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RESEARCH ARTICLE

Biocontrol potential of Moringa leaf extract on fungi causing postharvest deterioration of maize (*Zea mays* L.) seeds during storage in Makurdi

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ABSTRACT

Maize (*Zea mays* L.) is an important cereal crop ranked second to wheat and a source of protein and energy in human diets throughout the world. The biocontrol potential of *Moringa* leaf extract on fungi causing deterioration of maize seeds in storage was carried out. The fungi were isolated using standard blotter method and *Moringa* leaf extract at concentrations of 25, 50, 75 and 100%w/v were used to inhibit the growth of the fungi from the maize seeds both *in vitro* and *in vivo*. Four species of fungi namely; *Rhizopus stolonifer, Aspergillus flavus, Aspergillus niger* and *Curvularia affini*s were isolated from the maize seeds. There was no significant difference in percentage occurrence of *Rhizopus stolonifer* across the markets (P>0.05). For *Aspergillus flavus*, High Level market recorded significantly higher (P<0.05) occurrence (18.90) compared with Wadata market (0.00) which had the lowest incidence. However, there was significant difference between Wadata and High Level markets. For *Aspergillus niger* and *Curvularia affini*s, there was no significant difference across the four markets (P>0.05). For seed germination, Wadata market recorded the highest (98.90) while Wurukum reported the lowest (41.20).In the biocontrol experiment, all extract concentrations inhibited the growth of the different fungi at varying degrees with 100%w/v showing significantly higher inhibition compared to all other concentrations. The relatively high prevalence of *Aspergillus niger* on the maize seeds points to the possibility of aflatoxin contamination. Application of *Moringa* leaf extract inhibits greatly the growth of fungi on maize seeds and the degree of inhibition increases with increasing concentration.

Keywords: *Moringa*, Maize Seed, storage, Fungi, Postharvest, Biocontrol

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INTRODUCTION

Maize (*Zea mays* L.) is an important annual cereal crop of the family Poaceae. *Zea* is an ancient Greek word which means “sustaining life” and *mays* is a word from Taino language meaning “life giver” (Kumar and Jhariya, 2013). In terms of world grain production, it is ranked second next to wheat. It is a source of energy and protein in humans’ diet throughout the world (Rehman, 2006). It is also the most important cereal in the world after wheat and rice with regards to cultivated areas and total production. In Nigeria, it is widely grown and intercropped with other crops like

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cassava and melon (Agboola and Makinde, 2008). Maize is a multipurpose crop, providing food and fuel for human beings, feeds for animals, poultry and livestock, its grains have nutritional value and are used as raw materials for manufacturing many industrial products (Afzal et al., 2009). It started as a subsistence crop in Nigeria and has gradually risen to a commercial crop on which many agro-based industries depend as a raw material (Iken and Amusa, 2014). Nigeria is currently the tenth largest producer of maize in the world and the largest maize producer in Africa (IITA, 2012). Fungi are natural enemies to farm produce both at pre-harvest and post-harvest levels, causing severe damage in both quantity and quality of the produce. They account for yield losses of up to 67%. It was reported that fungi caused about 50-80% damage to farmers' maize during storage period or when conditions are favorable for their development, resulting in significant losses both quantitatively and qualitatively (IITA, 2012). In addition, fungi produce mycotoxins which are hazardous to man and animals. They are significant destroyers of foodstuffs and grains during storage, rendering them unfit for human consumption by retarding their nutritive value and often by producing mycotoxins (Marin et al., 1999). A large number of pathogenic fungi, bacteria, virus and insects infecting and infesting maize grains cause combined worldwide annual losses of 9.4% (Ali, 2007). Fungi affect the quality of grain and as a result there is increase in its mustiness, production of toxins and finally spoilage of grain in many ways. They are the second important cause of deterioration and loss of maize next to insects (Ali, 2007). Owing to the economic importance of the pathogen (fungi) in causing yield loss of maize which is a threat to food security, it becomes mandatory to devise means through which their growth and development will be hindered. Therefore, their prevention and control becomes obligatory. At present, many fungicides are used to control the disease but these are not environmentally friendly and are causing health hazards.

