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Isolation and Identification of Fungal Post-harvest Rot of Some Fruits in Yemen

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Abstract
The physiological studies on fruits rotting fungi in Yemen are scarce. This work was designed to study the biodiversity of fungal post-harvest decay of apple, orange, banana, mango and grape fruits sold in local markets in Sana'a city, Yemen. A total of 150 rotted fruits samples were collected from different local markets, small pieces of mouldy part were inoculated on prepared plates of Potato dextrose agar (PDA), after 7 days of incubation, pure isolated fungi were identified according to the recommended references. The most common spoilage fungi which isolated and identified was *Penicillium expansum* (apple), *Colletotrichum musae* (banana), *Aspergillus terreus*, (orange), *Aspergillus niger* (mango) and *Penicillium glabrum* (grape). Several fungal species (39) belonging to 16 fungal genera could be regarded as post-harvest decay of apple, orange, banana, mango and grape fruits in Sana'a market. Proper measures should be adopted to protect fruits from fungal decay.
Keywords: Post-harvest, *Aspergillus*, *Penicillium*, *Colletotrichum*, rot and spoilage.

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INTRODUCTION

It has been recognized that fruits are commercially and nutritionally important food product. Fruits play an important role in human nutrition by contributing the necessary growth factors such as vitamins and essential minerals in human daily diet maintaining a good and normal health. Rot diseases caused by fungal pathogens provoke severe losses of agricultural and horticultural crops every year (Salman 2005; Parveen *et al*., 2016). Fruits have wide distribution in nature. The relatively short shelf-life period provoked by pathogens is one of the most important limiting factors that impact the economic value of fruits. Approximately 20-25% of the harvested fruits are deteriorated by pathogens during post-harvest handling even in advanced countries (Droby, 2006; Zhu, 2006).

The postharvest losses are often more harsh in developing countries due to lack of storage and transportation facilities. Fruit infections by fungi may appear during the growth period, harvesting, handling, transportation and post-harvest stockpile and marketing conditions, or after procuring by the consumer. Fruits incorporate high levels of nutrients element and sugars and their low pH values make them exceptionally desirable to fungal decay (Singh and Sharma, 2007). Fungi are considered as an essential post-harvest losses agent of different fruits, based on cultivar, season and production area amid other factors (Valiuskaite *et al*., 2006; Ewekeye *et al*., 2016).

Fungi are the most crucial and common pathogens and the mean cause of crop diseases. It Infect a wide range of fruits and vegetables during storage and transportation (Sommer, 1985).

Rotted fungi are ubiquitous biological agents that are able to infect fruits because of their ability to produce a wide range of hydrolytic enzymes. Mould growth depends on many factors such as pH, water activity (aw), temperature, atmosphere, time, etc (Magan, and Aldred, 2007).

As reported by Yemeni Ministry of Agriculture and Irrigation, (2011) the total cultivated area of fruits was reported to be 93,989 acre yielding 991,091 tons per year.

This study was aimed in isolation and identification of the mycobiota associated with post-harvest decay of apples, oranges, bananas, mangoes and grapes from different localities in Sana'a markets. Yemen.

MATERIALS AND METHODS

Collection of samples

One hundred and fifty samples of mouldy fruits including thirty samples of each of apples, banana, oranges, mangos and grapes were randomly collected from some markets in Sana'a city from December 2011 to august 2012. The weight of each sample was nearly 250 g. Samples were separately kept inside clean plastic bags, transferred to the laboratory and stored in a refrigerator until mycological analysis.

Isolation and identification of fungi

The direct plating technique described by Pitt and Hocking (1985) was employed. Four small pieces from the margin of lesion of each sample were directly inoculated on prepared plates of Potato dextrose agar which contain (g/L): peeled potato100.0g, glucose 20.0g, agar 15.0g, water 1000.0 ml. The medium was supplemented with chloramphenicol (250 mg per liter) as a bacteriostatic agent (Smith and Dawson, 1944). The plates were inoculated at 28 ± 1 ^{o}C for 5 to 7 days. Three replicates were prepared for each sample. The resulting fungi were isolated, purified and identified according to their macro and micro characteristics.

