Experimental results and Discussion

IV- RESULTS AND DISCUSSION

4.1. Isolation and Identification of fungi (Mold):

4.1.1. From soybean seeds (variety Crawford):

Table (1) shows the total counts and percentage of incidence of (mold) fungi isolated from soybean (*Glycine max L.*, variety Crawford) seeds on different media.

4.1.1.1. Firstly from finely ground soybean seeds:

Results in table (1) indicate that 74 fungal isolates were obtained from seeds of soybean (finely ground) on three different media , of which 23 isolate on Dox medium , 22 isolate on sabouraud medium and 29 isolate on potato-dextrose agar (PDA) medium.

Aspergillus niger was the most dominant fungus in the Dox medium, it was represented by (39.13%) of the isolated fungi, then followed by *Penicillium corlophilum* (34.78) and *Aspergillus flavus* (26.08) in a decreasing order. While in the sabouraud medium, the dominant fungus is *Aspergillus niger*, it was represented by (63.63%) of the isolated fungi, then followed by *penicillium corylophilum* (27.27%) and *Aspergillus flavus* (9.09%) in a decreasing order.

In PDA medium, the dominant fungus is *Aspergillus niger*, it was represented by (51.72%) of the isolated fungi, followed by *Penicillium corylophilum* (37.93%) and *Aspergillus flavus* (10.34%) in a decreasing order.

4.1.1.2. Secondly From whole soybean seeds:

The results showed that 46 fungal isolates were obtained from seed of soybean (whole seed) on three different media, of which 17 isolate on Dox. medium, 9 isolate on sabouraud and 20 isolate on PDA medium.

Aspergillus niger was the dominant fungus in the Dox. medium, it was represented by (82.35%) of the isolated fungi, following by *Aspergillus flavus* (11.76%) and *Penicillium corylophilum* (5.88%) in a decreasing order.

While in sabouraud medium, the *Aspergillus niger* was the dominant fungus, it was represented by (66.66%) of the isolated fungi, then followed by *Aspergillus flavus* (22.22%) and *Mucor racemosus* (11.11%) in a decreasing order.

In PDA medium, the *Aspergillus niger* was the most dominant fungus, it was represented by (75%), then followed by *Aspergillus flavus* (25%) in a decreasing order.

The obtained data, with the line of ***Kennedy (1964)***, he found that predominant genera of fungi isolated from soybean seeds were *Aspergillus, Alternaria, Fusarium* and *Penicillium*.

Table (1) : Total counts and percent of incidence (mold) fungi isolated from soybean (*Glycine maxL.*, variety Crawford) seeds on different media.

Mold species	Media (Percent of incidence)						Total count[d]
	Dox		Sab.		PDA		
a- From finely ground seeds							
Aspergillus spp.							
A. flavus **Link:Fr.**	6	(26.08%)	2	(9.09%)	3	(10.34%)	11
A. niger **Van Tieghem**	9	(39.13%)	14	(63.63%)	15	(51.72%)	38
Penicillium spp.							
P. corylophilum **Dierckx**	8	(34.78%)	6	(27.27%)	11	(37.93%)	25
Total count[c]	23		22		29		74
b- From whole seeds							
Aspergillus spp.							
A. flavus **Link:Fr.**	2	(11.76%)	2	(22.22%)	5	(25%)	9
A. niger **Van Tieghem**	14	(82.35%)	6	(66.66%)	15	(75%)	35
Penicillium spp.							
P.corylophilum **Dierckx.**	1	(5.88%)	-	-	-	-	1
Mucor spp.							
M. racemosus **Fresenius**	-	-	1	(11.11%)	-	-	1
Total count[c]	17		9		20		46

Dox, Czapek-Dox agar medium *(Thom & Raper, 1945);* Sab., Sabouraud agar medium *(Oxoid, 1982)* and PDA, potato Dextrose-agar medium *(Christensen, 1957);* c, total count for mold species grown on one tested medium and d, total count for mold species grown on the three different tested media.

4.1.2. From sorghum (low tannin, variety Dorado) grains:

Table (2) shows the total counts and percentage of incidence of (mold) fungi isolated from sorghum (*sorghum bicolor*, low tannin, variety Dorado) grains on different media.

4.1.2.1. Firstly from finely ground low sorghum grains:

Results in table (2) indicate that 63 fungal isolates were obtained from grains of sorghum (finely ground) on three different media of which 20 isolate on Dox. medium, 18 isolate on sabouraud medium and 25 isolate on PDA.

Alternaria alternatia was the most dominant fungus in the Dox media, it was represented by (50%) of the isolated fungi then followed by *Aspergillus niger* (25%), then followed by *Aspergillus flavus* & *Penicillium corylophilum* (10%) and *Fusarium solani* (5%) in a decreasing order.

While in the sabouraud medium, the dominant fungus is *Alternaria alternatia*, it was represented by (66.66%) of the isolated fungi, then followed by *Aspergillus niger* & *Fusarium solani* (11.11%) and *Aspergillus flavus* & *Penicillum corylophilum* (5.55%) in a decreasing order. In PDA medium, the dominant fungus is *Alternaria alternatia*, it was represented by (88%) of the isolated fungi, then followed by *Aspergillus niger*; *Fusarium solani* and *Penicillium corylophilum* (4%) in a decreasing order.

4.1.2.2. Secondly from whole low sorghum grains:

The results showed that 55 fungal isolates were obtained from grains of sorghum (whole grain) on three different media, of which 15

isolate on Dox. medium, 20 isolate on sabouraud medium and 20 isolate on PDA medium.

Alternaria alternatia was the dominant fungus in the Dox medium, it was represented by (53.33%) of the isolated fungi, then followed by *Aspergillus niger* (26.66%) and *Aspergillus flavus*, *Fusarium solani*, *Stemphylium botryosum* (6.66%) in a decreasing order.

While in the sabouraud medium, the dominant fungus is *Alternaria alternatia*, it was represented by (70%) of the isolated fungi, then followed by *Aspergillus niger* (10%), then followed by *Cladosporium cladosporiods*; *Curvularia spicifera*; *Fusarium solani* and *Stemphylium botryosum* (5%) in a decreasing order. Whereas in PDA, the dominant fungus is *Alternaria alternatia*, it was represented by (100%) of the isolated fungi.

Table (2): Total counts and percent of incidence of (mold) fungi isolated from sorghum[a] (*sorghum bicolor* low tannin, variety Dorado) grains on different media

Mold species	Media (percent of incidence)						Total count[d]
	Dox		Sab.		PDA		
a- From finely ground grains.							
Alternaria spp.							
A. alternatia	10	(50%)	12	(66.66%)	22	(88%)	44
Aspergillus spp.							
A. flavus	2	(10%)	1	(5.55%)	-	-	3
A. niger	5	(25%)	2	(11.11%)	1	(4%)	8
Fusarium spp.							
F.solani	1	(5%)	2	(11.11%)	1	(4%)	4
Penicillium spp.							
P. corylophilum	2	(10%)	1	(5.55%)	1	(4%)	4
Total count[c]	20		18		25		63
b- From whole grains							
Alternaria spp.							
A. alternata (Fries.) keissler	8	(53.33%)	14	(70%)	20	(100%)	42
Aspergillus spp.							
A. niger Van Tieghem	4	(26.66%)	2	(10%)	-	-	6
A. flavus Link: Fr.	1	(6.66%)	-	-	-	-	1

Continued Table (2):

Cladosporium spp.							
C. cladosporiods **(Fres.) de Vries.**	-	-	1	(5%)	-	-	1
Curvularia spp.							
C. spicifera **(Bainier) Boedijn.**	-	-	1	(5%)	-	-	1
Fusarium spp.							
F.solani **(Mart.) Sacc.**	1	(6.66%)	1	(5%)	-	-	2
Stemphylium spp.							
S.botryosum **Wallroth**	1	(6.66%)	1	(5%)	-	-	2
Total count [c]	15		20		20	(100%)	55

Dox, Czapek-Dox agar medium *(Thom & Raper, 1945);* Sab., Sabouraud agar medium *(Oxoid, 1982)* and PDA, potato Dextrose-agar medium *(Christensen, 1957);* c, total count for mold species grown on one tested medium and d, total count for mold species grown on the three different tested media.

4.1.3. From sorghum (high tannins, variety Framida) grains:

Table (3) shows the total counts and percentage of incidence of (mold) fungi isolated from sorghum (*sorghum bicolor*, high tannins, variety Framida) grains on different media.

4.1.3.1: Firstly from finely ground high sorghum grains:

Results in table (3) indicate that 16 fungal isolates were obtained from grains of sorghum (finely ground) on three different media of which 3 isolate on Dox. medium, 6 isolate on sabouraud and 7 isolate on PDA. *Penicillium corylophilum* was the most dominant fungus in the Dox medium, it was represented by (66.66%), of the isolated fungi, then followed by *Alternaria alternatia* (33.33%) in a decreasing order.

While in Sab., medium, the dominant fungus is *Pencillium corylophilum*, it was represented by (50%) of the isolated fungi, then followed by *Aspergillus niger* (33.33%) and *Penicillium funculosum* (16.66%) in a decreasing order.

In PDA medium, the dominant fungus is *Penicillium corylophilum*, it was represented by (57.14%), then followed by *Aspergillus niger* (28.57%), and *Alternaria alternatia* (14.28%) in a decreasing order.

4.1.3.2. Secondly from whole high sorghum grains:

The results showed that 27 fungal isolates were obtained from grains of sorghum (whole seed) on three different media, of which 6 isolate on Dox. medium, 9 isolate on Sab., and 12 isolate on PDA.

A.niger is the dominant fungus is Dox medium, it was represented by (50%), then followed by *Alternaria alternatia* (33.33%) and *A.flavus* (16.66%) in a decreasing order.

While in Sab., medium, the dominant fungus is *A.niger*, it was represented by (55.55%), then followed by *Stemphylium botryosum* (22.22%), *A.flavus* and *Cladosporium cladosprodies* (11.11%) in a decreasing order.

In PDA, the dominant fungus is *Alternaria alternatia*, it was represented by (41.66%) , then followed by (*Aspergillus niger* and *Stemphylium botryosum*), its were represented by (25%), and followed by *Fusarium solani* (8.33%).

The obtained da`ta, in tables (3&4) with the line of those ***Amein (1970)*** and ***D'ercole and Nipoti (1979). Amein (1970)*** stated that the seed borne fungi of sorghum grains consist of *Alternaria alternatia*, *Aspergillus niger*, *Curvularia spp* and *Fusarium spp.* while, ***D'ercole and Nipoti (1979)***, reported that from surface sterilized sorghum grains, *Penicillium spp.*, *Alternaria spp.*, *Rhizopus* and *Trichoderma spp.*, were most oftenly encountered followed by *Fusarium spp.*

Table (3): Total counts and percent of incidence of (mold) fungi isolated from sorghum (*sorghum bicolor*, high tannins, variety Framida) grains on different media.

Mold species	Media (Percent of incidence)						Total count[d]
	Dox		Sab.		PDA		
a- From finely ground grains.							
Alternaria spp.							
A. alternatia **(Fries) keissler**	1	(33.33%)	-	-	1	(14.28%)	2
Aspergillus spp.							
A. niger **Van Tieghem**	-	-	2	(33.33%)	2	(28.57%)	4
Penicillium spp.							
P. corylophilum **Dierckx**	2	(66.66%)	3	(50%)	4	(57.14%)	9
P. funculosum **Thom**	-	-	1	(16.66%)	-	-	1
Total count[c]	3		6		7		16
b - From whole grains.							
Alternaria spp.							
A. alternatia **(Fries) keissler**	2	(33.33%)	-	-	5	(41.66%)	7
Aspergillus spp.							
A. niger **Van Tieghem**	3	(50%)	5	(55.55%)	3	(25%)	11
A. flavus **Link:Fr.**	1	(16.66%)	1	(11.11%)	-	-	2
Cladosporium spp.							
Cladosporium cladosporides **(Fres.) de Vries.**	-	-	1	(11.11%)	-	-	1
Fusarium spp.							
Fusarium solani **(Mart.) Sacc.**	-	-	-	-	1	(8.33%)	1
Stemphylium spp.							
Stemphylium botryosum **Wallroth**	-	-	2	(22.22%)	3	(25%)	5
Total count[c]	6		9		12		27

Dox, Czapek-Dox agar medium *(Thom & Raper, 1945);* Sab., Sabouraud agar medium *(Oxoid, 1982)* and PDA, potato Dextrose-agar medium *(Christensen, 1957);* c, total count for mold species grown on one tested medium and d, total count for mold species grown on the three different tested media.

Fig. (5):Non infected germinated (healthy) soybean seed (variety Crawford) 5 days at 25°c

Fig. (6):An infected germinated soybean seed (variety Crawford) 5 days at 25°c by *Aspergillus flavus* Note the *discoloration* (brownish) of the infected seed.

Fig. (7): An infected germinated soybean seed (variety Crawford) 3 days at 25°c by *Aspergillus niger*. Note the appearance of small radical, the cracks of the seed coat and the slight discoloration of the seed

Fig. (8): An infected non germinated soybean seed (variety Crawford) 4 days at 25°c by *Aspergillus niger* Note the cracks of seed coat.

Fig. (9):An infected non germinated soybean seed (variety Crawford) 5 days at 25°c infected by *Aspergillus flavus* Note the discoloration of the infected seed and the vigorous growth of invading fungus on the seed surface .

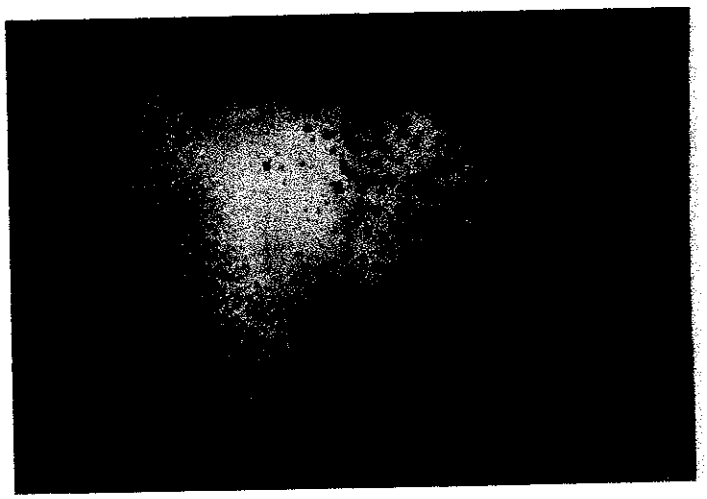

Fig. (10): An infected non germinated soybean seed (variety Crawford) infected by *Aspergillus flavus (*left) and *Aspergillus niger* (right) 5 days at 25°c.Note the brown discoloration of the infected seed

Fig. (11): Non infected germinated sorghum grain(healthy) sorghum grain (high tannin variety Framida) 2 days at 25°c.

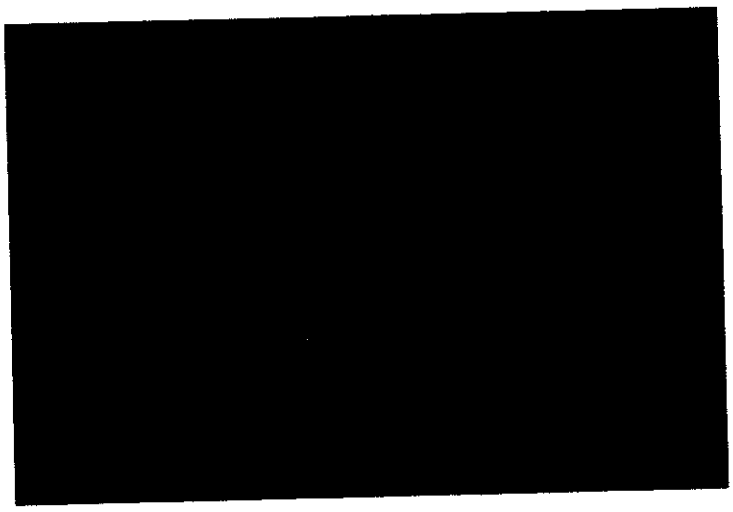

Fig. (12): Non infected germinated(healthy) sorghum grain (high tannin variety Framida) 4 days at 25°c.Note the elongated radical and the initiation of the hypocotyl formation

Fig. (13): Non infected germinated(healthy) sorghum grain (high tannin variety Framida), 5 days at 25°c. Note further of the hypocotyl as compared with fig (12).

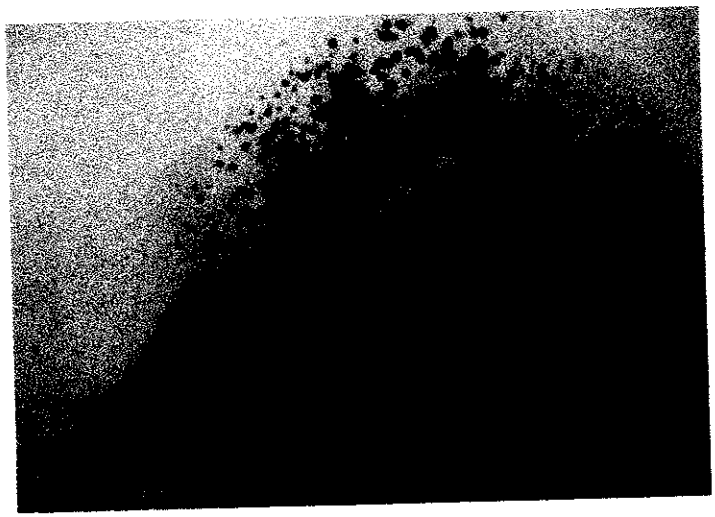

Fig. (14): An infected, germinated sorghum grain (high tannin variety Framida) by the (mold) fungus *Aspergillus niger*, 5 days at 25°c.Note the difference between the radical growth shown in figs 13 and 14.

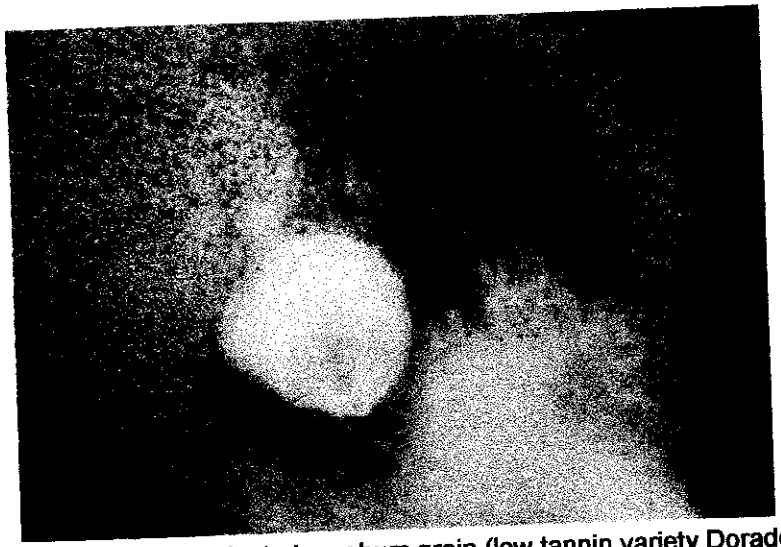

Fig. (15): An infected germinated sorghum grain (low tannin variety Dorado) by two species of the(mold) fungus *Aspergillus* i.e *niger* (top right) and *A. flavus*(left), 5 days at 25°c.Note the white "colony" mycelium associated with the fungal spores and the discoloration of the small radical.

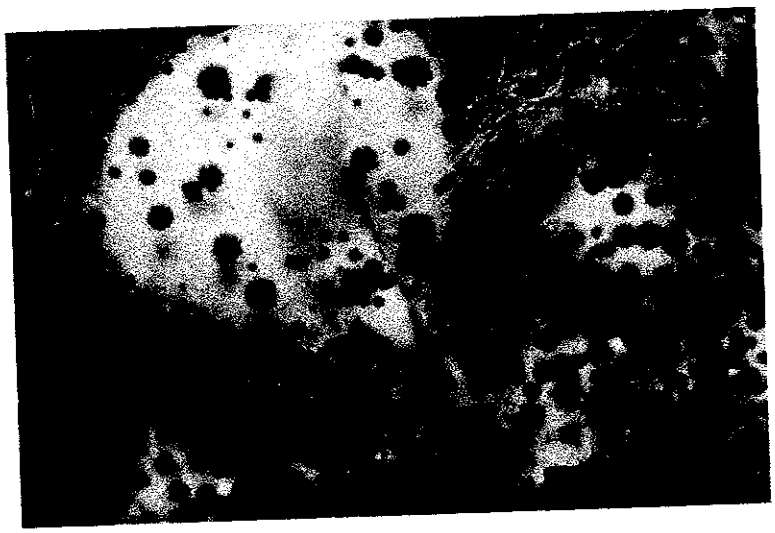

Fig. (16): An infected slightly germinated sorghum grain (low tannin variety Dorado) by *Alternaria alternata*, 5 days at 25°c. Note the:pattern of fungal colonies (rosette shape) on the grain coat and the brown discoloration of the radical and the grain coat.

