Zagazig J. Agric. Res., Vol. 34 No .(4) 2007 643-662

FUNGI ASSOCIATED WITH CARAWAY AND CUMIN SEEDS AND THEIR AFLATOXINS PRODUCTION

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Accepted 12/4/2007

ABSTRACT: Blotter and deep-freezing methods recommended by International Seed Testing Association (ISTA) were selected for detecting seed-borne fungi in caraway and cumin seeds. The total numbers of the detected fungi on caraway seeds were 26 and 22 species in blotter and deep freezing methods, respectively. However, on cumin seeds, were 25 species in blotter and 22 species in deep freezing method. Pericarp and mericarp seed parts contained most of the associated seed borne fungi, while embryo contained lower one. Seeds of caraway and cumin stored for one year in polyethylene bags and Jute sacks under lab. conditions revealed 20 species of fungi in caraway seeds,while in cumin seeds only 19 species were detected by using Blotter method. The moisture content, germinability and aflatoxins production were determined monthly in the stored seeds. Using synthetic medium, only 7 isolates out of 55 *Aspergillus flavus* isolates obtained from stored caraway seeds produced aflatoxins, while 3 isolates only among 28 isolates from stored cumin seeds produced aflatoxin.

Key words: Umbelliperous plants-seed health testing-seed borne fungi-aflatoxins

INTRODUCTION

The umbelliferous plants viz. coriander, cumin, aniseed, fennel, caraway and khillah etc. are grown as short annual herbs in the Mediterranean region for a long time, and was difficult to know the establish origin. The plants are propagated by seeds obtained from ripe fruits which collected during April to June. These crops are successfully grown in Middle and Upper Egypt. They classified as spicy and condiment plants, and also as aromatic and medicinal crops (Adel-Salam, 2000).

The most important of these crops in Egypt including:-

1. Caraway (*Carum carvi* L.), the fruits of this plant were known to the Arabian physicians from the thirteen century.

Medicinally, the fruits and their oil are used as antispasmodic carminative, mild somatic, disinfectant, digestive, relieve spasms and flatulence. Also, caraway fruits are used to treat rheumatism and pleurisy, and the plants are used in the manufacture of some spirits (Schavenbery and Paris, 1977).

2. Cumin (*Cumin cyminum* L.), the fruits of this plant and their oil are widely used as condiment for the flavoring of certain types of sausage, carminative, now chiefly used in veterinary medicine and culinary preparations.

The most cultivated area of caraway and cumin in Egypt are located in some Upper Egypt governorates (El-Minia and Assiout)

Fusarium solani and *F. semitectum* were recorded on cumin and coriander seeds using the blotter test. (Ram Nath *et al.* 1970). Narayan and Prasad (1981) isolated 45 fungi from *Foeniculum vulgars* seeds stored for three years. Numbers of fungi were gradually increased up to the second year and remained constant during the third year. In (2002), Szczeponek and Mazur isolated 383 fungi from caraway seeds. Among complex of different isolated pathogens *Alternaria* spp., *Fusarium* spp., and *Epicoccum* spp. were predominant.

Sharma and Sharma (1984) isolated the fungi associated with fennel fruits stored in muslin bags for 180 days at 28 °C and RH between 66% and 75%. *Aspergillus* spp. and *Penicillium* spp. were the predominated genera among the 26 isolated ones.

Ranu and Singh (1990) found that, 89 % of samples of fennel, coriander, cumin, and ammi were contaminated with aflatoxin B1 at the levels of 3000 ppb, 1640 ppb, 1580 ppb and 2550 ppb respectively. The natural occurrence of moulds, specially *Aspergillus flavus* incidence, and levels of aflatoxins (B1, B2, G1 and G2) production in 212 samples of medicinal plants and spices were studied by Soliman and Ismail (1999). The highest frequency of *A. flavus* was recorded in caraway (39.2%); karkade (38.3%) and peppermint (11.6%). Screening *A.flavus* strains showed that, some strains were toxigenic, and producing different combinations of aflatoxins. The total amount of aflatoxins ranged between 0.42 – 12.34 µg/kg.

The aim of the present work is to detect and identify the seed-borne fungi associated with caraway and cumin seeds. The effect of different storage methods (jute sacks and polyethylene bags) on the fungi population and the possibility of aflatoxin production in stored seeds were also studied.

MATERIALS AND METHODS

Seed Health Testing

Seed sampling

Seed samples of cumin and caraway were collected from both commercial markets and different fields in Assiout, El-Minia, El-Sharkia, El-Gharbia and El-Dakhlia governorates of Egypt during the years from 2000 up till 2004.

Isolation from whole seeds

Detection of seed-borne fungi was carried-out using the rules of International Seed Testing Association (ISTA, 1993). Four hundred seeds from each sample taken randomly were tested using standard blotter and deep freezing methods as follows:

Blotter method

Two hundred seeds of each sample were tested. Twenty-five seeds were plated in 9 cm diameter sterilized Petri-dish containing 3 layers of moistened filter papers. The dishes were incubated at 20 ± 2 °C for 7 days under fluorescent light with a 12 hours cycle light and darkness.

