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Studies on Mycological Status of Sundried Jew's-Mallow Leaves and Okra Fruits in Egypt

M.S. Youssef
Department of Botany, Faculty of Science, Sohag University, Egypt

Abstract: Thirty samples of each of sundried jew's mallow leaves and okra fruits collected from six Governorates in Egypt were analyzed for their mould contamination and potential presence of mycotoxins. Mycological investigation revealed that twenty-six species and two varieties belonging to 13 genera of fungi were identified on Czapek's-dextrose and potato-dextrose agar media at 28±2°C using dilution-plating method. Okra fruit samples were highly contaminated with fungal spores (total counts were 47523 and 30563 colonies g^{-1} sample) than jew's mallow leaves samples (16608 and 6045 colonies), while the relative diversity and broad number of fungal genera and species was recorded on jew's mallow leaves (10 genera, 20 species + one variety and 6 genera, 10 species) than okra fruit samples (8, 16 + 2 and 3, 9 + 1) on the two used media, respectively. *Aspergillus* was the highest occurrence (100% of the samples) and represented by 13 species + one variety of which, *A. flavus*, *A. niger*, *A. fumigatus*, *A. awamori*, *A. foetidus* and *A. ficuum* were the predominant. *Mucor, Rhizopus, Fusarium, Myrothecium*, *Emericella* and *Cochliobolus* were fungal genera isolated with different occurrences in high or/and moderate from the two plants samples tested on the two used media. Mycotoxin analysis proved that jew's mallow leave samples were free from any detectable mycotoxins, while five samples of dried okra fruits out of 30 tested (16.7%) were proved to be toxic. It is the first record of mycotoxins contamination of okra fruits in Egypt. The ability of 347 isolates of recovered fungi was screened for production of mycotoxins and extracellular cellulase enzymes.

Key words: Fungi, Mycotoxins, Jew's mallow leaves, okra fruits, cellulase enzymes

INTRODUCTION

The genus *Corchorus* comprises certain herbs and shrubs. It contains about 100 species, which are distributed in the tropics and subtropics, chiefly South East Asia and South America (Ahmad *et al*., 1998). One of these species is *Corchorus olitorius* L. plant (Fam. Tiliaceae), which is cultivated to provide bark for the production of fibres (Jute) and mucilaginous leaves for using in food as a vegetable. *C. olitorius* is known by many names jute in Bengal, jew's-mallow leaves, alias molokhia in Japan and molochia in Egypt (Gupta and Mukerji, 1982; Tanda, 1998; Hasan, 2002).

The seeds are used as a purgative and the leaves as a tonic and diuretic (Oliver-Bever, 1986). The leaf powder and its water-soluble viscous solution were found to decrease total serum and liver cholesterol concentrations and increase the fecal excretion of bile acids, total neutral sterols and cholesterol (Inammi *et al*., 1995). Two digitoxigenin glycosides (coroloside and glucoeva tromonoside), as well as, four strophanthidine glycosides (erysimoside, olitoriside, corchorosid A and helveticoside) were identified as main cardiac glycosides in the methanol extract of leaves (Goda *et al*., 1998). Three cardenolides were isolated from *C. olitorius* seed-leaves and their cytotoxic activities were evaluated against six cancer cell lines (Abdel-Wahab *et al*., 1999). Four flavonoid glycosides; astragaline,

tolifolin, isoquercetin and juglanin, as well as oleanolic acid glyceryl monopalmitate, β-sitosterol and β-sitosterol-3-glycoside, were isolated from the leaves of *C. olitorius* (Kohoda *et al*., 1994).

Six phenolic antioxidative compounds were identified in the leaves and their antioxidant activities were measured and caeffeoylquinic acid was proved to be a predominant phenolic antioxidant in *C. olitorius* leaves (Azuma *et al*., 1999). Phytoalexins are antimicrobial compounds synthesized by a plant in response to infection or stress. Five coumarins (scopoletin, fraxinol, isopimpinellin, xanthotoxol and peucedanol) were found to be produced as phytoalexins (stress metabolites) from the fresh young leaves of *C. olitorius* L. plant, in response to inoculation with biotic stress agent such as the spore suspension of the fungus *Helminthosporium turcicum* and with chemical stress agents, such as aqueous solution of mercuric chloride and cupric chloride (Abou-Zeid, 2002).

Okra, *Abelmoschus esculentus* L. Moench (Syn. *Hibiscus esculentus* L.) is of the Malvaceae or mallow family. It is known by many names: Lady fingers, okro, ochro, okoro, quimgombo, bhindi, bindi, bamia, bamiya, bamieh (Rattray, 2001). Okra edible part is the fruit pod, which varies in color from yellow to green. Okra can be served raw, marinated in salads or cooked on its own and goes well with tomatoes, onions, peppers and eggplant. Whole, fresh okra pods also make excellent pickles. Its subtle flavor can be compared to eggplant, though the texture is somewhat unusual. Many people prefer breaded and fried okra, because the slippery substance is less pronounced (Bodin, 1995).

Okra, fruit of a large vegetable plant thought to be of African origin, was brought to the United States three centuries ago by African slaves. The word, derived from the West African nkruma, was in use by the late 1700s. Okra grows wild in the upper watershed of the Nile and domestication of the plant began in the Nile basin in Egypt, where Egyptians have cultivated it for centuries. It spread through North Africa and onto the Mediterranean and India and first appeared in new world in Brazil and Dutch Guiana. Today, the major US centers for okra production are in the southeast: Texas, Georgia, Florida, California, Tennessee and Alabama (Raid and Palmateer, 2006)

In Egypt, okra fruits and jew's mallow leaves are important fresh vegetable crops in spring and summer for cooking, also used as dry vegetables for all year round cooking (EL-Shaikh, 2005). Large poverty population sundried okra fruits and jew's mallow leaves in dusty humidity weather and stored in unhealthy conditions, therefore, many microbes especially fungi can grow and secrete their toxic substances with human health, hazardous risk.