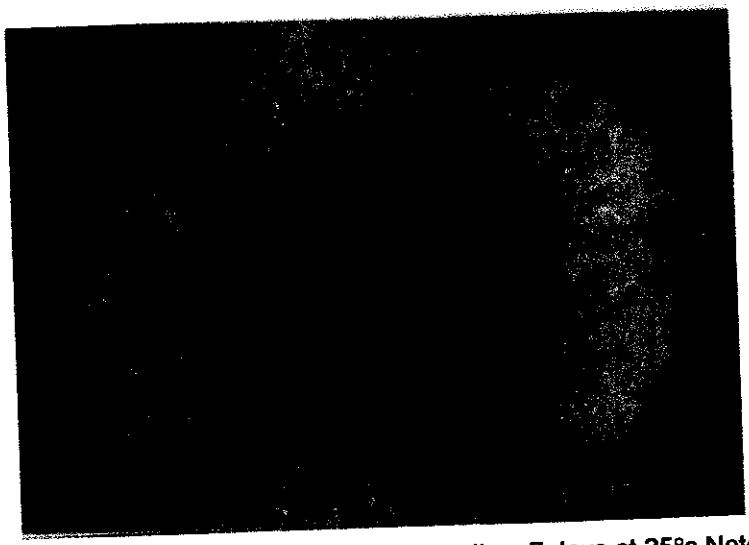

Fig. (17): *Aspergillus niger* grow on Dox medium 7 days at 25°c.Note, mycelium white at the margins, but black centrally. Tested using the finely ground soybean (variety Crawford) .

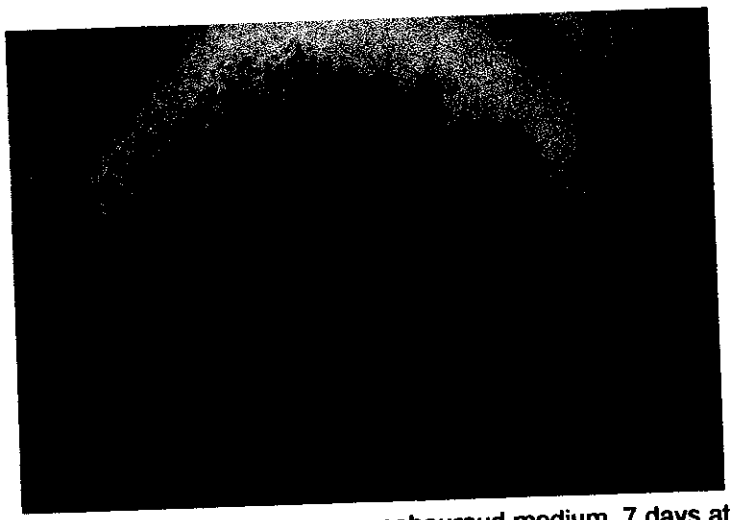

Fig. (18): *Aspergillus niger* grow on sabouraud medium, 7 days at 25°c. Tested using the finely ground soybean . Note, mycelium white at the margins, but black centrally

4.2. Chemical composition of plant commodities (soybean & sorghum).

4.2.1. Chemical composition of soybean seeds and sorghum grains, stored under different gaseous conditions.

4.2.1.1. Soybean

Table (4) shows the chemical composition of soybean seeds (variety Crawford) stored under atmospheric air, carbon dioxide and nitrogen gases for 3 and 6 months at room temperature. The freshly harvested soybean seeds characterized with 34.23% protein content, 22.12% total lipids, 6.10% crude fiber, 5.15% ash, 32.40% total carbohydrates, 6.2% total soluble sugars, 3.50% reducing sugars and 2.70% non reducing sugars on dry basis. When soybean seeds were stored under atmospheric air, CO_2 or N_2 for 3 and 6 months, protein content showed a slight increase ranged from 0.41 to 3.56%. Total lipids increased also in the range of 0.8 to 7.60%. Crude fiber showed similar trend which increased in the range of 1.97 to 11.64%. Ash increment ranged from 0.5 to 4.27%.

The maximum increment of protein, total lipids, crude fiber and ash were found in the stored soybean seeds under air followed by CO_2 and N_2 compared with control. Total soluble sugars and reducing sugars showed similar trend as the abovementioned chemical composition. An opposite trend was found due to total carbohydrates and non reducing sugars compared with control. The decrease in total carbohydrates ranged from 1.23% to 13.83% which is parallel with the increasing with the other chemical composition. From the above mentioned data, it could be concluded that storage under atmospheric air caused some deterioration in the chemical composition of soybean seeds especially

carbohydrates due to the seed respiration, followed by CO_2. Meanwhile, storage under N_2 keep the quality of soybean seeds.

The obtained data were in the line with the findings of ***El-Habbal et al., (1991)***, found that, crude protein concentration was increased in the inoculated seeds while the oil and carbohydrates concentrations decreased. And ***Glass et al., (1959)***, found that, storage grains in nitrogen prevented mold growth at all moisture level.

4.2.2. Chemical composition of soybean seeds and sorghum grains, inoculated with *Aspergillus flavus* and *Penicillium corylophylium* and stored under different gaseous conditions.

4.2.2.1. Soybean (inoculated with *A.flavus*).

Table (4) shows also the chemical composition of soybean seeds inoculated with *A.flavus* and stored under atmospheric air, CO_2 and N_2 gases for 3 and 6 months at room temperature compared with the freshly harvested seeds. The protein content increased by about 9.73% and 13.5% when the seeds stored under air for 3 and 6 months, respectively. While it was 5.05% and 5.76% respectively for the same periods of storage under N_2. Crude fiber, ash, total soluble sugars and reducing sugars showed similar trend as that of protein and it could be explained as follows. The increment of crude fiber was 18.03% and 25.74% under air, 12.3% and 18.85% under CO_2, 4.1% and 6.88 under N_2 for 3 and 6 months of storage respectively.

Ash resulted in 10.29% and 15.15% under air, 4.27 and 5.83% under CO_2, 0.97% and 3.3% under N_2, respectively after the storage periods.

Total soluble sugars showed an increment by about 21.77% and 28.4% under air; 0.65% and 12.9% under CO_2, 0.81% and 9.2% under N_2 for storage periods, respectively.

Reducing sugars showed also an increment by about 37.1% and 70.86% under air, 20.3% and 68% under CO_2 ; 20.57 and 32.3% under N_2 for the above mentioned storage periods, respectively.

An opposite trend was found concerning total carbohydrates, lipids and non-reducing sugars, which showed a decrease in its amounts due to inoculation with *A.flavus* and storage conditions. The decrease in total carbohydrates was 8.77% and 13.83% under air, 5.96% and 7.41% under CO_2, 1.23% and 2.72% under N_2 respectively for storage periods.

Total lipids decreased also by 9.04% and 11.26% under air, 3.48% and 4.61 under CO_2 , 1.22% and 1.65 under N_2 , respectively for the storage periods .

Non reducing sugars showed a decrease by about 0.0% and 26.7% under air, 24.8% and 36.3% under CO_2 , 24.8% and 20.74 under N_2 for the storage periods, respectively.

From the above mentioned data it could be observed that the inoculation with *A.flavus* deteriorated soybean seeds and this deterioration can be arranged in the following descending order.

Storage under air $>CO_2>$ N_2. The deterioration was slight because of the fungal growth depends on temperature, relative humidity and available nutrients of which the storage conditions were at room temperature and the atmospheric relative humidity.

The obtained data were in the line with those of ***Robertson et al., (1973)***, found that, there was a small decrease in total oil content of the field damaged sample and the protein content was higher in the damaged beans than in the undamaged ones.

Table (4) : Chemical composition of soybean seeds (variety Crawford) inoculated with *Aspergillus flavus*[a], stored under different gases and incubated at room temperature (25°C).

Storage conditions / Chemical composition	Zero time	Chemical analysis of samples (months) after inoculation											
		Atmospheric air				Carbon dioxide				Nitrogen			
		(NI)		(I)		(NI)		(I)		(NI)		(I)	
		3months	6months	3months	6months	3months	6months	3months	6months	3months	6months	3months	6months
Crude protein	34.23	35.20	35.45	37.56	38.85	34.75	34.92	35.96	36.20	34.37	34.55	34.60	34.88
Total lipids	22.12	23.30	23.80	20.00	19.63	22.41	22.62	21.35	21.10	22.30	22.36	21.85	21.76
Crude fiber	6.10	6.60	6.81	7.20	7.67	6.32	6.47	6.85	7.25	6.22	6.28	6.35	6.52
Ash	5.15	5.30	5.37	5.68	5.93	5.20	5.28	5.37	5.45	5.18	5.20	5.20	5.32
Total carbohydrates*	32.40	29.60	28.57	29.56	27.92	31.32	30.71	30.47	30.00	31.93	31.61	32.00	31.52
Total soluble sugars	6.20	4.92	3.53	7.52	7.96	5.84	5.63	6.24	7.00	6.00	5.94	6.25	6.77
Reducing sugars	3.50	2.63	1.23	4.82	5.98	4.30	4.15	4.21	5.28	3.40	3.36	4.22	4.63
Non reducing sugars	2.70	2.29	2.30	2.70	1.98	1.54	1.48	2.03	1.72	2.60	2.58	2.03	2.14

a, soybean seeds (variety Crawford) derived aflatoxin producing isolate , isolated in this study ; (NI) non inoculated , (I) inoculated samples and *, total carbohydrates calculated by difference method, not including fiber, ***(Tadrus 1989).***

4.2.2.1.1. Soybean (inoculated with *Penicillium corylophylium*).

Table (5) shows the chemical composition of soybean seeds inoculated with *Penicillium corylophilum* and stored for 3 and 6 months under atmospheric air, CO_2, or N_2 compared with the freshly harvested seeds. The obtained results revealed an increase in crude protein, crude fiber, ash, total soluble sugars and reducing sugars and a decrease in total lipids, total carbohydrate and non reducing sugars during storage periods. The increment was 10.43% and 13.67% ; 7.9% and 9.38%; 2.83 and 3.16% for protein; 13.93% and 23.6%; 11.8 and 18.36% , 3.6% and 4.9% for crude fiber; 11.06% and 13.4% ; 7.77% and 10.1%, 1.17 and 1.9% for ash , 11.45 and 19.03%, 7.26 and 18.9% , 3.55 and 6.77% for total soluble sugars, and 39.14 and 68.57% , 26.86 and 67.43%, 25.14 and 46.86% for reducing sugars when the soybean seeds stored under air, CO_2 and N_2 for 3 and 6 months respectively compared with the freshly harvested seeds. The decrease in total lipids was 10.44 and 15.24%, 8.14 and 9.13% , 1.31 and 1.54% when the seeds was stored under air, CO_2 and N_2 for 3 and 6 months respectively.

The decrease in total carbohydrates was 8.27 and 10.27% , 6.33 and 8.73% , 2.96 and 3.52 % while non reducing sugars showed a decrease by about 22.44 and 52.60% , 18.15 and 44.10% , 24.44 and 45.20% when the soybean seeds stored under the abovementioned conditions, respectively.

From the above mentioned data, it could be observed that inoculation with *Penicillium corylophilum* resulted in some deterioration of soybean seeds and this may be due to the unsuitable conditions for fungal growth such as temperature, relative humidity and available nutrients.

The obtained data with the line of ***James and Phillip (1989)*** found that both the moisture content of the grain sample and relative humidity of the surrounding air affect microbial growth and grain spoilage.

El-Gendy (1982) revealed that storage of cereals for long periods of time caused increase of ash content by increasing the storage period due to the decrease in carbohydrates because of its use in respiration and consumption.

Table (5): Chemical composition of soybean seeds (variety Crawford) inoculated with *Penicillium corylophylium*[a] , stored under different gases and incubated at room temperature (25°C).

Storage conditions / Chemical composition	Zero time	Chemical analysis of samples (months) after inoculation											
		Atmospheric air				Carbon dioxide				Nitrogen			
		(NI)		(I)		(NI)		(I)		(NI)		(I)	
		3months	6months	3months	6months	3months	6months	3months	6months	3months	6months	3months	6months
Crude protein	34.23	35.20	35.45	37.80	38.91	34.75	34.92	36.93	37.44	34.37	34.55	35.20	35.31
Total lipids	22.12	23.30	23.80	19.81	18.75	22.41	22.62	20.32	20.10	22.30	22.36	21.83	21.78
Crude fiber	6.10	6.60	6.81	6.95	7.54	6.32	6.47	6.82	7.22	6.22	6.28	6.32	6.40
Ash	5.15	5.30	5.37	5.72	5.84	5.20	5.28	5.55	5.67	5.18	5.20	5.21	5.25
Total carbohydrates*	32.40	29.60	28.57	29.72	28.96	31.32	30.71	30.38	29.57	31.93	31.61	31.44	31.26
Total soluble sugars	6.20	4.92	3.53	6.91	7.38	5.84	5.63	6.65	7.37	6.00	5.94	6.42	6.62
Reducing sugars	3.50	2.63	1.23	4.87	5.90	4.30	4.15	4.44	5.86	3.40	3.36	4.38	5.14
Non reducing sugars	2.70	2.29	2.30	2.04	1.28	1.54	1.48	2.21	1.51	2.60	2.58	2.04	1.48

a, soybean seeds (variety Crawford) derived aflatoxin producing isolate, isolated in this study ; (NI) non inoculated , (I) inoculated samples and * , total carbohydrates calculated by difference method, not including fiber, *(Tadrus 1989).*

4.2.1.2. Sorghum

4.2.1.2.1. Low tannin, variety Dordao :

Table (6) shows the effect of storage under atmospheric air, carbon dioxide or nitrogen gases for 3 and 6 months compared with unstored ones (control). The data revealed that the freshly harvested grains were characterized by protein content of 11.45%, total lipids 2.93%, crude fiber 2.61%, ash 2.54%, total carbohydrates 80.47%, total soluble sugars 1.03%, reducing sugars 0.3% and non reducing sugars 0.73%. When the grains stored under atmospheric air for 3 and 6 months, the above mentioned chemical composition were 11.93%, 3.46%, 2.88%, 3.0%, 78.73%, 1.25%, 0.42% and 0.83%, respectively after 3 months of storage while it was 13.58%, 3.85%, 2.9%, 3.53%, 76.14%, 1.73%, 0.81% and 0.92%, respectively after, 6 months of storage.

The chemical composition of sorghum grains stored under CO_2 for 3 and 6 months, resulted in crude protein 11.8 and 11.88%, total lipids 3.32 and 3.63%, crude fiber 2.73 and 2.78%, ash 2.58 and 2.67%, total carbohydrates 79.57 and 79.04%, total soluble sugars 1.07 and 1.1%, reducing sugars 0.35 and 0.4% and non reducing sugars 0.7 and 0.69%.

When the sorghum grains were stored under nitrogen gas for 3 and 6 months, crude protein amounted in 11.76 and 11.8%, total lipids 3.16 and 3.24%, crude fiber 2.62 and 2.63%, ash 2.55 and 2.60%, total carbohydrates 79.91 and 79.73%, total soluble sugars 1.06 and 1.07%, reducing sugars 0.37 and 0.42% and non reducing sugars 0.69 and 0.65%. From the above mentioned data, it could concluded that storage under atmospheric air increased protein content by about 4.2% and 18.6% after 3 and 6 months of storage while total carbohydrates

decreased by about 2.2 and 5.7% for the same storage periods, respectively. Meanwhile, storage under CO_2 or N_2 resulted in a slight increase or decrease in protein and total carbohydrates respectively.

It could be mentioned that respiration of the grains consumed some of total carbohydrates. The slight increase and decrease in protein and carbohydrates content during storage under CO_2 or N_2 reflect the inhibition of the respiration enzymes of the grains which need oxygen. Generally, storage under N_2 resulted in high quality grains as that of freshly harvested followed by CO_2. The obtained data are in the line with the findings of ***Saker (1995)*** who found that ash and protein contents of stored sorghum grains increased with increasing storage period (6 months). This may be partially due to the carbohydrates decrement.

4.2.2.2. Sorghum

4.2.2.2.1. Low tannin content, variety Dorado, inoculated with *A.flavus*.

Table (6) shows also the chemical composition of sorghum grains inoculated with *A.flavus* and stored under atmosphere air, carbon dioxide or nitrogen gases for 3 and 6 months compared with uninoculated grains at zero time (control). The inoculated grains showed protein content of 13.35% and 14.65% when stored under atmospheric air for 3 and 6 months, respectively. Storage under CO_2 or N_2 resulted in protein content of 12.74%, 13.86% and 11.98 and 12.2% after 3 and 6 months, respectively.

Total lipids reached about one half as a result of inoculation with *A.flavus* under atmospheric air while it slightly decreased under either CO_2 or N_2.

It is found that crude fiber of inoculated grains reached its maximum values being 8.21% and 10.33% when the grains stored under air for 3and 6 months, respectively, meanwhile a slight increment was found due to storage under CO_2 or N_2. Ash showed a similar, trend as that of fiber. On the contrary, total carbohydrates reached its minimum value after 6 months of storage under air followed by 3 months. (68.04 and 72.33%, respectively). A great increase in total soluble sugars was found under atmospheric air being 4.6 and 6.17% after 3 and 6 months, respectively. On the other hand, a slight increase or decrease was found in the chemical composition of inoculated sorghum grains stored under CO_2 or N_2 compared with the uninoculated control. From the aforementioned data, it could be concluded that inoculation with *A.*

flavus caused conclusive effect on the chemical composition of sorghum grains stored under atmospheric air. Meanwhile, storage under either CO_2 or N_2 of the inoculated grains resulted in slight effect. It is worth mentioning that storage under atmospheric air encourage to some extent the grains respiration which resulted in degradation the polysaccharides to monosaccharides. The later is considered as suitable medium for fungal growth, then the fungal enzymes degraded the grain components to simple ones for fungal utilization. This mechanizm did or very slight occur regarding storage under CO_2 or N_2.

The obtained data are in agreement with those of ***Parster et al. (1991)*** found that the fat content of moistened grains (16%) after storage of 80 and 96 days was not changed after 80 days but decreased after 96 days. And with ***Baran et al., (1992)***, they observed a decrease in nitrogenous substances and fat content and an increase in fiber content due to contaminate the maize by fungi and the effect of different concentration CO_2 on maize grain during storage.

Table (6): Chemical composition of sorghum grains (low tannin, variety Dorado) inoculated with *Aspergillus flavus*[a], stored under different gases and incubated at room temperature (25°C).

Storage conditions / Chemical composition	Zero time	Chemical analysis of samples (months) after inoculation											
		Atmospheric air				Carbon dioxide				Nitrogen			
		(NI)		(I)		(NI)		(I)		(NI)		(I)	
		3months	6months	3months	6months	3months	6months	3months	6months	3months	6months	3months	6months
Crude protein	11.45	11.93	13.58	13.35	14.65	11.80	11.88	12.74	13.86	11.76	11.80	11.98	12.20
Total lipids	2.93	3.46	3.85	1.58	1.24	3.32	3.63	2.66	2.32	3.16	3.24	2.87	2.83
Crude fiber	2.61	2.88	2.9	8.21	10.33	2.73	2.78	2.95	3.94	2.62	2.63	2.75	2.84
Ash	2.54	3.00	3.53	4.53	5.74	2.58	2.67	3.25	3.56	2.55	2.60	2.61	2.77
Total carbohydrates*	80.47	78.73	76.14	72.33	68.04	79.57	79.04	78.40	76.32	79.91	79.73	79.79	79.36
Total soluble sugars	1.03	1.25	1.73	4.60	6.17	1.07	1.10	2.53	3.12	1.06	1.07	1.44	1.53
Reducing sugars	0.30	0.42	0.81	3.23	3.84	0.35	0.40	1.20	1.82	0.37	0.42	0.34	0.45
Non reducing sugars	0.73	0.83	0.92	1.37	3.33	0.72	0.70	1.33	1.30	0.69	0.65	1.10	1.08

a, sorghum grains (low tannin, variety Dorado) derived aflatoxin producing isolate, isolated in this study ; (NI) non inoculated , (I) inoculated samples and * , total carbohydrates calculated by difference method, not including fiber, *(Tadrus 1989).*

4.2.2.2.1.1. Low tannin content, variety Dorado, inoculated with *Penicillium corylophilum.*

Table (7) shows the chemical composition of sorghum grains inoculated with *Penicillium corylophilum* and stored under atmospheric air, carbon dioxide or nitrogen gases for 3 and 6 months compared with uninoculated grains at zero time.

The inoculated grains showed protein content of 13.28% and 14.60% when stored under atmospheric air for 3 and 6 months, respectively. Storage under either CO_2 or N_2 resulted in protein content of 12.70 and 13.82%; 11.95 and 12.16% after 3 and 6 months storage periods, respectively. Total lipid content showed its minimum values under atmospheric air (1.54% and 1.18%) which was about 50% and 40% of the control, meanwhile a slight effect was found due to storage under either CO_2 or N_2.

Crude fiber resulted in about 2.8 and 3.6 fold as that of control, while storage under other gases resulted in slight increase in fiber content. Ash showed an increase by about 1.8 and 2.7 times as high as that of control when the inoculated grains stored under atmospheric air. Concerning the total carbohydrates, the inoculated grains with *Penicillium corylophilum* and stored under atmospheric air showed a greatest reduction after 6 months of storage (68.04%). The total carbohydrates content of sorghum grains inoculated with *Penicillium corylophilum* and stored under CO_2 or N_2 for 3 and 6 months ranged from 77.02 to 79.39% which showed a slight effect due to inoculation compared with control (80.47%). Total soluble sugars amounted in 4.66 and 7.22% for the inoculated grains and stored under atmospheric air for

3 and 6 months, respectively which was about 4.52 and 7 times as high as control, while it was 2.5 and 3.1 times as control when stored under CO_2 for 3 and 6 months, respectively.

