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RESEARCH ARTICLE

Screening and Characterization of Fungi and their associated Mycotoxins in some Fruit Crops

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Abstract

The mycological analysis of five fruit crops samples revealed that, the two genera *Alternaria* with nine species and *Fusarium* with eight species were found to be the most dominant fungi on all selected fruits. Pathogenicity test on pomegranate fruits revealed that *A. alternata* reproduced the typical symptoms of black spots while *A. alternata*, *A. arbusti*, *P. funiculsoum* and *A. niger* were pathogenic and reproduced the typical symptoms of heart rot. On guava fruits, *C. dematium*, *A. alternata, A. raphani*, *Phoma* sp., *Fusarium sporotrichioides*, *F. proliferatum* and *F. culmorum* caused visible symptoms of fruit spot and fruit rot while, fruits inoculated with *B. theobromae* and *Phomopsis* sp. exhibited stylar end rot symptoms. Additionally, according to available literatures, this is the first report of *A. alternaria* and *P. funiculosum* as causal pathogen of black spot and heart rot of pomegranate fruits in Egypt respectively. Also, pathogenicity trails reported for the first time stylar end rot disease of guava and *C. dematium* as a causal pathogen of anthracnose in Egypt. Mycotoxin assay revealed that eight *Fusarium* species were capable of producing detectable levels of four major mycotoxins *i.e.* Deoxynivalenol (ranging from 7.8-405.3µg/L), Fumonisin (35.9-1121.0 µg/L), T-2 toxin (0.0-55.2 µg/L) and Zearalenone (0.0-456.9 µg/L). Nine *Alternaria* species was tested for produce four kinds of mycotoxins *i.e.* Alternariol, Tenuazonic acid, altenuene and Alternariol monomethyl ether. *A. alternata* associated with fruit rot of guava was able to produce the four mycotoxins and other remaining species was varied in mycotoxins production profile. All four mycotoxins were apparently not produced by *A. zinniae* and *A. infectoria*.

1. Introduction

In the past 20 years international trade in fresh fruits and vegetables has grown greatly and is presently a multi-billion dollar business representing the major export for many developing countries (Barkai-Golan and Paster, 2008). One of the limiting factors that influence the fruits economic value, the relatively short shelf-life period caused by attacked pathogens. Plant pathogens may infect fruits either prior to harvest under field conditions or after harvest during transit and storage. The symptoms of infection may be observed at different period after harvest but many pathogens may remain dormant for varying periods until favorable conditions become available for their development, leading to visible symptoms. It is estimated that about 20-25% of the harvested fruits are decayed by pathogens during post-harvest handling even in developed countries (Droby,2006 and Zhu, 2006). It should be noted that for a total of 100,000 fungi, less than 10% are pathogenic for plants and around 100 species are responsible for the majority of postharvest damage (Eckert and Ratnayake, 1983).Storage diseases lead to economic loss by reducing quality and marketability of damaged fruit, or may result in complete loss of the stored fruit. Fruits contain

high levels of sugars and nutrients element and their low pH values make them particularly desirable to fungal decayed (Singh and Sharma, 2007). The common postharvest and storage fungi of fruits are *Alternaria* spp., *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp. (Bhale, 2011). Besides the losses in income to the fruit marketers, in some cases host pathogen interactions provide a favorable environment and source for production of many different compounds. Mycotoxins are a structurally diverse group of mostly small molecular weight compounds, produced mainly by the secondary metabolism of some filamentous fungi, or molds under suitable conditions (Zain,2011). The major mycotoxin-producing fungi are not aggressive pathogens in plants; however, mycotoxins are produced by several genera in plants during the growing season when portals of entry are provided and environmental conditions are appropriate and be continued or initiated in postharvest and stored products.
The majority of these toxins are produced by fungi of the genera, *Aspergillus, Penicillium* and *Fusarium* (Barkai-Golan and Paster, 2008). The toxigenic *Fusarium* and *Alternaria* species are often classified as field fungi, because they require very high moisture content in the substrate for growth and mycotoxin synthesis. The storage fungi, primarily species of AspergillusandPenicillium also grow well at lower moisture contents. Thus, *Fusarium* and *Alternaria* usually represent a high mycotoxicological risk in preharvested or freshly harvested plant that are drying, whereas toxigenic species of *Aspergillus* and *Penicillium* represent a higher risk for products in storage or being used in food and feed processing (Harris and Mantle,2001; Harvey et al., 2001; Hussein and Brasel, 2001 and Logrieco et al., 2002). In Egypt and under local markets in Najran, Saudi Arabia, there is relatively little information related to the natural occurrence of fungi and mycotoxins in fruits. The present investigation was undertaken to find out the association of field and storage fungal diseases with some important fruits and the mycotoxins mainly synthesised by strains of toxigenic fungi.

2. Materials and Methods

2.1. Samples Collection

A survey of crop fungi were conducted on five economically important fruits *i.e.* Promegranate (*Punica granatum*), Guava (*Psidium guajava*), apple (*Pyrus malus*), mango (*Mangifera indica*) and Citrus (*Citrus* sp.) during 2012, 2013 seasons. Naturally infected fruits of Guava, Pomegranate and Citrus were collected from orchards at Biehera governorate, Egypt. Also, infected fruits were collected from local markets in Najran, Saudi Arabia. Samples were brought to the laboratory in separate sterilized polythene bags, examined critically with respect to symptomatology and sorted out for the isolation of the causal agents.

