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ROLE OF *Aspergillus* spp. IN BIOCHEMICAL CHANGES OF BEAN SEEDS DURING STORAGE

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ABSTRACT: *Seeds of various plants harbor a great variety of microflora plonging to fungi which are the most prevalent. Isolation trials from naturally diseased bean seeds of 4 cultivars yielded several species of fungi belonging to 8 genera. The most frequent fungal genera was Aspergillus. Storage fungi might act independently or in combination to cause a reduction of bean seed viability and germ discoloration.*

Seed moisture content (mc), storage temperature and storage period are the main factors affecting the development of storage fungi and hence seed deterioration rate. Storage of infected seeds of two bean cultivars induced several biochemical changes. There was a straight line relationship between the changes in reducing and non-reducing sugar and relative humidity levels. The extent of change in the seeds biochemical composition differed according to the prevailing environmental factors. Infection with each tested fungus caused a noticeable reduction in total sugars, reducing sugars, non-reducing sugars, protein and fat contents accompanied by an increase in fat acidity value (FAV) if compared with the control treatment. The effect of A. flavus on the fat acidity value was more obvious in comparison with A. ochraceus.

Key Words: *bean, seeds, Aspergillus spp., biochemical*

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most important vegetable crops. It is favourable to the Egyptian people as dry seeds and /or green pods. Most seeds used in agriculture can be stored several years if they kept at moisture content (mc) of 5-8 %, while storage fungi usually grow on stored products without free water and on media with a high osmotic pressure (Neergaard, 1977).On the other hand, the chemical constituents of bean seeds are suitable to storage fungi growth as they contain 22% protein, 64% carbohydrates, 1.6% lipids and limited amounts of sulfur. Most of the seed-borne organisms are economically important because they can cause severe diseases in field planted with infected seeds, others have also, effects on seed quality.

Storage conditions, seed moisture content and storage duration are considered important. If the moisture content of seeds was maintained at sufficiently low level, seeds could be stored for many years with little deterioration (Zeleny, 1954). Also, low temperature is an important as

moisture content in the preservation of high quality stored seeds. Due to infection by storage fungi, many biochemical changes occur in seed component as air relative humidity increases from 75 to 100 %.

The aim of the present studies was to study the effect of fungi associated with bean seeds on some biochemical change under various storage factors.

MATERIALS AND METHODS

1-Isolation, purification and identification of the associated fungi:

Seeds of four bean cultivars namely, Nebraska, Serbo, Giza 3 and Giza 6 stored for 3 months, were obtained from the Vegetable Production Unit, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt. Two hundred discolored seeds (probably diseased) of each cultivar were surface sterilized by immersing in 2 % sodium hypochlorite solution for two mins, washed thoroughly in three changes of sterilized distilled water, then dried between sterilized filter papers. The seeds were aseptically transferred to plates of malt salt agar (MSA) medium (Christensen and Drechsler, 1954). Ten seeds of each cultivar were placed in each dish and ten dishes were used for each treatment .All plates were incubated at 25 ±1°c for 7 days, after which the emerged fungi were picked up and purified using a single spore technique and / or hyphal tip method .The pure cultures were maintained on PDA slants and kept at 5-8 °c in a refrigerator for subsequent studies. The purified fungi were identified to either genera or species level according to the descriptions of Barnett (1960).

2. Pathogenicity parameters

The pathogenic capability of the most frequent fungal genera *i.e.* Aspergillus was estimated *in vitro*. Two different fungi, namely. *Aspergillus flavus* and *A. ochraceus* were tested.

2.1. Preparation of fungus –free seeds:

Apparently disease –free seeds were chosen from the bulk of two bean cultivars, *i.e.* Giza3 and Nebraska. The seeds were disinfested by dipping in 2%sodium hypochlorite solution for two mins, then washed thoroughly in sterilized distilled water, dried in a hot air oven at 40 °C for 48 hr. and stored in disinfested glass jars till use.

2.2. Inoculum preparation:

The inoculum of each pure culture of the isolated fungi was prepared as a dense conidial suspension taken from 7-day old cultures, grown on MSA medium. The spore concentration was adjusted to approximately 10^4 spores / ml using a haemocytometer slide.

2.3. Inoculation:

Seeds of each cultivar (1.2 kg) were inoculated with the aforementioned adjusted spore suspension of the most frequent isolated fungi, *i.e. A. flavus* and *A. ochraceus*. The moisture content of seeds was raised to 20 %. Inocula

were added and mixed with seeds at rates providing 2000-2500 spores/g seed. Uninoculated seeds were treated with the same amount of sterilized distilled water and used as control .All seeds were stored in sterile glass jars (100 gm/each) at 25 °C for 30 days. The percentage of infection and seed viability as well as germ discoloration was determined according to Christensen and Drechsler (1954).

2.4. Seed invasion determination:

One hundred surface-sterilized seeds were plated onto malt – agar medium supplemented with 7.5 NaCl in Petri dishes (10 seeds /dish) and incubated at 28 °C. The plates were examined daily for 7th days. The seeds which exhibited growth of one fungus or more were counted and the percentage of seed invasion was calculated.

2.5. Seed viability testing:

One hundred seeds were maintained between moist filter papers in Petri dishes (10 seeds / dish). The plates were kept at 25 °C for 7 days. The number of seeds which produced normal sprouts was counted and the percentage of germination was calculated.