Meanwhile, storage under N_2 showed a slight effect on total soluble sugars. As mentioned before, the fungal needed carbon source to stimulate growth. Therefore, fungal enzymes play an important role in the degradation of the grain components to produce easy compounds for its consumption. The first easy compounds were total soluble sugars followed by other carbohydrates.

For this reason, a decrease in total carbohydrates was found especially under atmospheric air which encourage the fungal growth because of the presence of oxygen which enhance enzyme activities.

The obtained data were in the line with those of ***Farag (1990)***, reported that the activities of amylase, lipase and protease enzymes were much higher in infected seeds than healthy ones. And ***Farag et al., (1985)***. Found that, the fungi produced lipase besides the seeds lipase, which hydrolyzed the triglycerides to glycerol and fatty acids, the glycerol was consumed at first, and the fungi utilized the lipids as a source of energy besides the carbohydrates.

Table (7) : Chemical composition of sorghum grains (low tannin, variety Dorado) inoculated with *Penicillium corylophilum*[a], stored under different gases and incubated at room temperature (25°C).

Storage conditions / Chemical composition	Zero time	Chemical analysis of samples (months) after inoculation											
		Atmospheric air				Carbon dioxide				Nitrogen			
		(NI)		(I)		(NI)		(I)		(NI)		(I)	
		3months	6months	3months	6months	3months	6months	3months	6months	3months	6months	3months	6months
Crude protein	11.45	11.93	13.58	13.28	14.60	11.80	11.88	12.70	13.82	11.76	11.80	11.95	12.16
Total lipids	2.93	3.46	3.85	1.54	1.18	3.32	3.63	2.62	2.26	3.16	3.24	2.85	2.82
Crude fiber	2.61	2.88	2.9	7.25	9.38	2.73	2.78	3.14	3.32	2.62	2.63	2.70	2.81
Ash	2.54	3.00	3.53	4.58	6.80	2.58	2.67	3.27	3.58	2.55	2.60	2.75	2.82
Total carbohydrates*	80.47	78.73	76.14	73.35	68.04	79.57	79.04	78.27	77.02	79.91	79.73	79.75	79.39
Total soluble sugars	1.03	1.25	1.73	4.66	7.22	1.07	1.10	2.58	3.15	1.06	1.07	1.20	1.36
Reducing sugars	0.30	0.42	0.81	1.33	4.15	0.35	0.40	1.22	1.83	0.37	0.42	0.36	0.48
Non reducing sugars	0.73	0.83	0.92	3.33	3.07	0.72	0.70	1.36	1.32	0.69	0.65	0.84	0.88

a, sorghum grains (low tannin, variety Dorado) derived aflatoxin producing isolate, isolated in this study ; (NI) non inoculated , (I) inoculated samples and *, total carbohydrates calculated by difference method, not including fiber, ***(Tadrus 1989).***

4.2.1.2. Sorghum

4.2.1.2.2. High tannin content, variety Framida:

Sorghum grains (variety Framida) were stored under atmospheric air, CO_2 and N_2 for 3 and 6 months. The stored grains and freshly harvested ones were subjected to the chemical analysis and the data presented in **table (8).** The freshly harvested sorghum grains contained protein in the amount of 13.95%, total Lipid 3.9%, crude fiber 2.7%, ash 3.3%, total carbohydrates 76.15%, total soluble sugars 1.53%, reducing sugars 0.86%, and non reducing sugars 0.67%. The sorghum grains stored under atmospheric air for 3 months showed the following chemical composition percentage: crude protein 14.24%, total lipids 4.22%, crude fiber 2.97%, ash 3.4, total carbohydrates 75.17, total soluble sugars 1.92, reducing sugars 1.26 and non reducing sugars 0.66, meanwhile, the above mentioned chemical composition were 15.45, 4.56, 3.33, 3.50, 73.16, 2.3, 1.82 and 0.48, respectively when the grains stored for 6 months. Concerning storage under either CO_2 or N_2, a slight change were found in the chemical composition of sorghum grains.

It could be concluded that the respiration of grains consumed some of carbohydrates under atmospheric air, which lead to decrease carbohydrates content and increase other components. Storage under CO_2 or N_2 inhibit enzyme activities responsible for respiration and in subsequently carbohydrates consumption.

The obtained data were in the line with the findings of ***Sakr (1995)*** who found that ash and protein content of stored sorghum grains

increased with increasing storage period (6 months). This may be partially due to the carbohydrate decrement.

4.2.2.2. Sorghum

4.2.2.2.2. High tannin content, variety Framida, inoculated with *A.flavus*:

The inoculated sorghum grains (variety Framida) with *A. flavus* were stored under atmospheric air, CO_2 or N_2 for 3 and 6 months. The stored grains were analyzed to its chemical composition and the data presented in **table (8)** the freshly harvested grains (uninoculated) showed protein content of 13.95%, total lipid 3.9%, crude fiber 2.7% , ash 3.3%, total carbohydrates 76.15%, total soluble sugars 1.53%, reducing sugars 0.86% and non- reducing sugars 0.67%. The inoculated grains with *A.flavus* and stored for 3 months showed protein content of 15.38%, 14.25% and 13.97% under air, CO_2 and N_2 respectively, while it showed 15.85%, 14.33% and 14.0% under the same conditions but for 6 months of storage. It could noticed that the protein content increased under air by about 10.25% and 13.62% after 3 and 6months of storage, meanwhile it was 2.15 and 2.72%. Under CO_2 compared with the freshly harvested grains. Storage under nitrogen showed a slight increase in protein content at the end of storage period. Total lipids resulted in decreasing of its amounts due to the inoculation with *A.flavus*. For instance, the inoculated grains when stored under air for 3 and 6 months showed a decrease in the total lipids by about 10.26% and 14.36%, respectively while under CO_2 its decreased by about 7.7% and 8.97%, respectively. On the other hand, storage under N_2 showed a slight decrease in oil content due to inoculation with *A. flavus*. Concerning carbohydrates, it showed a slight decrease due to inoculation and storage being 3.33 and

3.93% under air, while under either CO_2 or N_2 the loss in carbohydrates showed no valuable variation compared with control which in parallel with the total soluble and reducing sugars. Crude fiber and ash showed a similar trend as that of protein.

From the above mentioned data, it could be concluded that storage under air for the sorghum grains inoculated with *A. flavus* resulted in some deterioration of the grains and this depends upon the temperature and relative humidity which encourage the fungal growth. On the contrary, storage under either CO_2 or N_2 slightly affected grains deterioration, which may be due to the inhibition of fungal growth. The obtained data were in the line with those obtained by ***Glueck et al., (1977)*** stated that, in deteriorated sorghum grains, the content of carbohydrates is reduced because they are utilized to provide energy for the growth and development of the fungi and respiration of grain.

Whereas ***Glass et al., (1959)*** found that storage grains in nitrogen prevented mold growth.

Table (8) : Chemical composition of sorghum grains (high tannin, variety Framida) inoculated with *Aspergillus flavus*[a] , stored under different gases and incubated at room temperature (25°C) .

Storage conditions / Chemical composition	Zero time	Chemical analysis of samples (months) after inoculation											
		Atmospheric air				Carbon dioxide				Nitrogen			
		(NI)		(I)		(NI)		(I)		(NI)		(I)	
		3months	6months	3months	6months	3months	6months	3months	6months	3months	6months	3months	6months
Crude protein	13.95	14.24	15.45	15.38	15.85	14.15	14.28	14.25	14.33	14.11	14.21	13.97	14.00
Total lipids	3.90	4.22	4.56	3.50	3.34	4.00	4.16	3.60	3.55	3.96	4.12	3.88	3.85
Crude fiber	2.70	2.97	3.33	4.13	4.21	2.82	2.88	3.40	3.58	2.76	2.80	2.84	2.92
Ash	3.30	3.40	3.50	3.38	3.44	3.36	3.42	3.33	3.40	3.33	3.38	3.33	3.38
Total carbohydrates*	76.15	75.17	73.16	73.61	73.16	75.67	75.26	75.42	75.14	75.84	75.49	75.98	75.85
Total soluble sugars	1.53	1.92	2.30	2.10	2.32	1.60	1.80	1.90	1.98	1.58	1.60	1.65	1.68
Reducing sugars	0.86	1.26	1.82	1.15	1.10	0.96	1.20	1.04	0.94	0.92	0.80	0.96	0.98
Non reducing sugars	0.67	0.66	0.48	0.95	1.22	0.64	0.60	0.86	1.04	0.66	0.80	0.69	0.70

a, sorghum grains (high tannin, variety Framida) derived aflatoxin producing isolate, isolated in this study ; (NI) non inoculated , (I) inoculated samples and *, total carbohydrates calculated by difference method, not including fiber, *(Tadrus 1989).*

4.2.2.2.2.1.High tannin, variety Framida, inoculated with *Penicillium corylophilum*:

Table (9) shows the chemical composition of sorghum grains (variety Framida) inoculated with *Penicillium corylophilum* and stored for 3 and 6 months under atmospheric air, CO_2 or N_2 compared with freshly harvested grains. The data revealed that the protein content increased by about 7.17% and 12.9% when the inoculated grains stored under air for 3 and 6 months, respectively, while the increment percentage was 7.1 and 10.4 due to storage under CO_2 for the same periods, respectively, than the control. Concerning, storage under N_2, protein content of the inoculated grains resulted in the same amount as compared with control (freshly harvested grains), crude fiber and ash showed similar trend as that of protein, crude fiber showed 1.9 and 2.02 fold as that of control when the inoculated grains stored under air for 3 and 6 months, respectively while it represented 1.52and 1.64 fold as control when stored under CO_2 for the same periods. A slight increase was found in crude fiber due to storage under N_2. Ash content resulted in remarkable increase due to storage conditions. It could be observed also that total soluble sugars, reducing and non reducing sugars, increased in large amounts due to inoculation and storage under air followed by CO_2 while under N_2 it showed no valuable change compared with control.

On the contrary, total lipids and total carbohydrates decreased depending on the storage conditions.

From the above mentioned data, it could be concluded that the inoculated sorghum grains showed the maximum deterioration when stored under air followed by CO_2 while storage under N_2 resulted in stability of the chemical composition of the grains. This may be due to the fact that the presence of N_2 may be reduced or prevent the fungal growth and therefore no enzymatic activity was found. The obtained data were in the line with the findings of ***Glueck et al., (1977)*** and ***Glass et al., (1959).***

Table (9): Chemical composition of sorghum grains (high tannin, variety Framida) inoculated with *penicillium corylophilum*[a] , stored under different gases and incubated at room temptation (25°C).

Storage conditions / Chemical composition	Zero time	Chemical analysis of samples (months) after inoculation											
		Atmospheric air				Carbon dioxide				Nitrogen			
		(NI)		(I)		(NI)		(I)		(NI)		(I)	
		3months	6months	3months	6months	3months	6months	3months	6months	3months	6months	3months	6months
Crude protein	13.95	14.24	15.45	15.28	15.75	14.15	14.28	14.94	15.40	14.11	14.21	13.97	13.97
Total lipids	3.90	4.22	4.56	3.41	3.21	4.00	4.16	3.61	3.53	3.96	4.12	3.86	3.84
Crude fiber	2.70	2.97	3.33	5.13	5.46	2.82	2.88	4.11	4.44	2.76	2.80	2.83	2.95
Ash	3.30	3.40	3.50	3.41	3.47	3.36	3.42	3.38	4.15	3.33	3.38	3.32	3.36
Total carbohydrates*	76.15	75.17	73.16	72.77	72.11	75.67	75.26	73.96	72.48	75.84	75.49	76.02	75.88
Total soluble sugars	1.53	1.92	2.30	3.16	3.58	1.60	1.80	2.00	2.04	1.58	1.60	1.68	1.72
Reducing sugars	0.86	1.26	1.82	2.11	2.17	0.96	1.20	1.06	0.97	0.92	0.80	1.00	1.02
Non reducing sugars	0.67	0.66	0.48	1.05	1.41	0.64	0.60	0.94	1.07	0.66	0.80	0.68	0.70

a, sorghum grains (high tannin, variety Framida) derived aflatoxin producing isolate, isolated in this study ; (NI) non inoculated , (I) inoculated samples and * , total carbohydrates calculated by difference method, not including fiber, ***(Tadrus 1989).***

4.3. Tannin content of sorghum grains (variety Dorado and Framida) inoculated with *Aspergillus flavus* or *Penicillium corylophilum* and stored under different gases.

Table (10) shows the effect of inoculation with either *A. flavus* or *Penicillium corylophilum* on the tannin content of sorghum grains (variety Dorado or Framida) stored for 3 and 6 months under air, CO_2 or N_2. Firstly, it could be mentioned that Dorado variety characterized with low tannin content while other variety namely Framida characterized with high tannin content. The sorghum grains (variety Dorado) contained 0.06% tannins while Framida variety contained 2.92 % at zero time . Storage of the uninoculated grains of Dorado variety showed slight reduction in tannin content due to storage conditions when the grains inoculated with *Aspergillus flavus* and stored under air, a slight increase in tannin content was found while under CO_2 , tannin content decreased by about 46 . 6% and 58. 3% than that of control. Storage under N_2 resulted in the same amount of tannin as that of control . Concerning inoculation with *Penicilliuim corylophilum.*, it could be observed that tannin content increased by about 18.3 and 33.3%, due to storage under air for 3 and 6 months, respectively, compared with control.

An opposite trend was found due to storage under CO_2 which resulted in decrement by about 20% and 43.3% after the same periods compared with control. Storage under N_2 showed no variation in tannin content than the freshly harvested sorghum grains. Regarding to Framida variety, tannins content showed a slight increase being 1.3 and 6.16% when stored under air for 3 and 6 months, respectively. Storage under

CO_2 resulted in 11.6 and 27.4% decreasing than control when stored for 3 and 6 months, respectively. Meanwhile storage under N_2 showed no variation in tannin content compared with control.

Inoculation with *Aspergillus flavus* showed the highest tannin content when the grains stored under air for 3 and 6 months. The increment was about 19.2% and 24.66% more than control, respectively. Meanwhile, storage under CO_2 resulted in 26.71% and 29.45% decreasing than control after 3 and 6 months storage periods. The tannin content of inoculated grains and stored under N_2 showed almost the same amount as that of control.

Inoculation with *Penicillium corylophilum*. showed the highest increase in tannin content being 1.22 and 1.26 fold as that of control when stored for 3 and 6 months under air. A reduction in tannin content was found under CO_2 being 23.3 % and 25.3 % than the control after the same periods of storage , respectively.

Storage of the inoculated grains with *Penicillium corylophilum* under N_2 resulted in almost the same amounts of tannin as that of control.

From the above mentioned data , it could be concluded that storage under air increased tannin content while storage under CO_2 decreased it, while storage under N_2 showed no variation in its amounts. And from the obtained data, the storage under nitrogen on the tannin content, show there are no change between the control and that stored under nitrogen for 3 and 6 months. As a conclusion, storage under

nitrogen is considered a very suitable inert gas which prevent deterioration or infection. The data were in agreement with that of ***Waichungo and Holt (1994)*** which illustrated that ideally tannin level should remain high during plant growth and be reduced during processing.

The increase in the tannin content of grains may be due to that the fungi used endosperm as a feed and resulted in high fiber which contains most of tannins. In this respect, ***Reichert et al., (1980)*** found that, 81.6% the considered tannins was located in the pericarp and 3.3% in the endosperm plus germ.

Table (10): Tannin content of sorghum grains (varieties Dorado and Framida) inoculated with *Aspergillus flavus* or *penicillium corylophilum* and stored under different gases.

Storage conditions / Treatments	Zero time	Atmospheric air		Carbon dioxide		Nitrogen	
		3 months	6 months	3 months	6 months	3 months	6 months
"variety Dorado"							
Uninoculated sorghum grains	0.06	0.040	0.040	0.005	0.005	0.050	0.050
Inoculated with *Aspergillus flavus*	0.06	0.062	0.068	0.032	0.025	0.060	0.060
Inoculated with *Penicillium corylophilum*	0.06	0.071	0.080	0.048	0.034	0.060	0.050
"variety Framida"							
Uninoculated sorghum grains	2.92	2.96	3.10	2.58	2.12	2.90	2.90
Inoculated with *Aspergillus flavus*	2.92	3.48	3.64	2.14	2.06	2.93	2.91
Inoculated with *Penicillium corylophilum*	2.92	3.56	3.67	2.24	2.18	2.93	2.93

4.4. Fatty acids composition of soybean seeds and sorghum grains inoculated with *A.flauvs* and *P.corylophilum* and stored under different gaseous conditions.

4.4.1. Soybean seeds:

Table (11) shows the fatty acids composition extracted from soybean oil either freshly harvested or inoculated with *Aspergillus flavus* or *Penicllium corylophilum* and stored under air, CO_2 or N_2 for 3 and 6 months at room temperature. The freshly harvested soybean contained $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ and $C_{20:0}$ in the amounts of 2.71, 10.05, 3.84, 28.79, 49.91, 4.61 and 2.19% , respectively. When the seeds inoculated by *Aspergillus flavus* and stored under air, $C_{14:0}$ and $C_{20:0}$ decreased besides all unsaturated fatty acids such as $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$. On the contrary, $C_{16:0}$ and $C_{18:0}$ increased after 3 or 6 months of storage. Similar trend was found regarding storage under CO_2. Meanwhile, storage under N_2 resulted in slight increase and decrease in the saturated and unsaturated fatty acids. These results can be explained by the ratio between Tu/Ts which amounted in 4.327 for the freshly harvested soybean while it was 3.26 and 2.865 under air, 3.38 and 2.97 under CO_2 and 3.33 under N_2 after storage periods of 3 and 6 months, respectively. Concerning soybean seeds inoculated by *Pencillium corylophilum*, the results showed an increase in $C_{14:0}$ due storage under either air or CO_2 while it slightly decreased under N_2. It is also found that $C_{16:0}$ increased by about 50% than the control when stored under air or CO_2 while the increment was about 10% under N_2 . $C_{18:0}$ showed an increase by about 30-68% according to the storage condition. On the contrary, the unsaturated fatty acids decreased as a result of infection treatment-depending upon the storage condition. For instance, $C_{18:1}$ slightly

affected by infection and storage conditions, meanwhile a remarkable decreased was found in the amounts of $C_{18:2}$ and $C_{18:3}$. Tu/Ts ratio showed a values of 2.97 and 2.72 under air, 2.997 and 2.76 under CO_2 and 3.9 and 3.738 under N_2. From the above mentioned data, it could be concluded that *Pencillium corylophilum* affected soybean oil more than *Aspergillus flavus* under the investigated conditions. this may be due to the enzymes produced by *Pencillium corylophilum* , which deteriorate the soybean oil more than that of *Aspergillus flavus*. The obtained data were in the line with the finding of ***Robertson et al. (1973)*** found that there was a decrease in the linolenic acid content of the oil extracted from all storage damaged soybean, whereas there was only a small decrease in the linoleic acid content and no apparent change in the oleic acid content. The decrease in the polyunsaturated fatty acids indicate that oxidative deterioration took place during storage and is reflected in the lower total oil content of the storage damaged beans.

Whereas ***Farag et al. (1985)*** found that the pattern of free fatty acids of soybean oil extracted from healthy seeds showed that lipases hydrolysed the esterified fatty acids having different carbon atoms both saturated and unsaturated species. The most frequent free saturated and unsaturated acids were 16:0, 18:1 and 18:2 and these acids comprised over 70% of the total amount of free acids. Free fatty acid compositions of soybean oils infected with *A. flavus* are quite different than that of healthy oil, and the fungi caused an appearance, disappearance and changed the concentration of certain fatty acids. And the obvious effect of fungi on the bound fatty acids was found in 16:0 (the most predominant saturated acid), 18:1and 18:2 (the most prevalent unsaturated acids). The ratios between the total unsaturated fatty acids to the total saturated acids (TU/TS) and the degree of unsaturation (DU) indicated that fungal infection lowered these parameters compared to control oil.

Table (11) : Effect of inoculating, certain toxigenic molds on the fatty acid composition of soybean (*Glycine maxL.*, variety Crawford) under different gases.

Fatty acid (Name and chemical formula)	Zero Time "control"	Storage period (month)											
		Aspergillus flavus[a]						*Penicillium corylophilum*[b]					
		Air		CO_2		N_2		Air		CO_2		N_2	
		3m	6m	3m	6m	3m	6m	3m	6m	3m	6m	3m	6m
Myristic ($C_{14:0}$)*	2.71	1.97	1.94	2.05	1.93	2.69	1.20	3.20	3.10	3.52	3.28	2.21	1.89
Palmitic ($C_{16:0}$)*	10.05	13.81	15.06	13.45	14.45	11.30	13.38	15.05	15.97	14.83	15.66	11.26	11.67
Stearic ($C_{18:0}$)*	3.84	5.70	7.02	5.22	6.87	5.16	5.75	5.51	6.46	5.22	6.19	4.96	5.64
Oleic ($C_{18:1}$)	28.79	27.50	26.37	27.74	26.82	28.45	27.08	28.51	27.34	28.60	27.44	28.23	27.68
Linoleic ($C_{18:2}$)	47.91	45.16	44.41	45.44	44.56	47.88	45.41	43.44	43.30	43.48	43.45	47.44	47.27
Linolenic ($C_{18:3}$)	4.61	3.89	3.31	4.00	3.45	4.53	4.42	2.88	2.52	2.91	2.54	3.98	3.96
Arachidic ($C_{20:0}$)*	2.19	1.97	1.88	2.10	1.92	-	1.97	1.41	1.34	1.45	1.43	1.92	1.91
TS	18.79	23.46	25.86	22.82	25.18	19.16	23.10	25.18	26.85	25.02	26.57	20.34	21.11
TU	81.31	76.54	74.10	77.19	74.84	80.85	76.91	74.84	73.15	74.99	73.43	79.66	78.899
TU/TS	4.327	3.26	2.87	3.38	2.98	4.22	3.33	2.97	2.72	2.997	2.76	3.9	3.74

a and b represent the toxigenic isolates (aflatoxin producing isolates of *Asperillus flavus* and *Penicillium corylophilum*) which were isolated in this study; —, not determined; TS, total saturated fatty acids ;TU, total unsaturated fatty acids and parentheses with star represent the saturated fatty acids .

