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Mesophilic Fungi and Mycotoxins Contamination of Libyan Cultivated Four Fabaceae Seeds

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Abstract: One hundred and forty three species in addition to 9 varieties belonging to 32 fungal genera were isolated and identified from 15 samples of each of broad bean, chickpea, kidney bean and sweet pea seeds collected from eight Shabias in Libya on 1% dextrose-Czapek's agar medium at 28±2°C using seed-plate method (3792 colonies 25 seeds⁻¹, 29 fungal genera, 111 species and 2 varieties) and dilution-plate method (2330 colonies g⁻¹ dry weight sample, 23 genera, 100 species and 7 varieties). The fungal genera of highest occurrence and their respective number of the species were *Aspergillus* (*A. flavus*, *A. niger* and *A. fumigatus*), *Penicillium* (*P. chrysogenum*), *Mucor* (*M. hiemalis*), *Alternaria* (*A. alternata*), *Fusarium* (*F. oxysporum*), *Rhizopus* (*R. stolonifer*) and *Eurotium* (*E. chevalieri* and *E. repens*). Mycotoxin assay proved that 16 samples (26.7%) out of 60 tested were toxic with different toxins and varying degrees of toxicity. No mycotoxins tested were detected in any chickpea seed samples investigated. It is the first report on mycoflora and mycotoxins of Fabaceae seeds in Libya, as well as 85 species in addition to 7 varieties belonging to 20 genera are new records to Libyan mycoflora.

Key words: Seed-borne fungi, mycotoxins, broad bean, kidney bean, chickpea, sweet pea

INTRODUCTION

Broad bean (*Vicia faba* L.), chickpea (*Cicer arietinum* L.), kidney bean (*Phaseolus vulgaris* L.) and sweet pea (*Pisum sativum* L.) are Fabaceae seeds cultivated in Libya and considered of the basic proteinous foods of people. Production of these seed crops were 1300, 200, 1000 and 6000 metric tons, respectively less than or not sufficient for consumption, therefore, about 4522 metric tons of Fabaceae seeds were imported (FAO, 2003).

High production of crops requires using high quality of seeds. However, seed-borne fungi play an important role in deterioration of seed quality, which leads to high economic losses in crop yield. Poor harvesting practices, improper storage and less than optimal conditions during transportation, marketing and processing can also contribute to fungal growth and increase the risk of mycotoxin production. The climatic conditions as well as the food production chains are characteristic in most parts of Africa and the largest mycotoxin-poisoning epidemic in a decade was reported in Africa during the last 5 years (Lewis *et al.*, 2005; Wagacha and Muthomi, 2008). Mycotoxins attract worldwide attention because of the significant economic losses associated with their impact on human health, animal productivity and trade (WHO, 2006). Mycotoxins are toxic secondary metabolites produced by many filamentous fungi and contaminated various agricultural commodities either before harvest or under post-harvest conditions. The nature occurrence of these mycotoxins mainly as (hepatotoxic, mutagenic, carcinogenic and teratogenic agents) on agricultural commodities and feed stuffs has become of increasing interest. Among these mycotoxins are aflatoxins, as secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*. Their recognition as potent carcinogens in human and some animals has made them the subject of government legislation as well as valuable tools in the study of cancer (WHO, 2006, Richard, 2007; Wagacha and Muthomi, 2008).

Numerous investigations have been carried out on Fabaceae seed-borne fungi and mycotoxins contamination all over the world (El-Kady and Youssef, 1993; Tseng and Tu, 1997; Costa and Scussel, 1998, 2002; Youssef *et al.*, 2002; Ibrahim, 2007; Gonçalves *et al.*, 2008; Kumar *et al.*, 2008). In Libya, no published studies exist on mycoflora and mycotoxins contamination of legume seeds, therefore, the purpose of this research was to study mycoflora and mycotoxins of four cultivated Libyan Fabaceae seed crops.

MATERIALS AND METHODS

Collection of Fabaceae Seed Samples

Fifteen seed samples of each of broad bean, chickpea, kidney bean and sweet pea of the crops of 2006, 500 g, each were collected from eight Shabias in Libya namely; Banighazy, Missrata, Tripoli, El-Mergab, Al-Zawia, Gefara, El-Nekat El-Khams and Naloot. Each sample was put in a sterile polyethylene bag sealed and put in another one which was also sealed to minimize the loss of water-content, transferred to the mycological laboratory and kept in a cool place (3-5°C) till fungal cultivation, isolation, identification and mycotoxins assay.

Determination of Moisture Content of Fabaceae Seeds

Twenty grams of each seed sample were milled and dried in an oven at 105°C for 24 h, then cooled in a desiccator and re-weighted to a constant weight. The moisture content was calculated as percentage of the dry weight according to the technique of International Seed Testing Association (1966).

Determination of Germinability of Seeds

One hundred seeds of each sample were incubated at 25°C over a pad of moist sterile filter paper placed in sterile Petri dishes for 7 days. The seeds with healthy roots and plumules were counted and the counts were expressed as percentages of the numbers of tested seeds.

Determination of Seed-Borne Fungi

Seed plate method as employed by El-Kady and Youssef (1993) and dilution plate method as described by Johnson and Curl (1972) were used for isolation of fungi. Modified 1% dextrose-Czapek's agar medium (g L⁻¹; Sodium nitrate 3.0, magnesium sulphate 0.5, potassium chloride 0.5, di-potassium hydrogen phosphate 1.0, iron sulphate 0.01, dextrose 10.0, agar agar 15.0-20.0, pH 7.3±0.1) was used as cultivation and isolation medium. Chloramphenicol (0.5 mg mL⁻¹) and rosebengal (30 ppm) were added to the medium as bacteriostatic agents (Al-Doory, 1980; Martin, 1950). Ten plates, five for each method were used for each sample. The plates were incubated at 28±2°C for 7- 15 days and the developing fungi were identified, counted and calculated per 25 seeds and per gram dry weight of each tested sample using the previous two isolating methods, respectively. The colonies of slow growing fungi as well as mycelial bits were transferred to slants with special media to ensure precise counting, then to plate for identification.

The following references were used for identification of fungi (based on purely morphologically macro- and microscopic characteristics): (Ellis, 1971, 1976; Booth, 1971, 1977; Raper and Fennel, 1977; Christensen and Raper, 1978; Pitt, 1979, 1991; Domsch *et al.*, 1980; Kozakiewicz, 1989; Klich and Pitt, 1992; Moubasher, 1993; Samson *et al.*, 1998).

Sample Preparation for Mycotoxins Analysis

Extraction Procedures

Fifty grams of each sample were defatted by extraction with cyclohexane (150 mL) for 10 h using Soxhlet type extractor. The defatted residue was extracted with ethyl acetate (three times, 50 mL/each).

The extracts were combined, dried over anhydrous sodium sulphate, filtered and then concentrated under vacuum to near dryness, transferred into brown glass vial and evaporated under nitrogen stream. For cleaning up the crude extracts; it was suspended in 1 mL chloroform and applied to 14×0.8 cm column containing 2.5 g kiesel gel 60, 70/230 silica gel. The washing and eluting solvents (8 mL, each) were carried out according to AOAC (1984).

Bioassay of Mycotoxins

Brine shrimps (*Artemia salina* L.) larvae were used for mycotoxins bioassay test according to Korpinen (1974).

Thin Layer Chromatography (TLC)

For qualitative detection of mycotoxins, thin layer chromatography technique was employed using precoated silica gel plates type 60 F₂₅₄ TLC (E, Merck, Germany). Aflatoxins B₁, B₂, G₁ and G₂, ochratoxins A and B, sterigmatocystin, citrinin, patulin, rubratoxin B, diacetoxyscirpenol, T₋₂ toxin, terrin, gliotoxin, roquefortin and zearalenone were applied as standard references. The developing solvent system was ethyl acetate-hexane (v/v, 30:70) and the developed plates were viewed under short wave length UV (252 nm) light according to AOAC (1984) and Dorner (1998).

Enzyme Linked Immuno Sorbent Assay (ELISA)

For quantitative determination of aflatoxin B₁ (AFB₁), ELISA technique was employed according to Rodriguez *et al.* (2003) because World Health Organization (WHO, 2006) has cited aflatoxins as the most potent naturally occurring carcinogens as well as International Agency for Research on Cancer (IARC) placed aflatoxin B₁ on the list of human carcinogens (Wu, 2004) and presence of aflatoxin B₁ kits.

High Performance Liquid Chromatography (HPLC)

HPLC analysis was done using spherisorb 5 sil column (250×4.6 mm). Mobile phase was chloroform-methanol (v/v, 97:3) with flow rate 1.2 mL min⁻¹ for 20 min. The quantitative determination of mycotoxins was carried out compared with standard mycotoxins (Sigma).