Extensive studies have been carried out on mycoflora and mycotoxins of several agricultural commodities such as seeds, grains, medicinal plants, dried raisins (EL-Kady and Youssef, 1993; Youssef, 1995, 1999; Youssef *et al*., 2000, 2002). On the other hand no published studies exist on mycoflora and mycotoxins contamination of dried okra fruits or jew's mallow leaves, therefore the purpose of this research is to study fungal and mycotoxins contamination of these dried vegetables, which used in cooking as cheapest and popular foodstuffs in Egypt.

MATERIALS AND METHODS

Collection of Samples

Thirty samples of each of dried okra fruits and dried jew's mallow leaves about (250-300 g, each) were collected at March 2006 after 20-22 storage weeks from different retail markets of vegetable crop seeds and plants of six Governorates in Egypt namely; EL-Faiyum, EL-Minia, Assiut, Sohag, Qena and Aswan. Each sample was placed in a sterile polyethylene bag, transferred to the mycological laboratory and kept in a cool place (3-5°C) until fungal determination and mycotoxin analysis.

Determination of Moisture Content

Twenty gram of each sample were ground in an electric mill and dried in an oven for 24 h and 105°C, then cooled in a desiccator and re-weighted to a constant weight. The moisture content was

calculated as percentage of the dry weight according to the technique of the International Seed Testing Association (1966).

Determination of Fungi

The dilution-plate method as described by Johnson and Curl (1972) was used for isolation of fungi. Modified Czapek's-dextrose agar medium (g L^{-1}: sodium nitrate 3.0, magnesium sulphate 0.5, potassium chloride 0.5, di-potassium hydrogen phosphate 1.0, iron sulphate 0.01, glucose 10.0, agar agar 15.0-20.0, pH 7.3±0.1) and potato-dextrose agar medium(g L^{-1}: 200 g potatoes infusion, 20 g dextrose, agar agar 20.0 g) were used as isolating media. Chloramphenicol (0.5 mg mL^{-1}) and rosebengal (30 ppm) were added to the medium as bacteriostatic agents (AL-Doory, 1980; Martin, 1950). Twelve plates, six for each medium were used for each sample. The plates were incubated at 28±2°C for 7-15 days and the developing fungi were identified, counted and calculated per g dry weight of tested sample. The colonies of slow growing fungi as well as mycelial bits were transferred to slants to ensure precise counting then to plate for identification.

The following references were used for identification of fungi (based on purely morphologically macro-and microscopic characteristics): Raper and Thom (1949), Gilman (1975), Booth (1971, 1977), Raper and Fennell (1977), Ellis (1971, 1976), Pitt (1979, 1991), Domsch *et al*. (1980), Ramirez (1982), Sivanesan (1984), Kozakiewicz (1989), Klich and Pitt (1992), Moubasher (1993) and Samson *et al*. (1995).

Sample Preparation for Mycotoxins Analysis

Extraction Procedures

Fifty gram of each sample were defatted by extraction with cyclohexane (150 mL) for 10 h using Soxhlet-type extractor. The defatted residue was extracted with ethyl acetate (three times, 50 mL/each). The extracts were combined, dried over anhydrous sodium sulphate, filtered and then concentrated under vacuum to near dryness, transferred into brown glass vial and evaporated under nitrogen stream. For cleaning up the crude extracts; the crude extract was suspended in 1 mL chloroform and applied to 14×0.8 cm column containing 2.5 g Kiesel gel 60, 70/230 silica gel. The washing and eluting solvents (8 mL, each) were carried out according to AOAC (1984).

Mycotoxins Production by Fungal Isolates

A number of 347 fungal isolates (represented 26 species + 2 varieties appertaining to 13 genera) were firstly grown on potato-dextrose agar slants at 28°C for 7-10 days. For each isolate, an inoculum (10^6~10^7 spores mL^{-1}) was cultivated on Czapek's-dextrose medium protified by 2 g yeast extract and 10 g peptone per liter and incubated at 28°C for 12 days. After the incubation period had finished, the culture in each flask (medium + mycelium) homogenized for 5 min in a high-speed blender (1,600 rpm) with chloroform (three times, 75 mL, each). The combined extracts were dried over anhydrous sodium sulphate, filtered, then concentrated under vacuum and transferred into a brown vial with a small amount of chloroform.

Bioassay of Toxins

Brine shrimps (*Artemia salina* L.) larvae were used for mycotoxins bioassay test according to Korpinen (1974).

Thin Layer Chromatography (TLC)

For qualitative detection of mycotoxins, thin layer chromatography technique was employed using precoated silica gel plates type 60 F_{254} TLC (E, Merck, Germany). Aflatoxins B_1, B_2, G_1 and G_2, ochratoxins A and B, sterigmatocystin, citrinin, T_2- toxin, diacetoxyscirpenol, patulin, fumigillin, terrin,

gliotoxin, rubratoxin B, roquefortin and zearalenone were applied as standard references. The developing solvent system was ethyl acetate-hexane (v/v, 30: 70) and the developed plates were viewed under short wave length UV (252 nm) light according to AOAC (1984) and Dorner (1998).

High Performance Liquid Chromatography (HPLC)

HPLC analysis was done using Spherisorb 5sil column (250×4.6 mm). Mobile phase was chloroform-methanol (v/v, 97:3) with flow rate 1.2 mL $min^{-1}$for 20 min. The quantitative determination of mycotoxins was carried out compared with standard mycotoxins (Sigma).

Screening for Extracellular Cellulase Enzymes Production by Isolated Fungi

Agar overlay assay technique was used as described by Schneider and Kubelka (1989) and Zani *et al.* (1991) to determine the fungal cellulolytic activity. Different sterilized Czapek's-cellulose agar media containing 2% of carboxy-methyl cellulose or avecil or cellulose powder instead of 1% glucose were used. Four cylinder reservoirs (7×7 mm) were made using a narrow cork-borer. Similar volume 100 µL of tested fungus filtrates (pre-cultivated in the three Czapek's-cellulose liquid media for 12 days transferred into each reservoir. One hundred microliter sterilized distilled water were transferred into the last reservoir as control. The dishes were incubated for 48 h at 30°C. After incubation, the congo-red solution was transferred into each reservoir for 15 min, the clear zone diameters around the reservoirs were determined using Vernier caliper as indication of cellulase enzyme activity of each fungus on different cellulolytic agar media.

