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EFFECT OF STORAGE DURATIONS ON FUNGAL INFECTION, AFLATOXIN CONTAMINATION, QUALITY AND YIELD OF SUNFLOWER SEEDS

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ABSTRACT

Ten fungi associated with four sunflower seed genotypes (Sakha53, Giza102, L92 and L120) were isolated and identified. These fungi were *Alternaria alternata* (Fries) Keissler, *Aspergillus flavus* Link, *Aspergillus niger* V. Tiegh, *Aspergillus sp.*, *Cladosporium herbarum* (Perssen) Link, *Fusarium equestii* (Corda) Saccardo, *Humicola grisea* Traaen, *Paecilomyces variotii* Bainier, *Penicillium sp.* and *Rhizopus oryzae* Went and Prinsen. The highest percentage of *A. flavus* infection was noticed with seeds of sunflower genotype L120 at two years after storage. The presence of aflatoxin was determined in contaminated sunflower seeds using HPLC methods. Four genotypes sunflower seeds were stored for two years with aflatoxin contamination except L92 genotype seeds. Increasing storage duration led to significant gradual decrease in seed oil and in fatty acids *i.e.* oleic and linoleic which associated with increasing moisture content. Significant differences were found among the tested sunflower genotypes, seed storage durations and their interaction for percentage germination, seed yield and its components under field conditions. The genotype L92 had the best storability which gave the highest percentage values of field germination and seed yield/fad., at field experiments as combined data over locations. Increasing storage durations caused relatively large reductions in yield and its components. The effective interacting treatment gave the highest seed yield/fad when genotype L92 was sown without storage (pre storage), since its yield remained high with increasing storage durations till two years after storage compared with other ones.

Keywords: Sunflower genotypes, storage duration, fungi, aflatoxin, quality, yield.

INTRODUCTION

Sunflower (*Helianthus annuus*, L.) is an important member of the family Asteraceae and is one of the major oil seed crops grown for the edible oil in the world (Anon., 2007). The importance of sunflower seeds increased because seed contains 25-32% edible oil and rich source of polyunsaturated fatty acids used for human consumption (Neergaard, 1977).

Sunflower seeds are highly exposed to fungi, which attack the plants at different stages of development and subsequently during harvesting and storage (Vaidehi, 2002). Sharfun-Nahar *et al*. (2005) found that a large number of fungi were associated with sunflower seeds *i.e., Aspergillus flavus*, *A. niger*, *A. ocheraceus, Alternaria alternata, Fusarium solani, Penicillium digitatum, Rhizopus arrihizus*, *Acremonium fusidioides*, *Arthrobotrys oligospora*, *Bipolaris bisepta*, *Cephaliophora tropica*, *Chaetomium spinosum*, *Cladobotryum varium*, *Cladosporium cladosporioides*, *Emericella nidulans*, *Gonatobotrys simplex*, *Humicola grisea*, *Memnoniella echinata*, *Mucor mucedo*, *Myrothecium verrucaria*, *Phialophora verrucosa* and *Syncephalastrum racemosum*. During storage, sunflower seeds are exposed to various infections by fungi. Which may lead to various damage including reduce yield of seed in both qualitatively and quantatively, besides

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these decrease in germination percentage, mycotoxin production and total decay (Godika *et al*., 1996; Ramesh and Avitha, 2005). Nine *Aspergillus* spp. were isolated from sunflower seed samples and all of them are reported to produce different groups of afltoxins which are natural toxins and hazardous animal and human (Abdel-Mallek *et al*., 1994). *Aspergillus flavus* is one of the major producers of aflatoxin and can contaminate wide range of agricultural commodities either in field or in storage. Fifteen presumptive *Aspergillus flavus* has been isolated from 30 feed and grain samples. All the isolates were found to be aflatoxigenic (Fakruddin *et al*., 2015). The economic value of sunflower is greatly influenced by associated saprotrophic fungi, which may reduce oil quality due to produce mycotoxins (Abdel-Mallek *et al*., 1994; Abdullah and Al-Mosawi, 2009). Patharkar *et al*. (2013) observed that, as the storage prolonged the fungal incidence increased ultimately the seed germination (%) decreased. According to Sisman (2005) during storage period the oil content of sunflower decreased progressively. The proportions of different fatty acids of seed oil during storage are dependent on the degradation rate of ones, which convert to each other (Ahmadkhan and Shahidi, 2000). Increasing storage periods of sunflower seeds is greatly influenced plants number per unit area which considered as one of the three main components of sunflower yield (Ahmed, 2001). For that the response of sunflower seeds to storage period is considered factor affecting quality and yield of sunflower seeds.