2.6. Germ discoloration estimation:

The seed embryo was dissected by immersing the seeds in 2% sodium sulphate and 2% lactic acid solution for 3 days at 35°C. The germ color was rated and the degree of germ discoloration was estimated.

3. Biochemical changes in the bean seeds due to infection:

Fifty grams of the aforementioned prepared bean seeds of two cultivars, *i.e.* Giza 3 and Nebraska were distributed in a series of sterile glass containers (25 gm/each). Seeds of each cultivar were inoculated with each tested *Aspergillus* spp. using the previous technique. The spore suspension of each fungus was adjusted to contain approximately 10^4 spores /ml as mentioned before. The calculated amount of water needed to raise the seed moisture content to 15 and 21 % was added as spore suspension. The adjusted spore suspension was employed to inoculate the seeds of each cultivar. Uninoculated seeds were treated in the same way with only sterilized distilled water and used as control. All the seeds were stored at 15 and 30 °C for three months. The total soluble sugars, reducing sugars, non-reducing sugars, total protein, fat content and fat acidity were determined after one and three months. Each analysis was repeated three times and the average was calculated. Four containers were used for each treatment and the samples representing each treatment were taken from each container and thoroughly mixed before each chemical analysis. All the chemical determinations were calculated on dry weight basis.

3-1-Determination of sugar contents:

Total soluble and reducing sugars were determined colorimetrically using the picric acid method described by Thomas and Dutcher (1924) as follows:

Ethanol extraction:

Fifteen grams of dried ground bean seeds of each cultivar, representing each treatment, were plunged immediately into 95 % boiling ethanol for 10 mins to kill the living tissues. The samples were then resumed for 10-12 hrs in Soxhelt unit using 75% ethanol. The obtained ethanol extracts were filtered and evaporated to near dryness on a rotary evaporator at 60 °C. The dried residue was re-dissolved in 6 ml of isopropanol 50 % and used to determine sugar contents.

Reagents:

1. Picrate-picric reagent: prepared by adding thirty six grams of picric acid to 500 ml of 1.0 %solution of sodium hydroxide and 400 ml of hot (90-100 °C)distilled water. The mixture was shaken until the picric acid was dissolved, then cooled and diluted to one liter.
2. Sodium carbonate reagent: prepared by dissolving twenty grams of sodium carbonate in 100 ml of distilled water.

3-1-1-Determination of total sugars:

Approximately, 0.8 ml of each seed sample extract was placed in a test tube containing 5 ml of distilled water and 4 ml of picrate/picric reagent. Then, the mixture was boiled for 10 mins in a water- bath. After cooling, one ml of sodium carbonate reagent was added and the mixture was boiled again for 10 min. After cooling, the tubes were diluted to 50 ml with distilled water. The developed color was measured using a spectrophotometer at 540 nm.

3-1-2-Determination of reducing sugars:

The previously mentioned technique was also applied except that picrate picric reagent and sodium carbonate were added together at the same time and boiled only for 10 mins. Reducing sugar contents was calculated using a glucose standard curve at the same previous wave length.

3-1-3- Determination of non-reducing sugars:

Non–reducing sugar contents was determined from the difference between the total soluble sugars and reducing ones.

3-2-Determination of total protein content:

The method proposed by Bradford (1976) was used as follows: One gm of the dried bean seeds was ground in liquid nitrogen, then one ml of phosphate buffer solution (pH 7.2) and antioxidant ascorbic acid (one gm / liter) were added. The mixture was centrifuged at 18000 rpm for 20 min. About 30 µl of the supernatant were added to 3 ml of "Serva blue G" stain and measured using spectrophometer at 595 nm. The blank was serva blue G stain only. The bovine serum albumin was used as a standard protein. The concentration of protein (mg/gm dry weight) was calculated using the following equation:

Protein content (mg/gm dry weight) = (2 x optical density) / 0.727
The reading was then transferred into percentages.

3-3-1-Determination of fat content:

The lipid content was determined according to the method described by A.O.A.C. (1990) as follows:15 gms of dried ground seeds of each sample were accurately weighed, then extracted in Soxhelt unit using petroleum ether (40-60°C) for 16 hrs. The solvent was removed by evaporation under reduced pressure and the percentage of total fat content was calculated.

3-3-2-Determination of fat acidity value (Acid number):

Acid number was measured according to the method described by A.O.A.C. (1990) as follows: A known weight of oil (2.5 mg) was dissolved in neutralized ethyl alcohol mixture (25 ml). The content of each flask was heated on a steam bath for 2 mins. and then titrated with alcoholic KOH (0.1N) in the presence of phenolphthalein as an indicator. Acid number was calculated using the following equation:

Acid number = V x N x 56.1/ Sample weight (gm)
Where: N = Normality of KOH V = milliliter of KOH

Experimental design and statistical analyses:

Data obtained were statistically analyzed using either the factorial experiment design method or the completely randomized design technique according to Snedecor and Cochran (1967). Treatment averages were compared at 0.05 level of probability using L.S.D. procedure.

