Identification and control of specific aflatoxin-producing fungi in stored maize seeds in awka using *azadirachta indica* (neem) and *garcinia kola seeds*

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Abstract: Four fungal isolates were identified in this study of which three were *Aspergillus* species with *Aspergillus flavus* having the highest frequency followed by *A.* parasiticus. The result of high frequency of *Aspergillus flavus* and *Aspergillus parasiticus* in the *Zea mays* sample revealed production of aflatoxins. Maize sample in Awka was found to contain aflatoxin B1 (9.60ppb) and B2 (13.3ppb). Inhibition of *A. flavus* and *A. parasiticus* with *Azadirachta indica* and *Garcinia kola* seed extracts showed that the test plant extracts were effective for reducing mycelial growth on the test organism. Methanolic extract of *G. kola* showed antifungal inhibitory activity on the test organisms and the highest at 10% concentration. With ethanol extracts of *G. kola*, the antifungal activity was effective i.e. for inhibition of *A. flavus* and *A. parasiticus*, with *A. parasiticus* having the higher percentage inhibition at 10%. Inhibiting growth of *Aspergillus flavus* and *Aspergillus parasiticus* using methanolic and ethanolic extracts of neem seeds was effective in the inhibition of the test organism at 10%. The methanolic and ethanolic extracts of combined *Garcinia kola* and neem seeds revealed effective inhibition of *A. flavus* and *A. parasiticus* with ethanolic extracts of the combined test plants exerting the highest inhibition against *A. flavus* (80.43±3.62). The extracts from this plant show the ability to suppress growth of toxigenic *A. flavus* and *A. parasiticus*. Phytochemical analysis showed that the methanolic and ethanolic extracts of *G. kola* and neem seeds showed the presence of secondary metabolites and this may be a reason for the inhibitory activity on *A. flavus* and *A. parasiticus*. Results from this study will be important in planning a management strategy against aflatoxin-producing fungi and other fungi associated with spoilage of stored food products.

Keywords: Aflatoxin, *Aspergillus*, plant extracts, maize seeds.

INTRODUCTION

Aflatoxins are a group of highly poisonous, deadly and cancer-causing agents produced by *Aspergillus flavus* and *A. parasiticus* (Mawcha and Assefa, 2014). *Aspergillus flavus* is the prevalent fungus that produces aflatoxin in maize and cotton seeds while in groundnuts, *Aspergillus parasiticus* is more regular fungus to produce aflatoxins than in maize. Aflatoxins B1, B2, G1 and G2 are produced by strains of *Aspergillus parasiticus* while aflatoxins B1 and B2 are produced by *A. flavus* strains (Yan-ni *et al*., 2008). The fungi *Aspergillus flavus* Link, *A. parasiticus* Speare are the fundamental producers of aflatoxins and *A. nomius* to a smaller range (Hell and Mutegi, 2011). The word aflatoxin was derived from the genus *Aspergillus* 'A' and the species *flavus* 'fla' added to toxin. Aflatoxin B1, B2, G1, G2, M1 and M2 are toxins identified in the 18 diverse classes of toxin found in the aflatoxin family with aflatoxin B1 being the most toxic in the group (Theddeus, 2009). Aflatoxins B and G are categorized as group1 which causes cancer in man while aflatoxin M1 is categorized as group 2B and has a probability to cause cancer in man (Krishnamurthy and Shashikala, 2006). Bankole and Adebanjo (2003), Shephard (2003), Bankole *et al*. (2006), Wagacha and Muthomi, (2008) reported that the weather conditions in some parts of Africa make one conducive for growth of fungi and production of deadly compounds and this makes aflatoxins to thrive and cause food spoilage across the continent. This problem is aggravated as a result of infestation by pest, drought, poor aeration and immoderate heat enhancing the production and dissemination of fungi in the environment. Consequently, to produce good quality and quantity food crops, various applications have been introduced to reduce the effect on food shortage (Hell *et al*., 2008). Some authors have reviewed the efficacy of some common plants to control these fungi and have found them efficient (Hsieh *et al*., 2001). In the past, fungicides and pesticides have been used to control fungi and pests of food crops, but their usage has been discouraged because of being non-biodegradable, causing adverse effect to man's health and environment (Unnikrishnan and Nath, 2002). Therefore, a more suitable approach need to be introduced that will preserve food crops and also pose an insignificant danger to humans and the environment (Phongpaichit *et al.,* 2005). Some plants that are used to cure disease have been found to be curative not only to humans but also for