RESULTS AND DISCUSSION

The moisture content of broad bean, chickpea, kidney bean and sweet pea seed samples (on oven dry basis) was relatively low and ranged between 7-9.7, 7.1-9.8, 6-12.9 and 6-9.8, respectively. There is no appreciable difference between germinability of the four types of seeds tested and the germination rates ranged between 35-100%.

Mycological analysis of Fabaceae seed samples tested based on seed- and dilution-plate methods using 1% dextrose-Czapek's agar medium at 28±2°C revealed that 143 species and 9 varieties belonging to 32 genera were identified. The gross total viable count, as well as the spectrum of fungal genera and species collected from tested samples using seed-plate method (3792 colonies 25 seeds⁻¹, 29 genera, 111 species+2 varieties) was broader than that by dilution-plate method (2330 colonies g⁻¹ dry weight sample, 23 genera, 100 species + 7 varieties), but the order of occurrence frequency of fungal genera and species was basically similar in the two methods used. Also, fungal genera, species and means of fungal infection varied between Fabaceae types tested (16 genera, 43 species +one variety and 3.7 fungal colonies per seed tested), (15, 50+1 and 2.9 colonies), (19, 63+2 and 1.7 colonies) and (25, 71+2 and 1.8 colonies) using seed-plate method, while (13, 55 + 3 and 37.4 colonies per gm dry weight sample), (17, 60+6 and 42.9 colonies), (16, 59+4 and 37.4 colonies) and (14, 52+3 and 39.9 colonies) using dilution-plate method in broad bean, chickpea, kidney bean and sweet pea seed samples, respectively as shown in Table 1.

Table 1: Number of species (NS), percentage counts (%C, calculated per total counts of fungi) and percentage frequency of occurrence (%F, calculated per 15 samples) of various genera recovered from broad bean, chickpea, kidney bean and sweet pea on 1% dextrose-Czapek's agar medium at 28±2°C using seed- and dilution-plate methods

Isolation method	Seed-plate method											
Fabaceae seed kind (samples)	Broad bean (1-15)			Chickpea (16-30)			Kidney bean (31-45)			Sweet pea (46-60)		
Genera	NS	%C	%F	NS	%C	%F	NS	%C	%F	NS	%C	%F
<i>Aspergillus</i> (46 spp.+6 var.)	17+1	54.5	100.0	22+1	47.3	100.0	24+1	49.2	100.0	21+1	44.8	100.0
<i>Penicillium</i> (46 spp. +1 var.)	9	3.8	73.3	10	8.5	73.3	14+1	14.8	80.0	20+1	14.9	80.0
<i>Mucor</i> (3 spp.)	2	19.2	100.0	2	24.4	93.3	2	15.2	53.3	1	11.4	66.7
<i>Alternaria</i> (1 sp.)	1	3.3	46.7	1	2.8	60.0	1	6.4	60.0	1	6.7	73.3
<i>Fusarium</i> (7 spp.)	3	4.7	60.0	4	4.1	60.0	3	2.6	40.0	5	3.8	26.7
<i>Rhizopus</i> (1 sp.)	1	10.6	53.3	1	7.0	33.3	1	3.0	26.7	1	4.4	20.0
<i>Eurotium</i> (6 spp.+1 var.)	1	0.1	6.7	1	0.1	6.7	2	1.1	13.3	1	0.3	6.7
<i>Emmericella</i> (1 sp.+1 var.)	1	2.1	46.7	1	1.5	26.7	1	1.1	20.0	1	1.2	33.3
<i>Cladosporium</i> (2 spp.)	1	0.2	6.7	1	0.1	6.7	1	2.9	40.0	2	1.8	40.0
<i>Talaromyces</i> (6 spp.)	1	0.1	13.3	2	0.6	13.3	5	0.9	26.7	3	4.3	33.3
<i>Syncephalastru</i> (1 sp.)	1	0.1	6.7	-	-	-	1	0.8	13.3	1	0.5	6.7
<i>Thamnidium</i> (1 sp.)	1	0.2	6.7	1	3.2	26.7	-	-	-	1	1.2	6.7
<i>Femmelia</i> (1 sp.)	-	-	-	-	-	-	-	-	-	-	-	-
<i>Phoma</i> (2 spp.)	-	-	-	1	0.1	6.7	1	0.3	13.3	1	0.2	6.7
<i>Trichoderma</i> (1 sp.)	1	0.1	6.7	1	0.5	6.7	-	-	-	1	0.8	6.7
<i>Acremonium</i> (1 sp.)	1	0.7	13.3	-	-	-	1	0.5	6.7	1	0.5	6.7
<i>Paecilomyces</i> (2 spp.)	-	-	-	-	-	-	1	0.2	6.7	1	0.9	6.7
<i>Gymnoascus</i> (1 sp.)	-	-	-	-	-	-	1	0.5	13.3	1	0.3	6.7
<i>Scopulariopsis</i> (1 sp.)	-	-	-	-	-	-	1	0.2	6.7	-	-	-
<i>Stachybotrys</i> (1 sp.)	-	-	-	-	-	-	1	0.2	6.7	-	-	-
<i>Cochilobolus</i> (1 sp.)	-	-	-	1	0.2	6.7	-	-	-	-	-	-
<i>Ulocladium</i> (1 sp.)	-	-	-	1	0.1	6.7	1	0.2	6.7	1	0.3	6.7
<i>Circinella</i> (1 sp.)	1	0.1	6.7	-	-	-	-	-	-	1	0.8	6.7
<i>Eupenicillium</i> (1 sp.)	-	-	-	-	-	-	-	-	-	-	-	-
<i>Myrothecium</i> (1 sp.)	-	-	-	-	-	-	1	0.2	6.7	1	0.2	6.7
<i>Spicaria</i> (1 sp.)	-	-	-	-	-	-	-	-	-	1	0.2	6.7
<i>Trichothecium</i> (1 sp.)	-	-	-	-	-	-	-	-	-	1	0.2	6.7
<i>Botryotrichum</i> (1 sp.)	1	0.1	6.7	-	-	-	-	-	-	-	-	-
<i>Chaetomium</i> (1 sp.)	-	-	-	-	-	-	-	-	-	1	0.2	6.7
<i>Humicola</i> (1 sp.)	-	-	-	-	-	-	-	-	-	1	0.2	6.7
<i>Macrophomina</i> (1 sp.)	-	-	-	-	-	-	-	-	-	1	0.2	6.7
<i>Setosphaeria</i> (1 sp.)	-	-	-	-	-	-	-	-	-	-	-	-
No. of genera (32)	16			15			19			25		
No. of species +var. (143+ 9)	43+1			50+1			63+2			71+2		
Total count of fungi	1400 colonies			1077 colonies			656 colonies			659 colonies		
Total count (%)	22.9			17.6			10.7			10.8		
No. of seeds or g dry weight sample tested	375 seeds			375 seeds			375 seeds			375 seeds		
Mean of fungal infection	3.7 colonies/seed			2.9 colonies/seed			1.7 colonies/seed			1.8 colonies/seed		
Isolation method	Dilution-plate method											
Fabaceae seed kind (samples)	Broad bean (1-15)			Chickpea (16-30)			Kidney bean (31-45)			Sweet pea (46-60)		
Genera	NS	%C	%F	NS	%C	%F	NS	%C	%F	NS	%C	%F
<i>Aspergillus</i> (46 spp.+6 var.)	25+2	70.4	100.0	21+4	66.4	100.0	24+4	70.1	100.0	18+3	58.3	100.0
<i>Penicillium</i> (46 spp. +1 var.)	12	10.9	60.0	18	11.7	73.3	17	18.1	60.0	16	24.9	73.3
<i>Mucor</i> (3 spp.)	3	3.7	46.3	3	8.5	40.0	2	4.5	26.7	2	5.8	26.7
<i>Alternaria</i> (1 sp.)	1	3.7	46.3	1	1.7	33.3	1	1.9	40.0	1	1.3	26.7
<i>Fusarium</i> (7 spp.)	3	3.2	33.3	3	0.6	20.0	2	0.4	13.3	2	0.3	13.3