RESULTS AND DISCUSSION

1-Isolation, purification and identification of the associated fungi:

Seeds of four bean cultivars were used for isolating the most common associated fungi. Data presented in Table (1) show that total number of 758 fungal isolates was isolated from bean seeds of four cultivars. The isolation trials yielded several species of fungi belonging to 8 genera; *Aspergillus flavus, A. ochraceus, Penicillium* spp., *A. niger, Alternaria alternata, Mucor* spp. *Nigrospora* spp., *Trichothecuim roseum, Chaetomium* spp., *A. parasiticus* and *Epicocum* spp. The percentage of their frequency was 30.74, 20.84, 16.62, 15.30, 4.88, 4.09, 3.83, 1.45, 1.06, 0.92 and 0.26 %, respectively. Limit variation was noticed between tested cultivars through fungal occurrence. In this respect, the total number of isolated fungi from the, *i.e.* Nebraska, Giza 6, Serbo and Giza3 was 183, 186, 190, 199, respectively. Data in Table (1) also reveal that *A. flavus, A. ochraceus, A. niger* and *penicillium* spp. were isolated from all cultivars. Among the different species of *Aspergillus, A. flavus* and *A. ochraceus* were the most common fungi. These results are in agreement with those reported by Bean and Fernando (1986) who mentioned that *Aspergillus* spp. was most frequently present in the seeds of 8 varieties and in more than 96 % of winged bean seeds. From the

obtained results, it could be concluded that cv. Giza3 was more infected than cv. Nebraska. Other bean cultivars were intermediate in this concern. These results are somewhat in agreement with those obtained by Sarhan (2000) who stated that the highest total number of the different fungal isolates were found on Contender (39.83) and Giza 3 (34.79).

2-Pathogenicity tests:

Inoculation experiments with two *Aspergillus* spp., proved that *A. flavus* was the most pathogenic fungus which caused the highest infection of bean seeds accompanied with a severe reduction in germination and increase of germ discoloration than that recorded by *A. ochraceus* (Table2). The analogous values on both tested cultivars, *i.e.* Nebraska and Giza3 induced by *A. flavus* were 97.0, 0.3, 99.7and 99.0, 0.7, 99.7%, respectively after 30 days of storage at 25 °C. The recorded figures caused by the other tested fungus, *i.e. A. ochraceus* were 95.3, 1.7, 98.3 and 97.0, 1.3, 97.0 %, respectively. The corresponding values in the control treatment on cvs. Nebraska and Giza3 were 5.3, 96.6, 1.0, 7.2, 94.0, and 1.0 %, respectively. The differences between any two treatments were significant, except in case of the interaction between fungi and cultivars in seeds infection it was not significant.

Table (1): Isolation and frequency of fungi associated with dry bean seeds of four cultivars after 7 days on PDA medium at 25 ± 1°C

Isolated fungi	Total number of isolates obtained from different bean cultivars					% Frequency
	Nebraska	Giza 6	Serbo	Giza 3	Total isolates	
Aspergillus flavus	43	63	48	79	233	30.74
Aspergillus ochraceus	52	21	56	29	158	20.84
Aspergillus niger	35	58	0	23	116	15.30
Aspergillus parasiticus	0	3	2	2	7	0.92
Pencillium spp.	43	10	46	27	126	16.62
Alternaria alternata	0	0	26	11	37	4.88
Nigrospora spp.	1	10	10	8	29	3.83
Epicocum spp.	0	0	0	2	2	0.26
Chaetomium spp.	0	8	0	0	8	1.06
Mucor spp.	0	13	2	16	31	4.09
Trichothecuim roseum	9	0	0	2	11	1.45
Total	183	186	190	199	758	99.99

Table (2): Pathogenic potentialities of two Aspergillus spp. on seeds of two bean cultivars after 30 days incubation at 25 °C seeds were infested by spore suspension technique.

Isolated fungi	% Seed infection			% Seed germination			% Embryo discoloration		
	Nebraska	Giza3	X̄	Nebraska	Giza3	X̄	Nebraska	Giza3	X̄
Aspergillus lavus	97.0	99.0	98.0	0.3	0.7	0.5	99.7	99.7	99.7
Aspergillus ochraceus	95.3	97.0	96.15	1.7	1.3	1.5	98.3	97.0	97.65
Control	5.3	7.2	6.25	96.6	94.0	95.3	1.0	1.0	1.0
X̄	65.86	67.73	66.78	32.86	32.0	32.43	66.3	65.9	66.1
$LSD_{(0.05)}$ for :	Fungi (F) = 0.90			0.37			0.12		
	Cultivars (C) = 0.73			0.31			0.10		
	F x C = N.S.			0.53			0.18		

The germ discoloration of stored bean seeds was somewhat proportional to the invasion or decrease in germination. These findings suggest that the reduction in germinability depends not only on germ infection but might be attributed to the interior changes in the seed constituents due to the extensive growth of fungi as mention by Aly, 1982.

3-Effect on total sugars, reducing and non-reducing sugar contents:

3-1-Effect on total sugar contents:

Data presented in Table (3) show that the total soluble sugars content was decreased with increasing storage conditions. The reduction was slightly higher for cv. Nebraska than cv. Giza 3. This component was 10.85 and 10.76 % on the average, when bean seeds of cv. Nebraska were stored at 15 °C and 15 % moisture content, respectively. These figures decreased to 9.58 and 9.68 % when the seeds were kept at 30 °C and 21 % moisture content, respectively. The corresponding percentages for cv. Giza 3 were 11.34, 11.10, 10.11 and 10.03 %, respectively. It was noticed that the longer period of storage caused a decline in seed TSS content. The average percentage of total sugar for cv. Nebraska seeds stored for one month was 11.54 % while it decreased to 8.91 after three months. The analogous values for cv. Giza3 were 12.24 and 9.21 %, respectively. In both cultivars, data also showed that increasing storage temperature at any level of mc or the reverse was accompanied by a decrease in this parameter. Meanwhile, it was found that *A. ochraceus* decreased the total sugar contents of both cultivars, followed by *A. flavus*. The difference between the effects of these fungi on this component was very little. This result was in the same trend with those recorded by Glueck *et.al*, (1977).Also, increasing of storage period led to a decrease of total sugar contents.

