Research Journal of Agriculture and Biological Sciences, 3(6): 631-637, 2007

Occurrence of Aflatoxins in Some Rotted Apricot Fruit in Egypt

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Abstract: Three fungal genera were isolated from rotted apricot fruits. These are *Alternaria, Aspergillus* and *Rhizopus. Aspergillus* spp. were the most frequently fungi than the others. *Aspergillus niger* was higherly occurred than *A. parasiticus. Rhizopus stolonifer* (*nigricans*) was occurred in moderate numbers. But, *Alternaria* spp. were presented in lower numbers. Two isolates of *Aspergillus parasiticus* were found to be produced one or more of aflatoxins i.e. B_1, B_2, G_1 and G_2. Neither *Alternaria spp.* nor *Rhizpus spp.* produced any of the aflatoxins. Analysis of rotted apricot fruits indicated that *Aspergillus parasiticus* reduced significantly fresh weight, fruit quality and all chemical contents. Higher loss percent were recorded with total soluble solids (TSS%), followed by total titrable acidity (TTA) and ascorbic acid (Vitamin C) but TSS/TTA ratio was not significant and showed lowest loss.

Keywords: Apricot (*Pruns armeniaca* L.), fruit rots, *Aspergillus,* mycotoxin, aflatoxins.

INTRODUCTION

Apricot (*Prunus armeniaca* L.), family Rosaceae (stone fruits) is one of the most important fruit crop in Egypt. Fruit rots are the important factor (s), which limits the storage life and resulted in appreciable losses at whole sale, retail and consumer levels. Several fungi attack apricot fruit causing rots and reduced their quality and cause yield losses. *Rhizopus stoloniefer* and *R. arrhizus* were isolated from fresh apricot fruits. *Alternaria alternata* cause red spot on apricot fruits[15]. On apricot fruits, *Penicillium expansum* was more prevalent than *R. stolonifer* and *A. niger* [11]. *Rhizopus* was identified as the main pathogen causing apricot and peach rots in orchards[14]. *Rhizopus nigricans* was the softening agent of apricot[18]. The most frequent pathogens of apricot fruit rots are *Rhizopus stolonifer, Botrytis cinerea, Monilinia Laxa, M. fructigena* and *Penicillium expansum* while less common pathogens were *Alternaria alternata, Aspergillus niger* and *Cladosporium herbarum*[17]. *Alternaria tenuis (A. atternata)* was identified as the causal agent of apricot black spot. It attacks mainly leaves and fruits[21].

Aspergillus parasiticus, produces B_1, B_2, G_1, G_2 aflatoxins, aspergillic acid and kojic acid[13]. Three isolate of *A. flavus* produced aflatoxin B_1 and B_2. One *A. parasiticus* isolates produced B_1, B_2, G_1 and G_2[12]. Four strains of *A. parasiticus* were also producing aflatoxins. Detection of agar plates of *A. flavus* and *A. parasiticus* showed aflatoxins after 21 days. It confirmed by TLC[1]. High levels of aflatoxin (> 100 ng/g) were detected in 83% of the figs infected by *A. parasiticus* and in 32% of the figs infected by *A. flavus* [6]. *Aspergillus parasiticus* groups showed significant differences in total aflatoxin (aflatoxins B_1 + B_2 + G_1 + G_2), ratio of G_1 + G_2/ B_1 + B_2 and Kojic acid[10]. The ability of some isolated fungi to produce mycotoxins in some legume seeds was studied and aflatoxins were detected with some isolate of *Aspergillus*[7].

Apricot fruits, uninoculated and inoculated with *Rhizopus arrhizus* spores, were stored for different periods. The ascorbic acid content was declined as the days of inoculation increased, but the rate of decline was higher in diseased tissue[8]. Change in the protein, reducing and non-reducing sugars were observed in seeds of cowpea infested with either *Aspergillus nidulants* or *A. tereus*[16]**.** Chemical contents of cowpea seeds were changed by *A. flavus, A. niger, Fusarium oxysporum*. *Tracheiphilum* and *Macrophomina phaseolina.* The reduction in protein content due to *A. flavus, A. niger* and *F. oxysporum* infection was also significant[20]. *Aspergillus flavus* utilizes carbohydrate of seeds for its growth and aflatoxin production,[3]. Chemical analysis of artificially infected legume seeds with a mycotoxin producer isolate (*Aspergillus flavus*) decreased the percentage of protein, carbohydrates, fat, fibers and ash content compared with non infected ones[7].