Moringa (*Moringa oleifera*) is considered as one of the world's most useful trees, as almost every part of the tree has an impressive effect of food, medication and industrial purpose (Khalafalla et al.,2010). *Moringa* leaves are potential sources of vitamins A and C, Iron, Calcium, Riboflavin, b-carotene, phenolics and powerful natural antioxidant (Njoku and Adikwu, 1997). The use of plant extracts such as *Moringa* has played a significant role in the improvement of seed quality and field emergence of plant seeds. The development of non-toxic, safe and effective biodegradable alternatives to synthetic fungicides has in recent years led to the global screening of various plants for bioactivity against plant pathogenic organisms. Plants generally contain a wide variety of free radicals scavenging molecules including phenols, flavonoids, vitamins and terpenoids that are rich in antifungal activity. Plant leaves offer potentials for fungi control through the production and release of toxic mixtures of phytocompounds in them (Murty and Renard, 2001). Hence, the objective of the research was to evaluate the biocontrol potential of aqueous extracts of *Moringa* leaves on fungi associated with stored maize seeds.

MATERIALS AND METHODS

Study area

The study was carried out in the Botany Laboratory of the Benue State University Makurdi. Makurdi is the capital of Benue state and it lies between latitude 07°15° and 07^{o} 45'N and longitude 08°15'and 08°, 40'E. The town lies in the Guinea Savanna vegetative belt and on the bank of the 2nd largest River in Nigeria, River Benue. The river divides the town into North and South banks and the town covers an area of 16 km^{2}.

Collection of maize seed samples

Maize Seed samples were collected from their storage containers in four different markets (Wurukum, Wadata, High level and Modern market), put in polythene envelopes and transported to the Botany laboratory of the Benue State University for the commencement of the experiment.

Preparation of potato dextrose agar (PDA)

Potato Dextrose Agar was prepared by suspending 39 grams of potato dextrose agar in 1000ml of distilled water. This was then heated using a heating mantle to dissolve the medium completely for a few minutes. The agar was afterwards sterilized by autoclaving at 15psi at 121 °C for 15 minutes and was allowed to cool before dispensing into sterile petri dishes.

Collection of *Moringa oleifera* leaves

Fresh leaves of *Moringa oleifera* were collected from different locations in Makurdi metropolis. A cutlass was used to cut the branches while the leaves were harvested by hand picking. The leaves were put in clean polythene bags and taken to the laboratory. In the laboratory, the leaves were first pre-washed carefully under a gentle stream of tap water for 1-2 minutes in order to remove surface dirt. This was followed by washing in sterile distilled water containing 1% Sodium hypochloride for thirty seconds. The leaves were removed and rinsed in three successions of sterile distilled water as reported by Liamngee et al.,(2015).

Extract concentrations

Concentrations of the plant species were prepared to give 25%w/v, 50%w/v, 75%w/v and100%w/v respectively. Extract concentration of 40%w/v was obtained by dissolving 40g of the plant leaf powder of each plant species respectively in 100mls of sterile distilled water in a beaker. Extract concentration of 60%w/v was obtained by dissolving 60g of the plant leaf powder of each plant species respectively in 100mls of sterile distilled water in a beaker. The same principle was applied to all other extract concentrations.

Preparation of crude extractions

Moringa oleifera leaves collected were weighed and the leaves were washed with sterilized distilled water and rinsed with distilled water. The leaves were then crushed using a clean mortar and pestle. The macerates were then transferred to beakers, each containing 100ml of sterile distilled water. The set up was left to stand for three hours and thereafter sieved using a muslin cloth to obtain the extract.

Experiment 1

Assessment of fungi species on maize seeds

The experiment was set up in a Completely Randomized Design (CRD) with markets being the treatments while the number of samples per market formed the replication. Detection of seed-borne fungi was done by standard blotter method as highlighted in Liamngee et al 2015. In this method, three pieces of blotter papers were soaked in distilled water and placed in 9cm sterile plastic petri dishes after draining out excess water. For each treatment, 120 seeds were plated (40 seeds per replication). Working samples of these seeds were plated out on the moisture blotter

papers after surface sterilization with 0.5 % Sodium hypochlorite solution for 1 minute and incubated at room temperature. After seven days of incubation, the seeds were examined for fungal growth. Slides of different fungal spores encountered were prepared and examined with Olympus microscope at 40x magnification to establish the identity of individual fungi. Percentage occurrence of fungi was determined by counting the number of times each individual fungus occurred and dividing by the total number of fungi and then expressing the answer as a percentage (Muhammad et al*.,* 2012) That is,

$$\text{Percentage occurrence of Fungi} = \frac{\text{number of times each Fungus occurred}}{\text{Total number of seeds per plate}} \times 100$$

