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*Original Research Article*

# Effects of Botanicals on Growth and Aflatoxin Production by *Aspergillus flavus* Infecting Maize

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ABSTRACT

Finding a cost-effective measure to prevent fungal deterioration of grains in the global world has become imperative. Effects of selected botanicals on growth and aflatoxin (AF) production by *Aspergillus flavus* (*A. flavus*) in maize were investigated. *A. flavus* isolated from field samples and stored samples of maize, collected in 18 batches of five varieties, were cultured with various edible plant: methanol extracts (*Annona muricata* and *Moringa oleifera)* and essential oils (*Cymbopogon citrates* and *Ocimum gratissimum*) onto mycological media using standard microbiological methods. Aflatoxins levels in all the maize batches were assayed using competitive enzyme immunoassay. The total natural aflatoxin was reduced to various levels by all the botanicals, with percentage reduction ranging from 0 to 91.8% for *Cymbopogon citratus*,7.1 to 88.3% for *Ocimum gratissimum*, 0 to 77.9% for *Moringa oleifera* and 23.3 to 90% for *Annona muricata*, which had the highest effect; the difference in percentage reduction was statistically significant ($p<0.05$). Similarly, in *in-vitro* studies using plate experiments, the various plant extracts showed significant reductions in counts/mean clearance zone diameter according to the extract concentration on the fungus. The botanicals studied significantly inhibited the growth of *A. flavus*; hence reduction in aflatoxin production.

***Keywords:*** Aspergillus species, Aflatoxin-contaminated food, *Cymbopogon citratus*, *Ocimium gratissimum*, *Annona muricata*, *Moringa oleifera*.

## Introduction

Fungi have been associated with man from ancient times. Among these fungi, *Aspergillus* was first described in 1729.[1,2] For over three centuries approximately 250 species have been recognized in the genus.[3,4] Some species of *Aspergillus* have negatively affected man in so many areas of life, both medically and commercially. Constant inhalation of spores of *Aspergillus* species had resulted in some predisposition of humans and other animals to Aspergillosis. Mechanism of toxin production by *Aspergillus* species in foods are still being studied as some *Aspergillus* species may also produce toxic compounds (Mycotoxins) which presents a serious health risk for both man and animals.[5,6]

There are different types of mycotoxins identified, with some being harmful to man and animals, e.g. zearalenone, fuminosins, aflatoxins, ochratoxins, vomitoxins, etc.[7-9]Aflatoxin was discovered in 1960 in England where close to 100,000 turkey poults died from aflatoxin-poisoning after eating peanut meal contaminated with *Aspergillus flavus*.[10-12] The discovery led to a growing awareness of the potential hazards (aflatoxicosis) of these substances as contaminants of food and feed; causing illness and even death in humans and animal.[10]

Maize (*Zea mays* L.), along with wheat (*Triticuma estivum* L.) and rice (*Oryza sativa* L.), are the primary staple food cereals in many countries.[13]

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The effective storage of these cereals and grains is being challenged with fungal contamination and the deterioration, globally. Pre and post harvested grains and cereals could be colonized by various species of *Aspergillus* when conditions are favourable, leading to deterioration and production of mycotoxins. Aflatoxin problem is grave world wild, and remedies are needed urgently as information is disjointed with lots of paucity. There is need to disseminate information about the dangers and management aspect of aflatoxins, especially on the use of some botanicals to prevent aflatoxin-contamination of foods and food quality. Today, there are strict regulations on chemicals and pesticides used during storage and harvesting. In recent years, the priority of scientists worldwide, therefore, is to find practical, cost-effective and non-toxic measures to prevent fungal deterioration of stored cereals and grains. The use of natural plant extracts provides an opportunity to avoid chemical preservatives, in order to formulate a safe management regimen towards aflatoxin problem in grains; hence this work aimed to feel the gap.

## Materials and Methods

*Study area and collection of maize*

The study area was Nigeria. Nigeria, based on geographical and cultural backgrounds, is divided into two regions: North (10.3010° N, 9.8237° E) and South (6.4584° N, 7.5464° E). Maize samples were collected differently from these regions during the two main seasons (dry and rainy seasons) in the field and upon storage. Eighteen batches of the five maize varieties (yellow, white, variegated, pop and mixed), weighing 1kg each, collected from different States, were used in this study. The distribution according to Northern and Southern Maize Varieties is shown in Table 1.

*Study periods*

The study period was from June 2017 to December 2019. The method of maize sample collection was done in such a way to give chances for

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positive cases. The sample collection periods and the number of maize samples collected are categorized as shown in Table 1; in addition, the distribution of maize batches sampled between 2017 and 2019 is shown in Table 2. Table 1 also shows the distribution according to number of the maize samples collected. Here the number of maize varieties collected was counted using this method: (number of maize seedlings implanted in a tube) × (number of tube cultures for each sample) × (number of times that particular maize was collected) e.g. 3 seeds were implanted in three culture tubes separately into 9 different collection times (3 x 3 x 9 = 81 seedlings).

This was done to create chances of positive samples.