The Present Study Includes:

- Isolation and identification of fungi found on rotted apricot fruits.
- Determination of mycotoxins.

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- Study the changes in fruit quality i.e. a- total soluble solids (TSS%), b- total titratable acidity (TTA%), c- TSS/TTA ratio % and d- Ascorbic acid mg/ml of juice.

MATERIALS AND METHODS

Fruit Samples: Samples of apricot fruits (*Prunus armeniaca*), showing disease symptoms i.e. decay and rot were collected after harvest from commercial orchards in different localities (Delta-Egypt) during summer (June) of 2005-2006.

Isolation, Purification and Identification of the Causal Agents: Collected apricot fruits were surface sterilized with 70% ethanol, then incubated in glass moist champers with 85-95% RH under room temp. Fruits were examined daily. All fungal growth was transferred and purified using hyphal tip method and/or single spore techniques on potato dextrose agar (PDA) medium[9] in the presence of antibiotic (traces of streptomycin). Developing fungi were transferred to PDA slant and after growth period were kept at 5°C for further studies. Pure fungal cultures (7 days old) were identified in Plant Pathology Dept., National Research Centre (NRC) Dokki, Egypt according to Barnett and Hunter[4], Domsch *et al.*[5] and Kulwant Singh *et al.*[13].

Mycotoxins Determination: Mycotoxins were determined in food toxicology and contaminants Dept., National Research Centre (NRC) according to[2] *Aspergillus, Alternaria and Rhizopus* isolates were grown in flasks (500 ml), each containing 4 of healthy appearance disinfected apricot fruits without stone (free stone) with enough moisture, then incubated at 26 ± 2°C for two weeks. After incubated period, the treated fruits were prepared for toxin examination and extracted for determination of the mycotoxins using B_1, B_2, G_1 and G_2 standard of aflatoxin as comparison on thin layer chromatography (TLC) plates and examined under ultraviolet detector (UV) wavelength 365 nm[7]

Chromatogram of tri fluro acetic acid (TFA) derivatives of the analysis aflatoxins in apricot fruits comparing with standard aflatoxins were recorded according to Sharman *et al.*,[19]. Mobile phase, methanol 0.1 M sodium dihyrogen phosphate, (77.23) adjusted to pH 3.35 by the addition of phosphoric acid, flow rate, 1 ml/min, column $ODSC_{18}$ 30 cm x 2.5 mm, fluorescence detector 335 nm excitation and 440 nm emission.

Effect of *Aspergillus parasiticus* on the Fruit Quality: The effect of *Aspergillus parasiticus* on chemical content of the apricot fruits were studied under laboratory condition. Freshly-harvested mature apricot fruits, were obtained from farm market at Kalubia Governorate with healthy appearance and surface sterilized with 70% ethanol. Then, fruits were washed with sterilized water and dried with filter paper. Four apparent healthy fruits were used as replicate and three replicates for each particular treatment. Fruit surface was then wounded with syringe. Nearly five microlitres of the suspension of conidia (1×10^5 CFU/ml) with sterile distilled water were injected into each wound. Control treatment was injected with sterilized water only. All treatments were incubated in glass moist chamber with 90-100% RH at 25 ± 2°C for 2 weeks. Loss assessment of apricot fruits was estimated after incubation period in comparison with un-inoculated ones. Percentage of loss was calculated as follows:

Loss % = (Wu) weight of un-inoculated – (Wi) weight of inoculated

$$\text{Loss \%} = \frac{Wi}{Wu} \times 100$$

$$\text{Decreasing \% (D \%)} = \frac{Wu - Wi}{Wu} \times 100$$

Fruit Quality: Some chemical characters of apricot fruits which infected with *Aspergillus parasiticus* were determined compared with free from disease in Pomology Dept., National Research Centre (NRC) according to Association of official Agricultural Chemists.