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plant diseases and so are used as bio-fungicides to replace synthetic chemicals. The use of plant extracts screened for antifungal properties as against synthetic chemicals has brought a remarkable improvement in managing fungi that affect food crops (Theddeus, 2009). Parekh and Chanda (2007), Ramkumar *et al*. (2007) reported that secondary metabolites of these medicinal plants and other free hydroxyl groups contribute as bio-fungicides. The ability of these plants to curb fungal invasion is the result of phytochemicals found in the plant (Nascimento *et al.*, 2000).

MATERIALS AND METHODS

Plant extracts used in the control of aflatoxin-producing fungi include: *Azadirachta indica* A. Juss (neem) seeds and *Garcinia kola* H. seeds procured from Awka market. The plants were identified by Mr Ugwuozor (Herbarium number: *Garcinia Kola*-NAUH27A, *Azadirachta indica* – NAUH14).

Media preparation and isolation

Sabouraud Dextrose Agar was used for the isolation of fungi associated with maize seeds and for the sub-culture, growth and maintenance of the fungal isolates. The SDA was prepared according to the manufacturer's prescription and autoclaved at 121ºC for 15 minutes 15psi (Cheesbrough, 2000 and Jawetz *et al*., 2004). All measurements were done using the electronic balance (Acculab Sartorius group 1809001). Direct isolation method was employed. Twenty relatively healthy maize seeds were dipped in 10% Sodium hypochlorite (NaOCl) solution for 10minutes and washed thrice with sterile distilled water and left to dry on sterile filter papers (Ritiche, 1991). Five maize seeds were inoculated on SDA (Petri) plates with three replicates and incubated at 28±2^{o}C for 5 days. The Petri plates were sealed with paraffin to prevent contamination.

Plant extract

Fresh and healthy seeds of *Azadirachta indica* A. Juss were de-pulped and washed with sterile distilled water while *Garcinia kola* H. seeds were peeled and sliced with a sterile blade. Moisture contents of these plant samples were removed at room temperature (25ºC), powdered using a manual grinder. Solvents for extraction included ethanol and methanol. Cold solvent extraction technique was employed (Harbone, 1973). Five hundred grams (500g) of each plant sample was dissolved in 1 litre of ethanol and methanol separately and left for 48 hours. The mixture was filtered and the filtrate was concentrated with a rotary evaporator at 75ºC (for ethanol) and 65ºC (for methanol). The crude extracts were collected in sterile sample bottles and stored at 4ºC.

Frequency of aflatoxin-producing fungi in maize seeds

The determination of frequency was a completely randomized design. Isolates were identified based on colony characterization, strain morphology and macroscopic features. The aflatoxin-producing fungi isolated from the maize seed were identified and their frequencies determined by calculating the percentage occurrence of each fungus. Other fungi found were also identified.

Antifungal analysis

Different concentrations of the test extracts were prepared by dissolving the extracts in the solvent; 25mg/10ml, 50mg/10ml, 75mg/10ml and 100mg/10ml. In every Petri dish, 1ml of each plant extract solution was distributed and 9ml of sterile SDA was added to each of the extracts to correspond to 2.5%, 5.0%, 7.5% and 10.0% extract concentration. Petri dishes were placed on a shaker for 10 minutes for even dispersion. At the middle of the Petri dishes was placed a five millimetre (5mm) disc of 5-day old pure culture of each test fungus and maintained at 28±2ºC. The negative control set up consisted of singly agar plates while the positive control consisted of the solvents mixed with the agar. Completely Randomized Design (CRD) was the experimental design applied and was replicated thrice. All plates were sealed with paraffin and the growth along the radius was taken in millimetre for five days. Two pre-drawn perpendicular lines were made at the back side of the plates and the fungal growth was taken as the mean along the two lines.