*Mycological studies*

Fresh and dry ear corns were collected from fields and stored batches during pre-harvest and post-harvest periods for culturing respectively. Using a pair of dissecting forceps, the three maize seedlings were collected from each maize cob and the surface was sterilized with 0.1% sodium hypochlorite in a 250 mL pyrex beaker. These seedlings were rinsed in another 250 mL Pyrex beaker containing enough sterile water.

**Table 1:** Distribution according to Northern and Southern maize varieties and according to the maize samples collected

| Northern variety | No of samples | Stored | Field | Rainy season | Dry season |
|---|---|---|---|---|---|
| **Maize varieties** | | | | | |
| Dent | 6 | 5 | 1 | | |
| Semi flint | 1 | 1 | - | | |
| Pop | 1 | 1 | - | | |
| Sweet | 2 | 1 | 1 | | |
| Total | 10 | 8 | 2 | | |
| **Southern variety** | | | | | |
| Maize varieties | | | | | |
| Floury | 2 | - | 2 | | |
| Semi flint | 1 | 1 | - | | |
| Pop | 1 | 1 | - | | |
| Sweet | 4 | 2 | 2 | | |
| Total | 8 | 4 | 4 | | |
| **Grand total** | 18 | 12 | 6 | | |
| | | | | | |
| **Batch** | | | | | |
| M | | * | - | 90 | - |
| A | | - | * | 63 | - |
| H | | - | * | 63 | - |
| J | | - | * | 63 | - |
| N | | - | * | 18 | - |
| B | | - | * | - | 81 |
| C | | - | * | - | 81 |
| D | | - | * | - | 81 |
| E | | - | * | - | 36 |
| F | | * | - | - | 45 |
| G | | - | * | - | 45 |
| I | | * | - | - | 63 |
| K | | * | - | - | 90 |
| L | | * | - | - | 90 |
| O | | - | * | - | 54 |
| P | | * | - | - | 54 |
| Q | | * | - | - | 72 |
| R | | - | * | - | 54 |
| Total | | | | 297 | 846 |

Key: * = Collected from; - = Not collected from

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**Table 2:** Distribution of maize batches sampled between 2017 and 2019

| **Northern Maize Varieties** | | | **Southern Maize Varieties** | | |
|---|---|---|---|---|---|
| **Yellow maize varieties** | **Botanical name** | **Number of batches** | **Yellow maize varieties** | **Botanical name** | **Number of batches** |
| Sweet local maize | *Zea mays saccharata* | 2 | Sweet local maize | *Zea mays saccharata* | 3 |
| Yellow dent maize | *Zea mays indentata* | 3 | Semi flint maize | *Zea mays indurate* | 1 |
| Pop corn | *Zea mays everta* | 1 | Pop corn | *Zea mays everta* | 1 |
| **White maize varieties** | **Botanical name** | **Number of batches** | **White maize varieties** | **Botanical name** | **Number of batches** |
| Flint local maize | *Zea mays indurata* | 1 | Sweet local maize | *Zea mays saccharata* | 1 |
| White dent maize | *Zea mays indentata* | 3 | White flour maize | *Zea mays amylacea* | 2 |
| **Total No of batches** | | **10** | | | **8** |

Using a pre-sterilised inoculating needle, the implantation of seeds was done by pressing onto the Sabaroud agar slants (SDA-Biotec) and potato dextrose agar (PDA-Oxoid) each. These were incubated at room temperature (25$^{o}$C) for two weeks for each batch of collection. Each different colonial type was aseptically picked onto a freshly prepared SDA and PDA media and re-incubated before being subjected to full identification as described by Kwon-Chung and Bennett.[14]

*Preparation of maizesamples for measurement of Aflatoxin*
A representative sample each of the eighteen different batches of the samples in the study was triturated (pulverised) using the Victoria® manual grinding machine and the electronic blender machine and thoroughly mixed in a mixer. Two (2) grams of the ground sample was weighed into a screw cap glass vial, then 10 mL of methanol/distilled water (70/30; v/v) was added and mixed for 10 min at room temperature (25-27$^{o}$C) using a shaker. After this, the entire extract was filtered using a filter paper Whatman no 1. Then 100 µL of the filtrate was diluted with 600 µL of the dilution buffer. Finally, 50 µl of the diluted solution was added to each of the wells.

*Assay of natural occurrenceof aflatoxinin maize samples*
This was pretreatment screening carried out on the different maize samples.All the maize batches were screened for aflatoxin level using commercially prepared test kits RIDASCREEN®Aflatoxin Total and it was carried out according to the standard procedure outlined in the kit.