Table (3): Average percentage of total sugar contents of two bean cultivars after one and three months of inoculation with two *Aspergillus* spp. under the effect of two levels of seed moisture content and storage temperature

Inoculated fungi (*Aspergillus* spp.)	Seed moisture content %	Storage temperature °C	Average percentage of total sugars						Grand mean
			Giza3 cv.			Nebraska cv.			
			1	3	X`	1	3	X`	
Aspergillus flavus	15	15	11.45	9.90	10.68	12.35	9.75	11.05	10.87
		30	11.00	7.50	9.25	11.05	6.15	8.60	8.93
		X`	11.23	8.70	9.97	11.70	7.95	9.83	9.90
	21	15	10.00	7.90	8.95	10.63	7.40	9.02	8.98
		30	13.85	5.00	9.43	8.73	6.10	7.42	8.42
		X`	11.93	6.45	9.19	9.73	6.75	8.24	8.72
Grand mean			11.58	7.58	9.58	10.72	7.35	9.04	9.31
Aspergillus ochraceus	15	15	12.90	8.90	10.90	11.65	8.40	10.03	10.47
		30	11.70	5.80	8.75	11.35	6.55	8.95	8.85
		X`	12.30	7.35	9.83	11.50	7.48	9.49	9.66
	21	15	11.85	8.50	10.18	11.05	6.90	8.98	9.76
		30	9.00	4.95	6.98	9.00	5.60	7.30	7.14
		X`	10.43	6.73	8.58	10.03	6.25	8.14	8.36
Grand mean			11.37	7.04	9.20	10.77	6.87	8.82	9.01
Control	15	15	14.15	13.45	13.80	13.55	12.80	13.18	13.49
		30	13.55	13.00	13.28	12.95	12.55	12.75	13.02
		X`	13.85	13.23	13.54	13.25	12.68	12.97	13.25
	21	15	13.95	13.15	13.55	13.15	12.60	12.88	13.22
		30	13.50	12.50	13.00	12.90	12.10	12.50	12.75
		X`	13.73	12.83	13.28	13.03	12.35	12.69	12.98
Grand mean			13.79	13.03	13.41	13.14	12.52	12.83	13.12

Giza 3 cv. : X` for temperature 15 °C= 11.34 30 °C= 10.11 Nebraska cv. :X` for temperature 15 °C= 10.85 30 °C= 9.58

X` for seed moisture content 15% = 11.10 21% = 10.03 X` for seed moisture content 15% = 10.76 21% = 9.68

3-2-Effect on reducing and non-reducing sugar contents:

Results obtained are presented in Table (4). It was found that reducing sugars decreased in the inoculated seeds with increasing seed mc from 15 to 21%, temperature from 15 to 30 °C and storage period from one to three months. The obtained percentages of reducing sugars under the effect of these storage conditions were 4.65, 4.43, 4.69, 4.30, 5.36 and 3.64 % for cv. Giza 3, respectively. The analogous values for cv. Nebraska were 4.85, 4.45, 4.88, 4.49, 5.29 and 4.08%. Also, data show that the lowest value of reducing sugars (3.80 %) was recorded in the seeds of cv. Giza 3 inoculated with *A. ochraceus* kept at 21% mc and 30 °C for 3 months if compared with uninoculated seeds. Generally, it was noticed that reducing sugars content was decreased when a given inoculated samples kept for prolonged time (3 months) at 21% mc and 30°C if compared with the uninoculated ones.

Data presented in Table (5) show that non- reducing sugars content was decreased with increasing storage conditions. The reduction was somewhat higher for cv. Nebraska than Giza3. The percentage of non-reducing sugars was 5.97 and 5.87 %, on the average, when seeds of cv. Nebraska were stored at 15 °C and 15 % mc, respectively. These figures decreased to 5.09 and 5.20 % when the seeds of the same cultivar were kept at 30 °C and 21 % mc, respectively. The corresponding percentages for cv. Giza 3 were 6.63, 6.45, 5.74 and 6.00 %, respectively. It was noticed that the longer period of storage caused a decline of this component. The average percentage of non-reducing sugars of cv. Giza3 stored for one month was 6.85 %, while it decreased to 5.56 % after 3 months. The analogous values for cv. Nebraska were 6.24 and 4.83 %, respectively. In both cultivars, data also clearly show that increasing storage temperature at any level of mc or the reverse was accompanied by a decrease in this component. These results were in a harmony with Bottomley *et al.*, (1950).The reduction in total, reducing and non reducing sugar may be due to its utilization in respiration, fungal growth and/or fungal origin formation after cellulose and pectin degradation in seeds by the hydrolysis enzymes produced by these fungi as mentioned before. Therefore, these isolates may play an active role in the seed deterioration.

