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Control of the Toxigenic Fungi Affecting Fig Fruits Quality

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Abstract

Over 25 to 30 percent loss of fruits is caused by fungal diseases. Several fungi invade and damage fig fruits and alter all the biochemical contents affecting the quality. So this study aimed to survey of mycoflora and mycotoxins affecting fig fruit quality in Egypt. Fig fruits from five different orchards in different localities in Egypt were analyzed for mycological examination and mycotoxins association. Three organic acids *i.e.* Ascorbic acid, Benzoic acid and Citric acid were evaluated as alternative fungicides against the selected toxigenic fungus. Four hundreds eighty fungal isolates were isolated from five different orchards of Fig fruit samples. Ten fungal species were identified. These are *Alternaria alternata* (Fr.) Keissler, *Aspergillus niger* Van Tiegh, *A. parasiticus, A. flavus* Link., *A. terreus* Thorn, *Botrydiplodia theobromae*, *Cladosporium* sp., *Penicillum* sp., *Rhizopus stolonifer* and *Fusarium* sp.. All tested fungi *i. e. Alternaria alternata, Aspergillus flavus* and *A. parasiticus* were found to decrease fruit quality which reduces the fruit weight (g), size (cm^3), length (cm) and diameter (cm^3), total soluble solids (TSS %), and increase the moisture percent of tested samples. Only five isolates of *A. parasiticus* gave a positive reaction for aflatoxins production. It could be concluded that, all tested organic acids substrates used i.e. Ascorbic acid, Benzoic acid and Citric acid (as an alternative fungicide) had antifungal effects against *A. parasiticus* which were able to reduce significantly the disease incidence of fig fruits compared with untreated control.

Keywords: Fig Fruits; Fungi; Fruit Quality; Mycotoxins; Organic acids.

1. Introduction

Twenty five to thirty percent of fruit loss is due to fungal diseases during transportation and storage [1]. Fresh figs (*Ficus carica* L., family Moraceae) contain high levels of sugars and other nutrients, as well as have perfect water activity for microbial growth; their low pH makes them particularly susceptible to fungal spoilage. A number of these molds can produce mycotoxins as they grow on the fruit, even during refrigeration [2]. Figs are an economically important crop in the Mediterranean region, the infection of fungi can be noticed on figs on the tree, after they wilt, after they have fallen to the ground, and within the drying process [3]. The critical periods of the formation of aflatoxin in fig fruits begin with the figs ripening on the tree, and continue through the overripe period after losing water, wilting and crumbling on the bottom, and until they are completely dried in drying trays. Some pests of insects that are active in the stage of fruit ripening may act as vectors in the transfer of aflatoxigenic fungi into the cavity of fruit. In figs, the production of mycotoxins begins on the tree and depends on some external and internal factors, including stress and physical injure. The aflatoxins formation in dried figs is mainly due to the contamination with *Aspergillus* species especially *A. flavus* and *A. parasiticus*. Toxic fungi can grow and produce aflatoxins on the outside or inside the lumen even if no damage occurs to the skin [3].

Microbial pathogens can affect fresh fruits and vegetables throughout the production chain [4]**.** To date, in many countries, the control of post-harvest diseases is led by the use of chemical fungicides. However, at this time, awareness of fungus resistance,

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environmental and health issues has led to the search for environmentally friendly and effective alternatives to disease management. Alternative regimens such as essential oils, salts, natural compounds (plant extracts), edible coatings, etc., among others for chemical use are suitable approaches to managing post-harvest disease. Organic acids have been known for years for their antifungal and antibacterial properties which are widely used in the food industries and agriculture [5,6]. Organic and inorganic salts are generally recognized as safe food additive chemicals (GRAS). It is widely used in the foodstuff industry due to its environmental impact and its low toxicity [7, 8]. Acetic, malic, tartaric, citric, benzoic, succinic, and sorbic acids are the most naturally occurring organic acids found in many fruits and vegetables. Some organic acids are found naturally in fruits and vegetables, and they basically behave as fungi stats [6]. This work aimed to survey of natural fungi associated fig fruits collected from five orchards in different localities in Egypt to determine the decay of fig fruit quality in contaminated fruits, analyze of mycotoxins production and prevention the major of toxigenic fungi by using some alternative fungicides (organic acids).

