



Fungi Associated with the Spoilage of Water Melon Fruits (*Citrullus lanatus* Thumb.) in Jega Local Government Area, Kebbi State, Nigeria

Abubakar, M., Singh, D., Hamisu, A. and Bashar, G.B.

Department of Plant Science and Biotechnology

Kebbi State University of Science and Technology Aliero, Kebbi State, Nigeria

ABSTRACT

Fungal pathogens lead to deterioration and reduction in yield of many fruits produced in Nigeria. This study was carried out to determine the fungal pathogens associated with spoiled watermelon fruits in Jega Local Government Area, Kebbi State. A total of fifty (50) watermelon fruits were collected from five locations viz; Jega, Dangamaji, Kimba, Gindi and Basaura markets. The fungi were isolated using agar plate method and pure isolates were identified in accordance with a standard chart. A total of nine (9) fungal isolates were associated with the spoilage of the fruits namely; *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus wentii*, *Scopulariopsis brevicaulis*, *Scopulariopsis fusca*, *Debaryomyces hanseni*, *Kluyveromyces marxianus*, *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii*. From the results obtained, *Aspergillus flavus* had the highest percentage of occurrence (23.53%) followed by *Aspergillus niger* (15.29%) while *Scopulariopsis brevicaulis* had the lowest (3.53%). Based on locations, Kimba had the highest percentage of the fungal loads (28.6%) followed by Jega Central Markets (22.4%) with Gindi having the lowest (10.2%). The fungi isolated in this study may use compromised surfaces such as wounds to cause rots on these fruits. There is therefore need for proper and careful check-up for deep and even light scratches on these fruits before storage as these pathogens can lead to considerable damage and fruit loss if not properly managed.

Keywords: Fungi, Identification, Watermelon, Jega and Spoilage

INTRODUCTION

Watermelon (*Citrullus lanatus* Thunb.) belongs to the family Cucurbitaceae, which consists of nearly 100 genera and over 750 species (Amadi *et al.*, 2014). It is a flowering plant that is vine-like and whose fruits are neither fruits nor vegetables but berries (so-called “pepos”) which are also called watermelons. Berries have an interior which is sweet, juicy and full of water (hence the name) and exterior in a form of smooth hard rind which is green with dark green stripes or yellow spots (Nwachukwu *et al.*, 2008). Interior can be of different colors (red, pink, orange, yellow, or white) and it contains many seeds (although there are seedless variants).

Watermelon is grown annually and can be up to 3 meters long. They are widely distributed in the tropics and subtropics, and a few species occur in temperate region. Watermelon grows well in alluvial and sandy soils even in arid regions and coastal saline areas (FAO, 2016). The plant edible part is the endocarp which is sweet, juicy and red in color. This portion constitutes 60% of the fruit and contains water, protein, fat, carbohydrate, minerals, vitamins, fiber and ash (Udoh *et al.*, 2012). 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 (Udoh *et al.*, 2015).

The seed contains phytochemicals such as carbohydrate, phenol, flavonoids, protein, fibre, phosphorus and iron at different proportions. The Proximate analysis of the seed was discovered to contain fat content (47.9%), protein

(27.4%) and carbohydrate (9.9%). The presence of these phytochemicals helps the fruit to relieve inflammation, and increases digestive ability of the body system (Al-Hindi *et al.*, 2016). There has been a significant increase in the consumption of sliced water melon because it is nutritious, convenient, easy to obtain and cheaper than the whole fruits. Due to some factors such as poor handling, climate, insect attack and others, fruits contamination by pathogens is increasing on daily basis. In many unhygienic environments, fruits remain hygienically poor or contaminated since fungal loads are moderately high (Blomberg *et al.*, 2004).

Watermelon is cultivated by farmers in different parts of the World. It is used as vegetable (for consumption) and also for diverse Industrial applications. Despite the potentials and diverse uses to which it is subjected, its production is constrained by several biotic agents particularly fungi. Losses in fruit yield due to fungal pathogens can be as higher as 68% (Thiyam *et al.*, 2013). Similarly, \$3.5 million are lost annually worldwide as a result of fungal infections. The spoilage caused by fungi limits the fruits availability to consumers and also leads to economic waste and damage to farmers and the Country as a whole. Despite these damages, little information is available on fungal species causing the spoilage of these fruits in the region (Zhu *et al.*, 2006).

This study was carried out to determine the fungal pathogens associated with the spoilage of Watermelon fruits in the study area. Identifying these species would

help in mitigating management strategies thereby reducing the risk of these fruits spoilage by rots fungi.