*Screening of Aspergillus flavus isolates for aflatoxigenic potentials*
This was carried out following the procedure according to Rashid *et al*.[15]*A. flavus* strains, isolated from the maize sample, were refreshed on PDA medium (i.e. subcultured) and these strains were tested for their toxin producing ability on sterilized rice (negative for Aflatoxin as determined by ELISA). One millilitre (1 mL) of spore suspension of *A. flavus* isolates, standardized at ($10^3$ spores/mL), was inoculated in 15 g sterilized rice in 250 mL flasks separately. This was done in triplicates: Uninoculated (only media); Inoculated (with a known standard aflatoxigenic *A. flavus*- controls); and then the test flask (with each *A.flavus* isolate per flask).The inoculated flask was fixed in thermostatically-controlled water bath shaker at 27±2 $^{o}$C for eight days. ELISA test was performed on 8$^{th}$ day to check the aflatoxin produced in the inoculated media and also in the uninoculated control. The methods used were according to Rashid *et al.*[15]

*Collection and identification of plant materials for extraction*
The fresh leaves of *Moringa oleifera* (*M. oleifera*), *Cymbopogon citratus* (*C. citratus*) i.e. lemon grass, *Ocimum gratissimum* (*O. gratissimum*) i.e. scent leaves and *Annona muricata (A. muricata*) i.e. sour sop; were collected from two local government areas, each in Anambra State and Enugu State, Southeast Nigeria, in April 2017; and identified by Ugwozor P.O. of Herbarium Section at Department of Botany and Plant Biotechnology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu. A voucher specimen (UNH 301$^{C}$) was deposited at the herbarium for future reference.

*Extraction of essential oils (EOs) and methanolic extracts from theplant leaves*
Methanol extraction of two plants leaves (*Annona muricata* and *Moringa oleifera*) were carried out using maceration method. For the extraction of EOs from leaves of *Cymbopogon citratus* and *Ocimium gratissimum*, 100 g of the respective dried leaves of the plants was placed in a 2-liter flask containing distilled water (200 mL) and subjected to hydrodistillation for 4 hr using Clevenger type apparatus. The distillate oil was collected, dried over anhydrous sodium sulfate and stored in dark sealed vials at 4°C until when needed for use.[16]

*Preparation of sample plants for extraction*
These plant *materials* (*Annona muricata* Linn and *Moringa oleifera Lam*) were air-dried in the laboratory for two weeks and then pulverized into powdered form using manual grinder (VICTORIA®CORN MILL) and electronic blender. This was done in order to increase the surface area of particles, so that the extraction process can be more effective and for easier solvent penetration in attracting phytochemical compounds contained in the cell.

*Methanol extraction*
Extraction method was by maceration according to Sukhdev-Swami *et al*.[17]The powdered plant materials *M. oleifera* (130.55 g) and *A. muricata* (70.0 g) leaves were each percolated in 1250 mL methanol in 5 liters capacity beaker, stoppered and kept for 24 hours with intermittent stirring. The percolates were filtered with (Whatman's No. 1) filter paper. The extracts were concentrated at 40℃ under reduced pressure using rotary evaporator (R110). The concentrated extracts were weighed; (*Moringa* leaf methanol extract 25.46 g) and (*Annona* leaf methanolic extract 15.99 g) using electronic sensitive balance (OHAUS® HZ-K300AH, MAX-300 g, d-0.01 g).

*Essential oils (EOs) extraction*
Clevenger apparatus was used to determine the volatile oil content in the sample and this was carried out according to Sukhdev-Swami *et al*.[17]

*Antifungal activities of EOs and methanol extracts of the selected plants*
Antifungal activities were tested by treatment of *Aspergillus flavus* isolates with EOs and methanolic plant extracts on plate media.

*Evaluation of antifungal effects of the essential oil (EO) and plant extracts*

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Different doses of the EOs were made: About 250 µl each was dissolved in Analar grade ethanol to give the final concentration of 50, 25, 12.5, 6.25, 3.125 and 1.56 % v/v with molten potato dextrose agar (PDA) which was allowed to cool to 45$^{o}$C. They were poured in plates (sterile 90 mm petri dishes) and allowed to solidify. A fungal disc of 5mm diameter from the periphery of a 7-day old culture, grown on PDA, was aseptically placed on the media. The culture was incubated at ambient temperature 28±2$^{o}$C for 7 days. Samples without oil treatment (plain PDA) were used as controls. Observed growths were measured horizontally, vertically and diagonally and mean taken. Controls were compared with test plates. A similar preparation was also done for plant extracts but dissolved in distilled water. A control incorporating only ethanol and PDA was also set up. This methodology was according to Brophy *et al*.[18]

Percentage growth was calculated as $Q = \frac{P}{Z} x100\%$

Percentage inhibition was calculated as $M = \frac{Z-P}{Z} x100\%$

Where,
P= Mycelial Redial Growth on test plate
Z= Mycelial Redial Growth on control plate

*Use of botanicals (phyto controls) to inhibit growth of A. flavus and aflatoxin (AF) production*
The quantitative measurement of aflatoxin production of the effects of botanicals on growth and aflatoxin production by *Aspergillus flavus* infecting maize was done using the methodology described by Faria *et al*.[19]

*Treatment of maize with EOs and methanolic plant extracts*
Seeds of maize cultivar (representatives), pre-sterilized with 1% sodium hypochlorite showing natural infection with toxigenic *A.flavus* (identified as a producer of aflatoxin), and which have been screened for aflatoxin positively, were treated with EOs and plant extracts which were reconstituted with sterile distilled water (with addition of surfactant; 1 mL of Tween-80 to extracts of sour sop to allow complete dissolution of extracts). The maize was treated at 2.4 mg/g of grains. Controls were the untreated grains. These grains were left in sterile petri dishes for 10 days at ambient temperature and then pulverized and analyzed for aflatoxin production using the method of natural aflatoxin screening using the commercially available test kit RIDASCREEN®Aflatoxin Total as described previously. Percentage inhibition was calculated for each set of treated grains as compared with the corresponding untreated naturally contaminated grains.

