Incidence of *Aspergillus* species in Maize seeds in Konshisha Local Government of Benue State and their Control using Neem leaf extract

Fayinminu, A.O[a], Ati, T.T[b], Agbatar, B.L.[c], San, S.J.[d] and Orbunde, J.J.[e]

[a]akintadeojo@gmail.com, [b] theophilusati@gmail.com, [c] bobagbatar@gmail.com

[a]Benue State University, Makurdi, Nigeria
[b]Benue State University, Makurdi, Nigeria
[c]Benue State University, Makurdi, Nigeria

Abstract

Seed is the basic and most important structure through which majority of crops are grown. To increased produce, there is need for viable seeds and proper preservative measures which prevent the seeds from attack by fungal pathogens, thus enabling germination and increased productivity. It is to this effect a study was carried out to investigate the occurrence of *Aspergillus* species in maize seeds obtained from Konshisha Local Government Area of Benue State and the use of plant extract to control the seed borne fungi. The study was conducted at the Botany Laboratory of the Department of Biological Sciences, Benue State University, Makurdi. A total of five markets namely; Agia, Ati, Awajir, Koryan and Shiliki were used for the collection of maize samples. A total of Four hundred and fifty seeds (360) were collected across the five locations and were analyzed for seed borne infection. The blotter method of fungal detection and isolation was employed for the study. Neem leaf extract was prepared at different concentration of 10, 20, 30, 40 and 50% w/v for the *in vitro* control of fungi isolated. The result obtained showed the presence of two Aspergillus species namely; *Aspergillus flavus and Aspergillus niger*. There was no significant difference in the occurrence of *A. flavus* among the markets assessed. Although, the highest value was recorded in Ati market. Percentage occurrence of *A. niger* was significantly high at ($P \leq 0.05$) in Koryan markets compared with Ati and Awajir markets. Values for shiliki and Agia were not different from that of koryan but were also significantly ($P \leq 0.05$) higher compared with Ati which had the lowest occurrence of *A. niger*. There was no significant difference recorded across the five markets as regards percentage seed germination. Neem leaf extract was effective in inhibiting radial mycelia growth the test fungi. Radial growth inhibition at 50% w/v was significantly higher ($P<0.05$) compared to other concentrations. The use of Neem leaf extract can be used to control the growth of these fungi on maize seeds in storage and also enhance their germination potential. Therefore government should establish industries for the seed treatment of crops with plant botanicals as this will help reduce the amount of money spent on synthetic fungicides.

Keywords: Fungi; Incidence; radial growth; plant extract

1. Introduction

Maize *(Zea mays* L.) is one of the world's widely cultivated cereal and ranks third in the world production after wheat and rice (FAO, 2002). It is a staple food for most Nigeria households in both urban and rural areas. In Nigeria, maize is grown in the North, East, West and Southern region, either in large or small scale farms. Its low productivity has been associated with various constraints especially drought. Drought is a common phenomenon in tropical environments contributing to annual maize yield losses of between 17- 60 % in severe drought conditions (Zaidi *et al*., 2004).). Other constraints include storage pests and fungal infections that attack maize in storage (Gichuki *et al*., 2000). It has be grown to be local 'cash crop' most especially in the southwestern part of Nigeria where at least 30% of the crop land has been devoted to small-scale maize production under various cropping systems. In Nigeria, the demand for maize is increasing at a faster rate daily. This may be due to the fact that the grain is being used for feeding poultry and also serve as the main food for many households (Sadiq *et al.,* 2013).

Maize grows well in various agroecologies and is unparalleled to any other crop due to its ability to adapt in diverse environments. It has emerged as a crop of global importance owing to its multiple end uses as a human food and

livestock feed and serves as an important component for varied industrial products. Besides, maize serves as a model organism for biological research worldwide (Hossain *et al*., 2016). Fungi belonging to the genera *Fusarium*, *Aspergillus* and *Penicillium* are commonly encountered on maize and are capable of producing mycotoxins which has proven to be toxic to man and animals (Orsi *et al*., 2000). The most common seed-borne maize mycoflora include *Fusarium* spp., *Aspergillus flavus*, *Aspergillus parasiticus*, *Cladosporium* spp. and *Penicillium* spp. More so, in order to combat the attack of maize plants by these pathogens either on field or during storage, Hossain *et al*. (2016) reported that the use of chemicals is considered as one of the best options for managing these diseases in the field, in the greenhouse and in storage. However, there are concerns about the use of synthetic chemicals in agriculture and the potential hazards associated with their use. This can probably be regarded as an echo of public concern in this regard. Hence, there is elevated interest in finding alternative measures to manipulate either seed germination or seedling growth or both in an attempt to address both the plant stand problem and consumer concern. Thus, natural products are a source of new chemical diversity and are the choice of today's world as they poses less or no threat, ecofriendly and are readily available (Gurjar *et al*., 2012).