2.2. Isolation and identification of the associated fungi

The isolation and identification of the causal agents were performed for every single fruit. Fungal pathogens responsible for fruit rotting were isolated from the flesh beneath the peal while fruits exhibit fruit spots were isolated from the peel surface. Infected fruit tissues were surface sterilized in 1% sodium hypochlorite solution for 1 min and rinsed twice in sterilized water. Using a sterile scalpel, tissue pieces composed of spots, halo, and surrounding healthy tissue were placed onto potato dextrose agar (PDA) amended with tetracycline at 12µg/ml, and incubated at 25°C.In some cases, rotting fruit samples were incubated in a moist chamber and mycelium of individual fungal species transferred onto PDA plates and incubated for 3-7 days at a temperature of 25°C.Fungal colonies emerging from symptomatic tissue was picked up and transferred to new plates and left to grow for 5 to 7 days prior. Pure cultures were then obtained by single spore isolation and maintained on PDA slants for further study. The fungi were observed under a microscope and identification of the pathogens was made with the help of available literature (Barnett and Hunter, 1999 and Biligrami et al., 1991). The identification for all fungal isolates was re-confirmed by the Microloge system (Biolog, Inc., Hayward, CA) at the National Research Central Lab., GSFMO, KSA for further identification(Grizzle, 2006 and Singh, 2009). Different frequencies of fungi on same crop were noted.

2.3. Pathogenicity test

To fulfill Koch's postulates, fresh disease free samples were brought to the laboratory and surface sterilized with 5% Ethanol. PDA-plugs, 5 mm in diameter, with seven-day-old actively grown mycelium were transferred to skin wounds by pressing slightly pin-pricking the fruits to a 1 mm. depth with a sterile needle on superficially disinfected fruits (three plug per fruit, 3fruit per fungus). In case of fungi associated with stylar end rot of guava, mycelium growth plugs were transferred into the fruit calyx as well as on wounds made by a scalpel on previously sterilized fruit surfaces. Following inoculation, the fruits were placed in plastic boxes supplemented with watered tissue paper to maintain relative humidity and kept at 25 °C. Fruits inoculated in the same way using PDA disks were kept as control. Pathogenicity tests were conducted on fruits and were repeated three times (Tziros et al., 2007).The artificially inoculated samples were examined daily and the extent of damage was recorded. Lesion development on fruit was assessed by measuring the disease area in centimeters on each fruit. The pathogens were reisolated and disease symptoms were clearly evident, the culture and symptoms signs were compared with original.

2.4.Mycotoxins:

The different fungal isolates were propagated as pure culture in 100 ml SMKY broth (Sucrose 200 g, MgSO47H2O 0.5 g, KNO3 3 g, yeast extract 7 g) for 10 days. All fungal isolates had three replicates and incubated in dark condition at 25±2°C.

2.4.1.*Fusarium* mycotoxins assay

Microtitre plate enzyme-linked immunosorbent assay (ELISA) reader (automated Chem-well) and Fusarium mycotoxins test kit (r-biopharm, Germany) were used to ELISA analyses. The samples were analyzed using the Fumonisin, Zearalenone, T-2 and DON test procedure which was described by company(r-biopharm) producer (Enzyme Immunoassay for the quantitative analysis of aflatoxins, 1999and Leszczynska et al., 2001). Ten ml of blended fungal broth has been sub-sampled with 20ml of 70% methanol and vortex for 10 min by magnetic stirrer. The extract was filtrated by Whatman no.1 filter paper and then diluted as 5ml filtered solution, 15ml distilled water and 0.25ml Tween 20. The solution was mixed by magnetic stirrer for 2min. 50 µl toxins (5, 10, 20, 45 ppb) standard solutions and 50 µl prepared test samples were added into separate wells of micro-titer plate, in duplicate. Plates were incubated at room temperature. The liquid was then removed completely from the wells, the each well was washed with 250 µl washing PBS-Tween-Buffer (pH 7.2) and this was repeated two times. Subsequently, enzyme substrate (50 µl) and Chromogen (tetramethyl-benzidine, 50 µl) were added to each well and incubated for 30min at room temperature in the dark. 100 µl of the stop reagent (1M H2SO4) was added and the absorbance was measured at 450nm in ELISA reader.

2.4.2. *Penicillium* mycotoxins assay

Penicillium isolate were examined to produce each of the Patulin, Citrinin, citreoviridin and penicillic acid. Culture was blended for 2 min using a high speed homogenizer and filtered using glass filter paper. Patulin was extracted from homogenized filtrate using acetonitrile: water (5:95 v:v) solution. The solvent was then evaporated at 35°C under vacuum. The dried residues were dissolved in 1 ml of acetonitrile: water (5:95 v:v) solution. HPLC was used to quantify Patulin (Christian, 1990).

2.4.3. *Aspegillus nigar* mycotoxins assay

The tested isolate of *A. nigar* was examined to oxalic acid excretion. The tested isolate cultivated on SMKY broth medium and oxalic acid concentration was determined by high performance Liquid chromatography (HPLC). Separation of oxalic acid was carried out in a CLCC825 CM caption exchange column; mobile phase, 90% H2O and 10% CH3OH; flow rate, 1 ml/min and temperature 35°C according to Ghorbani et al., 2007.

2.4.4. *Aternaria* mycotoxins assay

The different species of *Alternaia* was examined for mycotoxins production. Alternariol (AOH), Tenuazonic (TeA), Altenuene (ALT), and Alternariol monomethyl ether(AME) was determined by Chromatography analysis according to Nawaz et al., 1997.