Three hundred grams of apricot fruits were homogenized with a blender, filtered through Whattman Filter paper No. 1, then centrifuged at 3000 rpm for 15 min and obtained clear juice was used to measure chemical quality of the apricot fruits as follow:

Total Soluble Solids (TSS): One hundred ml of juice filtrate(s) from each healthy and diseased fruits were used to determine the total soluble solids (TSS) by using a hand refractometer. The percentage of TSS were recorded.

Acidity: Clear filtrate of inoculated and un-inoculated apricot fruits were used to determine the total titratable acidity (TTA) using phenolphthaline as an indicator, after titration with NaOH (0.1 N). The percentage of acidity was calculated as mg citric acid per 100 g fresh weight of apricot fruit according to the following equation:

$$\text{Acidity \%} = \frac{\text{ml of NaOH used} \times \text{N of NaOH (0.1)} \times 0.064}{\text{Sample volume of apricot (ml)}}$$

TSS/Acid Ratio: The total soluble solids (TSS) / total titratable acidity (TTA) ratio was calculated directly by dividing TSS value on TTA value for each treatment.

Ascorbic Acid: One hundred ml C/100 ml juice of apricot fruit. Finally, chemical content losses and reduction percent were calculated as follow:

$$L\% = H - I \qquad R\% = \frac{H - I}{H} \times 100$$

L% = Loss; H=Healthy fruit; I=Infected fruit and R % =Reduction

RESULTS AND DISCUSSIONS

Isolation: Fruits showing rot symptoms (Figs. 1 & 2) were used in isolation. The frequency of some fungi obtained with some apricot fruit rots are tabulated in Table (1).

Data indicate that, 78 isolates were obtained in this study. Sixty six isolates are belonging to three genera and responsible on apricot fruit rots in Egypt. *Aspergillus* spp. were more frequently from the rotted fruits (51.28 %) followed by *Rhizopus* spp. and *Alternaria* spp. which gave 23.08% and 10.26 %, respectively. On the other hand, higher occurrence was found and recorded with *A. niger* compared with *A. parasiticus* which gave 41.03 % and 10.26 % respectively (Fig. 1). *Alternaria* was the lowest frequently fungi isolated from rotted fruits than the others. Moderate frequency was recorded with *Rhizopus* which gave 23.08 %.

Mycotoxin Determination: Aflatoxins were tested by using thin layer chromatography (TLC). Data in Table (2) show that, aflatoxins were detected with *Aspergillus parasiticus* only. Also, data show that, only 2 isolates of *A. parasiticus* were positive producer of aflatoxins (Figs. 3 & 4).

Isolates No. 25 and No. 31 were the most producing isolate of 40 *A. parasiticus*. Aflatoxin G_1 was not detected with one isolate No. 25 *A. parasiticus*. Another isolate No. 31 was less producer of aflatoxin B_1 and B_2 compared with the other which record 20.0 and 0.20 ug/kg and 11.75 and 0.39 ug/kg respectively. While this isolate No. 31 gave higher aflatoxins G_1 and G_2 with 50.0 and 8.30 ug/kg compared with *A. parasiticus* isolate No. 25 under the same condition.

Effect of *Aspergillus Parasiticus* on the Apricot Fruit:

Losses in Fruits: The effect of *A. parasiticus* on losses fruits were recorded in Table (3). Data show that, *A. parasiticus* reduced fresh weight of apricot fruit(s) compared with healthy (un-inoculated) with two isolates (No. 25 and No. 31) causing 119.6 g and 135.8 g losses respectively with 10.88 and 12.22 loss percent. Isolate 25 gave higher reduction (decreasing) than other isolates (31) which recorded 89.12 and 87.78.