RESULTS AND DISCUSSION

The moisture content of sundried jew's mallow leaves and okra fruit samples was considerably low and ranged between 3.6-8.1 and 5.2-6.8, respectively.

Mycological analysis of samples based on dilution-plating method using Czapek's-dextrose and potato-dextrose agar media at 28°C revealed that twenty-six species and two varieties belonging to 13 genera were identified. The gross total viable count, as well as number of fungal genera and species collected from sundried jew's mallow leaves samples were (16608 colonies g^{-1} dry weight sample, 10 genera and 20 species + one variety) and (6045 colonies, 6 genera and 10 species). While from okra fruit samples were (47523 colonies, 8 genera and 16 species + 2 varieties) and (30563 colonies, 3 genera, 9 species + 1 variety) on the two media, respectively (Table 1, 2).

The total count of filamentous fungi recovered from the samples tested widely fluctuated between 312-592 and 1120-1680 colonies g^{-1} dry weight sample on Czapek's-dextrose agar medium and from 186-280 and 740-1185 on potato-dextrose agar medium from sundried jew's mallow leaves and okra fruit samples, respectively. The lowest spectrum of fungal species may be related to the highly contamination of samples with *Aspergillus* species, which have a powerful competitive ability against other fungal species. This result agrees with the data recorded by Srivastava and Dayal (1982), Gupta and Mukerji (1982), Adebanjo and Shopeju (1993) and Youssef *et al.* (2000, 2002, 2003).

Aspergillus is the highest occurrence on the two media from all samples tested (100%) of jew's mallow leaves and okra fruits. It occupied the first order (13113 colonies g^{-1} dry weight, 78.95% of total count and represented by 11 species + one variety) and (4710, 77.91% and 5) from jew's mallow leaves, while (44811, 94.29% and 9 + 1) and (28312, 92.63% and 6 + 1) from okra fruits on Czapek's-dextrose and potato-dextrose agar media, respectively. *A. awamori*, *A. flavus*, *A. niger*, *A. fumigatus*, *A. foetidus*, *A. versicolor* and *A. ficuum* were the most common species (Table 1, 2). These results are in agreement with results obtained by Dayal and Srivastava (1973), Srivastava and Dayal (1982), Gupta and Mukerji (1982), Adebanjo and Shopeju (1993), Youssef *et al.* (2000, 2002) and Siddiqui *et al.* (2004).

Table 1: Number of species (NS), number of cases of isolation (NCI, out of 30 samples), total counts of 30 samples of each of sundried jew's mallow leaves and okra fruits (TC, calculated per g dry weight sample) and percentage of total counts (TC%) of fungal genera and species recovered on Czapek's-dextrose and potato-dextrose agar media at 28±2°C using dilution-plating method

	Sample name															
	Sundried jew's-mallow leaves								Sundried okra fruits							
	Used medium															
	Czapek's-dextrose agar				Potato-dextrose agar				Czapek's-dextrose agar				Potato-dextrose agar			
Genera and species	NS	NCI	TC	TC%	NS	NCI	TC	TC%	NS	NCI	TC	TC%	NS	NCI	TC	TC%
Aspergillus (13 + 1)	11 + 1	30	13113	78.95	5	30	4710	77.91	9 + 1	30	44811	94.29	6 + 1	30	28312	92.63
Mucor (1)	1	24	390	2.35	1	8	90	1.49	1	22	282	0.59	-	-	-	-
Myrothecium (1)	1	22	360	2.17	-	-	-	-	-	-	-	-	-	-	-	-
Emericella (1 + 1)	1	15	555	3.34	-	-	-	-	1 + 1	12	555	1.17	-	-	-	-
Fusarium (2)	1	15	165	0.99	-	-	-	-	1	16	468	0.98	2	23	376	1.24
Cochliobolus (1)	1	15	210	1.26	1	3	30	0.50	-	-	-	-	-	-	-	-
Penicillium (2)	-	-	-	-	1	7	15	0.25	1	10	282	0.59	-	-	-	-
Papluaspora (1)	1	7	1515	9.12	-	-	-	-	-	-	-	-	-	-	-	-
Drechslera (1)	1	6	240	1.45	1	6	45	0.74	-	-	-	-	-	-	-	-
Cladosporium (1)	1	2	45	0.27	-	-	-	-	1	6	562	1.18	-	-	-	-
Eurotium (1)	1	2	15	0.09	-	-	-	-	-	-	-	-	-	-	-	-
Rhizopus	-	-	-	-	1	30	1155	19.11	1	5	375	0.79	1	30	1875	6.13
Apoicera	-	-	-	-	-	-	-	-	1	3	188	0.40	-	-	-	-
Total count		16608				6045				47523				30563		
Number of genera (13)		10				6				8				3		
Number of species and varieties (27+ 2)		20+1				10				16+2				9+1		

-: No fungal genus isolated

On Czapek's-dextrose agar, *Mucor* ranked the second order in number of cases of isolation 24 and 22 cases in spite of its count is low 390 and 282 colonies g^{-1} from jew's mallow leaves and okra fruits, respectively. *Myrothecium* occupied the third order in jew's mallow, while *Fusarium* come in the third place from okra fruits. On the other hand on potato-dextrose agar *Rhizopus* occupied the second order in both tested plants, while *Mucor* ranked third place in case of jew's mallow leaves and *Fusarium* in case of okra fruits (Table 1, 2).