2.5. Data analysis

Different data was recorded and expressed as means ± S.E. Significance of mean differences were statistically compared using an analysis of variance (ANOVA) at the 5% probability level with individual pairwise comparisons made using Tukey's HSD test through an SPSS v. 15.0 software package in Microsoft Windows 7 operating system.

3. Results and Discussion

3.1. Survey and isolated fungi

Survey study was conducted on five fruits *i.e.* Pomegranate, guava, mango, apple and citrus which collected from mature fruits in the field of Egypt and from different markets of Najran region, Saudi Arabia. The mycological analysis of five fruit crops samples revealed that, 31 fungal species belonging to nine genera were associated with fruit symptoms. In general, the two genera *Alternaria* with 9 species and *Fusarium* with 8 species were found to be the most dominant fungi on all fruits (Table 1). The post-harvest fungal diseases are responsible for biodeterioration of tropical fruits pulp (Gadgile et al., 2009a,b). Guava and pomegranate fruits showed more fungal association than other fruits although, the presence of fungal diseases on these fruits received little attention and not well documented in Egypt so, our study received more attention to these fruits. Similarly some species were thought to attack fruits long before harvest and exhibited host specificity *i.e*. stylar end rot of guava associated with *B. theobromae* and *Phomopsis psidii* and black spots of pomegranate caused by *A. alternata* while others were observed to be responsible for the postharvest, decay and deterioration as general pathogens(Table 1).

3.2. Symptoms on fruits

In a survey of pomegranate, we reported the appearance of novel symptoms of *Alternaria* spp. These symptoms include black spots on fruit, ranging from a single lesion to lesions that cover more than 50% of the fruit surface (Fig. 1). The damage is restricted to the peel surface while the edible tissue remains unaffected. In contrast, heart rot

of pomegranate associated with *Alternaria* spp., and *Penicillium funiculosum*, in which the fruit rot, is restricted to the internal area whereas the peel remains unaffected. Another fungus that is also isolated from pomegranate is *Aspergillus niger* : symptoms on fruits appeared in two forms: spherical depressed spots occurred in scattered form on the pericarp only (Fig.1a) and black rot restricted to internal fruit tissues. The heart rot decay caused by *A. niger* is softer with exuded juice than that caused by Alternaria and symptoms reached the outer surface of fruit. In the Mediterranean region, *A. alternata, Penicillium* spp. and *A. niger* are an important pathogens associated with black spots and heart rot of pomegranate (Labuda et al., 2004; Tziros et al., 2007 and Pala et al.,2009).

Table(1)Relative frequency(%), Pathogenicity and symptoms of fungal species associated with four different fruit crops during pre/postharvest.

Host	Fungi	Source		Symptoms	% frequency*	Pathogenicity*
		Field	Storages			
Pomegranate (*Punicagranatum*)	*A. arbusti*	Egypt	-	Fruit canker	2.75±0.14^{e}	0.54±0.02^{f}
	A. blumeae	Egypt	-	Fruit canker	2.1±0.06^{d}	0.0±0.0^{a}
	A .alternata	Egypt	-	Fruit canker	0.69±0.05^{a}	0.0±0.0^{a}
	A. bumsii	Egypt	-	Fruit spots	1.38±0.02^{c}	0.0±0.0^{a}
	A. alternata	Egypt	-	Black spots	6.13±0.02^{k}	0.86±0.01^{i}
	A. alternata	-	Egypt	Heart rot	1.38±0.02^{c}	0.73±0.02^{j}
	A. gaisen	Egypt	-	Fruit canker	0.69±0.01^{a}	0.0±0.0^{a}
	A .alternata	-	Yemen	Fruit blotch	2.77±0.04^{e}	0.43± 0.02^{c}
	P. funiculosum	-	Egypt	Heart rot	2.77±0.04^{e}	5.54±0.02^{n}
	Aspergillus niger	-	unknown	Black rot; fruit spot	4.15±0.03^{j}	7.36 ±0.04^{q}
	C. trichellum	-	unknown	Fruit canker	2.77±0.04^{e}	0.0±0.0^{a}
Guava (*Psediumguajva*)	*C. dematium*	Egypt	Egypt	Anthracnose	1.38±0.05^{c}	0.76±0.02^{j}
	F. sporotrichioides	-	Egypt	Fruit spots; fruit rot	4.50±0.3^{h}	1.40±0.02^{L}
	F. oxysporum	-	Egypt	Fruit spots; fruit rot	0.69±0.03^{a}	0.0±0.0^{a}
	F. chlamidosporium	-	Egypt	Fruit spots; fruit rot	1.04±0.02^{b}	0.95±0.03^{j}
	F. proliferatum	-	Egypt	Fruit spots; fruit rot	1.04±0.02^{b}	0.75±0.03^{j}
	F. culmorum	-	Egypt	Fruit spots; fruit rot	1.04±0.02^{b}	1.25±0.03^{k}
	A. raphani	-	Egypt	Fruit spots; fruit rot	3.46±0.03^{f}	0.47 ±0.04^{c}
	A .alternaria	-	Egypt	Fruit spots; fruit rot	2.77±0.01^{e}	0.75±0.02^{j}
	Phoma sp.		Egypt	fruit rot	0.69±0.01^{a}	0.45±0.01^{c}
	A. zinniae	-	Egypt	Stylar end rot	2.77±0.02^{e}	0.0±0.0^{a}
	Phomopsis sp.	Egypt	-	Stylar end rot	5.19±0.01^{f}	1.20±0.01^{c}
	B. theobromae	Egypt	-	Stylar end rot	4.15±0.01^{e}	1.45±0.01^{j}
	Fusarium sp.	-	Egypt	Stylar end rot	0.69±0.0^{a}	0.83±0.01^{c}

*Values are the mean ±S.E. of three replicates.
In the same column, means followed by the same letters are not significantly different (P ≥0.05).
**All F-values are significant at P >0.001.