Deep freezing method

The seeds were plated as in blotter method and incubated at room temperature for 24 hours, then the dishes were deep frozen at -20°C for 24 hours under complete darkness, then incubated at 20 ±2 °C for five days under fluorescent light with a 12 hours cycle light and darkness.

At the end of the incubations period, for both of blotter and deep freezing methods, dishes were examined using stereoscopic binocular microscope (6-50X magnification) for the presence of seed-borne fungi and their morphological features. The compound microscope was also used for confirmation. The percentages of the infected sample were calculated using the following formulae:

% of infected sample = number of sample infected / total number of samples x 100. The infection range recorded as low and high frequency of fungi in samples. The developed fungi were isolated and purified through the hyphal-tip and/or single spore techniques (Hildebrand, 1938). The pure cultures were maintained on PDA slants and kept at 5°C.

Isolation from seed parts

Samples of one hundred seeds, of caraway and cumin, from each sample, were tested using the component plating method as described by Maden *et al.* (1975). The seeds were surface sterilized by rinsing in 1% NaOCl for 2 minutes. Sterilized seeds washed thoroughly with sterile water and soaked in sterilized water for four hours. Seeds were then dissected aseptically into different parts, i. e., pericarp, mericarp and embryo.

Each part as well as the whole seeds, treated and/or untreated were placed on sterile tissue paper until dryness then plated as in blotter method. After seven days of incubation under 12 hours fluorescent light alternated 12 hours darkness cycle at 25± 2°C, the whole seed and seed parts were examined for fungal infection. The detected fungi were isolated and purified as previously mentioned then stored at 5°C.

Identification of isolated seed-borne fungi

The isolated fungi were identified using the Commonwealth Mycological Institute Description Sheets, Danish Government Institute of Seed Pathology Publications, and research work of Raper and Fennel (1965), Ellis (1971), Chidambaram *et al.* (1973), Seung – Hum *et al.* (1982), Booth (1985), Burrges *et al.* (1988), Singh *et al.* (1991) and Barrnet & Hunter (1998).

Storage Studies

Source of stored seed samples

Seed samples were collected from Assiout governorate where the largest area for cumin and caraway cultivation in 2002 growing season. The collected commercial variety stored under lab. conditions at Tag El–Ezz Agric. Res. Station. The average temperature and relative humidity profiles of the store were regularly recorded, and tabulated in Table 1. Sample of each crop (10 kg) was stored in two types of storage methods viz; jute sacks and polyethylene bags during May, 2002. Seed lot was stored for 1 year started from May, 2002 and ended in April, 2003.

Seed health testing of stored seeds

During storage period, 400 seeds from each stored sample were examined every 2 months using Standard Blotter method as previously described. After 7 days of incubation at 20°C, seeds were tested for the presence of fungal species and their frequency. Percentage of recover and fungal frequency associated with caraway and cumin seeds were estimated.

Germinability tests

The germinability tests were carried out for cumin and caraway seed samples at the beginning of storage and also periodically every two months of storage. The international rule of seed testing suggested by ISTA, (1993) was followed. Four hundred seeds, used as four replicates each represent (100 seeds) were tested in Petri dishes (9 cm. diam.) containing three layers of water-soaked blotters (25 seeds /plate). The plates were incubated at 20 ± 2°C under illumination cool white fluorescent lamp incubater and alternating cycle of 12 hours light and darkness. The counts of germinated seeds were determined after 15 days.

Table 1. The average temperature and relative humidity of the store during storage period.

Store °C & R.H	2002								2003			
	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
Temperature (°C)	24.5	27.5	31.0	30.0	28.5	26.0	22.0	18.0	17.0	15.0	17.0	21.5
Relative humidity (RH)	63.0	68.0	70.0	70.5	67.5	70.5	70.5	73.0	70.0	68.0	69.5	65.0

Moisture content measurements

Moisture content of both caraway and cumin seeds were determined at harvest and two month periodically of the storage by the method of A. O. A. C., (1980). The moisture content of seeds was calculated according to the methods described by (ISTA, 1993).

Analysis of aflatoxins

The analysis of aflatoxins production of stored seeds was carried out by Central Laboratory of Residue Analysis of Pesticides and Heavy Metals in Food, Agriculture Research Center. The method applied was according to A.O.A.C (1995) using high pressure liquid chromatography assay to determine the quantity of aflatoxins.

Screening of *Aspergillus flavus* isolates for aflatoxin production

Isolates of *Aspergillus flavus* were screened for their mycotoxigenic potential according to the color test suggested by Beuchat (1984). The moulds colonies showed orange-yellow reverse coloration indicative of potentially aflatoxigenic of *Aspergillus* were recorded after 42 – 44 hours of incubation.

Statistical Analysis

Statistical analysis for all the previously mentioned experiments had been carried out according to the methods described by Snedecor and Cochoran (1980).

