



Isolation and Identification of Fungi Associated With Rot of Watermelon Fruit in Sokoto Metropolitan, Sokoto State, Nigeria

Y. I. Alhaji^{1*}, S. Y. Lema¹, J. Ibrahim¹, A. Umar¹ and M. Garba¹

¹Department of Biological Sciences, Sokoto State University, Sokoto, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors YIA and SYL designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JI and AU managed the analyses of the study. Author MG managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Watermelon fruit contain high vitamins and minerals. Watermelon is susceptible to many pathogenic microorganisms that results to unpleasant odours and cause foodborne diseases. This study was conducted to isolate and identify fungi that are associated with rot of watermelon fruit in Sokoto metropolitan. The presence of fungal organisms was examined in five different locations within Sokoto metropolitan. Isolated fungus was identified morphologically, microscopically and using molecular standard procedures. Results showed that (3- *Aspergillus* is one genus) 4 fungal genera were isolated and Identified. The isolated fungi were *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus stolonifer* and *Mucor spp*. Fungi isolated from this study are of economical and public health importance. This study was carried out to examine the fungi associated with the rot of watermelon fruit in Sokoto metropolitan. Careful handling of watermelon fruit are recommended to ensure longer shelf-life, prevent spread of rot pathogens and keep fruit in the highest quality before consumption.

*Corresponding author: Email: abusalima01@gmail.com;

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1. INTRODUCTION

The fungi encompass microorganism that fill a similar ecological niche and yet are distributed among several taxonomic groups [1]. The fungi found consistently in association with a particular plant disease are called pathogens [2,3]. Fungal disease affects the food supply and as such has a direct impact on human civilization, Fungal pathogens derive nutrition from plants as facultative saprophytes, facultative parasites, of biotrophs and in the process cause symptoms of disease, which broadly include abnormal growth, abscission, host tissue replacement necrosis [4]. Pathogens are classified as fungus like (plasmodiophoromycata and the stramenopiles) and as time fungi (*Chytridiomyeetazygomycota*, *Ascomycota* and *Basidiomycota*). Each fungal group differs in hyphal morphology, survival, dispersal and reproductive strategy [5].

Diagnosis of disease caused by these organisms is critical for appropriate management [6]. Watermelons (*Citrullus lanatus*) belong to the family Cucurbitaceae is a vine like flowering plant which originated from Southern Africa [7]. Its fruit, which is also called Watermelon is a special kind referred by botanists as pepo, a berry which has a thick rind (exocarp) and a fleshy center (mesocarp and endocarp) pepos are derived from inferior ovary, and are characteristics of the Cucurbitaceae [8]. The watermelon fruit has a smooth exterior rind (usually green with dark green stripes or yellow spots) a juicy, sweet interior flesh (usually deep red to pink, but sometimes orange, yellow, or white). There are more than 1200 varieties of Watermelon ranging in size from less than a pound, to more than 200 pounds - Watermelon contains about 61% sugar and 92% water by weight, as with many other fruits, it is a source of vitamin C. it is not a significant source of other vitamins and minerals [9].

However, most times only the fleshy pulp of Watermelon is consumed leaving the seed end - the rind. Watermelon contain a high percentage of water averaging 92%, fats, and protein in very small varying amounts, a fair proportion of carbohydrate present as cellulose, starch in small quantity and sugar. Beside their low energy value, they are known for their high nutrients concentration [10]. Watermelon fruit is made up to 95% moisture, 0.5% Ash, 0.1% oil. 0.5% fibre, 5% carbohydrate, 255.000 mg vitamin A, 0.04

mg thiamine, 0.03 mg Riboflavin 0.8 mg calcium, 9.00 mg phosphorus, 0.200 mg iron, 0.6 mg Niacin, 15.0 mg Ascorbic Acid, and 0.0mg potassium [11].