Continued: Table (1) Relative frequency (%), Pathogenicity and symptoms of fungal species associated with four different fruit crops during pre/postharvest.

Host	Fungi	Source		Symptoms	% frequency*	Pathogenicity*
		Field	Storages			
Mango (*Mangife raindica*)	*C. fragariae*	-	Pakistan	Anthracnose	2.08±0.05^{d}	0.35±0.03^{b}
	C. gleosporioides	-	Pakistan	Anthracnose	4.15±0.02^{j}	0.50±0.03^{d}
	C. arachidis	-	Pakistan	Anthracnose	3.46±0.01^{f}	0.0±0.0a
	A. infectoria	-	Egypt	Black Spot	7.27±0.04^{L}	0.40±0.02^{b}

Apple(Pyrusmalus)	*C. musae*	-	France	Fruit rot	1.04±0.02[b]	0.0±0.0[a]
	Botrytis cineria	-	France	Gray rot	2.77±0.03[e]	2.45±0.01[m]
	F. equisti	-	France	Fruit rot	1.04±0.02[b]	0.67±0.01[j]
	F. langsethiae	-	France	Fruit rot	0.69±0.01[a]	0.58±0.02[f]
	A. alternata	-	France	Black spot	1.38±0.02[c]	0.38±0.02[b]
	Penicillium sp.	-	France	Fruit rot	2.08±0.05[d]	ND
	A.niger	-	France	Fruit rot	0.69±0.05[a]	ND
Citrus(*Citrus* spp.)	*A. citri*	-	Nourth Africa	Black rot	4.15±0.03[j]	ND
	P.digitatum	Egypt	-	Green rot	5.19±0.02[j]	ND
	A.niger	Egypt	-	Fruit rot	4.84±0.02[i]	ND

ND: not determined
*Values are the mean ±S.E. of three replicates.
In the same column, means followed by the same letters are not significantly different (P ≥0.05).
**All F-values are significant at P >0.001.

Based on the findings of this study, the most important causal agent responsible for the fruits diseases of guava are the fungi. Similarly some species were thought to attack fruits before harvest in the field as stylar end rot symptoms which observed in Egypt on the nearly-matured fruits in the region lying just below and adjoining the persistent calyx as brownish water-soaked area. Such area gradually increases in size and turn brown with translucent margins and covered with concentric zones of dark colour pycnidia embedded in the disease tissues (Fig. 2.a) or covered with fungal growth (Fig. 2. , b,c). One of the pathogenic fungi that are associated with fruit rot of guava is *Fusarium* spp. while, *C. dematium* was found to be associated with anthracnose symptoms of guava fruits(Fig.2e).In general, the following fruit diseases have been reported: gray mold caused by *Botrytis cinerea* on apple, Alternaria black spot (*Alternaria alternata*) of stored mango and apple fruits(Fig.2i), fruit rots on citrus and apple(*Penicillium* spp. and *Aspergillus niger* respectively) and anthracnose on apple and mango(Fig.2.f) (*Colletotrichum* spp.).