*Statistical analysis*
All generated data were subjected to statistical analysis using Statistical Package for Social Science (SPSS version 20.0, California Inc.). Data was compared using the Students't-test and one-way analysis of variance (ANOVA). Values of $p<0.05$ were considered statistically significant.

## Results and Discussion

Figure 1 shows the incidence of *Aspergillus flavus (A.flavus)* (aflatoxigenic & non-aflatoxigenic strains) isolated according to maize varieties. It was observed that the yellow maize had the highest incidence of non-aflatoxigenic *A. Flavus* (n=34; 54.8%) and aflatoxigenic *A. Flavus* (n=22; 35.5%) followed by the mixed variety with 1(1.6%) non-aflatoxicgenic *A. Flavus* and 3(4.8%) aflatoxigenic *A. flavus*. The least number of aflatoxigenic *A. Flavus* (n=1; 1.6%) was seen in the variegated variety. The pop variety had only 1(1.6%) non-aflatoxigenic *A. flavus* while the white variety had neither non-aflatoxigenic*A. flavus* nor the aflatoxigenic *A. flavus*. There was statistical significant difference between distribution of non-aflatoxigenic *A.flavus* and aflatoxigenic *A. flavus* in the yellow maize variety ($p < 0.05$) while this was not statistically significant in the three other varieties ($p > 0.05$).

Figure 2 shows the distribution of *A. flavus* according to maize type. It was observed that out of a total of 62(17.3 %) *A. flavus* isolated from the 1143 maize samples analysed, 53 (85.5 %) *A. flavus* isolates were from field samples while 9 (14.5 %) *A. flavus* isolates were from stored maize samples. Hence, there was a statistical significant difference between distribution of *A. flavus* isolates from field and stored samples ($p<0.05$). Amongst the field *A. flavus* isolates, 36 (58.1%) were non-aflatoxigenic while 17(65.4%) were aflatoxigenic*A. flavus*. There was a statistically significant difference between the proportion of aflatoxigenic *A. flavus* and non-aflatoxigenic *A. flavus* in the field samples ($p<0.05$). All the 9(34.6%) *A. flavus* isolates from stored samples were aflatoxigenic, there was no non-aflatoxigenic *A. flavus* isolated in the stored maize samples. In summary, a total of 36 (58.1%) non-aflatoxigenic *A. flavus* and 26(41.9 %) aflatoxigenic *A. flavus* isolates were encountered in the study.
Table 3 shows the growth inhibition M (mm) and the percentage of growth inhibition of *A. flavus* on *Cymbopogon citratus* in doubling concentrations of the EOs. Day 1 shows no growth but still in the standard measurement of the fungal agar block measuring 5mm. from Day 2 to Day 7 no growth was observed at concentrations of 50 %, 25 % and 12.5 % of EO rather there were complete inhibition (CI) of the *A. flavus*. But growth of *A. flavus* started to be observed at concentration of 6.25% increasingly from Day 2 to Day 5 and then becomes partially static on Days 6 and 7 when compared with the control tests. Table 4 shows the growth inhibition of *Aspergillus flavus* on *Ocimum gratissimum* and its corresponding percentage growth inhibition. It was observed that there was complete inhibition of *A. flavus* growth at concentrations of 50% and 25% unlike the EOs of lemon grass (*Cymbopogom citratus*) that showed complete inhibition from 50% to 12.5% concentration. Hence there was statistical significant difference ($p<0.05$) between the activity of the two EOs used. Also growth inhibition of *A. flavus* on *Ocimum gratissimum* showed lag phase control growth at 3.125% concentration of the EOs from days 3 to 6 before it started increasing in diameter again. Table 5 shows the growth inhibition M (mm) and the corresponding percentage growth inhibition M (%) of *A. flavus* on *Annona muricata* (sour sop). It was observed that there was complete inhibition (CI) at concentration of 50%, 25% and 12.5%. But *A. flavus* showed growth from concentrations of 6.25% increasingly to 1.56%, showing the lag phase of growth on days 5 and 6 at concentration of 6.25%. There was marked statistically significant difference between the growth inhibition from 12.5% (concentrations and above) and 6.25% (concentrations and below) ($p<0.05$). Table 6 shows the growth inhibition of *Aspergillus flavus* on *Moringa oleifera* and its corresponding percentage growth inhibition. It was observed that *Moringa oleifera* methanolic extracts inhibited the growth of *A. flavus*. Table 7 shows the total natural AF reduction according to maize specification using EO of *Cymbopogon citratus* (Lemon grass), EO of *Ocimum gratissimum* (scent leaf), methanolic extract of *Moringa oleifera* and methanolic extracts of *Annona muricata* (sour sop). For EO of *Cymbopogon citrates* (Lemon grass), it was observed that the highest percentage reduction was seen amongst the mixed species (91.8%) of the local sweet breeds followed by the Ezeabudu (90.8%) of the local sweet breeds and the least was seen amongst the Oka akpa (flour/dent) (0). This showed a statistically significant difference between percentage reduction in local sweet breeds and the four/dent maize varieties, ($p<0.05$). For EO of *Ocimum gratissimum* (scent leaf), it was observed that the highest percentage reduction was seen amongst the mixed species of the local sweet breeds (88.3 followed by the Ezeabudu's of the same local sweet breeds (85.6 %) and the least percentage reduction was seen amongs the Amiacha's of the same local sweet breeds (5.8%). There was no statistically significant difference in the percentage AF reduction between the EOs of *Ocimum gratissimum* and *Cymbopogon citratus* ($p>0.05$). For methanolic extract of *Moringa oleifera*, it was observed that the highest percentage reduction was seen amongst Oka akpa of the flour/dent (77.9%) followed by Igara species of the local sweet/flints breeds (74%). The least reduction was seen in the pioneer Hi-breed (0). There was a statistically significant difference in the percentage AF reduction between the methanolic extracts of *Moringa oliefera* and *Annona muricata* ($p<0.05$). For methanol extracts of *Annona muricata*

