SUITABILITY SOYBEAN CULTIVARS FOR *ASPERGILLUS FLAVUS* PATHOGENICITY AND AFLATOXIN PRODUCTION AND SOME AFFECTING FACTORS

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ABSTRACT

Nine Aspergillus' species were isolated from seeds of seven freshly harvested soybean cultivars. Aspergillus flavus and A. niger were the most frequent species from all tested cultivars. Seeds of H15L17 or Holladay soybean cultivars had the highest frequency of Aspergillus spp. while seeds of Giza 21 and Crawfored gave the lowest frequency. Only isolates of A. flavus and A. parasiticus could produce aflatoxin, however, other seven species could not. Artificial or natural inoculated seeds of soybean cultivars inoculated with spores of A. flavus caused the most decrease in seed germination and the highest increase in both seed invasion and/or aflatoxin production were found in the highly favourable of soybean cultivars (H15L17, Holladay and Toamo). In contrast, seeds of Giza 21, Giza35 and Giza 83 were partial resistance to establishment of A. flavus and aflatoxin accumulation. The cultivar (Crawford) was highly resistant without shown aflatoxin formation. The best vigor (mineral contents) was obtained from healthy seed of partially and highly unsuitable cultivars comparable to highly suitable cultivar for fungus infection.

The results also revealed the absence of significant variation in either total nitrogen or magnesium contents of the suitable, partially and highly unsuitable cultivars. Calcium, potassium and total phosphate content of both testa and kernels of the susceptible seeds were low as compared to those partially and highly resistant seeds, and vice versa with sodium and zinc contents.

INTRODUCTION

One of the major factors for low productivity of soybean seeds in Egypt is poor seed germination and early seedling mortality due to seed-borne fungi. Harvested soybean seeds have been found to be infected with many kinds of fungi, of which, *Aspergillus* spp. were the predominant pathogens isolated from seeds of soybean cultivars (Shotwell *et al.*, 1978; Diab, 1980 and Shatla *et al.*, 1982). These fungi are usually associated with poor seed quality and seed germination (Dorworth and Christensen 1968 and Arafa 1994). The correlation between reduction of germination and fungal invasion of soybean seeds was studied by several investigators (Kabeere and Taligoola 1983; Anderson, 1985 and Arafa *et al.*, 1996).

Aspergillus flavus., A. glaucus., A. niger., A. ochraceus., A. tamarii., A. amestelodami., A. candidus., A. cheralieri., A. melleus., A. sydowii., A. nidulans., A. quadrilineatus. and *A. parasiticus* were reported as common pathogen of soybean seeds (Shotwell *et al.*, 1978; Shatla *et al.*, 1982 and Chandra *et al.*, 1985).

The role of aflatoxin as carcinogenic substances is well known as stated by (Esuruoso, 1975 and Bullerman, 1979). Members of Aspergillus flavus group mainly produce these substances. The natural and artificial production of aflatoxin has been reported on various grains and seed especially in tropical and subtropical countries where the storage conditions are sub-optimal. The ideal way to prevent the formation of aflatoxin would be the development of plants varieties that produce seeds that are not suitable for fungal infection and toxin formation. Therefore, the present investigation was undertaken to evaluate seven susceptibility infections with different cultivars of soybean for their *Aspergillus flavus* and aflatoxin production. Also, factor affecting toxins formation was investigated.

MATERIALS AND METHODS

1-Seed samples: Seeds of seven soybean cultivars namely; Giza 35, Toamo, Giza 21, Holladay, Crowford, H15L17 and Giza 82, 1998/1999 growing season were obtained from Crops Department, Shandaweel Agriculture Research Station, Sohag, Egypt. All seed samples

were tested for natural contamination with aflatoxin and proved to be aflatoxin-free.