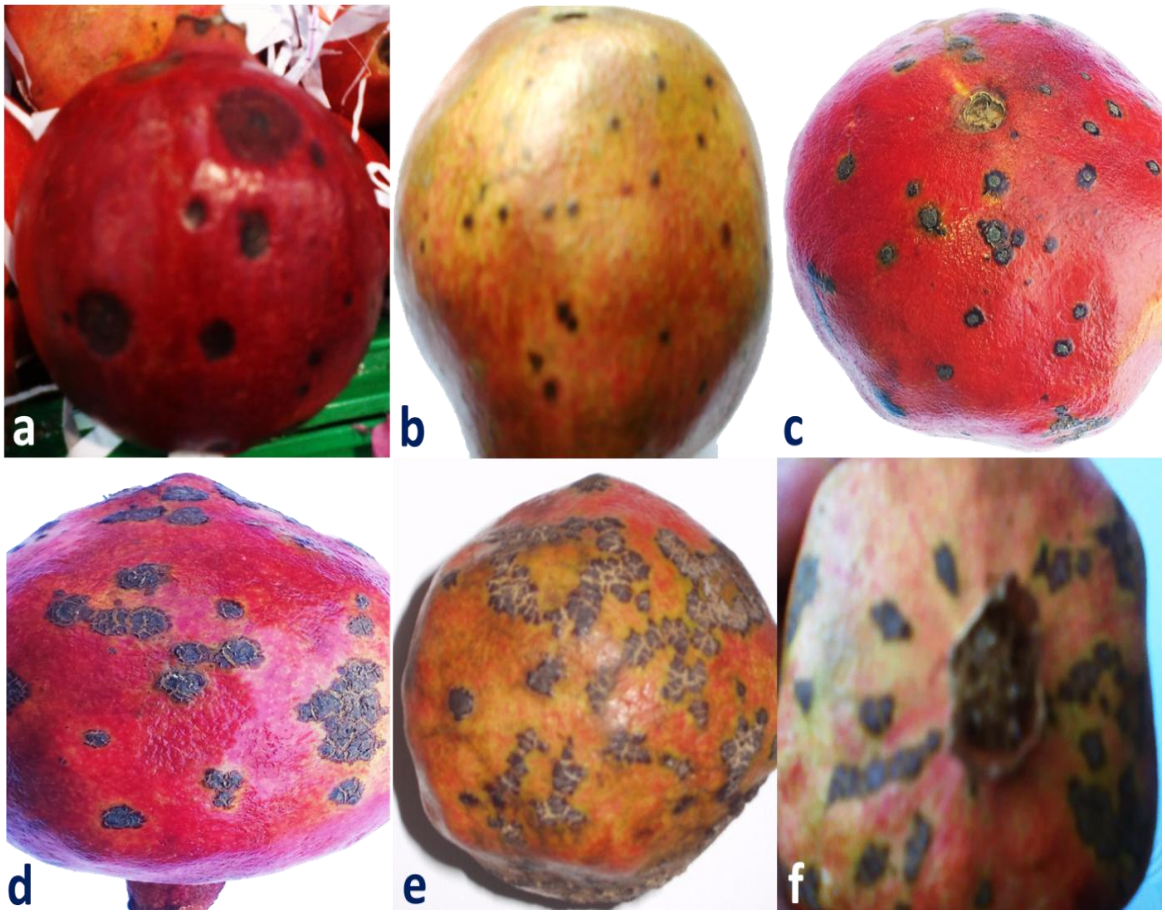

Fig.1. Disease symptoms of collected pomegranate fruits. a, fruit spots (*Aspergillus niger*); b, c, Black spots (*Alternaria alternata*) and d, e, f, fruit blotches (*Alternaria alternata, Alternaria* spp.).