2. Material and Methods

2.1. Materials

2.1.1.Collection of samples

Fig fruits which appeared any symptoms (up-normal fruits appeared rotten, lose water, shrivel and collapse) (as shown in Figure 1) were collected during 2019 / 2020 season from five orchards in different localities, Egypt. The collected samples (Each of them 10k.g) were put into sterile polythene bags then transferred to the laboratory of plant pathology, National Research Centre (NRC) for further processing.

Figure 1 Healthy and naturally infected of fig fruits symptoms showing over-ripe they lose water, shrivel and fall onto the ground under field condition

2.2. Methods

2.2.1. Isolation and identification

Randomly subsamples were washed aseptically with sterile water, surface-sterilized using 1% sodium hypo-chloride solution for 2 min., then rinsed with sterile water for several times and dried on sterilized filter papers. Prepared fruit samples were transferred and incubated into a sterile glass discator with (90% Relative humidity) at room temperature as shown in **Figure 2**, then examined for growth and sporulation of fungi after 3-5 days. All grown molds were isolated and purified on Potato Dextrose Agar (PDA) medium with traces of choloramephincol (antibiotic) to inhibit bacterial growth then identified in Plant Pathology Dept., National Research Centre (NRC) based on cultural and morphological characteristics and the availability of literature as compared with the description given by Raper and Funel [9] for genus *Aspergillus*, and Nelson et al. [10] for *Fusarium*, and Barnett and Hunter [11] for the genera of imperfect fungi. Total fungal count and frequency percent of naturally occurred fungi in fruit samples were calculated.

Figure 2 Fig fruit samples into a sterile glass discator with (90% RH.) before incubation.

2.2.2. The Decay of fig fruit quality

Healthy apparent of fresh fig fruits were inoculated with *Alternaria, Aspergillus flavus* and *A. parasiticus* then incubated at 25°C ± 2 for 4&10 days. Some Physico-chemical characters were determined in National Research Centre (NRC) according to The Association of Official Agricultural Chemists [12]. Fresh weight (g), dry weight (g), size (cm^3), length (cm), diameter (cm^3), total soluble solids TSS ($Birx^{\circ}$) by using a hand refract meter and moisture % were determined and recorded according to AOAC [12]; Embaby et al. [13,14].

2.2.3. Test of mycotoxin production

Mycotoxin standards from sigma, Chemical Co (St. Louis, MO, U.S.A.) were purchased from El-Nasr Co., Egypt. YES medium was inoculated with *Alternaria, Aspergillus flavus, A. parasiticus* and *Fusarium* sp., and incubated at 26°C ± 2 for 14 & 20 days. Alternariol toxin, Aflatoxins (AFs) and Fumonisin (FB_1) were tested by using (HPLC) according to AOAC [12] in Food Toxicology and

Contaminants Dept., National Research Center (NRC).

2.2.4. Control of the toxigenic *Aspergillus parasiticus* fungus

Three organic acids *i. e.* Ascorbic acid, Benzoic acid and Citric acid (Sigma Chemicals Company, St Louis, Mo.) were obtained from El-Nasr Co., Egypt as shown in **Table 1** then evaluated as alternative fungicides against the selected toxigenic fungus.

