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Isolation and Characterization of Post-Harvest Fungal Species and Pathogenicity Assessment of Spoilt Fruits Sold in Saudi Arabia Market

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Abstract: ***This study investigated the fungi associated with the spoilage of Mangoes and Tomatoes fruit, obtained from markets in Saudi Arabia. A total of 200 mixed samples, Mangoes and Tomatoes fruits were collected. The microorganisms were isolated, characterized and identified using standard methods. The fungi isolated and identified from the spoiled Mangoes were, Aspergillus niger , Aspergillus flavus, Alternaria spp., Rhizopus spp., Penicillium spp., Botryodiplodia and Phomopsis spp. On the other hand, the fungi isolated from Tomatoes were, Aspergillus niger, Fusarium oxysporum, Penicillium, Rhizopus, Cladosporium, Saccharomyces cerevisiae and Geotrichum candidum, and Aspergillus, Penicilliumand Rhizopus were the most prevalent fungi isolate from the samples and found in all samples collected from Mangoes and Tomatoes fruits and caused severe post-harvest losses.The study showed that the presence of these fungi associated with Mangoes and Tomatoes spoilage caused highly risk to humans and animals due to their production from spores and toxins that lead to food poisoning.***

Keywords: Isolation, Characterization**,** Mango, Tomato, Post harvest diseases, Retail level , wholesale, spoilage and Saudi Arabia

1. Introduction

Fruits play a vital sources in human nutrition by supplying the necessary growth factors such as vitamins, fats, oil and essential minerals in human daily diet and are known to reduce the risk of vitamin A. Man as well as animal relies on fruits as part of their diet and mean of survival deriving nutrient from it (Lewis et al, 2002). Diet consisting of fruits is important in maintaining overall body health due to phytochemicals which prom health that is present in them. Certain diseases such as cardiovascular diseases, stroke, type 2 diabetes can be prevented by eating a diet rich in fruits (Onyemata and Ibrahim, 2018). The relatively short shelf-life period caused by several limiting factors such as, inadequate rainfall, pests and microbial infection, are affected the fruits economic values. Fruits contain high levels of sugars and nutrient elements and their low pH values make them particularly desirable to microbial decay (Singh and Sharma, 2007 and Mairami et al, 2019) , hence most fruits of spoilage are caused by Fungi (Singh et al., 2007). Organisms such as yeasts, molds, bacteria, physiological factors as well as environmental factors play important role in fruits spoilage leading to fruit rot which is characterized by the change in texture, flavor, color, in an undesirable way (Onyemata and Ibrahim, 2018). Generally, spoiling microbes are considered toxigenic or pathogenic as a lot of toxigenic fungi have been isolated from spoilt fruits. Even during refrigeration some microbes such as moulds and other fungi produce mycotoxins of various types that are harmful to the consumers (Stinson et al 1981, Tournas and Stack 2001, and Bali, et al 2008). Microorganisms present in soil, dust or water which lead to the contamination of these fruits, these microbes contaminate fruits through the process of handling during harvest and postharvest period (Eni et al, 2010). Fruits and vegetables spoilage fungi such as, *Fusarium accuminatum, Fusarium oxysporum, Fusarium equiseti, Fusarium solani, Fusarium monoliforme, Aspergillus niger and Rhizopus stolonifer* were isolated from spoilt tomatoes (Iniekong et al 2015). In post-harvest conditions, mango get infected by several fungal diseases like *Rhizopus* rot, *Anthracnose*, stem end rot, *Aspergillus niger* rot, *Penicillium* rot, *Aspergillusfumigatus*, *Aspergillus flavus* rot etc. (Dasgupta and Bhatt, 1946) and stem-end rot caused by *Botryosphaeria parva* (Swart et al., 2002). *Aspergillus flavus* rot was maximum at different temperature in inoculated mango fruits (Gadgile and Chavan, 2010). Okereke et al. (2010) indicated that the fungi species *A. niger*,*Alternaria* spp. *Botryodiolodia theobromae*, *Colletotrichum gloeosporioides*. *Fusarium* spp, *A. flavus* and *Phoma* spp were isolated from the infected mangoes. (Gadgile and Chavan, 2017) found that, the infection of *Aspergillus niger* rot, anthracnose and *Rhizopus* rot in post-harvest mango fruit. In the tropics, the spoilage of post-harvest tomato fruits *Aspergillus niger*, *Rhizopus stolonifer*, *Fusarium oxysporum*, *Saccharomyces cerevisiae*, *Alternaria alternata*, *Penicillium digitatum* and *Geotrichum candidum* were identified (Ugwu et al. 2014, Onuorah andOrji, 2015).