The remaining fungal genera and species were less frequent on the two isolation media. Most of the recorded fungal species in this study had identified before from rhizosphere of okra fruits in India (Dayal and Srivastava, 1973; Srivastava and Dayal, 1982), from leaf surface of four varieties of jew's mallow leaves in Bengal (Gupta and Mukerji, 1982), from some sundried vegetables including okra fruits and jew's mallow leaves in Nigeria (Adebanjo and Shopeju, 1993) and from different seeds, grains, medicinal plants and other sources in Egypt (El-Kady and Youssef, 1993; Youssef, 1995, 1999; El-Maghraby *et al*., 1995; Youssef *et al*., 2000, 2002, 2003).

In case of jew's mallow leaves, 11 species and one variety belonging to 6 genera were isolated only on Czapek's-dextrose agar medium and completely missed on potato-dextrose agar medium and these were: *A. foetidus*, *A. versicolor*, *A. parasiticus*, *A. flavo-furcatis*, *A. ochraceus*, *A terreus*, *A. flavus* var. *columnaris*, *Myrothecium verrucaria*, *Emericella nidulans*, *Fusarium equiseti*, *Papluaspora immersa*, *Cladosporium cladosporioide*s and *Eurotium tonophilus*. While, *Penicillium* was represented by *P. purpurogenum* and isolated only on potato-dextrose agar medium and completely missed on Czapek's-dextrose agar medium (Table 2).

On the other hand in case of okra fruits (9 species + one variety and 6 genera) were recovered on Czapek's-dextrose and completely disappeared on the other medium and these were: *A. flavo-furcatis*, *A. foetidus*, *A. carbonarius*, *A. terreus*, *Emericella nidulans*, *E. variecolor* var. *astellata*, *Penicillium oxalicum*, *Cladosporium cladosporioides*, *Mucor racemosus* and *Apiocera chrysosperma*. While, *A. parasiticus* and *Fusarium oxysporum* were the only two species recovered on potato-dextrose agar medium only and completely missed on other medium (Table 2).

The results revealed that Czapek's-dextrose agar medium is better than potato-dextrose agar medium in cultivation and enrichment of fungi. Also, the gross total count of fungi from okra fruits is

Table 2: Total counts (TC, calculated per g dry sample), number of cases of isolation (NCI, out of 30 samples) and occurrence remarks (OR) of fungal genera and species isolated from sundried Jew's mallow leaves and okra fruits on Czapek's-dextrose and potato-dextrose agar media at 28±2°C using dilution-plating method

	Sample name															
	Sundried jew's-mallow leaves								Sundried okra fruits							
	Used medium															
	Czapek's-dextrose agar				Potato-dextrose agar				Czapek's-dextrose agar				Potato-dextrose agar			
Genera and species	TC	TC %	NCI	OR	TC	TC %	NCI	OR	TC	TC%	NCI	OR	TC	TC%	NCI	OR
Aspergillus (total count)	13113	78.95	30	H	4710	77.91	30	H	44811	94.29	30	H	28312	92.63	30	H
A. flavus Link	2585	15.56	28	H	495	8.19	20	H	12844	27.03	30	H	1032	3.38	24	H
A niger Van Tieghem	1925	11.59	27	H	2715	44.91	30	H	10218	21.50	26	H	3656	11.96	30	H
A. fumigatus Fresenius	485	2.92	22	H	105	1.74	12	M	5812	12.23	24	H	2438	7.98	20	H
A. awamori Nakazawa	7080	42.63	30	H	780	12.90	18	H	1688	3.55	22	H	15938	52.12	21	H
A. foetidus (Naka.) Thom and Raper	510	3.07	14	M	-	-	-	-	1125	2.37	15	M	-	-	-	-
A. versicolor (Vuillemin) Tiraboschi	90	0.54	12	M	-	-	-	-	-	-	-	-	-	-	-	-
A. ficuum (Reich.) Hennings	300	1.81	7	L	615	10.17	10	M	5062	10.65	12	M	2250	7.36	15	M
A. flavus var. *columnaris* Raper and Fennell	75	0.45	6	L	-	-	-	-	6844	14.40	6	L	2062	6.74	7	L
A. parasiticus Speare	18	0.11	5	L	-	-	-	-	-	-	-	-	936	3.06	14	M
A. flavo-furcatis Batista and Maia	15	0.09	4	L	-	-	-	-	844	1.78	3	R	-	-	-	-
A. ochraceus Wilhem	15	0.09	3	R	-	-	-	-	-	-	-	-	-	-	-	-
A. terreus Thom	15	0.09	2	R	-	-	-	-	188	0.40	2	R	-	-	-	-
A. carbonarius (Bainier) Thom	-	-	-	-	-	-	-	-	186	0.39	2	R	-	-	-	-
Mucor racemosus Fresenius	390	2.35	24	H	90	1.49	8	M	282	0.59	22	H	-	-	-	-
Myrothecium verrucaria Albertini and Schweinitz) Ditmer ex Steudl	360	2.17	22	H	-	-	-	-	-	-	-	-	-	-	-	-
Emericella (total count)	555	3.34	15	M	-	-	-	-	555	1.16	12	M	-	-	-	-
E. nidulans (Eidam) Vuillemin	555	3.34	15	M	-	-	-	-	375	0.79	8	M	-	-	-	-
E. variecolor var. *astellata* (Fennell and Raper) Benjamin	-	-	-	-	-	-	-	-	180	0.37	4	L	-	-	-	-
Fusarium (total count)	165	0.99	15	M	-	-	-	-	468	0.98	16	H	376	1.24	23	H
F. equiseti (Corda) Saccardo	165	0.99	15	M	-	-	-	-	468	0.98	16	H	188	0.62	13	M
F. oxysporum Scheldon	-	-	-	-	-	-	-	-	-	-	-	-	188	0.62	10	M
Cochliobolus lunatus Nelson and Haasis	210	1.26	15	M	30	0.50	3	R	-	-	-	-	-	-	-	-
Penicillium (total count)	-	-	-	-	15	0.25	7	L	282	0.59	10	M	-	-	-	-
P. oxalicum Currie and Thom	-	-	-	-	-	-	-	-	282	0.59	10	M	-	-	-	-
P. purpurogenum Stoll	-	-	-	-	15	0.25	7	L	-	-	-	-	-	-	-	-
Papluaspora immersa Hotson	1515	9.12	7	L	-	-	-	-	-	-	-	-	-	-	-	-
Drechslera halodes (Drechsler) Subram and Jain	240	1.45	6	L	45	0.74	6	L	-	-	-	-	-	-	-	-
Cladosporium cladosporioides (Fresen.) de Vries	45	0.27	2	R	-	-	-	-	562	1.18	6	L	-	-	-	-
Eurotium tonophilus Ohtsuki	15	0.09	2	R	-	-	-	-	-	-	-	-	-	-	-	-
Rhizopus stolonifer (Ehrenberq) Lind	-	-	-	-	1155	19.11	30	H	375	0.79	5	L	1875	6.13	30	H
Apoicera chrysosperma (Bulliard) Fries	-	-	-	-	-	-	-	-	188	0.40	3	R	-	-	-	-
Total count		16608				6045				47523				30563		
Number of genera (13)		10				6				8				3		
Number of species (27+ 2)		20+1				10				16+2				9+1		