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(sour sop), it was observed that the highest percentage reduction was seen amongst the species of the local sweet breeds (90%) followed by the mixed species of the local sweet breeds (89.9%). While the least percentage reduction was seen among the Igara specie (sweet/flint) of the local breeds (23.3%). There was a statistically significant difference in the percentage reduction of the total natural AF between *Annona muricata* and that of *Moringa oleifera* ($p<0.05$).

Aflatoxin contamination of crops is a world-wide concern. Aflatoxins aredifurano-coumarin compounds that exist as food-borne secondary toxic-metabolites, as well as a type of mycotoxin produced by *Aspergillus flavus* group of fungi.[5]They are the most widely studied mycotoxins that are of economic and health importance because of their ability to contaminate human food and animal feeds, particularly maize. It is likely that significant additional losses will occur amongst the human population because of morbidity and premature death associated with the consumption of aflatoxins.

A total of 1143 maize species (comprising yellow, white, variegated mixed and pop) samples, from both field 414 (36.2%) and stored 729 (63.8 %) samples, were analysed mycologically. Crops are infected in the field by fungi and these field fungi persist and proliferate during storage when favourable conditions prevail. Therefore, fungi incidence is likely to be higher in badly stored grains than those on the field as observed in this work. The exposure of stored samples to the ubiquitous fungi and their spores to a mildly anaerobic condition would encourage more fungal growth than the aerobic conditions of field.[20,21] This might explain the higher fungi occurrence in stored samples than those from the field.

It was observed in this study that the most active inhibitor of fungal growth was *Annona muricata*, and followed by *Ocimum gratissimum* and *C. citratus*. The least inhibitor was *Moringa oleifera*. These activities could be attributed to the phytochemical constitutuents that are contained in these botanicals. This study agrees with the work of Kpadonou-Kpoviessi *et al.*[22] which reveals the chemical variation of essential oil constituents of *Ocimum gratissimum L* from Benin and impact on antimicrobial properties and toxicity against *Artemia salina leach*. The antimicrobial potency of *O. gratissimum* plants is associated with the secondary metabolite. This corroborates the work done by Abdullahin[23], which reveals the phytochemical constituents and antimicrobial and gram protectant activities of Clove basil (*Ocimum gratissimum L.*). Meanwhile, the least inhibitor was *Moringa oleifera*. This invariably showed very little potency in fungicidal activity but more of fungiostatic activity because its chemical constituents are deficient in active constituents of *O. gratissimum, C. citrates* and *A. muricata* that are fungicidal.[24]

In order to control the various levels of AF concentrations, some natural control methods, using phytoremediation (using extracts and EOs) was employed in this study. For *C. Citrates*, the highest percentage reduction in total AF concentrations was 91.8% found in the mixed species (flour/dent) followed by 90.8% in the Ezeabudu of local sweet breeds. The least percentage reduction was Oka akpa of flour/dent corn (Enugu Ogbete) "0". In using the EO of *O. gratissimum* to check the reduction level of AF, it was observed that 88.3% was the highest percentage reduction which was also seen in the mixed species (flour/dent) followed by 85.6% in Ezeabudu and the least percentage reduction was seen in the Oka amiacha 1 (5.8%) amongst the local sweet breeds. There was marked statistically significant difference between the pretreated samples and the post-treated maize samples in the two EOs used for AF reduction ($p<0.05$). This difference in efficacy may be due to reduced insect pest infestation and the nature of the type of storage facilities.