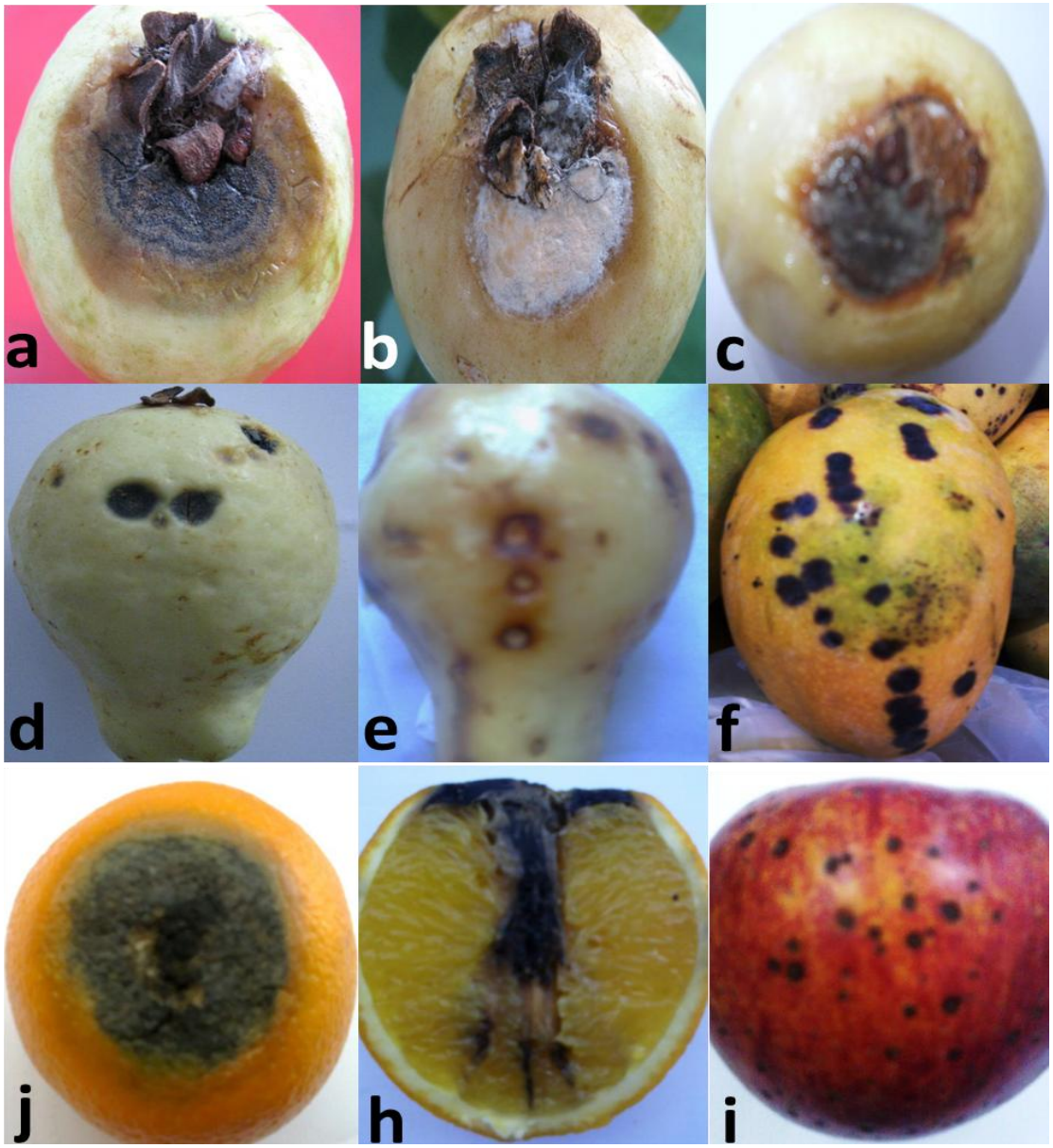

Fig. 2. Disease symptoms of different fruits collected from markets in Najran, Saudi Arabia and under field condition in Egypt. a ,b and c, Stylar end rot of guava (*Botryodiplodia theobromae* , *Phomopsis psidii*) ; d, fruit rot of guava (*Alternaria* spp.) ; e, Anthracnose of guava fruits (*Colletotrichum dematium*); f, Anthracnose of mango(*C. gleosporoides*, Pakistan); j,h, Black rot of citrus (*Alternaria citri*; south Africa) and i, Black spots of apple Royal Gala fruits (*Alternaria alternata*).

3.3. Proving pathogenicity

Different fungal species were established as the causative organisms of fruit diseases in the five crops tested fruits. Disease symptoms were observed after seven day of incubation at 25ºC on pomegranates inoculated with *Alternaria alternata, A. arbusti, A. niger* and *P. funiculosum*. No decay was observed on control fruit and fruit inoculated with *C. trichellum* and other *Alternaria* species. The first four fungi were consistently reisolated from decayed fruits. On fourth day of inoculation, *Alternaria alternata* reproduced the typical symptoms of black spot as brown to black spots on the peel surface of fruits (without any internal decay symptoms).Initial symptoms in case of *A. niger* and *P. funiculosum* revealed as necrosis on the fruit peel without sporulation. After 7days, more extensive necrosis surrounded by water soaked area was observed in case of *A. niger* and spore mass was associated with the central portion of necrosis area. Several *Penicillium* spp., *Alternaria alternata* and *A. niger* have been previously reported

as a causal pathogens of heart rot of pomegranate fruits (Tziros et al., 2007; Bardas et al., 2009; Ezra et al., 2010; Gat et al.,2012; Michailides et al., 2012 and Zhang et al., 2012). These species usually attack the fruit during bloom/fruit set and as the fruit develops, the fungus spreads to the interior of the fruit causing "black heart" or "heart rot" (Ezra et al., 2010; Gat et al., 2012 andYehia2013). According to available literatures, this is the first report of *A. alternaria* as causal pathogen of black spot of pomegranate fruits and *P. funiculosum*, as fruit rot causal agent in Egypt. Other *Alternaria* species from pomegranate fruits were non-pathogenic. *A. alternata* can be found as an epiphytic saprophyte on all parts of the plant without causing any damage. Isolation of *A. alternata* from plants does not indicate whether pathogenic isolates of this species are present (Farr et al., 1989). Based on literatures the specificity of *A. alternata* pathotypes causing black spot is unknown while, *Alternaria* sp. isolated from any part of the pomegranate plant and from other plant species can cause heart rot when it is introduced into the fruit (Ezra, et al.2010). Of the many species belonging to *Alternaria*, there are also include opportunistic plant pathogens which can affecting many cultivated plants in the fields and stored fruits and vegetables during post-harvest (Guo et al., 2004).

In inoculation of guava fruits, *C.dematium, A.alternata, A. raphani, Phoma sp.,Fusarium sporotrichioides, F. proliferatum* and *F. culmorum* caused visible symptoms as brown lesions on fruits appeared on the fourth day of inoculation (ranging from 0.45-1.40mm in diameter). With disease developed, infection progressed to the fruit flesh and fungal growth appears on the infected lesions (Sumia et al., 2006; Rao et al., 2012 and Zakaria et.al., 2012).Inoculated fruits with *B. theobromae* and *Phomopsis psidii* isolated from guava fruits exhibited stylar end rot symptoms showed disease symptoms similar to those found in field which showed on inoculated fruits 7 days after inoculation (Rai, 1956 and Quintero and Urdaneta, 1997). The rest genera didn't induce any visible symptoms. In the present study, *F. oxysporum* was found to be non-pathogenic to guava fruits however; *F. oxysporum* var. *psidi* was reported to cause post-harvest rot (Sumia et al., 2006) and causing Fusarium wilt disease of guava plant. *Fusarium* species causing fruit rot is considered opportunistic weak pathogen and often needed predisposing factors (Nishijima, 1998). Pathogenicity trails reported for the first time stylar end rot disease of guava and *C. dematium* as a causal pathogen of anthracnose in Egypt.

In general, pathogenicity test on other fruits revealed that, *C. gleosporioides* on mango, Botrytis cineria and A. alternata on apple were pathogenic and showed anthracnose, gray mold and black spots symptoms respectively (Singh and Sharma, 2007). Numerous cases have been reported in which single species of *Colletotrichum* infects multiple hosts as *C. domatium* (Freeman et al.,1998; Dillard, 1992 and Sutton, 1992). In contrast, it is common to find that several *Colletotrichum* species are associated with a single host as *C. gleosporioides* and *C. fragariae* (Howard et al., 1992 and Freeman et al.,1998).