4.4.2. Sorghum grains:

4.4.2.1. Low tannin content, variety Dorado :

Table (12) shows the fatty acids composition of sorghum grains , (low tannin, variety Dorado) inoculated with *Aspergillus flavus* and *Penicillium corylophilum* and stored under air, CO_2 and N_2 for 3 and 6 months at room temperature. The oil of freshly harvested sorghum grains contained $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ in the amounts of 2.23, 11.6, 0.82, 26.97, 53.91 and 4.48%, respectively. When the grains inoculated by *Aspergillus flavus* and stored under air, a decrease in $C_{14:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ was found depending upon the storage periods. For instance, $C_{14:0}$ decreased from 2.23% at zero time to 1.74 and 1.38, $C_{18:1}$ from 26.97 to 23.34 and 22.15% , $C_{18:2}$ from 53.91 to 52.57 and 51.33% and $C_{18:3}$ from 4.48 to 3.12 and 2.82% after storage for 3 and 6 months, respectively.

Meanwhile, the saturated fatty acids like $C_{16:0}$ and $C_{18:0}$ increased from 11.60 to 14.9 and 17.38 and from 0.82 to 1.85 and 2.9% after storage periods, respectively. Storage under CO_2 showed similar trend as that of air. When the inoculated grains with *Aspergillus flavus* under N_2 , a slight decrease and increase in the unsaturated fatty acids and saturated ones were found. For instance, $C_{18:1}$ decreased from 26.97% to 24.7% after 6 months of storage, while $C_{18:1}$ increased from 0.82% to 1.61%. $C_{20:0}$ appeared in amounts ranged from 2.04 to 3.25% due to infection of *Aspergillus flavus*. The ratio between total unsaturated fatty acids and total saturated ones (Tu/Ts) was 5.83:1 for the freshly harvested grains while it decreased to 3.77:1 and 3.27:1; 3.84:1 and 3.31:1 for the infected grains stored under air and CO_2 for 3 and 6 months respectively.

Meanwhile storage under N_2 showed slight decreased in the ratio. When the grains inoculated by *Penicillium corylophilum*, $C_{14:0}$ decreased than the control according to the storage condition and period.

The unsaturated fatty acids slightly affected due to *Penicillium corylophilum* inoculation. The Tu/Ts revealed the action of *Peinicillium* which showed 4.22 and 3.4 under air, 4.35 and 3.54 under CO_2 and 5.15 and 4.82 under N_2 when stored for 3 and 6 months respectively. From the above mentioned data, it could be concluded that infected sorghum grains showed no variable change in its fatty acids when stored under N_2 at room temperature. The obtained results are in agreement with the finding of ***Robertson et al.; (1973)*** and ***Farag et al., (1985).***

Table (12) : Effect of inoculating, certain toxigenic molds on the fatty acid composition of sorghum (*sorghum bicolor*, low tannins, variety Dorado) under different gases.

Fatty acid (Name and chemical formula)	Zero Time "control"	Storage period (month)											
		Aspergillus flavus[a]						*Penicillium corylophilum*[b]					
		Air		CO_2		N_2		Air		CO_2		N_2	
		3m	6m	3m	6m	3m	6m	3m	6m	3m	6m	3m	6m
Myristic ($C_{14:0}$)*	2.23	1.74	1.38	1.95	1.56	2.09	1.60	1.22	1.04	1.35	1.09	1.95	1.83
Palmitic ($C_{16:0}$)*	11.60	14.90	17.38	14.47	17.15	13.17	13.44	13.89	17.75	13.44	17.07	13.05	13.57
Stearic ($C_{18:0}$)*	0.82	1.85	2.90	1.69	2.42	1.06	1.61	1.93	2.03	1.70	1.85	1.26	1.80
Oleic ($C_{18:1}$)	26.97	23.34	22.15	23.49	22.33	26.20	24.69	24.45	23.35	24.65	23.64	25.78	25.45
Linoleic ($C_{18:2}$)	53.91	52.57	51.33	52.74	51.69	53.58	51.72	52.21	50.01	52.39	50.34	53.75	53.35
Linolenic ($C_{18:3}$)	4.48	3.12	2.82	3.16	2.90	3.91	3.71	4.11	3.92	4.28	4.02	4.23	4.12
Arachidic ($C_{20:0}$)*	-	2.50	2.04	2.56	2.09	-	3.25	2.10	1.92	2.19	2.06	-	-
TS	14.64	20.98	23.71	20.67	23.22	16.33	19.90	19.14	22.73	18.68	22.05	16.25	17.19
TU	85.36	79.02	76.29	79.39	76.92	83.69	80.11	80.78	77.27	81.32	77.99	83.75	82.92
TU/TS	5.83	3.77	3.22	3.84	3.31	5.13	4.03	4.22	3.40	4.35	3.54	5.15	4.82

a and b represent the toxigenic isolates (aflatoxin producing isolates of *Aspergillus flavus* and *Penicilluim corylophilum*) which were isolated in this study; — , not determined; TS, total saturated fatty acids ; TU, total unsaturated fatty acids and parentheses with star represent the saturated fatty acids.

4.4.2.2. High tannin content, variety Framida:

Table (13) shows the fatty acids composition of sorghum grains (high tannin) inoculated by *Aspergillus flavus* and *Penicillium corylophilum* and stored at room temperature for 3 and 6 months. The freshly harvested grains showed the following fatty acids $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ and $C_{20:0}$ in the amounts of 14.33, 1.04, 25.95, 51.3, 4.3 and 3.1% respectively. When the inoculated grains stored for 3 and 6 months, $C_{14:0}$ appeared in the amounts ranged from 1.03 to 1.26 except that of the grains inoculated with *Penicillium corylophilum* and stored under N_2 which showed to be as control. All inoculated grains resulted in increasing $C_{16:0}$ under air and CO_2 but slightly increased under N_2. Similar trend was found due to $C_{18:0}$ which showed increment by about 2-3 fold as that of control under all treatments. $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ decreased as result of inoculation by *Aspergillus flavus* and *Penicillium corylophilum* during storage. Meanwhile $C_{20:0}$ increased slightly according to the fungi infection and storage period. Concerning Tu/Ts, the freshly harvested sorghum grains showed a value of 4.415 while it decreased to 3.486 and 3.13 under air, 3.75 and 3.2 under CO_2 and 3.88 and 3.72 under N_2 when the grains inoculated by *Aspergillus flavus* and stored for 3 and 6 months, respectively. Inoculation with *Penicillium corylophilum* resulted in 3.58 and 3.29 under air, 3.63 and 3.34 under CO_2 and 4.14, and 3.92 under N_2 after storage periods of 3 and 6 months, respectively. From the above mentioned data it could be concluded that the high tannin sorghum grains affected with the *Aspergillus flavus* and *Penicillium corylophilum* infection during storage at room temperature. The obtained data were in the line with these of

From the data presented in table (13 and 14), it could be concluded that the deterioration of sorghum grains dependent upon the

storage conditions and not in the high tannin content. It could be recommended to use modified atmosphere to delay or prevent fungal growth and therefore, delay the deterioration of seeds ***Robertson et al. (1973)*** and ***Farag et al. (1985)***.

Table (13): Effect of inoculating, certain toxigenic molds on the fatty acid composition of sorghum (*sorghum bicolor*, high tannin, variety Framida) under different gases.

Fatty acid (Name and chemical formula)	Zero Time "control"	Storage period (month)											
		Aspergillus flavus[a]						*Penicillium corylophilum*[b]					
		Air		CO_2		N_2		Air		CO_2		N_2	
		3m	6m	3m	6m	3m	6m	3m	6m	3m	6m	3m	6m
Myristic ($C_{14:0}$)*	-	1.13	1.04	1.17	1.07	1.20	1.10	1.11	1.01	1.23	1.03	-	-
Palmitic ($C_{16:0}$)*	14.329	16.13	17.65	15.84	17.32	14.93	15.06	15.83	17.01	15.53	16.74	15.03	15.73
Stearic ($C_{18:0}$)*	1.043	2.29	3.31	2.05	3.04	1.32	2.01	2.43	3.02	2.18	2.83	1.73	2.09
Oleic ($C_{18:1}$)	25.946	24.60	24.21	24.67	24.28	25.19	24.66	24.52	24.25	24.61	24.31	25.88	25.84
Linoleic ($C_{18:2}$)	51.289	49.34	48.40	49.68	48.68	50.22	50.20	49.49	48.46	49.62	48.63	50.49	49.69
Linolenic ($C_{18:3}$)	4.299	3.77	3.20	3.80	3.26	4.11	3.96	4.17	3.99	4.18	4.03	4.20	4.17
Arachidic ($C_{20:0}$)*	3.094	2.73	2.21	2.82	2.37	3.06	3.03	2.44	2.27	2.66	2.43	2.69	2.51
TS	18.466	22.29	24.20	21.87	23.81	20.50	21.19	21.82	23.31	21.60	23.03	19.44	20.33
TU	81.534	77.71	75.80	78.15	76.21	79.51	78.81	78.18	76.69	78.41	76.97	80.56	79.71
TU/TS	4.415	3.49	3.13	3.57	3.20	3.88	3.72	3.58	3.29	3.63	3.34	4.14	3.92

a and b represent the toxigenic isolates (aflatoxin producing isolates of *Aspergillus flavus* and *Penicilluim corylophilum*) which were isolated in this study; _ , not determined ; TS, total saturated fatty acids ; TU, total unsaturated fatty acids and parentheses with star represent the saturated fatty acids .

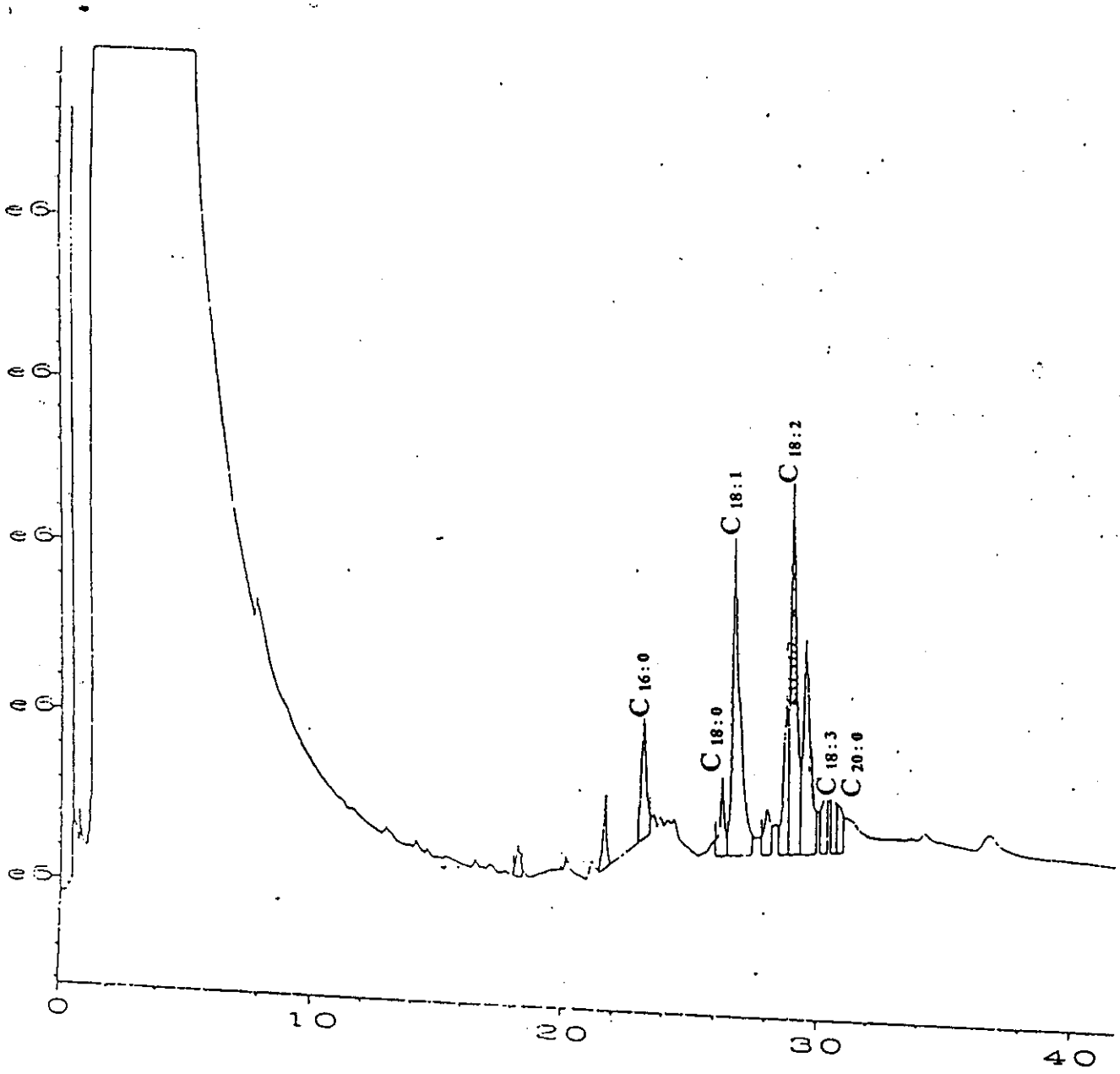

Figure (21) Fatty acids analysis profile of soybean seeds (variety Crawford) at zero time.

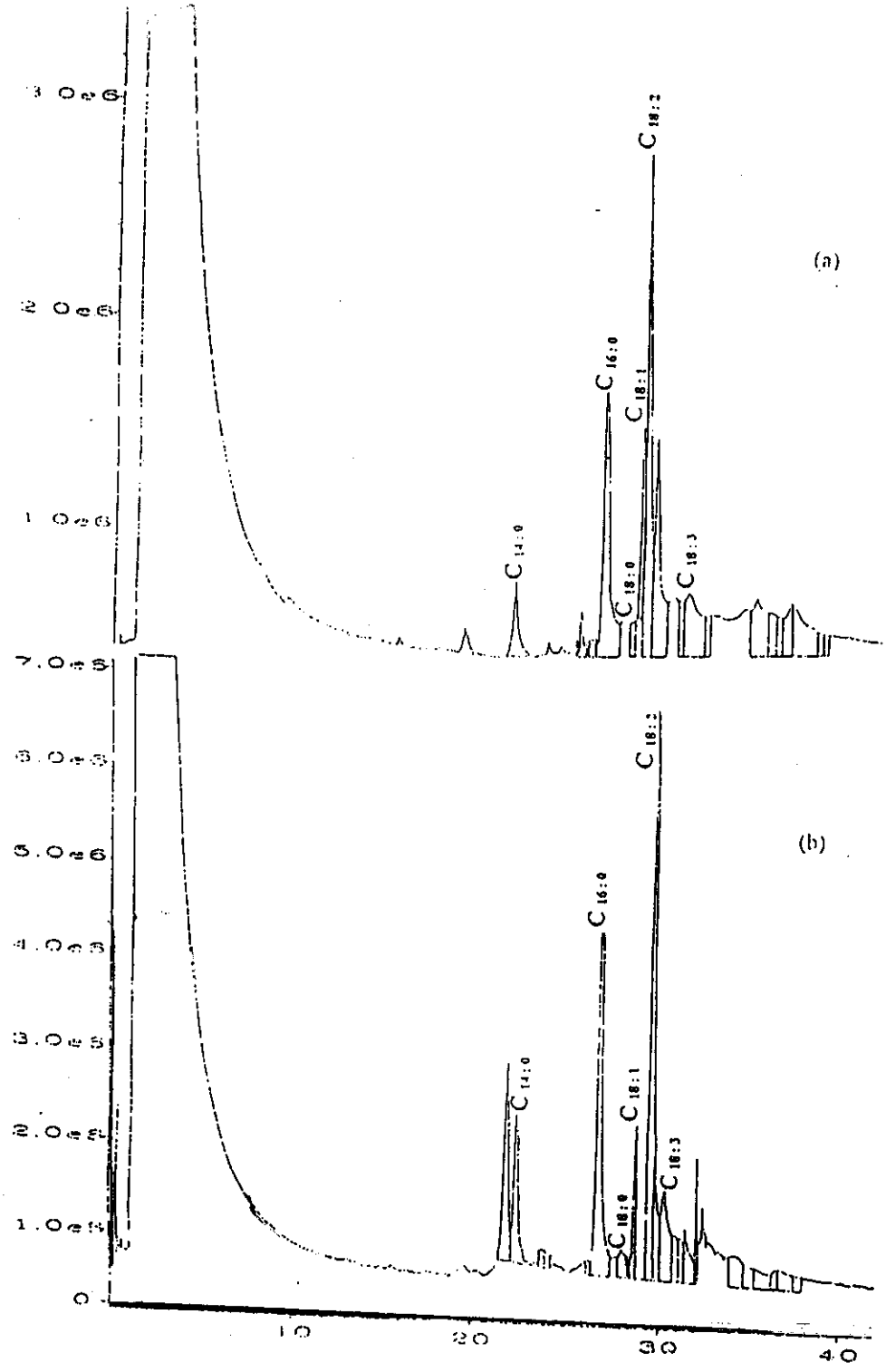

Figure (22) Fatty acids analysis profile of: a , soybean seeds (variety Crawford) inoculated with *Aspergillus flavus* and stored under CO_2 for 3 months and b , soybean seeds (variety Crawford) inoculated with *Aspergillus flavus* and stored under CO_2 for 6 months.

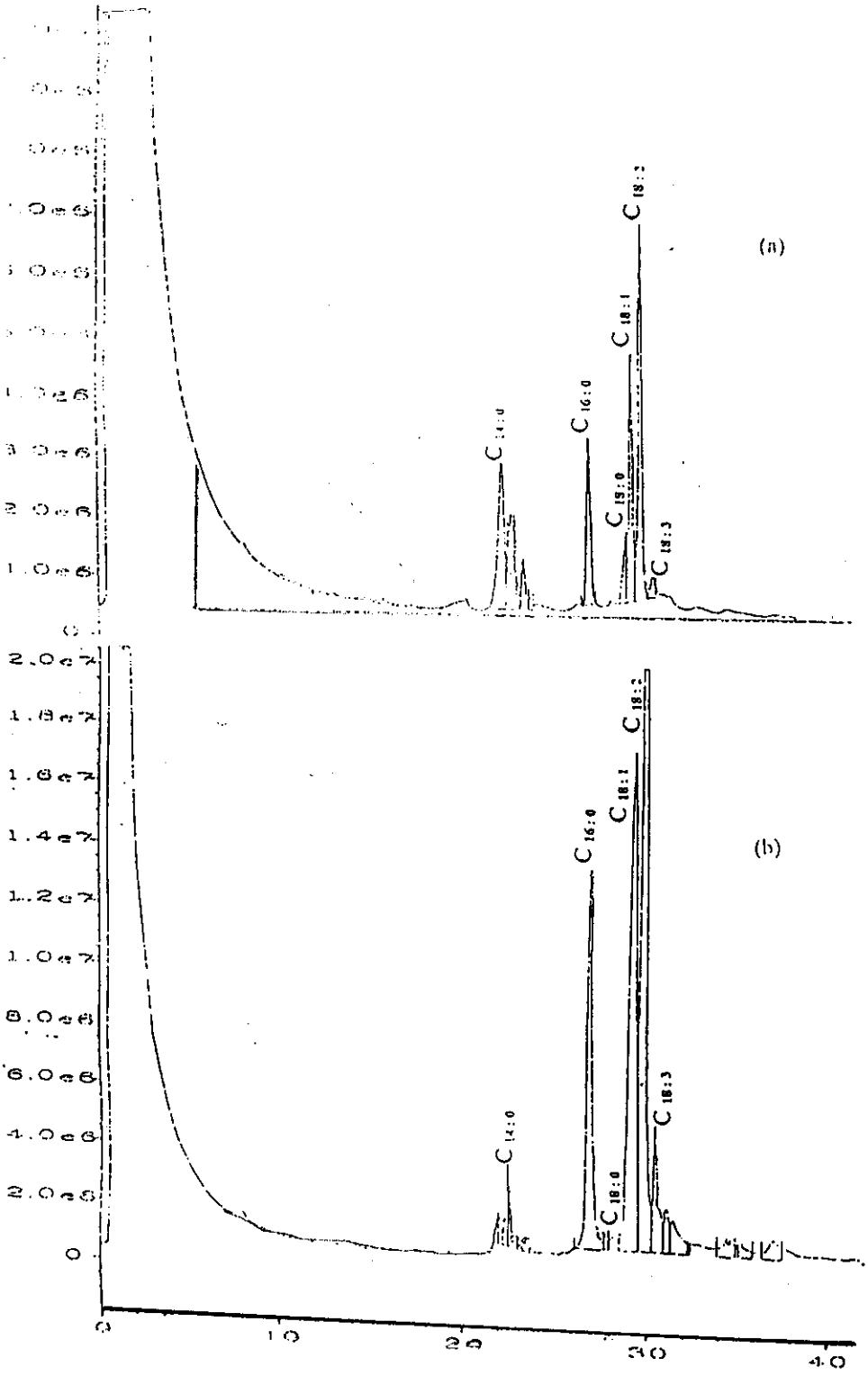

Figure (23) Fatty acids analysis profile of : a , soybean seeds (variety Crawford) inoculated with *Aspergillus flavus* and stored under N_2 for 3 months and b , soybean seeds (variety Crawford) inoculated with *Aspergillus flavus* and stored under N_2 for 6 months.

