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Inhibitory Effects of Bitter Kola (*Garcinia kola*) Seed Extract on *Aspergillus* Species Isolated from Maize

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Abstract
Maize is an important crop worldwide, serving as good source of food and income. Contamination by Aspergillus makes it less suitable for human intake as a result of discoloration and decrease in nutritional composition. This research sought to examine the inhibitory effect of different concentrations of bitter kola seed extract on Aspergillus species isolated from maize grains. The invitro experiment was carried out at the department of plant science laboratory, Modibbo Adama University, Yola in triplicate, using a Complete Randomized Design with a total of thirty plates. The test plant active components used were extracted using ethanol. 10, 30, 60 and 100% of bitter kola seed extract was added to Potato Dextrose Agar before inoculation. The effect of the extract on isolated Aspergillus species increased with increase in concentration. Inhibition percentage at the highest concentration (100%) of the extract was more effective in the control of Aspergillus niger (65.90%), an increased level of inhibition was also recorded against Aspergillus flavus (57.03%) at the highest concentration. There was notable variation in the suppression of fungal growth across the different concentrations of the extract. Phytochemical analysis showed the presence of alkaloids, flavonoids, tannins, saponins, phenol, terpenoids and glycosides. The findings of this study are very important in the control of maize rot as they are biologically based, affordable and ecofriendly; hence higher concentrations of bitter kola seed extract are recommended for use in the control of Aspergillus species causing maize rot.

Keywords: Mycelial, *Aspergillus*, Phytochemicals, Bitter kola, Maize

INTRODUCTION
Maize (*Zea mays* L.) belongs to the tribe Maydae, family Poaceae and originated in Mexico and Central America (Schnable *et al*., 2009). It is a staple diet in Sub-Saharan Africa (Kimanya *et al*., 2008) and consumed at a level of about 400 g per person per day (Shephard, 2008). In Nigeria, maize is an essential crop that provides millions of farmers with both food and revenue. It's the crop. mostly grown by small-scale farmers with sensitive cultivars and inexpensive technology (Mushongi, 2010).

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Maize is vulnerable to degradation by mycotoxigenic fungi belonging to *Aspergillus* genus (Shephard, 2008). Due to its rich nutrient values and large embryos, maize is easily infected by fungi in field and mainly, in post-harvest conditions (Nisar *et al.,* 2019). *Aspergillus* infection on maize grains in the field and in storage is a problem for maize farmers in Northern Nigeria, particularly in Yola, North Local Government Area, this reduces harvest output and increases food insecurity (Abbas *et al.,* 2020). Contamination of maize by this fungus renders the grain unfit for human consumption due to discoloration and reduction in nutritional value (Muthomi *et al*., 2012). Though synthetic fungicide may be effective and efficient for the control of seed-borne fungi, they cannot be safely applied to maize grains for reasons of pesticide toxicity (Achugbu *et al.,* 2016).

Bitter kola plant is a canopy tree that belongs to a large genus *Garcinia*, consisting of more than 250 species (Nuratu *et al*., 2024). The seed of bitter kola has been termed as a "wonder seed" by Nigerians because of its great medicinal importance. It is locally called "Agbilu" by Igbo citizens from the Eastern part of Nigeria, the Hausa people from the Northern part of Nigeria called it "Namijin goro" while the Yoruba people from the Southwest Nigeria called it "Orogbo" (Adegboye *et al*., 2008) It is a plant native to West Africa, known for its diverse pharmacological properties, including its potential as a natural antifungal agent, it also possesses antimicrobial activities, thus making it a promising candidate for controlling fungal pathogens such as *Aspergillus* spp. (Okeke *et al.,* 2020). *Garcinia kola* seed extract at different concentrations has been reported to inhibit the growth of *Candida albican*, *Aspergilus niger*, *Aspergilus flavus*, *Fusarium oxysparium* by Foorunsho *et al*. (2019). The effects of crude ethanolic plant extracts of *Garcinia kola* seeds on mycelial growth of seed-borne fungi of African yam bean at different concentrations have been investigated by Onyeke and Ugwuoke (2011). The seed borne fungi were *Aspergillus flavus*, *Fusarium moniliforme*, *Penicillium* sp. and *Absidia* sp. Bio-antifungal agents are ecofriendly, cheap and safe for use in preservation of maize grain, many research are in search of new agents to curb plant diseases caused by fungal pathogens. Despite their abundancy, fungal pathogens are still finding ways to resist antifungal treatment, making development of novel antifungals a permanent mission for scientists (Stojkovi´c *et al*., 2022). Thus, this research sought to examine the inhibitory effect of different concentrations of bitter kola seed extract on *Aspergillus* species isolated from maize grains in Yola North Local Government Area, of Adamawa state, Nigeria.