Identification of fungal genera and species

The pure isolated fungi were identified following the most documented keys in fungal identification (Raper and Fennell *et al.* 1965; Pitt 1979; 1985, Moubasher, 1993; Klich, 2002).

Statistical analysis

Data obtained was analyzed statistically using SPSS (Version 16).

RESULTS

The biodiversity of fungal species listed in table (1) could be regarded as common post-harvest decay agents of various studied fruits. Through this investigation at 28 oC thirty nine fungal species attributed to sixteen genera were isolated. Thirty-nine species belonging to 16 fungal genera were isolated from 150 samples of mouldy fruits collected from different localities in Sana'a markets. *Alternaria, Aspergillus* and *Penicillium* were the most common genera that colonized apple, orange, banana, mango and grape fruits with different incidences (Figure 1). In which *Aspergillus* were represented by (9 species), *Penicillium* (6 species) and *Alternaria* (4 species) contributed the broadest spectra fugal species.

Aspergillus species were classified according to Raper and Fennell. (1965) to the following species: *A. aculeatus, A. candidus, A. clavatus, A. flavus, A. fumigatus*, *A. japonicus, A. niger*, *A. parasiticus* and *A. terreus.*

Penicillium species were classified according to Raper and Thom (1949) to the following species: *P. chrysogenum, P. citrinum*, *P. corylophilum*, *P. expansum, P*. *glabrum* and *P. viridicatum*.

Alternaria contained 4 species namely *A. alternata*, *A. chlamydospora*, *A. phragmospora* and *A. tenuissima*. *Cochliobolus* and *Fusarium* were represented by 3 species for each namely *C. hawaiiensis*, *C. sativus, C. spicifer*, *F. chlamydosporum*, *F. mersimode* and *F. solani.*

Eupenicillium, Mucor and *Trichoderma* were represented by 2 species for each; *E. aegyptiacum, E. javanicum, M. circinelloides, M. hiemalis, T. harzianum* and *T. koningii***.**

The remaining genera *Cladosporium, Colletotrichum, Curvularia, Eurotium*, *Gliochladium*, *Rhizopus*, *Ulocladium* and *Verticillium* were represented by one species only namely *C*. *cladosporioides*, *C. musae, C. lunata, E*. *amstelodami, G*. *roseum, R. arrhizus***,** *U. botrytis* and *V. tenerum*.

It is worth to mention that *C. musae*, *G*. *roseum* and *E. javanicum* are new record to the microbial laboratory, biology department, faculty of science, Sana'a University.

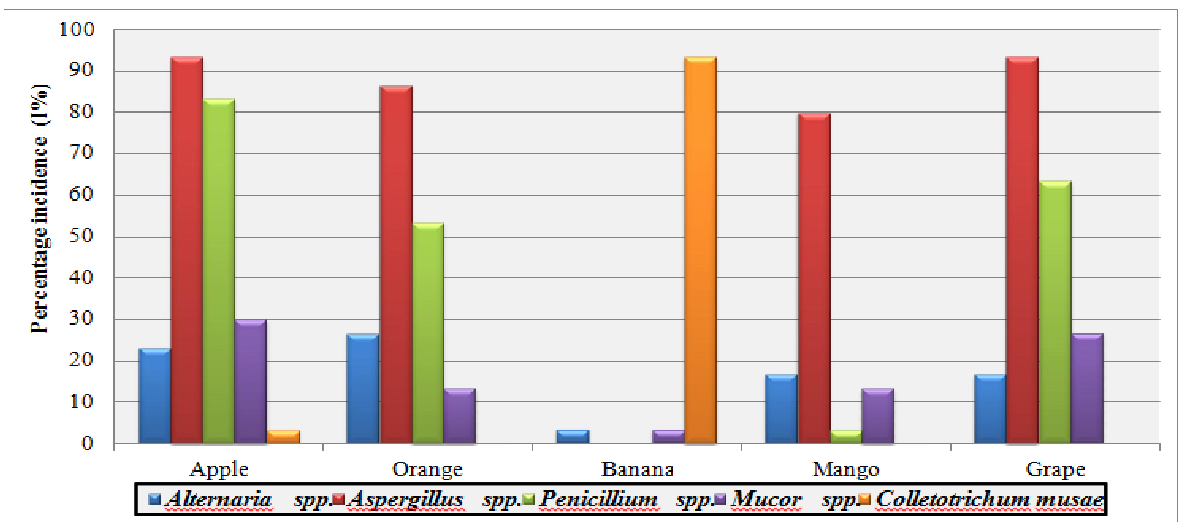


Fig. 1. Percentage incidence of cases of isolation (Percentage incidence I %) of the most common fungal genera isolated from different deteriorated fruits.