Additionally, seed germination was determined using the formula below: (Muhammad et al*.,* 2012)

$$\text{Percentage germination} = \frac{\text{number of seeds germinated}}{\text{total number of seeds per plate}} \times 100$$

Experiment 2

Evaluating the effect of *Moringa* leaf extract on radial growth of fungi *in vitro*

Two (2) mls of each extract concentration of the plant extract were dispersed in petri-dishes after which 15 - 20mls of molten PDA were added. The petri dishes were swirled gently on the work bench to ensure even dispersion of the extracts. The agar extract mixture were allowed to solidify and then used for the inhibition of mycelia growth of the test fungi. Four (4mm) diameter of mycelia obtained from the colony edge of 3-4 day old pure culture of fungi using a cork borer were inoculated centrally on to the medium using a sterile needle. Four replications were used for each extract concentration. Petri dishes containing PDA with no botanical extracts inoculated with fungi served as controls. The plates were arranged on laboratory desk following Completely Randomized Design and incubated at room temperature. Measurement of growth was done using a meter rule on days 3, 5, and 7. Inhibition of fungal growth was calculated using the formula:

$$\text{Growth inhibition of Fungi} = \frac{R_1 - R_2}{R_1} \times 100$$ (Becker et al., 2006)

Where:

R_1 = Radial Growth of the pathogen in the control plates and

R_2 = growth of the pathogen with treatment.

Experiment 3

Effect of seed treatment with *Moringa* leaf extract on occurrence of fungi associated with stored maize seeds (Biocontrol experiment*)*

The effect of seed treatment with *Moringa* leaf extract was assessed using *Moringa* leaf extract at rates of 25, 50, 75, and 100%w/v. Untreated maize seeds were used as control. Seeds were treated by soaking them in *Moringa* leaf extract for one hour and plating them using the standard blotter method as described in experiment 1. For each extract concentration, 10 maize seeds were plated and replicated 5 times in Completely Randomized Design. The

seeds were incubated for 7 days and then the percentage occurrence of fungi species and percentage seed germination were calculated and recorded as reported by Liamngee et al., (2015).

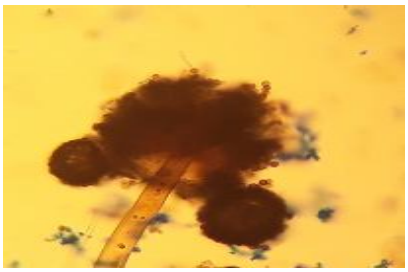
Data analyses

Data were analysed using SPSS (Statistical Package for Social Sciences) version 25. The Data obtained were subjected to One Way Analysis of Variance (ANOVA) and the Fisher's Least Significant Difference (FLSD) was used to separate the means at 5% level of significance.

RESULTS

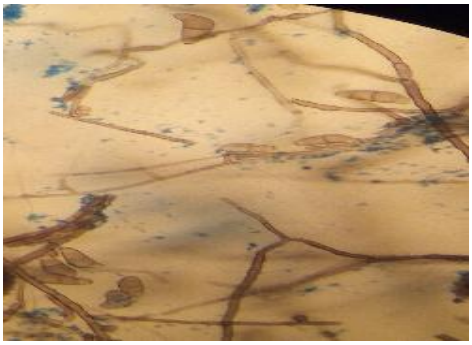
Occurrence of individual fungi on maize seeds from each market in Makurdi

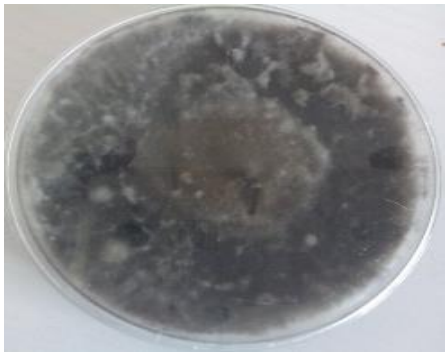
Occurrence of fungi species on maize seeds collected from four markets in Makurdi, Benue State revealed that four species of fungi namely; *Rhizopus stolonifer, Aspergillus flavus, Aspergillus niger* and *Curvularia affinis* were isolated from the maize seeds as shown in plates 1 to 8.