MATERIALS AND METHODS

Study location

The study was conducted in the Department of Plant Science laboratory of Modibbo Adama University, Yola, Adamawa State, Nigeria.

Source of Plant Materials

Ten gram each, of infected and healthy maize seeds as well as bitter kola (*Garcinia kola*) seeds was obtained from traders at Jimeta Ultra-Modern Market, Adamawa state, stored in air tight container and conveyed to the laboratory for further analysis. The identity of the samples was authenticated in the herbarium of Department of Plant Science, Modibbo Adama University Yola, Adamawa State, Nigeria.

Preparation of growth medium

Thirty-nine grams (39g) of Potato Dextrose Agar (PDA) (Oxoid CM0139, Hamphire, England) was weighed and suspended in 1 litre of distilled water, it was stirred gently to obtain a uniform mixture and autoclaved at 121 ˚C for 15 minutes. The autoclaved medium was allowed to cool to approximately 45 ºC, then supplemented with the broad-spectrum

antibiotic, Ampicillin (250 mg). Two capsules of Ampicillin were dissolved in 2 ml of sterilized distilled water and added to 500 ml of liquid Potato Dextrose Agar. The mixture was gently shaken to ensure a uniform blend before being poured into a petri dish. The resulting mixture was dispensed into 9 mm diameter Petri dishes (Smith and Onion, 1983).

Study Design

The laboratory experiment was carried out in triplicate with a total of 30 plates laid out using a Complete Randomized Design (CRD).

Isolation and identification of fungi

Contaminated maize seeds were disinfected with 70% ethanol and transferred to Petri dishes with solidified Potato Dextrose Agar. The inoculated plates were kept at room temperature (28 ± 2 °C) in the dark for 72 hours. The mycelial colonies that developed on the incubated plates were sub-cultured onto fresh Potato Dextrose Agar medium until a pure culture was achieved. Fungi were identified based on their colony characteristics in the culture media and structures under microscope with reference to the identification schemes of Snowdon (1990) and Watanabe (2002).

Pathogenicity test

The fungal isolates obtained from the infected maize seeds were tested for their ability to cause the same rot condition in healthy maize seeds. The fungi were sub-cultured into a fresh culture media and incubated for seven (7) days for better sporulation. A camel brush was used to collect the spores into a sterile distilled water in a beaker (25 ml of H_2O per plate) and incubated for 72 hours and a sieve was used to filter the spore suspension. The healthy maize seeds were washed with sterile water and the surface area was sterilized with 70% ethanol solution, dried using filter paper and then spread on the sterilized surface area. The suspension was sprayed all over the healthy maize seeds inside a plastic container and covered. It was incubated and observed daily for symptoms or fungal growth and fungi was isolated and compared with the original. The control group was inoculated with a disc of clean solidified Potato Dextrose Agar medium. The inoculations were performed in triplicates (Balogun *et al*., 2005)

Preparation of extract from Bitter kola seeds

Bitter kola seeds were peeled and cut into slices using a sterile blade, then air-dried at room temperature (25 ˚C) and processed into powder using a mortar and pestle. Cold solvent (ethanol) extraction technique was employed (Harborne, 1973). One hundred grams (100 g) of the plant sample were mixed with 1000 ml (1L) of ethanol and allowed to stand for 48 hours at 25 ˚C. The mixture was filtered using Whatman filter paper (No. 1) and the filtrate was diluted into 10, 30, 60 and 100% (Zakari *et al*., 2015).