Table 1. Summary of different types of fungal species isolated from deteriorated fruit samples during this investigation.

Genus	Species	Type of fruits Apple	Orange	Banana	Mango	Grape
	A. alternate	+	+	+	+	+
Alternaria	*A. chlamydospora*	-	+	-	-	-
	A. phragmospora	+	+	-	-	-
	A. tenuissima	-	+	-	-	-
	A. aculeatus	+	-	-	+	-
	A. candidus	+	-	-	-	-
	A. clavatus	+	-	-	-	+
	A. flavus	+	+	-	+	+
Aspergillus	*A. fumigatus*	-	+	-	-	+
	A. japonicas	-	-	-	+	-
	A. niger	+	+	-	+	+
	A. parasiticus	-	+	-	+	-
	A. terreus	-	+	-	-	-
Cladosporium	*C. cladosporioides*	-	+	-	-	-
	C. hawaiiensis	+	-	-	-	-
Cochliobolus	*C. spicifer*	-	+	-	-	-
	C. sativus	+	+	-	-	-
Curvularia	*C. lunata*	+	+	+	-	-
Colletotrichum	*C. musae*	+	-	+	-	-
Eurotium	*E. amstelodami*	-	-	-	+	-
Eupenicillium	*E. javanicum*	-	+	-	-	-
	E. aegyptiacum	-	+	-	-	-
	F.chlamydosporum	-	+	-	-	-
Fusarium	*F. mersimode*	-	-	+	-	-
	F. solani	-	-	+	-	-
Gliochladium	*G. roseum*	-	-	-	-	+
Mucor	*M. circinelloides*	+	+	+	-	+
	M. hiemalis	+	-	-	+	-
	P. chrysogenum	+	+	-	-	+
	P. citrinum	+	-	-	-	-
Penicillium	*P. corylophilum*	-	-	-	-	+
	P. expansum	+	-	-	-	-
	P. glabrum	+	+	-	-	+
	P. viridicatum	-	-	-	+	+
Rhizopus	*R. arrhizus*	-	-	-	+	-
Trichoderma	*T. koningii*	-	+	-	+	-
	T. harzianum	-	-	-	+	-
Ulocladium	*U. botrytis*	-	-	+	-	-
Verticillium	*V. tenerum*	-	-	-	+	-
No. of genera		**7**	**10**	**6**	**8**	5
No. of species		**17**	**20**	**7**	**13**	11

