aflatoxins were detected in 59.5% of the samples and yet they were free from fungi contamination while some (53.1%) of fungi contaminated samples had aflatoxins. In this case, aflatoxins were not detected in all the fungi contaminated samples likewise to the samples which were free from fungi contamination, aflatoxins were detected in some. For the samples free from fungi yet aflatoxins were detected could be because fungi were destroyed during herbal products processing. The study found that there was no significant association between ($p<0.05$) the presence of fungi and aflatoxins contamination. The occurrence of aflatoxins and other mycotoxins in herbal medicines as observed in the present study has been reported previously (Kneifel *et al.*, 2005; Sewaram *et al.*, 2006; Pavlovic *et al.*, 2006; Alwakeel, 2008). The mycotoxins pose great concern over consumer safety. Adriana *et al.*, (2006) in a study on fungal contamination of herbal products found that 49% of the isolates produced high levels of aflatoxins. Alwakeel, (2009) in a similar study reported the occurrence of toxigenic mycoflora and mycotoxins in medicinal plants and herbal products. The effects of mycotoxins especially aflatoxins on various organs have been reported in both experimental animals and man (Alwakeel, 2009). Impaired liver, kidney and brain function are well known consequences of ingesting even minute quantities of aflatoxins. Some studies claim that exposure to high levels of aflatoxins can lead to neurological problems and in

 

some cases may lead to death (Kneifel *et al.*, 2002; Sewaram *et al.*, 2006; Pavlovic *et al.*, 2006; Alwakeel, 2008). Since they are generally regarded as indestructible in all contaminated consumable items, they cannot be removed or destroyed, so prevention is the only real way forward. Aflatoxins are both hepatotoxic and hepatocarcinogenic (Orsi *et al*, 2007). Prolonged exposure, (especially daily home exposure), may be particularly harmful (Ezekwesili-Ofili *et al.*, 2014).

Although not all fungi produce aflatoxins, *Aspergillus flavus* and *A. parasiticus* are the two major genera reported to produce toxigenic aflatoxins according to Riba *et al.*, (2008). *Aspergillus flavus* and *A. parasiticus* have been reported to have the ability to produce aflatoxins in other previous studies (Tassaneeyakul *et al.*, 2004; Korir and Bii, 2012). Samples in the current study were contaminated with saprophytic fungi, pathogenic/mycotoxin producing fungi as well as pathogenic yeasts. Several environmental factors are reported to influence aflatoxins production, but temperature and humidity are considered to be the most critical according to a study by Simsek *et al.*, (2002). The prevalent weather conditions of Nairobi City where the herbal products were sampled from, are a predisposing factor for fungal growth which eventually produce aflatoxins and other mycotoxins as secondary metabolites.

Fumonisins are mycotoxins produced by fungi of the genus *Fusarium*. *Fusarium oxysporum*, *Fusarium moliniform, Fusarium solani* and *Fusarium spp* were isolated in this study and are associated with fumonisins contamination among the samples. Samples had fumonisins levels in varying degrees. Twenty one samples had fumonisins levels above 4.0ppm hence beyond accepted limits. Nine samples from herbal clinics, ten from streets vendors/hawkers and two from the supermarkets had fumonisins levels above 4.0ppb. These products were in form of liquids and powders. The presence of Fumonisins and fungal contamination among the samples showed that 47.4% of the samples were free from fungal contamination yet they had fumonisins contamination. Some (62.3%) were contaminated with both fungi and Fumonisins. Statistical analysis revealed that there was no significant association ($\chi^2$ test; p=0.081) between fumonisin detection and fungal contamination among the samples interms of sources/formulations.