Π-Isolation. Purification and identification of seed-borne fungi: the seeds of each cultivar were surface sterilized using 0.6% sodium hypochlorite solution for 1 minute, dried and then placed onto autoclaved Petri-dishes containing 20 ml of potato dextrose agar medium acidified with lactic acid (PH= 4.7). The plates were incubated at 22-26°C for 5-7 days. The developed fungal colonies were isolated, purified, using hyphal tip and/or single spore techniques (Anderson, 1985).

The isolated *Aspergillus* species were identified by standard mycological methods using the key, monograph and literature of (Kozakiewicz, 1989).

Π1- Determination of aflatoxin concentration:

Nine species of *Aspergillus*, isolated from soybean cultivars seeds, were tested to determine their ability to produce the toxins.

A-Artificial inoculation of soybean seeds by various species of *Aspergillus*:

Twenty gms of healthy soybean seeds for each specie of *Aspergillus* were used. For each sample, the seeds were surface disinfected with 25% aqueous solution of sodium hypochlorite as described by Anderson (1985). The disinfected seeds were transferred to sterile Petri dishes and equilibrated to 25% moisture content at a relative humidity of 90±2% at 28°C in chamber as described by Seenappa *et al.*, (1981). A conidial suspension in sterile distilled water was prepared from a 10-day-old culture of each *Aspergillus* species. The *Aspergillus* species were maintained in PDA slants. 20 g of each sample was inoculated with the conidial suspension of each *Aspergillus* specie containing about 1 X 10^6 spore/ml at a rate of 2ml/Petri dish. After inoculation, the Petri dishes were gently swirled to evenly spread the inoculums. The inoculated samples were incubated for 15 day at 25 ^{0}C.

B-Extraction and analysis of toxins: At the end of incubation period, each specific seeds sample seeds of each *Aspergillus* specie were extraction with n-hexane for 10h using Soxhlet unit extractor. The defatted residue was re-extracted for another 10h with chloroform. The chloroform extract was dried over anhydrous sodium sulphate, filtrated then distilled to near dryness. The residue was diluted with chloroform to 1ml. The chloroform solution was analyzed for the presence of aflatoxins (B1, B2, G1 and G2) using a thin layer chromatographic technique (TLC) as Gimeno, 1979.

C-Confirmation and estimation of aflatoxins: A chemical confirmatory test for aflatoxin B1 by direct formation of hemiacetal aflatoxins (B2a) on TLC plates before chromatography as described by Przybylski (1975) was used. The unknown and standard aflatoxin B1 spots were treated with trifluoroacetic acid directly on the sample plate. After reaction the plate is developed as usual and examined under UV-irradiation. The hemiacetal derivative has a blue fluorescence at a lower Rf than B1. Aflatoxin content of the toxic extract was estimated using the technique described by Coomes *et al.*, (1965).

IV -Soybean varietal response to *Aspergillus flavus* and aflatoxin formation: Two hundred gm of healthy seeds representing each cultivar were surface disinfected and artificial infected by *Aspergillus flavus* were described before. At the end of incubation period, the growth of the fungus (*A. flavus*) was visually assessed on each seed of soybean cultivar. (A) 20g of each cultivar were defatted by extraction and analysis for the presence of aflatoxin concentration and determination as mentioned before. (B) 100 seeds of each soybean cultivars were rinsed in 1% NaOCl for one minute, and placed on Czapek's agar medium + 500 ppm rose bengal + 100 ppm Streptomycin sulfate to detect *Aspergillus flavus* (Kulik, 1973). Another, 100 seeds were placed between moist paper towels at room temperature as studied by AOSA, 1965 to determine germinability. Moisture content determination as (wet weight basis) was carried out by drying the seeds at 105°C for 72-96h (Zinnen and Sinclair, 1982).