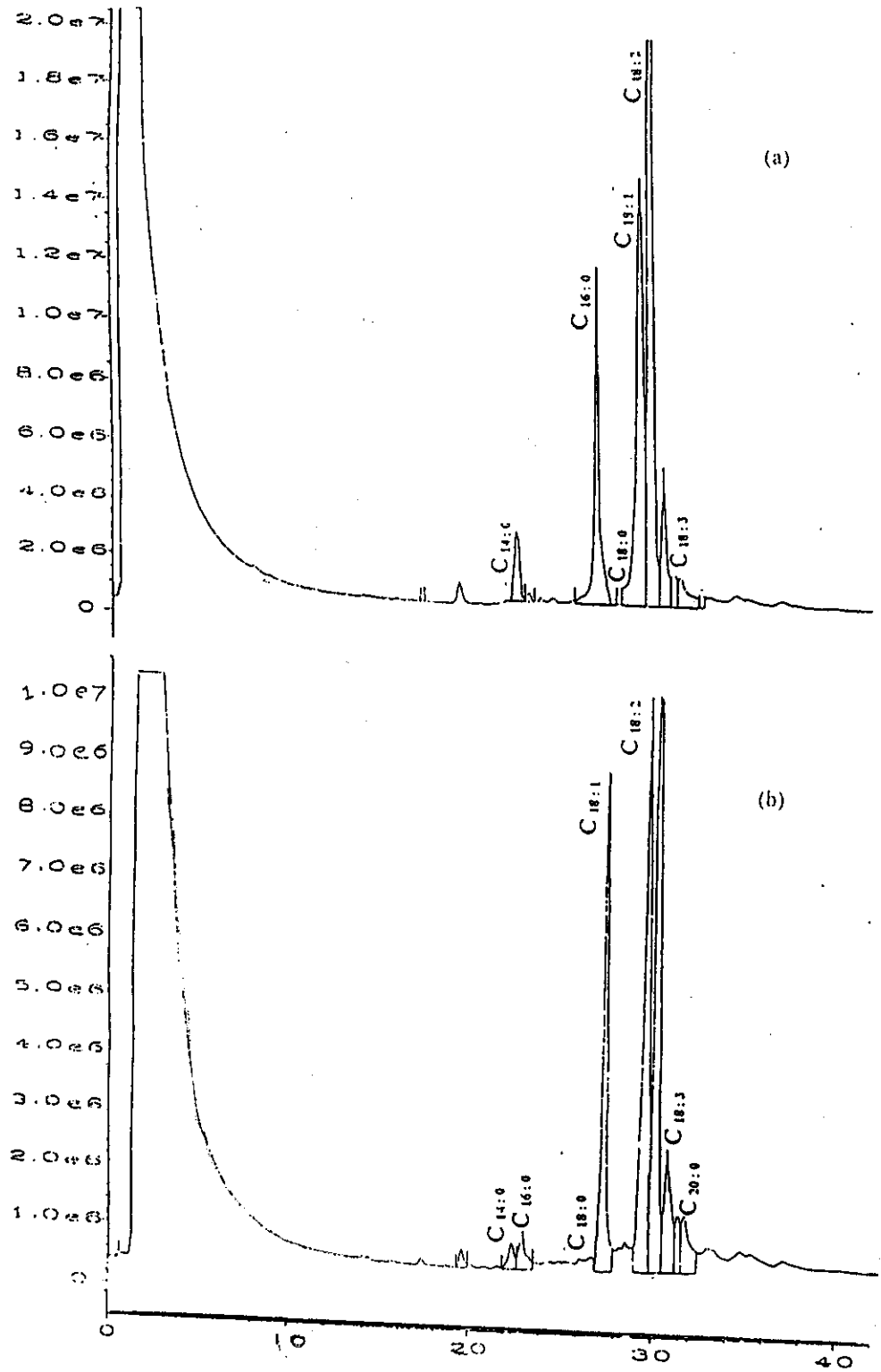

Figure (24) Fatty acids analysis profile of: a, soybean seeds (variety Crawford) inoculated with *Penicillium corylophilum* and stored under N_2 for 3 months and b, soybean seeds (variety Crawford) inoculated with *Penicillium corylophilum* and stored under N_2 for 6 months.

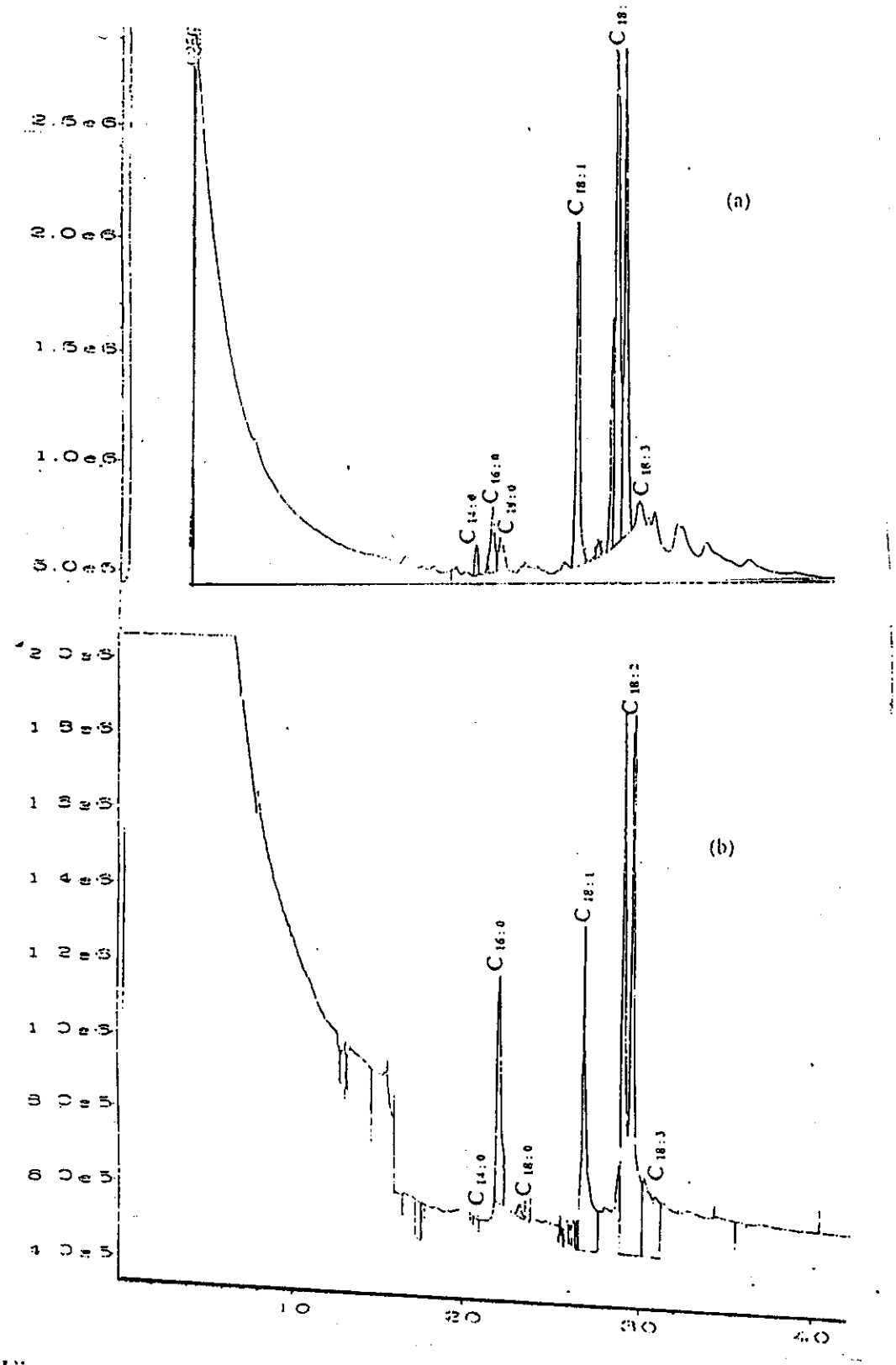

Figure (26) Fatty acids analysis profile of: a, sorghum low tannins (variety Dorado) inoculated with *Aspergillus flavus* and stored under N_2 for 3 months and b, sorghum low tannins (variety Dorado) inoculated with *Aspergillus flavus* and stored under N_2 for 6 months.

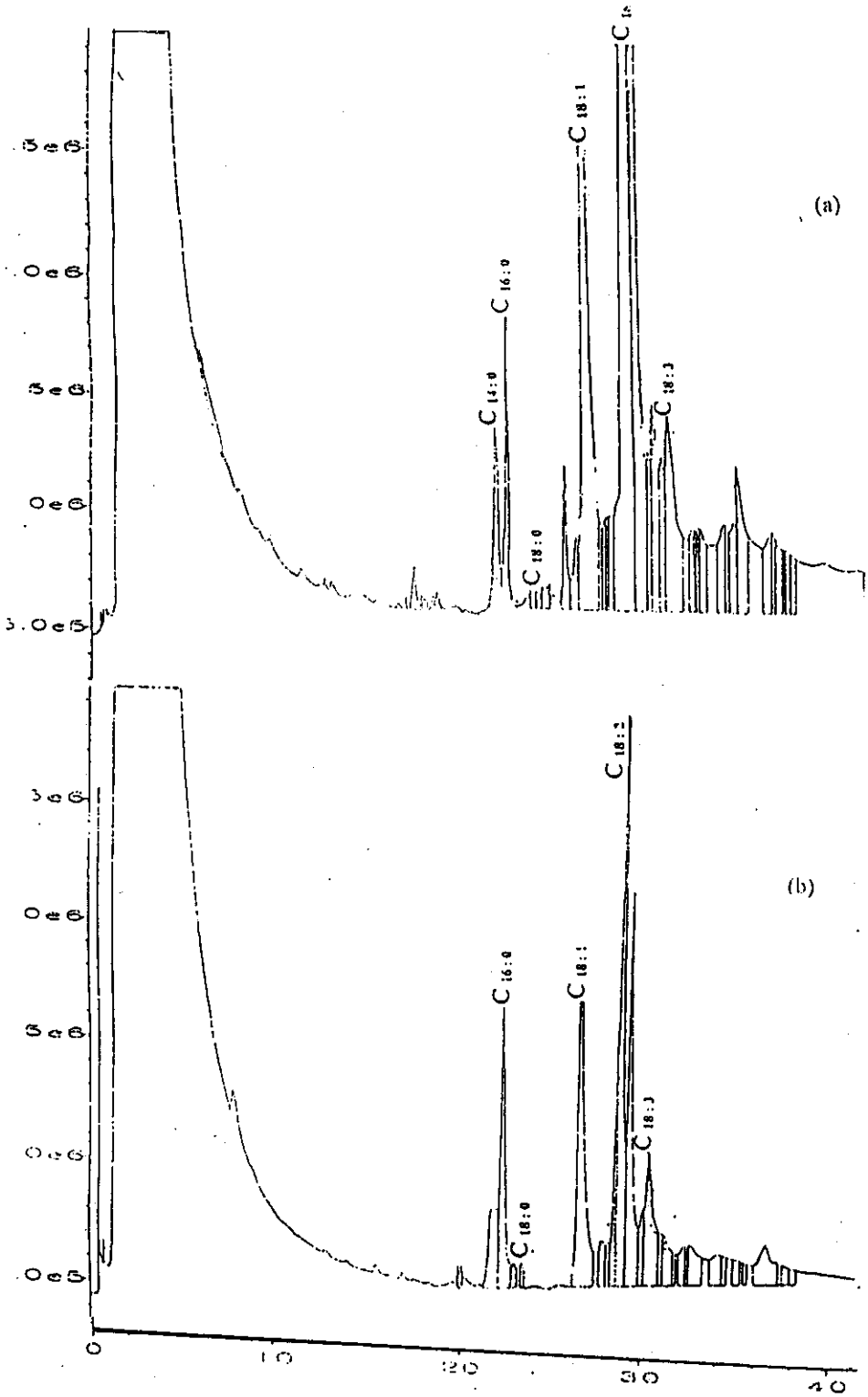

Figure (27) Fatty acids analysis profile of: a, sorghum low tannins (variety Dorado) inoculated with *Penicillium corylophilum* and stored under CO_2 for 3 months and b, sorghum low tannins (variety Dorado) inoculated with *Penicillium corylophilum* and stored under CO_2 for 6 months.

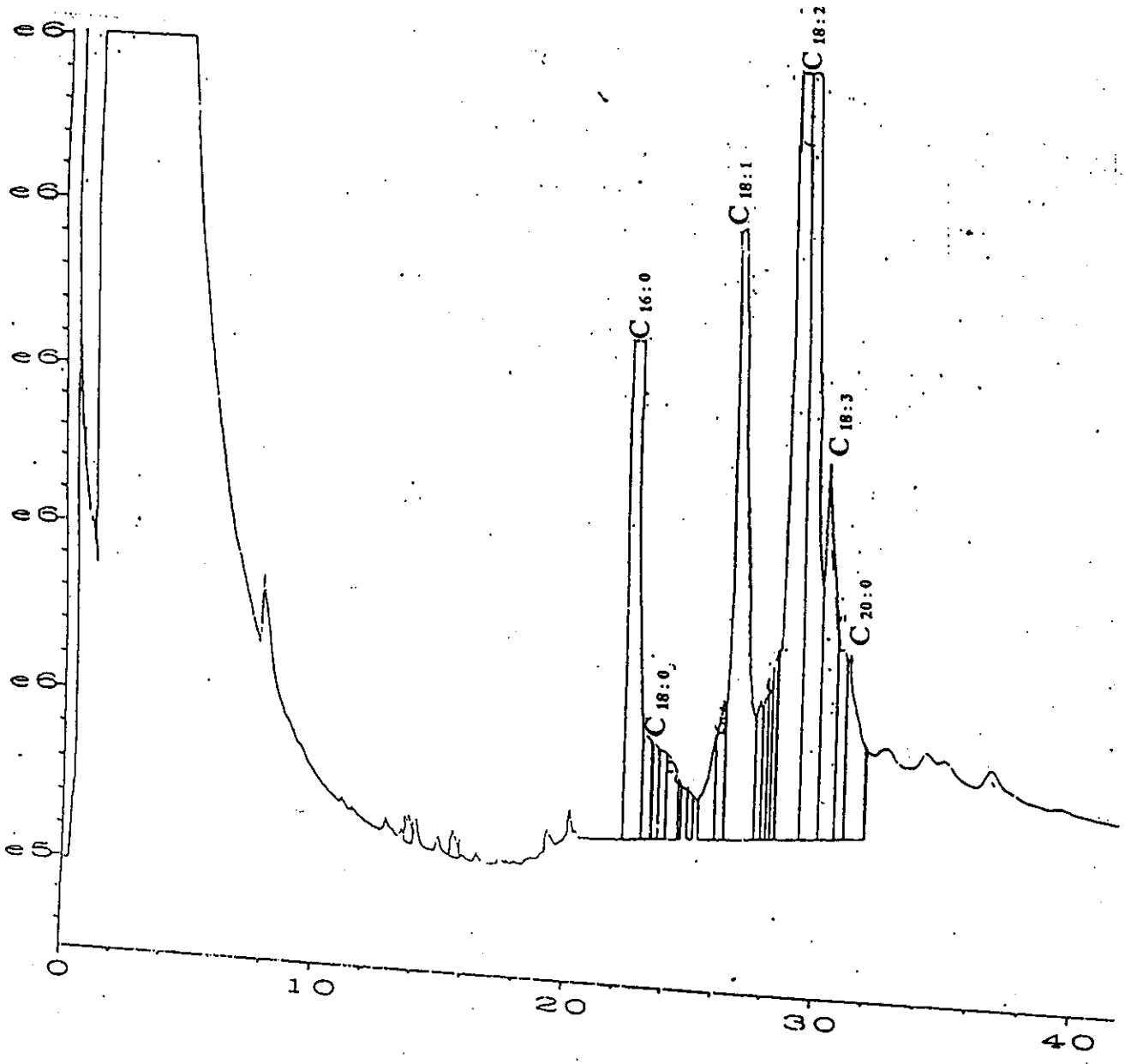

Figure (28) Fatty acids analysis profile of sorghum high tannin (variety Framida) at zero time.

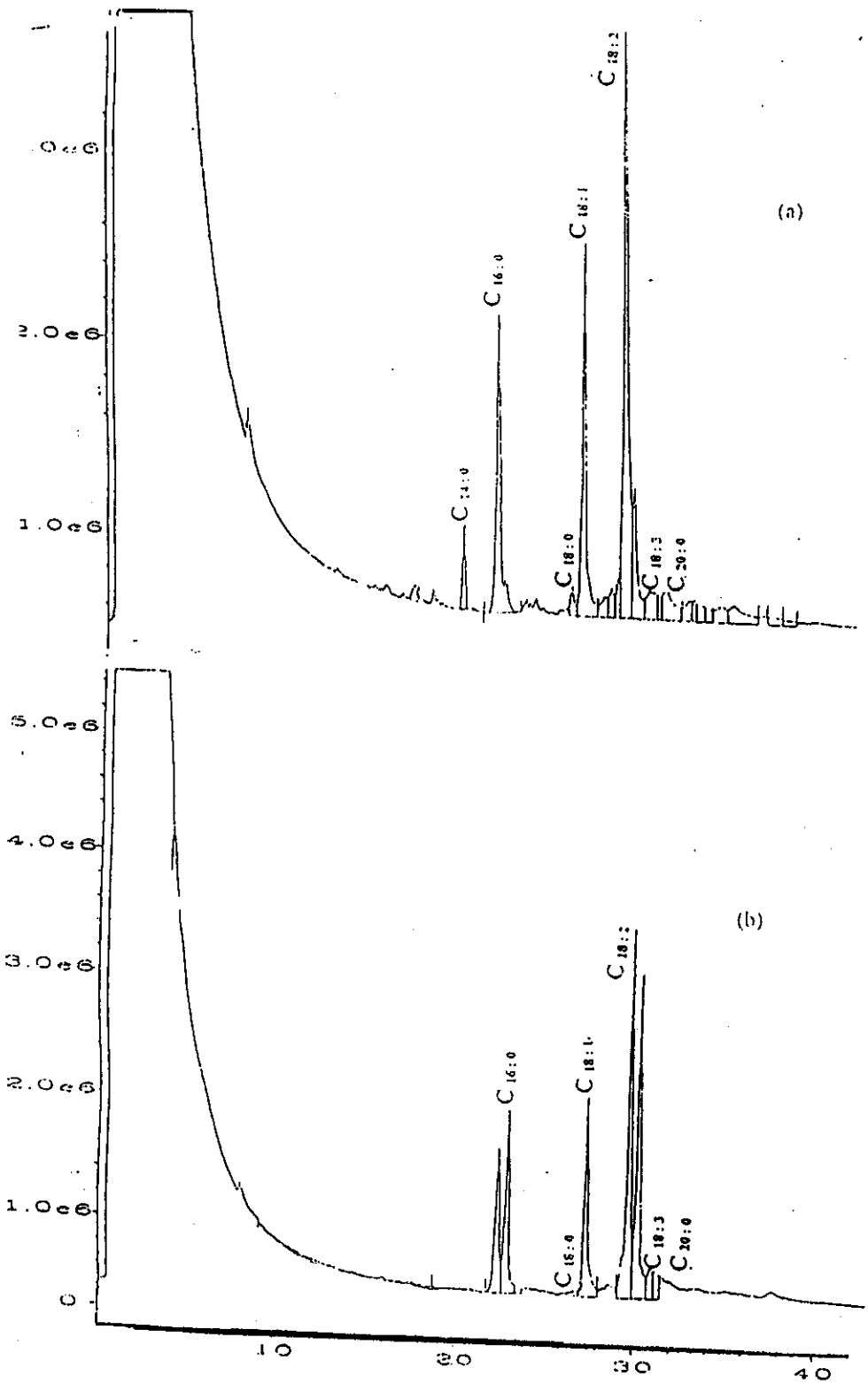

Figure (29) Fatty acids analysis profile of: a, sorghum high tannin (variety Framida) inoculated with *Aspergillus flavus* and stored under CO_2 for 3 months and b, sorghum high tannin (variety Framida) inoculated with *Aspergillus flavus* and stored under CO_2 for 6 months.