Therefore the main objectives of the present work was aimed to study the effect of storage duration on fungal infection and determined contamination with aflatoxin as well as its effect on quality and yield of some sunflower seed genotypes.

MATERIALS AND METHODS

Source of Seed Samples

Four samples of sunflower seed genotypes (*Helianthus annuus*, L.) *i.e.,* Sakha 53, Giza 102, L92 and L120, were obtained from stock breeding materials of Oil Crops Research Section, Field Crops Research Institute, ARC, Egypt. These samples were previously stored for one and two years in sterilized paper bags in store room at temperature ranged between 18± 2ºC in winter and ranged between 30± 2ºC in summer. The obtained samples were brought at the laboratory and kept at room temperature for the study. Samples were divided into two parts. The first part was used for laboratory test to isolate and identify the fungi associated during storage (one and two years), determine contamination with the aflatoxin in seeds and to assess the effect of storage duration on seed quality. The second part was used for field tests to study the effect of storage duration on yield and its components of some sunflower seed genotypes.

Laboratory Test

Isolation and identification

The selected seeds of Sakha 53, Giza 102, L92 and L120 were thoroughly washed with running tap water and surface sterilized by immersing in 2.0% clorox solution for 3 minutes, and washed several times with sterilized water. The surface sterilized seeds were dried between two sterilized filter papers. Twenty five sterilized sunflower seeds of each sample were randomly selected and plated on surface of PDA medium in sterile Petri dishes, each sample replicated four times. The plates were incubated at 25ºC for 7 days. After incubation the fungi developed from seeds were purified and identified according to Domch *et al.* (1980) and Moubasher (1993). Identification was carried out at Faculty of Science, Zagazig University. The percentage of infection with each fungus on seeds was calculated.

Detection of aflatoxins (AFs)

The tested sunflower seed genotypes (Sakha 53, Giza 102, L92 and L120) were placed in sterilized paper bags and stored for one and two years in storage room temperature. For each sample 50 g of sunflower seeds were used for analyzed aflatoxin contamination (B1, B2, G1 and G2). Detection of aflatoxin (AFs) was carried out at Mycotoxins Control Lab. and Food Safety, National Research Center according to AOAC (2007) as follows:

Sample extraction

1. Weigh 50 g ground sample with 5 g salt (NaCl) and place in blender jar.

2 Add to jar 100 ml methanol: water (80:20).

3. Cover blender jar and blend at high speed for 1 minute.
4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

Extract dilution

1. Pipet or pour 10 ml filtered extract into a clean vessel.
2. Dilute extract with 40 ml of distilled water and mix well.
3. Filter diluted extract through glass microfiber filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 10 ml.

Column chromatography

1. Pass 10 ml filtered diluted extract (10 ml = 1g sample equivalent) completely through Afla Test ®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
2. Pass 10 ml of distilled water through the column at a rate of about 2 drops/second.
3. Elute affinity column by passing 1.0 ml HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 ml) in a glass cuvette.
4. Dryness under a nitrogen stream, then determination with HPLC.

Determination of AFs by HPLC

Derivatization

The derivatives of samples and standard were done as follow: 100µl of trifluoroacetic acid (TFA) was added to sample and mixed well for 30 sec., and the mixture stand for 15 min., 900µl of water: acetonitrile (9:1 *V/V*) were added and mixed well by vortex for 30 sec., and the mixture was used for HPLC analysis.

The HPLC system consisted of Waters Binary Pump Model 1525, a Model Waters 1500 Rheodyne manual injector, a Waters 2475 Multi- Wavelength Fluorescence Detector, and a data workstation with Software Breeze 2. A phenomenex C_{18} (250 × 4.6mm i.d.), 5µm from Waters corporation (USA). An isocratic system with water: methanol: acetonitrile 240:120:40. The separation was performed at ambient temperature at a flow rate of 1.0 ml/min. The injection volume was 20µl for both standard solutions and sample extracts. The fluorescence detector was operated at wavelength of 360 nm for excision and 440 nm for emission (AOAC, 2007). Aflatoxin concentration was determined as ng/g dry weight.

Seed quality

A laboratory experiment was laid in a randomized complete block design with 3 replicates, where each sunflower genotype seeds were subjected to two storage durations (one and two years after storage) and pre storage sample served as control.

