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Fungi associated with spoiled fruits vended in local market

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Abstract

Fruits spoilage causes threat to human health and responsible for great economic loss worldwide. This study was conducted to isolate and identify fungi responsible for spoilage of fruit commonly sold in Panj Gula market, Khairpur, Sindh, Pakistan. A total of 60 spoiled fruits, 10 samples from each different fruit includingstrawberry (*Fragariaananassa)*, orange (*Citrus sinensis*), papaya (*Carica papaya*), melon (*Cucumis melo*), apple (*Malus domestica*) and guava (*Psidium guajava*) were collected aseptically in sterile polyethene bag and delivered to microbiology laboratory at refrigerated temperature. Fungi were isolated and identifiedby using culturing and microscopic methods. Seventy seven fungal isolates belonging to five different genera, *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., *Rhizopus* spp., and Yeastwere isolated from spoiled fruits. *Aspergillus* spp. had the highest occurrence in all the fruits tested with a frequency of 40.26% followed by *Penicillium* spp. (24.67%), *Alternaria* spp. (20.78%), *Rhizopus* spp. (9.1%), and Yeast (5.19%). The results of this study suggest that fruits are spoiled with variety of fungi which causes great economic loss of country. In addition, isolation of pathogenic fungi in this study highlights the risk to human and animal health associated with spoiled fruits. Therefore improved preservation methods are required to prolong the shelf life of fruits and minimize the health hazard.

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Introduction

Fruits have got an important role commercially as well as nutritionally. For balanced diet and keeping good health, fruits intake is necessary since they are source of vitamins and essential minerals. Although fruits are easily available worldwide, the limiting factor that affects their economic value is short shelf-life due to bacterial and fungal attack.

Due to containing high amount of sugar, low pH, and ideal water activity fruits are prone to bacterial and fungal spoilage (Droby, 2006; Singh and Sharma, 2007). On fruits, fungi overgrow bacteria, since they prefer to grow at low pH. Some fungi can start spoilage of fruits from the fields, while others can only contaminate and proliferate after harvesting when the plant natural defense system is reduced (Tournas and Katsoudas, 2005). It has been reported previously that about 20% of the harvested fruits is spoiled even in developed countries (Droby, 2006; Zhu, 2006). In developing countries, post-harvest losses of fruit is more severe due to less storage facilities.

Globally various researchers have reported post-harvest fungalspoilage. For example, Akhter *et al*., (2009) isolated *Colletotrichum* species from strawberry; Sharma *et al*., (2013) isolated 11 fungal species from local fruits; El-Gali, (2016) isolated 12 fungal species of 8 genera from various fruits; Mailafia*et al*., (2017) isolated 7 fungal isolates from variety of fruits; Biyik (2018)isolated 11 different fungal species from strawberries.

Fungal spoilage is responsible for organoleptic changes including change in texture and flavors of fruit. In addition, some moldshave been reported to produce hazardous metabolic products such as patulin, byssotoxin A, malformins, naphthopyrones and related mycotoxins (Frisvad and Samson, 1991; Pitt and Hocking, 1997; Beuchat and Pitt, 2001; Tournas and Stack, 2001), which poses threat to human and animal health. The use of synthetic fungicides mayreduce the fungal spoilage, however, use of same fungicides for long time could lead to resistance among fungiagainst fungicides (Spotts and Cervantes, 1986). Moreover, high residue of pesticides in fruits limits the use of pesticides.

There is limited published data on fungi which cause the post-harvest diseases in local fruits. Therefore, present study was conducted to study fungal pathogens responsible for post harvesting spoilage of some fruits available locally.

Material and methods

Sample collection

A total of 60 fruits samples showing rot symptoms comprising 10 samples of six different types of fruits such asstrawberry (*Fragariaananassa*), orange (*Citrus sinensis*), papaya (*Carica papaya*), melon (*Cucumis melo)*, apple (*Malusdomestica*)and guava (*Psidium guajava*)were collected aseptically in sterile polyethene bag from Panjgula market, Khairpur, Sindh, Pakistan. All samples were then delivered to microbiology laboratory at refrigerated temperature for fungal analysis.

Isolation of fungal species

The surface sterilization of sampled fruits was achieved with 1% sodium hypochlorite (for 3min)followed by five times wash in sterile distilled water. The fruit samples were then cut into small (approximately 3mm) pieces and inoculated on to sterilized potato dextrose agar (PDA) plates in three replicates. The plates were then kept in incubator at 28°C for five days. After incubation, plates were observed for fungal growth. Thepure cultur eswere prepared for each discrete colony by taking small portion of fungal hyphae with sterilized mycological needle and stab inoculation on fresh PDA plate. In case of yeast like colonies, the pure culture was prepared by inoculating culture on fresh PDA plate by streaking. Plates were incubated as mentioned earlier (Anwer *et al*., 2017).