V- Varietal differences of soybean cultivars in vigour and mineral components affecting aflatoxin production:

1-Vigour Tests: To determine some biological characters of healthy seeds in three soybean cultivars. i.e. susceptible (Holladay), Partially resistant (Giza 21), and highly resistant (Crawford), the following three analyses were performed as described by Hassanien (1985).

i - Electric conductivity test: The exudates excreted from ten seeds were measured using Electroconductivity meter model HACH44600.

ii-Overall dehydrogenase: Overall dehydrogenase activity was determined using 0.5% 2,3,5-triphenyltetrazolin chloride solution.

iii-Oxygen uptake: The respiration intensity was estimated as µl O2 taken up/minute by 5 sprouts after 24hr germination at 20°C by manometric method in the Warburg apparatus.

2- Mineral analysis: Total nitrogen contents were determined by use of Nessler reagent Vogel, 1968. Sodium and Potassium minerals were estimated by the flame photometer method (Williams & Twine, 1960). Calcium and Magnesium minerals were determined by the versene titration method (Schwarzenback and Biedermann, 1948). Total phosphorus was estimated colorimetrically according to Woods and Mellon (1941). Atomic absorption spectrophotometry (ZeissFMD3) was used for determination of zinc.

RESULTS AND DISCUSSION

I- Natural incidence of *Aspergillus* spp:

Results in table (1) indicated that nine *Aspergillus* species were isolated from seeds of the seven soybean cultivars but with significant various frequencies. *A. flavus* and *A. niger* gave the highest means of isolation (19.7%) and (16.75%), respectively. *A. melleus* recorded the least isolation (4.41%) but without any significant differences than *A. nidulans* (4.66%), *A. versicolor* (5.71%), *A. sydowi* (6.01%) and *A. parasiticus* (6.14%). *A. quadrilineatus* showed moderate frequency with the mean of 12.5%. The seven cultivars varied significantly in their mean frequency of the *Aspergillus species* isolated from them. The cultivar H^{15} L_{17} gave the highest mean, 18.16% followed by Holladay (17.4%) but with no significant differences among them. Toamo showed moderate mean (13.38%)while Crawford gave the least frequency (1.78%) with no significant difference than Giza 21 (1.86%). The rest cultivars means were 8.2% (Giza 82) and 4.68% (Giza 35) proved that there is a significant interaction between the isolated *Aspergillus species* and the soybean cultivars. Thus five species of *Aspergillus* were isolated from either the cultivar Crawford or the cultivar Giza 21. Eight species were obtained from the cultivar Giza 35, but the other cultivars gave the nine isolated *Aspergillus species*. On the other hand, the dominant isolated species significantly differ according to the soybean cultivars, as there was a significant interaction between the fungi and the cultivar. The most dominant species, in general, was *A. flavus* in case of H_{15} L17 (43.2%) followed by *A. niger* from the cultivar Holladay (33.1%). With the cultivar Toamo the dominant species was *A. flavus* (26.4%). Almost similar results were obtained by Christensen (1957 and Diab (1980) who reported that 661 fungal isolates representing six genera were obtained from Clark soybean cultivar. These fungi were *Aspergillus, Alternaria, Fusarium, Rhizopus, Penicillum* and *Cladosporium*. Shatla *et al.*, (1982) and Morsy *et al.*, (1982) showed that *Aspergillus terreus, A. niger, A. flavus, Fusarium spp* and *Alternaria tenuis* were the most commonly isolated fungi from seeds of Clark and Woodworth soybean cultivars. The same authors added that five species (*Aspergillus flavus, A. niger, A. alutaceus, A. terreus* and *A. fumigatus*) were generally the most common *Aspergillus* species recorded in Calland soybean cultivar (El-kady and Youssef, 1993).

II–Determination of aflatoxin production from *Aspergillus flavus*:

Nine species of *Aspergillus* were tested to determine their ability to produce toxins.

The preliminary data (untabulated) show that only *A. flavus* and *A. parasiticus* could produce aflatoxins. Similar results were reported by Shatla *et al.*, (1982), Calvert *et al.*, (1978) and El-Kady *et al.*, (1996).