Table 1 Formula of three organic acids used (as alternative fungicides)

No.	Compound	Formula	MWt(g/mol)
1	Ascorbic acid	$C_6H_8O_6$	176.12
2	Benzoic acid	C_6H_5COOH	122.12
3	Citric acid	$C_6H_8O_7$	192.12

Healthy fig fruits were obtained from the field and disinfected with 70% ethanol then, rinsed three times with sterilized water and blotted dry on sterilized filter paper. The fruits were wounded by a sterilized needle. Each treatment consisted of 12 fruits and replicated three times with 4 fruits per replicate. Efficacy of the tested chemical compounds as Ascorbic acid, Benzoic acid and Citric acid at different concentrations *i.e.* 500, 1000, 1500 and 2000 ppm were used to study the inhibitory effects on the selected *Aspergillus parasiticus* fungus was evaluated under *in vivo* conditions with the inoculated fig fruits. The fruits were dipped individually into the proposed concentrations of Ascorbic Benzoic and Citric acid (at 500, 1000, 1500 & 2000 ppm) for 5 minutes. Fruits were kept at room temperature and allowed to air dry. The fruits were artificially infected by spraying them with spore suspension ($2x10^5$ spore /ml) of *Aspergillus parasiticus* growth (10 days old) in sterilized distilled water. Thereafter, all treated fruits were placed into carton boxes, covered with plastic sheets to maintain a relative humidity RH (90-95%) at room temperature, for 10 days. In check treatment, fruits were dipped individually into sterilized water then inoculated with the pathogenic fungus without any chemicals. Three replicates were used for each treatment. Percentages of infected and decayed fruits were calculated according to Embaby [15, 16].

3. Results and discussion

3.1. Micological analysis of fig fruits

Analysis of mycoflora associated with rotted fig fruits yielded 480 fungal isolates as showing in **Table 2** and **Figure 3**. Data indicated that, fig fruit sample form location (D) orchard gave higher total fungal count comparing with other orchards, which record 222 isolates equal 46.25% followed by fig fruit sample form location (E) orchard which gave 105 isolates equal 21.87%, while fruit sample form location (A) orchard gave 60 isolates equal 12.5% and orchard (C) sample gave 48 isolates equal 10%. Fig fruit sample form location (B) orchard gave less total fungal count which record 45 isolates equal 9.38%.

Table 2 Total fungal count(s) associated with some rotted fig fruits

Fig fruits	Localities orchards					Total
	A	B	C	D	E	
Total Fungal Count	60	45	48	222	105	480
%	12.5	9.38	10	46.25	21.87	100

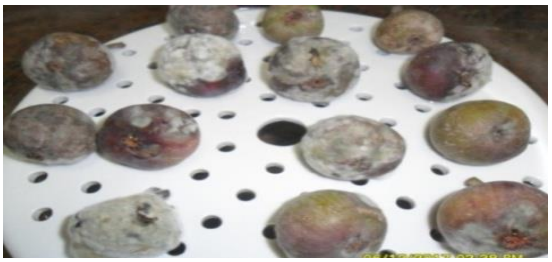

Figure 3 Fig fruit samples after incubation period (3-5 days) into a sterile glass discator

3.2. Fungal frequency

Ten fungal species were identified as showing in **Table 3**. These are *Alternaria alternata* (Fr.) Keissler, *Aspergillus niger* Van Tiegh, *A. parasiticus, A. flavus* Link., *A. terreus* Thorn, *Botrydiplodia theobromae*, *Cladosporium* sp., *Penicillum* sp., *Rhizopus stolonifer* and *Fusarium* sp., (**Figure 4**). *Aspergillus* genus (causing fig smut disease), was the most fungal frequency occurred which record 273 isolates (belong to 198 isolates equal 41.25% for *Aspergillus niger*, 57 isolates equal 11.87% for *Aspergillus parasiticus*, 15 isolates equal 3.12% for *Aspergillus terreus* and 3 isolates equal 0.62% for *Aspergillus flavus*) followed by *Rhizopus stolonifer* 69 isolates equal 14.37%, *Cladosporium* sp. 45 isolates (9.37%), *Fusarium* sp. 36 (7.5%), *Alternaria alternate* record 27 isolates equal 5.62% which causing *Alternaria* rot disease while *Penicillum* sp. record 18 isolates equal 3.75%. *Botrydiplodia theobromae* was less fungal frequency which record 12 isolates equal 2.5%. This results are in agreement with those which obtained by Bhale [17];