Evaluation of the efficacy of bitter kola seed extract on fungi isolates

It was done according to the method described by Zakari *et al*. (2015). Four equal sections were marked on each plate by drawing two perpendicular lines at the bottom. The center of the plates indicated the point of intersection of the inoculums. This was done before adding the prepared medium (PDA) to the plates. The extracts were poured into a flask, sealed with cotton wool and heated for about 10 minutes to prevent contamination. 2 ml of the extract at various dilution percentages were separately added to the Petri dishes containing 10 ml of Potato Dextrose Agar. Each plate was inoculated with a 5 mm plug of pure isolate taken from the edges of an actively growing fungal culture. The plates were incubated at 25 ± 2 °C. The control plates received only an equal amount of distilled water. The mycelial growth diameter of each isolate was measured and recorded once the growth in the control treatment was

complete. Mean radial mycelial growth of each isolate was recorded and data were transformed into inhibition percentage using the following formula:

Inhibition Percentage (%) = $\frac{D1-D2}{D1} \times 100$

Where: D1 - Average Diameter of fungal spore in control

D2 - Average diameter of fungal spore with treatment

Phytochemical analysis

The extracts were analyzed by the following procedures Harborne (1973); Sofowora (1993); Trease and Evans, (1989) to test for the presence phytochemicals present in bitter kola extract.

Data Analysis

Differences between the means of radial fungal growth inhibition were assessed using one way analysis of variance (ANOVA), and means that showed significant differences were separated using Tukey's Honest Significant Difference (HSD) test. (P≤0.05).

RESULTS AND DISCUSSION

Percentage occurrence of *Aspergillus* species from Maize seeds

Two fungi species were isolated from the infected maize seeds and were identified as *Aspergillus niger* and *Aspergillus flavus* based on their colonial and morphological characteristics with reference to identification schemes of Snowdon (1990). These fungi pathogens have previously been reported in maize seeds by Taiba and Zakari (2018) in the North Eastern region of Nigeria. The fungus *A. flavus* was the most prevalent, comprising 60%, while *A. niger* was the least frequent, with 40%. (Figure 1). Similar reports have been made by Achugbu *et al*. (2016) where *A. flavus* isolated from maize had a percentage occurrence of 88% while *F. oxysporum* was the lowest with 33.3%.