Watermelon has become synonymous with summer and picnics and for good reasons. Their refreshing quality and sweet taste help to combat the heat and also provide guilt free. Watermelon is an unsalted fruit source of carotenoid lycopene and a rich source of phenolic antioxidant [12]. Watermelon contain cucurbitacin, a triterpene anti-inflammatory phytonutrient, and usual amount of the amino acid citrulline, watermelon is a very good source of vitamin C. it is also good source of pantothenic acid, copper, biotin, potassium, vitamin A, (in the form of carotenoid), vitamin B1, vitamin B6, and magnesium [13].

Watermelon are used as a vegetable in China, they are stir-fried, stewed or more often pickled when stir-fried the skin and fruit is removed, and the rind is cooked with olive oil, garlic, chili peppers, scallions, sugar and rum. Watermelon is mildly diuretic and contains large amount of Carotenoids. Watermelon with red flesh is a significant source of lycopene [14]. This study aimed at isolating and identifying the fungi associated with rot of Watermelon fruit in Sokoto metropolitan considering its nutritional value and rate of consumption in our locality, while the objectives of this study are: To identify the common species of fungi associated with rot of Watermelon fruit in Sokoto metropolitan, To identify the effect of fungi on watermelon and to suggest possible ways of preventing and controlling Watermelon infection by fungi in Sokoto and Nigeria at large.

2. MATERIALS AND METHODS

2.1 Study Area

Sokoto is a city located in the extreme northwest of Nigeria, near to the confluence of the Sokoto River and the Rima Rivers. Sokoto is the modern day capital of Sokoto State. Sokoto is one of the seven northwestern states of Nigeria and is geographically located between Longitude 13°01'13"05' North and Latitude 5°10' 5"18' East. It covers a total land area of 51 km² (20 sq mi) [15].

It covers the two metropolitan local governments of Sokoto South and Sokoto North respectively,

the area is bordered by Kware local government to the North, Dange/Shuni Local Governments lies to the South. While it also shares boundaries with Wamakko local government in the West and Rabah Local Governemtn in the East. However, it is worthy to emphasize that some parts of the above mentioned local governments falls within the area that makes the Sokoto Metropolis [16].

The 2006 population and housing census put the population of Sokoto Metropolis at 232,846 (NPC 2006). This comprises the population of the, two metropolitan local governments of Sokoto North and Sokoto South together with the surrounding areas of the neighbouring local governments of Kware, Wamakko and Dange/Shuni [17]

2.2 Sterilization of Glass Wares

All the glass wares used, were washed with detergent water, rinsed very well and are allowed to air dry and were sterilized using a hot air oven at 160°C for 1 hour.

2.3 Collection of Sample

Fresh Watermelon was obtained from Sokoto Metropolitan, Sokoto State and was transported to microbiology laboratory in Sokoto State University for isolation and identification of fungi associated with rot watermelon fruits. The collection was done in five different location in the study area Viz, Kasuwan Daji market, Kofar Marke, Kofar Atiku, Kasuwar Dankure vegetable markets and Gawon Nama market.

2.4 Preparation of Media

7 g Potato dextrose agar (P.D.A) was weighed and dissolved in 250ml of distilled water, it was stirred using a stirring rod and allowed to dissolve by heating on a bursen burner flame. The conical flask containing the mixture was covered using cotton wool and aluminum foil, and was heated using a bursen burner flame, until it boils. The mixtures were autoclaved at 120°C for 15 minutes to ensure sterility after the autoclaving. The media is then incorporated with antibiotic (ampiclox) to prevent contamination by bacteria. The media was then allow to cool for about 40minutes, before being poured into sterile petri dishes and allow to gel at room temperature [18].

2.5 Surface Sterilization

The varieties of spoilt or infected parts of the Watermelon fruits were then washed thoroughly

with series of changes of sterile distilled water [19].