MATERIALS AND METHODS

Study Area

The study was conducted in Jega Local Government Area of Kebbi State, Nigeria. It has an estimated land mass of 1043 km, located on latitude 12° 13' 19.88"N, longitude of 4°22' 46.67" and has a population of 73, 495 during the 2006 census. The area is characterized with two seasons - wet and dry seasons. The dry season lasts between May and October with a unimodal peak of rainfall in August. The mean annual temperature is 31°C and annual rainfall is 500 mm. The people of Jega are mostly farmers and traders (Suleiman, 2008).

Field Survey and Sample Collection

Watermelon (*Citrullus lanatus*) fruits were obtained from five different locations which include Dangamaji, Kimba, Gindi, Basaura and Jega central markets. The samples were transported to Botany Laboratory, Department of Biological Sciences, Kebbi State University of Science and Technology, Aliero for the mycological analysis. A total of twenty (50) infected watermelon fruits were obtained from the five locations visited in the study area.

Mycological Analysis

Each of the infected samples 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 was cut out with flame 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 (Oyeleke and Manga, 2008).

When fungal growth from the tissue was visible, fungi were sub cultured onto freshly prepared sterile PDA plates to obtain a pure culture for identification. Where there is a mixed culture, fungi were continuously sub cultured until pure isolates were obtained. Stock cultures of the pure

isolates were prepared and preserved at 4°C in the refrigerator (Ricciardi *et al.*, 2012).

Identification of Fungal Isolates

The fungal isolates were subjected to comparative morphological studies by an image and analysis system using published descriptions in a mycological atlas obtained at the Department of Microbiology Laboratory Kebbi State University of Science and Technology, Aliero. This was followed by a slide mount of each isolate. The characteristics observed were matched with those available in the aforementioned mycological atlas and were identified accordingly (Adebayo and Okonko, 2012).

Determination of Percentage Occurrence of the Isolates

This was done to determine the percentage occurrence of the different fungal isolates. The number of occurrence for each of the isolates was recorded and calculated using the formula as described by Carlson (2014). % Frequency = Number of identified fungi/Total number of fungi x100

RESULTS

The result in Table 1 presents the nine (9) fungal isolates identified from the five locations in the study area namely: *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus wentii*, *Scopulariopsis brevicaulis*, *Scopulariopsis fusca*, *Debaryomyces hansenii*, *Kluyveromyces marxianus*, *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii*. Table 2 shows the occurrence of fungi associated with rotten watermelon collected from five different locations in the study area. The highest occurring isolate was *A. flavus* which occurred in about 60 % of the locations. The result in Table 3 indicates the frequency of occurrence of the several species of fungi associated with spoiled watermelon in the study area. *Aspergillus flavus* had the highest frequency of occurrence (23.53%) followed by *Aspergillus niger* (15.29%) with *Scopulariopsis brevicaulis* being the lowest (3.53%). Table 4 presents percentages of fungal isolates found in the surveyed locations. Samples collected from Kimba were the most heavily infested (28.6 %), followed by Jega and Basaura each with (22.4%) of fungal loads respectively while Gindi had the lowest percentage of fungal isolates (10.2%).

Table 1: Morphological and cultural characteristics of the isolated fungi.

Fungal isolates	Cultural and morphological features
<i>Aspergillus niger</i>	Colonies consist of a compact white or yellow basal felt covered by a dense layer of dark brown to black conidial heads. Conidial heads are large globose, dark brown. Conidiophores stripes are smooth walled, hyaline or turning dark towards the vesicle.
<i>Aspergillus wentii</i>	Colonies are typically plain green in colour with dark red brown cleistothecia developing within and upon the conidial layer. Reverse may be olive to drab grey. Conidiophores stripes are usually short, brownish and smooth walled. Conidia are globose and rough walled.