4-Effect on protein content:

Data in Table (6) reveal that each of the tested fungi caused a decrease in protein content in the seeds of both cultivars if compared with the control. The effect was to somewhat, higher in cv. Nebraska than in cv. Giza 3. Increasing each of storage temperature and period lead to more decrease in protein content. Protein content of cv. Giza 3 reached 13.99 and 14.31 % in seeds stored at 15 °C and after one month of storage with the two *Aspergillus* spp., respectively. When seeds kept at 30 °C or for 3 months, protein content decreased to 13.58 and 13.26 %, respectively. The analogous percentage for cv. Nebraska were 13.00, 13.43, 12.72 and 12.29 %, respectively.

Table (4): Average percentage of reducing sugar contents of two bean cultivars after one and three months of inoculation with two *Aspergillus* spp. under the effect of two levels of seed moisture content and storage temperature

Inoculated fungi (*Aspergillus* spp.)	Seed moisture content %	Storage temperature °C	Average percentage of reducing sugars						Grand mean
			Giza3 cv.			Nebraska cv.			
			1	3	X`	1	3	X`	
Aspergillus flavus	15	15	5.00	3.45	4.23	4.85	4.55	4.70	4.47
		30	5.25	2.60	3.93	5.30	2.10	3.70	3.82
		X`	5.13	3.03	4.08	5.08	3.33	4.20	4.14
	21	15	5.00	2.90	3.95	5.00	3.20	4.10	4.03
		30	4.35	1.95	3.15	4.50	2.95	3.73	3.44
		X`	4.68	2.43	3.55	4.75	3.08	3.92	3.73
Grand mean			4.90	2.73	3.82	4.92	3.20	4.06	3.94
Aspergillus ochraceus	15	15	4.70	2.75	3.73	5.15	3.75	4.45	4.09
		30	5.25	2.75	4.00	4.85	3.05	3.95	3.98
		X`	4.98	2.75	3.87	5.00	3.40	4.20	4.03
	21	15	5.55	2.50	4.03	4.45	2.55	3.50	3.77
		30	4.10	1.90	3.00	4.35	2.25	3.30	3.15
		X`	4.83	2.20	3.52	4.40	2.40	3.40	3.46
Grand mean			4.90	2.48	3.69	4.70	2.90	3.80	3.75
Control	15	15	6.50	5.90	6.20	6.45	6.25	6.35	6.28
		30	6.10	5.65	5.88	6.15	6.20	6.18	6.03
		X`	6.30	5.78	6.04	6.30	6.23	6.27	6.15
	21	15	6.45	5.75	6.10	6.25	6.15	6.20	6.15
		30	6.05	5.65	5.85	6.25	5.95	6.10	6.48
		X`	6.25	5.70	5.98	6.25	6.05	6.15	6.07
Grand mean			6.28	5.74	6.01	6.28	6.14	6.21	6.11

Giza 3 cv. : X` for temperature 15 °C= 4.69 30 °C= 4.30 Nebraska cv. :X` for temperature 15 °C= 4.88 30 °C= 4.49

X` for seed moisture content 15% = 4.65 21% = 4.43 X` for seed moisture content 15% = 4.85 21% = 4.45

Table (5): Average percentage of non- reducing sugar contents of two bean cultivars after one and three months of inoculation with two *Aspergillus* spp. under the effect of two levels of seed moisture content and storage temperature

Inoculated fungi (*Aspergillus* spp.)	Seed moisture content %	Storage temperature °C	Average percentage of non-reducing sugars						Grand mean
			Giza3 cv.			Nebraska cv.			
			1	3	X̄	1	3	X̄	
Aspergillus flavus	15	15	6.45	6.45	6.45	7.50	5.20	6.35	6.40
		30	5.75	4.90	5.33	5.75	4.05	4.90	5.12
		X̄	6.10	5.68	5.89	6.63	4.63	5.63	5.76
	21	15	5.00	5.00	5.00	5.75	4.20	4.98	4.99
		30	9.50	3.05	6.28	4.20	3.15	3.68	4.98
		X̄	7.25	4.03	5.64	4.98	3.68	4.33	4.98
Grand mean			6.68	4.85	5.77	5.80	4.15	4.98	5.37
Aspergillus ochraceus	15	15	8.20	6.15	7.18	6.50	4.65	5.58	6.38
		30	6.45	3.05	4.75	6.50	3.50	5.00	4.88
		X̄	7.33	4.60	5.97	6.50	4.08	5.29	5.63
	21	15	6.30	6.00	6.15	6.60	4.35	5.48	5.82
		30	4.90	3.05	3.98	4.65	3.35	4.00	3.99
		X̄	5.60	4.53	5.07	5.63	3.85	4.74	4.90
Grand mean			6.47	4.57	5.52	6.07	3.97	5.02	5.27
Control	15	15	7.65	7.55	7.60	7.10	6.55	6.83	7.22
		30	7.45	7.35	7.40	6.80	6.35	6.58	6.99
		X̄	7.55	7.45	7.50	6.95	6.45	6.70	7.10
	21	15	7.50	7.40	7.45	6.90	6.45	6.68	7.07
		30	7.45	6.85	7.15	6.65	6.15	6.40	6.78
		X̄	7.48	7.13	7.30	6.78	6.30	6.54	6.92
Grand mean			7.52	7.29	7.40	6.87	6.38	6.62	7.01

Giza 3 cv. : X̄ for temperature 15 °C= 6.63 30 °C= 5.74 Nebraska cv. :X̄ for temperature 15 °C= 5.97 30 °C= 6.9