Table 1: Continued

Isolation method	Dilution-plate method											
	Broad bean (1-15)			Chickpea (16-30)			Kidney bean (31-45)			Sweet pea (46-60)		
Genera	NS	%C	%F	NS	%C	%F	NS	%C	%F	NS	%C	%F
<i>Rhizopus</i> (1 sp.)	1	2.0	20.0	1	3.2	20.0	1	1.7	20.0	1	1.7	6.7
<i>Eurotium</i> (6 spp.+1 var.)	4	3.0	46.3	2+1	1.3	33.3	3	1.3	33.3	4	1.7	33.3
<i>Emmericella</i> (1 sp.+1 var.)	1+1	1.1	20.0	1+1	0.5	13.3	-	-	-	-	-	-
<i>Cladosporium</i> (2 spp.)	-	-	-	2	0.6	13.3	-	-	-	2	1.0	20.0
<i>Talaromyces</i> (6 spp.)	-	-	-	-	-	-	1	0.2	6.7	1	0.2	6.7
<i>Syncephalastru</i> (1 sp.)	1	0.5	13.3	1	1.6	20	1	0.6	13.3	-	-	-
<i>Thamnidium</i> (1 sp.)	-	-	-	-	-	-	-	-	-	-	-	-
<i>Femmelia</i> (1 sp.)	-	-	-	1	2.4	20	1	0.4	6.7	1	3.8	13.3
<i>Phoma</i> (2 spp.)	1	0.4	6.7	1	-	-	1	0.2	6.7	-	-	-
<i>Trichoderma</i> (1 sp.)	-	-	-	1	0.6	6.7	-	-	-	1	0.3	6.7
<i>Acremonium</i> (1 sp.)	-	-	-	1	0.2	6.7	-	-	-	-	-	-
<i>Paecilomyces</i> (2 spp.)	-	-	-	1	0.3	6.7	1	0.2	6.7	1	0.7	6.7
<i>Gymnoascus</i> (1 sp.)	1	0.5	6.7	-	-	-	-	-	-	-	-	-
<i>Scopulariopsis</i> (1 sp.)	1	0.5	13.3	1	0.2	6.7	-	-	-	-	-	-
<i>Stachybotrys</i> (1 sp.)	-	-	-	1	0.5	6.7	1	0.2	6.7	-	-	-
<i>Cochilobolus</i> (1 sp.)	-	-	-	1	0.2	6.7	1	0.2	6.7	-	-	-
<i>Ulocladium</i> (1 sp.)	-	-	-	-	-	-	-	-	-	-	-	-
<i>Circinella</i> (1 sp.)	-	-	-	-	-	-	-	-	-	-	-	-
<i>Eupenicillium</i> (1 sp.)	1	0.5	6.7	-	-	-	-	-	-	1	0.2	6.7
<i>Myrothecium</i> (1 sp.)	-	-	-	-	-	-	-	-	-	-	-	-
<i>Spicaria</i> (1 sp.)	-	-	-	-	-	-	1	0.2	6.7	-	-	-
<i>Trichothecium</i> (1 sp.)	-	-	-	-	-	-	-	-	-	1	0.2	6.7
<i>Botryotrichum</i> (1 sp.)	-	-	-	-	-	-	-	-	-	-	-	-
<i>Chaetomium</i> (1 sp.)	-	-	-	-	-	-	-	-	-	-	-	-
<i>Humicola</i> (1 sp.)	-	-	-	-	-	-	-	-	-	-	-	-
<i>Macrophomina</i> (1 sp.)	-	-	-	-	-	-	-	-	-	-	-	-
<i>Setosphaeria</i> (1 sp.)	-	-	-	-	-	-	1	0.2	6.7	-	-	-
No. of genera (32)	13			17			16			14		
No. of species +var. (143+ 9)	55+3									52+3		
Total count of fungi	561 colonies			634 colonies			536 colonies			599 colonies		
Total count (%)	9.2			10.4			8.8			9.8		
No. of seeds or g dry weight sample tested	15.0 g			15.0 g			15.0 g			15.0 g		
Mean of fungal infection	37.4 colonies/g			42.9 colonies/g			37.4 colonies/g			39.9 colonies/g		

-: No isolated fungal genera

The total count of filamentous fungi recovered from the different samples tested widely fluctuated between (56-166), (43-95), (15-78) and (13-69 colonies 25 seeds⁻¹) using seed-plate method, while (10-135), (12-85), (16-62) and (12-141 colonies g⁻¹ dry weight sample) using dilution-plate method from broad bean, chickpea, kidney bean and sweet pea, respectively. It is axiomatic that seed samples with high values of moisture content contained high numbers of fungi and vice versa. The highest count of fungi was recorded in broad bean samples tested (1400 colonies 25 seeds⁻¹, 22.9% of gross total fungi) followed by chickpea (1077, 17.6%), sweet pea (659, 10.8%) and kidney bean (656, 10.7%) using seed-plate method. On the other hand, in case of dilution-plate method the richest seed samples type in fungi was chickpea (634 colonies g⁻¹ dry weight seed sample, 10.4% of gross total count of fungi) followed by sweet pea (599, 9.8%), broad bean (561, 9.2%) and kidney bean (536, 8.8%) as shown in Table 1.

The relatively lowest spectrum of total fungal count recorded in case of dilution-plate method may be related to highly contamination of samples with *Aspergillus* species, which have a powerful competitive ability due to highly species diversity with heavy spores production and their highly productivity of different mycotoxins which prevent other fungal species growth. These results are in

harmony with data recorded by Abdel-Hafez (1988), El-Kady and Youssef (1993), Tseng and Tu (1997), Youssef *et al.* (2002), Ibrahim (2007), Youssef (2008) and Gonçalves *et al.* (2008).

Aspergillus was the most common genus, emerged from 100% of Fabaceae seed samples tested, contributing (54.5, 47.3, 49.2 and 44.8%) of total fungi using seed-plate method and (70.4, 66.4, 70.1 and 58.3%) using dilution-plate method in tested sample types, respectively. It was represented by 46 species and 6 varieties (17+1, 22+1, 24+1 and 21+1) and (25+2, 21+4, 24+4 and 18+3) using two isolation methods from seed samples of four Fabaceae types assayed, respectively (Table 1). Of which *A. flavus*, *A. niger* and *A. fumigatus* were the most prevalent. They occurred in 26.7-100% of the samples, comprising 2.8 – 32.4% of total *Aspergillus* and 1.4-22.8% of total fungi in different seed types. *A. parasiticus*, *A. flavus* var. *columnaris* and *A. ficuum* were emerged in moderate or low frequency of occurrence in samples tested (Table 2). The remaining *Aspergillus* species (41 species +5 varieties) were less frequent as listed in (Table 3).

Penicillium occupied the second order in case of dilution-plate method and the third place in case of seed-plate method after *Mucor*, encountered in 60-80% of the samples, accounting 3.8-24.9% of total count of fungi. Of *Penicillium*, 46 species in addition to one variety were isolated using the two methods, (32 species +1 variety) in case of seed-plate method, while (34 species) in the other method. Also, from the different types of Fabaceae seeds tested, respectively (9, 10, 14+1 and 20+1) and (12, 18, 17 and 16 species) were identified using seed- and dilution-plate methods, respectively (Table 1). *P. chrysogenum* was the dominant species occurred in 13.3-26.7% of the samples followed

Table 2: Average total counts (ATC), percentage (%ATC, of 60 samples tested), number of cases of isolation (NCI) and occurrence remarks (OR) of fungal genera and species recovered from four Fabaceae seeds tested on 1% dextrose-Czapek's agar medium at 28±2°C using seed and dilution-plate methods

Isolation method	Seed-plate method				Dilution-plate method			
	ATC	ATC%	NCI	OR	ATC	ATC%	NCI	OR
Genera and Species*								
Gross total count	3792	100.0	60		2330	100.0	60	
<i>Aspergillus</i> (Micheli) Corda	1890	49.8	60	H	1541	66.1	60	H
<i>A. flavus</i> Link	512	13.5	45	H	302	13.0	37	H
<i>A. niger</i> van Tieghem	372	9.8	27	M	178	7.6	31	H
<i>A. fumigatus</i> Fresenius	50	1.3	12	L	205	8.8	31	H
<i>A. parasiticus</i> Speare	68	1.8	22	M	187	8.0	14	L
<i>A. flavus</i> var. <i>columnaris</i>	90	2.4	17	M	28	1.2	12	L
Raper and Fennell								
<i>A. ficuum</i> (Reich.) Hennings	177	4.7	16	M	40	1.7	12	L
<i>A. terreus</i> Thom	19	0.5	10	L	39	1.7	13	L
<i>A. heteromorphus</i> Batista and Maia	147	3.9	8	L	56	2.4	8	L
<i>A. awamori</i> Nakazawa	67	1.8	12	L	9	0.4	4	R
<i>Penicillium</i> Link	341	9.0	46	H	379	16.3	40	H
<i>P. chrysogenum</i> Thom	45	1.2	13	L	30	1.3	9	L
<i>P. cyclophum</i> Westling	40	1.1	11	L	18	0.8	7	R
<i>Mucor</i> Micheli	707	18.6	47	H	134	5.8	21	M
<i>M. hiemalis</i> Wehmer	682	17.9	47	H	97	4.2	16	M
<i>Alternaria alternata</i> (Fr.) Keissler	162	4.3	36	H	50	2.1	22	L
<i>Fusarium</i> Link	152	4.0	28	M	26	1.1	12	L
<i>F. oxysporum</i> Schlecht	95	2.5	18	M	4	0.2	4	R
<i>Rhizopus stolonifer</i> (Ehrenb.) Lind	273	7.2	20	M	50	2.1	10	L
<i>Eurotium</i> Link	11	0.3	5	R	42	1.8	22	M
<i>Emmericella</i> Berkeley and Broome	60	1.6	19	M	9	0.4	5	R
<i>E. nidulans</i> (Eidam) Vuillemin	60	1.6	19	M	5	0.2	3	R
<i>Cladosporium</i> Link	35	0.9	14	L	10	0.4	5	R
<i>C. cladosporioides</i> (Fresen.) De Vries	31	0.8	13	L	8	0.3	3	R
<i>Talaromyces</i> Benjamin	43	1.1	13	L	2	0.1	2	R