Bashar et al.[1]; El-Gali [18] and El-Gali [19] ,they reported that, the common postharvest and storage fungi of fruits are *Aspergillus* spp., (*Aspergillus flavus, A. fumigatus, A. niger*), *Fusarium* spp., *Alternaria alternata, Geotrichum* sp., *Penicillium* spp., *Rhizopus nigricans, R. stolonifer,* and *Sclerotinia sclerotorum. Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Fusarium oxysporum, Geotrichum* sp. *Rhizopus nigricans, R. stolonifer* and *Penicillium* spp. Saadullah and Samir [20] isolated thirty one species assigned to 14 genera from dried figs. *Aspergillus* was represented by 12 species and showed the widest diversity among all recorded genera, followed by *Penicillium* which represented by five species. Three teleomorphic ascomycetes namely, *Emericella nidulans, E. qudrilineata* and *Eurotium amstelodami* were also detected. *A. flavus, A. carbonarius , A. niger, and A. parasiticus* were the most common species. Also, Heperkan et al. [3] reported that, the major fungal flora in dried figs were *Aspergillus niger, Fusarium* spp., *Aspergillus flavus* and *Penicillium* spp.

Table 3 Fungal frequency associated with some rotted fig fruits

Fungal isolate(s)		Localities orchards					Total
		A	B	C	D	E	
Alternaria alternata	T.C	NF	9	NF	NF	18	27
	%	0.0	1.87	0.0	0.0	3.75	5.62
Aspergillus niger	T.C	18	18	30	81	51	198
	%	3.75	3.75	6.25	16.87	10.62	41.25
Aspergillus parasiticus	T.C	NF	9	NF	30	18	57
	%	0.0	1.87	0.0	6.25	3.75	11.87
Aspergillus flavus	T.C	NF	NF	NF	3	NF	3
	%	0.0	0.0	0.0	0.62	0.0	0.62
Aspergillus terreus	T.C	NF	NF	NF	15	NF	15
	%	0.0	0.0	0.0	3.12	0.0	3.12
Botrydiplodia theobromae	T.C	3	NF	NF	9	NF	12
	%	0.62	0.0	0.0	1.87	0.0	2.5
Cladosporium sp.	T.C	30	NF	NF	15	NF	45
	%	6.25	0.0	0.0	3.12	0.0	9.37
Penicillum	T.C	NF	NF	NF	18	NF	18
sp.	%	0.0	0.0	0.0	3.75	0.0	3.75
Rhizopus stolonifer	T.C	9	9	18	24	9	69
	%	1.87	1.87	3.75	5	1.87	14.37
Fusarium	T.C	NF	NF	NF	27	9	36
sp.	%	0.0	0.0	0.0	5.62	1.87	7.5
Total	T.C	60	45	48	222	105	480
	%	12.5	9.4	10.0	46.2	21.9	100

T.C= Total count %= frequency percent, NF = Not found

Figure 4 *Aspergillus parasiticus, A. niger,* and *Penicillium* association

3.3. The Decay of fig fruit quality

Data presented that, all tested fungi *i. e. Alternaria alternata, Aspergillus flavus* and *A. parasiticus* are commonly found decaying figs and decrease all tested physicochemical parameters as shown in **Table 4**. Weights of fresh fig fruits were decreased from 39.89g to 33.24, 38.20 and 31.97g after 4 days when inoculated by *Alternaria alternata, Aspergillus flavus and A. parasiticus* respectively. Continue decreasing with increasing the time period of storage from 4 to 10 days which record 30.06, 20.61 and 27.51g with the same tested fungi respectively. Decrease dry weight (g) from 7.20g with un-inoculated fig fruits (control) to 5.01, 7.11 and 5.9g after 4 days and 2.61, 1.86 and 2.81 g after 10 days from inoculation with the same tested fungi respectively. The size also decreased from 36.6 to 34.6, 35.8 and 30.6 cm^3 after 4 days and 29.3, 35.2 and 30.0 cm^3 after 10 days from inoculation with the same tested fungi respectively. The decrease in the length and diameter was recorded with inoculated fig fruits, in which the length decreased from 3.7 cm to 3.3, 3.2 and 3.2 cm after 4 days and 3.2, 3.2 and 3.2 cm after 10 days respectively, while the diameter decreased from 4.5 cm to 4.3, 4.2 and 3.9 cm after 4 days and 3.7, 4.0 and 3.7 cm after 10 days with the same tested fungi respectively. The percentage of