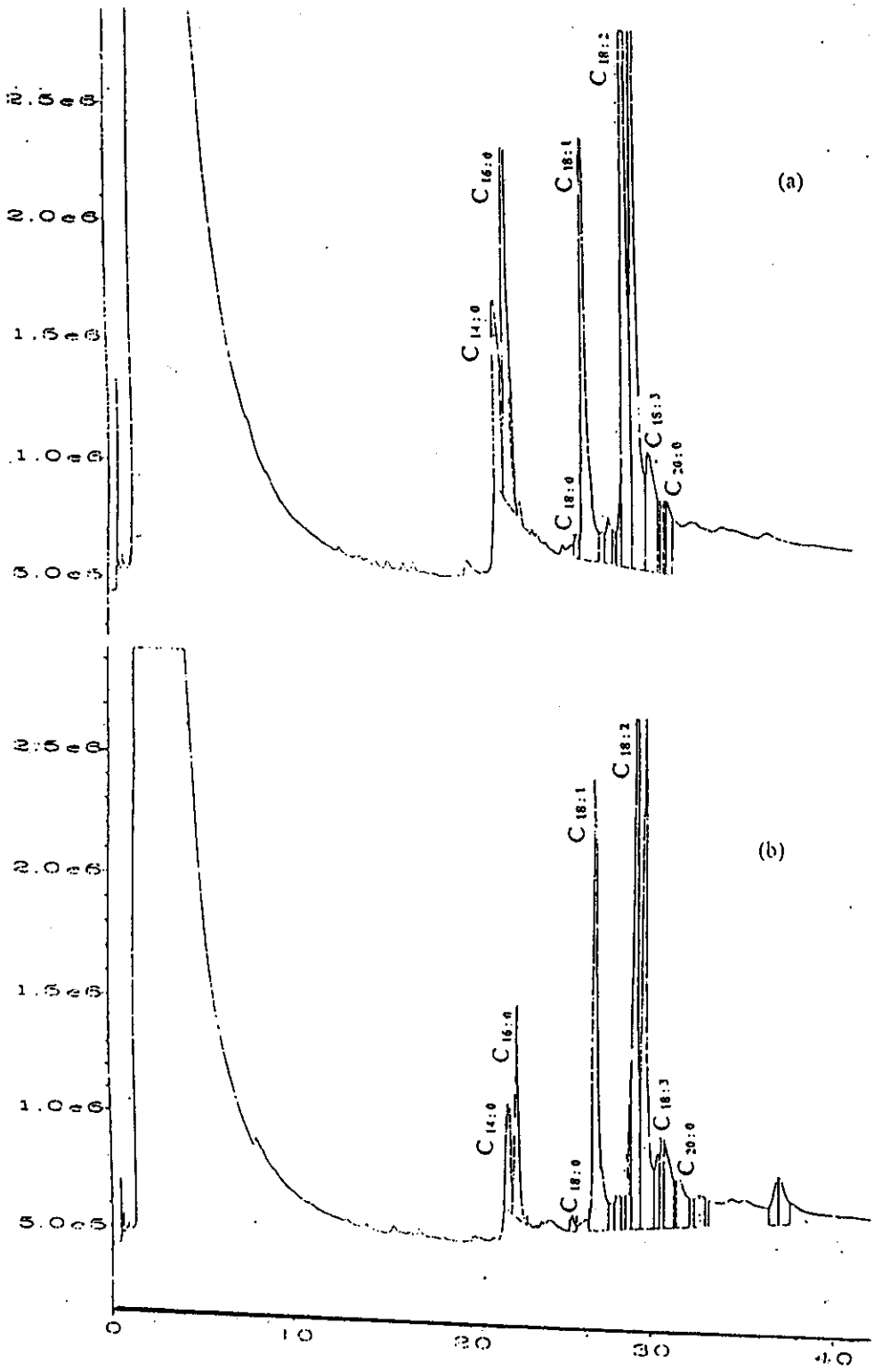

Figure (30) Fatty acids analysis profile of: a, sorghum high tannin (variety Framida) inoculated with *Aspergillus flavus* and stored under N_2 for 3 months and b, sorghum high tannin (variety Framida) inoculated with *Aspergillus flavus* and stored under N_2 for 6 months.

4.5. Aflatoxin production and accumulation :

4.5.1. Chemical and Physical properties of aflatoxin

Table (14) shows the chemical and physical properties of aflatoxin standards. The R_f values and colours of authentic aflatoxins B_1, B_2, G_1 and G_2 were chromatographed on silica gel DC-60 aluminum sheets, and viewed under u.v. light (366nm) and presented in table 14. The R_f determined by two solvent systems was as shown in the same table. In addition this table shows, the contain molecular formula, molecular weight and melting point of the aflatoxins (B_1, B_2, G_1 and G_2).

Table (14) : Chemical and physical properties of aflatoxin standards.

Aflatoxins	Colour	Molecular formula	Moleuclar weight	Melting point	R_f value*		References
					Solvent System I	solvent system II	
B_1	Blue	$C_{17} H_{12} O_6$	321	268-269	75.00	29.37	*Asao et al. (1965)*
B_2	Blue	$C_{17} H_{14} O_6$	314	286-289	67.85	26.57	*Chang et al. (1963)*
G_1	Green	$C_{17} H_{12} O_7$	328	244-246	60.71	22.37	*Asao et al. (1965)*
G_2	Green	$C_{17} H_{14} O_7$	330	237-240	53.57	17.48	*Hartiey et al. (1963)*

a, as detected under the UV (362-366 nm).

I, Chloroform : Acetone (90 : 10); II, Toluene: acetic acid: formic acid (6 : 3 : 1)

4.5.2.2.2.Quantitative determination of aflatoxins by HPLC with fluorescence detector:-

Table (15): shows aflatoxin production by toxigenic isolate of *Aspergillus flavus* in soybean seeds (variety Crawford), the aflatoxin levels quantified by high performance liquid chromatography (HPLC).

The seeds of healthy soybean inoculated by the toxigenic isolate of *Aspergillus flavus* , divided into three groups, each of three groups stored separately under the atmospheric air, CO_2 and N_2.

The results in table (24) indicated that, when soybean inoculated with *Aspergillus flavus* and stored under atmospheric air, for three months, the concentration of aflatoxin production (B_1, B_2 & G_2) were 33.82, 0.46 and 1.59. Whereas the total production 35.87. Note the absent of G_1 and the absent of all types of aflatoxin at zero time.

When soybean inoculated with *Aspergillus flavus* and stored under CO_2 , for three months, the concentration of aflatoxin production (B_1, B_2 & G_2) were 14.22, 0.42 and 0.89. Whereas the total production 15.54. Note the absent of G_1 and the absent of all types of aflatoxin at zero time.

While, when the soybean inoculated with *Aspergillus flavus* and stored under N_2, for three months, the concentration of aflatoxin production (B_2 & G_2) were 0.79 and 1.41. Whereas the total production 2.20. Note the absent of B_1 & G_1 and the absent of all types of aflatoxin at zero time.

From the above data, it is clear that, the concentration of production of aflatoxin(s) produced is higher in atmospheric air than in CO_2 and N_2. And the production of aflatoxin(s) is higher in CO_2 than N_2, because, N_2 may be, inhibit the fungal growth.

Table (15) : Aflatoxin production by toxigenic[a] isolate of *Aspergillus flavus* in soybean seeds (variety Crawford). The seeds inoculated by the toxigenic isolate of *Aspergillus flavus*, divided into three groups. Each of three groups stored separately, aflatoxin levels quantified by HPLC.

Aflatoxin type	Aflatoxin levels (concentration) (ng/g)				
	Host-toxigenic mold species interaction system**.				
	Soybean only (control)*		Soybean+ *A. flavus* + atmospheric air	soybean + *A. flavus* + CO_2	soybean + *A. flavus* +N_2
	Non inoculated (NI) Time zero	Non inoculated (NI) Time 3 months	Incubation (I)	Incubation (I)	Incubation (I)
B_1	-	x	33.82	14.22	0.00
B_2	-	x	0.46	0.42	0.79
G_1	-	x	0.00	0.00	0.00
G_2	-	x	1.59	0.89	1.41
Total	-	x	35.87	15.54	2.20

a, isolate derived from soybean (variety Crawford) which was isolated in this study; x, no data; * anlysis done at time zero (before inoculation) and ** analysis done at 3 months after inoculation.

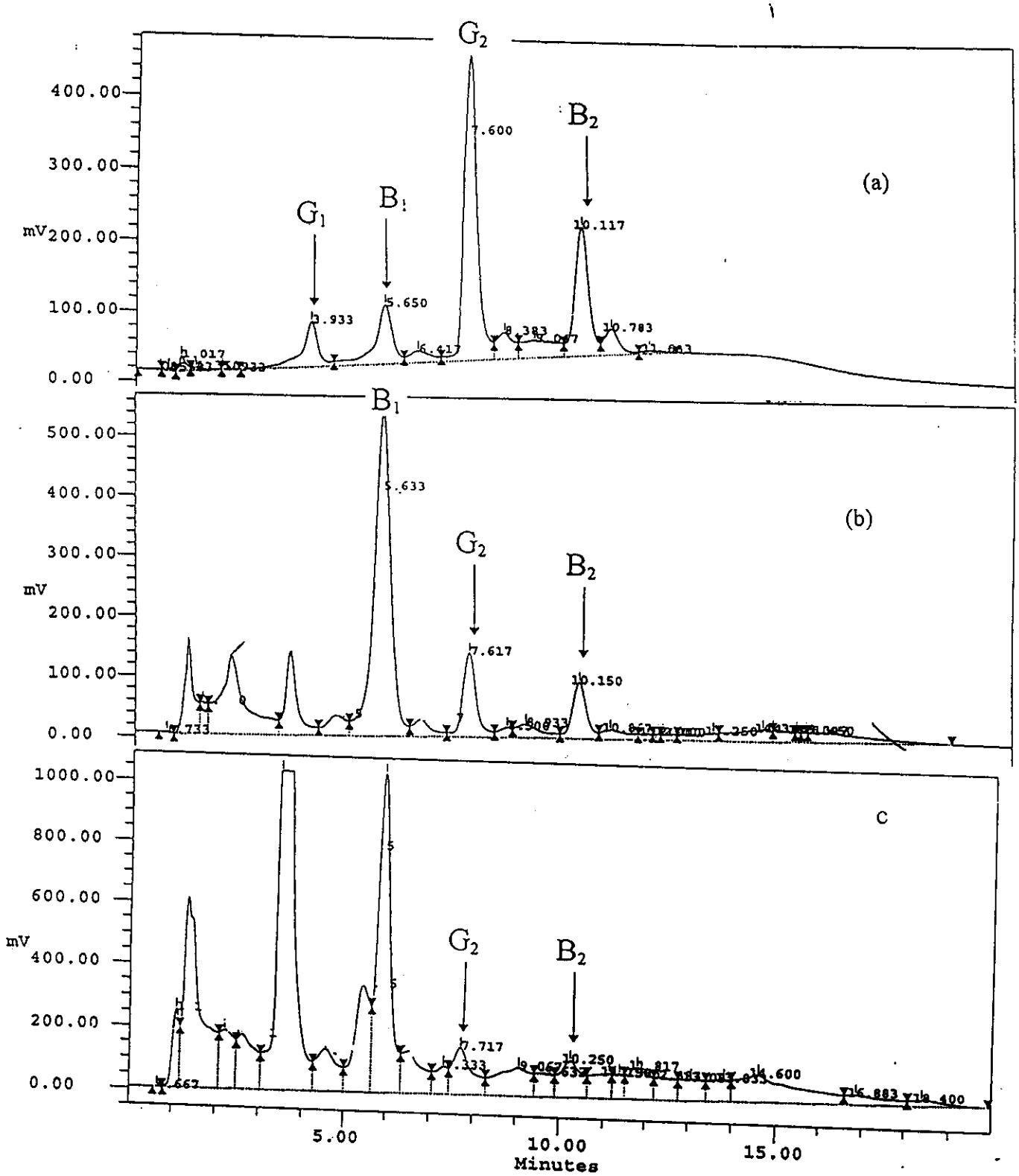

Figure (31) HPLC profile of : a , traces for aflatoxin standards, gradients as described in text. Times for B_1, B_2, G_1 and G_2 ; b , aflatoxin extract from inoculated soybean (*Glycine maxL.*, variety Crawford) by *Aspergillus flavus* and stored under CO_2 for 3 months and c , aflatoxin extract from inoculated soybean (*Glycine maxL.*, variety Crawford) by *Aspergillus flavus* and stored under N_2 for 3 months.

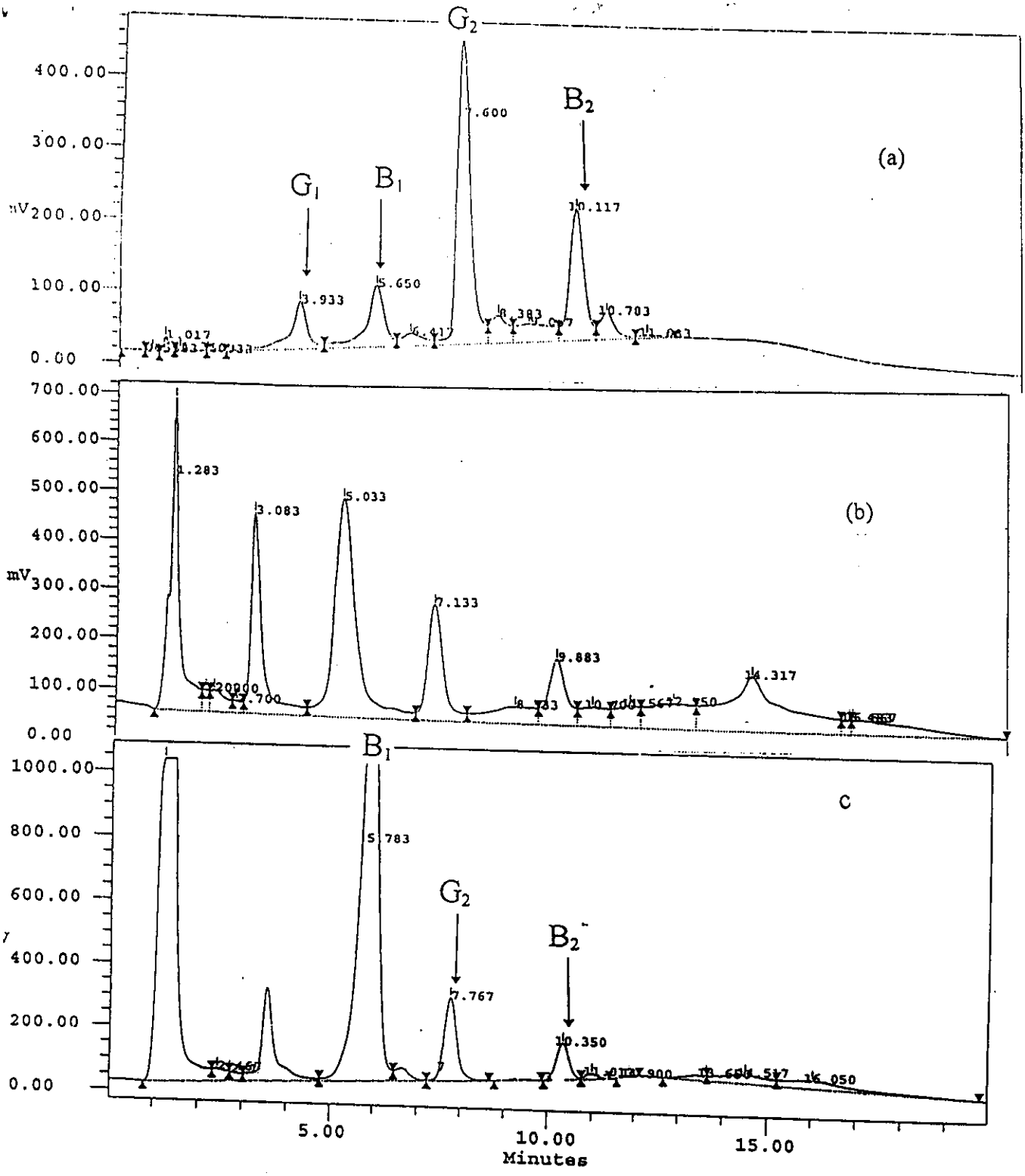

Figure (32) HPLC profile of : a , traces for aflatoxin standards, gradients as described in text. Times for B_1, B_2, G_1 and G_2 ;b, aflatoxin extract from soybean seeds (*Glycine maxL.*, variety Crawford), uninoculated at the time of incubation at room temperature 25° c and c, aflatoxin extract from inoculated soybean seeds (*Glycine maxL.*, variety Crawford) by *Aspergillus flavus* and stored under atmospheric air, for 3 months.

4.5. Aflatoxin production and accumulation:

4.5.2. Screening of aflatoxin produced by the isolated molds in soybean and sorghum

4.5.2.1. *In vitro*

4.5.2.1.1.Soybean seeds

Table (16) shows, screening for aflatoxin produced by various isolates of fungi isolated from soybean seeds, (variety Crawford), grown in yeast-extract-sucrose medium.

Therefore it can be concluded that the thin layer chromatographic analysis of the purified chloroform extracts of the fermentation medium of each of the aflatoxins-positive isolates studied revealed that one or more fluorescing spots with R_f values identical to those of standards aflatoxins were visible .

Table (16) showed that, a total of (26) isolates of *Aspergillus flavus; Aspergillus niger* and *Penicillium funciculosum* were the studied isolates which have been screened for aflatoxins production. Among the 26 isolates, (14) represent the genus *A.flavus*; (5) represent the *Aspergillus* species (*A.niger*) while (7) represent the fungal mold *Penicillium funiculosum*.

In addition, in case of *Aspergillus flavus*, (4) isolates produced aflatoxin B_1 (No.1, No.4, No.6 and No.14), the isolates (No.1 and No.4) produce low detection level of aflatoxin and isolates (No.6 and No.14) produce very low detection level of aflatoxin ; (6) isolates produced aflatoxin B_2 (No.1 , No.2 , No.3, No. 4, No.6 and No. 9), the isolates (No.9, No.3 and No.1) produce moderate detection level of aflatoxin B_2 , while the isolates (No. 2 and No.6) produce low detection level of

aflatoxin B_2 and the isolate (No.4) produce high level detection of aflatoxin B_2 ; (7) isolates produced G_1 (No.7, No.9, No.10, No.11, No.12, No.13 and No.14).

The isolates (No.9, No.10, No.11, No.12, No.13 and No.14) produce very low detection level aflatoxin G_1 and the isolate (No.7) produce moderate detection level of G_1. And (2) isolates produce G_2 (No. 8 and No.14), the isolate No.8 produce low detection level of G_2, while the isolate No.14 produce very low detection level of G_2.

There are (5) isolates only produced more than one aflatoxin, (No.1, No.4, No.6, No.9 and No.14).

The (5) isolates which produced more than one aflatoxin were classified as follows. (3) isolates produced B_1 & B_2 (No.1, No.4 and No.6); one isolate (No.9) produced B_2 & G_1 and one isolate (No.14) produced B_1 , G_1 and G_2.

While one isolate (No.5) showed no aflatoxins production in YES medium.

In case of *Aspergillus niger* , all the (5) isolates of *Aspergillus niger* failed to produce aflatoxins in YES medium. While with *Penicillium funiculosum* , (7) isolates of *Penicillium funiculosum* were screened for aflatoxin production and classified as follow, (3) isolates (No. 20, No.24 and No. 25) show no aflatoxin production in (YES) medium; the isolate (No.21) produces aflatoxin at low detection level of B_2 and very low detection level of (G_1 and G_2); the isolates (No.22 and No.23) produce very low detection level of G_2 and the sevenths isolate (No.26) produces very low detection level of B_2.

Table (16): Screening for aflatoxin produced by various isolates of (mold) fungi, isolated from soybean seeds, (variety Crawford), grown in the liquid (YES) medium.

Fungal (genera & species)	Code	Aflatoxin form (type)				Aflatoxin standard (ng/ml)*			
		B_1	B_2	G_1	G_2	B_1	B_2	G_1	G_2
Aspergillus spp. *A. flavus* link:Fr.	1	+ (+)	+ (++)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A. flavus link:Fr.	2	- (-)	+ (+)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A.flavus link:Fr.	3	- (-)	+ (++)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A. flavus link:Fr.	4	+ (+)	+ (+++)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A. flavus link:Fr.	5	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A. flavus link:Fr.	6	+ (-+)	+ (+)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A. flavus link:Fr.	7	- (-)	- (-)	+ (++)	- (-)	(+++)	(+++)	(+++)	(+++)
A.flavus link:Fr.	8	- (-)	- (-)	- (-)	+ (+)	(+++)	(+++)	(+++)	(+++)
A. flavus link:Fr.	9	- (-)	+ (++)	+ (-+)	- (-)	(+++)	(++)	(+++)	(+++)
A. flavus link:Fr.	10	- (-)	- (-)	+ (-+)	- (-)	(+++)	(+++)	(+++)	(+++)
A. flavus link:Fr.	11	- (-)	- (-)	+ (-+)	- (-)	(+++)	(+++)	(+++)	(+++)
A. flavus link:Fr.	12	- (-)	- (-)	+ (-+)	- (-)	(+++)	(+++)	(+++)	(+++)
A. flavus link:Fr.	13	- (-)	- (-)	+ (-+)	- (-)	(+++)	(+++)	(+++)	(+++)
A. flavus link:Fr.	14	+ (-+)	- (-)	+ (-+)	+ (-+)	(+++)	(+++)	(+++)	(+++)

Continued Table (16)

Fungal (genera & species)	Code	Aflatoxin form (type)				Aflatoxin standard (ng/ml)"			
		B_1	B_2	G_1	G_2	B_1	B_2	G_1	G_2
Aspergillus spp. *A. niger* Van Tieghem	15	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A. niger Van Tieghem	16	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A.niger Van Tieghem	17	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A. niger Van Tieghem	18	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A. niger Van Tieghem	19	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
Penicillium spp. *P.funiculosum* Thom	20	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
P. funiculosum Thom	21	- (-)	+ (+)	+ (-+)	+ (-+)	(+++)	(+++)	(+++)	(+++)
P. funiculosum Thom	22	- (-)	- (-)	- (-)	+ (-+)	(+++)	(+++)	(+++)	(+++)
P.funiculosum Thom	23	- (-)	- (-)	- (-)	+ (-+)	(+++)	(+++)	(+++)	(+++)
P. funiculosum Thom	24	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
P. funiculosum Thom	25	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
P. funiculosum Thom	26	- (-)	+ (-+)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)

YES, yeast extract sucrose medium; a, level of standard type of aflatoxin as detected on TLC plates under U.V. light (365nm); known quantities of standard aflatoxins was ng/ml; - ; + represent negative and positive response of aflatoxin production (forms B_1, B_2, G_1 and G_2); (-+) represents very low detection level of aflatoxin (ng/ml); (+), low (ng/ml) ; (++), moderate (ng/ml) and (+++), high (ng/ml) based on comparable levels with aflatoxin standards.