*Fungal genera and species isolated in high occurrence (more than 59 cases out of 120 tested), moderate (30-59 cases), and low (15-29 cases) were enclosed in this table, while fungi with rare occurrence (less than 15 cases out of 120 tested) using the two isolation methods were omitted from this table and enclosed in Table 3. ATC: Average Total Count (60 Fabaceae seed samples), %ATC: Percentage of Average Total Count, NCI: No. of Cases of Isolation, OR: Occurrence Remarks, H: High occurrence, more than 29 cases out of 60 samples tested, M: Moderate occurrence (15-29 cases), L: Low occurrence (8-14 cases), R: Rare occurrence (less than 8 samples)

Table 3: Average total counts (ATC), percentage (%ATC, of 60 samples tested), number of cases of isolation (NCI) and occurrence remarks (OR) of fungal genera and species recovered from four Fabaceae seeds tested on 1% dextrose-Czapek's agar medium at 28±2°C using seed- and dilution-plate methods

Isolation method	Seed-plate method				Dilution-plate method			
	ATC	ATC%	NCI	OR	ATC	ATC%	NCI	OR
Genera and Species								
Gross total count	3792	100.0	60		2330	100.0	60	
<i>Aspergillus</i> (Micheli) Corda	1890	49.8	60	H	1541	66.1	60	H
<i>A. flavus</i> Link	512	13.5	45	H	302	13.0	37	H
<i>A. niger</i> van Tieghem	372	9.8	27	M	178	7.6	31	H
<i>A. fumigatus</i> Fresenius	50	1.3	12	L	205	8.8	31	H
<i>A. parasiticus</i> Speare	68	1.8	22	M	187	8.0	14	L
<i>A. flavus</i> var. <i>columnaris</i> Raper and Fennell	90	2.4	17	M	28	1.2	12	L
<i>A. ficuum</i> (Reich.) Hennings	177	4.7	16	M	40	1.7	12	L
<i>A. terreus</i> Thom	19	0.5	10	L	39	1.7	13	L
<i>A. heteromorphus</i> Batista and Maia	147	3.9	8	L	56	2.4	8	L
<i>A. awamori</i> Nakazawa*	67	1.8	12	L	9	0.4	4	R
<i>A. aculeatus</i> Iizuka*	56	1.5	6	R	48	2.1	8	L
<i>A. candidus</i> Link	11	0.3	3	R	45	1.9	10	L
<i>A. foetidus</i> (Nakazawa) Thom and Raper	64	1.7	8	L	22	0.9	4	R
<i>A. tubingensis</i> (Schober) Mosseray*	45	1.2	5	R	41	1.8	6	R
<i>A. sydowi</i> (Bainier and Sartory) Thom and Church	21	0.6	8	L	6	0.3	3	R
<i>A. ochraceus</i> Wilhelm	17	0.4	8	L	1	0.04	1	R
<i>A. viride-nutans</i> Ducker and Thrower*	-	-	-	-	84	3.6	8	L
<i>A. montivivensis</i> Talice and Mackinnon*	-	-	-	-	39	1.7	8	L
<i>A. flavor-furcatis</i> Batista and Maia	9	0.2	5	R	3	0.1	2	R
<i>A. eburneo-cremus</i> Sappa	9	0.2	6	R	1	0.04	1	R
<i>A. versicolor</i> (Vuillemin) Tiraboschi	8	0.2	4	R	26	1.1	2	R
<i>A. silvaticus</i> Fennell and Thom*	7	0.2	2	R	9	0.4	4	R
<i>A. oryzae</i> (Ahlb.) Cohn*	-	-	-	-	13	0.6	6	R
<i>A. carbonarius</i> (Bainier) Thom	6	0.2	1	R	7	0.3	4	R
<i>A. fumigatus</i> var. <i>ellipticus</i> Raper and Fennell	-	-	-	-	11	0.5	5	R
<i>A. terreus</i> var. <i>africanus</i> Fennell and Raper*	-	-	-	-	7	0.3	5	R
<i>A. ustus</i> (Bainier) Thom and Church	2	0.05	2	R	3	0.1	3	R
<i>A. sulphureus</i> (Fres.) Thom and Church*	37	1.0	4	R	-	-	-	-
<i>A. phoenicis</i> (CDS.) Thom*	-	-	-	-	27	1.2	4	R
<i>A. aureolatus</i> Munt-Cvet. and Bata.	9	0.2	3	R	1	0.04	1	R
<i>A. funiculosus</i> Smith*	-	-	-	-	31	1.3	3	R
<i>A. japonicus</i> Saito*	26	0.7	2	R	1	0.04	1	R
<i>A. ellipticus</i> Raper and Fennell *	26	0.7	3	R	-	-	-	-
<i>A. wentii</i> Wehmer*	2	0.05	1	R	4	0.2	2	R
<i>A. zonatus</i> Kwon and Fennell*	2	0.05	2	R	3	0.1	1	R
<i>A. fruticulosus</i> Raper and Fennell	3	0.1	1	R	2	0.1	1	R
<i>A. tamarii</i> Kita	1	0.03	1	R	3	0.1	1	R
<i>A. penicilloides</i> Spegazzini*	-	-	-	-	38	1.6	1	R
<i>A. foetidus</i> var. <i>pallidus</i> Nakazawa, Simo and Watanabe*	-	-	-	-	18	0.8	1	R
<i>A. clavato-nanica</i> Batista, maia and Alecrim*	10	0.3	1	R	-	-	-	-
<i>A. avenaceus</i> Smith*	5	0.1	1	R	-	-	-	-
<i>A. crustosus</i> Raper and Fennell *	4	0.1	1	R	-	-	-	-
<i>A. cervinus</i> (Masse) Neill*	3	0.1	1	R	-	-	-	-
<i>A. clavatus</i> Desmazieres	2	0.05	1	R	-	-	-	-
<i>A. thecicus</i> Raper and Fennell *	1	0.03	1	R	-	-	-	-
<i>A. biplanes</i> Raper and Fennell *	1	0.03	1	R	-	-	-	-
<i>A. foetidus</i> var. <i>acidus</i> Nakazawa, Simo and Watanabe*	-	-	-	-	1	0.04	1	R
<i>A. melleus</i> Yukawa*	1	0.03	1	R	-	-	-	-
<i>A. multicolor</i> Sappa*	1	0.03	1	R	-	-	-	-
<i>A. proliferans</i> Smith*	-	-	-	-	1	0.04	1	R
<i>A. subolivaceus</i> Fennell and Raper*	-	-	-	-	1	0.04	1	R
<i>A. terreus</i> var. <i>aureus</i> Thom and Raper*	-	-	-	-	1	0.04	1	R
<i>A. terricola</i> Marchal*	1	0.03	1	R	-	-	-	-

