

Isolation, Identification and characterization of Fungal Species Associated with the spoilage of some selected fruits

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ABSTRACT

Spoilage of fruits imparts great economic loss and a potential health hazard to the general populace. A research was conducted to identify the different types of fungal flora responsible for the spoilage of fruits in Bwari market, Abuja, Nigeria. A total number of seventy (70) different types of fruits that showed decayed symptomatology and thirty healthy ones were purchased from Bwari market, Abuja. The samples were surfaced sterilized with ethanol and the homogenates. Sections of the rotten and healthy fruits were cut of using a sterile blade and were inoculated onto petri dishes containing Potato Dextrose Agar, the plates were incubated at 27°C for five days. The pure cultures obtained were identified morphologically and microscopically using a standard microbial Atlas for verification. The result showed the presence of *Aspergillus niger*, *Rhizopus nigricans*, *Mucor mucedo*, and *Fusarium oxysporum*. However, *Aspergillus niger* (58.06%) was found to be the most dominant spoilage causing organism on fruits vended in Bwari market while *Fusarium oxysporum* (9.68%) was the least abundant fungal flora. However, the pathogenicity of *Rhizopus sp.* was found to be higher than that of all the remaining fungal species. This can be attributed to the predisposal and nature of fruits handling and storage in the market. These findings are indicative that fungal species are associated with the spoilage of fruits. Based on the fact that, all the fungal species identified in this research were known to produce toxins that could impart severe food poisoning.

Keywords: Isolation, characterization, identification, fruits and spoilage

1.0 INTRODUCTION

Fruits play a vital role in human nutrition by supplying necessary growth factors such as vitamins and essential minerals in daily diet which help to live a healthy life. Fruits are the consumable part of mature ovary of flowering plants which are normally eaten raw and they include many structures that are not commonly called fruits such as beanpods, corn kernels, tomatoes, and wheat grains (Ikhiwili, 2012). Besides all these, there is an increasing concern on the use of fruits in diet therapy for certain clinical diseases. However, despite all these tremendous benefits of fruits to human well-being, pathogenic attacks threatened the shelf-life of fruits and degrade their economic value. Fruits are mainly exposed to microbial contamination through contact with soil, dust and water and by poor handling or during post-harvest processing. This makes them to harbor a wide range of microorganisms (Oforet *et al.*, 2009; Eni *et al.*, 2010) especially fungi. The principle of spread of fungal infection in fruits supports that a single infected fruit can infect other healthy fruits during storage and on transit (Jay, 2003). Fruits and vegetables generally give the body every necessary vitamin, fats, minerals and oil in the right proportion to maintain growth and development in humans (Mojtaba *et al.*, 2019). Over the years, there has been an increase in the reported spoilage of fruit resulting in change in the condition of fruit in which the fruit becomes less palatable, or even toxic, these changes may be accompanied by alteration in taste, smell, appearance or texture (Singh and Sharma, 2007). Annual reports have shown that 20% of fruits and vegetables produced are lost to spoilage especially during post-harvest stages and this has been associated pathogenic fungi (Singh and Subali, 2007). Toxin-producing fungi have been identified and isolated from spoiled fruits and have been

reported in cases of infections or allergies (Thiyam and Sharma, 2013)

The fungi can be introduced into the crop on the seed itself, during crop growth in the field, during harvesting and post-harvest handling, or during storage and distribution. Post-harvest losses and decay of fruits can be traced mostly to infections that occur during harvesting and subsequent handling and storage activities. Pre-harvest infections are mainly caused by fungal pathogens such as *Phytophthora sp.*, and *Colletotrichum gloeosporioides* (Browning *et al.*, 1995; El Ghaouth *et al.*, 2002). Isolation and identification of the decay-causing fungi are desirable in order to strategize the control measures with a view to reducing losses due to spoilage or infections (Singleton *et al.*, 1992). Thus, this study was aimed at isolating and identifying fungi associated with fruits spoilage in Bwari market, Abuja, Nigeria.

2.0 MATERIALS AND METHODS

2.1 Sample Collection

Samples of fruits that showed signs of spoilage (discoloration, softening, visible mold) were collected from the fruits retailers randomly using sterile techniques. A total number of one hundred samples comprising of seventy fruits that showed decay-symptomatology and thirty healthy fruits were procured. The fruits were placed in sterile plastic bags and labeled accordingly. The samples were kept in the refrigerator at 4°C till when use for mycological analysis.

Surface Sterilization

The fruits were cut into smaller segments (3-5 mm) from the margin of the rotten area using a sterile scalpel. The segments were surface-sterilize using 70% ethanol or 1% sodium hypochlorite solution for a short duration, followed

by rinsing multiple times with sterile distilled water to remove surface contaminants while keeping the internal pathogen viable

2.2 Isolation and identification of Fungal isolates

The surface-sterilized fruit segments were aseptically placed onto the surface of a solidified Potato Dextrose Agar (PDA) amended with an 30 mg/l chloramphenicol to inhibit bacterial growth. The inoculated plates were incubated at 30°C for seven days.