4.5.2.1.2. Sorghum grains

4.5.2.1.2.1. Sorghum, low tannin content

Table (17) shows screening for aflatoxin produced by various isolates of (mold) fungi, isolated from sorghum grains, (low tannin, variety Dorado), grown in the liquid (YES) medium.

The results obtained from **Table (17)** revealed that (3) isolates of *Aspergillus flavus*; (2) isolates of *Aspergillus niger* and (7) isolates of *Alternaria alternatia* which were screened for aflatoxins production.

Of the isolates of *Aspergillus flavus*, tested one isolate (No.31) produces very low detection level of aflatoxin B_1 ; a second isolate (No.32) produce B_1 and B_2 (low level and very low detection level of aflatoxin), respectively.

In addition, there is one isolate (No.30) produce low detection level of aflatoxin B_1.

However all the isolates of *Aspergillus niger* and *Alternaria alternatia* failed to produce aflatoxins in the used YES medium.

Table (17): Screening for aflatoxin produced by various isolates of (mold) fungi, isolated from sorghum grains, (low tannin, variety Dorado), grown in the liquid (YES) medium.

Fungal (genera & species)	Code	Aflatoxin form (type)				Aflatoxin standard (ng/ml)*			
		B_1	B_2	G_1	G_2	B_1	B_2	G_1	G_2
Aspergillus spp. *A.flavus* link:Fr.	30	+ (+)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A. flavus link:Fr.	31	+ (-+)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A. flavus link:Fr.	32	+ (+)	+ (-+)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A.niger Van Tieghem	33	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A. niger Van Tieghem	34	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
Alternaria spp. *A.alternatia* (Fries) keissler	35	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A. alternatia (Fries) keissler	36	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A. alternatia (Fries) keissler	37	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)

Continued Table (17):

Fungal (genera & species)	Code	Aflatoxin form (type)				Aflatoxin standard (ng/ml)[a]			
		B_1	B_2	G_1	G_2	B_1	B_2	G_1	G_2
A. alternatia (Fries) keissler	38	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A. alternatia (Fries) keissler	39	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A. alternatia (Fries) keissler	40	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A. alternatia (Fries) keissler	41	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)

YES, yeast extract sucrose medium; a, level of standard type of aflatoxin as detected on TLC plates under U.V. light (365nm); known quantities of standard aflatoxins was ng/ml; - ; + represent negative and positive response of aflatoxin production (forms B_1, B_2, G_1 and G_2); (-+) represents very low detection level of aflatoxin (ng/ml); (+), low (ng/ml) ; (++), moderate (ng/ml) and (+++), high (ng/ml) based on comparable levels with aflatoxin standards.

4.5.2.1.2.2. Sorghum, high tannin, content

Table (18) shows the screening for aflatoxins produced by various isolates of (mold) fungi, isolated from sorghum grains, (high tannin, variety Framida), grown in the liquid (YES) medium.

The results from table **(18)** revealed that (3) isolates of *Aspergillus niger*; (4) isolates of *Alternaria alternatia*; (3) isolates of *Penicillium oxalium* and (2) isolates of *Penicillium funiculosum* were screened for aflatoxins production.

Of the isolates of *Aspergillus niger*, one isolate (No. 50) did not produce aflatoxin in YES medium; one isolate (No. 51) produced very low detection level of aflatoxin B_1 and one isolate (No.52) produce B_1 and B_2 (low level and very low detection level of aflatoxin), respectively.

While all isolates of *Alternaria alternatia*; *Penicillium oxalium* and *Penicillium funiculosum* did not produce these aflatoxins in the YES medium.

Table (18): Screening for aflatoxin produced by various isolates of (mold) fungi, isolated from sorghum grains, (high tannin, variety Framida), grown in the liquid (YES) medium.

Fungal (genera & species)	Code	Aflatoxin form (type)				Aflatoxin standard (ng/ml)[a]			
		B_1	B_2	G_1	G_2	B_1	B_2	G_1	G_2
Aspergillus spp. *A.niger* Van Tieghem	50	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A.niger Van Tieghem	51	+ (-+)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A. niger Van Tieghem	52	+ (+)	+ (-+)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
Alternaria spp. *A.alternatia* (Fries) keissler	53	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A.alternatia (Fries) keissler	54	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A.alternatia (Fries) keissler	55	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
A.alternatia (Fries) keissler	56	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)

Continued Table (18):

Fungal (genera & species)	Code	Aflatoxin form (type)				Aflatoxin standard (ng/ml)"			
		B_1	B_2	G_1	G_2	B_1	B_2	G_1	G_2
Penicillium spp. *P.oxalicum* **Currie & Thom**	57	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
P.oxalicum **Currie & Thom**	58	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
P. oxalicum **Currie & Thom**	59	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
P.funiculosum **Thom**	60	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)
P. funiculosum **Thom**	61	- (-)	- (-)	- (-)	- (-)	(+++)	(+++)	(+++)	(+++)

YES, yeast extract sucrose medium; a, level of standard type of aflatoxin as detected on TLC plates under U.V. light (365nm); known quantities of standard aflatoxins was ng/ml; -; + represent negative and positive response of aflatoxin production (forms B_1, B_2, G_1 and G_2); (-+) represents very low detection level of aflatoxin (ng/ml); (+), low (ng/ml) ; (++), moderate (ng/ml) and (+++), high (ng/ml) based on comparable levels with aflatoxin standards.

4.5.2.2. Production of aflatoxins by the isolated toxigenic mold isolates *in vivo* under different gaseous conditions.

4.5.2.2.1. Semiquantitative determination by T.L.C.

4.5.2.2.1.1. In soybean

4.5.2.2.1.1.1. For three months after inoculation and storage

Table (19) shows the semiquantitative analysis of aflatoxin in seeds of soybean (variety Crawford) inoculated by isolates of aflatoxin producing mold, and there are three groups, each of the three groups stored separately under normal atmospheric air, carbon dioxide and nitrogen, then incubated at room temperature at 25°C, for three months.

Group (I): which was stored under atmospheric air for 3 months and divided into three sub groups.

The first sub group contains healthy soybean (variety Crawford) which were inoculated by *Aspergillus flavus*. The results indicated that, there are (high level) of B_1 after three months and (very low level) of G_2 three months of storage after inoculation. Note the aflatoxin (B_1, B_2, G_1 and G_2) were absent at zero time.

The second sub group, contains healthy soybean (variety Crawford) which were inoculated by *Penicillium corylophilum*. The results indicated that B_1 produced at moderate level after three months and (very low detection level) of G_2 after three months. Note the aflatoxin (B_1, B_2, G_1 and G_2) were absent at zero time. While the third sub group, contains only healthy soybean (variety Crawford) which non inoculated (control). The results indicated that the absent of any type of aflatoxins (B_1, B_2, G_1 and G_2), in YES m ım used in this study , before and after the storage months.

Group (II): which was stored under carbon dioxide for 3 months and divided into three subgroups.

The first subgroup contains soybean (variety Crawford) which was inoculated by *Aspergillus flavus* and incubated at (25°C) for 3 months, the results indicate that there are (moderate detection level) of B_1 and (very low detection level) of G_2 after 3 months. Note the absent of aflatoxin (B_1, B_2, G_1 and G_2) at zero time.

The second subgroup contains soybean (variety Crawford) which was inoculated by *Penicillium corylophilum* and stored under CO_2 for 3 months at (25°C). The results indicate that, there are (very low level of B_1) and (very low level) of G_2. Note the aflatoxin (B_1, B_2, G_1 and G_2) were absent at zero time. The third subgroup contains healthy soybean (variety Crawford), non inoculated (control). The results indicated the absent of aflatoxins (B_1, B_2, G_1 and G_2) when stored under the same condition for 3 months.

Group (III): which was stored under nitrogen for three months and divided into three subgroups.

The first subgroup contains healthy soybean (variety Crawford) which inoculated by *Aspergillus flavus* and stored under N_2 for three months at room temperature (25°C). The results indicate that, the presence of (very low detection level) of B_2 and (low detection level) of G_2 after 3 months.

The second subgroup contains healthy soybean (variety Crawford) which was inoculated by *Penicillium corylophilum* and stored under N_2 for 3 months. The results indicate that, the presence of very low level of (B_1 & G_2) after three months. Whereas the third subgroup contains healthy soybean (variety Crawford), non inoculated (control). The results indicate the absent of aflatoxins (B_1, B_2, G_1 and G_2) when stored under the same condition for 3 months.

Table (19): Semiquantitative analysis[a] of aflatoxin produced in seeds of soybean (variety Crawford) seeds inoculated by isolates of aflatoxin producing mold, isolated in this study, divided into three groups, subsequently each of the three groups stored separately under normal atmospheric air, CO_2 and N_2, respectively and were incubated at room temperature (25 °C), for three months.

Host/cultivar-aflatoxin[b] producing mold species Interaction	storage conditions	Levels[c] of aflatoxin types (months) after inoculation							
		B_1		B_2		G_1		G_2	
		zero	3	zero	3	zero	3	zero	3
"Group I" Soybean (*Glycine maxL.*, variety Crawford) + *Aspergillus flavus*	Atmospheric air	-	+++ (+++)*	-	-	-	-	-	-+ (+++)*
Soybean (*Glycine maxL.*, variety Crawford) + *Penicillium corylophilum*		-	+-+ (+++)*	-	-	-	-	-	-+ (+++)*
Soybean (*Glycine maxL.*, variety Crawford) only, non inoculated (control)*		-	x	-	x	-	x	-	x
"Group II" Soybean (*Glycine maxL.*, variety Crawford) + *Aspergillus flavus*	Carbon dioxide	-	-+- (+++)*	-	-	-	-	-	- + (+++)*
Soybean (*Glycine maxL.*, variety Crawford) + *Penicillium corylophilum*		-	-+ (+++)*	-	-	-	-	-	- + (+++)*
Soybean (*Glycine maxL.*, variety Crawford) only, non inoculated (control)*		-	x	-	x	-	x	-	x

Continued Table (19).

Host/cultivar-aflatoxin[b] producing mold species interaction	storage conditions	Levels[c] of aflatoxin types (months) after inoculation							
		B_1		B_2		G_1		G_2	
		zero	3	zero	3	zero	3	Zero	3
"Group III" Soybean (*Glycine maxl..*, variety Crawford) + *Aspergillus flavus*	Nitrogen	-	-	-	-+ (+++)*	-	-	-	+ (+++)*
Soybean (*Glycine maxL.*, variety Crawford) + *Penicillium corylophilum*		-	-+ (+++)*	-	-	-	-	-	-+ (+++)*
Soybean (*Glycine maxL.*, variety Crawford) only, non inoculated (control)*		-	x	-	x	-	x	-	x

a, analysis was done on samples of the three month-old seeds of each of the three inoculated groups; b, soybean derived isolate; c, levels are evaluated semiquantitatively based on the arbitrary grading scores for the aflatoxin standards concentrations which were determined under UV (363nm) as compared to the equivalent levels of the aflatoxin in the observed tested samples; note, (-), absent ; (-+), very low level ; (+), low level ; (++) moderate level and (+++), high level ; (+++)* The parentheses with asterese represent the level of the developed standard type of aflatoxin and x , no data.

4.5.2.2.1.1.1.1. For six months after inoculation and storage

Table (20) shows the semiquantitative analysis of aflatoxin produced in seeds of soybean (variety Crawford) inoculated by isolates of aflatoxin producing mold, and there are three groups, each of the three groups stored separately under normal atmospheric air, CO_2 and N_2, respectively and were incubated at room temperature (25°C), for six months.

Group (I): which was stored under atmospheric air for six months and divided into three sub groups.

The first sub group contains healthy soybean, (variety Crawford) which inoculated by *Aspergillus flavus*. The results in sub group indicated that, there are high detection level of aflatoxin B_1 after six months of inoculation.

The second sub groups contains healthy soybean (variety Crawford) which was inoculated by *Penicillium corylophilum*. The results in sub group showed that, the presence of very low detection level of aflatoxins (B_1 and G_2) after 6 months. Whereas the third sub group contains healthy soybean (variety Crawford), which non inoculated, the data, in this section, showed that, absent of aflatoxin before and after of storage for 6 months.

Group (II): which was stored under carbon dioxide for six months and divided into three sub groups.

The first sub group include, healthy soybean (variety Crawford) which inoculated by *Aspergillus flavus*. The results in sub group showed that, the presence of very low detection level of aflatoxin (B_1 and G_2) after storage for 6 months.

The second sub group contains healthy soybean (variety Crawford) which was inoculated by *Penicillium corylophium*. The results indicate that, the presence of very low detection level of aflatoxins (B_1 and G_2) after 6 months of storage. Whereas the third sub group include, healthy soybean (variety Crawford) which non inoculated, and storage for 6 months, the data, show that, absent of aflatoxins before and after storage.

Group (III): which was stored under nitrogen for six months and divided into three sub groups.

The first sub group contains healthy soybean (variety Crawford) which was inoculated by *Aspergillus flavus*. The results showed that the presence of very low detection level of aflatoxin (B_2 and G_2) after storage for six months.

The second sub group include healthy soybean (variety Crawford) which inoculated by *Penicillium corylophilum*, the results, indicate that, the presence of very low detection level of aflatoxin G_2 after storage for six months. Whereas the third sub group contains healthy soybean (variety Crawford) which was non inoculated, the data showed that, absent of aflatoxin, before and after storage for 6 months.

Table (20): Semiquantitative analysis[a] of aflatoxin produced in seeds of soybean (variety Crawford) inoculated by isolates of aflatoxin producing mold, isolated in this study, divided into three groups, subsequently each of the three groups stored separately under normal atmospheric air, CO_2 and N_2, respectively and were incubated at room temperature (25 °C), for six months.

Host/cultivar-aflatoxin[b] producing mold species Interaction	Storage Conditions	Levels[c] of aflatoxin types (months) after inoculation							
		B_1		B_2		G_1		G_2	
		zero	6	zero	6	zero	6	zero	6
"Group I" Soybean (*Glycine maxL.*, variety Crawford) + *Aspergillus flavus*	Atmospheric air	-	+++ (+++)*	-	-	-	-	-	-
Soybean (*Glycine maxL.*, variety Crawford) + *Penicillium corylophilum*		-	-+ (+++)*	-	-	-	-	-	-+ (+++)*
Soybean (*Glycine maxL.*, variety Crawford), only non inoculated (control)*		-	x	-	x	-	x	-	x
"Group II" Soybean (*Glycine maxL.*, variety Crawford) + *Aspergillus flavus*	Carbon dioxide	-	-+ (+++)*	-	-	-	-	-	-+ (+++)*
Soybean (*Glycine maxL.*, variety Crawford) + *Penicillium corylophilum*		-	-+ (+++)*	-	-	-	-	-	-+ (+++)*
Soybean (*Glycine maxL.*, variety Crawford), only non inoculated (control)*		-	x	-	x	-	x	-	x

Continued Table (20).

Host/cultivar-aflatoxin[b] producing mold species interaction	storage conditions	Levels[c] of aflatoxin types (months) after inoculation							
		B_1		B_2		G_1		G_2	
		zero	6	zero	6	zero	6	zero	6
"Group III" Soybean (*Glycine maxL.*, variety Crawford) + *Aspergillus flavus*	Nitrogen	-	-	-	-+ (+++)*	-	-	-	-+ (+++)*
Soybean (*Glycine maxL.*, variety Crawford) + *Penicillium corylophilum*		-	-	-	-	-	-	-	-+ (+++)*
Soybean (*Glycine maxL.*, variety Crawford), only non inoculated (control)*		-	x	-	x	-	X	-	x

a, analysis was done on samples of the three month-old seeds of each of the three inoculated groups; b, soybean derived isolate; c, levels are evaluated semiquantitatively based on the arbitrary grading scores for the aflatoxin standards concentrations which were determined under UV (363nm) as compared to the equivalent levels of the aflatoxin in the observed tested samples; note, (-), absent ; (-+), very low level ; (+), low level ; (++) moderate level and (+++), high level ; (+++)* The parentheses with asterese. represent the level of the developed standard type of aflatoxin and x , no data.

4.5.1.2.1.2. In Sorghum:

4.5.2.2.1.2.1. Low tannin, variety Dorado (for three months after inoculation and storage

Table (21) shows the semiquantitative analysis of aflatoxin produced in grains of sorghum (low tannin, variety Dorado) inoculated by isolates of aflatoxin producing mold, and divided into three groups, each of the three groups stored separately under normal atmospheric air, carbon dioxide and nitrogen, respectively and were incubated at room temperature (25°C), for three months.

Group (I): which was stored under atmospheric air, and divided into three sub groups.

The first sub group contains healthy sorghum (low tannin, variety Dorado) which was inoculated by *Aspergillus flavus*. The results, showed that, there are moderate detection level of B_1 after three months and low detection level of aflatoxin G_2 after three months of storage of inoculation.

The second sub group contains, healthy sorghum (variety Dorado) which inoculated by *Penicillium corylophilum*. The results indicated that, the presence of very low detection level of aflatoxins (B_1 and G_2) after storage for 3 months. Whereas the third sub group contains healthy sorghum (variety Dorado) which was noin inoculated, the data, indicate that, absent of aflatoxins, after and before storage.

Group (II): which was stored under carbon dioxide for 3 months and divided into three sub groups.

The first sub group contains healthy sorghum (low tannin, variety Dorado) which was inoculated by *Aspergillus flavus*, the results show that, there are low detection level of aflatoxin B_1 and very low detection level of aflatoxins (B_2 and G_2) when storage for 3 months.

The second sub group contains, healthy sorghum (low tannin, variety Dorado) which was inoculated *Penicillium corylophilum*. The results indicated that, presence of low detection level of (B_1 and G_2) after storage for 3 months of inoculation. Whereas the third sub group contains, healthy sorghum (low tannin, variety Dorado), which was non inoculated, the results, indicate that, absent of aflatoxin after and before storage.

Group (III): which was stored under nitrogen, and divided into three sub groups.

The first sub group contains, healthy sorghum (variety Dorado) which was inoculated by *Aspergillus flavus*. The results indicated that, aflatoxin B_1 at very low detection level and low detection level of G_2 when storage for 3 months.

The second sub group contains healthy sorghum (variety Dorado) which was inoculated by *Penicillium corylophilum*. The results show that, presence of very low detection level of aflatoxin B_1 when storage for 3 months. Whereas the third sub group contains healthy sorghum, non inoculated, the data show that, absent of aflatoxin of any type, when storage for three months.

Table (21): Semiquantitative analysis[a] of aflatoxin produced in grains of sorghum (low tannin, varitey Dorado) inoculated by isolates of aflatoxin producing mold, isolated in this study, divided into three groups, subsequently each of the three groups stored separately under normal atmospheric air, CO_2 and N_2, respectively and were incubated at room temperature (25 °C), for three months.

Host/cultivar-aflatoxin[b] producing mold species Interaction	storage conditions	Levels[c] of aflatoxin types (months) after inoculation							
		B_1		B_2		G_1		G_2	
		zero	3	zero	3	zero	3	zero	3
"Group I" Sorghum (low tannin, varitey Dorado) + *Aspergillus flavus*	Atmospheric air	-	++ (+++)*	-	-	-	-	-	+ (+++)*
Sorghum (low tannin, varitey Dorado) + *Penicillium corylophilum*		-	-+ (+++)*	-	-	-	-	-	-+ (+++)*
Sorghum (low tannin, *sorghum bicolor*, variety Dorado), only non inoculated (control)*		-	x	-	X	-	x	-	x
"Group II" Sorghum (low tannin, variety Dorado) + *Aspergillus flavus*	Carbon dioxide	-	+ (+++)*	-	-+ (+++)*	-	-	-	-+ (+++)*
Sorghum (low tannin, variety Dorado) + *Penicillium corylophilum*		-	+ (+++)*	-	-	-	-	-	+ (+++)*
Sorghum (low tannin, *sorghum bicolor*, variety Dorado), only non inoculated (control)*		-	x	-	X	-	x	-	x

4.5.2.2.1.2.2. Low tannin, variety Dorado (for six months after inoculation and storage)

Table (22) shows the semiquantitative analysis of aflatoxin produced in grains of sorghum (low tannin, variety Dorado) inoculated by isolates of aflatoxin producing mold, and divided into three group, each of the three groups, stored separately under normal atmospheric air, carbon dioxide and nitrogen, respectively and were incubated at room temperature (25°C), for six months.