Table 3: Continued

Isolation method	Seed-plate method				Dilution-plate method			
	ATC	ATC%	NCI	OR	ATC	ATC%	NCI	OR
<i>Penicillium</i> Link	341	9.0	46	H	379	16.3	40	H
<i>P. chrysogenum</i> Thom	45	1.2	13	L	30	1.3	9	L
<i>P. cyclopium</i> Westling	40	1.1	11	L	18	0.8	7	R
<i>P. purpurogenum</i> Stoll*	18	0.5	8	L	4	0.2	3	R
<i>P. varians</i> Smith*	18	0.5	6	R	5	0.2	2	R
<i>P. verruculosum</i> Peyronel*	5	0.1	2	R	8	0.3	6	R
<i>P. palitans</i> Westling*	8	0.2	3	R	17	0.7	4	R
<i>P. puberulum</i> Bainier*	13	0.3	5	R	5	0.2	2	R
<i>P. citrinum</i> Thom	14	0.4	6	R	2	0.1	1	R
<i>P. duckauxi</i> Delacroix*	12	0.3	6	R	4	0.2	1	R
<i>P. steckii</i> Zaleski*	-	-	-	-	43	1.8	5	R
<i>P. stoloniferum</i> Thom	33	0.9	4	R	4	0.2	1	R
<i>P. brevis-compactum</i> Dierckx*	3	0.1	2	R	13	0.6	3	R
<i>P. expansum</i> Link*	-	-	-	-	54	2.3	4	R
<i>P. islandicum</i> Sopp	24	0.6	3	R	5	0.2	1	R
<i>P. funiculosum</i> Thom	4	0.1	3	R	1	0.04	1	R
<i>P. jensenii</i> Zaleski*	4	0.1	1	R	21	0.9	2	R
<i>P. variable</i> Sopp*	12	0.3	1	R	9	0.4	2	R
<i>P. viridicatum</i> Westling	-	-	-	-	16	0.7	3	R
<i>P. corymbiferum</i> Westling*	5	0.1	1	R	9	0.4	2	R
<i>P. frequentans</i> Westling	12	0.3	2	R	1	0.04	1	R
<i>P. corylophilum</i> Dierckx*	10	0.3	3	R	-	-	-	-
<i>P. paxilli</i> Bainier*	8	0.2	2	R	2	0.1	1	R
<i>P. canescens</i> Sopp	-	-	-	-	5	0.2	3	R
<i>P. janthinellum</i> Biourge	7	0.2	1	R	2	0.1	1	R
<i>P. piscarium</i> Westling*	-	-	-	-	5	0.2	2	R
<i>P. rugulosum</i> Thom*	-	-	-	-	5	0.2	2	R
<i>P. nigricans</i> (Bainier) Thom	4	0.1	2	R	-	-	-	-
<i>P. canemberti</i> Thom*	2	0.05	1	R	1	0.04	1	R
<i>P. rubrum</i> Stoll*	-	-	-	-	3	0.1	2	R
<i>P. cyclopium</i> var. <i>echinulatum</i> Westling*	2	0.05	2	R	-	-	-	-
<i>P. aurantio-candidum</i> Dierckx*	-	-	-	-	63	2.7	1	R
<i>P. italicum</i> Wehmer*	-	-	-	-	12	0.5	1	R
<i>P. digitatum</i> Saccardo	8	0.2	1	R	-	-	-	-
<i>P. roqueforti</i> Thom*	8	0.2	1	R	-	-	-	-
<i>P. cyaneo-fulvum</i> Biourge*	5	0.1	1	R	-	-	-	-
<i>P. lanosum</i> Westling*	-	-	-	-	5	0.2	1	R
<i>P. oxalicum</i> Currie and Thom	4	0.1	1	R	-	-	-	-
<i>P. claviforme</i> Bainier*	-	-	-	-	2	0.1	1	R
<i>P. herquei</i> Bainier and Sartory*	2	0.05	1	R	-	-	-	-
<i>P. lanoso-griseum</i> Thom*	-	-	-	-	2	0.1	1	R
<i>P. lanoso-viride</i> Thom*	-	-	-	-	2	0.1	1	R
<i>P. martensii</i> Biourge*	2	0.05	1	R	-	-	-	-
<i>P. olivino-viride</i> Biourge*	1	0.03	1	R	-	-	-	-
<i>P. sublateralitium</i> Biourge*	1	0.03	1	R	-	-	-	-
<i>P. terrestre</i> Jensen*	-	-	-	-	1	0.04	1	R
<i>P. urticae</i> Bainier*	1	0.03	1	R	-	-	-	-
<i>P. waksmani</i> Zaleski*	1	0.03	1	R	-	-	-	-
<i>Mucor</i> Micheli	707	18.6	47	H	134	5.8	21	M
<i>M. hiemalis</i> Wehmer	682	17.9	47	H	97	4.2	16	M
<i>M. circinelloides</i> van Tieghem*	25	0.7	4	R	6	0.3	2	R
<i>M. racemosus</i> Fresenius	-	-	-	-	31	1.3	5	R
<i>Alternaria alternata</i> (Fr.) Keissler	162	4.3	36	H	50	2.1	22	L
<i>Fusarium</i> Link	152	4.0	28	M	26	1.1	12	L
<i>F. oxysporum</i> Schlecht	95	2.5	18	M	4	0.2	4	R
<i>F. solani</i> (Mart.) Saccardo	28	0.7	7	R	16	0.7	4	R
<i>F. equiseti</i> Corda) Saccardo	15	0.4	3	R	4	0.2	2	R
<i>F. xylarioides</i> Steyaert*	11	0.3	3	R	1	0.04	1	R
<i>F. tabacinum</i> (Beyma) W.Gams*	2	0.05	1	R	-	-	-	-
<i>F. moniliforme</i> Sheldon	1	0.03	1	R	-	-	-	-
<i>F. sulphureum</i> Schlecht*	-	-	-	-	1	0.04	1	R

Table 3: Continued

Isolation method	Seed-plate method				Dilution-plate method			
	ATC	ATC%	NCI	OR	ATC	ATC%	NCI	OR
Genera and Species								
<i>Rhizopus stolonifer</i> (Ehrenb.) Lind	273	7.2	20	M	50	2.1	10	L
<i>Eurotium</i> Link	11	0.3	5	R	42	1.8	22	M
<i>E. chevalieri</i> Mangin*	4	0.1	2	R	17	0.8	10	L
<i>E. repens</i> De Bary*	5	0.1	1	R	15	0.6	9	L
<i>E. rubrum</i> (Konig, Spieckermann and Bremer)*	-	-	-	-	6	0.3	3	R
<i>E. amstelodami</i> Mangin*	-	-	-	-	2	0.1	2	R
<i>E. chevalieri</i> var. <i>intermedius</i>	-	-	-	-	2	0.1	2	R
(Thom and Raper) Malloch and Cain*								
<i>E. echinulata</i> Fennell and Raper*	1	0.03	1	R	-	-	-	-
<i>E. niveoglaucum</i> (Thom and Raper)	1	0.03	1	R	-	-	-	-
Malloch and Cain*								
<i>Emmericella</i> Berkeley and Broome	60	1.6	19	M	9	0.4	5	R
<i>E. nidulans</i> (Eidam) Vuillemin	60	1.6	19	M	5	0.2	3	R
<i>E. nidulans</i> var.	-	-	-	-	4	0.2	2	R
<i>acistata</i> (Fennell and raper) Subram*								
<i>Cladosporium</i> Link	35	0.9	14	L	10	0.4	5	R
<i>C. cladosporioides</i> (Fresen.) de Vries	31	0.8	13	L	8	0.3	3	R
<i>C. herbarum</i> (Pers.) Link ex S.F.Gray	4	0.1	2	R	2	0.1	2	R
<i>Talaromyces</i> Benjamin	43	1.1	13	L	2	0.1	2	R
<i>T. luteus</i> (Zukal) Benjamin	16	0.4	7	R	2	0.1	2	R
<i>T. thermophilus</i> Stolk*	8	0.2	5	R	-	-	-	-
<i>T. wortmanni</i> (Klöcker) Benjamin*	16	0.4	1	R	-	-	-	-
<i>T. bacillisporus</i> (Swift) Benjamin*	1	0.03	1	R	-	-	-	-
<i>T. flavus</i> (Klöcker) Stolk and Samson*	1	0.03	1	R	-	-	-	-
<i>T. stipitatus</i> (Thom) Benjamin	1	0.03	1	R	-	-	-	-
<i>Syncephalastrum racemosum</i> (Cohn) Schroeter*	10	0.3	4	R	16	0.7	7	R
<i>Thamnidium elegans</i> Link*	48	1.3	6	R	-	-	-	-
<i>Fennellia flavipes</i> (Bain. and Sart.)	-	-	-	-	40	1.7	6	R
Thom and Church								
<i>Phoma</i> (Fries) Desmazieres	4	0.1	4	R	3	0.1	2	R
<i>P. humicola</i> Gilman and Abbott*	4	0.1	4	R	2	0.1	1	R
<i>P. glomerata</i> (Corda) Wollenweber and Hochapfel*	-	-	-	-	1	0.04	1	R
<i>Trichoderma viride</i> Pers. Ex S.F. Gray	12	0.3	3	R	6	0.3	2	R
<i>Acremonium rutilum</i> W.Gams*	16	0.4	4	R	1	0.04	1	R
<i>Paecilomyces</i> Bainier	7	0.2	2	R	7	0.3	3	R
<i>P. variotii</i> Bainier*	7	0.2	2	R	5	0.2	2	R
<i>P. lilacinum</i> (Thom) Samson	-	-	-	-	2	0.1	1	R
<i>Gymnoascus reesii</i> Baranetzky*	5	0.1	3	R	3	0.1	1	R
<i>Scopulariopsis brevicaulis</i> (Sacc.) Bainier*	1	0.03	1	R	4	0.2	3	R
<i>Stachybotrys atra</i> Coda	1	0.03	1	R	4	0.2	2	R
<i>Cochliobolus lunatus</i> Nelson and Haasis	2	0.05	1	R	2	0.1	2	R
<i>Ulocladium atrum</i> Preuss	4	0.1	3	R	-	-	-	-
<i>Circinella simplex</i> van Tieghem*	7	0.2	2	R	-	-	-	-
<i>Eupenicillium crustaceum</i> Ludwig*	-	-	-	-	4	0.2	2	R
<i>Myrothecium roridum</i> Tode ex Steudel	2	0.05	2	R	-	-	-	-
<i>Spicaria violaceae</i> Abbott*	1	0.03	1	R	1	0.04	1	R
<i>Trichothecium roseum</i> (Pers.) Link ex Gray	1	0.03	1	R	1	0.04	1	R
<i>Botryotrichum piluliferum</i> Saccardo and Marchal*	1	0.03	1	R	-	-	-	-
<i>Chaetomium globosum</i> Kunze*	1	0.03	1	R	-	-	-	-
<i>Humicola grisea</i> Traaen	1	0.03	1	R	-	-	-	-
<i>Macrophomina phaseolina</i> (Tassi) Goid	1	0.03	1	R	-	-	-	-
<i>Setosphaeria rostrata</i> Leonard*	-	-	-	-	1	0.04	1	R
Sterile mycelia (white color)	-	-	-	-	2	0.1	1	R
No. of genera, species+varieties								
(32 genera, 143 species+9 varieties)	29 genera, 111 species+				23 genera, 100 species+			
	2 varieties				7 varieties			
Total count	3792 colonies per 25 seeds				2330 colonies per gm dry weight sample			