X̄ for seed moisture content 15% = 6.45 21% = 6.00 X̄ for seed moisture content 15% = 5.73 21% = 5.20

Table (6): Average percentage of protein content of two bean cultivars after one and three months of inoculation with two *Aspergillus* spp. under the effect of two levels of seed moisture content and storage temperature

Inoculated fungi (*Aspergillus* spp.)	Seed moisture content %	Storage temperature °C	Average percentage of protein content						Grand mean
			Giza3 cv.			Nebraska cv.			
			1	3	X`	1	3	X`	
Aspergillus flavus	15	15	13.35	12.55	12.95	12.39	10.80	11.60	12.27
		30	13.47	12.36	12.92	10.56	10.46	10.51	11.71
		X`	13.41	12.46	12.93	11.48	10.63	11.05	11.99
	21	15	13.50	12.36	12.93	10.68	10.52	10.60	11.77
		30	13.56	11.37	12.47	13.68	9.96	11.82	12.14
		X`	13.53	11.87	12.70	12.18	10.24	11.21	11.95
Grand mean			13.47	12.16	12.82	11.83	10.44	11.13	11.97
Aspergillus ochraceus	15	15	13.20	12.00	12.60	12.75	11.28	12.02	12.31
		30	12.66	10.98	11.82	12.18	10.17	11.18	11.50
		X`	12.93	11.49	12.21	12.47	10.73	11.60	11.90
	21	15	12.90	10.98	11.94	12.39	10.23	11.31	11.63
		30	12.00	9.87	10.94	11.64	9.15	10.40	10.67
		X`	12.45	10.43	11.44	12.02	9.69	10.85	11.15
Grand mean			12.69	10.96	11.82	12.24	10.21	11.22	11.52
Control	15	15	16.95	16.68	16.82	16.26	16.26	16.26	16.54
		30	16.71	16.69	16.70	16.26	16.24	16.25	16.48
		X`	16.83	16.69	16.76	16.26	16.25	16.26	16.51
	21	15	16.71	16.67	16.69	16.23	16.22	16.23	16.46
		30	16.68	16.64	16.66	16.20	16.17	16.19	16.42
		X`	16.70	16.66	16.68	16.22	16.20	16.21	16.44
Grand mean			16.76	16.67	16.72	1.24	16.22	16.23	16.47

Giza 3 cv. : X` for temperature 15 °C= 13.99 30 °C= 13.58 Nebraska cv. :X` for temperature 15 °C= 13.00 30 °C= 12.72

X` for seed moisture content 15% = 13.97 21% = 13.60 X` for seed moisture content 15% = 12.97 21% = 12.76

Increasing seed mc of cv. Nebraska from 15 to 21% resulted in a decrease in protein content from 12.97 to 12.76 %. On the other hand, increasing seed mc of cv. Giza 3 caused a higher reduction in this component from 13.97 to 13.60. Also, data show that protein content was affected by mc, storage temperature and storage period. The decline in protein content probably may be due to its decomposition by the tested fungi. Seeds of cv. Nebraska inoculated with either *A. flavus* or *A. ochraceus* contain low amount of protein content by increasing the temperature from 15 to 30 °C as compared with the uninoculated seeds and cv. Giza3. Also, results in table (6) indicate that, the lowest protein content (9.15%) was noticed in bean seeds of cv. Nebraska inoculated with *A. ochraceus* and kept at 21 % mc and 30 °c for three months. These results were agreed with results of (Dixit *et. al.*, 1997). The decline in protein content probably may be due to its decomposition by enzymes which produced by the tested fungi.

5-Effect on fat content:

Data in Table (7) reveal that storage temperature, seed mc and storage period has affected seed fat content. The percentage of fat content was decreased from 0.88 to 0.80 % in cv. Giza 3 by increasing the storage temperature from 15 to 30 °C. The analogous figures for cv. Nebraska were 0.91 and 0.83 %, respectively. The same trend was also true for storage period. When storage period was increased from one to three months, % seed fat content, decreased from 0.93 to 0.76 % in cv. Giza 3, while it decreased from 0.95 to 0.79 % for cv. Nebraska as compared with the uninoculated seeds. The lowest percentage of this component of cv. Giza 3 (0.47 %) was recorded when the seeds were inoculated with *A. ochraceus* and stored for 3 months at 21 % mc and 30 °C The greatest reduction in fat content may have been caused by the lipolytic activity of the seed-borne fungi to increase the amounts of the free unsaturated acids can be used as source for energy and main materials for feeding and mycelium building.

6-Effect on fat acidity value (F.A.V):

Data in Table (8) indicate that F.A.V. of the inoculated seeds was higher than that recorded in the control treatment. It is evident that this value was increased in the seeds with increasing each of mc, temperature and storage time. Value of F.A.V. increased from 49.34 to 51.33 after storage of the seeds of cv. Nebraska at 15 and 21 % mc, respectively. The corresponding figures for cv. Giza 3 were 47.56 and 50.52, respectively. Also, it was found that increasing storage temperature from 15 to 30 °C resulted in an increase in this value in seeds of cv. Nebraska, being 48.48 and 52.18, respectively. The analogous values of cv. Giza 3 were 46.96 and 51.11, respectively. The same trend was also found in the seeds of both cultivars due to storage time. The highest value of F.A.V. (75.50) was noticed in seeds of cv. Nebraska inoculated with *A. flavus* and stored at 21 % mc and 30 °C for 3 months.