Aspergillus niger

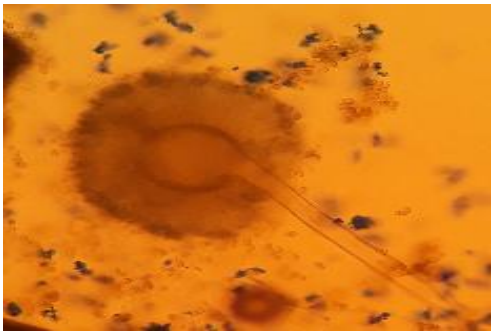
Plate 1: Conidiophore is smooth and crowded conidia structures colour **Plate 2:** Colony is compact and bears Hyaline. Conidia is very dark in erect and

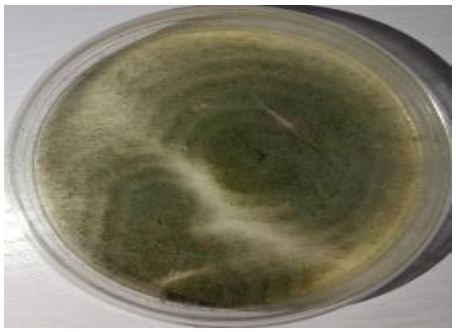




Curvularia affinis

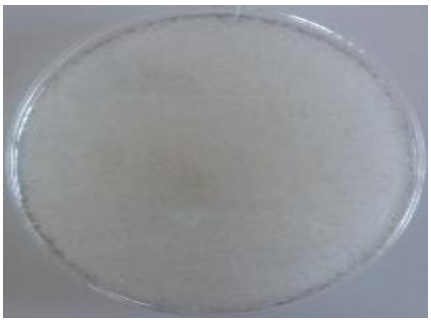
Plate 3: Conidia often curved, broadly, fusiform to ellipsoidal and spread loosely. **Plate 4**: Colony is blackish brown Conidiophores arise singly.

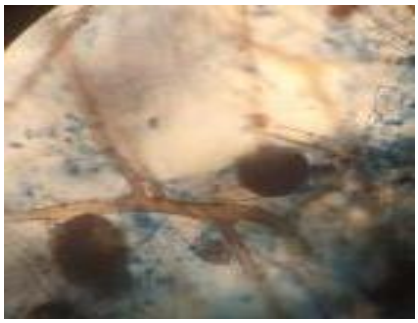




Aspergillus flavus

Plate 5: Conidia head is spherical, with a hyaline conidiophore. **Plate 6**: Colony is spreading and Light green in colour.





Rhizopus stolonifer

Plate 7: Colony is white in Colour and becomes brownish **Plate 8:** Presence of rhizoids in the mycelium With the formation of spores

There was no significant difference in the occurrence of *Rhizopus stolonifer* across the markets (P>0.05). For *Aspergillus flavus*, High Level market recorded significantly higher (P<0.05) occurrence of the fungus compared with Wadata market which had the lowest incidence. However, there was no significant difference between Wurukum and Modern markets. For *Aspergillus niger* and *Curvularia affinis,* there was no significant difference across the four markets (P>0.05) as shown in Table 1

Seed germination of Maize and occurrence of fungi from each market in Makurdi

In percentage seed germination of maize, Wadata market recorded significantly higher seed germination (98.90) compared to High level (58.90) and Wurukum (41.20) but this was not significantly different compared to modern market (86.70). The markets showed no significant difference in the occurrence of seed borne fungi as seen in Table 2.

Effect of *Moringa* extract on the Radial Growth of various Fungi *in vitro*

The effect of *Moringa* leaf extract on the radial growth of fungi *in vitro* is shown in Tables 3, 4, 5 and 6. Varying concentrations of *Moringa* leaf extract resulted in the inhibition of growth of *Aspergillus flavus*, *Aspergillus niger, Curvularia affinis* and *Rhizopus stolonifer*. For *A. flavus* on days 3, 5 and 7, all the concentrations of *Moringa* extract; 25, 50, 70 and

100% (w/v) significantly (P<0.05) reduced radial growth of *A. flavus* compared with the control. There was significant difference across the concentrations on the radial growth of *A. flavus* in all the days as seen in Table 3. However 100%w/v showed significantly higher inhibition on fungi growth compared to the lower concentrations on all the days of the study.

