Rot Inducing Fungi of Watermelon (*Citrullus lanatus*) Fruits in Storage and Fruit Stalls in Maiduguri, Nigeria

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ABSTRACT

Fungi associated with decay of watermelon (*Citrullus lanatus* [Thunb.] Matsum. & Nakai) fruits in five different locations in Maiduguri Metropolis were investigated. Isolation of fungi associated with decayed watermelon fruits was carried out on acidified potato dextrose agar (PDA). A total of nine fungal isolates comprised of three *Aspergillus* spp., two *Scopulariopsis* spp. and four yeast species were isolated. *S. cerevisiae* occurred most often in about 26% of the spoilt samples and 60% of the locations. The location with the highest fungal load was Gamboru vegetable market. *D. hansenii* and *Z. bailii* were the least isolated from the samples. Pathogenicity test conducted showed that *A. niger* was the most pathogenic, followed by *S. cerevisiae* and *Z. bailii*. These fungal isolates likely used compromised surfaces of the fruits such as wounds to cause rots. The fruits must therefore, be properly checked for deep and even light scratches prior to shelving on fruit stalls or packing in storage as these rot pathogens can cause considerable fruit loss if they remain on wound sites.

Keywords: Watermelon, Aspergillus spp., Yeast species, Maiduguri.
INTRODUCTION

Watermelon (*Citrullus lanatus* [Thunb.] Matsum. & Nakai) fruits’ edible part is the endocarp which is sweet, juicy and red in colour. This portion constitutes 60% of the fruit and contains water, protein, fat, carbohydrate, minerals, vitamins, fiber and ash (Schippers, 2000). It is also commonly used to make a variety of salads, most notably fruit salad, fruit giving, fruit basket and fruit banquet (Motes et al., 2005). Like any other fruits, it supplies some necessary nutritional substances such as vitamins and essential minerals in human daily diet that keeps the body in a good and healthy condition (Ewekeye et al., 2013).

Borno state is a leading producer of watermelon in Nigeria. It is mainly cultivated in Kaga, Konduga, Marte Monguno, Kukawa, Mafa, Dikwa, Ngala and Magumeri Local Government Areas of the State and distributed to different parts of the country. It is also retailed out to vendors who in turn sell it whole or slice and sell to final consumers within the state. When sliced and packaged fresh into ready-to-eat form, the fruits were found to be contaminated by various fungi (Nwachukwu et al., 2008; Nwachukwu and Osuocha, 2014; Titilayo and Salome 2014; Odebisi-Omokanye et al., 2015; Udoh et al., 2015).

These fruits also get rotten either in storage or on retail shop shelves. It was against this background that the present study was set to determine the amount and species of fungi responsible for deterioration of watermelon fruits awaiting distribution or sale on retail shop shelves in Maiduguri, the Borno State Capital.

MATERIALS AND METHODS

The experiment for the assessment of fungal pathogens associated with infected watermelon fruits was conducted at the Plant Pathology Laboratory, Department of Crop Protection, University of Maiduguri, Borno State, Nigeria during the months of March to July, 2015.

Sample collection

For the laboratory assessment, five different locations comprised of four fruit stalls and one fruit storage site were used for collection of the samples. Fifteen (15) samples of the infected watermelon fruits were obtained from each of the five locations made up of Baga motor park, Gamboru vegetable market (storage site), Bama motor park, University of Maiduguri and Monday market in Maiduguri Metropolis.

Culturing procedure

Each of the infected sample was washed and surface sterilized in 1% commercial bleach for one minute. These were then rinsed in three successive changes of sterile distilled water and blotted dry with sterile filter paper. From each sample, five pieces of segments measuring 3mm³ from the advancing margins of rotted lesions and the healthy tissues were cut out with sterile scalpel and forceps, and plated on acidified potato dextrose agar (PDA) in 90 mm Petri dishes. The plates were incubated at room temperature (28 ± 2°C) for seven days.

Identification of the Isolated Fungi

During incubation of the plated samples, developing fungal colonies were sub-cultured continuously on fresh PDA plates to obtain pure cultures of the isolates. Fungal isolates were identified based on growth pattern, colour of mycelia and microscopic examinations of vegetative and reproductive structures of the fungi, according to IMI, 1992; Barnett and Hunter, 1998.
Pathogenicity test
The isolated fungi were tested for their ability to induce rot in healthy watermelon fruits. Healthy samples of the fruits were washed and surface sterilized with 1% commercial bleach and then washed in running tap water. The fruits were weighed before inoculation to serve as initial weight in kilogrammes. With the aid of a sterile cork borer, 5mm diameter cylindrical holes were dug into the healthy cucumber fruits and the plugs were pulled out. In each hole, a 3mm diameter mycelial disc of pure culture of each of the fungal isolates was introduced by placing it at the bottom of the hole. The plugs were carefully replaced and the wounded area sealed with sterile petroleum jelly to prevent external infection. The inoculated fruits were incubated at room temperature (28 ± 2°C) for 7 days. Inoculated watermelon fruits were subsequently observed for rot development. After which, final weight of the individual watermelon fruit and extent of rot (severity) were determined. The degree of pathogenicity of each fungus on the inoculated samples was determined by calculating the percent severity of rot (Kassim, 1986) as follows:

\[
\text{% severity} = \frac{W - w}{w} \times 100
\]

where, \( W \) = initial wt. of healthy fruits
\( w \) = final wt. of rotten fruits

RESULTS

Table 1. Occurrence of fungi associated with rotten watermelon collected from five different locations in Maiduguri Metropolis

<table>
<thead>
<tr>
<th>Fungal isolates</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>-</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>+</td>
</tr>
<tr>
<td>Aspergillus wentii</td>
<td>-</td>
</tr>
<tr>
<td>Debaryomyces hansenii</td>
<td>-</td>
</tr>
<tr>
<td>Kluyveromyces marxianus</td>
<td>+</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>+</td>
</tr>
<tr>
<td>Scopulariopsis brevicalis</td>
<td>-</td>
</tr>
<tr>
<td>Scopulariopsis fusca</td>
<td>-</td>
</tr>
<tr>
<td>Zygosaccharomyces bailii</td>
<td>-</td>
</tr>
</tbody>
</table>

I= Bama motor Park; II= Gamboru Market; III= University of Maiduguri; IV= Monday Market; V= Baga motor Park

Distribution of fungal species associated with spoiled samples of watermelon collected from five different locations in Maiduguri metropolis are presented in Table 1. Five moulds (A. flavus, A. niger, A. wentii, S. brevicalis and S. fusca) and four yeast species (D. hansenii, K.
marxianus, S. cerevisiae and Z. bailii) were found distributed among the five locations surveyed. The highest occurring isolate was S. cerevisiae which appeared in about 60% of the locations. While the location with the highest fungal load was Gamboru vegetable market with three genera of fungal isolates (Aspergillus spp., Scopulariopsis sp. and Zygosaccharomyces sp.). Baga motor park contained only S. cerevisiae out of the nine fungal isolates.

Table 2. Percentage of fungal isolates associated with locations of sample collection

<table>
<thead>
<tr>
<th>Location</th>
<th>Fungal isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bama motor Park</td>
<td>18.4</td>
</tr>
<tr>
<td>Gamboru Market</td>
<td>22.4</td>
</tr>
<tr>
<td>University of Maiduguri</td>
<td>28.6</td>
</tr>
<tr>
<td>Monday Market</td>
<td>20.4</td>
</tr>
<tr>
<td>Baga motor Park</td>
<td>10.2</td>
</tr>
</tbody>
</table>

Percentages of fungal isolates found in the surveyed locations are presented in Table 2. Watermelon fruit samples collected from University of Maiduguri fruit stalls were the most heavily infested (28.6%), followed by Gamboru vegetable market samples and Monday market Maiduguri with percent fungal load of 22.4 and 20.4, respectively. Baga motor park sample had the lowest percent fungal isolates.

Fig. 1. Percentage occurrence of fungi associated with spoiled watermelon fruits collected from five different locations in Maiduguri metropolis.
Fig. 1 shows the frequency of the fungi isolated from the spoiled samples of watermelon across the five locations surveyed in Maiduguri Metropolis. Nine fungal isolates were found associated with rots of watermelon fruits. Three *Aspergillus* spp. (*A. flavus*, *A. niger* and *A. wentii*), two *Scopulariopsis* spp. (*S. brevicaulis* and *S. fusca*), and four yeast species (*D. hansenii*, *K. marxianus*, *S. cerevisiae* and *Z. bailii*) were isolated. *S. cerevisiae*, *K. marxianus* and *S. brevicaulis* occurred most often in descending order of frequency 25.9, 16.7, and 14.8%, respectively. *A. niger* was more prevalent than other *Aspergillus* spp. isolated from the samples. *D. hansenii* and *Z. bailii* were isolated least often from the samples.