Moisture content (%)

The moisture content percentage of the seed was calculated according to the International Seed Testing Association Rules (ISTA, 1996) by hot air oven method maintaining 103°C ± 2°C for 17 hr., and then at 105°C till constant dry weight. The moisture content was calculated on wet basis and expressed in percentage by using the following formula, Seed moisture percentage (%) = [(M2 – M3)/ (M2 - M1)] × 100.

Where, M1 = weight of empty moisture tin in (g), M2 = weight of moisture tin and seed material before drying (g) and M3 = weight of moisture tin and seed material after drying (g).

Seed oil percentage (%)

Soxhelt apparatus was used for the determination of ether extract percent, heating by electric heaters; cold water was used through the condenser. Petroleum ether (60-80°C) was preferred for extractions which continued for not less than eight hours (rate of siphoning was 6-7 /hr.) according to AOAC (1990). Moreover, oil percentage was calculated by multiplying ether extract percent × yield of dry matter (g).

Fatty acids composition

Gas liquid chromatography (Agilent 6890 GU, USA) was used for determination and identification of the fatty acids methyl esters, in Central Laboratory of Food Technology Research Institute, ARC, Egypt, according to Zygadlo *et al.* (1994).

Field Experiments

Complementary field experiments were conducted along successive summer season 2014 at Kafr El-Hamam and Tag Al-Ezz Research Stations, ARC, Egypt to study the effect of three storage durations (pre storage- 1 year after storage and 2 years after storage) on seed yield and its components of four sunflower genotypes (Sakha 53, Giza 102, L92 and L120). In each location, the seeds of the above mentioned treatments were sown simultaneously at the field.

Recorded data

Field experiments were arranged in a split plot design with 3 replicates, where the main plots had four sunflower genotypes (Sakha 53, Giza 102, L92 and L120), and sub plots comprised three storage durations (pre storage,1 years after storage and 2 years after storage). Since, the field experiment, each subplot (experimental unit) had 5 ridges, each of 60 cm in width and 4.0 m in length and 30 cm as hill spacing, occupying an area of 12 m^2. Agricultural practices were applied as usually done in the ordinary sunflower fields. Soil samples (0-30 depth cm) collected from the experimental site were analyzed for physic-chemical characteristics as suggested by Jackson (1973) and results are summarized in Table 1.

Field germination percentage (%) and number of days to flowering (day) were determined. Then at harvest, five guarded plants were taken from 2nd ridge, harvested, tied and left to head dry, thereafter the following characters were estimated: plant height (cm), stem and head diameters (cm), 100-seed weight (g), seed yield $plant^{-1}$(g) and seed oil percentage (was determined as previously described). Plants in the two central ridges in each subplot were harvested for seed yield per square meter and converted to recorded seed yield in kg/4200 m^{2}.

Statistical Analysis

Analysis of variance according to Gomez and Gomez (1984) for each variable was done using randomized complete block design for seed quality at laboratory, and split plot design for seed yield and its components at field experiments. At field experiments, homogeneity of variance between two locations was checked as described by Gomez and Gomez (1984). Therefore, the proper combined analysis of variance (over the two locations) of the split plot design was done according to Snedecor and Cochran (1989). The least significant difference (LSD) test at 5% level was used for assigning differences among means.

RESULTS AND DISCUSSION

Isolation of Fungi

Ten fungi were isolated from four sunflower seed genotypes. These fungi were identified as *Alternaria alternata* (Fries) Keissler, *Aspergillus flavus* Link, *Aspergillus niger* V. Tiegh, *Aspergillus sp., Cladosporium herbarum*, Perseen Link, *Fusarium equestii* (Corda) Saccardo, *Humicola grisea* Traaen, *Paecilomyces variotii* Bainier, *Penicillium sp.* and *Rhizopus oryzae* Went and Prinsen (Table 2). The obtained results are in harmony with those reported by Sharfun-Nahar *et al.* (2005). The highest percentage of infection was noticed with *A. flavus* (25.0%) isolated from L120 sunflower seeds after two years of storage, while, with L92 *A. flavus* was not isolated. On the other hand, the percentage of *A. flavus* infection was recorded as 13.0 and 16.0% with Sakha 53 and Giza 102 two years after storage, respectively. *A. alternata* was recorded as 18.0, 10.0 and 15.0 with Sakha 53, Giza102 and L120, respectively. Two years after storage *Humicola grisea* was recorded 15.0 and 13.0% for Sakha 53 and Giza 102 while, *Cladosporium herbarum* recorded 7.0, 9.0 and 13.0% with sunflower genotypes Sakha 53, Giza102 and L120, respectively.