Total Soluble Solids (TSS) was found to decrease from 20.5% with uninoculated fig fruits (control) to 15.3, 16.8 and 17.5% after 4 days and from 20.5% to 7.5, 8.0 and 8.3% after10 days from inoculation with *Alternaria alternata, Aspergillus flavus* and *A. parasiticus* respectively. Moisture percent was found to increase from 80.20% with un-inoculated fig fruits (control) to 83.33, 81.86 and 81.74% after 4 days and from 80.20% to 92.14, 90.97 and 89.7% after10 days with the same tested fungi respectively. Tournas et al. [21] and Abbas et al. [22] mentioned that, 18.9 billion pounds of fruits and vegetables are lost every year because of the microbial spoilage according to USDA-Economic Research Service. *Alternaria, Aspergillus, Candida, Fusarium, Mucor, Rhizopus, Penicillium* fungi etc., cause spoilage of various foodstuffs. Doster and Michailides [23] reported that, the three main diseases of fig fruit decay are fig smut which caused by *Aspergillus niger*. *Alternaria* rot which caused by *Alternaria alternata* and *Ulocladium atrum* , and fig endosepsis which caused by *Fusarium* spp. Many other fungi, especially in the genus *Aspergillus*, also decompose figs. At least sixteen different *Aspergillus* spp. were found to decay figs crop. Fig smut a dangerous disease caused by *Aspergillus niger* has usually been present at the same level as the main crop. Fungal decay often develops inside the fig fruits because the ostiole allows fungal spores to reach the inner cavity. Embaby et al. [14] reported that, *Aspergillus parasiticus* reduced all physical parameters of fig fruit samples, in which it decreased significantly the fresh, dry weight, size, length and diameter compared with healthy ones. It also reduced the total soluble solids (TSS %) significantly from 26.0 to 11.0%, but increased significantly moisture percent from 81.6to 83.3 %.

Table 4 Effect of some fungal association on fig fruit quality causing decaying fig fruits.

Fungal isolates	Type of mycotoxins	Orchards localities				
		A	B	C	D	E
Alternaria alternata	Alternariol toxin	ND	ND	ND	ND	ND
Aspergillus flavus	Aflatoxins B_1, B_2, G_1, G_2	ND	ND	ND	ND	ND
A. niger		ND	ND	ND	ND	ND
A. parasiticus		ND	ND	ND	++	+++
Fusarium sp.	Fumonisin B_1	ND	ND	ND	ND	ND

%D = Decrease percent

3.4.Test of mycotoxin production

Data presented that, five isolates of *A. parasiticus* gave positive reaction which produced one or more aflatoxins. *Alternaria alternata, Aspergillus niger, A. flavus* and *Fusarium* sp., gave negative reaction as showing in **Table 5**.

Table 5 Test of mycotoxin production:

Characters	Period/ days	*Alternaria alternata*	*Aspergillus flavus*	*A. parasiticus*	Control
Fresh Weight (g)	4 days	33.24	38.20	31.97	39.89
	%D	16.67	4.24	19.86	
	10 days	30.06	20.61	27.51	
	%D	24.64	48.33	31.04	
Dry Weight (g)	4 days	5.01	7.11	5.91	7.20
	%D	30.42	1.25	17.17	
	10 days	2.61	1.86	2.81	
	%D	63.75	74.17	60.97	
Size (cm^3)	4 days	34.6	35.8	30.6	36.6
	%D	5.46	2.19	16.39	
	10 days	29.3	35.2	30.0	
	%D	19.95	3.83	18.03	
Length (cm)	4 days	3.3	3.2	3.2	3.7
	%D	10.81	13.51	13.51	
	10 days	3.2	3.2	3.2	
	%D	13.51	13.51	13.51	
Diameter (cm)	4 days	4.3	4.2	3.9	4.5
	%D	4.44	6.67	13.33	
	10 days	3.7	4.0	3.7	
	%D	17.78	11.11	17.78	
TSS %	4 days	15.3	16.8	17.5	20.5
	%D	25.37	18.05	14.63	
	10 days	7.5	8.0	8.3	
	%D	63.42	60.98	59.51	
%Moisture	4 days	83.33	81.86	81.74	80.20
	%D	3.76	16.21	1.88	
	10 days	92.14	90.97	89.7	
	%D	12.96	11.84	10.59	

ND: Not detected

3.5. Determination of aflatoxins

Data in **Table 6** and **Figure (5-10)** showed that, two isolates of *A. parasiticus* from orchard (D) samples and three isolates from orchard (E) samples were found to produce one or more aflatoxins. Orchard (E) samples gave less aflatoxins comparing with orchard (D) samples. On the other hand, determination of aflatoxins produced by *A. parasiticus* isolated from orchard (D) sample (No.8) (Fig.6) resulted in aflatoxin B_2 with 10.7 ng/g concentrations and aflatoxin G_1 with 0.20 ng/g conc**.** Also, Isolate No.10 (Fg.7) from location (D) sample was found to produce aflatoxin B_1 0.10 ng/g conc., and aflatoxin G_1 0.20 ng/g conc**.** *A. parasiticus* isolate No.19 (Fig.8) from orchard (E) sample gave aflatoxin G_1 in the concentration of 0.14 ng/g**.** Aflatoxin B_1 was found in the concentration of 0.36 ng/g., aflatoxin G_1 was 0.23 ng/g and aflatoxin

AFG_2 was 0.10 ng/g conc., with *A. parasiticus* orchard (E) sample isolate No.44 (Fig.9), while *A. parasiticus* from orchard (E) sample isolate No. 59 (Fig.10) gave 1.9 and 0.15 ng/g conc., for both aflatoxins B_1 and G_1 respectively. Shundo et al. [24] and Lewis and Schneider [25], proved that, aflatoxins B_1 and G_1 occurred most frequently and in high quantities in fruit and their products. Aflatoxin contamination has been found to affect figs, peanuts, pistachios, almonds, dates, hazelnuts, and raisins, and also recorded that, contamination of figs with aflatoxin has reached levels above 600 $\mu g/kg^{-1}$ in 83% of figs contaminated with *A. parasiticus* and 38% with *A. flavus*. Only figs contaminated by *A. parasiticus* contained both B and G toxins, while *A. flavus* contamination only yielded B toxins. Heperkan et al. [3] reported that, The major fungal flora in dried figs consisted of *Aspergillus niger* and *Fusarium* spp. and *Aspergillus flavus*, and *Penicillium* spp. Fungal infections can cause mycotoxins contamination such as aflatoxin, patulin, citrinin, fumonisin, and ochratoxin A. Olsen et al. [26] and Saadullah and Samir [20] proved that, *A. flavus* and *A. parasiticus* which grow on many agricultural commodities are the most significant aflatoxigenic fungi. Abyaneh et al.[27] and Rodrigues et al. [28] found that, *A. parasiticus* strains are usually strongly aflatoxigenic, producing both AFBs and AFGs.