RESULTS AND DISCUSSION

Isolation and Identification of Seed Borne Fungi

Caraway seed

A total of 26 fungal species belongs to 16 genera were isolated and identified from 82 collected samples as shown in Table 2. The occurrence of each fungus was recorded in terms of percentage and range of infection.

The data obtained showed that the dominant observed fungi were

Table 2. Percentage of fungi isolated from caraway seed samples (82 samples) using standard plotter and deep freezing methods.

Fungi	Blotter method			Deep freezing method		
	NIS	% of infection	range of infection	NIS	% of infection	range of infection
Alternaria alternata	75	91.5	(0.5–90)	68	83	(0.5–80)
Alternaria burnsii	22	26.8	(0-10)	25	30.4	(0-15)
Aspergillus flavus	36	43.9	(1–8)	24	29.26	(1–5)
A. fumigatus	28	34.1	(0–5)	16	19.5	(0–2.5)
A. niger	58	70.7	(0–15)	28	34.14	(1–12)
A. ochraceus	15	18.3	(0–3.5)	7	8.5	(1–4)
A. tamarii	9	11.0	(0–1)	6	7.3	(0–2.5)
A. versicolor	12	14.6	(0–3)	9	11.0	(0–2)
Botrytis sp.	4	4.8	(0–3)	0	0	0
Chaetomium sp.	7	8.50	(0–1)	4	4.8	(0–0.5)
Cladosporium sp.	15	18.3	(0–3)	10	12.0	(0–5)
Curvularia lunata	2	2.40	(0–1)	2	2.4	(0–0.5)
Epicoccum sp.	1	1.2	0.5	1	1.2	0.5
Fusarium equiseti	1	1.2	0.5	0	0	0
F. moniliforme	25	30.4	(0–1.5)	12	14.6	(0–0.5)
F. oxysporum	24	29.3	(0–2.5)	17	20.7	(0–1)
F. semitectum	5	6.0	(0–1)	3	3.6	(0–0.5)
F. solani	18	22.0	(0–2)	10	12.2	(0–2)
Helminthosporium spp.	31	37.8	(0–15)	26	31.7	(0–20)
Mucor spp.	15	18.3	(0–2)	13	15.8	(0–1)
Myrothecium sp.	4	4.9	(0–1)	0	0	0
Nigrospora sp.	2	2.4	(0–0.5)	0	0	0
Penicillium spp.	12	14.6	(1–3)	18	22.0	(1–5)
Phoma sp.	10	12.0	(0–8)	12	14.6	(0–6)
Rhizopus spp.	25	30.5	(0–12)	15	18.3	(0–5)
Stemphilium spp.	5	6.0	(0–7)	3	3.6	(0–3)
Chi-square value			559.487**			474.517**
Coefficient of variance (c.v)			99.34			112.64

NIS = Number of Infected Samples

Alternaria alternata, Aspergillus niger, A.flavus, Helminthosporium spp., *Aspergillus fumigatus, Rhizopus* spp., *Fusarium moniliforme, Fusarium oxysporum* and *Alternaria burnsii.*

Comparing the efficiency of two tested methods, the blotter method showed higher number of infected samples by fungi than the deep-freezing one except for *Alternaria burnsii.* Data presented in Table 2, also show that, blotter method was more accurate than deep-freezing one for detecting *Fusarium* spp. through percentage and range of infection. The percentage of such fungi was reduced when deep-freezing method was followed. These results are in agreement with the findings of Neergaard, (1979) and Ghoneem (1998) and differed

with the findings of Mathur, *et al.* (1975) who found that deep freezing method was superior to the blotter and agar plate methods for the detection of *Fusarium* spp. and *Chaetomium* sp. from sorghum seeds. Also blotter method was more accurate for detecting *Botrytis* sp. and *Helminthosporium* spp. On the other hand, the deep-freezing method was more accurate for detecting *Alternaria burnsii* and *Penicillium* spp. comparing with the blotter one.

It was also noticed from data in Table 2 that both methods were equally effective in detecting seed-borne *Curvularia lunata and Epicoccum sp.*

Four fungal species were recorded by blotter method and disappeared in deep-freezing technique i.e., *Botrytis* sp., *Fusarium equiseti, Myrothecium* sp. and *Nigrospora* sp. These results might be attributed to that the blotter method was known to provide a favourable conditions suit the development of mycelial growth and conidial sporulation of many imperfect fungi. Similar results were obtained by Neergaard, (1979) and Ghoneem (1998).

Cumin seeds

A total of 25 fungal species belongs to 15 genera were isolated and identified from 82 collected cumin samples. Comparing the composition of seed-borne fungi of caraway and cumin in Tables 2 and 3 data show that, *Botrytis* sp. and *Nigrospora* sp. were not detected in cumin seeds and detected in caraway seeds. While, *Trichoderma* spp. was detected in cumin seeds and not in caraway seeds.