Group (I): which was stored under atmospheric air, and divided into three sub groups.

The first sub group contains healthy sorghum (low tannin, variety Dorado) which was inoculated by *Aspergillus flavus*. The results indicate that, presence of very low detection level of aflatoxin (B_1 and G_2), when storage for six months.

The second sub group contain healthy sorghum (low tannin, variety Dorado) which was inoculated by *Penicillium corylophilum*. The results, indicated that, presence of very low detection level of aflatoxin G_2, after six months of storage. Whereas the third section, contain, healthy sorghum (variety Dorado) which non inoculated, the data, show that, absent of aflatoxin before and after storage for 6 months.

Group (II): which was stored under carbon dioxide, for 6 months and divided into three sub groups.

The first sub group contains, healthy sorghum (variety Dorado) which was inoculated by *Aspergillus flavus*. The results indicated that there are very low detection level of aflatoxins (B_1, B_2 and G_2), after storage for 6 months.

The second sub group contains healthy sorghum (variety Dorado) which inoculated by *Penicillium corylophilum*. The results indicated

that, presence of very low detection level of aflatoxins (B_1 and G_2), when storage for 6 months.whereas the third sub group contains, healthy sorghum (variety Dorado) which non inoculated, the data, showed that, absent of aflatoxin before and after storage for 6 months.

Group (III): which was stored under nitrogen, for 6 months and divided into three sub groups.

The first sub group contains healthy sorghum (variety Dorado) which was inoculated by *Aspergillus flavus*. The results, indicated that, there are very low detection level of (B_1 and G_2), after storage for 6 months.

The second sub group contains, healthy sorghum (variety Dorado) which was inoculated by *Penicillium corylophilum*. The results, indicate that, presence of very low detection level of aflatoxin B_1, after storage for 6 months. Whereas the third sub group contains, healthy sorghum (variety Dorado) which was non inoculated, the data, showed that, absent of any type of aflatoxin before and after storage for 6 months.

Table (22): Semiquantitative analysis[a] of aflatoxin produced in grains of sorghum (low tannin, variety Dorado) inoculated by isolates of aflatoxin producing mold, isolated in this study, divided into three groups, subsequently each of the three groups stored separately under normal atmospheric air, CO_2 and N_2, respectively and were incubated at room temperature (25 °C), for six months.

Host/cultivar-aflatoxin[b] producing mold species interaction	Storage Conditions	Levels[c] of aflatoxin types (months) after inoculation							
		B_1		B_2		G_1		G_2	
		zero	6	zero	6	zero	6	zero	6
"Group I" Sorghum (low tannin, variety Dorado) + *Aspergillus flavus*	Atmospheric air	-	-+ (+++)*	-	-	-	-	-	- (+++)*
Sorghum (low tannin, variety Dorado) + *Penicillium corylophilum*		-	-	-	-	-	-	-	- (+++)*
Sorghum (low tannin, *sorghum bicolor*, variety Dorado), only non inoculated (control)*		-	x	-	x	-	x	-	x
"Group II" Sorghum (low tannin, variety Dorado) + *Aspergillus flavus*	Carbon dioxide	-	-+ (+++)*	-	-+ (+++)*	-	-	-	- (+++)*
Sorghum (low tannin, variety Dorado) + *Penicillium corylophilum*		-	- (+++)*	-	-	-	-	-	- (+++)*
Sorghum (low tannin, *sorghum bicolor*, variety Dorado), only non inoculated (control)*		-	x	-	x	-	x	-	x

Continued Table (22).

Host/cultivar-aflatoxin[b] producing mold species interaction	Storage Conditions	Levels[c] of aflatoxin types (months) after inoculation							
		B_1		B_2		G_1		G_2	
		zero	6	zero	6	zero	6	zero	6
"Group III" Sorghum (low tannin, variety Dorado) + *Aspergillus flavus*	Nitrogen	-	-+ (+++)*	-	-	-	-	-	-+ (+++)*
Sorghum (low tannin, variety Dorado)+ *Penicillium corylophilum*		-	-+ (+++)*	-	-	-	-	-	-
Sorghum (low tannin, *sorghum bicolor*, variety Dorado), only non inoculated (control)*		-	x	-	x	-	x	-	x

a, analysis was done on samples of the three month-old seeds of each of the three inoculated groups; b, soybean derived isolate; c, levels are evaluated semiquantitatively based on the arbitrary grading scores for the aflatoxin standards concentrations which were determined under UV (363nm) as compared to the equivalent levels of the aflatoxin in the observed tested samples; note, (-), absent ; (-+), very low level ; (+), low level ; (++) moderate level and (+++), high level ; (+++)* The parentheses with asterese. represent the level of the developed standard type of aflatoxin and x , no data.

4.5.2.2.1.2.2.1. High tannin, variety Framida (for three months after inoculation and storage)

Table (23) shows the semiquantitative analysis of aflatoxin produced in grains of sorghum (high tannins, variety Framida) inoculated by isolates of aflatoxin producing mold, and divided into three groups, each of the three groups stored separately under normal atmospheric air, CO_2 and N_2, respectively and were incubated at room temperature (25°C), for three months.

Group (I): which was stored under atmospheric air, for 3 months and divided into three sub groups.

The first sub group contains healthy sorghum (high tannins, variety Framida), which was inoculated by *Aspergillus flavus*. The results, indicated that, presence of low detection level of B_1 after 3 months.

The second sub group contains healthy sorghum (variety Framida), which was inoculated by *Penicillium corylophilum*. The results, showed that, there are very low level of aflatoxins (B_1 and B_2) after 3 months of storage. Whereas the third sub group contains healthy sorghum (variety Framida) which was non inoculated, the results, indicated that, absent of aflatoxin before and after 3 months of storage.

Group (II): which was stored under carbon dioxide, for 3 months and divided into three sub groups.

The first sub group contains healthy sorghum (variety Framida) which was inoculated by *Aspergillus flavus*. The results, indicated that, there are very low detection level of aflatoxins (B_1 and G_2) when storage for 3 months.

The second sub group contains healthy sorghum (variety Framida), which was inoculated by *Penicillium corylophilum*. The

results indicated that, presence of very low level of B_1 and low level of G_2. Whereas the third sub group contains healthy sorghum (variety Framida) which was non inoculated. The results, indicated that, absent of any type of aflatoxin before and after 3 months of storage.

Group (III): which was stored under nitrogen for 3 months, and divided into three sub groups.

The first sub group contains healthy sorghum (high tannins, variety Framida) which was inoculated by *Aspergillus flavus*. The results indicated that, presence of very low detection level of B_1 after storage for 3 months.

The second sub group contains healthy sorghum (variety Framida) which was inoculated by *Penicillium corylophilum*. The results, showed that, there are very low level of B_1 after storage for 3 months. Whereas the third sub group contains healthy sorghum (variety Framida) which was non inoculated. The results, indicate that, absent of aflatoxin before and after 3 months of storage.

Table (23): Semiquantitative analysis[a] of aflatoxin produced in grains of sorghum (high tannins, variety Framida) inoculated by isolates of aflatoxin producing mold, isolated in this study, divided into three groups, subsequently each of the three groups stored separately under normal atmospheric air, CO_2 and N_2, respectively and were incubated at room temperature (25 °C), for three months.

Host/cultivar-aflatoxin[b] producing mold species interaction	storage conditions	Levels[c] of aflatoxin types (months) after inoculation							
		B_1		B_2		G_1		G_2	
		zero	3	zero	3	zero	3	zero	3
"Group I" Sorghum (high tannins, variety Framida)+ *Aspergillus flavus*	Atmospheric air	-	+ (+++)*	-	-	-	-	-	-
Sorghum (high tannins, variety Framida)+ *Penicillium corylophilum*		-	-+ (+++)*	-	-+ (+++)*	-	-	-	-
Sorghum (high tannins, *sorghum bicolor*, variety Framida), only non inoculated (control)*		-	x	-	x	-	x	-	x
"Group II" Sorghum (high tannins, variety Framida)+ *Aspergillus flavus*	Carbon dioxide	-	-+ (+++)*	-	-	-	-	-	-+ (+++)*
Sorghum (high tannins, variety Framida)+ *Penicillium corylophilum*		-	-+ (+++)*	-	-	-	-	-	+ (+++)*
Sorghum (high tannins, *sorghum bicolor*, variety Framida), only non inoculated (control)*		-	x	-	x	-	x	-	x

Continued Table (23).

Host/cultivar-aflatoxin[b] producing mold species interaction	storage conditions	Levels[c] of aflatoxin types (months) after inoculation							
		B_1		B_2		G_1		G_2	
		zero	3	zero	3	zero	3	zero	3
"Group III" Sorghum (high tannins, variety Framida)+ *Aspergillus flavus*	Nitrogen	-	-+ (+++)*	-	-	-	-	-	-
Sorghum (high tannins, variety Framida)+ *Penicillium corylophilum*		-	-+ (+++)*	-	-	-	-	-	-
Sorghum (high tannins, *sorghum bicolor*, variety Framida), only non inoculated (control)*		-	x	-	x	-	x	-	x

a, analysis was done on samples of the three month-old seeds of each of the three inoculated groups; b, soybean derived isolate; c, levels are evaluated semiquantitatively based on the arbitrary grading scores for the aflatoxin standards concentrations which were determined under UV (363nm) as compared to the equivalent levels of the aflatoxin in the observed tested samples; note, (-), absent ; (-+), very low level ; (+), low level ; (++) moderate level and (+++), high level ; (+++)* The parentheses with asterese represent the level of the developed standard type of aflatoxin and x , no data.

4.5.2.2.1.2.2.2. High tannin variety Framida (for six months after inoculation and storage)

Table (24) shows semiquantitative analysis of aflatoxin produced in grains of sorghum (high tannins, variety Framida) which was inoculated by isolates of aflatoxin producing mold and divided into three groups; each of the three groups were stored separately under normal atmospheric air, carbon dioxide and nitrogen, respectively and were incubated at room temperature (25°C) for six months.

Group (I): which was stored under atmospheric air for 6 months and was divided into three sub groups.

The first sub group, contains healthy sorghum (variety Framida) which was inoculated by *Aspergillus flavus*. The results indicated that presence of very low level of B_1, when storage proceeds for 6 months.

The second sub group contains healthy sorghum (variety Framida) which was inoculated by *Penicillium corylophilum*. The results, indicated that presence of very low level of B_1, after storage for 6 months. Whereas the third sub group contains healthy sorghum (variety Framida), which was non inoculated. The results indicated that, absent of any type of aflatoxins before and after storage for 6 months.

Group (II): which was stored under carbon dioxide, for 6 months, and was divided into three sub groups.

The first sub group contains healthy sorghum (variety Framida), which was inoculated by *Asperigllus flavus*. The results indicated that, presence of very low level of G_2.

The second sub group contains healthy sorghum (variety Framida) which was inoculated by *Penicillium corylophilum*. The results showed that, presence of very low level of (B_1 and G_2) after 6 months of storage. Whereas the third sub group contains healthy sorghum (variety

Framida), which was non inoculated. The results indicated that, absent of any type of aflatoxins before and after storage for 6 months.

Group (III): which was stored under nitrogen, for 6 months, and divided into three sub groups.

The first sub group contain healthy sorghum (high tannin, variety Framida) which was inoculated by *Aspergillus flavus*. The results indicated that, absent of any type of aflatoxins, after storage for 6 months.

The second sub group contains healthy sorghum (variety Framida), which was inoculated by *Penicillium corylophilum*. The results, indicated that, presence of very low detection level of B_1. Whereas the third sub group contains healthy sorghum (variety Framida), which was non inoculated, the results indicated that, absent of any type of aflatoxins before and after storage for 6 months.

Table (24): Semiquantitative analysis[a] of aflatoxin produced in grains of sorghum (high tannins, variety Framida) inoculated by isolates of aflatoxin producing mold, isolated in this study, divided into three groups, subsequently each of the three groups stored separately under normal atmospheric air, CO_2 and N_2, respectively and were incubated at room temperature (25 °C), for six months.

Host/cultivar-aflatoxin[b] producing mold species interaction	storage conditions	Levels[c] of aflatoxin types (months) after inoculation							
		B_1		B_2		G_1		G_2	
		zero	6	zero	6	zero	6	zero	6
"Group I" sorghum (high tannins, variety Framida)+ *Aspergillus flavus*	Atmospheric air	-	-+ (+++)*	-	-	-	-	-	-
Sorghum (high tannins, variety Framida)+ *Penicillium corylophilum*		-	-+ (+++)*	-	-	-	-	-	-
Sorghum (high tannins, *sorghum bicolor*, variety Framida), only non inoculated (control)*		-	x	-	x	-	x	-	x
"Group II" Sorghum (high tannins, variety Framida)+ *Aspergillus flavus*	Carbon dioxide	-	-	-	-	-	-	-	-+ (+++)*
Sorghum (high tannins, variety Framida)+ *Penicillium corylophilum*		-	-+ (+++)*	-	-	-	-	-	-+ (+++)*
Sorghum (high tannins, *sorghum bicolor*, variety Framida), only non inoculated (control)*		-	x	-	x	-	x	-	x

Continued Table (24).

Host/cultivar-aflatoxin[b] producing mold species interaction	storage conditions	Levels[c] of aflatoxin types (months) after inoculation							
		B_1		B_2		G_1		G_2	
		zero	6	zero	6	zero	6	zero	6
"Group III" Sorghum (high tannins, variety Framida)+ *Aspergillus flavus*	Nitrogen	-	-	-	-	-	-	-	-
Sorghum (high tannins, variety Framida)+ *Penicillium corylophilum*		-	-+ (+++)*	-	-	-	-	-	-
Sorghum (high tannins, *sorghum bicolor*, variety Framida), only non inoculated (control)*		-	x	-	x	-	x	-	x

a, analysis was done on samples of the three month-old seeds of each of the three inoculated groups; b, soybean derived isolate; c, levels are evaluated semiquantitatively based on the arbitrary grading scores for the aflatoxin standards concentrations which were determined under UV (363nm) as compared to the equivalent levels of the aflatoxin in the observed tested samples; note, (-), absent ; (-+), very low level ; (+), low level ; (++) moderate level and (+++), high level ; (+++)* The parentheses with asterese. represent the level of the developed standard type of aflatoxin and x , no data.

The results from tables (19-24) indicate that, when the grains and seeds of sorghum and soybean, stored under atmospheric air, the concentration of aflatoxin production is higher than storage under CO_2 or N_2, because the atmospheric air, stimulate the growth of the tested fungi (strains producing aflatoxin), while, storage under CO_2 inhibit, the growth of fungi and when the storage period increase, the rate of aflatoxin production decreased. The obtained data with the line of ***Glass et al., (1959)*** who have found that storage grains in nitrogen prevented mold growth at all moisture levels. Also with the line of ***Dukes and Apple (1965)*** who has been reported the need to reduce oxygen atmosphere to low level up to 1% or less to achieve a striking suppression of fungal growth.

Ciegler et al. (1966) reported that production and peak yields of aflatoxins were usually obtained in 72 hours after which the aflatoxin concentration declined rapidly. Degradation of aflatoxins depend primarily on mycelial lysis, and high aeration conditions.

Landers et al., (1967) studied the influence of atmospheric gases on aflatoxin production by *Aspergillus flavus* in peanut. It was found no reduction in growth and sporulation of the fungus occurred when the CO_2 concentration was increased from 0.03% (air) to 20%, but they were reduced with 20% increase in CO_2 from 20 to 80%. Striking reductions occurred when O_2 was reduced from 5 to 1% with 0, 20, or 80% CO_2.

Aflatoxin production in peanut kernels decreased with increasing concentrations of CO_2. Aflatoxin was lower in peanut stored at 15°C under 20% CO_2 for 6 weeks when O_2 was reduced from 20 to 5%.

The amount of oxygen required for spore germination, vegetable growth and sporulation might be highly variable, ***Mateles and Wogan, (1967).*** Likewise, fungi are available in their tolerance for high concentrations of carbon dioxide, ***Stozky and Goos, (1965). Diener and Davis (1968)*** reported that fungus growth, sporulation and aflatoxin were reduced with each successive 20% increase in CO_2 from 20% to 100%. No growth or aflatoxin production occurred in 100% CO_2. However, in general, reducing the oxygen concentration decreased aflatoxin production. Especially when O_2 was reduced from 5 to 1%.

Sauer, (1987) found that the aflatoxin producing fungus; *Aspergillus flavus* grow in maize in the field and also in stored grain after harvest. The most important factors affecting fungal growth and toxin production are moisture content and temperature, drying and cooling of the grain are the principal means of control. Other factors that affect, *Aspergillus flavus* growth are oxygen and carbon dioxide concentration, physical damage to the grain, initial levels of mold contamination, insect activity and genetic differences.

V-Summary

The present work included the results achieved from storing soybean seeds (variety Crawford) and two varieties of sorghum grains (variety Dorado and variety Framida) which characterized by low tannin content and high tannin content, respectively.

The inoculated soybean seeds and sorghum grains stored under atmospheric air, carbon dioxide and nitrogen for 3 and 6 months. The isolates of aflatoxin-producing fungi have been chosen to inoculate the tested samples of soybean seeds and sorghum grains. They were isolated from the soybean seeds and sorghum grains which have been used in this study. The aim of this work is to study the effect of storage conditions under different gaseous condition on the chemical composition of soybean and sorghum and on the production of aflatoxins.

The obtained data for soybean seeds and sorghum grains are summarized as follows:

1- Soybean seeds:

1- *Aspergillus niger* was the dominant fungus from fungi which were isolated from soybean (variety Crawford).

2-Chemical composition of soybean seeds has been determined before and after storage under carbon dioxide and nitrogen in the presence of fungi compared with storage of seeds under atmospheric air. Total carbohydrate was decreased under atmospheric air.

3-The percentage of oil is high under atmospheric air.

4-Each of proteins, total soluble sugars were increased.

5-Each of ash and fiber were increased directly with increase storage periods.

Conclusion: storage under N_2 is best than storage under CO_2 and atmospheric air.

6-The separation, identification and determination of fatty acids extracted from soybean seeds before and after storage under air, CO_2 and N_2 in the presence of fungi for 3 and 6 months were determined and the results revealed the following points.

When soybean seeds stored under air for 3 and 6 months, short chain fatty acids were appeared and each of oleic, linoleic and linolenic were decreased but palmitic, and stearic acids increased under these conditions.

Storage of soybean seeds under CO_2 for 3 and 6 months showed an increment in palmitic and stearic acid. Showed an increment after 3 months of storage and it decreased after 6 months of storage, also oleic acid decreased when soybean seeds stored under CO_2 months, each of linoleic and linolenic remained constant.

Storage of soybean seeds under N_2 for 3 and 6 months caused an increase in palmitic and stearic acids, but there is a slight decrease in oleic acid. On other hand, each of linoleic and linolenic remain constant.

2- Sorghum grains

1- *Alternaria alternatia* is the dominant fungus in case of sorghum (low tannins, variety Dorado). *Penicillium corylophilum; Aspergillus niger* and *Alternaria alternatia* were the most dominant fungi, in case of sorghum (high tannins, variety Framida).

2- Chemical composition of sorghum grains has been determined before and after storage under carbon dioxide and nitrogen, in the presence of fungi, compared with storage of seeds under atmospheric air. Total, a carbohydrate was decreased under atmospheric air.

3- The percentage of oil is high under atmospheric air.

4- Each of proteins, total soluble sugars were increased.

5- Each of ash and fiber were increased directly with increase storage periods.

Conclusion: storage under N_2 is best than storage under CO_2 an atmospheric air.

6- The separation, identification and determination of fatty acids extracted from sorghum grains before and after storage under air, CO_2 and N_2 in the presence of inoculated fungi for 3 and 6 months were determined and the results revealed the following points.

When sorghum grains stored under air for 3 and 6 months, short chain fatty acids were appeared and each of oleic, linoleic and linolenic were decreased but palmitic, and stearic acids increased under these conditions.

Storage of sorghum grains under CO_2 for 3 and 6 months showed an increment in palmitic and stearic acid. Showed an increment after 3

months of storage and it decreased after 6 months of storage, also oleic acid decreased when sorghum grains stored under CO_2 months, each of linoleic and linolenic remained constant.

Storage of sorghum grains under N_2 for 3 and 6 months caused an increase in palmitic and stearic acids, but there is a slight decrease in oleic acid. On other hand, each of linoleic and linolenic remained constant.

7- The tannin content when sorghum grains were stored under nitrogen, showed no variation compared with the control and the percentage of tannin content, remained constant, (because N_2 may be inhibit fungal or prevent fungal growth).

While the content of tannins increased in sorghum grains which were stored under atmospheric air, the tannins content decreased in sorghum grains which were stored under CO_2

Conclusion: The storage under N_2 is best than storage under CO_2 for the content of grains for tannins.

8- The isolates of fungi which were screened for the production of aflatoxins in the yeast-extract sucrose-medium (YES) showed variation for the response of aflatoxin production.

9- Analysis of aflatoxins which produced by the isolated fungi, which was performed by thin layer chromatography or high performance-liquid-chromatography (HPLC), showed that nitrogen prevents the production of aflatoxins and that the type of fungus involved affects the type and amount of aflatoxins produced.