According to Whipps (1987), the percentage inhibition was calculated as:

$$\% \text{ inhibition} = \frac{R1-R2}{R1} \times 100$$

Where R_1 is the farthest growth along the radius of fungus in the control plates

R_2 is the farthest growth along the radius of fungus in experimental plates

To get the minimum inhibition concentration (MIC), the inhibition percentage was set as a guideline to choose the best plant extract concentration that will be effective in controlling the test fungi. This was done using the method of Sangoyomi (2004).

≤ 0% no inhibition
> 0-20% slight inhibition
> 20-50% moderate inhibition
> 50-100% inhibition (effective)
100% high degree inhibition

Aflatoxin analysis

Thin layer chromatography method was used for aflatoxin analysis. Twenty grams (20g) of the powdered maize sample was dissolved in 100 millilitres (ml) of 70% methanol and placed on a shaker (VWR, DS2-5002 Orbital Shaker) for 30 minutes. The mixture was filtered into a separating funnel and 20ml of de-ionized water was added into the filtrate followed by 25ml of absolute di-chloromethane. The organic layer was collected into sterile beakers over 5g of anhydrous sodium sulphate. Ten milliliter of di-chloromethane was added to the separating funnel and the procedure was repeated. The organic solvent was placed under the fume cupboard for 24hours after which the beaker was washed with 100µl of di-chloromethane, vortexed and dispensed in sterile micro bottles. To quantify the toxin content in the sample, the sample was spotted on thin layer chromatography (TLC) plates and placed in the developing machine. The dried TLC plates were then placed under the UV Scanner (Lamag Spectrolite Model ENE-260C/FE NEW YORK, USA) for qualitative analysis for the presence of toxins and quantitatively under the TLC Scanner3 027.6481 Switzerland (read in ppb). The B1, B2, G1 and G2 constitute bands that reflect aflatoxins.

Phytochemical Screening

Crude extracts of the test plants used in this study were analyzed qualitatively and quantitatively for presence of alkaloids, tannins, saponins, flavonoids, terpenoids, steroids, cardiac glycosides, phlobatannis and reducing sugars following the method of Jeyaseelan, *et al*., 2012.

STATISTICAL ANALYSIS

Data were analyzed using ANOVA via SPSS and least significance difference (LSD) was used to separate means at p value ≤ 0.05.

RESULTS

Identification of fungal isolates

Fungi isolated from the maize sample included: *Aspergillus flavus*, *A. parasiticus*, *A. niger* and *Fusarium oxysporum*. Pure cultures of *A. flavus* formed yellow colonies on SDA (fig. 1a.) and the morphological features revealed that *A. flavus* forms mycelia that are white with spreading yellow colonies. The microscopic characteristics of the fungus include long non-septate cells borne on the hyphae (fig. 1b.)

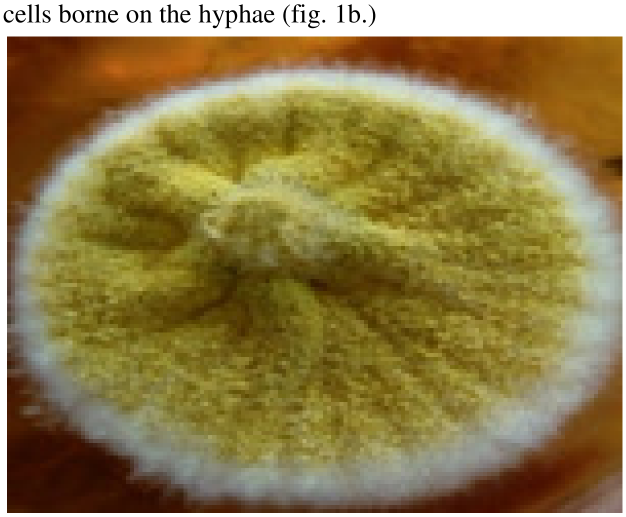

Fig. 1a: Culture plate of *A. flavus*

The pure culture of *A. parasiticus* showed morphological features that revealed greenish mycelia (fig. 2a.) and the microscopic characteristics included spore cells that are wide and globose (fig. 2b.).