Data in Table 3 shows that deep freezing method was more suitable for the detection of *Alternaria burnsii.* However, blotter method was also more accurate than deep freezing method for detecting most of fungi including *Alternaria alternate*, *Fusarium* spp. and *Trichoderma* sp. Data also show that *Aspergillus flavus* and *A. ochraceus* were dominant on seeds.

In Blotter method used the infection percentage of most fungi were higher than deep freezing method revealing more effective detection of seed borne fungi. The results of isolated seed-borne fungi from caraway and cumin were generally in agreement with Randhawa *et al.* (1995), Kishor Chand *et al.* (2000) and Szczeponek and Mazur (2002). Therefore, the researchers recommended blotter method when the purpose of seed examination is to survey all the mycoflora associated with the seed.

Table 3. Percentage of fungi isolated from cumin seed samples (82 samples) using standard blotter and deep freezing methods.

Fungi	Blotter method			Deep freezing method		
	NIS	%of infection	range of infection	NIS	%of infection	range of infection
Alternaria alternata	77	93.3	(5 - 80)	70	85.3	(9 - 75)
Alternaria burnsii	35	42.7	(1-10)	45	54.8	(1-22)
Aspergillus flavus	39	47.6	(3 - 25)	28	34.1	(1-3.5)
A. fumigatus	22	26.8	(0 – 9)	16	19.5	(1 - 9)
A. niger	62	75.6	(1 - 17)	35	42.6	(1- 5.5)
A. ochraceus	12	14.6	(2 - 5)	8	9.7	(1 – 2.5)
A. tamarii	5	6.0	(0.5 - 2)	5	6.0	(0 – 0.5)
A. versicolor	5	6.0	(0 - 2)	1	1.2	0.5
Chaetomium sp.	3	3.6	(0 - 1)	2	2.4	(0 – 0.5)
Cladosporium sp.	25	30.4	(0 - 12)	20	24.3	(1 – 5)
Curvularia lunata	2	2.4	(0 - 4)	1	1.2	0.5
Epicoccum sp.	8	9.7	(1 – 2.5)	3	3.6	(0 – 1)
Fusarium equiseti	2	2.4	(0 - 5)	0	0	0
F. moniliforme	15	18.5	(0 - 2)	5	6.0	(0 – 0.5)
F. oxysporum	22	26.8	(2 -15)	11	13.4	(1 – 5)
F. semitectum	1	1.2	0.5	0	0	0
F. solani	42	51.2	(1– 3.5)	12	14.6	(0 – 2.5)
Helminthosporium spp.	25	30.4	(2 - 20)	22	26.8	(2 – 18)
Mucor spp.	6	7.3	(0 – 0.5)	1	1.2	0.5
Myrothecium sp.	1	1.2	0.5	0	0	0
Penicillium spp.	32	39.0	(2 - 5)	18	22.0	(1 – 2.5)
Phoma sp.	2	2.4	(0 – 0.5)	1	1.2	0.5
Rhizopus spp.	22	26.8	(3 - 4)	10	12.0	(0 – 90)
Stemphilium spp.	10	12.0	(3 - 5)	8	9.7	(0 – 3)
Trichoderma sp.	2	2.4	(0 – 0.5)	0	0	0
Chi-square value		652.566*			627.31**	
Coefficient of variance (c.v)		104.55			130.74	

NIS = Number of infected samples

Seed parts of cumin and caraway

Data in Tables 4 and 5 revealed that, blotter method showed lower percentage of detected fungi in surface sterilized seeds with 1% NaOCl. On the other hand *Fusarium solani,* and *F. semitectum* showed similar infection for both surface sterilized and non- sterilized seeds. While, *F. oxysporum* showed an apparent increase in its incidence by the disinfestation treatment. The disinfestation decreased fast growth saprophytes on seeds. These results are in agreement with the findings of

Table 4. Percentage of seed-borne fungi in whole and different parts of caraway surface sterilized and non-surface sterilized seeds with sodium hypochlorite using standard blotter method.

Fungi	Whole seed		Treated seed parts		
	Non-surface sterilized seeds	Surface sterilized seeds	pericarp	Mericarp	Embryo
Alternaria alternata	56.00	35.00	22.00	10.00	3.00
Alternaria burnsii	25.00	14.00	8.00	5.00	1.00
Aspergillus spp.	12.00	8.00	4.25	2.75	1.25
Chaetomium sp.	2.25	1.75	2.25	1.00	0.00
Cladosporium spp.	4.50	3.25	3.00	0.75	0.00
Curvularia lunata	3.25	2.25	2.00	0.50	0.00
Epicoccum sp.	0.50	0.25	0.00	0.00	0.00
Fusarium equiseti	1.00	0.75	0.75	0.25	0.00
F. moniliforme	2.25	1.50	1.50	0.50	0.25
F. oxysporum	1.25	1.00	0.50	0.50	0.25
F. solani	1.00	1.00	1.00	0.00	0.00
F. semitectum	1.25	1.25	1.00	0.50	0.25
Helminthosporium sp.	22.00	12.00	11.00	8.00	1.75
Mucor spp.	1.00	0.50	0.50	0.00	0.00
Penicillium spp.	1.75	1.00	1.00	0.75	0.00
Phoma sp.	3.00	2.25	1.25	1.00	0.00
Rhizopus spp.	2.25	1.00	1.00	1.00	0.00
Stemphillium spp.	5.00	4.50	4.00	2.75	1.25
Mean	8.07	5.07	3.60	1.95	0.5

L.S.D at 5 % for:

Treatments (T) = 0.815 Fungi (F) = 1.547 T × F =3.459

Dawar Shahnaz and Abdul Ghaffar (1990). Yousef Safaa (2001) proved that surface disinfestations provides a chance for the internally seed-borne fungi to appear in greater number.