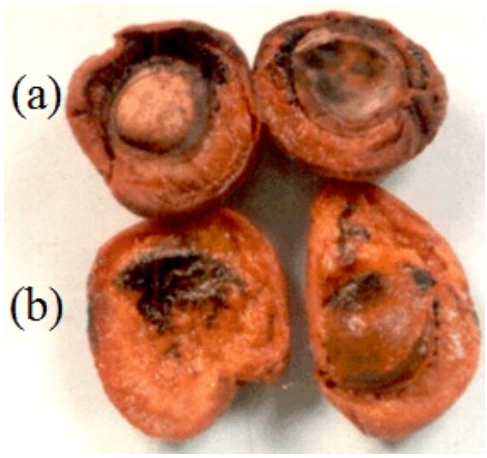

Fig. 1: Apricot fruit rot (Internal syptoms).
a - Black colour with Alternaria.
b - Greenish colour with Aspergillus parasiticus.

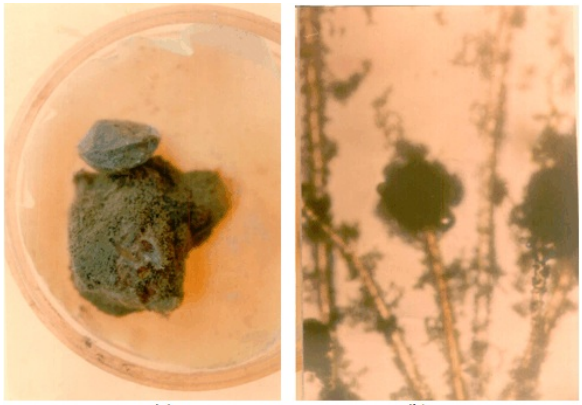

Fig. 2: Aspergillus parasiticus.
a - Natural contaminated *apricot* fruit and stone (external symptom).
b - Under light microscope (400 X), Grenish conidia and conidiophora.

Fruit Quality: The effect of *A. parasiticus* on some chemical content of rotted apricot fruits were studied and recorded in Table (4). Fruit quality i.e., total soluble solids (TSS), total titratable acidity (TTA), total soluble solids (TSS) / total titratable acidity (TTA) ratio and ascorbic acid (Vitamin C) were determined and recorded after two weeks from inoculation as quality characters. Chemical analysis of apricot fruits in both inoculated and un-inoculated (rotted fruits)

Table 1: Frequency of some fungi associated with some apricot fruit rots.

	Aspergillus niger	Aspergillus *parasiticus*	*Alternaria alternata*	*Rhizopus stolonifer*	Un Known	Total
T.C	32	8	8	18	12	78
%	41.02	10.26	10.26	23.08	15.38	100
Total	40		8	18	12	78
%	51.28		10.26	23.08	15.38	100

T.C. = Total count

Table 2: Mycotoxin determination.

Type of aflatoxin	*Aspergillus parasiticus*		*A. niger*	*Alternaria alternata*	*Rhizopus stolonifer*
	No. 25	No. 31			
B_1	11.75	20.00	ND	ND	ND
B_2	0.39	0.20	ND	ND	ND
G_1	ND	50.00	ND	ND	ND
G_2	2.35	8.30	ND	ND	ND

ND = Not detected; Aflatoxins were determined by ug/kg.

Table 3: Effect of *Aspergillus parasiticus* on apricot fruit losses after 2 weeks.

A. parasiticus	Wu	Wi	L (gms)	L%	D%
Isolate No. 25	134.2	14.6	119.6	10.88	89.12
Isolate No. 31	154.7	18.9	135.8	12.22	87.78

Wu = Weight of un-inoculated; Wi = Weight of inoculated (*A. parasiticus*).
L (gms) = Loss (gms); L% = Loss percent
D% = Decreasing percent.

Table 4: Effect of *Aspergillus parasiticus* on chemical content of apricot fruit.