2. Materials and Method

2.1. Collection of Samples

Maize seed samples of the white variety were collected from five major markets in Konshisha namely; Agia, Ati, Awajir, Koryan and Shiliki markets. Thirty (30) seeds were collected from three different sale points in each market making a total of ninety (90) seeds per market and four hundred and fifty (450) across the five markets. The collected maize seed samples were packaged in polythene bags, labeled properly and conveyed to the Botany Laboratory of Benue State University for isolation of the seed borne fungi. Also, Neem leaf was collected within the premises of the Benue State University packaged in polythene bags and taken to the Botany Laboratory for Neem leaf extract preparation.

2.2. Preparation of Media

Potato Dextrose Agar was prepared by dissolving 39g of PDA in 1000 ml of water. The content of the flask was stirred vigorously to homogenize. The flask content was heated until the solution became clear and no layers were formed. After heating, the mouth of the flask was covered with cotton wool wrapped in foil paper and introduce into the autoclave. Autoclaving was done for 15 minutes at a temperature of 121^0C at 760mm/Hg.

2.3. Assessment of the occurrence of Aspergillus species and seed germination of Maize samples Collected From different Markets in Konshisha.

The standard blotter method recommended by the international seed Testing Association (ISTA) (1996) was employed for isolation of the seed borne fungi in maize samples. Three layers of Whatman filter papers were soaked in sterile water and placed at the bottom of 9 cm diameter Petri dishes. Ten seeds were taken from each sale point in the market and replicated three times to make a total of thirty seeds. The seeds were surface sterilized in 0.5% Sodium hypochlorite for 1 minute and were rinsed in three successive changes of sterile distilled water as reported by Ekefan *et al*. (2018). The sterilized seeds were placed on the moist filter paper in the Petri dishes and incubated at ambient temperature for 7 days. After 7 days of incubation, the seeds were examined for fungal growth and germination of seeds. On appearance of fungi, little quantity of each fungi colony was picked with the aid of inoculation needle and inoculated on prepared Potato Dextrose Agar (Agar). The Petri plates shall be incubated at ambient condition of light and temperature for 3-4 days and observed daily for fungal growth. After 3-4 days, sub-culturing was done to obtain pure culture of the isolates. The data collected include; occurrence of fungi and seed germination rate.

Percentage occurrence of fungi

Seeds shall be observed for growth and the occurrence of fungi shall be determined by counting the number of times each individual fungus occurred divided by the total number of fungi and expressed as a percentage using the formula adopted by Liamngee *et al*. (2016).

$$\frac{\text{Number of times each fungus occurred}}{\text{Total number of fungi per plate}} \times 100$$

Percentage Seed Germination

Total number of seeds germinated shall be recorded and the percentage seed germination shall be calculated using the formula adopted by Liamngee *et al*. (2016).

$$\frac{\text{Number of seeds germinated}}{\text{Total number of seeds per plate}} \times 100$$

2.4. Preparation and Concentration of Neem leaf Extract

Fresh leaves of Neem plant (middle age) were obtained and grams of 10, 20, 30, 40 and 50 were weighed differently using electronic weighing scale. Each weighed grams of Neem leaf was washed thoroughly and pounded with laboratory mortar and pestle to obtain fine paste. Each of the measured Neem leaf was poured in a clean and sterilized 75cl container and labeled. 100ml of distilled water was added into each of the Neem leaf paste and allowed to stand 3 hours. After 3 hours the paste was filtered to obtain pure Neem leaf extract to give 10% w/v, 20% w/v, 30% w/v, 40% w/v and 50% w/v extract concentrations.

2.5. Effect of Neem Leaf Extracts on the Radial Growth of *Aspergillus* species *In vitro*

Two milliliters each of 10% w/v, 20% w/v, 30% w/v, 40% w/v and 50% w/v of the extract was dispensed in sterile Petri dishes after which 20mls of molten Potato Dextrose Agar (PDA) was added. The mixtures in the Petri plates was shaken gently to mix the content and then allowed to solidify and then used for inhibition of mycelia growth of the test fungi. A 5mm agar plug from a 7 day old culture of the fungi isolates was placed centrally on the medium in Petri plates. Controls were Petri dishes containing Potato Dextrose Agar with no plant extract. All plates were properly labeled and incubated at room temperature for 5-7 days during which measurement of growth of fungal colony was carried out using a metre rule at intervals of 48 hours. Radial growth of fungal mycelia was calculated using the formula below;

$$\frac{R_1 - R_2}{R_1} \times 100$$

Where R_1 = radial growth of the pathogen in the control plate

R_2 = growth of the pathogen with treatment

2.6. Data Analysis

The data collected was analyzed using Analysis of Variance and treatment means were separated using Fisher's Least Significant Difference at 5% level of significance.