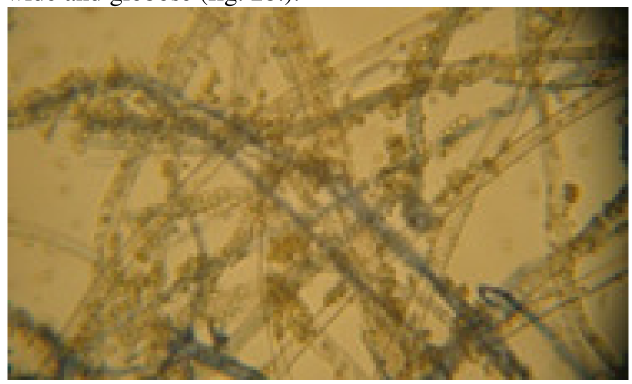

Fig. 1b: *A. flavus* mycelia

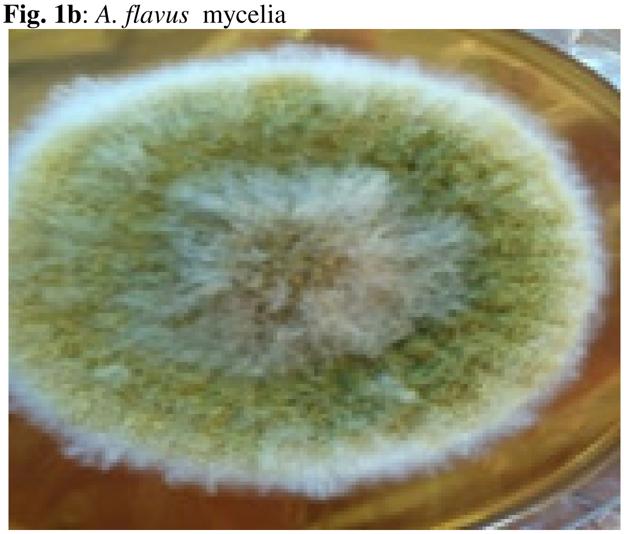

Fig. 2a: Culture plate of *A. parasiticus*

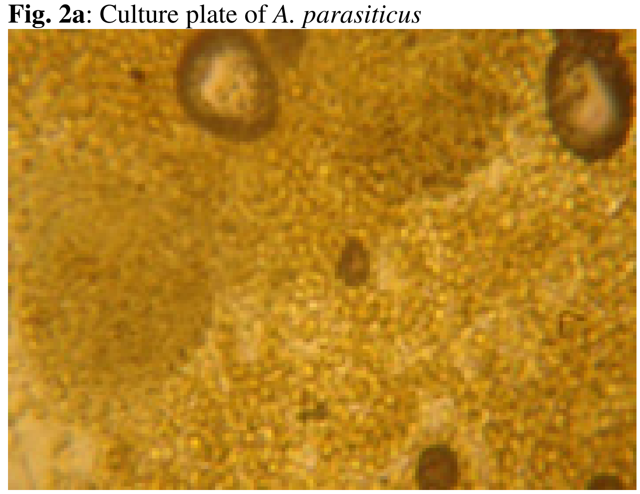

Fig. 2b: *A. parasiticus* spores

Percentage occurrence of fungi from maize seeds

Aspergillus flavus, A. parasiticus, A. niger and *Fusarium oxysporum* were fungal isolates associated with maize observed in this study. The most frequent fungi were *A. flavus* (88.8%) while *F. oxysporum* was isolated with 33.3 percentile (fig. 3).