III–Infection of *Aspergillus flavus* and germinability of soybean cultivar seeds stored at 25% moisture content and 26 ± 2 °C for 10 days:

The invasion of seven-soybean cultivar seeds by spores of *A. flavus*, and their germinability after storage for 10 days at 90% RH (26 ± 2 °C) was given in Table (2). Based on seed reaction, data in Table (2) indicate that soybean cultivars could be behaved as four groups:

1. Giza 21 and Crawford cultivar seeds remained almost free from *A. flavus* and gave very high germinability (92 and 90%, respectively).

2. Giza 82 and Giza 35 seeds were moderately invaded by *A. flavus*, being 12% and 16%, respectively and when compared to the control seeds (95 and 92%), respectively there was decline in germination 84 and 80%, respectively at compared to the control seed.

3. *Aspergillus flavus* invaded 22% of the Toamo soybean seeds. Consequently a great decline in germinability took place (62%).

Table (1): Frequency of *Aspergillus* species isolated from the seeds of seven soybean cultivars.

Fungi	Frequency (%)							Mean
	Giza 82	Giza 35	Giza 21	Toamo	Holla day	Crawfo rd	H15L 17	
Aspergillus flavus (Link) Fr.	18.5	10.6	4.2	26.4	33.1	2.0	43.2	19.71
A. niger Van Tieghem	12.4	8.2	6.1	16.2	40.9	5.2	28.1	16.75
A. versicolor (Wuill) Tiraboschi	4.6	0.0	0.0	10.5	9.8	0.0	11.4	5.71
A. sydowi (Bain & Sart) Thom & Church	3.3	1.9	1.2	7.5	14.2	0.0	14.0	6.01
A. quadrilineatus Thom & Raper	13.2	7.7	4.0	19.0	16.1	6.5	21.0	12.5
A. nidulans (Eidam) Wint	2.2	3.1	1.2	6.6	8.2	1.1	10.2	4.66
A. melleus Yukawa	1.4	1.0	0.0	7.4	10.2	0.0	10.9	4.41
A. ochraceus Wilhelm	11.6	5.4	0.0	17.9	13.4	0.0	13.2	8.79
A. parasiticus Speare	6.6	4.2	0.0	8.9	10.7	1.2	11.4	6.14
Mean	8.20	4.68	1.86	13.38	17.4	1.78	18.16	

Percentage was calculated on the basis of 400 seeds.

L. S. D at 5%: Fungi (F) = 2.39

Cultivar (C) = 3.09

F x C = 6.14

4. Holladay soybean seeds were readily invaded by *A. flavus*, this extensive invasion followed by a greatly reduce germinability, and

there was only a slight reduction in non-inoculated seeds About half of the H15L17 soybean seeds were invaded by *A. flavus*, and also H15L17 seeds suffered a serious decline in germinability, if compared with the control seeds. The positive correlation between the reduction in germination and fungal invasion of soybean seeds was studied by several investigators, Chamberlain and Gray (1974), and Kabeere and Taligoola (1983). They reported that (*A. flavus, A. niger, A. terreus, Alternaria tenuis*, and *Fusarium* sp.) were to invade soybean seeds leading to a reduction in germination. On the other hand, Morsy *et al.*, (1982) reported that *Alternaria tenuis* and *Aspergillus flavus* showed the highest percentage of seed invasion in both soybean cultivars (Clark and Woodworth), also, the same authors found that the most aggressive fungi were *Aspergillus terreus* and *A. flavus* which penetrated the seeds of various soybean cultivars in high proportion during the first 10 days after inoculation. The percentage of invaded seed required to reduce germination may depend on a number of factors such as duration of storage (Wallen and Seaman, 1963), degree of infection of each seed (Kulik and Schoen, 1981), fluctuation in moisture content of seed, susceptibility of each cultivar to the fungal infection occurring in the field (Bechtel *et al.*, 1985), and /or the chemical structural of seeds and the interior changes in their constituents during fungal invasion (Arafa *et al.*, 1999).