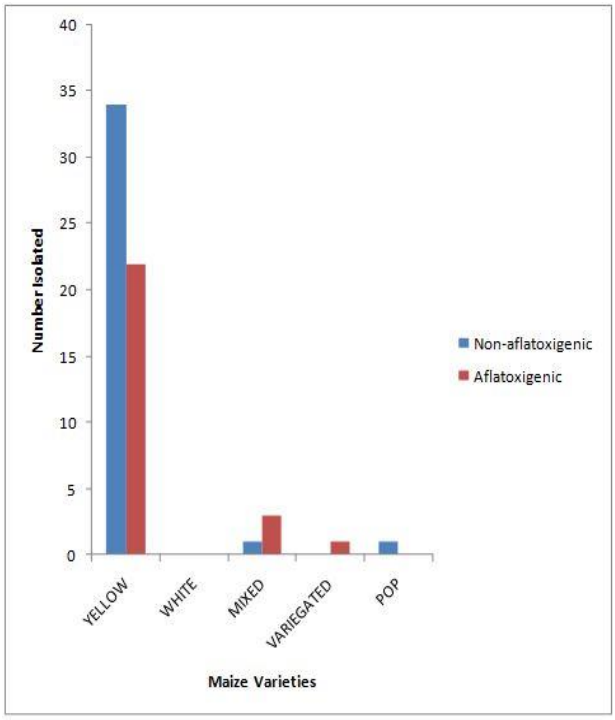


**Figure 1:** Incidence of *Aspergillus flavus* (Aflatoxigenicand Non-Aflatoxigenic Strains) Isolated According to Maize Varieties.

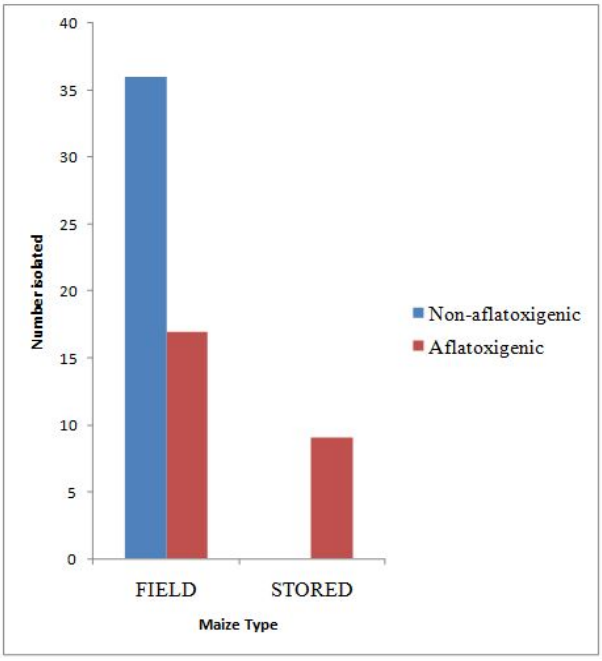


**Figure 2:** Incidence of *Aspergillus flavus* (Aflatoxigenicand Non-Aflatoxigenic Strains) Isolated According to Maize Type

**Table 3:** Growth inhibition M (mm) and Percentage growth inhibition M (%) of *Aspergillus flavus* on *Cymbopogon citratus*

| **Concentration (%)** | **Day** | **1** | **Day** | **2** | **Day** | **3** | **Day** | **4** | **Day** | **5** | **Day** | **6** | **Day** | **7** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M (mm) | M(%) | M (mm) | M(%) | M (mm) | M (%) | M (mm) | M(%) | M (mm) | M(%) | M (mm) | M(%) | M (mm) | M(%) |
| 50 | 5 | 0 | CI | CI | CI | CI | CI | CI | CI | CI | CI | CI | CI | CI |
| 25 | 5 | 0 | CI | CI | CI | CI | CI | CI | CI | CI | CI | CI | CI | CI |
| 12.5 | 5 | 0 | CI | CI | CI | CI | CI | CI | CI | CI | CI | CI | CI | CI |
| 6.25 | 5 | 0 | 10.2 | 38.6 | 13.4 | 47.7 | 13.9 | 56 | 14.1 | 65 | 14.3 | 70.1 | 14.3 | 74.5 |
| 3.125 | 5 | 0 | 13.3 | 19.9 | 15.1 | 41 | 15.5 | 50.9 | 17.6 | 56.3 | 16.9 | 64.6 | 17.1 | 69.5 |
| 1.56 | 5 | 0 | 18.2 | 0 | 19.6 | 23.4 | 21.2 | 32.9 | 21.9 | 45.7 | 24 | 49.8 | 24.2 | 56.9 |
| **Control** | **5** | **100** | **16.6** | **100** | **25.6** | **100** | **31.6** | **100** | **40.3** | **100** | **47.8** | **100** | **56.1** | **100** |

**Table 4:** Growth inhibition M (mm) and Percentage growth inhibition M (%) of *Aspergillus flavus* on *Ocimum gratissimum*

| **Concentration (%)** | **Day** | **1** | **Day** | **2** | **Day** | **3** | **Day** | **4** | **Day** | **5** | **Day** | **6** | **Day** | **7** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M (mm) | M(%) | M (mm) | M(%) | M (mm) | M (%) | M (mm) | M(%) | M (mm) | M(%) | M (mm) | M(%) | M (mm) | M(%) |
| 50 | 5 | 0 | CI | CI | CI | CI | CI | CI | CI | CI | CI | CI | CI | CI |
| 25 | 5 | 0 | CI | CI | CI | CI | CI | CI | CI | CI | CI | CI | CI | CI |
| 12.5 | 5 | 0 | 4.7 | 71.7 | 4.9 | 80.9 | 5.4 | 82.9 | 6.2 | 84.6 | 6.1 | 87.2 | 6.3 | 88.8 |
| 6.25 | 5 | 0 | 6.1 | 63.3 | 6.4 | 75 | 6.9 | 78.2 | 7.1 | 82.4 | 8.4 | 82.4 | 9.8 | 82.5 |
| 3.125 | 5 | 0 | 10.3 | 38 | 10.7 | 58.2 | 10.7 | 66.1 | 10.7 | 73.4 | 10.7 | 77.6 | 10.9 | 80.6 |
| 1.56 | 5 | 0 | 13.8 | 16.9 | 15.2 | 40.6 | 18.6 | 41.1 | 21.1 | 47.6 | 24 | 49.8 | 25.7 | 54.2 |
| **Control** | **5** | **100** | **16.6** | **100** | **25.6** | **100** | **31.6** | **100** | **40.3** | **100** | **47.8** | **100** | **56.1** | **100** |

