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REGULAR ARTICLE

ISOLATION AND MORPHOLOGICALLY IDENTIFICATION OF ASPERGILLUS FLAVUS INCIDENCES FROM MAIZE SEEDS IN ABUJA, NIGERIA

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ABSTRACT

Food safety and security well implemented could help in making more crops available for consumption. Maize seed is a crop well known to be attacked by fungi such as Aspergillus flavus and reduce its nutrients. This study intends to isolate and morphologically identify the Aspergillus flavus from maize seeds from Abuja, Nigeria. The experimental design was complete randomized design involving untreated maize seeds from 7 locations in Abuja, Nigeria. Pure culture of fungal isolate was prepared using Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA). Isolates obtained were characterized and identified on the basis of their colonial and morphological characteristics which include macroscopic and microscopic examinations. All maize seeds from the Abaji had no fungi incidences in both SDA and PDA, while all maize seeds from the experimental field show fungi incidences in both SDA and PDA. On SDA, the maize color yellow and white had F (2, 6) static values of 7.083 and 0.212 at p=0.129 and 0.941 respectively. For the PDA, white maize seeds and yellow maize seeds from all the locations had F (2, 6) static values of 0.377 and 0.521 at p=0.850 and 0.773. Aspergillus flavus was isolated in this study. The maize seeds from Kuje district and the experimental field 2 show a high incidence records. All growth of the Aspergillus in the two media used were not significant at p>0.05.

Keywords: Maize seed, Aspergillus flavus, food safety, fungal incidences, Aflatoxin

INTRODUCTION

Fungi are known to cause deterioration and loss of nutrients in maize after insect (Debnath et al., 2012). The fungi genera Aspergillus, Bipolaris, Curvularia, Fusarium, and Penicillium which are well known fungi that attack seeds have been linked with maize seeds (Hussain et al., 2013). Aspergillus flavus is the most common member of the Aspergillus species in West African and the United States soils (Gachara et al., 2018). Systemically, fungal attack on maize replete its viability, nutrient quality and quantity, seedling blight, failure in germination, subdued seedling and unappreciable crop performance (Enyiwku and Ononuju, 2016). Aspergillus flavus is a saprophytic pathogen that thrives largely on many organic nutrient sources with sugars (Amaike and Keller, 2011). It is a fungus with wide economic impact which cut across been a pathogen of animals and insects, cause of storage rots in large number of crops, production of highly regulated mycotoxin, aflatoxin B1 (Klich, 2007). Its aflatoxin contaminants had been reported in some agricultural products (Perrone et al., 2014). A. flavus a well-known and cosmopolitan fungus could survive some series of environmental conditions (Abbas et al., 2009). They have the tendency to survive temperatures within 12°C to 48°C, an optimal growth temperature of 28°C to 37°C and a high humidity above 80% (Hell and Mutegi, 2011; Yu, 2012). As a storage mold on plants products, Maize seeds have been reported to be infected by A. flavus in the field prior to their harvest and in storage (Klueken et al., 2009).

Maize (Zea mays L.) a cereal crop belongs to the Poaceae family and it is rich in vitamins A, C and E, carbohydrate, protein, essential minerals, fibre and calories (Salako et al., 2019). Millions of maize about 8.63 million Metric Tons (MT) is produced annually in Nigeria (Sule et al., 2014). It is a staple for over 1.2 million individuals in Africa and Americas (IITA, 2009; USDA, 2016). Maize is used as raw material for some industrial production, feed, fodder, and vegetable. It has been reported that poor storage condition, storage period, temperature, humidity levels and suitable climate could lead to infection caused by various storage fungi, such as Aspergillus species (Ezekiel et al. 2008).

This study intended to isolate and morphologically identify the Aspergillus flavus from maize seeds from Abuja, Nigeria.

MATERIALS AND METHODS

Study area

The maize seeds used in this study was obtained from seven (7) different locations across Abuja, Nigeria, Table 1 and Figure 1 illustrate their geographical location.

Table 1: Location where maize seeds were obtained with their geographical location

<table>
<thead>
<tr>
<th>Maize seed source</th>
<th>Region in Abuja</th>
<th>Abbreviation</th>
<th>Latitude, Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bwari (BR) Market</td>
<td>Abuja North</td>
<td>BR</td>
<td>9.3046N, 7.3768E</td>
</tr>
<tr>
<td>Goza (GZ) Market</td>
<td>Abuja North</td>
<td>ABN</td>
<td>8.9307 N, 7.2994 E</td>
</tr>
<tr>
<td>Gwagalada(GL) Market</td>
<td>Abuja North</td>
<td>ABN</td>
<td>8.9308 N, 7.0969 E</td>
</tr>
<tr>
<td>Experimental Field</td>
<td>UNIABUJA</td>
<td>-</td>
<td>F 8.9807 N, 7.1805 E</td>
</tr>
<tr>
<td>Kuje Market</td>
<td>Abuja South-ABS</td>
<td>ABN</td>
<td>8.8810 N, 7.2281 E</td>
</tr>
<tr>
<td>Kwali Market</td>
<td>Abuja South-ABS</td>
<td>ABN</td>
<td>8.8153 N, 7.0363 E</td>
</tr>
<tr>
<td>Abaji Market</td>
<td>Abuja South-ABS</td>
<td>ABN</td>
<td>8.5082 N, 7.0348 E</td>
</tr>
</tbody>
</table>