2.3 Sub-culturing

The plates were observed daily for fungal growth. Different colonies emerged distinguished by color and morphology, a small portion of the aerial mycelia from each distinct colony was transferred to fresh Potato Dextrose Agar plates using a sterile inoculating needle to obtain pure cultures.

2.4 Identification of the isolates

2.4.1 Macroscopic Examination

Cultural characteristics of the pure cultures, including colony color (e.g., black, green, white), texture, growth rate, and pigmentation were observed and recorded

2.4.2 Microscopic Examination:

Slides were prepared using the Lactophenol Cotton Blue (LPCB) stain method. A small amount of mycelium was mounted in a drop of the stain on a clean slide, covered with a cover slip, and viewed under a light microscope (at 10x and 40x magnification)

2.5 Characterization:

The fungal isolates were characterized based on the morphology of their hyphae (septate or non-septate), conidiophores, sporangia, and spores by comparing observations with a standard mycological atlas or identification manual

2.6 Pathogenicity Test

Healthy fruits were surface sterilized with 0.1% mercury chloride using cotton wool. Cylindrical tissues were cut out from the fruits using a sterilized 3mm sized cork borer. Agar discs containing one week old fungal culture were aseptically placed in these holes, then covered and sealed off by means of petroleum jelly. The procedure was repeated separately across each of the fungal isolates. The inoculated samples and the control were placed in sterile polythene bags and incubated at 30°C for 14 days. The point of inoculation of each type of fungus was examined and recorded accordingly. The diameter of the rotten portion of the fruits was measured. The fungi were later re-isolated from the inoculated fruits and compared with the initial isolates.

3.0 RESULTS

The result for the prevalence of fungal isolates on the fruits sold in Bwari market is presented in Table 1. The result showed that, four different fungal species were found associated with the fruits sold in Bwari market. The result indicated that *Aspergillus niger* is present in all the eight different fruits from Bwari market and is the most predominant species (58.06%) abundance. However, *Fusarium oxysporum* is present only in water melon, tomato and pepper and is the least predominant fungal species (9.68%). However, the result for the prevalence of the

fungal flora on the eight fruits vended in Bwari market is presented in

Table 2. The result showed the presence of four different fungal species in the fruits vended in Bwari market. The fungal species are: *Aspergillus niger*, *Rhizopus nigricans*, *Mucor mucedo*, and *Fusarium oxysporum*. The result indicated that, *Aspergillus niger* has the highest occurrence of 58.06%; while *Fusarium oxysporum* has the least abundance of 9.68%. Furthermore, the result of the pathogenicity test (Table 3) shows that, *Rhizopus nigricans* induces the highest rotten diameter on the fruits (36 mm in diameter). However, *Fusarium oxysporum* was found to have the lowest rotten diameter of 22 mm in diameter.

4.0 DISCUSSION

The presence of four different fungal species (*Aspergillus niger*, *Rhizopus nigricans*, *Mucor mucedo*, and *Fusarium oxysporum*) identified in the spoiled fruits obtained from Bwari market have provided an impetus upon the dangers associated with the consumption of such fruits without proper washing or treatment. The findings of this research were in conformity with those of (Sulaiman *et al.*, 2024) who isolated, *A. niger*, *R. stolonifer*, *R. oryzae*, *A. alternata*, *Mucor*, and *Fusarium*). *A. niger* is the most frequent with over 30% of the total.

More so, these fungal pathogens impart severe losses of marketable quality and posed threat to public health resulting in major economic problem in Nigeria. The species isolated in this study were previously identified in different fruits obtained in Nigeria by Amadi and Oso, Oyetunji *et al.* (2012), Amadi *et al.*, Mbajuka *et al.* and Ezikanyi. These fruits might likely be contaminated via infestation in the field prior to harvest, handling during harvesting and methods of packaging and distribution of produce to the market as stressed by Amadi *et al.*. The high prevalence of *Aspergillus niger* in the spoiled fruits vended in Bwari market is in conformity with the findings of Mailafia *et al.* who reported high prevalence of *Aspergillus niger* among fruits vended in Gwagwalada market. The finding also is in consistent with that of Samuel *et al.* who identified *Aspergillus niger* as the most prevalent fungal species causing spoilage of carrot (*Daucus carota*). This indicated the risks associated with the consumption of these fruits as *Aspergillus niger* was highly toxigenic known to produce several toxic metabolites such as malformins, nathopyrones as reported by Frisvad and Samson and ochratoxin which are popular for their harmful effects to human and animal health as reported by Peraica *et al.* and Petzinger and Weldenbach. However, this finding contradicts the work of Amadi *et al.* who reported *Fusarium oxysporum* as the most prevalent fungal species in the spoilage of guava fruits. Proof of pathogenicity is the single most reliable criterion used in implicating associated microorganisms in the causal processes of disease development as stressed by Amadi *et al.*. All the species proved to be pathogenic on the fruits and this is consistent with the work of Udoh *et al.* who reported similar finding

among the fungal species isolated from different fruits in Enugu, Nigeria.