2.6 Identification of Isolated Fungi

A drop of lactophenol cotton blue stain was placed on the centre of clean and greace free slide. With a sterile inoculating needle, a small portion of the fungal growth was cut out and is transferred directly into the drop of lactophenol cotton blue on the slide. Clean cover slip was placed on top of the teased portion of the isolate. The prepared slides were then examined microscopically using X40 objectives lens. Identification of the fungal species were based on their nature of the Mycelium, the type of fruiting bodies, and spore structure and the cultural characteristics. The observed structures were then compared with that in the mycological atlas and identification manual by Mueller and Schmit [20].

3. RESULTS

The result of this study has indicated that, the Watermelon is infected with fungi either during the process of storing or at the selling point. From the samples obtained in five different locations within Sokoto metropolis, four genera of fungi were isolated from the watermelon and Identified as *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus stolonifer* and *Mucor species*. Tables 1 and 11 below indicate the microscopic and macroscopic analysis of the sample and the locations where the samples were obtained. In Kofar Marke *Rhizopus stolonifer* organisms were found, *Aspergillus flavus* were found in Kasuwar Daji market and Kofar Atiku while *Aspergillus niger* were found in Gawon Nama market and *Mucor spp* were found in Dankure vegetable market.

4. DISCUSSION

Fungi associated with the rot of watermelon spoilage from five different locations within Sokoto metropolis was isolated and identified. The high prevalence of fungi isolates in the selected locations showed that Fungi are the major cause of watermelon spoilage. This was in agreement with the result of Singh and Sharma [9] where fungi have been found causing fruit rot. Fruits contain high levels of sugars and nutrients and their low pH values make them particularly desirable to fungal infection. Previous study also showed that the prevalence of fungi as the spoilage organism of some edible fruits

Table 1. Culture characteristic of fungal isolate

S/N	Sample	Macroscopy	Microscopy	Fungi/identifie
1.	Sample A	Colon compact and became darkening cotton after few days	Sprongia contain spores, have rhizods	<i>Rhizopus stolonifer</i>
2.	Sample B	Green surface reverse side black	Speta, hyphae conidioshores with single branch	<i>Aspergillus flavus</i>
3.	Sample C	Green surface reverse side black	Septa, hyphae conidiosphores with single branch	<i>Aspergillus flavus</i>
4.	Sample D	Pick like black growth	Non-branched conidiophore with bulb and carries conidia like sun rays	<i>Aspergillus niger</i>
5.	Sample E	Greenish cotton like, colony like, growth spotted blackish colour	Sporangia contain spore, do not have rhizolds.	<i>Mucor</i>

Table 2. Result of the samples

S/N	Sample	Location	Organisms identified
1.	Sample A	Kofar Marke	<i>Rhizopus stolonifer</i>
2.	Sample B	Kasuwani Daji Market	<i>Aspergillus flavus</i>
3.	Sample C	Kofar Atiku	<i>Aspergillus flavus</i>
4.	Sample D	Gawon Nama	<i>Aspergillus niger</i>
5.	Sample E	Dankure Vegetable Market	<i>Mucor spp</i>

abundant in different locations in Nigeria. Fruits and vegetables are exposed to contamination by microorganisms either by direct contact with soil, dust and water or by handling at harvest or during postharvest processing. This could also cause loss of profit from the fruits to farmers. Earlier researchers such as in the work of [6] which studied Fungi playing role in the spoilage of some Fruits and vegetables, he was able to isolate *Rhizopus stolonifer*, *Aspergillus niger*, *Fusarium accuminatum*, *Fusarium oxysporum*, *Fusarium equiseti*, *Fusarium solani*, as well as *Fusarium moniliforme* from spoiled tomatoes while *Fusarium accuminatum*, *Rhizopus stolonifer* and *Aspergillus niger* were found to be responsible for the spoilage of watermelon and [7] in their study of Fungi assessment in some spoiled fruits sold in Gwagwalada market of Abuja, Nigeria isolated *A. niger*, *F. avanaceum*, *P. digitatum*, *R.stolonifer*, *Saccharomyces* species, *A. flavus*, and *F. solani* with *Aspergillus niger* being prevalent with the percentage of 38% in fruits while *Fusarium avanaceum* had the frequency of 31% in watermelon, pineapples, oranges, pawpaw, and tomatoes *Penicillium digitatum* and