The results of isolated seed-borne fungi from caraway and cumin showed that both of *Aspergillus flavus* and *A. ochraceus* were dominant on seeds. These data attract the attention to their capability to produce aflatoxins and ochratoxins in seeds

Presence of fungi in different parts of caraway and cumin seeds showed that pericarp and mericarp contained most of fungi, while embryo contained lower numbers of seed- borne fungi. Also, percentage of fungi isolated from treated seed parts was higher than treated whole seeds. The

Table 5. Percentage of seed-borne fungi in whole and different parts of cumin surface sterilized and non-surface sterilized seeds with sodium hypochlorite using standard blotter method.

Fungi	Whole seed		Treated seed parts		
	Non-surface sterilized seeds	Surface sterilized seeds	Pericarp	Mericarp	Embryo
Alternaria alternata	62.00	42.00	24.00	12.00	2.50
Alternaria burnsii	25.00	15.00	11.00	8.00	1.75
Aspergillus spp.	18.00	11.00	5.00	2.00	0.25
Chaetomium sp.	1.75	0.25	0.25	0.25	0.00
Cladosporium spp.	7.00	2.50	2.25	1.25	0.00
Curvularia lunata	2.75	1.25	1.00	0.25	0.00
Fusarium equiseti	1.75	0.75	0.50	0.25	0.25
F. moniliforme	2.25	1.25	0.25	0.25	0.25
F. oxysporum	3.75	4.25	2.50	2.25	0.25
F. solani	1.25	1.25	0.75	0.25	0.00
F. semitectum	0.25	0.50	0.25	0.00	0.00
Helminthosporium sp	18.00	2.50	2.50	1.00	1.00
Mucor spp.	1.25	0.25	0.25	0.25	0.00
Penicillium spp.	3.75	1.25	1.75	1.50	0.25
Phoma sp.	0.25	0.00	0.25	0.00	0.00
Rhizopus spp.	7.00	1.00	1.25	0.50	0.00
Stemphilium spp.	8.50	4.25	3.25	1.50	0.25
Trichoderma sp.	2.25	2.00	1.25	0.75	0.00
Mean	9.26	5.06	3.23	1.79	0.37

L.S.D at 5 %
Treatments (T) = 1.225 Fungi (F) = 1.283 T × F =2.872

explanation of such results might be attribute to the infection of seeds coat in untreated seeds during many handling operations especially at threshing time. On the other hand, treated seeds decreased saprophytic fungi on seed surfaces than in untreated ones. These results are in agreement with those reported by Shaarawy (1987), Dawar Shahnaz and Abdul Ghaffar (1990), Jain and Jain (1995), Nwachukwu and Umechuruba (1997) and Shrestha *et al.* (2000). Fungi recovered from cumin seed parts are in harmony with that obtained by Shaarawy (1987) who isolated *Fusarium oxysporum, Alternaria alternata , Penicillium* spp., *Cladosporium* spp. and *Aspergillus flavus* from endospermic tissues of cumin seeds.

The percentage of *Alternaria alternata* infected cumin seed parts were 24, 2 and 2.50% in pericarp ,

mericarp and embryo, respectively (Table, 5) . Similar results were obtained by Shaarawy (1987) and also Chand *et al.* (1999) who found that percentage seed borne nature of *Alternaria alternata* in the pericarp, mericarp and embryo were 22, 9 and 2%, respectively in cumin seeds.

Storage Studies

Effect of jute sacks, polyethylene bags and periods of storage on associated fungi frequency with caraway and cumin seeds

Data present in Tables 6 and 7 revealed that, 20 species belongs to 14 genera of different fungi were isolated from caraway seeds and 19 species belongs to 12 genera were isolated from cumin seeds.

At the beginning of storage the dominant fungi on caraway and cumin seeds were *Alternaria* spp., *Cladosporium* spp., *Helmintho-sporium* spp., *Stemphillium* spp. and *Fusarium* sp. These fungi decreased in species and abundance with prolonging the storage period. Such fungi are considered as field fungi and require high moisture content mostly present in seeds at the time of harvest. *Aspergillus* spp., *Botrytis* sp., *Mucor* spp., *Myrothecium* sp., *Penicillium* spp. and *Rhizopus* spp. increased with increasing storage period. These mentioned fungi considered as storage fungi that require relatively low moisture content compared with field fungi. Increasing of such storage fungi might be due to the elimination of field fungi through decreasing moisture content and more compitition between storage fungi and field fungi. Species of *Aspergillus* and *Penicillium* increased gradually regarding the species and infection percentages with advancement of storage, in both polyethylene bags and jute sacks stored under ambient conditions. However, *Botrytis sp.*, *Fusarium oxysporum* and *F. solani* were disappeared at the end of storage. Similar results were obtained by Giridhar and Reddy (1997), Bankole *et al.* (1999) as well as Sharma and Sharma (1984).