Table 1: Percentile inhibition concentration for methanol extract of *G. kola* seed against *A. flavus* and *A. parasiticus*

Concentration of Extract	% Inhibition	
	Aspergillus flavus (mm)	*Aspergillus parasiticus* (mm)
2.5%	45.61±8.60	43.81±5.77
5.0%	68.41±6.57	46.19±6.60
7.5%	73.68±3.64	53.33±0.83
10.0%	77.54±1.21	54.28±1.43
p-value	**	ns

Table 2: Percentile inhibition concentration for ethanol extract of *G. kola* seed against *A. flavus* and *A. parasiticus*

Concentration of Extract	% Inhibition	
	Aspergillus flavus (mm)	*Aspergillus parasiticus* (mm)
2.5%	31.39±3.26	54.65±9.84
5.0%	38.16±1.56	65.50±2.81
7.5%	45.65±6.71	76.74±5.07
10.0%	53.62±1.72	79.45±5.85
p-value	ns	ns

Table 3: Percentile inhibition concentration for methanol extract of neem seed against *A. flavus* and *A. parasiticus*

Concentration of Extract	% Inhibition	
	Aspergillus flavus (mm)	*Aspergillus parasiticus* (mm)
2.5%	35.08±22.48	40.00±4.92
5.0%	54.73±12.93	30.47±9.30
7.5%	68.06±13.37	37.99±6.94
10.0%	64.91±5.40	54.76±3.60
p-value	ns	ns

Table 4: Percentile inhibition concentration for ethanol extract of neem seeds against *A. flavus* and *A. parasiticus*

Concentration of Extract	% Inhibition	
	Aspergillus flavus (mm)	*Aspergillus parasiticus* (mm)
2.5%	53.14±6.96	40.69±1.20
5.0%	57.00±1.09	43.41±3.74
7.5%	67.87±2.68	49.61±7.74
10.0%	75.11±4.43	61.84±6.70
p-value	ns	ns

Table 5: Percentile inhibition concentration for methanol extract of combined (*G. kola* and Neem) seed against *A. flavus* and *A. parasiticus*

Concentration of Extract	% Inhibition	
	Aspergillus flavus (mm)	*Aspergillus parasiticus* (mm)
2.5%	44.20±5.99	38.09±7.75
5.0%	55.78±4.72	39.04±5.16
7.5%	61.05±6.15	55.23±8.59
10.0%	69.82±2.65	54.76±5.41
p-value	ns	ns

Table 6: Percentile inhibition concentration of *A. flavus* and *A. parasiticus* by ethanol extract of combined (*G. kola* and Neem) Seed

Concentration of Extract	% Inhibition	
	Aspergillus flavus (mm)	*Aspergillus parasiticus* (mm)
2.5%	58.93±7.04	26.64±5.55
5.0%	69.56±3.16	44.18±4.57
7.5%	73.42±4.60	39.28±6.42
10.0%	80.43±3.62	48.06±4.47
p-value	**	ns

**$p <0.05$, ns: not significant, each result represents mean± std

Table 7: Summary of maize sample analyzed for aflatoxins B1, B2, G1, G2 (ppb)

Sample Code	B1	Aflatoxins(ppb) B2	Sum
EA-1	8.73	13.62	22.35
EA-2	9.54	12.72	22.26
EA-3	10.52	13.58	24.10
Average	9.60	13.31	

EA- maize from Awka

Table 8: Bioactive agents of methanol and ethanol extract of *Garcinia kola* and neem Seeds

Sample	Alkaloid	Saponin	Tannin	Flavonoid	Steroid	Terpenoids	Cardiac glycoside
Neem (methanol)	++	-	-	+	-	+++	++
Neem (ethanol)	+	+	-	-	-	-	++
Garcina kola (methanol)	+	+	+++	-	+	-	-
Garcina kola (ethanol)	++	-	++	-	+	-	+

