Isolation of *Aspergillus niger* from Deteriorating Sweet Oranges (*Citrus sinensis*) and their Effect on Fresh Oranges

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**ABSTRACT:** Sweet oranges (*Citrus sinensis*) are the most well-known fruit in the citrus family. It is a good source of vitamin C, phenolic compounds, flavonoids, and pectins. Although, fruits are now stored well but concerns for spoilage of fruits particularly due to fungal diseases still present. Fungi can grow on a variety of substrates, and hence responsible for various diseases in humans and are quite harmful to crops and fruits as well. The present study was conducted for isolation, identification and characterization of fungi associated with spoilage of orange fruit (*Citrus sinensis*). The samples of *Citrus sinensis* were collected from fruit markets located near Sanda, Lahore. Pure cultures of *Aspergillus niger* were obtained, after colonial and morphological (shape, structure, color) characterization of the isolates. The isolated fungal strain (*Aspergillus niger*) was applied on the skin of fresh *Citrus sinensis* and pathogenicity test was carried out. It was noticed the isolated strain was pathogen and cause disease in fresh specimens of sweet oranges.

**Keywords:** Sweet orange, *Aspergillus niger*, spoilage, isolation, diseased

**INTRODUCTION**

Sweet orange (*Citrus sinensis*) is one of the most important crops in the world and well known for health and economical values (Talon and Gmitter, 2008). It belongs to ‘Rutaceae’ family. It is mainly used for the extraction and consumption of its fresh juice. *Citrus sinensis* are a rich source of vitamin C along with vitamin B (Thiamin, Riboflavin, Pantothenic acid, Pyridoxine, Niacin, and Folate) and has great potential to act as an antioxidant (Di Majo et al., 2005; Okwu and Emenike, 2006; Liu et al., 2012; Etebu and Nwauzoma, 2014). They are rich in phytochemicals such as carotenoids, flavonoids, alkaloids, phenols, tannins and can be used against cardiovascular and cancerous diseases in man. The presence of minerals in the fruits is helpful in lowering the blood pressure and risk of stroke (Okwu and Emenike, 2006; Peterson et al., 2006; Liu et al., 2012). However, a number of fungal diseases responsible for a significant loss in harvested fruits (Adaskaveg et al., 2002; El-Ghaouth et al., 2002; Liu et al., 2013).
Aspergillus niger is a fungus and one of the most common species of the genus Aspergillus (Bennett, 2010; Sharma, 2012). It widely spread due to spore-forming ability (Raghukumar, 2017). It is a versatile fungus that is cosmopolitan in distribution and has been observed in a wide range of habitats such as soil, air, water, and on decaying plant materials (Sharma, 2012; Dijksterhuis et al., 2013). It is commonly found as a saprophyte growing on dead fruits, leaves, compost piles, stored grains, and other decaying vegetations (Ashraf et al., 2007; Gautam et al., 2011; Sharma, 2012). A. niger can cause various plant diseases which leads to great economic loss. A. niger can produce aflatoxins, ochratoxin A in stored products which seems to be very inevitable (Soares et al., 2013). Production of Fumonisin B2 and Ochratoxin A OTA was also reported from A. niger (Frisvad et al., 2007; Logrieco et al., 2009; Noonim et al., 2009). Fumonisin B2 has the ability to cause diseases both in man and animals, moreover, it is regarded as carcinogenic (Frisvad et al., 2007; Logrieco et al., 2009). Aspergillus spp. along with plants is responsible for human fungal infections particularly in immunocompromised people (Diba et al., 2007).

There is raising public concerns regarding contamination of perishables with fungicidal residues. The present study is designed to trace the path of pathogenicity in fresh sweet oranges (Citrus sinensis) by isolating fungal species from rotten oranges.

**MATERIALS AND METHODS**

**Physical examination and collection of sample**

Ten diseased or spoiled oranges were collected from the market of Lahore located near Sanda. The oranges were transported in polyethylene bags to the lab for analysis. Physical examinations of diseased or spoiled oranges have been done and oranges with green rot and black lesions were selected.