Table (7): Average percentage of fat content of two bean cultivars after one and three months of inoculation with two *Aspergillus* spp. under the effect of two levels of seed moisture content and storage temperature.

Inoculated fungi (*Aspergillus* spp.)	Seed moisture content %	Storage temperature °C	Average percentage of fat content						Grand mean
			Giza3 cv.			Nebraska cv.			
			1	3	X`	1	3	X`	
Aspergillus flavus	15	15	0.94	0.67	0.81	0.96	0.75	0.86	0.83
		30	0.87	0.57	0.72	0.89	0.64	0.77	0.74
		X`	0.91	0.62	0.76	0.93	0.70	0.81	0.79
	21	15	0.89	0.62	0.76	0.90	0.71	0.81	0.78
		30	0.65	0.54	0.60	0.75	0.62	0.69	0.64
		X`	0.77	0.58	0.68	0.83	0.67	0.75	0.71
Grand mean			0.84	0.60	0.72	0.88	0.68	0.78	0.75
Aspergillus ochraceus	15	15	0.86	0.56	0.71	0.88	0.63	0.76	0.73
		30	0.79	0.52	0.66	0.78	0.54	0.66	0.66
		X`	0.83	0.54	0.68	0.83	0.59	0.71	0.70
	21	15	0.83	0.54	0.69	0.81	0.57	0.69	0.69
		30	0.55	0.47	0.51	0.63	0.50	0.57	0.54
		X`	0.69	0.51	0.60	0.72	0.54	0.63	0.61
Grand mean			0.76	0.52	0.64	0.78	0.56	0.67	0.65
Control	15	15	1.19	1.17	1.18	1.20	1.19	1.20	1.19
		30	1.17	1.16	1.17	1.19	1.14	1.17	1.17
		X`	1.18	1.17	1.17	1.20	1.17	1.18	1.18
	21	15	1.18	1.14	1.16	1.19	1.15	1.17	1.17
		30	1.16	1.13	1.15	1.19	1.07	1.13	1.14
		X`	1.17	1.14	1.15	1.19	1.11	1.15	1.15
Grand mean			1.18	1.15	1.16	1.19	1.14	1.17	1.16

Giza 3 cv. : X` for temperature 15 °C= 0.88 30 °C= 0.80 Nebraska cv. :X` for temperature 15 °C= 0.91 30 °C= 0.83

X` for seed moisture content 15% = 0.87 21% = 0.81 X` for seed moisture content 15% = 0.90 21% = 0.84

Table (8): Fat acidity value of two bean cultivars after one and three months of inoculation with two *Aspergillus* spp. under the effect of two levels of seed moisture content and storage temperature

Inoculated fungi (*Aspergillus* spp.)	Seed moisture content %	Storage temperature °C	Average percentage of fat acidity value						Grand mean
			Giza3 cv.			Nebraska cv.			
			1	3	X`	1	3	X`	
Aspergillus flavus	15	15	56.90	66.13	61.52	57.65	69.25	63.45	62.48
		30	61.30	67.32	64.31	63.41	74.78	69.10	66.70
		X`	59.10	66.73	62.91	60.53	72.02	66.27	64.59
	21	15	60.20	66.43	63.32	59.50	68.18	63.84	63.58
		30	64.10	73.30	68.70	66.23	75.50	70.87	69.78
		X`	62.15	69.87	66.01	62.87	71.84	67.35	66.68
Grand mean			60.63	68.30	64.46	61.70	71.93	66.81	65.64
Aspergillus ochraceus	15	15	51.70	56.20	53.95	54.83	64.50	59.67	56.81
		30	64.10	66.21	65.15	63.01	66.45	64.73	64.94
		X`	57.90	61.21	59.55	58.92	65.48	62.20	60.88
	21	15	63.23	62.25	62.73	63.10	66.45	64.78	63.75
		30	62.13	70.80	66.47	65.20	69.25	67.23	66.85
		X`	62.68	66.52	64.60	64.15	67.85	66.00	65.30
Grand mean			60.29	63.86	62.08	61.54	66.66	64.10	63.09
Control	15	15	19.13	20.00	19.57	18.13	19.93	19.03	19.30
		30	20.02	21.70	20.86	19.00	21.13	20.07	20.46
		X`	19.58	20.85	20.21	18.57	20.53	19.55	19.88
	21	15	20.10	21.31	20.17	19.30	21.00	20.15	20.43
		30	19.48	22.90	21.19	19.74	22.50	21.12	21.16
		X`	19.79	22.11	20.95	19.52	21.75	20.64	20.79
Grand mean			19.68	21.48	20.58	19.00	21.14	20.09	20.43

Giza 3 cv. : X` for temperature 15 °C= 46.96 30 °C= 51.11 Nebraska cv. :X` for temperature 15 °C= 48.48 30 °C= 52.18

X` for seed moisture content 15% = 47.56 21% = 50.52 X` for seed moisture content 15% = 49.34 21% = 51.33

Generally, it could be concluded that *A. flavus* caused the highest percentage of F.A.V., being 65.64 followed by *A. ochraceus*, being 63.09. The results were in the same trend with result of (Hafez *et al.*, 1996). lipolytic activity of the *Aspergillus* fungi increase the amounts of the free unsaturated acids which may effect directly on pH and also fat acidity value. The production free unsaturated acids were effect with cultivar, storage condition and lipolytic activity of fungal species.