OR: Occurrence remarks, H: High occurrence, more than 15 cases out of 30 tested, M: Moderate occurrence, between 8-15 cases, L: Low occurrence, between 4-7 cases, R: Rare occurrence, less than 4 cases, -: No fungal genus isolated

very highly (47523 and 30563 colonies g^{-1} dry weight) in comparison of jew's mallow leaves (16608 and 6045) on the two media used, respectively. This result is agreed with Bailey and Mansfield (1982) and Abou-Zeid (2002) that fresh young leaves of jew's mallow produced phytoalexins in response to

microbial infection, which are antimicrobial compounds played an important role in inhibition growth and secondary metabolites production of field and storage fungi.

The toxicity test using brine shrimp larvae (*Artemia salina* L.) revealed that the ethyl acetate extracts of five samples (16.7%) out of 30 dried okra fruits proved to be toxic. Based on TLC and HPLC analysis, aflatoxin B_1 was detected in two samples with concentration 8.8 and 10.2 μg kg^{-1} of dried sample. While, aflatoxins $B_1$and B_2 were detected in one sample (9.8 and 8.2 μg kg^{-1}) whereas aflatoxins B_1, B_2, G_1 and G_2 were recorded in two samples (8.9-12.2 μg kg^{-1}). These toxic samples were heavily contaminated with many members of *Aspergillus flavus* group (*A. flavus*, *A. parasiticus*, *A. flavo-furcatis* and *A. flavus* var. *columnaris*). On the other hand one sample was contaminated with zearalenone (9.6 μg kg^{-1}) and another sample was contaminated with diacetoxyscirpenol (DAS, 10.2 μg kg^{-1}) and these samples were contaminated with *Fusarium* species (*F. oxysporum* and *F. equiseti*) highly toxins-producers (Table 3, Fig, 1).

It is worth to mention that in case of jew's mallow leaves, no mycotoxin could be detected in any sample using TLC and HPLC, in spite of the toxicity test using brine shrimp revealed that all samples are very toxic (100% death). This result may be due to the highly phytoalexins content of jew's mallow leaves, which played an important role in prevent mycotoxins production and lead to death of larvae. The obtained results are in harmony to those recorded by Bailey and Mansfield (1982) and Abou-Zeid (2002).

The present study shows the correlation of aflatoxin B_1 concentration as effective toxic agent and mortality of brine shrimp larvae (Fig. 2). In addition, it is clear that identified mycotoxins have been produced by infecting toxigenic fungi during long drying period of okra fruits in dusty humidity warm weather contaminated with aspergilli spores. These results are in harmony to those recorded by

Table 3: Sample number (SN), sample source, biological assay (Brine shrimp), natural occurring of mycotoxins identified and common mycotoxin-producing fungi isolated from the toxic okra fruit samples*

SN	Sample source	Dead larvae (%)	Mycotoxins identified (μg kg^{-1})	Mycotoxin producing-fungi
3	El-Minia	82	Aflatoxin B_1, (10.2 μg kg^{-1}) Zearalenone (9.6 μg kg^{-1})	*A. flavus, A. parasiticus* *F. oxysporum, F. equiseti*
6	El-Fayium	74	Aflatoxin B_1 (8.8 μg kg^{-1}) DAS (10.2 μg kg^{-1})	*A. flavus, A. parasiticus* *F. oxysporum. F. equiseti*
9	El-Fayium	85	Aflatoxins B_1, B_2, G_1 and G_2 (8.9-10.8 μg kg^{-1})	*A. flavus, A. parasiticus, A. flavo- furcatis, A. flavus* var. *columnaris*
17	Sohag	78	Aflatoxins B_1 and B_2 (9.8 and 8.2 μg kg^{-1})	*A. flavus, A. parasiticus, A. flavus* var. *columnaris*
22	Qena	89	Aflatoxins B_1, B_2, G_1 and G_2 (10.4-12.2 μg kg^{-1})	*A. flavus, A. parasiticus*