Detection of Aflatoxin

Results obtained after one year storage, using HPLC revealed that Sakha 53, Giza 102, L92 and L120 showed no evidence of contamination with aflatoxins and also L92 seeds showed no contamination with aflatoxin after two years of storage (Table 3). The stored sunflower seed genotypes for two years were contaminated with aflatoxin except L92 sunflower seed.

Total aflatoxin (B1, B2, G1 and G2) were detected as 3.69 ng/g in Sakha53, 4.22 in Giza102 and 25.9 in L120 two years after storage. On the other hand, maximum level of

Table 1. Mechanical and chemical analyses of soil properties at 0-30 cm depth

Location	Available (ppm)			pH	EC mmh/cm	$CaCO_3$ (%)	Clay (%)	Silt (%)	Fine sand (%)	Texture
	N	P	K							
Kafr El-Hamam	42	15.8	246.2	7.9	1.38	2.6	55.60	25.43	18.97	Clay
Tag Al- Ezz	35.4	7.8	222	7.8	2.3	2.52	35.6	25.4	39	Clay loam

Table 2. Percentage of infection for sunflower seed genotypes during different storage durations

Fungus	sunflower genotype							
	Sakha 53		Giza 102		L 92		L 120	
	1 y	2y	1 y	2y	1 y	2y	1 y	2y
Alternaria alternata	13.0	18.0	6.0	10.0	0.0	0.0	5.0	15.0
Aspergillus flavus	0.0	13.0	0.0	16.0	0.0	0.0	0.0	25.0
Aspergillus niger	2.0	6.0	3.0	14.0	0.0	5.0	4.0	10.0
Aspergillus sp.	0.0	0.0	0.0	8.0	0.0	0.0	0.0	5.0
Cladosporium herbarum	5.0	7.0	8.0	9.0	0.0	0.0	10.0	13.0
Fusarium equestii	0.0	0.0	0.0	3.2	0.0	0.0	0.0	0.0
Humicola grisea	12.0	15.0	10.0	13.0	0.0	0.0	0.0	2.0
Paecilomyces variotii	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0
Penicillium sp.	0.0	0.0	0.0	0.0	0.0	3.0	0.0	5.0
Rhizopus oryzae	3.0	12.0	2.0	10.0	0.0	0.0	0.0	00

Based on examination of 100 sunflower seeds inoculated on PDA medium
1y= One year 2y= Two years

Table 3. Aftlatoxin contamination of sunflower seed genotypes during different storage durations

sunflower genotype	storage duration	Aflatoxin concentration (ng/ g)				
		AFG1	AFB1	AFG2	AFB2	Total
Sakha 53	After 1 year of storage	ND	ND	ND	ND	ND
	After 2 years of storage	ND	2.54	ND	1.15	3.69
Giza 102	After 1 year of storage	ND	ND	ND	ND	ND
	After 2 years of storage	ND	4.22	ND	ND	4.22
L 92	After 1 year of storage	ND	ND	ND	ND	ND
	After 2 years of storage	ND	ND	ND	ND	ND
L 120	After 1 year of storage	ND	ND	ND	ND	ND
	After 2 years of storage	13.3	8.47	1.25	2.88	25.9

ND = Not detected

contamination with aflatoxin in L120 sunflower seeds was obtained at maximum storage duration of two years. Beheshti and Asadi (2013) reported that contaminated sunflower seeds with aflatoxin detected at mean level of 40.68 ng/g. The deleterious biological effect of these toxin contaminated sunflower seed materials on these results raise concerns with respect to the health of human consumers of sunflower seeds after prolonged storage. This including immune system disorder, permanent damage of spleen, liver, kidney and brain functions (Truckess *et al.,* 1991).

Effect of Storage Duration on Moisture and Oil Contents of Sunflower Seed Genotypes

Moisture content (MC) is one of the most important factors effective on the losses during storage. Therefore, MC of sunflower seed was determined and changes in moisture contents with storage duration were presented in Table 4. From results it was clear that, increasing storage duration increased seeds MC. These data are in harmony with those reported by Turgut and Murat (2011). On the other hand, increased percentage of MC in all sunflower genotype seeds, which particularly significant after one and two years of storage. The high MC during storage duration was determined as 14.22 and 13.70% for Sakha 53 and L120 two years after storage, respectively.