2. Materials and Methods

2.1 Samples Collection

A survey was conducted to assess the extent of loss in Mangoes and Tomatoes fruits caused by fungal diseases during post-harvest. A total of 200 samples of infected fruits were purchased from different fruits market of Riyadh regions in Saudi Arabia. They were collected from both the whole sellers and retailers randomly. The samples were sorted to identify infected mango and tomatoes, and were transferred by using hand gloves into sterile polythene bags, labeled, and assessed in the laboratory. The glass wares were properly washed, dried and sterilized in the oven at 160 0C for one hour. The loss due to fungal diseases was assessed at weekly intervals for four months (April to September 2019).

Prevalence

The Prevalence of each disease was estimated by counting the number of fruit disease location with respect to total locations based on symptoms and calculated using the following formula (Tucho *et al*., 2014).

$$\text{Prevalence (\%)} = \frac{\text{Number of locations with diseased fruits}}{\text{Total number of locations}} \times 100$$

Isolation of fungi from spoilt fruits

The fruits were washed with sterile water then A sterile blade and forceps was used to cut small section of the tissue containing both the healthy and the rotten portion (3 mm diameter) and plated on solidified Potato Dextrose Agar (PDA) containing chloramphenicol (30 mg mL–1) to prevent bacterial growth. The colonies that developed were counted and subcultured repeatedly on PDA to obtain pure cultures. The subculturing was carried out by using a sterile fresh medium of potato dextrose agar (PDA) and incubated at 28°C for 5-7 days until fungal proliferation on medium surface. The isolation of pure fungal colony in culture medium was performed by using slants of a sterile fresh medium of PDA and incubated at 28°C for 5-7 days. The isolated fungi were maintained at 4°C (Iniekong et al., 2015).

Identification and classification of fungi from spoilt fruits

Fungal isolates obtained from the slant were identified based on their gross morphology such as colony growth pattern, conidial morphology and pigmentation by slide culture techniques(De Lange et al 1998 and Ezikanyi D.N. 2016), and A small portion of the mycelia was picked from culture media by using a sterile inoculating needle and inoculated on a slide containing a fraction of a prepared solidified potato dextrose agar and incubated for 48 h, then viewed under the light microscope to detect spore, hyphae and other special structures. The morphological characteristics and appearance of the fungal isolates from the rotten fruits were classified and confirmed according to the conventional guidelines of fungus identification (Ellis, 1971, Samson and Varga, 2007).

3. Results and Discussion

Fruits are affected by a wide array of microorganisms causing its decay. Different colonies and a mass of mycelia growing on the surface of the fruits were observed at the end of the procedure associated with the isolation and identification of fungi associated with spoilage of post-harvest Mangoes and Tomatoes. The commonly fungal species isolated from the infected fruits were identified as follows *Aspergillus niger* , *Aspergillus flavus*, *Alternaria* spp., *Rhizopus* spp., *Penicillium* spp., *Botryodiplodia* and *Phomopsis* spp. (mango) (Tables1&2). *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium*, *Rhizopus*, and *Cladosporium*, *Saccharomyces cerevisiae* and *Geotrichum candidum* (tomatoes) (Tables3&4). Studies have shown that Mangoes fruit were susceptible to infection and to a number of diseases at all stages of its development from the seedling to the fruits (Alemu, 2014 and Palejwala et al., 1987). In post-harvest conditions, mango get infected by several fungal diseases like *Rhizopus* rot, *Anthracnose*, stem end rot, *Aspergillus niger* rot, *Penicillium* rot, *Aspergillusfumigatus*, *Aspergillus flavus* rot etc. (Dasgupta and Bhatt, 1946) and stem-end rot caused by *Botryosphaeria parva* (Swart et al., 2002). *Aspergillus flavus* rot was maximum at different temperature in inoculated mango fruits (Gadgile and Chavan, 2010). Okereke et al. (2010) indicated that the fungi species *A. niger,Alternaria* spp. *Botryodiolodia theobromae* , *Colletotrichum gloeosporioides. Fusarium* spp, *A. flavus* and *Phoma* spp were isolated from the infected mangoes. (Gadgile Dhondiram and Chavan Ashok, 2017) found that, the infection of *Aspergillus niger* rot, anthracnose and *Rhizopus* rot in post-harvest mango fruit.In the tropics, *B. theobromae* is an economically important fungus known to cause major losses to mango, and cause stem end rot in mango fruits (Rieger, 2006; Amusa et al., 2003). (Sangeetha et al., 2011; Rossel et al., 2008; Khanzada et al., 2004b; Arjunan et al., 1999; Sangchote, 1988). It showed that, various fungal diseases occurring on post-harvest tomato surfaces , *Aspergillus niger, Rhizopus stolonifer, Fusarium oxysporum, Saccharomyces cerevisiae, Alternaria alternata, Penicillium digitatum and Geotrichum candidum* were invaded tomato tissue and black rot lesion was observed. Over all, Aspergillus species were the most frequently isolated fungi. This was followed by *Penicillium, Rhizopus, Fusarium, Alternaria, Botryodiplodia, Phomopsis* spp , *Cladosporium*, *Saccharomyces cerevisiae* and *Geotrichum candidu,* respectively. They were the commonest fungal diseases affecting Mangoes and Tomatoes fruits.Aflatoxins are associated with some diseases in livestocks and humans throughout the world. *Aspergillus flavus* is the main producer of the well known carcinogenic aflatoxins and its presence in food is of huge concern in terms of food safety , they are toxic at low concentrations (Rodriques *et al*., 2007 and Ezikanyi D.N. 2016). *Penicillium* spp, were found among other fungi species involved in deterioration of tomatoes fruit (Mbajiuka and Enya (2004). *Penicillium* and *Fusarium* are among the most important genera of mycotoxigenic fungi (Zain, 2011). *Aspergillus niger* as one of the major fungi responsible for the production of volatile compounds in spoilt tomatoes and they are pathogenic (Baker 2006) also, *Rhizopus* spp were associated with the spoilage of tomatoes Akinmusire 2011). *Aspegillius* spp, *Penicillum* spp, *Fusarium* spp and *Saccharomyces* spp from spoilt tomato fruits (Wogu and Ofuase 2014 , Mbajiuka and Enya 2014 and Fatih et al 2005)