IV-Moisture content and aflatoxin production of *A. flavus* soybean seeds:

Results in Table (3) show that the moisture content of noninoculated seed control in all cultivars increased slightly with the storage time, the same trend was obtained from inoculated seeds, except seeds of H15L17, Holladay and Toamo cultivars. The affected by aflatoxin was detected only in the controls of cultivars, H15L17, Holladay, and Toamo and traces of aflatoxin were found in Giza 35 only. Therefore, there might be a correlation between the high moisture content and high seed fungi invasion. Moisture content of 13 - 14 % might be the most suitable for seed invasion with storage fungi and this was reported by

Table (2): Invasion of *Aspergillus flavus* and germinability of soybean seed stored at 25% moisture and 26 ± 2 °C for 10 days

Soybean cultivar seeds	Treatment	Fungus seed invasion (%) at 10 days storage	Germinability	
			Control (%)	*A. flavus* inoculated %
Giza 82	Inoculated [b]	12	93 (95) [c]	84
	Control [d]	0		
Giza 35	Inoculated	16	91 (92)	80
	Control	2		
Giza 21	Inoculated	1	96 (98)	92
	Control	0		
Toamo	Inoculated	22	87 (90)	62
	Control	1		
Crawford	Inoculated	3	94 (96)	90
	Control	0		
Holladay	Inoculated	28	84 (89)	44
	Control	2		
H15L17	Inoculated	46	85 (88)	51
	Control	4		
L. S. D. at 5%		4.6	2.1 3.4	6.7

i) Data from three lots of inoculated seeds and one lot of control seeds for each cultivar.

ii) Each lot was inoculated with spores of *A. flavus*.

iii) Germinability at 0 time.

iv) Seeds inoculated with fungus-free agar discs.

Table (3): Moisture content and detected aflatoxin of control and inoculated seed stored at 25% moisture and 26 ± 2 °C for 10 days.

Cultivar seeds	Control			Seeds inoculated with *A. flavus*.	
	Moisture content %[a]		Aflatoxin mg/kg seeds	Moisture content (%)[a]	Aflatoxin mg/kg seeds
	Initial	Final			
Giza 82	12.1[b]	12.9[c]	0	13.6[c]	25
Giza 35	11.9	12.7	Traces	13.2	30
Giza 21	12.2	12.9	0	13.0	25
Toamo	12.5	13.1	15	13.9	120
Crawford	11.8	12.5	0	12.4	0
Holaday	12.6	13.2	20	13.8	140
H15L17	12.8	13.4	25	14.0	130

i) Wet weight basis. Moisture content (M. C) at 0 time.

ii) Moisture content after storage time.

(Dorworth and Christensen, 1968), and this invasion may be accompanied or followed by a decrease in germination and an increase in mycotoxin production (Zohri, 1993 and Arafa *et al.*, 1999).

In concern of aflatoxin production. Also, the results presented in Table (3) show that the amount of aflatoxin produced varied among the different cultivars tested. One cultivar (Crawford) no aflatoxin was detected. Three cultivars (Toamo, Holladay, and H15L17) were highly susceptible to the aflatoxin accumulation where (the amount of the aflatoxin given ranged from 120 µg/kg to 140 µg/kg seeds). Similar observations were reported by Shotwell *et al.*, (1978) on where aflatoxin accumulation was dependent on the variety of soybean. Zohri, (1993), showed that three cultivars of cowpea were highly resistant to seed invasion and aflatoxin production, while 8 cultivars showed seems to be partial resistance (aflatoxin produced was about 25 µg/kg seeds). The remaining cultivars were highly susceptible to the establishment of *A. flavus* and aflatoxin accumulation (the amount of formed aflatoxin ranged from 55 µg/kg to125 µg/kg seeds).