There are significant effects of storage duration on seed oil content of sunflower seeds. The lowest oil percentage was recorded from seed sample that stored for longer periods (Table 4). Similar results were reported by Ghasemnezhad *et al*. (2007) in evening primrose. They showed that as the storage time increased, a rapid decrease of the oil percentage compared to the control. During storage sunflower seed are exposed to microorganism infection like fungi which may lead to various damage including reduce yields of seed in qualitatively and quantitatively (Godika *et al*., 1996 ; Ramesh and Avitha, 2005).

The growth of *Alternaria* spp., *Aspergillus* spp., *Rhizopus* spp. and other fungi on oil seeds has been reported to reduce oil quality (Zimmer and Zimmerman, 1972). The ageing process naturally affects the quality of seeds during storage at various conditions, particularly the oil content which is sensitive to deterioration as a results of the oxidation process- a reaction between unsaturated fatty acids and oxygen. It is well established that, the rate of oxidation increase with increasing in oxygen concentration and the duration of the exposure. The oxidation of oil requires the presence of atmospheric oxygen. The longer storage time lead to the higher oxygen availability and *vice versa*. This might be a reason why the oil percentage of stored seeds tends to reduce during storage (Suriyong, 2007). On the other hand, the metabolism of seed during storage to provide energy for its physiological activities could be another reason of seed oil reduction during longer storage duration (Ghasemnezhad and Honermeier, 2009).

Effect of Storage Duration on Fatty Acids (Oleic and Lineolic Acids) of Sunflower Seed Genotypes

Increasing storage duration decreased the fatty acids; oleic and linoleic in all tested sunflower genotypes seeds. Sakha 53 followed by Giza 102 were given the lowest content of oleic and linoleic acids after storage for two years (Table 5). The proportions of different fatty acids of seed oil during storage are dependent on the degradation rate of ones, which convert to each other (Ahmadkhan and Shahidi, 2000). The strong reduction in oleic acid could be related to the production of free fatty acid and conversation to other fatty acids. Fatty acids in free forms are more susceptible to oxidiation. It can conclude that, increased in free forms of oleic acid and its oxidation during storage could be the reason why the content of this fatty acid decreases (Ghasemnezhad and Honermeier, 2009).

Field Germination Percentage and Seed Yield Parameters

It is clear from the results of combined data analysis over locations (Table 6) that, significant differences among the studied sunflower genotypes were found for all studied traits. In a general among genotypes, L92 followed by L120 gave the highest values of field germination (%) and seed yield/fad., L92 followed

Table 4. Effect of storage durations on seed moisture content and Oil (%) of sunflower seed genotypes

Storage duration	Seed moisture content (%) sunflower genotype seeds					Seed oil (%) sunflower genotype seeds				
	Sakha 53	Giza 102	L92	L120	Mean	Sakha 53	Giza 102	L92	L120	Mean
Pre-storage (control)	7.14	4.93	5.15	6.16	5.84	40.13	42.31	44.96	41.54	42.24
After 1 year of storage	9.14	8.16	7.11	8.53	8.24	33.03	35.95	34.84	33.96	34.45
After 2 years of storage	14.22	12.26	11.74	13.70	12.98	28.19	29.17	28.96	27.79	28.53
Mean	10.17	8.45	8.00	9.46	9.02	33.78	35.81	36.25	34.43	35.07
LSD 5%	0.29	0.78	0.68	0.66		1.27	0.98	1.51	1.49	

Table 5. Effect of storage durations on fatty acids (oleic and linoleic) of sunflower seed genotypes

Storage duration	Oleic					Linoleic				
	Sunflower genotype seeds					Sunflower genotype seeds				
	Sakha 53	Giza 102	L92	L120	Mean	Sakha 53	Giza 102	L92	L120	Mean
Pre-storage (control)	29.03	31.59	31.88	31.16	30.91	55.77	58.33	59.85	57.73	57.92
After 1 year of storage	27.96	31.00	29.00	28.78	29.19	45.03	48.45	43.18	48.79	46.36
After 2 years of storage	26.75	29.82	29.02	27.47	28.26	39.34	44.13	39.45	44.18	41.78
Mean	27.91	30.80	29.97	29.14	29.45	46.72	50.30	47.49	50.23	48.69
LSD 5%	0.72	1.22	1.22	1.39		6.40	1.26	2.11	2.02	