Key: + =Trace amount, ++ =Moderately present, +++ =Abundantly present, - =Absent

Table 9: Mean phytochemical composition of test plant

	% Composition			
Test Plants	Tannins	Alkaloids	Flavonoids	Saponins
G. kola seeds	5.55±0. 071	14.8±0.000	1.9±0.028	10.5±0.014
Neem seeds	1.11±0.028	18.6±0.071	3.8±0.000	9.15±0.028
p-value	**	**	**	**

Results are in Mean ± Std, **p<0.05

Inhibition of test organism by different concentrations for methanol extracts of G. kola seed

Methanol extract of *G. kola* against A. *flavus* indicated that at 2.5% concentration of extract, *G. kola* showed moderate inhibition of *A. flavus* but inhibition was effective at 5.0%, 7.5% and 10.0% concentration (table 1). At 2.5% and 5.0% concentration, *G. kola* against *Aspergillus parasiticus* was moderate and effective at 7.5% and 10.0% concentration. In this study, it was observed that the concentrations of methanol extract of *G. kola* significantly inhibited the growth of *Aspergillus flavus* (p<0.05) but not of *Aspergillus parasiticus*.

Inhibition of test organism by different concentrations for ethanol extract of garcinia kola seed

Antifungal activity of ethanol extract of *G. kola* against *A. flavus* and *A. parasiticus* showed that at 2.5%, 5.0% and 7.5% concentration, *G. kola* showed moderate inhibition against *A. flavus* but effective at 10.0% concentration. Against *A. parasiticus*, all concentrations of *G. kola* extract showed an effective inhibition. There was no significant difference at p>0.05 to inhibit growth of *A. flavus* and *A. parasiticus* with concentrations of ethanol extract of *G. kola* (table 2).

Inhibition of test organism by different concentration for methanolic extracts of neem seed

Results of antifungal activity using methanolic extracts of neem seed against *A. flavus* and *A. parasitic us* indicated at 2.5% concentration of extract, neem seed showed a moderate inhibition against *Aspergillus flavus* but effective inhibition at 5.0%, 7.5% and 10.0% concentrations. At 2.5%, 5.0% and 7.5% concentrations, neem seed showed a moderate inhibition against *A. parasiticus* and effective inhibition at 10.0% concentration (table 3). Methanol extract of neem seed showed no significant difference in the inhibition of *A. flavus* and *A. parasiticus* (p>0.05) within the columns.

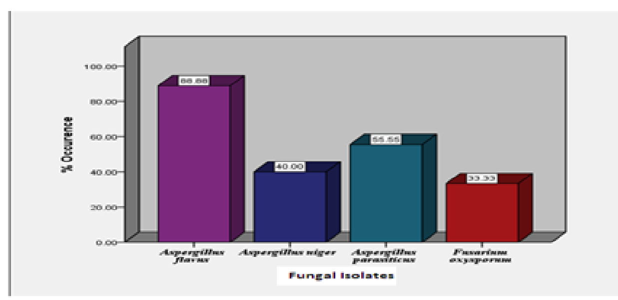

Fig. 3: Percentile occurrence of fungi isolated from maize sample

Inhibition of test organism by different concentration for ethanol extract of neem seeds

Effect of ethanol extract of neem seed on *A. flavus* indicated that all concentrations (2.5%, 5.0%, 7.5% and 10.0%) of neem seeds extract, showed effective inhibition of *Aspergillus flavus*. At 2.5%, 5.0% and 7.5% concentrations, ethanol extract of neem seeds showed a moderate inhibition of *Aspergillus parasiticus* and an effective inhibition at 10.0% concentration. There was no significant difference at $p>0.05$ to inhibit the growth of *A. flavus* and *A. parasiticus* with concentration of ethanol extract of neem seeds (table 4).