4. Conclusion

This study showed that the profile of spoilage fungi which caused pathogenecity of some local fruits in Saudi Arabia. It also showed that fungi were involved in the spoilage of two types of fruits Mangoes and Tomatoes. It is clear that fungal species of *Aspergillus*, *Fusarium*, *Penicillium*and *Rhizopus* were the commonest fungal diseases affecting Mangoes and Tomatoes fruits and caused severe post-harvest losses. Mechanical injuries that occur during harvesting or post-harvesting could provide infection. Precaution in preventing fruit spoilage and infection should be taken during harvesting, sorting, cleaning, packaging, transporting, in storage and market. Also,lowering storage temperatures, proper fungicides, sanitation of warehouses and disinfection of packaging and transit containers are very important to reduce contamination and infection, and to enhance reduction the risk of aflatoxin and other mycotoxins which

are produced by these fungi. Refrigeration is essential for keeping quality and extending lifetime of these fruits. However, several yeasts and moulds can grow at low temperatures, therefore fruits that are very sensitive to low storage temperatures should be marketed quickly to avoid fungal spoilage.

Table 1: Symptoms of post-harvest fungal diseases in Mangoes fruit

Fruit	Pathogen	Disease	Symptoms
Mango	*Aspergillus niger*	rot	light-brown circular spots, that enlarges into darker lesion
	Aspergillus flavus	rot	Powdery yellow green spores
	Alternaria alternata	rot	Brown to dark brown circular lesions later extend to pulp Lesions.
	Rhizopus sp.	rot	Water soaked lesions, soft decay
	Penicillium sp.	Blue mould rot	Watery spots, changes into bluish green at later stages
	Botryodiplodia sp.	rot	Black lesions extends to pulp with color, water-soaked spot
	Phomopsis sp.	rot	Brown to black spots

Table 2: Prevalence of post-harvest diseases in Mangoes fruit

Diseases	Prevalence (%)
Aspergillus niger rot	100
Aspergillus flavus rot	95
Alternaria rot	85
Rhizopus rot	90
Blue mould rot	75
Botryodiplodia rot	65
Phomopsis rot	70

Table 3. Symptoms of post-harvest fungal diseases in Tomatoes fruit

Fruit	Pathogen	Disease	Symptoms
Tomatoes	*Aspergillus niger*	rot	light-brown circular spots, that enlarges into darker lesion
	Aspergillus flavus	rot	Powdery yellow green spores
	Rhizopus sp.	rot	Water soaked lesions, soft decay
	Penicillium sp.	Blue mould rot	Watery spots, changes into bluish green at later stages
	Geotrichum candidum.	rot	Lightly creamy and white
	Saccharomyces cerevisiae	rot	White turned to creamish / yellowish

Table 4: Prevalence of post-harvest diseases in Tomatoes fruit

Diseases	Prevalence (%)
Aspergillus niger	100
Aspergillus flavus	90
Rhizopus sp.	95
Penicillium sp.	95
Geotrichum candidum	85
Saccharomyces cerevisiae	*75*

5. Conflict of Interest

The authors declare that they have no conflict of interest.

6. Acknowledgement

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