REFERENCES

Aly, H. Y. (1982). Studies on fungi associated with sorghum grains during storage Ph .D. Thesis Pl. Path. Fac. Agric. Cairo Univ. Egypt.

A.O.A.C. (1990). Official Methods of Analysis of the Association of Official Analytical Chemists. 15th ed., Washington D.C, USA.

Barnett, H. L. (1960). Illustrated genera of imperfect fungi. Burgess publishing Co., Mineoplis, Minn., USA. 225 P.

Bean,G. and T. Fernando (1986). Winged bean (*Psophocarpus tetragonolobus*, L.) as a substrate for growth and aflatoxin production by aflatoxigenic strains of Aspergillus spp. Mycopathologia (90),141-145.

Bottomley, R. A., C. M. Christensen and W. F. Geddes (1950). Grain storage studies: the influence of various temperatures, humidities and oxygen concon mold growth and biochemical changes in stored yellow corn .Cereal Chemistry, 27: 271-296.

Bradford, M. M. (1976). A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein dye binding. Analytical Biochemistry, 72:248-254

Christensen, C. M. and R. F. Drechsler (1954). Grain storage studies.14: Changes in moisture content germination percentage and moldiness of wheat samples stored in different portions of bulk wheat in commercial bins. Cereal Chem., 31,206 and 216.

Dixit, V. R. Tiwari, and S.C.Tripati (1997). Change in the protein content of groundnut due to fungal infestion. Journal of living world 4 (2) 33-35.

Glueck, J. A., L. W. Rooney, D. T. Rosenow and F. R. Miller (1977). Physical and structural properties of field deteriorated sorghum grain 102-112 In Third Annual Progress Report, Texas A. and M. Univ. College station Texas, U.S.A. (c.f. Sorghum Diseases. A world Review proceedings of the International workshop. International Gropes Research Institute for the semi-Arid tropics, India, 469 pp., 1978.

Hafez, S. A., (Amal) H. Mahmoud and M. I. E. Barakat (1996). Effect of growth of fungi on the fatty acid composition of stored soybean seeds. Egypt .J. Agric. Res.74,(2),429-442.

Neergaard, P. (1977). Seed Pathology vol. 1: 839 pp. The Machillan press Ltd., London and Basing stoke.

Sarhan, E. M. (2000). Studies on some seed borne disease of bean and their control. M.Sc. Thesis, Fac. of Agriculture ,Zagazig University.

Snedercor, G.W. and W.G. Cochran (1967). Statistical Method 6th ed. The Iowa State, Unive., Press Ames, Iowa, USA.

Thomas, W. and R. A. Dutcher (1924). The colorimeteric determination of Carbohydrates in plants by picric reduction method ,The estimation of reducing sugars and sucrose J. A. M. Soc ., 46: 1662-1669.

Zeleny, L. (1954). Chemical physical and nutritive changes during storage.P. 46-76 in 3. A. Anderson and A.W. Alcock (eds.), storage of cereal grains and their products. Am. Assoc. Cereal Chem., st. paul., Minn.

دور فطريات الاسبرجيلس في التغيرات البيوكيمائية لبذور الفاصوليا خلال التخزين

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الملخص العربي

تعتبر بذور المحاصيل النباتية عرضة للاصابة بعدد كبير من الفطريات .وتجارب العزل التى تمت باستخدام أربعة أصناف من نبات الفاصوليا وهى نبراسكا ,جيزة ٦, سربو ,جيزة ٣ أدت إلى ظهور أنواع عديدة من الفطريات شملت ثمانية أجناس ، وكان جنس الاسبرجيلس أكثرها تكراراً والذى يعتبر من الفطريات التى تتواجد بالحقل ولكن تتواجد أيضا بشكل اكبر فى المخزن.

وتمثل فطريات التخزين سببا رئيسيا فى نقص حيوية البذور وارتفاع حرارتها وتلون الأجنة بها.والمحتوى الرطوبى للبذور ودرجة حرارة التخزين وطول فترة التخزين من العوامل المؤثرة والتى تلعب دوراً كبيراً فى نمو فطريات التخزين علاوة على تأثيرها على زيادة معدل تلف البذور حيوياً .وعندما تم تخزين بذوراً (معداه صناعيا بالفطرين اسبرجليس فلافيس، واسبرجليس اوكراشيس) لصنفين من الفاصوليا أحداهما كان أكثر إصابة بالفطريات والأخر كان أقل إصابة أدى ذلك إلى حدوث العديد من التغيرات الكيموحيوية في التركيب البيوكيمائى لهذه البذور مقارنة بالعينات السليمة (الكنترول). حيث وجدت علاقة خطية بين نقص محتوى السكريات المختزلة والغير مختزلة ومستوى الرطوبة النسبية .وكان اختلاف معدل التغيير فى مكونات البذور تبعا للعوامل التخزين السائدة والصنف ونوع الفطر .علاوة على إن الإصابة بالفطريات المختبرة أدى إلى نقص ملحوظ فى محتوى السكريات الكلية وأيضا محتوى البروتين بينما محتوى الدهون فقد صاحبه زيادة فى رقم الحموضة مقارنة بمعاملات المقارنة .وقد لوحظ أن الفطر اسبرجليس فلافيس كان أكثر تأثيرا من الفطر اسبرجليس اوكراشيس على الخصائص البيوكيمائية للبذور.