Also, low levels of aflatoxin were obtained when toxigenic fungi artificially infected other leguminous crops. Hitokoto *et al.*, (1981) examined 604 samples of six different types of beans to determine their suitability for use as soiled substrates for mycotoxin production. The authors found that the produced aflatoxin levels were 0.25, 0.5, 1.0, 2.0, 2.0 and 4.0 mg/kg seeds of pea beans, red beans, lima beans, kidney beans, green peas and cowpea, respectively.

In meantime, El-Kady *et al.*, (1991) examined 100 different cultivars and lines of broad bean seeds to determine varietal differences which may support or resist aflatoxin production and found that 11 cultivars /lines were highly resistant to seed invasion and aflatoxin production while 9 cultivars/lines showed partial resistance.

V-Varietal differences of soybean cultivars in aflatoxin production related to vigour tests and some chemical seed components:

The elucidation of precise chemical mature of factors responsible for varietal differences in susceptibility to aflatoxin production will help plant geneticists to breed strains with such physiological and biological parameters. A systematic examination of the different constituents (total nitrogen, total phosphate, Ca^{++}, Mg^{++}, Zn^{++}, K++ and Na^{+} contents) of both testas and kernels of susceptible, partially and highly resistant soybean cultivars seed were carried out.

1- Vigour tests:

a- Electric conductivity and overall dehydrogenase:

Results in Table (4) revealed that cultivar Holladay gave the highest amount of exudates (1.48 µs/5seeds) but with no significant differences with the two others (0.6 and 0.5 µs/5seeds).

In concern with the overall dehydrogenase, Crawford and Giza 21 gave more significant activity than Holladay (9.60, 9.05 and 5.40), respectively.

b- Oxygen uptake:

Results of oxygen uptake (Table, 4) showed that the cultivars Crowford and Giza 21 took up significant more oxygen than Holladay as the recorded figures were successively 0.52, 0.48 and 0.24 (02(µl)/min/1 sprout).

Table (4): Some biological characters in healthy seeds of three soybean cultivars that are susceptible (Holladay), partially resistant (Giza 21), and highly resistant (Crawford) with respect to aflatoxin production.

Tested soybean cultivar	Electroconductivity of exudates after 24 hrs. of soaking (µs/5seeds)	Over-all dehydrogenase after 24 hrs. soaking (mg/ phenyl formazane/seed)	Amount of O2 (µL) taken up per min. by one sprout
Giza 21	0.60	9.05	0.48
Crawford	0.50	9.60	0.52
Holladay	1.48	5.40	0.24
L.S.D at 5%	0.90	2.71	0.22

2- Mineral analysis:

Examination of the different seed constituents revealed an absence of a significant variation in total nitrogen and magnesium in susceptible, partially and highly resistant cultivars (Table, 5). The susceptible seed contained the lower calcium concentration, followed by the partially resistant and the highest level was recorded in the highly resistant ones. These results in agreement with those obtained by Howell (1970) who examined 3114 different seed and grain samples and found that soybeans did not constitute a good substrate for aflatoxin production. He speculated that the levels of calcium in soybeans, which are higher than those of cereals, might be inimical to toxin production. Mashaly and El-Deeb (1983) showed that calcium stimulated aflatoxin production and enhanced the growth rate of all seven *Aspergillus flavus* and two *A. parasiticus* strains tested in synthetic media. The effect gradually increased with increasing of concentrations calcium in the culture medium.