Table 6 Determination of aflatoxins ng/g produced by *A. parasiticus* fungus

Orchards	Isolate No.	The concentration of Aflatoxin (ng/g)				Total Aflatoxin (ng/g)
		AFB_1	AFG_1	AFB_2	AFG_2	
D	8	NF	0.20	10.7	NF	10.27
D	10	0.10	0.20	NF	NF	0.30
E	19	NF	0.14	NF	NF	0.14
E	44	0.36	0.23	NF	0.10	0.69
E	59	1.9	0.15	NF	NF	2.05

NF = Not found

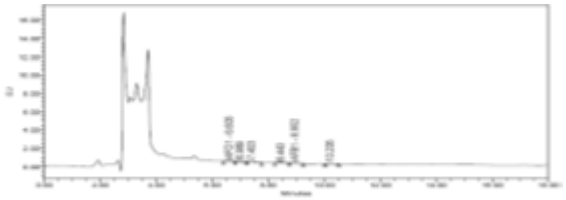

Figure 5 Standard chromatogram of aflatoxins (B_1, B_2, G_1 and G_2)

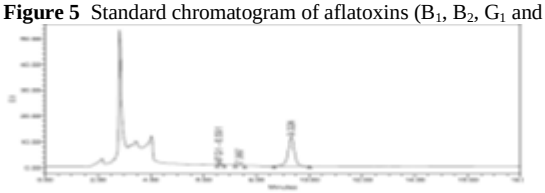

Figure 6 HPLC chromatogram of aflatoxins produced by *Aspergillus parasiticus* (No. 8)

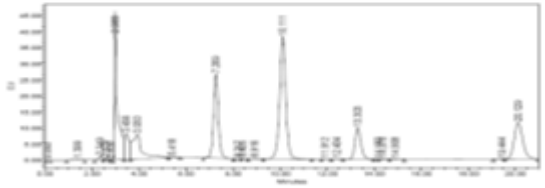

Figure 7 HPLC chromatogram of aflatoxins produced by *Aspergillus parasiticus* (No. 10)

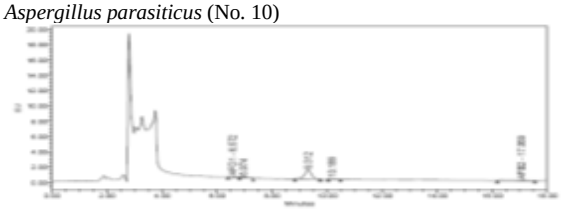

Figure 8 HPLC chromatogram of aflatoxins produced by *Aspergillus parasiticus* (No. 19)

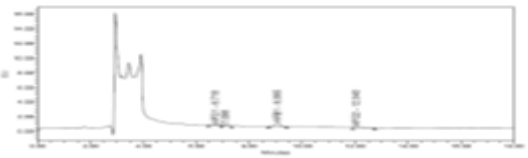

Figure 9 HPLC chromatogram of aflatoxins produced by *Aspergillus parasiticus* (No. 44)

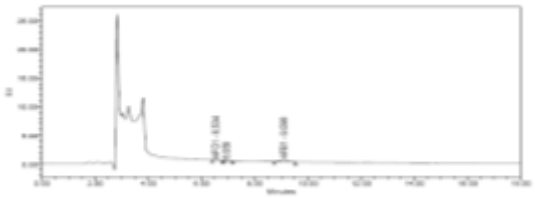

Figure 10 HPLC chromatogram of aflatoxins produced by *Aspergillus parasiticus* (No. 59)

3.6. Control of the toxigenic fungus (*Aspergillus parasiticus*) by alternative fungicides

Data in **Table 7** indicated that, all the three tested alternative fungicides used *i. e.* Ascorbic, Benzoic and Citric acids (all of them are organic acids) had an antifungal effect and significantly decrease the infection percent of fig fruits inoculated by the toxigenic *Aspergillus parasiticus* fungus. On the other hand, Ascorbic acid and Benzoic acid were the best alternative fungicides used while; Citric acid was less effective in controlling *A. parasiticus* and decreasing the infection percent of fig fruits. Continue decreasing