Rhizopus stolonifer was also isolated from fruits such as tomato and oranges and they have the least occurrence with the frequency of 4%. *Saccharomyces* species, *Fusarium solani*, and *A. flavus* were also isolated in this study with the frequency of 10%, 8%, and 5% respectively. Fungi names to be in italics-entire text

This was in contrast with the result of [12] who reported that no fungi was isolated from the spoiled fruits such as watermelon and tomatoes and this could be as a result of physiological factors such as temperature, oxygen, or enzymes present in the fruits. The result of pathogenicity test also revealed that the fungi isolated from spoiled fruits of the study were able to induce the same disease symptom present in healthy fruits with the same fungi being reisolated from the inoculated healthy fruits and thus show that the fungi were responsible for the spoilage of the fruits. The mycotoxins are not limited to their areas of infections; they diffuse rapidly throughout the fruit through its fluid, contaminating all parts and otherwise pose a potential health hazard and less desirable for

human consumption. No growth was observed in the control fruits. *Aspergillus niger*, popularly known as the black mould and several other species cause decay of foodstuffs. *A. flavus* and *A. niger* parasitizes man and animals. They cause a number of diseases grouped under the name Aspergillosis. Most people breathe in *Aspergillus* spores every day without getting sick. However, people with weakened immune systems or lung diseases are at a higher risk of developing health problems due to *Aspergillus*. Health issues related to *Aspergillus* include infection in the organs of the body such as the lung as well as allergic reaction. This infection may also be seen in human ear and is called Otitis media. *A. flavus* is reported to produce mycotoxin known as Aflatoxin which is a potent carcinogen and has been directly correlated with adverse health effects, such as liver cancer, in many animal species [4].

Particularly common clinical syndromes associated with *A. flavus* include chronic granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infections and osteomyelitis following trauma and inoculation. *Aspergillus flavus* outbreak differs from *A. fumigatus* outbreak in that that outbreak that are of *A. flavus* is found to be related to just one strain or closely related strains [5]. [2] Isolated Ochratoxin A Produced by Strains of *Aspergillus niger* var. *Niger* OA contaminates a variety of plant and animal products but is most often found in stored cereal grains. As a nephrotoxin, it has long been of particular importance to the poultry and swine industries. In healthy humans, *Mucor* rarely causes disease, but in the host compromised by immune deficiency, immune suppression, or a serious underlying disease, the incidence of *Mucor* infections is much higher. *Fusarium* is reported to produce trichothecenes, which are most strongly associated with chronic and fatal toxic effects in animals and humans. The adverse effect of fungi in plants and fruits has resulted in the shortage of fruits for consumption. Spoilage of fruits by fungi leads to a shortage of consumption and loss of profits to the farmers and industries whose raw material is fruits. In this study, six fruits spoilage fungi were isolated and identified as follows: *Aspergillus niger*, *Aspergillus flavus*, *Mucor racemosus*, *Mucor* species and *Fusarium* species. The isolated fungi are of economic and public health importance. Some species of these fungi have been reported to produce potent mycotoxins called ochratoxins that can be harmful to human beings and animals.

5. CONCLUSION

Based on the result of this study, three fungal genera have been successfully isolated and identified from the rot of watermelon within Sokoto metropolis including *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus stolonifer* and *Mucor species*. The finding of this study implies tough the realities for the farmers in guaranteeing safe produce and require an adaptation appropriate control measures against farms infection during the harvesting and postharvest in order to avoid cut and reduce losses. Researchers should also embark on similar related studies so as to improve the economy of the farmers and country at large.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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