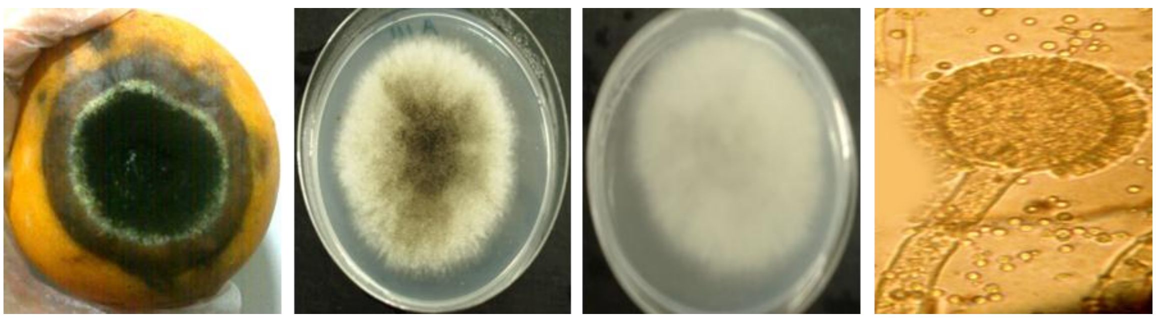
+ : Present, - : Absent

Aspergillus was by far the most common genus affecting the different kinds of fruits. It appeared on 93.3 % of each of apple and grape fruits. 86.6% of orange fruits and 80.0 % of mango fruits. *A. flavus* and *A. niger* were the dominant species on the tested fruits. The highest incidence of *A. niger* was observed on orange fruits followed by grape fruits (60.0% and 56.6%, respectively). Whereas *A. flavus* was found to be common on apple fruits followed by grape fruits (60.0% and 30.0%, respectively). Figure (2) show the macro and microscopic characters of *A. aculeatus*.

Penicillium was the second most common genus especially on apple (83.3%) followed by grape (63.3%) and orange (53.3%), *P. chrysogenum* and *P. glabrum* appeared with variable incidences on most kinds of tested fruits. Other species showed higher affinity towards certain fruits such as *P. expansum* on apple fruits. Figure (3) show the macro and microscopic characters of *P. glabrum*.

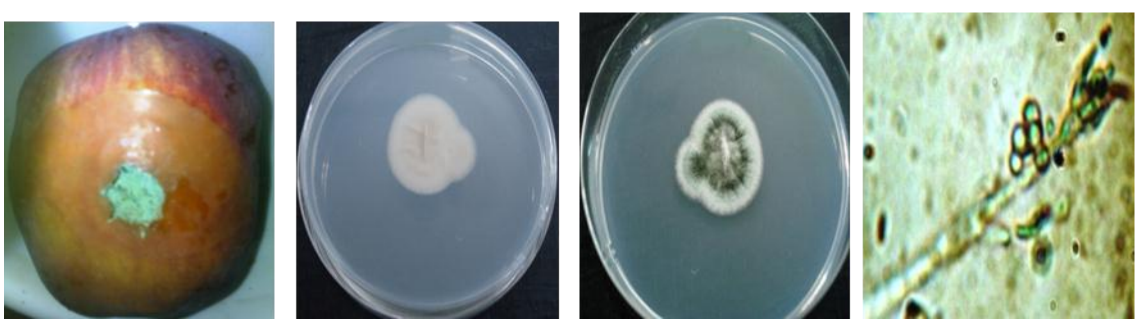
Figure (4) showed that *Alternaria* (represented by *A. alternata*, *A. chlamydospora*, *A. phragmospora* and *A. tenuissima*) ranked third in abundance on decayed fruits. It was more common on orange (26.7%) followed by apple (23.3 %) and finally on mango and grape (16.6 % for both). *A. alternata* was occurred more frequently than other species. Figure (5) showed the macro and microscopic characters of *A. alternate.*

During this study, *Colletotrichum musae** proved to be the main causative agent of banana rot where it was found on 93.3% of its tested samples. Figure (6) show the macro and microscopic characters of *C. musae*.



(a) (b) (c) (d)

Fig. 2. Images of Fungal infection in mango fruit (a), surface view of old colony on Potato dextrose agar (b), reverse view of old colony on Potato dextrose agar (c) and coinidial head and conidia of *Aspergillus aculeatus* (d).



(a) (b) (c) (d)

Fig. 3. Images of fungal infection in grape fruit (a), reverse view of old colony on Potato dextrose agar (b), surface view of old colony on Potato dextrose agar of penicillin of *Penicillium glabrum* × 400 (d)