the infection percent by increasing the concentration used with all tested alternative fungicides used. The highest concentration (2000 ppm) was the best concentration. The Ascorbic acid was the best alternative fungicide used which made completely fruit protection at the highest concentration used (2000 ppm) followed by Benzoic acid, while Citric acid was less effective. Similar results that had been explained by Abd El-Magid et al. [29] and Ramparasad et al. [30], they reported that, antioxidants were had the potentials to delay the onset, to inhibit, and to control *Botrytis* grey mould on several vegetable crops. Citric acid exhibited the lowest suppressive effect while Benzoic acid and Ascorbic acid were the most pronounced effects**.** Also, EL-Korany and Mohamed [31] proved that, Benzoic acid, ascorbic acid, and citric acid gave *in vitro* an inhibitory effect in different degrees on colony growth (2.87% -21.17%), sporulation (17.06 - 42.93 %) and conidia germination (9.26 - 64.89 %) of *Botrytis cinerea* compared to control. El-Saidy and Abd El-Hai [32] found that the acids had effectively controlled the fungi. The inhibitory effect of the evaluated antioxidants was within the range of 25.62 to 100% (salicylic acid), 18.29 to 95.96% (citric acid). Embaby et al. [15] found that, *in vitro*, all used alternative substrates *i. e.* Ascorbic acid, Benzoic acid, Citric acid and Potassium sorbate could reduce *C. acutatum* linear growth.

Table 7 Prevention of *Aspergillus parasiticus* by alternative fungicides

Type of alternative fungicides	Conc. ppm.	Infection percent (%) Period / days 2	4	6	8	10	LSD 5%
Ascorbic acid	500	25 ± 1.73 b	50 ± 3.46 c	100 ±2.88 d	100 ±2.30 c	100 ±3.46b	18.671 A
	1000	25 ± 1.15 b	25 ±1.15 b	75 ±2.30 c	75 ±1.15 b	75 ±0.57a	
	1500	25 ± 1.15 b	25 ±2.30 b	50 ±2.88 b	50 ±4.61 a	75 ±0.00a	
	2000	0.0 ± 0.00 a	0.0 ±0.00a	25 ±1.73 a	50 ±4.04 a	75 ±1.15a	
Benzoic acid	500	25 ± 0.57 b	50 ±1.73 c	100 ±1.73 d	100 ±4.61 c	100 ±2.88b	18.671 AB
	1000	25 ± 1.15 b	25 ±1.73 b	75 ±0.57c	100 ± 0.57c	100 ±0.57b	
	1500	25 ± 2.30 b	25 ±1.15 b	75 ± 0.00c	75 ± 1.15b	75 ±2.88a	
	2000	0.0 ± 0.00 a	0.0 ±0.00 a	50 ±2.30 b	75 ±2.30b	75 ±2.30a	
Citric acid	500	25 ± 1.73 b	75 ±2.88 d	100 ±1.15 d	100 ±0.00c	100 ±1.73b	18.671 C
	1000	25 ± 0.57 b	75 ±2.30 d	100 ±2.88 d	100 ±4.61c	100 ±0.57b	
	1500	25 ± 0.00 b	50 ± 0.57 c	100 ±0.00d	100 ±1.73c	100 ±4.04b	
	2000	25 ± 0.57 b	50 ± 2.30 c	100 ±3.46 d	100 ±0.00c	100 ±0.00b	
Control		50 ± 2.88 c	75 ±1.15 d	100 ±4.04 d	100 ±5.00 c	100 ±2.88 b	18.671 C

Results are mean values of three replicates ± standard deviation. The different letters in each column indicate significant differences at P<0.05

4. Conclusions

Rotten fruits contaminant caused by various types of fungi. This may be the result of the infected fruits pre-harvest (in the field). A survey for fungal contamination in fig fruits leading to the detection of some toxigenic fungi such as *A. parasiticus*. Ascorbic, Benzoic and Citric acids were potent antifungal agents for fig treatment against *A. parasiticus* fungus. These compounds could be considered as economical and environmental friendly materials.

Data Analysis

Data obtained in this study were analyzed using software (IBM SPSS Statistics v.16. USA). Statistical significance was performed using one way Analysis of Variance (ANOVA) test. A value of $p<0.05$ was considered statistically significant. Least significant difference (LSD) was calculated at $P \leq 0.05$ according to Gomez and Gomez [33].

Conflicts of interest

"There are no conflicts to declare".

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