In both liver and kidney, fumonisin B-induced toxicity is often characterized; initially by increased apoptotic and oncotic necrosis, regeneration and in the case of liver, bile-duct hyperplasia (Bhandari *et al.*, 2001). Experimental evidence for synergistic interactions between aflatoxin $B_1$ and fumonisin $B_1$ (Gelderblom *et al.*, 1999; WHO, 2000a; Carlson *et al.*, 2001) and between aflatoxin $B_1$ and nivalenol (Ueno *et al.*, 1992; Cohen *et al.*, 2000) in inducing hepatic cancer in rats has been reported. According to WHO (2000b) an association has been established between the occurrence of the *Fusarium verticillioides*, *Fusarium moniliforme* and other Fusarium mycotoxins on maize and the incidence of oesophageal cancer in various regions of the world. In this study, Fusarium producing mycotoxins were detected hence, the products are not safe for human consumption to some degree.

## 5. Conclusion

Samples were contaminated with Fumonisins and Aflatoxins at varying degrees. Among the samples, 15.2% were contaminated with fumonisins beyond the accepted European Pharmacopoeia accepted limits while 5.2% had aflatoxins levels beyond this limits (4ppb) hence were not safe for human consumptions. Herbs from street vendors and herbal clinics, which were in form of powders and liquids, were highly contaminated (beyond 4.0ppb). There was no significant association between the presence of fungi and aflatoxins/fumonisin contamination among the herbal product ($\chi^2$ test; p=0.779 and $\chi^2$ test; p=0.081, respectively).

## 6. Recommendation

There were varieties of molds isolated in this study, which are associated with mycotoxins production, the current study only focused on aflatoxins and fumonisins and therefore other mycotoxins should be investigated so as to ensure overall fungi toxins safety among the herbal products.

**Competing interests:** We the authors declare that there is no conflict of interest regarding publication of this paper whatsoever.

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**List of Tables**

**Table 1: Association between aflatoxin contamination and source of herbal products**

| Source | Aflatoxin Contamination Level (ppb) | | | n/Freq | $\chi^2$ |
|---|---|---|---|---|---|
| | <LOD | 0.001-4.0 | >4.000 | | |
| Chemist | 2(100.0%) | 0(0.0%) | 0(0.0%) | 2 | |
| Health Food Store | 0(0.0%) | 2(100.0%) | 0(0.0%) | 2 | |
| Herbal Clinic | 8(23.5%) | 22(64.7%) | 4(11.8%) | 34 | 0.001 |
| Manufacturer/ Wholesaler | 5(71.4%) | 2(28.6%) | 0(0.0%) | 7 | 1.000 |
| Street vendor/ Hawker | 37(50.0%) | 34(45.9%) | 3(4.1%) | 74 | 0.001 |
| Supermarket/ Shop | 12(63.2%) | 7(36.8%) | 0(0.0%) | 19 | 1.000 |
| Total | 64(46.4%) | 67(48.6%) | 7(5.1%) | 138 | |

**Key:** LOD-Limit of detection, 0.001-4.00-fumonisin range, >4.000- fumonisin more than 4.0ppb, n-Number of samples per category, $\chi^2$ - Chi square test

**Table 2: Association between aflatoxin contamination and type of herbal formulation**

| Formulations | Aflatoxins Contamination Level (ppb) | | | n/Freq | $\chi^2$ |
|---|---|---|---|---|---|
| Capsules | 0(0.0%) | 2(100.0%) | 0(0.0%) | 2 | |
| Liquid | 7(43.8%0 | 8(50.0%) | 1(6.3%) | 16 | 0.001 |
| Cream/lotion | 2(66.7%) | 2(33.3%) | 0(0.0%) | 3 | .100 |
| Juice | 0(0.0%) | 2(100.0%) | 0(0.0%) | 2 | |
| Powder | 48(45.2%) | 52(49.0%) | 6(5.8%) | 106 | 0.00 |
| Syrup | 7(87.5%) | 1(12.5%) | 0(0.0%) | 8 | 1.001 |
| Total | 64(46.4%) | 67(48.6%) | 7(5.1%) | 138 | |

**Key:** LOD-Limit of detection, 0.001-4.00-fumonisin range, >4.000- fumonisin more than 4.0ppb, n-Number of samples per category, $\chi^2$ - Chi square test