Identification of isolated fungi

The molds were identified byobserving cultural characteristics (colony growth pattern, colony color, presence of aerial mycelium, wrinkles and furrows)

and microscopic characteristics (Hyphae and spores morphology).The wet mount technique by using lactophenol cotton blue was performed as suggested by Oyeleke and Manga, (2008). Concisely, lactophenol cotton blue (one drop) was placed on the clean glass slide, where a small portion of mycelia of fungal culture was placed with the help of mycological needle. A coverslip was placed over the specimen by applying a little pressure.

The slide was observed on 10x and 40x objective lenses of microscope formoldsidentifications in accordance with Samson and Varga,(2007),Adebayo-Tayo*et al.*,(2012), Tafinta *et al.*, (2013) and Samuel*et al.*,(2015).Bacteria like colonies were identified as yeast by performing simple staining method as suggested by Anwer *et al.*,
(2017).

Statistical analysis

All experiments were performed in triplicates. Prevalence of each fungal isolate was calculated in percentage.

Results and discussion

Fungi responsible for spoilage of fruits after harvesting were studied. Seventy seven fungal isolates belonging to five differentgenera were isolated from various fruits collected from Panjgula market, Khairpur, Sindh, Pakistan.

Table 1. Prevalence of fungal isolates on the spoiled fruits.

No.	Fungi	No. of isolates	Occurrence (%)	Fruit sample					
				Strawberry	Orange	Papaya	Melon	Apple	Guava
1.	*Aspergillus* spp.	31	40.26	+	+	+	+	+	+
2.	*Penicillium* spp.	19	24.67	+	+	+	-	-	-
3.	*Alternaria* spp.	16	20.78	-	-	+	+	+	+
4.	*Rhizopus* spp.	07	9.1	+	-	+	+	-	-
5.	Yeast	04	5.19	+	-	-	+	+	-

The fungi identified in this study were *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., *Rhizopus* spp., and Yeast (Fig. 1.). The fungal isolates associated with spoiled fruitsand their frequency of occurrence in the various fruits analyzed is shown in Table1. Control PDA plates did not show growth.

Aspergillus spp. was found to be more frequent prevalent (40.26%) and was isolated from each fruit sample tested followed by *Penicillium* spp. (24.67%) in strawberry, orange and papaya; *Alternaria* spp. (20.78%) in papaya, melon, apple and guava, *Rhizopus* spp. (9.1%) in strawberry, papaya and melon, and Yeast(5.19%) in strawberry, melon and apple (Table 1). Similar results have been obtained by other researchers, for example, *Aspergillus niger* has been reported to be most frequent fungal isolate in tomato (Ibrahim *et al.*, 2011; Mailafia *et al.*, 2017).Kalyoncu, (2005) found *Alternaria alternata* in spoiled fruit. Ghosh, (2009) and Akinmusire,(2011) have reported the presence of *Rhizopus* spp. in fruit. The study conducted by Ayoola, (2007); Chukwuka *et al.*, (2010); Wogu and Ofuase,(2014); Mbajiuka and Enya, (2014) and Mailafia *et al.*, (2017) has revealed the presence of *Aspegillus* spp., *Penicillum* spp., and Yeast in spoiled fruits.

In various countries fungi are among most commonly occurring organisms on fruits and their prevalence have been recorded by various researchers (Booth, 1976; Amadi and Oso, 1996; Bukar, 2009; Akhter *et al.*, 2009; Oyetunji *et al.*, 2012; Sharma *et al.*, 2013; El-Gali, (2016); Mailafia *et al.*, (2017).

Generally, there could be different factors responsible for contamination of agricultural products such as

pre-harvesting invasion in field, improper handling of fruits during harvesting, poor sanitation, careless packaging and delivery of fruits to the market. Therefore, efforts should be made to minimize wounding of fruits. This would prolong the shelf life of fruits and make them available for more time at least in season. In addition, care must be taken for field sanitation to reduce the load of contaminant microbes carried from the field to post harvest stage.

In this study, strawberries were found to be spoiled with all the fungi isolated.

This could be because of their soft peel which could make them prone to physical damage and easy penetration by fungi. Other fruits showed growth of some fungi and absence of other fungi. This could be due to difference in sugar content, pH and water content of fruits analyzed in this study.