Seed collections

Two different color of maize seeds were obtained from six different markets across Abuja and experimental field, University of Abuja (UNIABUJA). Distribution of the location where the maize seeds were obtained is as illustrated in Fig. 1.
Preparation and sterilization of media

Sabouraud Dextrose Agar (SDA)
SDA was used in this study and prepared according to the manufacturer’s instructions thus, 65g of SDA is dissolved in 1000 ml of sterile water and then sterilized (autoclaved) at 121°C and pressure of 15pa for 15 minutes.

Potato Dextrose Agar
PDA was also used in this study and prepared according to the manufacturer’s instructions thus; 39g of PDA powder was added to 1 liter of distilled water and boiled while mixing to dissolve completely. The sterilization was done at 121°C for 15 minutes using the autoclave. The sterilized prepared media was dispensed aseptically into petri dishes.

Preparation of pure culture of fungal isolate

The young fungal colony were aseptically picked up and transferred to fresh sterile SDA and PDA plates to obtain pure culture. The pure cultures on SDA and PDA plates were grown at 25 ± 2°C in a refrigerator. The isolates were subculture to obtain young cultures for further studies (Klich, 2000).

Identification of the fungal isolate

Cultural identification

Twelve isolates obtained from subculture were characterized and identified on the basis of their colonial and morphological characteristics which include macroscopic and microscopic examinations. Among the characteristics used were colonial characteristics such as size, surface, appearance, texture, and reversed pigmentation of the colonies of sporing structures. Appropriate references were done by using mycological identification keys and taxonomic description (Harrigan and McCance, 2006).

Morphological Characterization of Aspergillus flavus

Morphological attributes as described by Klich (2002) and Clayton in Thathana et al., (2017) were then utilized for further verification the isolates. Attributes such as colony color, colony growth, colony texture exudation which could be classified as macroscopic characteristics were studied. For microscopic analysis, attributes such as vesicles, ascomidophores, phialides, matulae and conidia were observed under the microscopic analysis of the isolate. Riddell’s classic slide culture method (Thathana et al., 2017) and a method described by Diba et al., (2007) were used for the cultivation of the isolate the microscopic slides. Motic BA210 Basic Biological Light Microscope (Motic Instruments Inc., Richmond, BC, Canada) were used to examine the prepared slides using the immersion oil (100x) objective lens.

Incidence of fungi

Incidences of fungal infection on each sample were calculated by using the following formula:

\[ \text{In} (\%) = \frac{\text{Number of infected seeds}}{\text{Total number of seeds}} \times 100 \]

RESULTS AND DISCUSSION

The incidence of the fungi was calculated and stated as indicated in Table 1. In this study, from findings stated in Table 2, there was more fungi incidences with the potato dextrose agar (PDA) compared to the Sabouraud Dextrose Agar (SDA).

Table 2: Mean Incidence of Fungi on yellow and white maize seeds collected from field and farmer store across Abuja

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample code</th>
<th>Incidence %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SDA</td>
<td>PDA</td>
</tr>
<tr>
<td>1</td>
<td>FY</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>FW</td>
<td>2.9</td>
</tr>
<tr>
<td>3</td>
<td>GLW</td>
<td>0.0</td>
</tr>
<tr>
<td>4</td>
<td>GLY</td>
<td>10.2</td>
</tr>
<tr>
<td>5</td>
<td>BRW</td>
<td>0.0</td>
</tr>
<tr>
<td>6</td>
<td>BRY</td>
<td>0.0</td>
</tr>
<tr>
<td>7</td>
<td>GZW</td>
<td>0.0</td>
</tr>
<tr>
<td>8</td>
<td>GZY</td>
<td>0.0</td>
</tr>
<tr>
<td>9</td>
<td>KJW</td>
<td>0.0</td>
</tr>
<tr>
<td>10</td>
<td>KJY</td>
<td>30.4</td>
</tr>
<tr>
<td>11</td>
<td>ABW</td>
<td>0.0</td>
</tr>
<tr>
<td>12</td>
<td>ABY</td>
<td>0.0</td>
</tr>
<tr>
<td>13</td>
<td>KLW</td>
<td>2.9</td>
</tr>
<tr>
<td>14</td>
<td>KLY</td>
<td>5.8</td>
</tr>
</tbody>
</table>


All maize seeds from the Abaji (AB Y and AB W) had no fungi incidences in both SDA and PDA, while all maize seeds from the experimental field (F Y and F W) show fungi incidences in both SDA and PDA. The yellow maize seed overall show more fungi incidence than the white maize seeds. On SDA, from Table 3 the maize color yellow and white had F (2,6) static values of 7.083 and 0.212 at p=0.129 and 0.941 respectively.