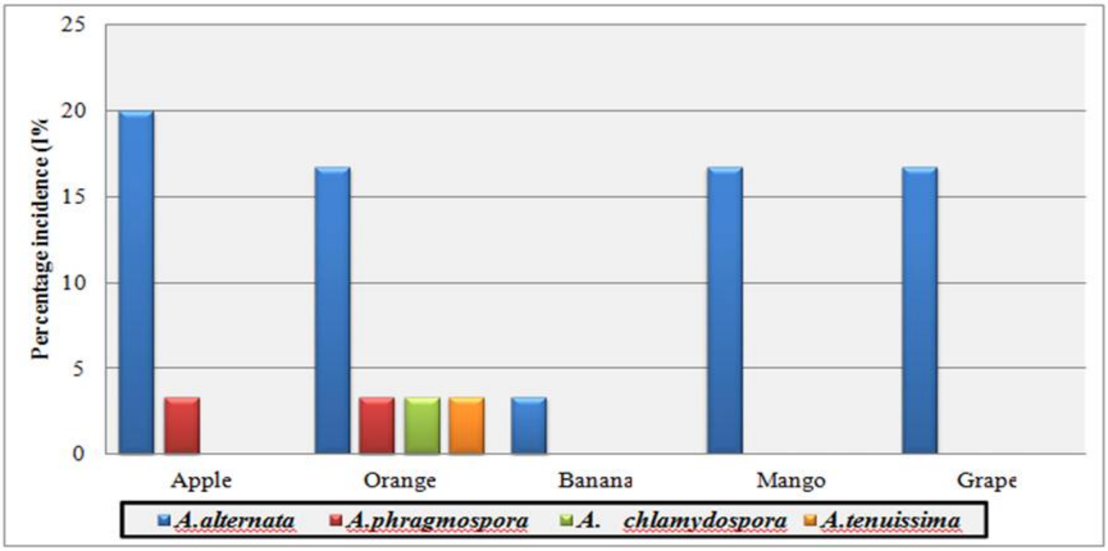
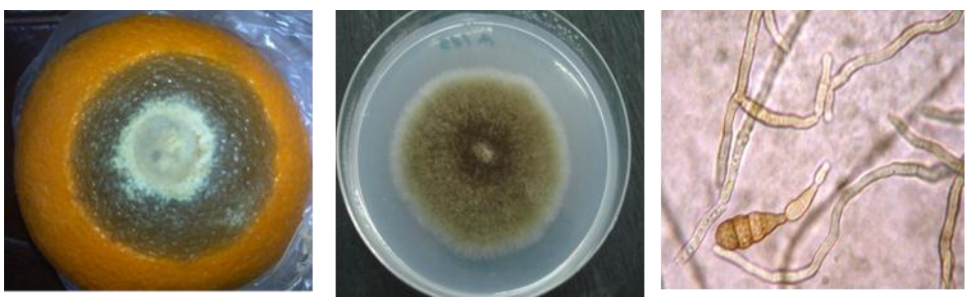
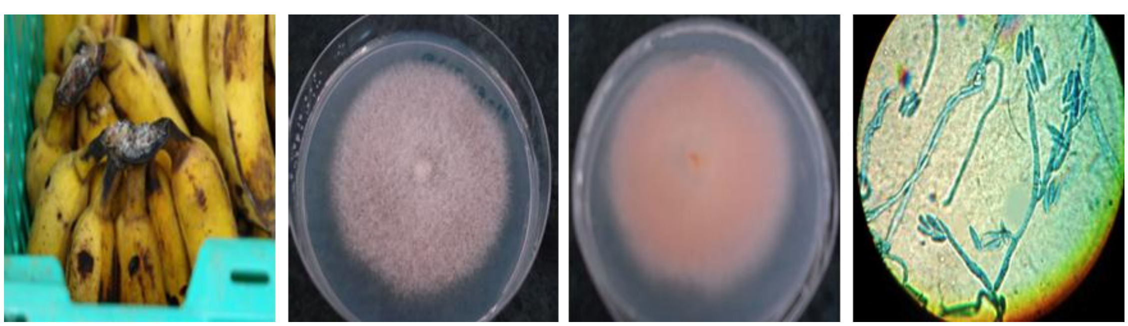


Fig. 4. Percentage incidence of cases of isolation (I %) of the most common *Alternaria* species isolated from different deteriorated fruits.



(a) (b) (c)

Fig. 5. Images show fungal infection in orange fruit (a), surface view of old colony potato dextrose agar (b). and conidial chain of *Alternaria alternata* × 600 (c).



(a) (b) (c) (d)

Fig. 6. Crown rot of banana fruits (a), surface view of old colony on potato dextrose agar (b), reverse view of old colony on potato dextrose agar (c) and conidia of *Colletotrichum musae* (d) × 600.