Characters	H	I	L	R%
TSS%	av. 13.7	2.0	11.7	85.4
	13.5, 13.6, 14.0	1.9, 2.0, 2.1		
TTA%	av. 0.53	0.08	0.5	84.9
	0.4, 0.51, 0.67	0.07, 0.09, 0.09		
TSS/TAA ratio %	av. 27.1	23.4	3.7	13.7
	33.8, 26.7, 20.9	27.1, 22.2, 21.0		
V.C	av. 17.1	6.2	10.9	63.7
	15.2, 16.0, 20.0	3.8, 7.0, 7.7		
L.S.D. 5%	TSS%	TTA%	TSS/TTA	V.C
	0.266	0.133	6.80	3.11
	(S)	(S)	(N.S)	(S)

H = Healthy of apricot fruits.
I = Infected.
L = Loss.
R% = Reduction.
TSS% = Total soluble solids.
TTA% = Total titratable acidity (Acidity).
TTS/TTA ratio = Total soluble solids / Total titratable acid ratio.
V.C = Vitamin C / 100 ml.
S = Significant.
N.S. = Not significant.

showed that, *Aspergillus parasiticus* reduced all chemical contents in all inoculated fruits compared with un-inoculated ones. Higher reduction and loss percent were recorded with total soluble solids (TSS %) followed by total titratable acidity (TTA) and ascorbic acid as vitamin C, while TSS/TTA ratio gave the lowest reduction and loss percent.

- Total soluble solids (TSS %) percent was reduced significantly from 13.7% in un-inoculated apricot fruits to 2.0% in inoculated fruits with 85.4% reduction.

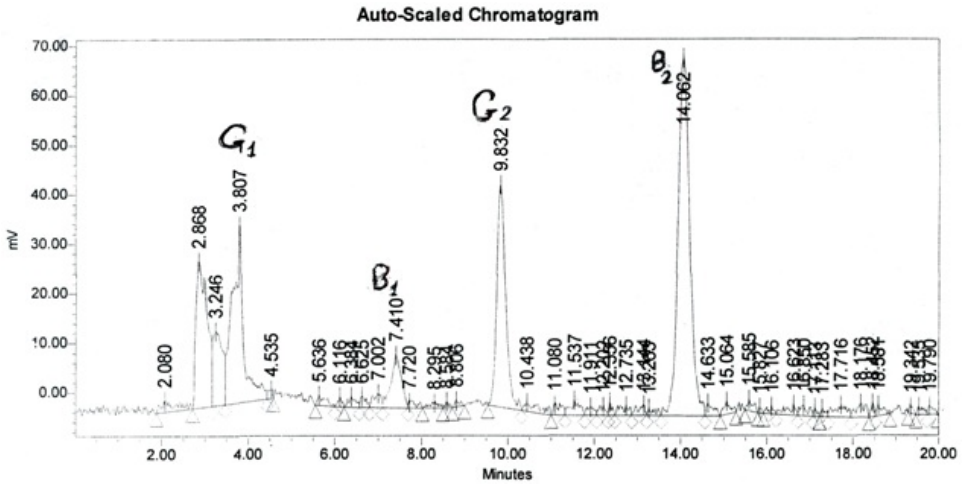

Fig. 3: shows a chromatogram of derivatives aflatoxins standard G1, B1, G2 & B2.