Increase of seed-borne fungi incidence in stored Jute sacks was relatively slow compared to those of polyethylene bags. The explanation of such results might attributed to less ventilation and accumulation of high relative humidity at the ambient condition around seeds stored in the polyethylene bags, which might encourage and suitable for fungal spore germination, infection and further development (El-Shehaby *et al.*, 1997). Thus, perforated jute sacks might be much suitable for storing seeds.

Table 6. Percentage of isolated fungi during storage of caraway seeds for one year under laboratory conditions in Jute and Polyethylene bags.

Isolated Fungi	Zero Time	2002						2003						Mean	
		June		Aug.		Oct.		Dec.		Feb.		April			
		A*	B**	A	B	A	B	A	B	A	B	A	B	A	B
Alternaria alterata	85	75	70	50	45	40	45	25	30	25	15	25	15	40.00	36.67
Alternaria burnsii	60	45	45	30	30	20	25	20	15	15	12	10	8	23.33	22.50
Aspergillus flavus	1	2	1.5	15	10	17	18	24	27	28	30	30	35	19.33	20.25
A. fumigatus	0	0	0	3	0	7	7	20	18	26	15	32	8	14.67	8.00
A. niger	2	10	7	12	12	18	15	25	18	30	20	30	25	20.83	16.17
A. ochraceus	0	0	0	5	3	6	5	7	7	12	12	10	15	6.67	7.00
A. tamarii	0	0	0	1	3	3	8	7	9	12	9	12	10	5.83	6.50
Botrytis spp.	1	1	1	1.5	0.5	2.5	1	3	1.5	2.5	0	0	0	1.75	0.67
Chaetomium sp.	0	0	0	0	1	2	2	3	3	2.5	2	1	0	1.42	1.33
Cladosporium sp.	8	4	5	7	4	3	3	0	2	0	2	0	1	2.33	2.83
Curvularia lumata	3	2.5	2	3	2	1.5	1	1	0	1	0	0	0	1.50	0.83
Epicoccum sp.	0	0	0	0.5	0	0	0	0	0	0	0.5	0	1.5	0.08	0.33
Fusarium moniliforme	0	0	0	0.5	1	0.5	1.5	1	2	1.5	1	0.5	1	0.67	1.08
F. oxysporum	3	2.5	3	1.5	2	1	2	0	1	0	1	0	0	0.83	1.50
Helminthosporium spp.	5	7	6	8	7	7.5	4	6	3	5	2	1.5	2	5.83	4.00
Mucor spp.	1	1	1	1	1	0.5	1	1	1	1.5	1	2	1.5	1.16	1.08
Myrothecium sp.	0	0	0	1	0	1	1.5	0.5	1.5	1	0	0.5	0	0.67	0.50
Pencillium spp.	1	1.5	0	5	12	16	13	22	20	23	25	20	25	14.58	15.83
Rhizopus spp.	1	1	1	2.5	3	7	4	12	5	9	7	5	8	6.08	4.67
Stemphillium spp.	5	4.5	5	5	6	7	5	4.5	3	2	0	0	0	3.83	3.17

L.S.D at 5% for:							
Storage Time (A) :	n.s	n.s	0.456	n.s	n.s	n.s	0.560
Fungi (B):	1.257	2.741	1.234	1.240	1.455	1.526	1.769
AXB :	n.s	ns	1.671	1.752	2.058	2.158	2.504

* Jute sacks ** Polyethylene

Table 7. Percentage of isolated fungi during storage of cumin seeds for one year under laboratory conditions in Jute and Polyethylene bags.