DISCUSSION

This investigation embraces an extensive survey of the mycobiota associated with post-harvest rot of fruits in 150 samples collected from different localities in Sana'a markets. The tested samples comprised apple, banana, orange, mango and grape fruits (30 samples for each).

In this respect, Akinmusire (2011) and Chukwuka *et al.* (2010) mentioned that fruits can affected by a wide range of microorganisms such as fungi which have a serious threat to production of fruits. Spoilage attributes to any change in the condition of food making it less palatable, or even toxic; these alterations may be accompanied by changes in taste, smell, appearance.

During the first part of this investigation, it was possible to isolate 39 species belonging to 16 fungal genera from the 150 samples of the mouldy fruits. The broadest spectrum of fungal species was recovered from orange fruits (20 species).

The findings of this study showed that, *Aspergillus* was the genus most frequently isolated from the different kinds of mouldy fruits being recovered from 0 % to 93.3% of samples with the highest incidence observed on apple and grape and the comparatively no incidence from banana. On orange and mango fruits *Aspergillus* was recovered in 86.7% and 80% of the samples. Among the 9 species of *Aspergillus, A. niger* and *A. flavus* were the most common species. They were respectively recovered from 20% - 60% and 13.3% - 60.0% of the different mouldy fruits sample.In India, the isolation of these pathogens confirmed by Fatima *et al.* (2012) who found that the long time that taken in transportation of fruits through country as well as increasing in humidity, favors the development of *Aspergillus* rot of fruits. Al-Hindi *et al.* (2011) reported that *Aspergillus* spp. were found to be the most spoilage fungi among all examined spoilage fruits. And he found that *A. niger* was a commonly fungus on grapes (Chulze, 2006), apples (Oelofse, 2006) and orange (Gadgile and Chavan, 2010).

In Ibadan, Oyo State, South Western Nigeria, Baiyewu *et al*. (2007), Bali *et al*. (2008) and Chukwuka *et al.* (2010) found that *A. niger* and *A flavus* were the main responsible for deterioration of orange fruits in major markets. These two species were previously recorded as causative agents of citrus fruits decay during storage in the same country (Fawole and Odunfa, 1992). In addition, in Greece, Egypt and Morocco Paola *et al*. (2008) found that *Aspergillus* rot of grape that caused by *A. niger* and *A. flavus* usually severe in the warmer grape-producing areas.

The most common fungal species isolated from infected mangoes in Saudi Arabia were identified as *A. niger* and *Alternaria* sp. (Al-Hindi *et al.*., 2011). However, Fatima *et al*. (2012) found that the main fungi that responsible for post-harvest deterioration of fresh fruits in Pakistan were identified as *A. flavus, A. niger*. In other studies, *A. niger* and *A. flavus* were the responsible for decay of mango fruits in post-harvest phase (Prusky and Yakaby, 2003; Diedhiou, *et al.*, 2007 and Muhammad *et al.*, 2011).

Penicillium was the second most frequent genus on the tested fruit samples. It showed its highest incidence on apple fruits, followed by grape and orange fruits. *Penicillium* was rare on mango fruits, while there was no occurrence of *Penicillium* on banana fruits examined during this study. The genus was represented by 6 species of which, *P. chrysogenum* was the most common especially on orange, apple and grape fruits. *P. glabrum* was fairly common on grape, apple and orange fruits. *P. expansum* and *P. citrinum* were appeared only on apple fruits and was missed on other kinds of tested fruits.

In agreement with our results, Snowdon (1990) and Benkhemmar *et al.* (1993) found that *Penicillium* spp. such as *P. chrysogenum, P. citrinum, P. glabrum* and *P. viridicatum* are common pathogens of stored grapes. In a previous study, Narayanasamy (2006) reported that *Penicillium* appeared to be the most fungal genus infected fruit crops such as apple, citrus; grapes, pear, and strawberry. In addition, Barkai-Golan. (2001) and Paola *et al.* (2008) reported that many *Penicillium* species were the causative agent of post-harvest diseases in which they infect a wide range of crop.