The mean values of zinc in both the testa and kernel of the partially and highly resistant seeds were significantly low as compared to those obtained from susceptible soybean seed cultivars (Table, 5). The stimulatory effect of zinc on a aflatoxin formation is well documented by Mateles and Adye, 1965; Lee *et al.*, 1966; Davis *et al.*, 1967; Lillehoj *et al.*, 1974; Marsh *et al.*, 1975 and Zohri, 1993. Of all the trace elements previously investigated, zinc seems to play a role in the biosynthesis of secondary fungal metabolites including aflatoxins. At least, twenty enzymes have been found to be zinc dependent (Parisi and Valee, 1969), which may partly account for its role. Adye and Mateles (1964) and Mateles and Adye (1965) showed that deletion of zinc (2 µg/g) reduced aflatoxin yield by 88% without restricting the growth of the fungus. However, Obadiah and Dubois (1981) reported that absence of zinc completely blocks fungal growth and aflatoxin production. Zohri (1993) found that the mean values of zinc in both the testa and kernel of the partially and highly resistant seeds were significantly low as compared to those obtained from susceptible cowpea seed cultivars.

Also, the results obtained in the present study clearly show that there was a significant difference in the total phosphate concentrations of the different cultivars (Table, 5). The total phosphate content of the susceptible, partially and highly resistant seeds were 5.87, 7.78 and 8.01 mg/g of seeds, respectively. In terms of phosphate content, the phytic acid present (80% of total phosphate) constitutes 4.69, 6.22 and 6.41 mg g of susceptible, partially and highly resistant seeds, respectively. These results are in agreement with the finding of Gopalan *et al.*, (1971); Gupta and Venkitasubramanian, (1975); and Zohri, 1993 who recorded that the phytic acid concentrations in soybean, groundnut, wheat, peas, rice and cowpea were 6.90, 3.90, 3.06, 2.98, 1.90 and 6.26 mg/g, respectively. Gupta & Venkitasubramanian, (1975)

attributed the negligible amount of aflatoxin obtained with non-autoclaved soybean (0.335 mg/100g of seeds) to the binding of zinc with phytic acid that then rendered it unavailable. In case of autoclaved soybeans, a large amount of aflatoxin was obtained being 6.85 mg/100 g of seeds. They added that at high temperatures the phytic acid is broken down and the zinc would be released.

Table (5): The mean values (MV) ± standard deviation (SD) of calcium, magnesium, total nitrogen, zinc, potassium, sodium and total phosphate contents (mg/g of dry seed) in different seeds of soybean cultivars which are susceptible (Holladay), partially resistant (Giza 21) and highly resistant (Crawford) with respect to aflatoxin production.

Elements	Soybean cultivars seed	Kernel MV±SD	Testa MV±SD	Total MV±SD
Calcium	A*	1.120±0.65(a)	2.600±1.44(a)	
	B	1.450±0.52(a)	4.120±1.21(ac)	
	C	2.720±0.69(c)	4.260±1.50(c)	
Magnesium	A	0.516±0.14(a)	0.842±0.19(a)	
	B	0.426±0.10(a)	0.990±0.22(a)	
	C	0.394±0.12(a)	1.001±0.11(a)	
Total nitrogen	A	3.059±0.31(a)	3.370±0.23(a)	
	B	4.166±0.24(a)	4.490±0.80(a)	
	C	4.277±0.27(a)	4.881±0.35(a)	
Zinc	A	0.070±0.02(a)	0.084±0.02(a)	
	B	0.068±0.01(1)	0.062±0.01(c)	
	C	0.105±0.01(c)	0.119±0.01(c)	
Potassium	A	1.242±0.09(a)	0.884±0.04(a)	
	B	1.614±0.08(c)	0.749±0.06(a)	
	C	1.992±0.10(c)	0.924±0.05(a)	
Sodium	A	0.840±0.08(a)	1.074±0.18(a)	
	B	0.620±0.09(c)	1.044±0.14(a)	
	C	0.550±0.07(c)	1.000±0.14(a)	
Total phosphate	A	4.170±0.49(a)	1.700±0.36(a)	5.87±0.44(a)
	B	5.840±0.80(a)	1.940±0.40(a)	7.78±0.74(c)
	C	5.920±0.81(a)	2.090±0.66(a)	8.01±055(a)

* A: Holladay, B: Giza 21, and C: Crawford soybean cultivars.