**Table 5:** Growth inhibition M (mm) and Percentage growth inhibition M (%) of *Aspergillus flavus* on *Annona muricata*

| **Concentration (%)** | **Day** | **1** | **Day** | **2** | **Day** | **3** | **Day** | **4** | **Day** | **5** | **Day** | **6** | **Day** | **7** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M (mm) | M(%) | M (mm) | M(%) | M (mm) | M (%) | M (mm) | M(%) | M (mm) | M(%) | M (mm) | M(%) | M (mm) | M(%) |
| 50 | 5 | 0 | CI | CI | CI | CI | CI | CI | CI | CI | CI | CI | CI | CI |
| 25 | 5 | 0 | CI | CI | CI | CI | CI | CI | CI | CI | CI | CI | CI | CI |
| 12.5 | 5 | 0 | CI | 71.7 | CI | 80.9 | CI | 82.9 | CI | 84.6 | CI | 87.2 | CI | 88.8 |
| 6.25 | 5 | 0 | 5.2 | 63.3 | 5.5 | 75 | 5.5 | 78.2 | 5.8 | 82.4 | 5.9 | 82.4 | 6.1 | 82.5 |
| 3.125 | 5 | 0 | 6 | 38 | 6.4 | 58.2 | 6.7 | 66.1 | 6.9 | 73.4 | 7.2 | 77.6 | 7.6 | 80.6 |
| 1.56 | 5 | 0 | 7.2 | 16.9 | 7.7 | 40.6 | 7.9 | 41.1 | 8.6 | 47.6 | 9.2 | 49.8 | 10.6 | 54.2 |
| **Control** | **5** | **100** | **37.4** | **100** | **38.9** | **100** | **42.9** | **100** | **48.6** | **100** | **49.3** | **100** | **53.7** | **100** |

**Table 6:** Growth inhibition M (mm) and Percentage growth inhibition M (%) of *Aspergillus flavus* on *Moringa oleifera*

| **Concentration (%)** | **Day** | **1** | **Day** | **2** | **Day** | **3** | **Day** | **4** | **Day** | **5** | **Day** | **6** | **Day** | **7** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M (mm) | M(%) | M (mm) | M(%) | M (mm) | M (%) | M (mm) | M(%) | M (mm) | M(%) | M (mm) | M(%) | M (mm) | M(%) |
| 50 | 5 | 0 | 14.4 | 61.5 | 20 | 48.6 | 22.9 | 46.6 | 23.7 | 51.2 | 24.1 | 51.1 | 24.9 | 53.6 |
| 25 | 5 | 0 | 18.2 | 51.3 | 20.8 | 46.5 | 24.6 | 42.7 | 24.7 | 49.2 | 25.5 | 48.3 | 27.2 | 49.3 |
| 12.5 | 5 | 0 | 20.2 | 46.5 | 26.4 | 32.9 | 27.5 | 35.9 | 28.9 | 40.5 | 29.8 | 39.6 | 32.1 | 40.2 |
| 6.25 | 5 | 0 | 20.7 | 44.7 | 28.4 | 27 | 28.9 | 32.6 | 32.4 | 33.3 | 35.3 | 28.4 | 37.6 | 30 |
| 3.125 | 5 | 0 | 22.2 | 40.6 | 30.1 | 22.6 | 33.3 | 22.4 | 35.6 | 26.7 | 38.9 | 21.1 | 41.7 | 22.3 |
| 1.56 | 5 | 0 | 27.4 | 26.7 | 39.6 | 0 | 41.8 | 2.6 | 44.4 | 8.6 | 48.7 | 1.2 | 50.9 | 5.2 |
| **Control** | **5** | **100** | **37.4** | **100** | **38.9** | **100** | **42.9** | **100** | **48.6** | **100** | **49.3** | **100** | **53.7** | **100** |