<i>Aspergillus flavus</i>	Growth is recognized within three days Consisting of dense felt or yellow-green colouration with of septate hyphae. Consisting of dense felt or yellow-green conidiospores. Conidial heads typically radiate, later splitting into several loose columns, yellow-green becoming d a rudimentary yellow-green.
<i>Kluyvermyces marxianus</i>	Colonies are cream to brown with occasional pink pigmentation. Produce iron chelate pigments and pulcherrimin. Cells appear globose or ellipsoidal, 2-6x3-11 um in size. It forms a rudimentary to branched pseudo mycelium with few blastopores. A thin pellicle may also be present.
<i>Scopulariopsis fusca</i>	Colonies are white velvety to powdery with a central tuft. Occasionally the powdery appearance of the colony is disturbed by small white sterile over growths. Conidiogenous cells annellate, borne singly on aerial hyphae. Conidia sub globose to broadly ovate with a truncate base
<i>Saccharomyces cerevisiae</i>	Growth is observed within few days. Appear creamy- yellowish green. Appear in green flat circular colony. Spherical spores often in group of fours. Appear spherical or ovoid in shape. Have simple pseudo hyphae.
<i>Scopulariopsis brevicaulis</i>	Colonies brownish tan center, when stain with lactophenol cotton blue, numerous branched conidiophores with chains of conidia. Rough walled lemon shaped conidia with small protrusion is present. Conidial heads radiate, tending to split into loose columns with age.
<i>Zygosaccharomyces bailii</i>	Colonies usually ellipsoidal, produce ascospores that are smooth, thin walled. On various nutrients ager, colonies are smooth round convex and white to cream colored with a diameter of 2-3mm at 3-7 days.
<i>Debaryomyces hansenii</i>	Colonies are fast growing, powdery to suede-like, funiculose or tufted, and yellow- brown or sand-coloured. Yellow-brown colony pigmentation, cylindrical phialides, and presence of chlamyospores.

Table 2: Occurrence of fungi associated with rotten watermelon collected from five different locations in Jega Local Government Area

Fungal Isolates	Location				
	I	II	III	IV	V
<i>Aspergillus flavus</i>	+	+	-	+	-
<i>Aspergillus niger</i>	+	-	+	-	-
<i>Aspergillus wentii</i>	-	+	-	-	-
<i>Debaryomyces hansenii</i>	-	-	-	+	-
<i>Kluyveromyces marxianus</i>	+	-	-	+	-
<i>Saccharomyces cerevisiae</i>	+	-	-	-	-
<i>Scopulariopsis brevicaulis</i>	-	+	+	-	-
<i>Scopulariopsis fusca</i>	-	-	-	+	-
<i>Zygosaccharomyces bailii</i>	-	+	-	-	-

Key: I =Dangamaji Market, II=Jega Central Market, III=Kimba Market, IV=Basaura, V=Gindi

Table 3: Frequency of occurrence of fungi associated with spoilt watermelon in Jega Local Government.

Fungal Isolates	Frequency of occurrence	Percentage of occurrence (%)
<i>Aspergillus flavus</i>	20	23.53
<i>Aspergillus niger</i>	13	15.29
<i>Aspergillus wentii</i>	10	11.76
<i>Debaryomyces hansenii</i>	5	5.88
<i>Kluyvermyces marxianus</i>	7	8.24
<i>Saccharomyces cerevisiae</i>	12	14.12
<i>Scopulariopsis brevicaulis</i>	3	3.53
<i>Scopulariopsis fusca</i>	6	7.06
<i>Zygosaccharomyces bailii</i>	9	10.59

Total	85	100
-------	----	-----

Table 4: Frequency of fungal isolates with respect to locations of sample collection

Location	Fungal Isolates (%)
Dangamaji Market	18.40
Jega Central Market	22.40
Kimba Market	28.60
Basaura	22.40
Gindi	10.20

DISCUSSION

This study was conducted to determine the fungal species associated with spoilage of watermelon in Jega Local Government Area of Kebbi State. A total of nine fungal species were identified in this study of which *A. flavus*, had the highest frequency of occurrence (23.53.67%) while *Scopulariopsis brevicaulis* had the lowest (3.53%). The higher number of fungi species identified may be due to climatic conditions such as high temperature and air humidity which favor the growth of microorganism particularly fungal pathogens leading to deterioration of the fruits (Aladele *et al.*, 2008). Fruits contents of high levels of sugars and nutrient elements and their low pH values make them particularly desirable to fungi (Singh and Sharma, 2007). Similar study was carried out by Ezekiel and Sombie (2014), in Ogun State-Nigeria where eleven (11) fungal pathogens were identified on watermelon fruits.

The occurrence of the isolated fungi with respect to locations of sample collection revealed that Kimba had the highest percentage of the fungal loads (28.6%) followed by Jega with (22.4%) while Gindi had the lowest (10.2%). The variations in the fungal load may be due to the handling methods of the fruits during packaging, transportation or storage (Aladele *et al.*, 2008). Deterioration of fruits by *Aspergillus* species have been reported earlier in watermelon and also in other fruits (Al-Hindi *et al.*, 2011; Tafinta *et al.*, 2013; Amadi *et al.*, 2014; Udoh *et al.*, 2015 and Aminu and Ali, 2017). The presence of these fungal pathogens on freshly cut watermelon fruits suggest 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 and condition that favours the growth of rot pathogens especially, the yeasts (Aminu and Ali, 2017).

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 (Nwachukwu *et al.*, 2008).