ATC: Average Total Count (60 Fabaceae seed samples), %ATC: Percentage of Average Total Count, NCI: No. of Cases of Isolation, OR: Occurrence Remarks, H: High occurrence, more than 29 cases out of 60 samples tested, M: Moderate occurrence (15-29 cases), L: Low occurrence (8-14 cases), R: Rare occurrence (less than 8 samples), -: No isolated fungus

*New record to Libyan mycoflora

by *P. cyclopium* (Table 2). The remaining *Penicillium* species (44+1) were less frequent as shown in Table 3. These obtained results are in full agreement with those previously recorded by Costa and Scussel (1998, 2002), Youssef *et al.* (2002), Ibrahim (2007) and Gonçalves *et al.* (2008) that *Aspergillus* and *Penicillium* were the most dominant genera in different cultivars of kidney bean (white, black and colored), chickpea seeds, Egyptian peanut seed types (untreated, roasted and roasted salted) and Brazilian peanut seeds, respectively. Also, Creepy (2002) reported that seeds and grains are more liable to fungal infection particularly *Aspergillus*, *Penicillium* and *Fusarium* species in tropical and sub-tropical regions dependent on high levels of moisture content. Also, Christensen (1991) reported that *Aspergillus* and *Penicillium* species are the main components of storage fungi play an important role in seed deterioration and they have competitive ability against other fungi due to heavy spores and mycotoxins production of their diversity species.

Mucor was the most second genus in case of seed-plate method, isolated in high frequency of occurrence (47 cases out of 60 tested), accounting (78.3%) of the samples and (18.6%) of total fungi, while in case of dilution-plate method retarded to the fifth place with moderate frequency of occurrence (21 cases out of 60 tested), encountered in (35%) of the samples and (5.8%) of total fungal count. It emerged in 26.7- 100% of the samples of different Fabaceae seed types tested. It was represented by 3 species of which, *M. hiemalis* was the most prevalent, while *M. circinelloides* and *M. racemosus* were less frequent as shown in (Table 2, 3). These results are in harmony with those obtained by El-Maghraby *et al.* (1992), El-Kady and Youssef (1993), Tseng *et al.* (1995), Youssef *et al.* (2002), Ibrahim (2007), Gonçalves *et al.* (2008) and Kumar *et al.* (2008).

Alternaria (*A. alternata*) occupied the fourth place among isolated fungi in both isolation methods used. It appeared in high (36 cases out of 60 tested, 4.3% of total fungi) and moderate (22 cases, 2.1%) frequency of occurrence using seed- and dilution-plate methods, respectively. *Fusarium* ranked the fifth order with moderate frequency of occurrence (28 cases, 46.7% of the samples and 4.01% of total fungi) and the sixth place with low frequency (12 cases, 20% and 1.1%) using the two isolating methods, respectively. It was represented by 7 species of which, *F. oxysporum* was the most dominant, while *F. solani*, *F. equiseti*, *F. xylarioides*, *F. tabacinum*, *F. moniliforme* and *F. sulphureum* were less frequent (Table 2, 3).

El-Maghraby *et al.* (1992) reported that *Alternaria* (*A. alternata*) occupied the second, third and fourth most frequent genus in green, frozen and dry pea seeds (55, 55 and 50%) of the samples and (25.6, 11.9 and 1.7%) of total fungi, respectively. While, *Fusarium* was the fourth, fifth and sixth most frequent isolated genera in green, dry and frozen pea seed samples (30, 45 and 30%) of the samples and (4.1, 3.2 and 4.6%) of total fungi, respectively. Bullerman (1979) reported that *A. alternata* was listed as one of potentially toxic molds in American seeds and grains. Thomas (1984) stated that species of *Fusarium* cover spectrum of activity from those which are fairly specific plant pathogens to those which are saprophytic on senescent plant materials or even biodegradation of industrial products. Smith and Moss (1985) recorded that *Fusarium* species especially *F. moniliforme* is the most common species in tropical and sub-tropical climates.

Rhizopus (*R. stolonifer*) was isolated in moderate and low frequency of occurrence, occupied the sixth and seventh places among recovered fungi, emerged in (33.3 and 16.7%) of the samples and (7.2 and 2.1%) of total fungal count using the two isolation methods, respectively as shown in (Table 2, 3). El-Kady and Youssef (1993) reported that *Rhizopus* (*R. stolonifer*) was isolated in moderate frequency of occurrence accounting (7.4 and 0.8%) of total fungi of soybean seeds using seed- and dilution-plate methods, respectively.

Eurotium occupied the third and eighth places with moderate and rare frequencies of occurrence emerged in 22 and 5 samples out of 60 tested, accounting (36.7 and 8.3%) of the samples and (1.8 and 0.3%) of total fungi using dilution- and seed-plate methods, respectively (Table 2). It was represented by 6 species and one variety, of which *E. chevalieri* and *E. repens* were the most prevalent, while

E. rubrum, *E. amstelodami*, *E. chevalieri* var. *intermedius*, *E. echinulata* and *E. niveoglaucum* were less frequent (Table 3). El-Maghraby *et al.* (1992) recorded that *Eurotium* was isolated in moderate and low frequency of occurrence in dry and frozen pea seeds. It represented by five species, of which *E. amstelodami* was the most predominant. This genus is generally osmophilic (or osmotolerant) or halophilic (or halotolerant) as reported by Raper and Fennel (1977) and have been isolated on sucrose and/or NaCl-Czapek's or -malt agar medium all over the world (El-Kady *et al.*, 1986; Weidenborner and Kunz, 1994; Tseng *et al.*, 1995; Abdel-Sater and Saber, 1999; Youssef *et al.*, 2000, 2003).

The remaining fungal genera and species were less frequent on the plates of the two isolation methods used as shown in (Table 1, 3). The obtained results are in agreement with those obtained by several investigators around the world (Abdel-Hafez, 1988; Tseng *et al.*, 1995; Costa and Scussel, 1998, 2002; Youssef *et al.*, 2002; Youssef, 2008).

Generally, the results revealed that the gross total count of fungal population as well as the spectrum of genera and species isolated from samples tested using seed-plate method (3792 colonies, 29 genera, 111 species+2 varieties) was broader than those by dilution-plate method (2330 colonies, 23 genera, 100 species+7 varieties). On the other hand, the results showed that the direct-plating method was substantially more effective in detecting individual mould genera and species than dilution plate method. In this respect, the frequency of occurrence of fungal genera and species using seed-plate method was relatively better than that in dilution-plate method; such as *Penicillium* isolated from 46 samples out of 60 tested in case of seed-plate method, while from 40 samples using dilution-plate method as well as *Mucor* (47 and 21), *Alternaria* (36 and 22), *Fusarium* (28 and 12), *Rhizopus* (20 and 10), *Emericella* (19 and 5), *Cladosporium* (14 and 5) and *Talaromyces* (13 and 2) using seed and dilution-plate methods, respectively, except *Eurotium* only isolated in moderate occurrence (22 samples) using dilution-plate method, while in rare occurrence (5 samples) using seed-plate method. Also, (41 species +1 variety) appeared using seed-plate method and completely disappeared using dilution-plate method, whereas (36 species + 2 varieties) identified only from plates of dilution-plate method and completely missed using seed-plate method (Table 3).