Table 1: Occurrence of individual fungi on Maize seeds from each Market in Makurdi

Markets	*Rhizopus stolonifer*	*Aspergillus flavus*	*Aspergillus niger*	*Curvularia affinis*
Wadata	9.22[a]	0.00[b]	16.00[a]	8.50[a]
High Level	8.59[a]	18.90[a]	11.70[a]	8.70[a]
Wurukum	4.07[a]	13.90[ab]	18.00[a]	4.40[a]
Modern	9.89[a]	11.00[ab]	13.80[a]	9.00[a]
LSD (0.05)	6.35	15.47	10.83	6.79

Table 2: Seed germination of Maize and occurrence of fungi from each Market in Makurdi

Markets	Seeds germination	Seed borne fungi
Wadata	98.90[a]	64.00[a]
High Level	58.90[b]	58.90[a]
Wurukum	41.20[b]	65.60[a]
Modern	86.70[a]	84.40[a]
LSD (0.05)	21.59	30.96

Table 3: Effect of *Moringa* extract on the Radial Growth of *Aspergillus flavus* in vitro

Concentration (w/v)	Radial growth (cm)		
	Day 3	**Day 5**	**Day 7**
0	3.02[a]	5.05[a]	7.02[a]
25	2.02[b]	2.92[b]	3.43[b]
50	1.62[c]	2.48[c]	3.00[c]
75	1.23[d]	1.87[d]	2.68[d]
100	0.75[e]	1.35[e]	1.95[e]
LSD (0.05) 0.07	0.07	0.06	0.07

For *A. niger* on days 3, 5 and 7, all the concentrations of *Moringa* extract; 25, 50, 70 and 100% (w/v) significantly (P<0.05) reduced radial growth of *A. niger* compared with the control. There was significant difference in the radial growth of *A. niger* in all the days. The degree of inhibition increased with increasing concentrations with 100%w/v showing significantly higher inhibition compared to all other concentrations as seen in Table 4.

Table 4: Effect of *Moringa* extract on the Radial Growth of *Aspergillus niger in vitro*

Concentration (w/v)	Radial growth (cm)		
	Day 3	Day 5	Day 7
0	2.74[a]	4.73[a]	6.22[a]
25	1.81[b]	2.07[b]	2.97[b]
50	1.53[c]	1.83[c]	2.58[c]
75	1.02[d]	1.35[d]	2.05[d]
100	0.68[e]	0.93[e]	1.45[e]
LSD(0.05)	0.08	0.07	0.07

For *Curvularia affinis* on days 3, 5 and 7, all the concentrations of *Moringa* extract; 25, 50, 70 and 100% (w/v) significantly (P<0.05) reduced radial growth of *Curvularia affinis* compared with the control. There was significant difference in the radial growth of *Curvularia affinis* across the concentrations on all the days. The degree of inhibition increased with increasing concentrations as with 100%w/v showing significantly higher inhibition of the fungi compared to other concentrations as seen in Table 5.

Table 5: Effect of Moringa extract on the Radial Growth of *Curvularia affinis in vitro*

Concentration (w/v)	Radial growth (cm)		
	Day 3	Day 5	Day 7
0	2.60[a]	4.50[a]	6.57[a]
25	1.98[b]	3.18[b]	4.02[b]
50	1.62[c]	2.72[c]	3.50[c]
75	1.20[d]	2.20[d]	3.03[d]
100	0.78[e]	1.83[e]	1.92[e]
LSD (0.05)	0.07	0.04	0.06

For *Rhizopus stolonifer,* on days 3, 5 and 7, all the concentrations of *Moringa* extract; 25, 50, 70 and 100% (w/v) significantly (P<0.05) reduced radial growth of *Rhizopus stolonifer* compared with the control. There was significant difference in the radial growth of *Rhizopus stolonifer* on all the days of the study. The degree of inhibition increased with increasing concentrations with 100%w/v showing significantly higher inhibition on fungi growth as seen in Table 6.