Table 6. Germination (%) and seed yield parameters of four sunflower seed genotypes as affected by storage duration and their interaction (combined data of over locations)

Storage duration (S)	Genotype (G)					Genotype (G)				
	Sakha 53	Giza 102	L92	L120	Mean	Sakha 53	Giza 102	L92	L120	Mean
	Field germination (%)					Flowering date				
Pre-storage (control)	88.0	90.5	92.0	89.8	90.1	51.5	47.5	47.3	50.0	49.1
1 year after storage	62.2	70.0	74.2	69.8	69.0	54.0	51.0	47.2	50.8	50.8
2 years after storage	55.3	60.7	66.5	63.8	61.6	54.2	51.0	50.7	55.5	52.8
Mean	68.5	73.7	77.6	74.5	73.6	53.2	49.8	48.4	52.1	50.9
LSD 5%	4.8	1.6	1.0	1.7		1.9	1.0	1.8	1.4	
LSD 5% (G)			1.0					0.6		
LSD 5% (S)			0.9					1.1		
LSD 5% (G × S)			2.1					1.2		

Table 6. Continued

	Plant height (cm)					Stem diameter (cm)				
Pre-storage (control)	168.8	214.3	250.7	171.3	201.3	1.4	1.3	1.0	1.4	1.3
1 Years after storage	162.3	199.7	221.7	163.2	186.7	1.7	1.7	1.4	2.2	1.8
2 Years after storage	149.3	171.7	192.8	154.2	167.0	2.8	2.6	2.2	3.0	2.6
Mean	160.2	195.2	221.7	162.9	185.0	2.0	1.9	1.5	2.2	1.9
LSD 5%	4.7	12.9	11.5	7.0		0.2	0.1	0.2	0.3	
LSD 5% (G)			3.7					0.1		
LSD 5% (S)			4.6					0.1		
LSD 5% (G × S)			7.3					0.2		
	Head diameter (cm)					**100-seed weight (g)**				
Pre-storage (control)	19.1	16.8	16.3	18.2	17.6	6.6	5.6	4.3	6.2	5.7
1 Years after storage	21.8	17.9	17.5	19.1	19.1	7.4	5.7	4.9	6.8	6.2
2 Years after storage	22.8	19.1	18.9	19.2	20.0	8.0	5.9	5.5	7.6	6.8
Mean	21.2	17.9	17.5	18.8	18.9	7.3	5.8	4.9	6.9	6.2
LSD 5%	1.8	0.5	0.6	0.4		0.2	0.2	0.2	0.1	
LSD 5% (G)			0.4					0.1		
LSD 5% (S)			0.3					0.2		
LSD 5% (G × S)			0.7					0.1		
	Seed yield/plant (g)					**Seed yield/fad. (kg)**				
Pre-storage (control)	80.1	54.2	51.6	59.9	61.4	856.2	1024.3	1269.8	943.9	1023.5
1 Years after storage	82.8	63.3	58.4	60.3	66.2	716.8	955.3	1084.1	837.9	898.5
2 Years after storage	95.4	68.4	60.2	73.6	74.4	644.7	795.7	979.7	734.0	788.5
Mean	86.1	62.0	56.7	64.6	67.4	739.2	925.1	1111.2	838.6	903.5
LSD 5%	5.9	4.6	2.3	4.1		21.3	10.1	56.0	34.2	
LSD 5% (G)			1.7					13.3		
LSD 5% (S)			1.9					11.9		
LSD 5% (G × S)			3.4					26.6		

	Oil (%)				
	Sakha 53	**Giza 102**	**L92**	**L120**	**Mean**
Pre-storage (control)	40.0	42.3	42.4	41.5	41.6
1 Years after storage	29.2	38.3	30.8	34.6	33.2
2 Years after storage	28.2	31.5	29.2	30.0	29.7
Mean	32.5	37.4	34.1	35.4	34.8
LSD 5%	1.6	1.6	2.2	1.2	
LSD 5% (G)		0.6			
LSD 5% (S)		0.8			
LSD 5% (G × S)		1.3			

by Giza 102 behaved as the earliest and tallest ones; L120 expressed the largest diameter of stem; Sakha 53 followed by L120 exhibited the largest diameter of head, the heaviest 100-seed weight and seed yield/plant and Giza 102 followed by L120 possessed the highest seed oil (%). These results are in harmony with those reported by El-Sayed *et al.* (2004) in Egypt, they observed that, under different storage periods significant differences in germination percentage, moisture percentage, oil percentage, yield and its components of five seed flax cultivars.