On other hand *P. expansum*, was the main causes of blue mould rot of apples, pears and other fresh fruits, it is an example of the destructive post-harvest pathogens that cause a large part of the economic losses during post-harvest phase. Dov Prusky *et al.* (2010) found that *P. expansum* responsible for rotting apple fruits, in which it lead pH to decreases from 3.95 to 4.31 in the healthy mesocarp to values ranging from 3.64 to 3.88 in the rotting tissue.

Our data showed that *Alternaria* could be regarded as the third most frequent genus on the tested fruits. It occurred with different incidences in all kinds of fruits. Among 4 species of *Alternaria, A. alternata* was the commonest. These results conform with findings of Barry *et al.* (2003) who showed that from 218 fungal isolates isolated from lesions on different fruits, *A. alternata* resembled 96% of fungi responsible for *Alternaria* rot. In addition, *A. alternata* appeared to be the most common decay organism of post-harvest apple, where this species represented 81.9% 0f 110 strains of *Alternaria* isolated from decayed apples (Vinas *et al.,* 1992).

In similar studies, Mansour *et al.* (2006), Haggag *et al*. (2009) and Muhammad *et al.* (2011) found that *Alternaria* black spot (*A. alternata*) had become the most important disease of fresh and stored fruits. Peever *et al.* (2005) stated that post-harvest black rot of fruit occurred mainly in citrus fruits and caused by different species of *A. alternata*.

The two species of *Mucor* identified during this investigation, were *M. circinelloides* and *M. hiemalis.* They occurred in low or rare incidence on the different kinds of fruits. In this respect*, M. hiemalis* in addition to *M. mucedo* and *M. piriformis* were found to cause decay of numerous fruits and vegetables including apples, guava, pears, peaches, strawberries and sweet potatoes (Moline, 1984). Different Mucor species were previously recorded to cause decay of different fresh fruits (El-Tahtawi, 2005).

During this study, *Colletotrichum musae* proved to be the main causative agent of banana rot. Scot. (2001) found that the two primary post-harvest rots of banana fruits in Hawai'i were crown rot and anthracnose caused by the fungus *C. musae*. Raut and Ranade (2004) and Ranasinghe *et al.* (2005) reported that, banana suffer from serious post-harvest losses caused by fungal infections, especially *C. musae*. Sulali *et al.* (2004) mentioned that from nine localities in Sri Lanka the fungal pathogen isolated from the anthracnose lesions of banana was identified as *C. musae.* In Taiwan, Chuang and Yang (1993) reported that banana anthracnose induced by *C. musae* was an important post-harvest disease and caused serious loss during transport. Similar findings were also reported in Sri Lanka.

Fungal species belonging to *Cladosporium*, *Eupenicillium, Eurotium*, *Gliochladium, Fusarium*, *Rhizopus, Trichoderma, Ulocladium* and *Verticillium* were generally rare on the different kinds of the tested fruits.

CONCLUSION

Post-harvest pathology, earlier termed “market pathology”, refers to the science of, and practices for, the protection of harvested produce during harvesting, packing, transportion, processing, storage, and distribution**.** Several fungal species (39) belonging to 16 fungal genera could be regarded as most common causes of post-harvest deterioration of apple, orange, banana, mango and grape fruits in Sana'a market. Common species were *Aspergillus niger, A. flavus*, *Alternaria alternata*, *Mucor circinelloides, Penicillium chrysogenum, P. expansum* (on apple), *Colletotrichum musae* (on banana).

RECOMMENDATIONS

Considering the fact that the isolated fungi found on a group of fruits under- study, possess the ability to produce enzymes that could change the nutritious value of these fruits, leading to economical loss. Furthermore, these fungi can also produce several toxins within the examined fruits, resembling a public health hazard. This issue is of importance as the targeted fruits are priceless and mostly consumed by poor people (economically low-class people). For that we recommended the following:

1. Fruits sales in markets should be under a clean-safe environment that would be unfavorable environment for the growth of such fungi.
2. The concerning bodies should hold their responsibilities in following-up fruit-sales markets to ensure high-quality and toxic-free fruits.
3. Proceeding further studies in this field, as this issue related to consumers health.

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CONFLICT OF INTEREST

The authors declare that this article content has no conflict of interest.

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