**Preparation of media and isolation of fungi**

Potato dextrose agar (PDA) was used for isolation. The PDA media was prepared by autoclaving for 15 min at 121°C under a pressure of 15lb/inch². After this media was cooled and dispensed into sterile Petri dishes. For the prevention of bacterial growth Streptomycin was added to the medium. The infected or diseased tissue of fruit was cut and inoculated on PDA media. The media with inoculated samples were incubated at room temperature for 5-7 days. The isolated fungal strains were identified on the basis of micro and macro-morphological characteristics.

**Pathogenicity test**

Fresh orange fruits (five in numbers) collected from the same markets were used and the pathogenicity test was applied. The fresh fruits were cleaned with tap water and finally, the surface of fresh oranges was sterilized with 75 % ethanol. A 3 mm sterile cork borer was used to make holes in fruits. A loop full of fungi isolated from pure culture was used to inoculate the oranges and sealed with petroleum jelly to prevent contamination. A similar set of fresh oranges act as a control that was bored with sterilized cork but not inoculated. Both experimental sets were placed in polythene bags. The cotton wool balls were used for absorbance of humidity and incubated for 5 days at 30 ± 3°C. The samples were starting to observe for the appearance of symptoms after 72 h by following methods of Akinmusire, (2011). The infected oranges were noticed and the
causal agent was re-isolated. Later on, the original isolates were compared with re-isolated agents.

**RESULTS AND DISCUSSION**

The rotten specimens of sweet oranges (*Citrus sinensis*) were collected from two markets of Lahore. The samples were finally treated for the isolation of fungal isolates. Isolates were identified on the basis of micro and macro morphological characteristics. The isolated species was found as *Aspergillus niger* (Table 1). The morphological features such as stipes color, surface, shape, and conidia surface noticed in *Aspergillus niger* were same as reported by Diba et al., (2007) and Silva et al., (2011). The decaying effects of *Aspergillus niger* isolated from decaying sweet oranges were noticed on fresh oranges and a diameter range of rotting was noticed as 13-21 mm and was in agreement with Tafinta et al. (2013).

Table 1: Morphological Characteristic of *Aspergillus niger* and diameter of rotting recorded in mm on fresh oranges

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Size µm</th>
<th>Stipes colour</th>
<th>Surface</th>
<th>Shape</th>
<th>Conidia Surface</th>
<th>Diameter of Rot</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus niger</em></td>
<td>400-1100</td>
<td>Slightly brown</td>
<td>Smooth walled</td>
<td>Globose</td>
<td>Very rough irregular</td>
<td>13-21 mm</td>
</tr>
</tbody>
</table>

Table 2 revealed the results of the pathogenicity test on fresh healthy citrus fruit samples. *Aspergillus niger* was found as a decaying agent of fresh fruits and consistent with the results of Chukwuka et al. (2010). Though, it was considered as a causative agent for deterioration in sweet oranges, however; a very least pathogenic effect compared to other fungal strains was noticed on fresh oranges as reported by Tafinta et al. (2013). The pathogenic activity of the *Aspergillus niger* may be due to the presence of secondary metabolites that they produced in plant tissues. Furthermore, a number of studies support the formation of certain toxic chemicals from this *Aspergillus* species such as aflatoxins, Ochratoxin A and Fumonisins B2 (Frisvad et al., 2007; Logrieco et al., 2009; Noonim et al., 2009; Soares et al., 2013). All these compounds particularly, Fumonisins B2 had the ability to cause diseases both in man and animals especially cancer (Frisvad et al., 2007; Logrieco et al., 2009). The bad effect of metabolites from *Aspergillus spp.* was found high in immunocompromised people (Diba et al., 2007). Bukar et al., (2009) reported about mycological pathogenicity in sweet oranges was due to various fungal species and almost 90% orange samples were infected with one or more than one fungal species. The most commonly isolated species belongs to *Aspergillus* (32.5%), Mucor (25%), Penicillium (15%), Rhizopus (15%), Fusarium (7.5%), and Alternaria (5%). Similary, Fatima et al. (2009) also agreed that *Aspergillus sp.* along with various other fungi was also a major cause for post harvest diseases.
Table 2: Pathogenicity test on healthy fresh oranges

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

CONCLUSION

Aspergillus niger was found as a decaying agent for the deterioration in sweet oranges. The pathogenic activity of the Aspergillus niger may be due to the presence of secondary metabolites that they produced in plant tissues.

REFERENCES