Isolated Fungi	Zero Time	2002 June		2002 Aug.		2002 Oct.		2002 Dec.		2003 Feb.		2003 April		Mean	
		A*	B**	A	B	A	B	A	B	A	B	A	B	A	B
Alternaria alternata	80	65	62	45	40	30	20	30	20	30	15	35	10	39.17	27.83
Alternaria burnsii	50	40	42	35	25	20	15	15	15	10	11	10	5	20.00	18.83
Aspergillus flavus	0	2	2	5	7	15	20	18	25	28	28	30	37	16.33	19.83
A. fumigatus	0	0	0	0	1.5	2.5	4	5	17	10	22	15	20	5.42	10.75
A. niger	1	2.5	0.5	15	3	17	10	20	15	25	20	27	28	17.75	12.75
A. ochraceus	0	0	0	1.5	2	7	5	8	12	10	10	8	13	5.75	7.00
A. tamarii	0	0	0	2	1	0	2.5	3	4	3.5	5	5	5	2.25	2.92
Chaetomium spp.	0	0	0	0	1	0.5	2	1	2	1.5	1	0	0.5	0.50	1.08
Cladosporium sp.	25	20	15	10	10	8	5	5	5	5	0	2.5	0	8.42	5.83
Curvularia lumata	3	2.5	2	1	2	2.5	1	2	0	1	0	0	0	1.50	0.83
Epicoccum sp.	5	3	0.5	4	1	2	3	2	5	0	3	0	2	1.83	2.42
Fusarium moniliforme	0.5	1.5	2	5	5	7	12	2	13	2	7	2	2	3.25	6.83
F. oxysporum	5	4	4	2.5	2	0.5	2	0	1	0	0	0	0	1.17	1.50
F. solani	4	3.5	3	2	2	2	1	1	1.5	0	1	0	0	1.42	1.42
Helminthosporium spp.	10	12	12	15	15	13	15	8	9	4.5	7	1.5	3	9.00	10.17
Mucor spp.	0	0	0	1	1.5	3	2	5	4	7	4.5	8	6	4.00	3.00
Pencillium spp.	1	2	2	5	4	6	10	10	15	17	17	17	18	9.50	11.00
Rhizopus spp.	2	2	2	4	2	5	3	7	5	9	3	5	3	5.33	3.00
Stemphillium spp.	8	8	7	5	8	6	5	5	2	3	2	0	1	4.50	4.17

L.S.D at 5% for:							
Storage Time (A) :	n.s	n.s	0.456	n.s	0.347	n.s	n.s
Fungi (B):	2.457	1.134	1.404	1.464	1.074	1.369	1.634
AXB :	ns	ns	1.987	2.072	1.519	1.938	2.313

* Jute sacks ** Polyethylene bags

Effect of jute sacks, polyethylene bags and periods of storage on germinability of caraway and cumin seeds

Results in Table 8 indicate that, caraway and cumin germinated seeds were 95% and 90%, respectively at the starting time of storage. However, at the end of storage period the percentage of germinated seeds that stored in Jute sacks was 90% and 80% respectively, while seeds stored in polyethylene bags were 80% and 45%, respectively.

Results in the same table also show that, storage process in polyethylene bags caused decrease in the germinability than storage in jute sacks, in caraway and cumin seeds. Such results are in agreement with those obtained by Kumar and Kumar (2001). Several explanations have been put forward to explain decreased germination of seed in storage. The invasion of seeds by storage fungi leads to an escalating process involving increase in respiration, moisture content and temperature, resulting in damage of embryo, and these ultimately affect seed germination (Christensen and Kaufmann 1969). The production of toxins by storage fungi which often results in death of tissues in embryonic axis has also been put forward as one of the probable reasons. (Harman and Nash, 1972 and Lacey, 1975)

Effect of jute sacks, polyethylene bags and periods of storage on moisture content of caraway and cumin seeds

Data in Table 8 show that, moisture content of caraway and cumin seeds were 12.2 % and 9%, respectively at the start time of the storage in May. The moisture content of storage seeds declined with prolonged storage in the two type of storage container, but the decrease was more pronounced in jute sacks. At the end of the storage period, the seed moisture content of caraway was 9.50% and 10 % in jute sacks and polyethylene bags, respectively. While it was 6.5 and 7.5% in cumin, respectively. A decrease in moisture content was more pronounced in jute sacks. This finding may be due to the free exchange of moisture through the jute materials between the atmosphere inside this bag and the outside environment. Similar results were obtained by Bankole *et al.* (1999) who reported that, differences in moisture content of seeds stored within jute and polyethylene bags is reflection of the nature of the two bags. It is also due to the nature porous of jute sacks, there is free exchange of moisture between the inside of the storage container and the outside environment. However, the polyethylene bags having fine pores do not readily permit such free exchange of moisture.

Table 8. Changes in different parameters of caraway and cumin seed health in storage for one year in Jute sacks and polyethylene bags, under laboratory conditions.

Crops	Seed measurements	Zero Time	Jute sacks						Polyethylene bags					
			June	August	October	December	February	April	June	August	October	December	February	April
Caraway	% moisture content	12.2	11.8	10.2	10.0	9.5	9.5	9.5	11.9	11.5	10.5	10.0	10.0	10.0
	% seed germination	95	95	95	95	90	90	90	95	90	85	85	80	80
	aflatoxin production µg/kg	0.0	Less than LOQ*						Less than LOQ					
Cumin	% moisture content	9.0	8.0	7.0	6.5	6.25	6.5	6.5	8.5	8.0	8.0	7.5	7.5	7.5
	% seed germination	90	90	85	85	85	80	80	85	75	70	62	54	45
	aflatoxin production µg/kg	0.0	Less than LOQ						Less than LOQ					

*LOQ (limit of quantification) of aflatoxins detected; B1 (2) , B2 (2), G1 (2), G2 (2) µg/kg.