Inhibition of test organism by different concentrations for methanol extract of combined (G. kola and neem) seed

Results of the antifungal activity of methanol extract of combined (*G. kola* and neem) seeds against *A. flavus* showed a moderate inhibition at 2.5% concentration and an effective inhibition at 5.0%, 7.5% and 10.0% concentrations. Against *A. parasiticus*, inhibition was moderate at 2.5% and 5.0% concentration and effective at 7.5% and 10.0% concentrations. The concentration of methanol extract of *G. kola* and neem seeds showed no significant difference in the inhibition of *A. flavus* and *A. parasiticus* ($p>0.05$) (table 5).

Inhibition of test organism by different concentrations for ethanol extract of combined (G. kola and neem) seed

Antifungal activity of ethanol extract of combined (G. kola and neem) seeds on A. flavus showed that inhibition was effective in all concentration while against A. parasiticus inhibition was moderate in all concentrations (table 6). The concentrations at 2.5%, 5.0%, 7.5% and 10.0% for ethanol extract of combined (G. kola and neem) seeds showed significant difference in the inhibition of Aspergillus flavus ($p<0.05$) and not significant for Aspergillus parasiticus.

Aflatoxin analysis

Qualitative analysis of TLC plates under the UV light suggests the presence of two types of aflatoxins i.e. B and G. Blue fluorescence indicates the presence of B aflatoxins while green spots reflect G aflatoxins. In this study, the maize associated fungal sample was found with aflatoxin B1 and B2. Quantitative estimation expressed the amount of aflatoxin in parts per billion (ppb) (table 7).

Phytochemical composition of garcinia kola and neem seeds

The results of the phytochemical screening of *Azadirachta indica* and *Garcinia kola* seeds revealed the presence of various bioactive agents. Both *Garcinia kola* and neem seeds extract were found to contain agents such as: alkaloid, saponins, tannin, flavonoid, steroid, terpenoids and cardiac glycosides (table 8) when analyzed qualitatively. The quantitative phytochemical analysis of the test plant indicated that the percentage of tannin was higher in *G. kola* seeds (5.55±0. 071) and lower in neem seeds (1.11±0.028). Neem seeds had a higher percentage of alkaloid (18.6±0.071) than *G. kola* (14.8±0.000). The percentage of flavonoids was higher in neem seeds (3.8±0.000) and lower in *G. kola* (1.9±0.028) while the percentage of saponins was higher in *G. kola* (10.5±0.014) and lower in neem seeds (9.15±0.028) (table 9).

DISCUSSION

Four fungal isolates were identified in this study, of which three were *Aspergillus* species with *A. flavus* having the highest frequency of 88.88%. According to Yan-ni *et al*. (2008), *Aspergillus* species are found to be associated to disease in maize. Bankole *et al*. (2006), Wagacha and Muthomi (2008) stated that high incidence of *Aspergillus flavus* in maize produces toxin, the result of high frequency of *A. flavus* in maize in this study agrees with the report.

In this study, the ability of the test plants extracts (individually and in combination) against *Aspergillus flavus* and *Aspergillus parasiticus* varied. Results showed that the test plants extracts were effective in reducing mycelial growth of the test organism. Methanolic extract of *G. kola* on the test organisms showed antifungal activity in the inhibition which was highest at 10% concentration. The result confirms the efficacy of *G. kola* and the *in vitro* activities of this plant extract and also provides evidence to support the use of this plant in the control of *A. flavus* and *A. parasiticus*. With ethanol extracts of *G. kola*, the antifungal activity was effective for the inhibition of *A. flavus* and *A. parasiticus*, with *A. parasiticus* having the higher percentage inhibition.

This study also showed that the methanolic and ethanolic extracts of neem seeds used against *Aspergillus flavus* and *Aspergillus parasiticus* was effective in which the result of phytochemical screened showed positive for saponins, alkaloids, terpenoids flavonoids and cardiac glycosides. This is supported by the report of Uzma and Shahida (2007) that neem seed powder is a very good biological fungicide. Also, methanolic and ethanolic extracts of combined *Garcinia* kola and neem seeds showed effective inhibition of *A. flavus* and *A. parasiticus* with ethanolic extracts of the combined test plants having the highest inhibition against *A. flavus* (80.43±3.62). The extracts from this plant show the ability to suppress growth of toxigenic *A. flavus* and *A. parasiticus*. It is possible that these bioactive compounds may be responsible for the antifungal activity and hence the efficacy against *A. flavus* and *A. parasiticus*. Phytochemical analysis showed that the methanolic and ethanolic extracts of *G. kola* and neem seeds showed the presence of secondary metabolites and this may be a reason for the inhibitory activity against *Aspergillus flavus* and *Aspergillus parasiticus*.