Using statistical analysis of obtained results according to Sendecor and Cochran (1980) and study the relationship between means of isolated fungi at low significant differences (0.01 and 0.05) and samples fungal contamination, the data revealed that recovered fungi with high and moderate frequencies of occurrence in addition to source and storage conditions of different samples tested played the essential role in sample fungal contamination and the obtained results of different Fabaceae types were significant.

The toxicity test using brine shrimp (*Artemia salina* L.) larvae revealed that ethyl acetate extracts of 16 samples (26.7%) out of 60 Fabaceae seed samples tested proved to be toxic. Based on TLC, ELISA and HPLC analyses, aflatoxins B₁ and B₂ were detected in 5 samples of broad bean with concentrations ranged from (2.1- 10.2 µg kg⁻¹), in addition to 3 samples of kidney bean (1.6 to 9.5 µg kg⁻¹), whereas aflatoxins B₁, B₂, G₁ and G₂ were recorded in 6 samples of sweet pea (1.8 to 12.2 µg kg⁻¹). These toxic samples were heavily contaminated with many members of *Aspergillus flavus* group (*A. flavus*, *A. parasiticus*, *A. flavus* var. *columnaris*) as aflatoxins-producers (Table 4).

Sterigmatocystin was detected alone in 2 samples of broad bean (4.8 and 8.2 µg kg⁻¹) and combined with aflatoxins B₁, B₂, G₁ and G₂ in one sample of sweet pea (2.8 µg kg⁻¹) and these samples were rich in *Emericella nidulans* and *Eurotium chevalieri* as sterigmatocystin-producing fungi as stated in (Table 4).

Citrinin combined with aflatoxins B₁ and B₂ was contaminated one sample of broad bean (6.2 µg kg⁻¹) and contaminated one sample of sweet pea (2.8 µg kg⁻¹) combined with aflatoxins B₁, B₂, G₁ and G₂, these samples were heavily contaminated with citrinin-producing fungi (*A. terreus*, *A. candidus*, *P. citrinum* and *P. viridicatum*) as recorded in (Table 4). The obtained results are relatively

Table 4: Sample number (SN), sample source, moisture content (%MC), biological assay (Brine shrimp larvae), naturally occurring of mycotoxins and common mycotoxin-producing fungi associated with toxic Fabaceae seed samples tested

SN	Sample source	MC%	Dead% larvae	Mycotoxins identified ($\mu\text{g kg}^{-1}$)	Mycotoxin-producing fungi
Broad bean seed samples					
2	Missrata	8.3	40.0	Sterigmatocystin (4.8)	<i>Emericella nidulans</i>
4	Tripoli	7.5	75.0	Aflatoxins B ₁ and B ₂ (3.8 and 8.9)	<i>A. flavus</i>
9	Al-Zawia	8.4	60.0	Sterigmatocystin (8.2)	<i>Emericella nidulans</i>
10	Al-Zawia	7.6	70.0	Aflatoxins B ₁ and B ₂ (3.6 and 7.8)	<i>A. flavus</i>
12	El-Nekat	8.6	63.0	Aflatoxins B ₁ and B ₂ (2.6 and 6.2)	<i>A. flavus</i> , <i>A. parasiticus</i>
14	El-Nekat El-Khams	7.3	84.0	Aflatoxins B ₁ and B ₂ (3.7 and 10.2) Citrinin (6.2)	<i>A. flavus</i> , <i>A. parasiticus</i> <i>A. terreus</i> , <i>A. candidus</i> <i>P. viridicatum</i>
15	Naloot	8.3	41.0	Aflatoxins B ₁ and B ₂ (2.1 and 4.3)	<i>A. flavus</i> , <i>A. parasiticus</i>
Kidney bean seed samples					
41	Al-Zawia	9.7	53.0	Aflatoxins B ₁ and B ₂ (2.9 and 6.1)	<i>A. flavus</i>
42	El-Nekat El-Khams	12.8	77.0	Aflatoxins B ₁ and B ₂ (3.8 and 9.5)	<i>A. flavus</i>
44	El-Nekat El-Khams	6.0	46.0	Aflatoxins B ₁ and B ₂ (1.6 and 4.8)	<i>A. flavus</i> , <i>A. flavus</i> var. <i>columbaris</i>
Sweet pea seed samples					
49	Tripoli	7.6	60.0	Aflatoxins B ₁ , B ₂ , G ₁ and G ₂ (2.4-8.8) Citrinin (4.6)	<i>A. flavus</i> <i>A. terreus</i> , <i>P. citrinum</i>
54	Al-Zawia	9.2	50.0	Aflatoxins B ₁ , B ₂ , G ₁ and G ₂ (1.8- 3.7) Sterigmatocystin (2.8)	<i>A. flavus</i> <i>Emericella nidulans</i> , <i>Eurotium chevalieri</i>
56	Al-Zawia	8.3	85.0	Aflatoxins B ₁ , B ₂ , G ₁ and G ₂ (4.3- 12.2)	<i>A. flavus</i> , <i>A. flavus</i> var. <i>columbaris</i>
57	El-Nekat El-Khams	8.2	57.0	Aflatoxins B ₁ , B ₂ , G ₁ and G ₂ (2.1- 4.4)	<i>A. flavus</i> , <i>A. flavus</i> var. <i>columbaris</i>
58	El-Nekat El-Khams	8.0	75.0	Aflatoxins B ₁ , B ₂ , G ₁ and G ₂ (3.5- 9.5)	<i>A. flavus</i> , <i>A. flavus</i> var. <i>columbaris</i>
60	Naloot	9.7	68.0	Aflatoxins B ₁ , B ₂ , G ₁ and G ₂ (2.9- 9.2)	<i>A. flavus</i>

similar to that previously recorded in different parts of the world (El-Maghraby *et al.*, 1992, 1995; El-Kady and Youssef, 1993; Costa and Scussel, 2002; Kumar *et al.*, 2008; Gonçalves *et al.*, 2008; Wagacha and Muthomi, 2008).

On the other hand, no tested mycotoxins were detected in any chickpea seed samples assayed. As well as, ochratoxins A and B, patulin, rubratoxin B, diacetoxyscirpenol, T₂-toxin, terrin, gliotoxin, roquefortin and zearalenone could not be detected in any Fabaceae seed samples tested (Table 4). This result is in full agreement with that reported by Youssef *et al.* (2002) that aflatoxins (B₁, B₂, G₁ and G₂), ochratoxin A, sterigmatocystin, simple microcyclic trichothecenes (DAS, T-toxin, HT-toxin), patulin, fumigillin and zearalenone could not be detected in any Egyptian roasted chickpea seed samples tested, but unfortunately the isolated fungi exhibited potentially mycotoxins production in favorite conditions.

It is the first report on mycoflora and mycotoxins of Fabaceae seed samples in Libya, as well as 85 species in addition to 7 varieties are new records to Libyan mycoflora (Table 3, 4).

In conclusion, it is clearly evident that Fabaceae seed samples tested were a vehicle for numerous heterogeneous fungal species contamination. As a result of mycological growth in the field and during storage of seeds, the risk of mycotoxins production especially aflatoxins as highly potent carcinogenic and hepatotoxic agents should be taken into consideration. So, for human public health, seeds in different stages of production chain must be subjected to quality control and microbiological examinations.