Table 3. Pathogenicity test

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Fresh weight before inoculation (kg)</th>
<th>Final weight after inoculation (kg)</th>
<th>Rot severity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. flavus</em></td>
<td>2.54</td>
<td>2.52</td>
<td>0.79</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>2.52</td>
<td>1.57</td>
<td>60.51</td>
</tr>
<tr>
<td><em>A. wentii</em></td>
<td>3.53</td>
<td>3.50</td>
<td>0.86</td>
</tr>
<tr>
<td><em>K. marxianus</em></td>
<td>2.59</td>
<td>2.54</td>
<td>1.97</td>
</tr>
<tr>
<td><em>S. cerevisiae</em></td>
<td>2.55</td>
<td>2.00</td>
<td>27.50</td>
</tr>
<tr>
<td><em>S. brevicaulis</em></td>
<td>3.20</td>
<td>3.10</td>
<td>3.23</td>
</tr>
</tbody>
</table>

Pathogenicity test conducted showed that all the fungal isolates tested were pathogenic to watermelon fruits (Table 3), though it varied from one isolate to another. *A. niger* was the most pathogenic resulting in spoilage of more than 60% of fresh samples of whole watermelon fruits inoculated with it. Whereas *S. cerevisiae* and *Z. bailii* resulted in rot severity of 27.5 and 20.2% over the final weight of the inoculated samples. The test showed that *A. flavus* and *A. wentii* were least pathogenic to watermelon fresh fruits.

**DISCUSSION**

The present study has found nine different fungal isolates associated with postharvest rots of watermelon fruits distributed among the five locations within Maiduguri Metropolis, Borno State. The yeast, *S. cerevisiae* appeared in more locations than other isolates and Gamboru market had more isolates than other locations. These are saprophytes growing on any substrates containing sugar (Singh and Sharma, 2007); possibly originated from the field and transported on surfaces of fruits to fruit stalls and stores. Gamboru market being the main vegetable market, where all supplies of fruits and vegetables are assembled before their distribution, may serve as a source of these rot pathogens.

*S. cerevisiae* and *K. marxianus* were the most prevalent fungal isolates on the rotten samples of watermelon fruits collected from different locations in the present study. Fruits contain high levels of sugars and nutrients element and their low pH values make them particularly desirable to fungal decay (Singh and Sharma, 2007). The same yeasts were similarly reported to be associated with spoilage of tomato (Mbajiuka and Emmanuel, 2014; Agbabiaka et al.,
2015), pepper (Ibrahim and Sada, 2015), and orange (Oviasogie et al. 2015). However, Nwachukwu et al. (2008), Nwachukwu and Osuocha (2014), Titilayo and Salome (2014) and Odebisi-Omokanye et al. (2015) found S. cerevisiae as contaminants of fresh sliced ready-to-eat watermelon fruits sold on the streets of some cities in Nigeria.

All the fungal isolates found in the present study caused rots on watermelon after subjecting them to pathogenicity test. However, the extent of pathogenicity varied considerably. A. niger, S. cerevisiae and Z. bailii being the most pathogenic, achieved 60.5, 27.5 and 20.2%, respectively, decay of fresh fruit as against other isolates. Similar deterioration of fruits by A. niger has been reported earlier in watermelon (Udoh et al., 2015) and also in other fruits (Al-Hindi et al., 2011; Tafinta et al., 2013; Amadi et al., 2014; Aminu and Ali, 2017). The least pathogenic fungal isolates were A. flavus and A. wentii that caused little damage to the fruits after inoculation.

The presence of these fungal pathogens on freshly cut watermelon fruits suggests that they used compromised surfaces of the fruits such as wounds to cause rots. Moreover, most watermelon fruit sellers use polythene sheets with poor ventilation to cover the fruits at the end of their sales period and before resuming sales or distributions; condition that favours growth of rot pathogens especially, the yeasts. The fruits must therefore, be properly checked for deep and even light scratches prior to shelving on fruit stalls or packing in storage as these rot pathogens can cause considerable fruit loss if they remain on wound sites. If only other fabrics are used to cover watermelon fruits on fruit stalls or in storage, this will reduce the rate of deterioration of the fruits as this will improve ventilation which slows down the growth of the anaerobes such as the yeasts.

CONCLUSION

The present study has found that all the tested fungal isolates were responsible for decay of watermelon fruits. The same isolates contaminate fresh cut ready-to-eat watermelon fruits. This suggests that they used compromised surfaces of the fruits such as wounds to cause rots. The fruits must therefore, be properly checked for deep and even light scratches prior to shelving on fruit stalls or packing in storage as these rot pathogens can cause considerable fruit loss if they remain on wound sites.

ACKNOWLEDGEMENT

We are grateful to the laboratory staff of the Department of Crop Protection, Faculty of Agriculture, University of Maiduguri for their assistance in the course of the study.
REFERENCES