** Values in the same column followed by the same letter are not significantly different at 5% level by Duncan's multiple range test.

The seeds of resistant soybean cultivars contain higher phytate concentrations (6.41 mg / g) and lower zinc contents (4.69 mg / g) as compared with the high susceptible seeds (Table, 5).

Also, results in Table, (5) reveal that there was a significant variation in potassium concentration in seed kernels of the different cultivars. The mean potassium content was low in the susceptible seeds compared to the partially and highly resistant ones. These results are in the line with those of Davis *et al.*,(1967) who showed that increasing the amount of potassium sulphate (K2SO5) inhibited production of aflatoxin without a corresponding decrease in growth. They also reported that potassium fluoride somewhat inhibited aflatoxin synthesis.

Significant differences in sodium concentration were recorded between the different cultivars. The susceptible seeds contained the higher sodium concentration, followed by the partially resistant and the lowest sodium level was recorded in the highly resistant seeds (Table, 5). These results are confirmed with these of Buchanan and Ayres 1976 and Mashaly and El-Deeb, (1983)) who showed a significant increases in both mycelial growth and aflatoxin production by *Aspergillus flavus* and/or *A. parasiticus* when the culture media were supplemented by 1-4% sodium chloride.

From the reviewed results of Uraih and Chipley, 1976 and that obtained from this investigation it could be concluded that low concentrations of sodium may stimulate some enzymes responsible for aflatoxin synthesis by the toxigenic fungi.

It appears that susceptibility or resistance of soybean cultivars for *A. flavus* colonization and aflatoxin formation is influenced by the complex interaction of several factors. Calcium, potassium, sodium, total phosphate and zinc are essential trace elements for aflatoxin synthesis. Zinc and sodium levels were markedly increased in susceptible cultivars as compared with those of resistant seeds. On the contrary, the highest levels of calcium, potassium and total phosphate were recorded in the highly resistant cultivars.

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ملائمة أصناف فول الصويا لقدرة الفطر اسبيرجيلس فلافس الممرضة وإنتاج الافلاتوكسين و بعض العوامل المؤثرة

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الملخص العربي

عزلت من بذور سبعة أصناف من فول الصويا بعد الحصاد مباشرة، تسعة أنواع من فطر الاسبيرجلس وكانت أكثرها سيادة عند العزل هما الفطرين اسبيرجلس فلافس و اسبيرجلس نيجر.

احتوت بذور صنف فول الصويا H15L17 وهوليداى على أعلى نسبة تكرار لعزل أنواع الاسبيرجلس، بينما جيزة 21 و كراوفورد كانت الأقل.

كان للفطرين اسبيرجلس فلافس، واسبيرجلس برازيلتكس فقط القدرة من بين كل الأنواع على إنتاج سموم الأفلاتوكسين.

عند الحقن الصناعي لبذور سبعة أصناف فول الصويا بجراثيم الفطر اسبيرجلس فلافس وجد أن ثلاثة أصناف وهى H15L17، هولياداي، وتومو ذات حساسية عالية لغزو الفطر ونموه وإنتاج الافلاتوكسين، أما الأصناف جيزة 21، جيزة 35، وجيزة 82 فكانت لها مقاومة جزئية بينما الصنف الباقي كراوفورد فكان عالي المقاومة. و قد كانت الصفات البيولوجية للبذرة هي الأفضل في الأصناف العالية أو الجزئية المقاومة عند المقارنة بالأصناف الحساسة. هذا ولم يلاحظ وجود اختلافات واضحة في المحتوى النيتروجيني أو كمية الماغنسيوم بين الأصناف المقاومة أو القابلة للإصابة، أما كمية الكالسيوم و البوتاسيوم والفوسفات فكانت في البذور القابلة للإصابة قليلة بالمقارنة بتلك البذور التي تكون مقاومة للفطر، وكانت عكس ذلك تماما في حالة عنصري الصوديوم والخارصين.