3. Results

The *Aspergillus* species isolated from Maize seeds in this study were *Aspergillus flavus* and *Aspergillus niger*. The growth of *A. flavus* on PDA has a bluish green colouration with rings formed along the spores as shown in Plate 1a and the microscopic view is shown in Plate 1b with a green or bluish green radiating conidial heads and a transparent conidiohores. Appearance of *A. niger* on PDA has a smooth dark or dark brown colouration on the surface as shown in Plate 2a and its microscopic view is shown in Plate 2b with a dark or dark brown conidial and has a hyaline conidiophores.

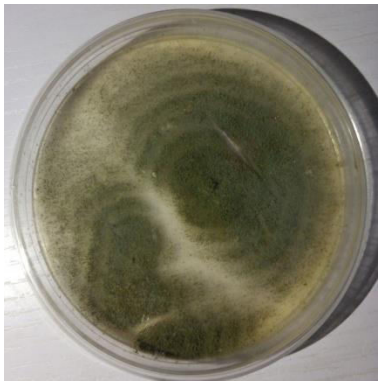

Plate 1(a) *A.flavus* on PDA

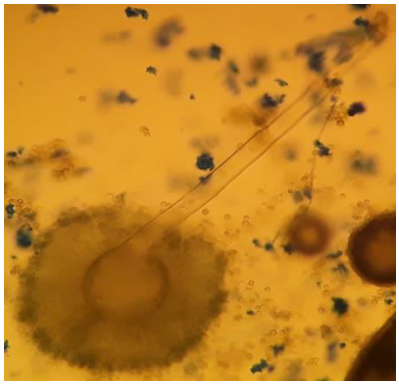

Plate 1 (b) *A.flavus* viewed under microscope

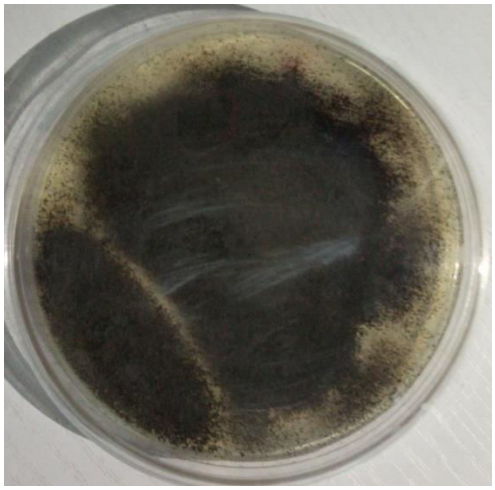

Plate 2(a) *A. niger* on PDA

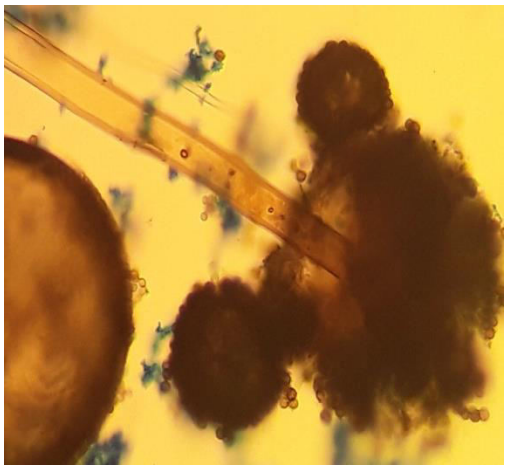

Plate 2 (b) *A. niger* viewed under microscope

Table 1. shows the percentage occurrence of *Aspergillus* species and percentage seed germination of maize samples collected from Konshisha Local Government Area of Benue State. There was no significant difference in the occurrence of *A. flavus* among the markets assessed. Although, the highest value was recorded in Ati market. Percentage occurrence of *A. niger* was significantly high at ($P \leq 0.05$) in Koryan markets compared with Ati and Awajir markets. Values for shiliki and Agia were not different from that of koryan but were also significantly ($P \leq 0.05$) higher compared with Ati which had the lowest occurrence of *A. niger.* For percentage seed germination, there was no significant difference recorded across the five markets.

Table 1**.** Percentage occurrence of *Aspergillus* species and percentage seed germination of maize samples collected from Konshisha Local Government Area f Benue State.