* 5 toxic okra fruit samples (16.7%) out of 30 tested

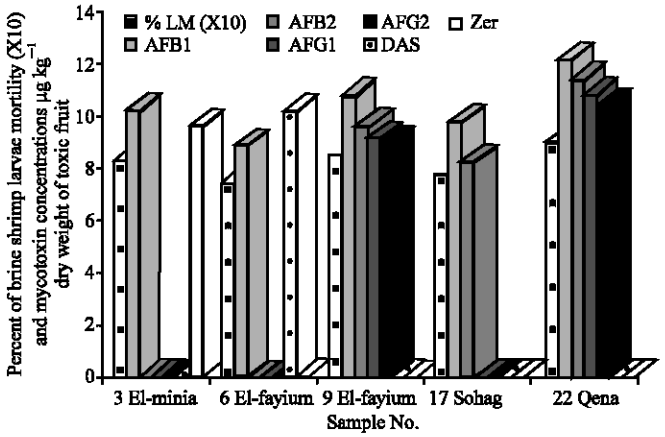

Fig. 1: Natural occurrence of mycotoxins in sundried toxic okra fruit samples

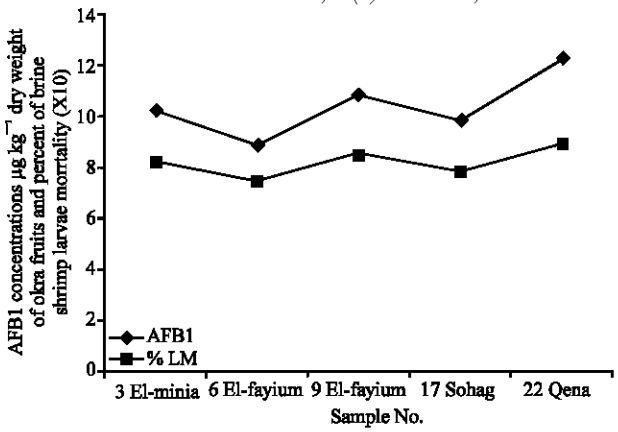

Fig. 2: Correlation between AFB1 as a common effective mycotoxin agent in toxic okra fruit samples and percentage mortality of Brine shrimp larvae (direct proportion)

Table 4: Production of secondary metabolites by fungi isolated from sundried Jew's mallow leaves and okra fruit samples in Egypt

Genera and species	TIT	Number of isolates able to produce cellulase enzymes	Count	Mycotoxins producing isolates (µg 50 mL^{-1} medium) Mycotoxins identified
Total isolates	347	285.0	128.0	
(%)	100	82.1	36.9	
Aspergillus (total count)	243	206.0	115.0	
A. flavus Link	40	40.0	16.0	Aflatoxin B_1 (220-380)
			18.0	Aflatoxins B_1,B_2,G_1,G_2 (180-320)
A. niger Van Tieghem	30	30.0	14.0	Kojic acid (300-380)
A. fumigatus Fresenius	24	24.0	16.0	Fumagillin (210-350)
A. awamori Nakazawa	20	16.0	8.0	Kojic acid (180-300)
A. foetidus (Naka.) Thom and Raper	18	12.0	-	-ve
A. versicolor (Vuillemin) Tiraboschi	18	10.0	5.0	Sterigmatocystin (160-240)
A. ficuum (Reich.) Hennings	16	11.0	-	-ve
A. flavus var. *columnaris* Raper and Fennell	15	15.0	-	-ve
A. parasiticus Speare	24	20.0	6.0	Aflatoxin B_1 (240-380)
			4.0	Aflattoxins B_1, B_2 (180-300)
			10.0	Aflatoxins B_1,B_2,G_1,G_2 (240-400)
A. flavo-furcatis Batista and Maia	10	8.0	-	-ve
A. ochraceus Wilhem	8	5.0	6.0	Ochratoxin A (150-220)
A. terreus Thom	8	3.0	5.0	Citrinin and terrein (120-180)
A. carbonarius (Bainier) Thom	12	12.0	2.0	Ochratoxin A (80 and 120)
			5.0	Kojic acid (100-240)
Mucor racemosus Fresenius	12	12.0	-	-ve
Myrothecium verrucaria (Albertini and Schweinitz) Ditmer ex Steudel	8	6.0	1.0	Verrucarin A (120)
Emericella (total count)	12	9.0		
E. nidulans (Eidam) Vuillemin	8	7.0	-	-ve
E. variecolor var. *astellata* (Fennell and Raper) Benjamin	4	2.0	-	-ve
Fusarium (total count)	16	16.0	7.0	
F. equiseti (Corda) Saccardo	8	8.0	2.0	T_{-2} (140 and180)
F. oxysporum Scheldon	8	8.0	2.0	DAS (150-210)
			3.0	Zearalenone (120-180)
Cochliobolus lunatus Nelson and Haasis	5	5.0	-	-ve
Penicillium (total Counts)	13	10.0	-	
P. oxalicum Currie and Thom	7	7.0	4.0	Oxalic acid (160-220)
P. purpurogenum Stoll	6	3.0	-	-ve
Papluaspora immersa Hotson	5	1.0	-	-ve
Drechslera halodes (Drechsler) Subram and Jain	6	3.0	-	-ve
Cladosporium cladosporioides (Fresen.) de Vries	6	3.0	-	-ve
Eurotium tonophilus Ohtsuki	5	1.0	1	Sterigmatocystin
Rhizopus stolonifer (Ehrenberg) Lind	12	12.0	-	-ve
Apoicera chrysosperma (Bulliard) Fries	4	1.0	-	-ve

TIT: Total isolates tested, -: No isolates of fungal species able to produce detectable mycotoxins

Adebanjo and Shopeju (1993), Youssef (1999) and Youssef *et al.* (2000, 2003). On the other hand, ochratoxin A and B, $T\text{-}_2$ toxin, patulin, terrin, gliotoxin, rubratoxin B, sterigmatocystin, citrinin, fumigillin and roquefortin could not be detected in any sample tested. It is the first record of mycotoxins in okra fruits in Egypt.

Testing the ability of fungal isolates (represented 26 species + 2 varieties appertaining to 13 genera) to produce mycotoxins revealed that 128 isolates (36.9%) proved to be mycotoxin-producers (Table 4). The ability of toxin production as well as the amount of released toxins differs not only among the fungal species but also among the different isolates of the same species. The obtained results are in harmony with those obtained by Youssef *et al*. (2000, 2003).

Since enzymes are important chemical weapons aiding the fungal pathogen to invade host tissues, it was essential to shed some light on the ability of isolated fungi to produce cellulase enzymes. These enzymes were detected in 28.1% of tested fungal cultures (Table 4). Hussein (2004) recorded similar results by testing the ability of fungi isolated from agricultural wastes (wheat straw, sugar-cane bagasse and water hyacinth) to produce cellulase enzymes. In Egypt, several investigations had been done for isolation of cellulolytic fungal species from different seeds and grains (Moubasher *et al*., 1972; Moubasher and Mazen, 1990; Samia-Soliman, 1999).