CONCLUSION

This study revealed the presence of several fungi associated with spoilage of watermelon fruits in Jega Local Government Area, Kebbi State Nigeria. Postharvest diseases of fruits in general and Watermelon in particular are of huge economic importance. Although the results of the present study may be considered preliminary, these fungi associated with this crop are potential threat to its production. Further research should be conducted on the pathogenicity of these fungi associated with spoilage of the fruits to confirm the primary pathogens and to differentiate them from secondary invaders. Similarly, Proper handling methods should be adopted by both farmers and consumers to minimize the fungal deterioration of watermelon fruit in the study area.

REFERENCES

- Adebayo, B.C. and Okonkwo, T.O. (2012). Microorganisms associated with spoilage of stored Fruits and vegetables in Uyo metropolis, Akwa Ibom state, Nigeria. *Journal of Natural Sciences* 10(3): 23- 32.
- Al-Hindi, R.R., Al-Najada, A.R. and Mohamed, S.A. (2016). Isolation and identification of somefruit spoilage fungi: Screening of plant cell wall degrading enzymes. *African Journal of Microbiology Researches* 5(4): 443-448.
- Aladele, S. E., Ariyo, O. J. and Lapena, R. (2008). Genetic Relationship among West African Okra (*Abelmoschus caillei*) and Asian Genotypes (*Abelmoschus esculentus*) using RAPD. *African Journal of Biotechnology*.7:1426-1431.
- Amadi, J. E., Adebola, M. O. and Eze, C. S. (2014). Isolation and Identification of a Bacterial blotch Organism from Watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai). *African Journal of Agriculture Research*. 4(11): 1291- 1294.
- Aminu, F. and Ali, M. (2017). Isolation and identification of microorganisms associated with spoilage of cabbage (*Brassica oleracea*) in Sabon-Gari Market Kano, Nigeria. *International Journal of Advance Academic Researches*. 3(2): 1-8.
- Blomberg, M. (2004). "In Season: Savory Summer Fruits." The Gainesville Sun Retrieved Jul. 17, 2005.
- Carlson, L.(2014). Species delimitation in Trametes: a comparison of ITS, RPBI, RPB2 and TEFI gene phylogenies. *Mycologia*, 106(10): 3852/13275.
- Ezekiel, C. N and Sombie, J. I. (2014). Survey of aflatoxins and fungi in some commercial breakfast

- cereals and pastas retailed in Ogun State, Nigeria. *Journal of Natural Science*, 6: 27-32.
- Food and Agricultural organization (2016) FAO Stat Database. Available from: <http://faostat.fao.org>
- Nwachukwu, E., Ezeama, C. F. and Ezeanya, B. N. (2008). Microbiology of polyethylene- packaged sliced watermelon (*Citrullus lanatus*) sold by street vendors in Nigeria. *African Journal of Microbiological Research*, 2: 192-195
- Oyeleke, A. and Manga, S.B. (2008). *Essential of Laboratory Practice*. 3rd ed. Tobest Publisher, Minna, Niger state, Nigeria. p12-29.
- Ricciardi, C., Castagna, R., Ferrante, I., Frascella, F. and Luigi-Marasso, S. (2012). Development of a microcantilever-based immunosensing method for mycotoxins detection. *Journal of Biosensors and Bioelectronics*, 40: 233- 239.
- Singh, D. and Sharma, R.R. (2007). *Postharvest diseases of fruit and vegetables and their Management*. In: Prasad, D., editor. Sustainable Pest Management. Daya Publishing House, New Delhi, India.
- Suleiman, K. (2008). *A social, economic and political history of Jega*. Kraft Books Ltd. Ibadan, Nigeria
- Tafinta, I.Y., Shehu, K., Abdulganiyyu, H., Rabe, A.M. and Usman, A. (2013). Isolation and identification of fungi associated with the spoilage of watermelon (*Citrullus lanatus*) fruit in Sokoto State. *Nigerian Journal of Basic and Applied Sciences*. 21(3): 193- 196.
- Thiyam, B. and Sharma, G.D. (2013). Isolation and identification of fungi associated with local fruits of Barak Valley, Assam. *Current World Environment*, 8(2): 319-322.
- Udoh, I. P., Eleazar, C. I., Ogeneh, B. O. and Ohanu, M. E. (2015). Studies on fungi responsible for the spoilage/deterioration of some edible fruits and vegetables. *Advanced. Microbiology*, 5: 285-290.
- Zhu, S.J. (2006). *Non-chemical approaches to decay control in postharvest fruit*. In: Nouredine, B. and Norio, S., editors. Advances in Postharvest Technologies for Horticultural Crops Research Signpost, Trivandrum, India. Pp297-313.