Aflatoxin Determination

The results in Table 8 show that, the amount of total aflatoxins (G_2 , G_1 , B_2 and B_1) in each storage sample of caraway and cumin after storage on different periods in polyethylene bags and jute sacks, were not detected or less than 2 µ g /Kg . The results obtained indicated that, the total level of aflatoxins in storage samples under natural conditions was below the limits of quantification (2µg /Kg) which was set consumption is 10µg /kg that were recommended by the commission regulation No. 472 of European Communities (2002) for the tolerance level of aflatoxins in seeds for human consumption. These results might be explained on the basis that changes in moisture contents of the seeds during storage were less to favor fungal growth and toxin production and drying the grains before storage were necessary. Similar results were obtained by Aziz *et al.* (1998), Soliman and Ismail (1999), Abdulkadir *et al.* (2002) and Keshri Seema and Monica Basu (2003).

Screening of isolated *Aspergillus flavus* for their mycotoxigenic potential

Data in Table 9 show that, only 7 isolates (12.7%), out of 55 isolates of *A.flavus* obtained from stored caraway seeds produced aflatoxin in synthetic medium, while among 28 isolates from stored cumin seeds only 3 isolates (10.7%) produced aflatoxin in synthetic medium.

Table 9. Screening of *Aspergillus flavus* isolates for their mycotoxigenic

Crop	No. of Isolated *A. flavus*	No. of toxigenic isolates	% of toxigenicity
Caraway	**55**	**7**	**12.7**
Cumin	**28**	**3**	**10.7**

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الفطريات المصاحبة لبذور الكمون والكراويا وإنتاج الأفلاتوكسينات

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أجريت اختبارات صحة وسلامة البذور على ٨٢ عينة لكل من حبوب الكمون والكراويا وتم جمعها من الأسواق المحلية والحقول في محافظات أسيوط والمنيا والشرقية والغربية والدقهلية .وذلك باستخدام طريقتي أوراق الترشيح المبللة وطريقة التجميد حيث تم اختبار ٤٠٠ بذرة من كل عينة بذور . - تم تعريف ٢٦ نوعاً من الفطريات تابعة لـ ١٦ جنساً من الفطريات المحمولة على بذور الكراويا و ٢٥ نوعاً تابعاً لـ ١٥ جنساً من الفطريات المحمولة على بذور نبات الكمون وقد وجد تشابهاً كبيراً في أنواع الفطريات المعزولة من نوعي البذور ووجد أن استخدام طريقة أوراق الترشيح المبللة كانت أكفأ في تحديد عدد أكبر من الفطريات المحمولة على البذور عن طريقة التجميد .و وجد أن معاملة البذور بـ ١% هيبوكلوريد صوديوم لمدة ٢ دقيقة ثم غسيل البذور بماء معقم ثلاث مرات يؤدي إلى خفض معنوي في عدد الفطريات المعزولة . تم تحديد مواقع بعض الفطريات المحمولة ببذور الكراويا والكمون بأجزاء البذرة المختلفة عن طريق استخدام طريقة أوراق الترشيح المبللة وقد وجد أن معظم الفطريات محمولة على الغلاف الخارجي والداخلي والقليل على الجنين .
تم تخزين بذور الكراويا والكمون في نوعين من العبوات وهي عبوات الجوت والبلاستيك لمدة عام كامل تحت الظروف الطبيعية. وتم تحديد الفطريات المصاحبه للبذور فى كل شهر من شهور التخزين لكل العينات . وقد تم عزل ٢٠ نوعا تابعه ل١٤ جنس من الفطريات من بذور الكرويا كما تم عزل ١٩ نوعا تابعة ل ١٢ جنس من الفطريات من بذور الكمون وذلك باستخدام طريقة ورق النشاف .ووجد ان عدد الفطريات المصاحبة للبذورتزداد وبخاصة فطريات المخزن بزيادة فترة التخزين بينما تقل فطريات الحقل تدريجيا وتم تحديد الرطوبة النسبية للبذور وكذلك نسبة الانبات فى كل شهر من شهور التخزين وقد وجد أن المحتوى الرطوبى وكذلك درجة الانبات تقل تدريجيا خلال فترة التخزين كما تم تحديد كمية السموم الفطرية (G1 , G2,B1 and B2) الموجودة طبيعيا فى البذرة فى كل شهر من شهور التخزين ووجد أنها أقل من الحد المسموح به المحدد بواسطة الاتحاد الاوربى وهو ١٠ميكروجرام /كجم بذرة فى كل العينات المخزنة فى نوعى العبوات. وقد تم إختبار جميع عزلات اسبرجلس فلافس لاظهار قدرتها على انتاج السموم الفطرية ووجد ان سبعة عزلات فقط من بين ٥٥ عزلة للفطراسبرجلس المعزوله من بذور الكراويا منتجة للأفلاتوكسين بينما من بين ٢٨ عزلة للفطر اسبرجلس المعزوله من بذور الكمون ٣ عزلات فقط هي المنتجة للأفلاتوكسين .