In conclusion, it is evident that sundried vegetables in unhealthy conditions and bad storage system in tropical and subtropical areas is considered a vehicle for numerous fungal pathogens contamination. As a result of mycological proliferation and the risk of mycotoxins as carcinogenic and hepatotoxic agents should be taken into consideration especially for babies, pregnant women and people with low immunity. So, for human public health, sundried vegetables in retail markets must be subjected to quality control and can be dried under antiseptic conditions and must be bagged in safety and healthy form.

REFERENCES

Abdel-Wahab, S.M., W.T. Islam and N.D. EL-Tanbouly, 1999. Cardenolides from *Corchorus olitorius* L. seed-leaves. Bull. Fac. Pharm. Cairo Univ., 37: 149-153.

Abou-Zeid, A.H.S., 2002. Stress metabolites from *Corchorus olitorius* L. leaves in response to certain stress agents. Food Chem. J., 76: 187-195.

Adebanjo, A. and E. Shopeju, 1993. Sources and mycoflora associated with some sundried vegetables in storage. Int. Biodet. Biodeg. J., 31 (4): 255-263.

Ahmad, V.U., A. Ali, Z. Ali, F.T. Baqai and F.N. Zafar, 1998. Cycloartane triterpene glycoside from *Corchorus depressus*. Phytochemistry, 49: 829-834.

AL-Doory, Y., 1980. Laboratory Medical Mycology. Lea and Febiger, Philadelphia Kimpton Publishers, London, pp: 410.

AOAC., 1984. Association of Official Analytical Chemists. Official Methods of Analysis. 13th Edn. Washington DC, USA., pp: 429.

Azuma, K., M. Nakayama, M. Koshioka, K. Ippoushi, Y. Yamaguchi, K. Kohata, Y. Yamauchi, H. Ito and H. Higashio, 1999. Phenolic antioxidants from the leaves of *Corchorus olitorius* L. J. Agric. Food Chem., 47: 3963-3966.

Bailey, J.A. and J.W. Mansfield, 1982. Phytoalexins. Glasgow, Blackie.

Bodin, M.P., 1995. One Legendary Southern Dish is Fried Okra, Acajun Family's Recipe Book. Photo-Courtesy of Oregon State University.

Booth, C., 1971. The genus *Fusarium*. Com. Mycological Inst., Kew, Surrey, England, pp: 237.

Booth, C., 1977. *Fusarium*, laboratory guide to the identification of the major species. Com. Mycological Inst., Kew, Surrey, England, pp: 58.

Dayal, R. and L.S. Srivastava, 1973. Studies on the rhizosphere mycoflora of *Abelmoschus esculentus*. I. Influence of varieties and age of the plant. Syd., Ann. Mycol. Ser., 11: 96-111.

Domsch, K.W., W. Gams and T.H. Anderson, 1980. Compendium of Soil Fungi. Academic Press. London, pp: 859.

Dorner, J.W., 1998. Chromatographic Analysis of Mycotoxins. In: Chromatographic of Environmental and Food Toxicants, Shibamato T. (Ed.). Marcel Dekker, Inc. New York, Hong Kong, pp:113-168.

EL-Kady, I.A. and M.S. Youssef, 1993. Survey of mycoflora and mycotoxins in Egyptian soybean seeds. J. Basic Microbiol., 33 (6): 371-378.

Ellis, M.B., 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Inst., Kew, Surrey, UK., pp: 608.

Ellis, M.B., 1976. More Dematiaceous Hyphomycetes. Commonwealth Mycological Inst., Kew, Surrey, UK., pp: 507.

EL-Maghraby, O.M.O., I.A. EL-Kady and A. Samia-Soliman, 1995. Microflora and *Fusarium* toxins of three types of corn grains in Egypt with special reference to production of trichothecene toxins. Microbiol. Res., 150: 225-232.

EL-Shaikh, K.A.A., 2005. Effect of phosphorus and potassium fertilization on green yield, seed production and quality of two okra cultivars in reclaimed soils. Assiut J. Agric. Sci., 36 (1): 51-68.

Gilman, J.C., 1975. A Manual of Soil Fungi. 2nd Indian Reprint, Published by Oxford and IBH Publishing Co., 66 Janpath, New Delhi, India, pp: 450.

Goda, Y., S. Sakai, T. Nakamura, H. Akiyama and M. Toyodo, 1998. Identification and analysis of main cardiac glycosides in *Corchorus olitorius* seeds and their acute oral toxicity to mice. Shok. Eise. Zas., 39: 256-265.

Gupta, R. and K.G. Mukerji, 1982. Microbial communities on the leaf surface of four varieties of *Corchorus olitorius* L., Angew. Bot., 56: 109-119.

Hasan, H.A.H., 2002. Gibberellin and auxin production by plant root fungi and their biosynthesis under salinity-calcium interaction. Rostlinna-Vyroba-Uzpi, 48 (3): 101-106.

Hussein, A.E.A., 2004. Safe recycling of environmental solid waste by biological system. M.Sc. Thesis, Ain Shams Univ., Egypt.

Inammi, S., K. Nakamura, K. Tabata, M. Wada and T. Takita, 1995. Soluble viscous substances of jew`s mallow leaves lowers serum and liver cholesterol concentrations and increase fecal steroid excretion in rats fed on rich cholesterol diet. J. Nut. Sci. Vetaminol., 41: 465-475.

International Seed Testing Association, 1966. International rules for seed testing. Proc. Int. Seed Test. Assoc., 23: 1-52.

Johnson, L.F. and E.A. Curl, 1972. Methods for Research on Ecology of Soil-Borne Pathogens. Minneapolis, Burgess Pub. Co.

Klich, M.A. and J.I. Pitt, 1992. A Laboratory Guide to Common *Aspergillus* Species and Their Teleomorphs. 2nd Edn. Published by Common. Sci. and Ind. Res. Organ., Div. Food Processing, North Rude, N.S.W.