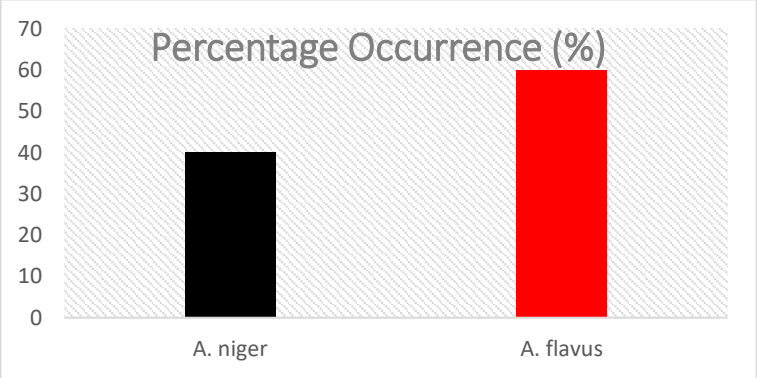

Figure 1: Percentage occurrence of isolated *Aspergillus* species

Effect of Bitter kola seed Extract against *Aspergillus* species

The antifungal activity of bitter kola (*Garcinia kola*) extract was evaluated against *Aspergillus niger* and *Aspergillus flavus* isolated from maize samples collected in Yola North Government Area of Adamawa State (Table1). The extract was tested at concentrations of 100%, 60%, 30%, and 10%. The highest (65.90 %) zone of inhibition for *A. niger* was recorded in 100% of the extract which differed significantly (P≤0.05) from the control with 0.00 inhibition. The !00% treatment was closely followed by 60% and 30% which had an inhibition value of 54.57% and 53.83% respectively for *A. niger*. The extract significantly (P≤0.05) inhibited the proliferation of *A, flavus*. The highest (37.03%) inhibition of *A, flavus* was observed at 100% extract application, which was followed closely by 60% (43.12%) and 30% (35.93%) while 10% extract application had the lowest (24.33%) inhibition and the control had 0.00% inhibition. In this

study the ability of bitter kola seed extract to inhibit growth of *A. flavus* and *A. niger* varied, the effect of the extract on the fungi isolated was directly proportional to the concentration of the extract, however the result showed that higher concentration (100%) of bitter kola seed extract had more inhibitory effect on *A. niger* compared to *A. flavus*, this corroborates the reports of Folorunsho *et al*. (2019) that 75 mg/ml of bitter kola seed extract had grater inhibitory effect on *A. niger* compared to *A. flavus*. This suggested that the use of bitter kola seed extract could be effective in curbing pathogenic *Aspergillus* infestation of maize based on the zone of inhibition observed in this study.

Table 1: Percentage inhibition of fungi isolates combined with the ethanolic plant extract of Bitter kola seed

Concentration	Growth Inhibition (%)	
	A. niger	***A. flavus***
Control	0.00±0.00 ^{c}	0.00±0.00 ^{d}
10%	25.70±4.30 ^{b}	24.33±3.60 ^{c}
30%	53.83±12.29 ^{a}	35.93±2.90 ^{b}
60%	54.57±3.50 ^{a}	43.12±0.50 ^{b}
100%	65.90±4.05 ^{a}	57.03±5.58^{a}
***P*- value**	1.86e-09***	1.38e-08***

Key: *** highly significant means, means with different superscripts are significantly different at P≤0.05.

Phytochemical Composition of Bitter Kola seed Extract

The qualitative assessment of the phytochemical composition of bitter kola (*Garcinia kola*) extract revealed the presence of various bioactive compounds which includes; alkaloids, flavonoids, tannins, saponins, phenol, terpenoids and glycosides (Table 2). It was suggested by Achugbu *et al*. (2016) that these bioactive compounds may be responsible for the antifungal activity and hence the efficacy against *A. flavus* and *A. niger*. The presence of phenol, tannin, flavonoids, glycosides and saponins in bitter kola seed extract as recorded in this study, is in line with the results of Idoko *et al*. (2022), which found that the ethanol extract of bitter kola included phenol, sterols, alkaloids, tannin, flavonoids, saponins, and terpenoids. Saponins have also been reported to contribute to the antifungal effects of medicinal plants against *Candida albicans* and *Aspergillus niger* (Gizaw *et al*., 2022)

Table 2: Qualitative determination of phytochemical group in bitter kola seed extract

Phytochemical	Inference
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Phenols	+
Terpenoids	+
Glycosides	+

+= Present

CONCLUSION

This research showed that *A. niger* and *A. flavus* are the species of *Aspergillus* associated with maize rot in Jimeta, Yola. Bitter kola seed extract possesses strong antifungal properties against *Aspergillus* species isolated from infected maize seeds. Higher concentrations of the extract are more potent at inhibiting the growth of the fungi; however, the inhibitory potency of the extract was higher in *A. niger* compared to *A. flavus*. The findings of this study are very

important in the control of maize rot as they are biologically based, affordable and ecofriendly; hence higher concentrations (60- 100%) of the extract are recommended for use in the control of *Aspergillus* species causing maize rot.

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