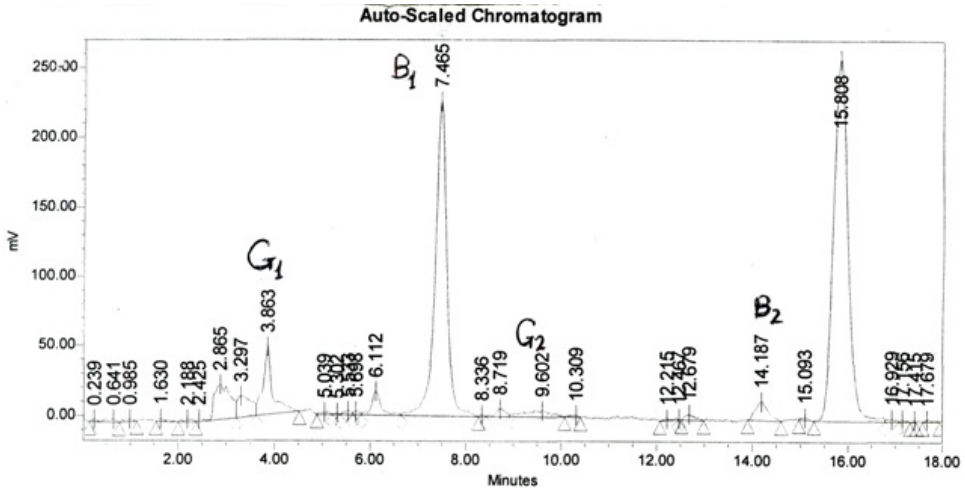

Fig. 4: is a chromatogram of natural contaminated sample of apricot fruits

- *Aspergillus parasiticus* decreased significantly the av. of total titratable acidity percent (TTA) from 0.53% to 0.08% with 84.9% reduction percent.
- The total soluble solids (TSS%) / total titratable acidity (TTA) ratio determination was reduced from 27.1 in healthy fruits to 23.4% in rotted fruits with 13.7 reduction. No significant difference in fruits between the TSS/TTA ratio percent.
- Also, data show that, the av. of ascorbic acid as mg of vitamin C was decreased significantly from 17.1 in un-inoculated fruits to 6.2 in inoculated fruits and gave 63.7 reduction percent.
- Percentage of chemical losses were calculated at the time (after 2 weeks) and recorded in Table (4). Data indicated that, the most losses percent were recorded with total soluble solids (TSS) (11.7) followed by ascorbic acid which gave 10.9 Also; *A. parasiticus* caused 0.5% loss of total titratable acidity.

Discussion: Apricot fruit is one of the most important fruit Crops for local consumption. Apricot fruit usually have short post-harvest life. Decay and/or fruit rot are the important factors which limits the storage life of fruits and resulted in appreciable losses at whole sale, retail and consumer levels. Isolation of fungi from rotted fruits yielded 78 fungal isolates belong to three genera of fungi. These genera are *Alternaria, Aspergillus* and *Rhizopus. Aspergillus* spp. were the most frequently than others. Also, *A. niger* was higher frequently when compared with *A. parasiticus* with 41.03% and 10.26 respectively. *Alternaria* was the lowest frequently isolation than others, while Rhizopus gave moderate frequency. Similar results were recorded by Lafuste[14] Ros *et al.*[18], Pratella[17] and Zu Jianlan *et al.*[10]. Two isolates of *Aspergillus parasiticus* were found to be aflatoxins producer (B_1, B_2, G_1 and G_2) in rotted apricot fruits. Similar results were obtained by Kulwant Singh *et al.*[19], Kheiralla[12],

Abramson and Clear[1], Horn *et al.*[10] and Embaby and Mona[7]. Neither *Alternaria* nor *Rhizopus* was aflatoxins producer.

Analysis of chemical content of rotted apricot fruits indicated that, *Aspergillus parasiticus* reduced fresh and fruit quality as well as decreased all chemical contents (total soluble solids (TSS), total titratable acidity (TTA) and TSS / TTA ratio in addition to ascorbic acid (Vitamin C)). Similar results were recorded by Ushamalini *et al.*[20], Aziz and Mahrous[3] and Embaby and Mona[7]. Total soluble solids (TSS %) were reduced significantly reduction from 13.7% to 2.0% in un-inoculated and inoculated fruits respectively, with 85.4% reduction. *Aspergillus parasiticus* decreased significantly the total titratable acidity (TTA) from 0.53% to 0.08% with 84.9% reduction, also reduced TSS/TTA ratio from 27.1% to 23.4% with 13.75 reductions. The av. of ascorbic acid was decreased significantly from 17.1 mg to 6.2 mg with 63.7% reduction. On the other hand, the most loss percent were recorded with total soluble solids (TSS) 11.7% followed by ascorbic acid (Vitamin C) 10.9% and total titratable acid (0.5% acidity). Finally total soluble solids (TSS) / titratable acidity (TTA) ratio were not significant and gave lowest losses which recorded 3.7%. Similar results were obtained by Gunasekaran and Weber[8], Aziz and Mahrous[3] and Embaby and Mona[7].

ACKNOWLEDGEMENT

The author would like to thank Dr. Diaa El-Din Hassanein Prof. of Plant Pathology, National Research Centre for his help and reviewing the work.

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