Kohoda, H., S. Tanaks, Y. Yamaoka, S. Morinaga and Y. Ohhara, 1994. Constituents of *Corchorus olitorius* L. Nat. Med., 48: 213-214.

Korpinen, E.L., 1974. Studies on *Stachybotrys alternans*. I-Comparison of rabbit skin, mouse fibroblast culture and brine shrimp tests as indicators of *Stachybotrys* toxins. Acta Pathol. Microbiol. Scan. Sect. Bull., 82: 465-469.

Kozakiewicz, Z., 1989. *Aspergillus* species on stored products (Mycological paper No. 161). C.A.B. Int. Mycological Inst., Kew, Surrey, UK.

Martin, J.P., 1950. Use of acid, rosebengal and streptomycin in the plate method for estimation of soil fungi. Soil Sci., 69: 215-233.

Moubasher, A.H., M.A. EL-Naghy and S.I.I. Abdel-Hafez, 1972. Studies on the fungus flora of three grains in Egypt. Mycopathol. Mycol. Applied, 47 (3): 261-274.

Moubasher, A.H. and M.B. Mazen, 1990. Competitive colonization of buried cellulose film by soil fungi in Egypt. Bull. Fac. Sci. Assiut Univ., 19 (1-D): 165-172.

Moubasher, A.H.,1993. Soil Fungi in Qatar and Other Arab Countries. Published by the Center for Scientific and Applied Research, Univ. of Qatar, Doha Qatar, pp: 568.

Oliver-Bever, B., 1986. Medicinal Plants in Tropical West Africa, with Illustration. Cambridge University Press, Cambridge, London, New York, pp: 375.

Pitt, J.I., 1979. The Genus *Penicillium* and its Teleomorphic States *Eupenicillium* and *Talaromyces*. Com. Sci. Ind. Res. Org., Div. Food Res., North Ryde, N.S.W. Australia, Academic Press, Inc. Ltd. London, pp: 634.

Pitt, J.I., 1991. A Laboratory Guide to Common *Penicillium* species. 2nd Edn. Com. Sci. Ind. Res. Org., Div. Food Processing, North Ryde, N.S.W. Australia, Academic Press, Inc. Ltd. London, pp: 187.

Raid, R. and A. Palmateer, 2006. Florida Plant Disease Management Guide: Okra. Univ. Florida, Inst. Food Agric. Sci. (UF/IFAS), pp: 1-3.

Ramirez, C., 1982. Manual and Atlas of Penicillia. Elsevier Biomedical Press, Amsterdam, The Netherlands, pp: 874.

Raper, K.B. and C. Thom, 1949. A Manual of *Penicillium*. Williams and Wilkins, Baltimore, USA., pp: 875.

Raper, K.B. and D.I. Fennell, 1977. The genus *Aspergillus*. R.E. Krieger Publishing Co., Huntington, NY. USA., pp.:686.

Rattray, D., 2001. Okra, Roselle, Aibika and Kenaf (Other Edible *Hibiscus*), Okra: Recipes and Information. Okra Photodisc, Inc., pp: 1-3.

Samia-Soliman, A., 1999. Mycoflora and mycotoxins of sorghum grains in Egypt. Ph.D Thesis, Sohag, South Valley Univ., Egypt.

Samson, R.A., E.S. Hoekstra, J.S. Frisvad and O. Filtenborg, 1995. Introduction to Food-Borne Fungi. 4th Edn. Centraalbureau Voor Schimmelcultures Baarn Delft. The Netherlands.

Schneider, K. and W. Kubelka, 1989. The antibacterial activity of *Alchornea cordifolia*. Planta Med., 55: 651.

Siddiqui, I.A., S.S. Shaukat and A. Khan, 2004. Differential impact of some *Aspergillus* species on *Meloidogyne javanica* biocontrol by *Pseudomonas fluorescens* strain CHAO. Lett. Applied Microbiol., 39: 74-83.

Sivanesan, A., 1984. The Bitunicate Ascomycetes and Their Anamorphs. Strauss and Cramer Gmbh, Hirschberg ISBN, Germany, pp: 701.

Srivastava, L. S. and R. Dayal, 1982. Studies on the rhizosphere mycoflora of *Abelmoschus esculentus* X-Effect of fungicidal spray on rhizosphere and rhizoplane mycoflora. Indian Phytopathol., 34 (4): 426-429.

Tanda, S., 1998. Two powdery mildew fungi found on Jew's mallow, an alias molokhia (*Corchorus olitorius* L.) J. Agric. Sci., 43 (3): 152-158.

Youssef, M.S., 1995. Mycoflora and mycotoxins of some medicinal plants and their antimicrobial activities in Egypt. Ph.D Thesis, Sohag, South Valley University, Egypt.

Youssef, M.S., 1999. Mesophilic fungi and mycotoxins associated with five cultivated Brassicaceae seeds in Egypt. Bull. Fac. Sci. Assiut Univ., 28 (2-D): 105-121.

Youssef, M.S., N.F. Abo-Dahab and A.A. Abou-Seidah, 2000. Mycobiota and mycotoxins contamination of dried raisins in Egypt. Afr. J. Mycol. Biotech., 8 (3): 69-86.

Youssef, M.S., M.B. Aboel-Nasr and N.F. Abo-Dahab, 2002. Mycoflora and mycotoxins of roasted cicer (*Cicer arietinum* L.) in Egypt. Proc. Third Int. Conf. Fungi (Hopes and Challenges), Fac. Sci. AL-Azhar Univ., Cairo, 30th Oct-1Nov., 1: 75-89.

Youssef, M.S., N.F. Abo-Dahab and R.M. Farghaly, 2003. Studies on mycological status of salted fish "Moloha" in Upper Egypt. Mycobiol., 31 (3): 166-172.

Zani, F., G. Massimo, S. Benvenuti, A. Bianchi, A. Albasini, M. Melegari, G. Vampa, A. Bellotti and P. Mazza, 1991. Studies on the genotoxic properties of essential oils with *Bacillus subtilis* recassay and *Salmonella* microsome reversion assay. Plant. Med., 57: 237-241.