3.4. Mycotoxins production:

Mycotoxigenic fungi belong mainly to *Fusarium, Alternaria, Aspergillus* and *Penicillium* genera,other species are known as uncommon toxigenic fungi. *Fusarium* and *Alternaria* usually represent a high mycotoxicological risk and often classified as 'field fungi' because they require very high moisture content for growth and their mycotoxinwere formed in preharvested or freshly harvested plant. The toxigenic species of *Aspergillus* and *Penicillium* represent a high horrible risk for products in storage or being used in food and feed processing and grow well at lower moisture contents (Logrieco et al., 2003 and Barakai-Golan and Paster, 2008).

3.4.1. Fusarium mycotoxins:

We examined eight Fusarium species recovered from guava and apple fruits in producing four major mycotoxins. Results of mycotoxin production by Fusarium species showed the quantification of four mycotoxins *i.e*. Deoxynivalenol(DON) (ranging from 7.8-405.3µg/L), Fumonisin (FUM)(35.9-1121.0 µg/L), T-2 toxin(0.0-55.2 µg/L) and Zearalenone (ZEA) (0.0-456.9 µg/L).i.e. DON, FUM, T-2 and ZEA.

Out of 8 Fusarium species recovered, four species produced detectable levels of four mycotoxins *in vitro i.e. F. oxysporum, F. sorotrichioides, F. proliferatum* and *F. equiseti*. All these species have been reported as producer of these mycotoxinsand all of these were in matching with (Logrieco et al., 2002; Logrieco et al., 2003 and Rai et al., 2012). In general Fusarium species synthesise different mycotoxins, some at very high concentration as FUM produce by *F. proliferatum* (up to 1121.0 µg/L) and others species *i.e. F. culmorum , chlamdosporium* and *F. langsethiae* were found unable to produce detectable amounts of T-2 toxin(Table 2).The economic importance of Fusarium species is enhanced by their ability to synthesize harmful mycotoxins and efforts to control Fusarium infections and prevent or eliminate the presence of its mycotoxins in foods have not met with a great deal of success (Desjardins et al., 2000 and Barakai-Golan and Paster, 2008).

Table (2).Profile of mycotoxins production by *Fusarium* strains from some fruit crops.

Fusarium isolates	Host	Origin	Mycotoxins concentration (µg/L)*			
			(DON)	(FUM)	(T-2)	(ZEA)
F.culmorum	Guava	Egypt	11.1±0.06^{e}	1025±14.4^{b}	0.0±0.0^{f}	253.3±.73^{b}
F.sporotrichioides	Guava	Egypt	23.5±0.31^{c}	985±2.89^{b}	73.6±0.35^{a}	121.6±0.35^{d}
F.oxysporum	Guava	Egypt	405.3±0.17^{a}	45.6±0.35^{f}	55.2±0.12^{b}	90.8±0.46^{e}
F.chlamdosporium	Guava	Egypt	17.4±0.23^{d}	645±2.89^{c}	0.0±0.0^{f}	0.0±0.0^{j}
F. proliferatum	Guava	Egypt	338.6±0.35^{b}	1121.0±0.58^{a}	17.0±0.58^{c}	456.9±0.52^{a}
Fusarium sp	Guava	Egypt	1.25±0.03^{j}	325.1±0.058^{e}	0.0±0.0^{f}	195.3±0.17^{c}
F. equiseti	Apple	France	11.4±0.23^{e}	546.8±32.9^{d}	14.5±0.29^{d}	30.6±0.35^{f}
F. langsethiae	Apple	France	7.8±0.46^{f}	35.9±0.52^{f}	11.2±0.12^{e}	0.0±0.0^{j}
F value**			390926.6	1137.01	10979.9	51808.16

-Deoxynivalenol(DON), Fumonisin (FUM), T-2 toxin, Zearalenone(ZEA) .
*Values are the mean ±S.E. of three replicates.
In the same column, means followed by the same letters are not significantly different (P ≥0.05).
**All F-values are significant at P >0.001.

3.4.2. Alternaria mycotoxins:

The results showed that all *Alternaria* species tested except of *A. zinniae* and *A. infectoria* in this study were capable to produce one and/or several kinds of four mycotoxins *i.e*. Alternariol (AOH) Tenuazonic acid (TA), altenuene (ALT) and Alternariol monomethyl ether (AME).

Only *A. alternata* from fruit rot of guava, produced *in vitro* considerable amounts of TA (3.2 µg/L), AOH (5.2 µg/L), AME (27.9 µg/L) and ALT (4.9 µg/L), while the black spot strain from pomegranate fruits produced TA (12.5 µg/L), AOH (7.5 µg/L) and ALT (1.7 µg/L) but unable to synthesize any detectable amount of AME (Table 3).In general, ALT (1.3 µg/L), TA (5.81 µg/L), AOH (4.8 µg/L) and AME (32.5 µg/L)were the only detectable mycotoxins in *A. arbusti, A. blumeae, A. A. bumstii* and *A. gaisen* respectively. *Alternaria alternata* is a ubiquitous necrotrophic fungus including at least seven pathogenic variants, each producing unique host-selective toxins which play an important role in the pathogenesis of plants and causing disease on specific host plants (Kohmoto et al., 1991; Hatta et al.,2002 and Ito et al., 2004). Five major *Alternaria* mycotoxins belong to three structural classes can be found as natural contaminants in foodstuffs: the benzopyrene derivatives alternariol (AOH), alternariol mono methyl ether (AME), altenuene (ALT), the tetramic acid tenuazonic acid (TA) and the perylene derivative alter toxin I (ATX I) (Bottalico and Logrieco, 1998 and Barkai-Golan and Paster, 2008). Several authors revealed that Alternariol and alternariol monomethyl ether have been isolated from many different cultures of the genus *Alternaria* as A. *alternata, A. arborescens, A. infectoria* and *A. tenuissima*, among others (Andersen et al., 2002). In general, only little toxicological data are available just for seven out of the 30 known *Alternaria* mycotoxins which is insufficient for an assessment of the health risk for the consumer (Bundesinstitut für Risikobewertung, BfR, 2003).