REFERENCES

- Abdel-Hafez, A.I.I., 1988. Mycoflora of broad bean, chick pea and lentil seeds in Egypt. *Crypto. Mycol.*, 9 (4): 335-343.
- Abdel-Sater, M.A. and S.M. Saber, 1999. Mycoflora and mycotoxins of some Egyptian dried fruits. *Bull. Fac. Sci. Assiut. Univ. Egypt*, 28 (1-D): 91-107.
- Al-Doory, Y., 1980. *Laboratory Medical Mycology*. Lea and Febiger, Philadelphia Kimpton Publishers, London, pp: 410.
- AOAC., 1984. Association of Official Analytical Chemists. *Official Methods of Analysis*. 13th Edn. Washington DC., pp: 429.
- Booth, C., 1971. The Genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England, pp: 237.
- Booth, C., 1977. *Fusarium* laboratory guide to the identification of the major species. Commonwealth Mycological Institute, Kew, Surrey, England, pp: 58.
- Bullerman, L.B., 1979. Significance of mycotoxins to food safety and human health. *J. Food Prot.*, 42: 65-86.
- Christensen, C.M. and K.P. Raper, 1978. Synoptic key to *Aspergillus nidulans* group species and related *Emericella* species. *Trans. Br. Mycol. Soc.*, 71: 177-191.
- Christensen, C.M., 1991. Fungi and Seed Quality in Handbook of Applied Mycology. In: Food and Feeds, Arora, D.K., K.G. Mukerji and E.H. March (Eds.). 8. Marcel Dekker, New York, pp: 99.
- Costa, L.L.F. and V.M. Scussel, 1998. Toxigenic fungi in beans (*Phaseolus vulgaris* L.). *Med. Vet.*, 149 (6): 518.
- Costa, L.L.F. and V.M. Scussel, 2002. Toxigenic fungi in beans (*Phaseolus vulgaris* L.) classes black and color cultivated in the state of Santa Catarina, Brazil. *Braz. J. Microbiol.*, 33 (2): 350-359.
- Creepy, E.E., 2002. Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicol. Lett.*, 127: 19-28.
- Domsch, K.W., W. Gams and T.H. Anderson, 1980. *Compendium of Soil Fungi*. Academic Press, London, pp: 859.
- Dorner, J.W., 1998. Chromatographic Analysis of Mycotoxins. In: *Chromatographic of Environmental and Food Toxicants*, Shibamoto, T. (Ed.). Marcel Dekker, Inc. New Yourk, Hong Kong, pp: 113-168.
- El-Kady, I.A., O.M.O. El-Maghraby and S.M. Saber, 1986. Halophilic or halotolerant fungi of four seeds from Egypt. *Crypt. Mycol.*, 7 (2): 289-293.
- El-Kady, I.A. and M.S. Youssef, 1993. Survey of mycoflora and mycotoxins in Egyptian soybean seeds. *J. Basic Microbiol.*, 33 (6): 371-378.
- Ellis, M.B., 1971. *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, Surrey, UK., pp: 608.
- Ellis, M.B., 1976. *More Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, Surrey, UK., pp: 507.

- El-Maghraby, O.M.O., S.M. Saber and M.B. Aboul-Nasr, 1992. Fungal contamination of pea (*Pisum sativum* L.) and resistance of cultivars to mycotoxin accumulation. Sohag Pure Applied Sci. Bull. Fac. Sci. Assiut. Univ. Egypt, 8: 137-155.
- El-Maghraby, O.M.O., I.A. El-Kady and S.M. Saber, 1995. Mycotoxins naturally and producing moulds of four leguminous seeds in Egypt. Kor. J. Mycol., 24 (3): 20-24.
- FAO., 2003. Food and Agriculture Organization of the United Nations, Rome, FAO Statistic Series, No. 176, 56: 106-115.
- Gonçales, E., J.H.C. Nogueira, H. Fonseca, J.D. Felicio, F.A. Pino and B. Correa, 2008. Mycobiota and mycotoxins in Brazilian peanut kernels from sowing to harvest. Int. J. Food. Microbiol., 123: 184-190.
- Ibrahim, Y.M.M., 2007. Mycobiota and mycotoxins of peanuts (*Arachis hypogaea* L.) in Egypt. M.Sc. Thesis, Sohag University, Egypt.
- International Seed Testing Association (ISTA), 1966. International rules for seed testing. Proc. Int. Test. Assoc., 32: 1-52.
- Johnson, L.F. and E.A. Curl, 1972. Methods for Research on Ecology of Soil-Borne Pathogens. Burgess Publication Co., Minneapolis.
- Klich, M.A. and J.I. Pitt, 1992. Laboratory Guide to Common *Aspergillus* Species and their Teleomorphs. 2nd Edn. Published by Commonwealth Scientific and Industrial Research Organization, Division of Food Research, North Ryde, NSW.
- Korpinen, E.L., 1974. Studies on *Stachybotrys alternans*, I-Comparison of rabbit skin, mouse fibroblast culture and brine shrimp tests as detectors of *Stachybotrys* toxins. Acta Pathol. Microbiol. Scan. Sect. Bull., 82: 465-469.
- Kozakiewicz, Z., 1989. *Aspergillus* Species on Stored Products (Mycological Paper No. 161), CAB., International Mycological Institute, Kew, Surrey, UK.
- Kumar, V., M.S. Basu and T.P. Rajendran, 2008. Mycotoxin research and mycoflora in some commercially important agricultural commodities. Crop Prot., 27 (6): 891-905.
- Lewis, L., M. Onsongon, H. Njapau, H. Schurz-Rogers, G. Luber, S. Kieszak, J. Nyamongo, L. Backer, A.M. Dahiye, A. Misore, K. Decoet, C. Rubin and The Kenya Aflatoxicosis Investigation Group, 2005. Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in Eastern and Central Kenya. Environ. Health Persp., 113: 1763-1767.
- Martin, J.P., 1950. Use of acid, rosebengal and streptomycin in plate method for estimation of soil fungi. Soil Sci., 69: 215-233.
- Moubasher, A.H., 1993. Soil Fungi in Qatar and Other Arab Countries. Published by the Center of Scientific and Applied Research, University of Qatar, pp: 566.
- Pitt, J.I., 1979. The genus *Penicillium* and its Teleomorphic States, *Eupenicillium* and *Talaromyces*. Commonwealth Scientific and Industrial Research Organization, Division of Food Research, North Ryde, NSW. Australia, Academic Press, Inc. Ltd. London, pp: 634.
- Pitt, J.I., 1991. A Laboratory Guide to Common *Penicillium* species. Commonwealth Scientific and Industrial Research Organization, Division of Food Research, North Ryde, NSW. Australia, Academic Press, Inc. Ltd. London, pp: 187.
- Raper, K.B. and D.I. Fennel, 1977. The Genus *Aspergillus*. R.E. Krieger Publishing Co., Huntington, NY. USA., pp: 686.
- Richard, J.L., 2007. Some major mycotoxins and their mycotoxicosis. An overview. Int. J. Food Microbiol., 119: 3-10.
- Rodriguez, V.M.L., D.M.M. Calonge and E.D. Ordonez, 2003. ELISA and HPLC determination of the occurrence of aflatoxin M₁ in raw cow's milk. Food Addit. Contamin., 3: 276-280.

- Samson, R.A., V.R. Hoekstra, J.C. Frisvad and O. Filtenborg, 1998. Introduction to Food-Borne Fungi. 4th Edn. Centraalbureau voor Schimmelcultures, Baarn Delft. The Netherlands, pp: 64.
- Sendecor, G.W. and G.W. Cochran, 1980. Statistical Methods. 7th Edn. Iowa State University Press, Ames, Iowa, USA.
- Smith, J.E. and M.O. Moss, 1985. Mycotoxins Formation, Analysis and Significance. John Wiley and Sons. New York, pp: 147.
- Thomas, J.L., 1984. *Fusarium* as a Biodeterioration Case History. In: The Applied Biology of *Fusarium*, Moss, M.O. and J.E. Smith (Eds.). Cambridge University Press, Cambridge, pp: 107-116.
- Tseng, T.C., J.C. Tu and S.S. Tzean, 1995. Mycoflora and mycotoxins in dry bean (*Phaseolus vulgaris* L.) produced in Taiwan and Ontario, Canada. Bot. Bull. Acad. Sin., 36: 229-234.
- Tseng, T.C. and J.C. Tu, 1997. Mycoflora and mycotoxins in adzuki and mung beans produced in Ontario, Canada. Microbiol., 90 (363): 87-95.
- Wagacha, J.M. and J.W. Muthomi, 2008. Review on mycotoxin problem in Africa: Current status, implications to food safety and health and possible management strategies. Int. J. Food Microbiol., 124 (1): 1-12.
- Weidenborner, M. and B. Kunz, 1994. Contamination of different muesli components by fungi. Mycol. Res., 98 (5): 583-589.
- WHO., 2006. Mycotoxins in African foods; implications to food safety and health. Afro Food Safety Newsletter, World Health Organization Food Safety (FOS), Issue No. July, 2006. www.afro.who.int/des.
- Wu, F., 2004. Mycotoxin risk assessment for the purpose of setting international regulatory standards. Environ. Sci. Technol., 38: 4049-4055.
- Youssef, M.S., N.F. Abo-Dahab and A.A. Abou-Seidah, 2000. Mycobiota and mycotoxins contamination of dried raisins in Egypt. Afr. J. Mycol. Biotechnol., 8 (3): 69-86.
- Youssef, M.S., M.B. Aboel-Naser and N.F. Abo-Dahab, 2002. Mycoflora and mycotoxins of roasted cicer (*Cicer arietinum* L.) in Egypt. Proceedings of the 3rd International Conference on Fungi: Hopes and Challenges, Cairo, 30-31 Oct., Egypt, (1): 75-89.
- Youssef, M.S., N.F. Abo-Dahab and R.M. Farghaly, 2003. Studies on mycological status of salted fish (Moloha) in Upper Egypt. Mycobiology, 31 (3): 166-172.
- Youssef, M.S., 2008. Studies on mycological status of jew's-mallow leaves and okra fruits in Egypt. Res. J. Microbiol., 3 (5): 375-385.