Markets	*A. Flavus*	*A. Niger*	% seed germination
Agia	19.2	24.7	18.3
Ati	20.0	16.67	19.2
Awajir	17.8	19.17	16.7
Koryan	18.5	30.0	21.7
Shiliki	12.8	24.17	23.3
$LSD_{(0.05)}$	NS	7.24	NS

There was no significant difference in the occurrence of *A. flavus, A. niger* and percentage seed germination as influence by seed treatment with Neem leaf extract.

Table 2**.** Effect of seed treatment with neem leaf extract on the percentage occurrence of *Aspergillus* species and percentage seed germination of maize samples collected from Konshisha Local Government Area of Benue State.

Extract concentration (w/v)	*A. Flavus*	*A. Niger*	% seed germination
0	12.0	10.0	6.0
10	12.0	6.0	6.0
20	22.0	8.0	6.2
30	22.0	14.0	2.0
40	14.0	10.0	2.0
50	14.0	6.0	8.0
$LSD_{(0.05)}$	NS	NS	NS

Table 3. shows the effect of Neem leaf extract on radial growth of *A. flavus* on Potato Dextrose Agar. At day 3 radial growth of *A. flavus* was significantly higher ($p \leq 0.05$) in the untreated control compared with other treatment. All the levels of Neem leaf extract treated significantly ($P \leq 0.05$) reduced radial growth of *A.flavus*. The same trend was observed at day 7 but on day 5 radial growth in the control and that of 10% w/v was not different.

Table 3. Effects of neem leaf extract on radial growth of *A. flavus* on Potato Dextrose Agar.

Extract concentration (w/v)	Day 3	Day5	Day 7
0	2.70	2.30	6.52
10	0.40	0.75	1.22
20	0.04	0.52	0.42
30	0.50	0.45	0.40
40	0.30	0.42	0.40
50	0.30	0.37	0.30
$LSD_{(0.01)}$	0.32	0.32	0.93

4. Discussion

Seed health is usually defined by the presence of pathogens like viruses, bacteria, fungi and animal pests such as insects. Among these pathogens fungi and are known to cause greater damage to seeds. In this study two main fungi were identified namely *A. flavus* and *A. niger* in maize in most markets and the percentage occurrence of *A. niger* was more than *A. flavus*. The occurrence of *A.niger* in maize in most of the market sampled indicate that *A. niger* is high in Konshisha local Government Area of Benue State and most of the maize species grown are liable to fungi and aflatoxin contamination. Low percentage occurrence of *Aspergillus* species was notice across the markets sampled and this differs with the works of Bankole and Adebanjo, (2003) who showed that percentage occurrence of *Aspergillus* species is higher in post-harvest maize of some states in West Africa countries. All these difference are

attributed to poor agronomic practice like poor storage facilities and pre-harvest infection that pose a major treat on maize seeds. Percentage seed germination in the maize seed sampled indicated that the highest percentage seed germination was in Shiliki market as compared to Agia, Ati, Awajir and koryan markets and this difference were as a result of seed viability and poor treatment of seeds after harvesting. The effect of seed treatment with Neem leaf on the percentage occurrence of *Aspergillus* species and percentage seed germination reveals that the varying concentration of Neem leaf did not inhibit the occurrence of *Aspergillu*s species and seed germination and this disagree with the works of Torres *et al*. (1980) that says Neem leaf constituent are known to potentially inhibit *Aspergillus* species. This difference in inhibiting potentials might be attributed to the concentration rates of the Neem leaf extract used which might not be effective enough to inhibit fungi infestation of maize seeds.

In testing the effect of Neem leaf extract on the growth of *A.flavus* on PDA, the result reveals that various concentration of Neem leaf extract reduced the radial growth of *A.* flavus in maize seed tested. The potential inhibitory power of Neem leaf extract revealed by this research shows that Neem leaf extract has a strong antifungal properties. These studies agree with similar works of Suleiman and Omafe (2014) that says that extract of botanicals reduces or inhibit radial growth of *A. flavus.* The presence of active ingredients such as saponin, tannin, alkaloids as pointed out by Vinoth *et al*. (2012) explained the antifungal potentials of this plant.

5. Conclusion

Maize at post-harvest stage contained high population *Aspergillus*s pp. The occurrence of *A. niger* was high in Ati and Agia market. This is a concern especially because the presence of *Aspergillus* in food can be directly associated with aflatoxin contamination*. A. flavus and A.niger* were detected in fungal isolates of the maize samples collected and the use of Neem leaf extract can be used to control the growth of these fungi on maize seeds in storage and also enhance their germination potential.

Acknowledgements

Our sincere appreciation goes to the Department of the Biological Sciences for granting permission to use the Botany Laboratory and equipment for carrying out this research.

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