**Table 3: Association between fungal contamination and aflatoxins**

| Samples | Aflatoxins Detection | | | Statistics | | |
|---|---|---|---|---|---|---|
| Contamination | >LOD (n) (%) | With toxins (n) (%) | Total (n) (%) | $\chi^2$ | df | p |
| No Fungi | 30 (40.5) | 44 (59.5) | 74 (100.0) | 0.078 | 1 | 0.779 |
| With Fungi | 30 (46.9) | 34 (53.1) | 64 (100.0) | | | |
| Total | 60 (43.5) | 78 (56.5) | 138 (100.0) | | | |

Key: $\chi^2$ - Pearson Chi-square Value, df – Degree of freedom, p-value, ppb-parts per billion, (n)-frequency, (%) - percentage

 

**Table 4: Fumonisins contamination of herbal product**

| Sources & formulations | Fumonisin Contamination Levels (ppb) | | | n/Freq | $\chi^2$ |
|---|---|---|---|---|---|
| | **<LOD** | **0.001-4.000** | **>4.000** | | |
| Chemist | 2(100.0%) | 0(0.0%) | 0(0.0%) | 2 | |
| Health Food Store | 0(0.0%) | 2(100.0%) | 0(0.0%) | 2 | |
| Herbal Clinic | 9(26.5%) | 16(47.1%) | 9(26.5%) | 34 | 0.001 |
| Manufacturer/ Wholeseller | 4(57.1%) | 3(42.9%) | 0(0.0%) | 7 | 1.000 |
| Street vendor/ Hawker | 38(51.4%) | 26(35.1%) | 10(13.5%) | 74 | 0.001 |
| Supermarket/ Shop | 11(57.9%) | 6(31.6%) | 2(10.5%) | 19 | 0.001 |
| Total | 64(46.4%) | 53(38.4%) | 21(15.2%) | 138 | |

**Key:** LOD-Limit of detection, 0.001-4.00-fumonisin range, >4.000- fumonisin more than 4.0ppb, n-Number of samples per category, $\chi^2$ - Chi square test

**Table 5: Fumonisins contamination of herbal product**

| Formulations | Fumonisin Contamination Levels (ppb) | | | n/Freq | $\chi^2$ |
|---|---|---|---|---|---|
| Capsules | 0(0.0%) | 2(100.0%) | 0(0.0%) | 2 | |
| Liquid | 9(56.3%) | 5(31.3%) | 2(12.5%) | 16 | 0.001 |
| Cream/lotion | 2(66.7%) | 1(33.3%) | 0(0.0%) | 3 | .100 |
| Juice | 1(50.0%) | 1(50.0%) | 0(0.0%) | 2 | |
| Powder | 44(41.3%) | 43(41.3%) | 19(17.3%) | 106 | 0.001 |
| Syrup | 7(87.5%) | 1(12.5%) | 0(0.0%) | 8 | 1.000 |
| Total | 64(46.4%) | 53(38.4%) | 21(15.2%) | 138 | |

**Key:** LOD-Limit of detection, 0.001-4.00-fumonisin range, >4.000- fumonisin more than 4.0ppb, n-Number of samples per category, $\chi^2$ - Chi square test

**Table 6: Relationship between fungi and fumonisin**

| Samples | Fumonisins | | | Statistics | | |
|---|---|---|---|---|---|---|
| Fungal Contamination | LOD (n) (%) | Detected (n) (%) | Total (n) (%) | $\chi^2$ | df | p |
| Not contaminated | 38 (52.6) | 38 (47.4) | 76 (100.0) | 3.035 | 1 | 0.081 |
| Contaminated | 23 (37.7) | 39 (62.3) | 62 (100.0) | | | |
| Total | 61 (46.0) | 77 (54.0) | 138 (100.0) | | | |

Key: $\chi^2$ - Pearson Chi-square Value, df – Degree of freedom, p-value, ppb-parts per billion, (n)-frequency, (%) - percentage