The study also shows that all the species isolated and identified are responsible for the fruits spoilage in Bwari market. This is in line with the work of Peter *et al.*, Amadi *et al.* and Renu and Lal that *Fusarium oxysporum* and

Aspergillus niger are notorious causal agents of rot in many fruits and vegetables including watermelon, carrot and guava. Similarly, Mathew *et al.* had reported *Aspergillus niger* and *Rhizopus stolonifer* in post-harvest diseases of guava.

Table 1. Percentage frequency of occurrence of fungal isolates

| Sample | <i>Aspergillus niger</i> (%) | <i>Rhizopus nigricans</i> (%) | <i>Mucor mucedo</i> (%) | <i>Fusarium oxysporum</i> (%) |
|--------------|------------------------------|-------------------------------|-------------------------|-------------------------------|
| O | 7 | 1 | - | - |
| B | 7 | - | 1 | - |
| PA | 4 | 2 | 2 | - |
| PI | 5 | 1 | 2 | - |
| WM | 2 | 2 | 1 | 2 |
| PU | 4 | 1 | 3 | - |
| TO | 2 | 1 | 2 | 2 |
| PE | 5 | 1 | - | 2 |
| Total | 36(58.06) | 9 | 11(17.74) | 6(9.68) |

Key:- O=Orange, B=Banana, PA=Pawpaw, PI

Table 2: Distribution of Fungal Species in Various Fruits Sold at Bwari Market, Abuja

| Sample | <i>Aspergillus niger</i> | <i>Rhizopus nigricans</i> | <i>Mucor mucedo</i> | <i>Fusarium oxysporum</i> |
|-----------------|--------------------------|---------------------------|---------------------|---------------------------|
| Orange (O) | 7 | 1 | - | - |
| Banana (B) | 7 | - | 1 | - |
| Papaya (PA) | 4 | 2 | 2 | - |
| Pineapple (PI) | 5 | 1 | 2 | - |
| Watermelon (WM) | 2 | 2 | 1 | 2 |
| Pumpkin (PU) | 4 | 1 | 3 | - |
| Tomato (TO) | 2 | 1 | 2 | 2 |
| Pear (PE) | 5 | 1 | - | 2 |
| Total | 36 (58.06%) | 9 (14.52%) | 11 (17.74%) | 6 (9.68%) |

=Pineapple, WM=Water melon, PU=Pumpkin, TO=Tomato, PE=Pepper

Table 2: Percentage Prevalence of Fungal Species in Fruits Sold in Bwari Market, Abuja

| S/N | Fungal Isolate | Frequency | Percentage (%) |
|--------------|---------------------------|-----------|----------------|
| 1 | <i>Aspergillus niger</i> | 36 | 58.06 |
| 2 | <i>Rhizopus nigricans</i> | 9 | 14.52 |
| 3 | <i>Mucor mucedo</i> | 11 | 17.74 |
| 4 | <i>Fusarium oxysporum</i> | 6 | 9.68 |
| Total | | 62 | 100 |

Table 3: Pathogenicity of Fungal Species on Fruits Obtained from Bwari Market, Abuja

| S/N | Fungal Species | Diameter of Rot (mm) |
|-----|---------------------------|----------------------|
| 1 | <i>Aspergillus niger</i> | 27 |
| 2 | <i>Rhizopus nigricans</i> | 36 |
| 3 | <i>Mucor mucedo</i> | 30 |
| 4 | <i>Fusarium oxysporum</i> | 22 |

Fruit spoilage can be prevented using physical methods as reported by Boyer and chemical method as reported by Msagati, but no efficient strategy has been proposed so far to reduce the microbial growth ensuring public health safety.

5.0 CONCLUSION

In this study, *Aspergillus niger*, *Rhizopus nigricans*, *Mucormucedo* and *Fusarium oxysporum* was identified in spoiled fruits was found in spoiled mango. Some of these fungal species have been reported to contain potential mycotoxins which can lead to health complication for both human and animals. Therefore, the use of biological control agents, proper packaging, storage facilities and fruit handling by fruit sellers to ameliorate postharvest fruit spoilage is necessary to ensure the availability of safe and healthy fruits and reduce health risk due to consumption of fungi infested fruits.

6.0 RECOMMENDATION

Mature fruits are better eaten fresh to avoid long term exposure that lead to spoilage. Immediate and proper discarding of fruits with symptomatic features such as alteration in color or taste of the fruit is noticed as this can be hazardous to human health.

7.0 COMPETING INTERESTS: Authors have declared that no competing interests exist.

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