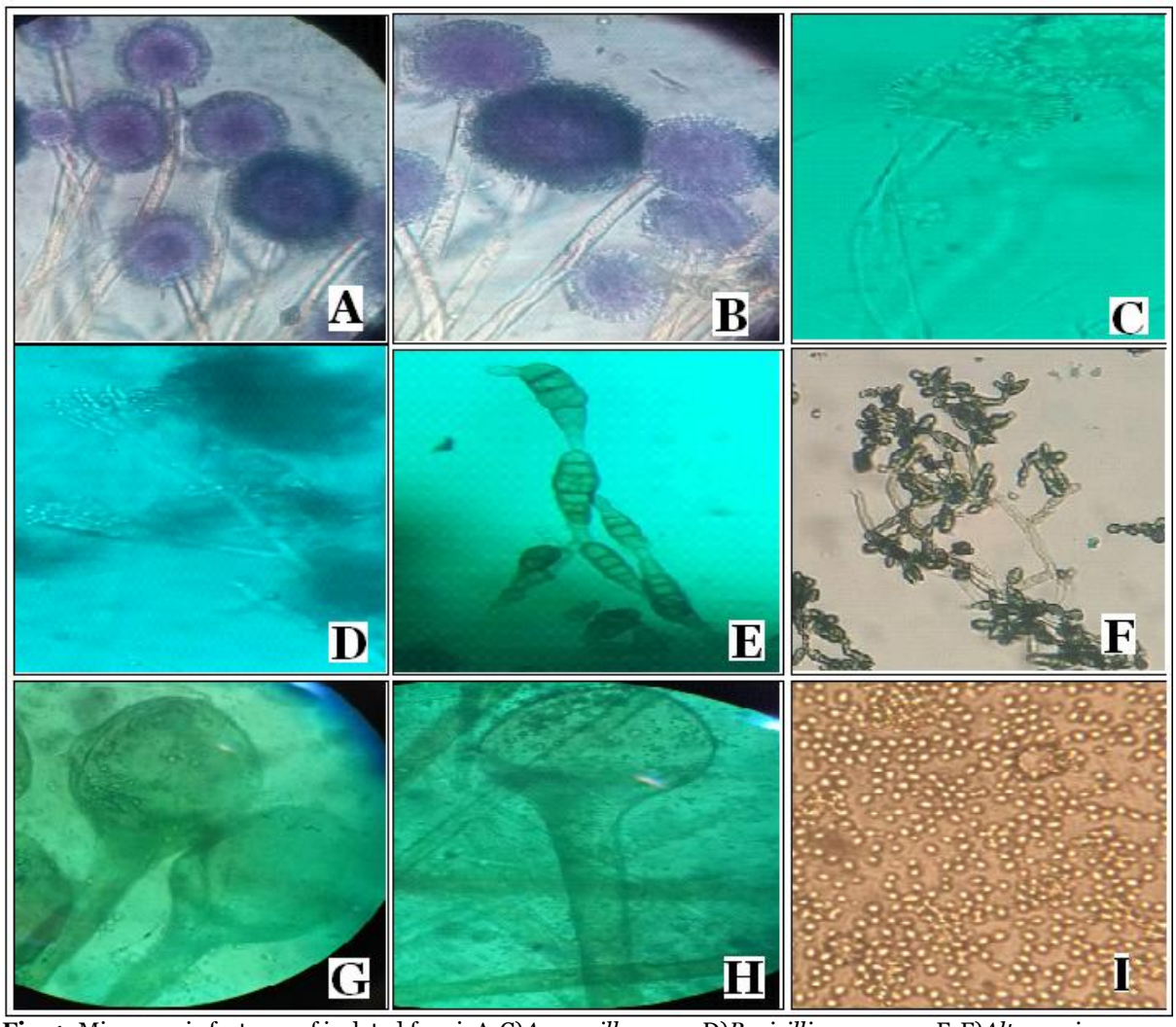

Fig. 1. Microscopic features of isolated fungi, A-C)*Aspergillus* spp., D)*Penicillium* spp., E-F)*Alternaria* spp., G-H) Rhizopus spp. and I) Yeast.

Since fruits spoiled with fungi can infect other fruits, it is very important to discard that fruit immediately to avoid the loss of other fruits. In addition, washing of fruits could reduce the microbial load however, after washing if fruits are not consumed they should be properly dried otherwise presence of moisture on fruits could encourage the growth of fungi. Some of the fungal genera isolated in this study such as *Aspergillus*, *Penicillium* and *Alternaria*are pathogenic and produce mycotoxins. These fungi are able to grow on fruits at room temperature and pose a risk to human and animal health. Fungal spoilage of fruits is minimizing hygiene of fruits and marketable quality, causing major economic loss in Pakistan,

therefore, strategies need to be devised for minimizing spoilage of fruits.

Conclusion

Fruits have high economic and nutritional value. Their spoilage is not only hazardous to health but an economic loss of country.Proper handling of fruits from pre-harvest to post-harvest is required to avoid spoilage of fruits with fungi.

The use of modern preservation methods such as radiation, vacuum packing, pasteurization, high-pressure food preservation and bio preservation isrecommendedto prevent fungal spoilage andimproveshelf life of fruits.

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Conflict of interest

The authors declare that they have no conflict of interest.

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