Table 3: Analysis of variance of the maize types from the different location on the two media

<table>
<thead>
<tr>
<th>Media type</th>
<th>Maize colour</th>
<th>F (2,6)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDA</td>
<td>White</td>
<td>0.212</td>
<td>0.941</td>
</tr>
<tr>
<td></td>
<td>Yellow</td>
<td>7.083</td>
<td>0.129</td>
</tr>
<tr>
<td>PDA</td>
<td>White</td>
<td>0.521</td>
<td>0.773</td>
</tr>
<tr>
<td></td>
<td>Yellow</td>
<td>0.377</td>
<td>0.850</td>
</tr>
</tbody>
</table>

For the PDA, white maize seeds and yellow maize seeds from all the locations had F (2, 6) static values of 0.377 and 0.521 at p=0.850 and 0.773. The study by Sowley et al., (2018) also reported a non-significant fungal incidence occurrence from maize samples.

Phenotypic Characterization of the Aspergillus flavus Isolates

Macroscopic Characteristics of the Isolates on PDA
The colony characteristics of the isolates are shown in Fig. 2 at the inception, the isolates were seen to have mycelia white color.

For the PDA, white maize seeds and yellow maize seeds from all the locations had F (2, 6) static values of 0.377 and 0.521 at p=0.850 and 0.773. The study by Sowley et al., (2018) also reported a non-significant fungal incidence occurrence from maize samples.

Figure 2: Colony morphology of Aspergillus flavus isolates on PDA

The isolates after three days were seen to produce olive and dark green conidia, which happen to be the predominant appearance of the colony. They look raised like a cerebri. Droplets of liquid that is brown or uncolored were produced by the isolates. Sclerotia that were deep brown in coloration were produced in the
isolates. The colonies were encircled by a white border, and the colony diameter ranged between 65 and 75 mm. The undersides of the colonies were slightly pale.

Microscopic Characteristics of the Isolates on SDA

The attributes of the isolated colony are shown in Fig. 3. On the SDA the isolate colony were at the inception white with a velvety soft surface. After four days of growth, a floccose was seen at the center with a raise.

**Figure 3**: Colony morphology of *Aspergillus flavus* isolates on SDA

Yellowish-green and olive conidia were produced by the colony during sporulation. The whole surface of the colony was covered by conidia the edges, where border whitish in color were seen. On the sixth day of incubation, the produced sclerotia which were white initially became deep brown. No droplet of liquid known as exudates was produced.

**Microscopic Characteristics of the *A. flavus* Isolates**

The isolates were examined to ascertain their definite identification, the microscopic attributes (conidiophores, conidia, metulae, phialides and vesicles) (Fig. 4). The conidiophores appeared uncolored, thick walled, roughened and vesicles bearing. Their diameter ranged between 800 and 1200 µm. Some isolates exhibit vesicles that were subglobose and globose in others with difference in diameter, ranging between 1800 and 2000 µm. There were uniseriate or biseriate or both kind of cells. The phialides were situated on the metulae with the biseriate cells, but attached to the vesicle, in uniseriate cells. The vesicles were covered with the metulae and radiated in all directions from the vesicles. Globose with thin wall with 250 and 450 µm range in diameter made up the conidia.

**Figure 4**: *Aspergillus flavus* sporix100

According to Da Gloria (2011), both field and storage fungus contamination incidences in maize may vary among farms or producers in the same regions. This study share this view, for instance all the maize from Abaji-AB (yellow and white) show no fungal incidence in both PDA and SDA medium as illustrated in Table 2. While the case of Gwagwalada-GL and Kuje-KJ yellow side there was fungal incidence in both PDA and SDA. The environmental situations and conditions could be a major determinant in the varying occurrences of *A. flavus* in the various districts of Abuja indicated in this study. Warm climate play a significant role in a huge chance of infection by aflatoxin producing fungal in some regions and this infection occurs only when there is drought with increase in temperature (Cotty and Jaime-Garcia, 2007).

**CONCLUSION**

*Aspergillus flavus* was isolated in this study and the maize seeds from Abuja South Kuje district and the experimental field shows a high incidence records. All growth of the *Aspergillus* in the media were not significant at p>0.05. The maize seeds that recorded more incidences of *Aspergillus flavus* could be infected by higher levels of aflatoxins that cause some ill-health issue to humans, animals and plants. It will be wise then for fungus to be fight to its mini mum in crops.

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Yusuf et al., in Fungal Territory