**Table 7:** Total natural aflatoxin (AF) (ng/kg) reduction according to maize specification using EO of *Cymbopogon citratus* (lemon grass), EO of *Ocimum gratissimum* (scent leaf),methanolic extract of *Moringa oleifera* and methanolic extracts of *Annona muricata* (sour sop)

| | **EO of *C. citratus*** | | | **EO of *O. Gratissimum*** | | | **ME of *M. oleifera*** | | | **M.E of *A. muricata*** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Maize type** | **Total AF (ng/kg) in pre-treated sample** | **Total AF (ng/Kg) in post-treated sample** | **(%) AF reduction** | **Total AF(ng/kg) in pre-treated sample** | **Total AF (ng/kg) in post-treated sample** | **(%) AF reduction** | **Total AF (ng/kg) in pre-treated sample** | **Total AF (ng/kg) in post-treated sample** | **(%) AF reduction** | **Total AF (ng/kg) in pre-treated sample** | **Total AF (ng/kg) in post-treated sample** | **(%) AF reduction** |
| **yellow dent** | | | | | | | | | | | | |
| yellow maize 1 | 5250 | 3850 | 26.7 | 5250 | 3150 | 40 | 5250 | 3325 | 36.7 | 5250 | 2345 | 55.3 |
| yellow maize 2 | 5215 | 4410 | 15.4 | 5215 | 3125 | 40.1 | 5215 | 2765 | 47 | 5215 | 1785 | 65.8 |
| yellow maize 3 | 2450 | 1890 | 22.9 | 2450 | 1750 | 28.6 | 2450 | 2380 | 2.9 | 2450 | 1470 | 40 |
| **(sweet/flint)** | | | | | | | | | | | | |
| **white dent** | | | | | | | | | | | | |
| white maize 1 | 4550 | 3220 | 29.2 | 4550 | 3150 | 30.1 | 4550 | 2975 | 34.6 | 4550 | 1925 | 57.7 |
| white maize 2 | 9450 | 6300 | 33.3 | 9450 | 2800 | 70.4 | 9450 | 5950 | 37 | 9450 | 3850 | 59.2 |
| white maize 3 (**flour)** | 9100 | 6475 | 28.8 | 9100 | 7700 | 15.4 | 9100 | 2905 | 68.1 | 9100 | 5250 | 42.3 |
| **pop** | | | | | | | | | | | | |
| pop corn 1 | 7700 | 5600 | 27.3 | 7700 | 5950 | 22.7 | 7700 | 5425 | 29.5 | 7700 | 4200 | 45.5 |
| pop corn 2 | > | 11200 | | > | 16800 | | > | 19250 | | > | 9100 | |
| **local breeds** | | | | | | | | | | | | |
| **(sweet)** | | | | | | | | | | | | |
| okaamiacha 1 | 14000 | 11200 | 20 | 14000 | 13195 | 5.8 | 14000 | 4900 | 65 | 14000 | 8400 | 40 |
| okaamiacha 2 | 7000 | 4200 | 40 | 7000 | 5740 | 18 | 7000 | 3325 | 52.5 | 7000 | 3255 | 53.5 |
| okaigbo | > | 14000 | | > | 19950 | | > | 35000 | | > | 9100 | |
| okabende**(flour)** | 8050 | 5775 | 28.3 | 8050 | 7000 | 13 | 8050 | 3325 | 58.7 | 8050 | 3850 | 52.2 |
| ezeabudu**(sweet)** | 112000 | 10325 | 90.8 | 112000 | 16100 | 85.6 | 112000 | 47250 | 57.8 | 112000 | 11200 | 90 |
| igara**(sweet/flint)** | 10500 | 7700 | 26.7 | 10500 | 9345 | 11 | 10500 | 2730 | 74 | 10500 | 8050 | 23.3 |
| **market** | | | | | | | | | | | | |
| okaakpa**(flour/dent)** | 9800 | 9800 | 0 | 9800 | 9100 | 7.1 | 9800 | 2170 | 77.9 | 9800 | 5600 | 42.9 |
| mixed species | 125300 | 10220 | 91.8 | 125300 | 14700 | 88.3 | 125300 | > | | 125300 | 12600 | 89.9 |
| **(flour/dent)** | | | | | | | | | | | | |
| **hibreeds (semi flint)** | | | | | | | | | | | | |
| pioneer hi-breed 1 | 9450 | 4900 | 48.1 | 9450 | 7000 | 25.9 | 9450 | 7350 | 22.2 | 9450 | 4025 | 57.4 |
| pioneer hi-breed 2 | 9450 | 5425 | 42.6 | 9450 | 7350 | 22.2 | 9450 | 11795 | 0 | 9450 | 4200 | 55.6 |

**Key:** EO of *C. citratus* = EO of*Cymbopogoncitratus* (lemon grass); EOof*O. gratissimum* (scent leaf) = EOof*Ocimumgratissimum* (scent leaf); ME of *M. Oleifera* = *M*ethanolic extract of *Moringa oleifera*
M.E *A. muricata* = Methanolic extract of *Annonamuricata;* > represents undetectable above the rang

## Conclusion

Fungal pathogens and also aflatoxigenic *A. flavus* occur more in field than in stored maize samples.Plant extracts; methanol extracts of *Annona muricata*, essential oils of *Cymbopogon citratus*, essential oils of *Ocimum gratissimum* and methanolic extracts of *Moringa oleifera* were able to significantly inhibit *A. flavus* growth hence aflatoxin production. Both the extracts were able to decontaminate an already existing aflatoxin in the maize samples in varying degrees. In many instances low concentrations of test compounds stimulated fungal growth and/ or toxin production, while higher concentrations completely inhibited them.

## Conflict of interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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