Table (3). Quantitative estimation of mycotoxins produces by *Alternaria* species *in vitro*.

Alternaria Isolates	Host	Origin	Mycotoxins concentration (µg/L)*			
			(AOH)	(TA)	(ALT)	(AME)
A. arbusti	Pomegranate	Egypt	0.0±0.0^{d}	0.0±0.0^{d}	1.3±0.30^{c}	0.0±0.0^{d}
A. blumeae	Pomegranate	Egypt	0.0±0.0^{d}	5.81±1.7^{b}	0.0±0.0^{d}	0.0±0.0^{d}
A. bumsii	Pomegranate	Egypt	4.8±0.12^{c}	0.0±0.0^{d}	1.9±0.40^{c}	0.0±0.0^{d}
A. gaisen	Pomegranate	Egypt	0.0±0.0^{d}	0.0±0.0^{d}	0.0±0.0^{d}	32.5±0.50^{a}
A. raphani	Guava	Egypt	6.9±0.23^{b}	0.0±0.0^{d}	2.6±0.31^{b}	17.8±0.80^{c}
A. alternata	Guava	Egypt	5.2±0.23^{c}	3.2±0.40^{c}	4.9±0.0^{a}	27.9±0.90^{b}
A. zinniae	Guava	Egypt	0.0±0.0^{d}	0.0±0.0^{d}	0.0±0.0^{d}	0.0±0.0^{d}
A. alternata	Pomegranate	Egypt	7.8±0.23^{a}	12.5±0.55^{a}	1.7±0.40^{c}	0.0±0.0^{d}
A. infectoria	Mango	Egypt	0.0±0.0^{d}	0.0±0.0^{d}	0.0±0.0^{d}	0.0±0.0^{d}
F value**			588.95	151.9	110.49	2922.4

- Alternariol (AOH) Tenuazonic acid (TA), altenuene (ALT) and Alternariol monomethyl ether (AME).
*Values are the mean ±S.E. of three replicates.
In the same column, means followed by the same letters are not significantly different (P ≥0.05).
**All F-values are significant at P >0.001.

3.4.3. *Aspergillus* and *Penicillium* Mycotoxins

Investigation carried out on the presence of mycotoxins in one isolate of *P. funiculosum* , the causal pathogen of heart rot of pomegranate revealed the occurrence of Patulin (PAT,25.6 µg/L) and Penicillic acid (PAC, 11.9 µg/L). On the other hand, *P. funiculosum* was found to be unable to produce any detectable amount of Citrinin and citreoviridin (Table 4). *A. niger* associated with black rot of pomegranate found to be capable of producing Oxalic acid (7.9 µg/L). Aflatoxins AFB1, AFB2, AFG1 and AFG2 were not produced by *A. niger* used in our study (Table 4).

Table (4). Profile of mycotoxin production by *A.niger* and *P. funiculosum* isolated from pomegranate fruits.

Fungal Isolates	Host	Origin	Mycotoxins concentration (µg/L)				
			(oxalic acid)	(AFB1)	(AFB2)	(AFG1)	(AFG2)
A. niger	Pomegranate	Egypt	7.9	0.0	0.0	0.0	0.0
			PAT	CIT	CITV	PAC	--
P. funiculosum	Pomegranate	Egypt	25.6	0.0	0.0	11.9	--

- Aflatoxins: AFB1, AFB2, AFG1 and AFG2

-Patulin (PAT), Citrinin (CIT), Penicillic acid (PAC), citreoviridin (CITV).

The ability of several *Penicillium* and *Aspergillus* species involved in postharvest decay of fruits to produce such mycotoxins has previously been demonstrated (Frisvad et al.,2006; Moslem et al., 2013 and Tancinová et al., 2013). Samson and Pitt (1990) have been reported *P. funiculosum* as mycotoxin producer. *Aspergillus niger* can produce a variety of mycotoxins, include oxalic acid crystals, kojic acid, and cyclic pentapeptides called malformins. *A.niger* may be does not have the genetic structure to Aflatoxins secretion, despite the ability of some species in same genus as *A. flavus, A. parasiticus* to Aflatoxins formation (Blumenthal, 2004). *A. niger* was found able to produce oxalic acid in liquid media and to secrete this acid into the medium (Blumenthal,2004).The production of oxalic acid may have a role in pathogenicity and ecology of fungi which reviewed by Dutton and Evans (1996). About the role of oxalic acid in pathogenesis, it is believed to play a role in facilitating plant cell wall degradation also, substrate mobilizing through plant cell wall i.e pectin(Tanaka and Nonaka, 1981; Ruijter et al.,1999 and Sharma et al., 2011).This fact may explain the symptoms associated with black heart rot of pomegranate and water soaked area associated with *A. niger* infection.

4. Conclusion

The investigation aimed to monitor and survey some fungal plant diseases on some fruit in the tropical region, which has recorded for the first time as well as study their pathogenicity, symptoms and their possibility of mycotoxins secretion that may be harmful to both humans and animals and adept the plant for infection.

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6. References

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