The results of combined data over locations (Table 6) clearly evident that any increase in storage duration from zero (pre storage) to two years after storage was followed by a respective

significant decrease in each of field germination (%), plant height (cm), seed yield/fad., (kg) and seed oil (%). But, on the contrary, it is worth noting that the other traits like, days to flowering (day), stem and head diameters (cm), 100-seed weight (g) and seed yield/plant (g) were gradually significant increase with increasing storage durations. The reduction in seed germination at field might be due to seed deterioration which reflected on the other traits under current study. In this respect, Crnobarac (1992) showed that seed quality also affects the rate and uniformity of emergency, and on the dynamics of initial plant growth.

The interaction effects between sunflower genotypes and storage durations on all studied traits are given in Table 6. Generally, under all studied sunflower genotypes in combined data over locations, further increase in storage durations from (pre storage) to two years after storage was associated with a sharp decline in field germination (%), plant height (cm), seed yield/fad., (kg) and seed oil percentage. Whereas, the highest values of days to flowering (day), stem and head diameters, 100-seed weight (g) and seed yield/plant (g) were, in general, associated with increasing storage durations from zero (pre storage) to two years after storage. On the other side, under all storage durations, genotypes L92 and Giza 102 had the highest values of field germination (%), the tallest ones and the heaviest seed for seed yield/fad. (kg) compared to other ones, whereas the latest in flowering, the largest stem and head diameters, 100-seed weight (g) and seed yield/plant (g) were recorded by Sakha 53 and L120 compared to L92 and Giza 102. The highest values of seed oil (%) was achieved by interaction between L92 and sowing without storage (pre storage) reaching 42.4% followed by sowing Giza 102 after 1 year of storage reaching 38.3%.

Conclusion

From above mentioned results, it can be concluded that increasing storage duration, in general, caused relatively large increased in percentage of fungal infection and contaminated with aflatoxin, as well as gradually decreased in seed oil (%), fatty acids compositions, seed yield and its components of four sunflower seed genotypes.

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تأثير فترات التخزين علي الإصابات الفطرية والتلوث بالأفلاتوكسين والجودة والمحصول لبذور دوار الشمس

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أوضحت الدراسة أنه تم عزل الفطريات المصاحبة لأربعة تراكيب وراثية لبذور دوار الشمس وهي سخا 53 وL92 وجيزة 102 وL120، تم تعريف الفطريات المعزولة علي أنها *Alternaria alternate, Aspergillus flavus, Aspergillus niger, Aspergillus* sp, *Cladosporium herbarum, Fusarium equestii, Humicola grisea, Pacilomyces variotii, Penicillium* sp. and *Rhizopus oryzae*، سجلت أعلى نسبة إصابة بالفطر *Aspergillus flavus* لبذور دوار الشمس L120 بعد سنتين من التخزين، وتم الكشف عن التلوث بالأفلاتوكسين لبذور دوار الشمس تحت الدراسة باستخدام HPLC، وجد أن بذورالتراكيب الوراثية قد احتوت علي الأفلاتوكسين فيما عدا L92 بعد سنتين من التخزين، وقد أدت زيادة فترة التخزين إلى تقليل نسبة الزيت والأحماض الدهنية (أوليك واللينوليك) مع زيادة نسبة الرطوبة، أشارت النتائج إلي وجود فروق معنوية بين التراكيب المدروسة من دوار الشمس وفترات التخزين وتفاعلهم لنسبة الإنبات الحقلي والمحصول ومكوناته في البيانات المجمعة للموقعين خلال التجربة الحقلية، وكان التركيب الوراثي L92 ذو أفضل قدرة تخزينية مع إعطاءه أعلي قيمة لنسبة الإنبات الحقلي ومن ثم محصول البذور/فدان، كما أدت الزيادة في فترات التخزين إلى نقص كبير نسبياً في المحصول ومكوناته، وكان التفاعل ذو تأثير فعال في أعطاء أعلي محصول بذور/فدان بزراعة التركيب الوراثي L92 بدون تخزين (قبل التخزين)، حيث ظل محصوله مرتفع مع زيادة فترات التخزين حتي سنتين من التخزين بالمقارنة بالتراكيب الوراثية الأخرى.

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