Percentage germination and fungi occurrence in maize seeds treated with different concentrations of *Moringa* extract

The effect of seed treatment with *Moringa* leaf extract on the occurrence of fungi species on maize seeds is shown in Table 7. All concentrations (25, 50, 70 and 100 % w/v) significantly reduced percentage occurrence of fungi. There was significantly higher difference in growth inhibition of the fungi between the 0% (w/v) concentration and the rest concentrations. However, there was no significant difference between concentrations 25 and 50% (w/v) as well as between concentrations 70 and 100% (w/v) on growth inhibition of the fungi. Seed germination was highest in the lowest concentration 0%w/v (99.2) and this was significantly higher compared to the other concentrations but was not significantly different from 25%w/v.

Table 6: Effect of Moringa extract on the Radial Growth of *Rhizopus stolonifer in vitro*

Concentration (w/v)	Radial growth (cm)		
	Day 3	Day 5	Day 7
0	3.05[a]	5.63[a]	7.45[a]
25	2.93[b]	3.98[b]	5.00[b]
50	2.40[c]	3.80[c]	4.92[c]
75	1.73[d]	3.03[d]	4.43[d]
100	1.03[e]	2.53[e]	2.72[e]
LSD (0.05)	0.06	0.05	0.32

Table 7: Percentage germination and fungi occurrence in maize seeds treated with different concentrations of *Moringa* extract

Concentrations (w/v)	Percentage Occurrence	
	Fungi	Percentage seed germination
0	55.1[a]	99.2[a]
25	27.8[b]	94.4[a]
50	25.6[b]	27.8[b]
70	10.0[c]	24.4[b]
100	3.3[c]	14.4[b]
LSD (0.05)	9.94	16.16

DISCUSSION

The result of this study revealed a percentage occurrence of fungi species from 11-30% in maize samples collected from four different markets during storage in Makurdi. The varying percentage of fungi species observed in this study can be attributed to poor storage structures and pre-harvest infections which greatly influenced the mycoflora in storage. The nature of these structures most times does not guarantee a moisture free storage, mould infection and hence low protection of grains against contamination (Afzal et al*.,* 2009). Occurrence of *A. flavus*, *A. niger*, *Rhizopus stolonifer* and *Curvularia affinis* in this study differed markedly from market to market with High level market having the highest percentage occurrence while Wadata market had the least percentage occurrence. Also, there was a significant difference in percentage occurrence between Wadata/Modern markets compared with High level/Wurukum. This might be due to improper handling and processing of seeds after harvesting, difference in storage conditions and kind of seed-borne fungi associated with them.

The result obtained showed that different concentrations of *Moringa* leaf extract reduced the radial growth of *A. niger* in the maize seeds examined. The ability of *Moringa* leaf extracts to inhibit the growth of *A. niger* implies that *Moringa* leaf extract possesses strong antifungal properties which are also dependent on the concentrations of the plant extract.

In terms of effect of concentration of *Moringa* leaf extract on the percentage occurrence of fungi species, it was observed that varying concentrations of *Moringa* leaf extract affected significantly, the percentage occurrence of four species (*Aspergillus flavus, Aspergillus niger, Rhizopus stolonifer*, *Curvularia affinis*). It was observed that increasing concentrations of *Moringa* leaf extract resulted in reduction in the percentage occurrence of fungal species (*Aspergillus flavus, Aspergillus niger, Rhizopus stolonifer*, *Curvularia affinis*). This is so since the control (maize seeds without *Moringa* leaf extract treatment) recorded the highest abundance of *Aspergillus* species, *Rhizopus stolonifer* and *Curvularia affinis*. The effect of concentrations of *Moringa*

leaf extract on percentage occurrence of *Aspergillus* species, *Rhizopus stolonifer* and *Curvularia affinis* also differed significantly. Ability of *Moringa* leaf extract to affect the relative abundance of *Aspergillus* species by lowering its abundance is similar to the report of Liamngee et al., (2015). The abundance of *Aspergillus* spp, *Rhizopus stolonifer* and *Curvularia affinis* was affected by the plant extract due to its antifungal nature.

CONCLUSION

Four major species of fungi (*Aspergillus flavus, Aspergillus niger, Rhizopus stolonifer*, and *Curvularia affinis*) are associated with maize seeds during storage in Makurdi, Benue State of which *Aspergillus niger* was the most abundant. The relatively high prevalence of *Aspergillus niger* points to the possibility of aflatoxin contamination. It can safely be concluded that application of *Moringa* leaf extract inhibits greatly the growth of fungi on maize seeds and the degree of inhibition increases with increasing concentration.

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