Aflatoxin analysis of *Zea mays* sampled in Awka, Anambra State, Nigeria was found to contain aflatoxin B1 (9.60ppb) and B2 (13.3ppb). This type of aflatoxin found in the maize sample is considered the most toxigenic and carcinogenic for humans. This agrees with Mawcha and Assefa (2014) that aflatoxins are a group of extremely poisonous, deadly and cancer causing compounds. Aflatoxins B1, B2, G1 and G2 are produced by strains of

Aspergillus parasiticus while aflatoxins B1 and B2 are produced by deadly *A. flavus* strains (Yan-ni *et al*., 2008). From this study, it can be reported that maize sample from Awka, Anambra State, Nigeria contains toxigenic *A. flavus* and toxigenic *A. parasiticus*, and exposure can increase the risk of lethal aflatoxicosis to humans as aflatoxins B1 and B2 are classified as Group I human carcinogens (Krishnamurthy and Shashikala, 2006). Mohammed (2011), noted that even a reduced degree exposure to aflatoxin can raise the risk of acute liver cancer in humans. According to The ddeus (2009), high dose exposures to aflatoxins lead to intense, chronic damage of the liver with high disease and death rate. Aflatoxin consumption of 2-6mg/day in a month can lead to severe liver disease and mortality. Cumulative levels of aflatoxin B1 and B2 could cause health problems to people in Awka, Anambra State, Nigeria. According to Lanyasunya *et al.* (2005), similar reports have associated aflatoxin B1 with acute hepatitis caused by consuming musty maize seeds reported in some places of Africa, Western India and Malaysia in which the people affected came from places liable to low rainfall and inadequate nourishment.

Having noted the health hazards of aflatoxin B1 and B2 exposure, we may call for urgent attention to guide food crops (maize) against contamination with aflatoxins and several efforts have been made. The European Union sets the level of aflatoxin consumption of food at 2ppb (Cotty and Mellon, 2006). This study observed that maize sample from Awka, Anambra State, Nigeria recorded aflatoxin level above 2ppb. Thus, maize should be thoroughly processed before consumption to reduce the level of aflatoxin.

CONCLUSIONS

From the result in this study, high level of aflatoxin B1 and B2 correlates with high incidence of *Aspergillus flavus* and *Aspergillus parasiticus*; this corresponds with previous studies that *Aspergillus flavus* and *A. parasiticus* produce aflatoxin B1 and B2. These fungi have been proved hazardous for human health. This study recommends that food should be well processed to reduce the level of aflatoxin in untreated food products.

The two plant extracts used in this study contained effective phytochemical compounds that could be used as inhibitors of *A. flavus* and *A. parasiticus* fungal growth and aflatoxin activity in *Zea mays* grains and also in other plant grains or cereals. The bioactive substance in these plants indicates that these plants are potential for managing deadly *Aspergillus flavus* and *Aspergillus parasiticus* production of *Zea mays* grains in Awka. To adopt methods used in this study to control *A. flavus* and *A. parasiticus*, consideration should be given to the use of different concentrations of the crude plant extracts, the solvents used and the test plant extracts as this had variations in inhibiting the growth of the organisms. This study also recommends the need to evaluate other medicinal plants as a bio-fungicides against *A. flavus* and *A. parasiticus*. Also extensive research on other food products in Awka, Anambra State, Nigeria should be conducted for aflatoxins and awareness should be created in communities of Awka regarding the health hazards of consuming aflatoxin contaminated food.

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