

Screening of Fungi Associated With Commercial Grains and Animal Feeds in Al-Bayda Governorate, Libya

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Abstract: Isolation and identification of fungi associated with food grains were carried out on 20 food grains including cereals: wheat, barley, corn, sorghum, fenugreek, millet; legumes: pea, peanut, chickpea, faba bean, kidney bean, cowpea, red and green lentil; oil-producing seeds, walnut, almond, hazelnut, sesame, pistachio and two animal feeds: diet and ration obtained from local markets during 2003 to 2006 in Al-Bayda, Libya. Blotter test technique and Agar plate technique were used to germinate the samples. Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Yeast Extract Agar (YEA) and Sabrand's Dextrose Agar (SDA) media were used for fungal isolation. Twenty fungi species belonging to 10 genera were identified. These include *Aspergillus flavus*, *A. ochraceus*, *A. terreus*, *A. niger*, *A. candidus*, *A. fumigates*, *Penicillium chrysogenum*, *P. canescens*, *P. waksmanii*, *Fusarium oxysporum*, *F. graminearum*, *F. sporotrichioides*, *Rhizopus stolonifer*, *Mucor piriformis*, *Alternaria tuniissima*, *Rhizoctonia solani*, *Pythium ultimum*, *Phyllactinia rigida* and *Saccharomyces cerevisiae*. Among the cereals, sorghum grains exhibited the highest fungal contamination (45%), while barley was the least contaminated (11.17%). Among the legumes, peanut seeds had the highest contamination (37.94%) followed by pea (37.50%), while and the least was in lentil (6%). Among the fatty seeds, walnut had the highest degree of contamination (71.17%), followed by almond (48.07%), while the least contaminated was hazelnut (15.48%). Of the animal feeds, ration (35%) was more contaminated than the diet (30.63%). PDA media using the Blotting test technique exhibited more *Aspergillus* and *Rhizopus* colonies compared to the same media using agar plate technique. The highest number of mycotoxin-producing fungus *Aspergillus* was isolated from peanut seeds (49.38%) followed by hazelnut (19.20%) and the laest in wheat (7.67%). *Penicillium* was isolated from all samples of walnut seeds and least isolated from hazelnut (6.34%). The highest contamination with *S. cerevisiae* was found in walnut seed samples (98%), while the least was in pea (10%).

Key words: Mycotoxigenic fungi · Food grains · Animal feeds · Libya · Blotter test · Agar plate technique · Potato Dextrose Agar · Malt Extract Agar · Yeast Extract Agar · Sabrand's Dextrose Agar

INTRODUCTION

Nine crops are of major importance namely wheat, rice, maize, barley, sorghum, sugar beet, common phaseolus bean, soybean and groundnut, which contribute to the greater part of food production worldwide. All these crops are attacked by devastating seed-borne fungi and cause losses of cereal grains which differ widely, amounting to about 10% worldwide, but may reach up to 50% in tropical countries [1]. Maize, soybean,

wheat, barley, rice and sorghum are the main cereals consumed in Libya [2].

Major groups of the fungal pathogens are seed borne and seed transmitted. Besides saprophytism and parasitism, fungi are also known to form other inherent associations with seeds of some members of Cistaceae, Ericaceae and Orchidaceae [3]. Fungal penetration of peanut seeds can occur through the central cavity of the exotesta cells, through the space between cells and through cracks in seed coat [4]. The effects of fungal

invasion include a reduced germination potential, development of visible moldiness, discolouration, unpleasant odour, loss of dry matter, heating, chemical and nutritional changes, loss of quality and production of mycotoxins [5, 2].

Many treatments such as drying in sun or treating with lime have been applied to exclude fungi from food seeds. Peanut seeds dried in the field and in shade had high levels of *Penicillium spp.* and *Aspergillus flavus* infection regardless of lime treatment. However, the effect of lime and drying method on *Rhizopus spp.* growth was evident [6]. After harvest, a longer exposure to the environment (field- and shade-drying) resulted in more fungal infection because of low rate of water loss [7]. The activity of *F. moniliforme* and *F. proliferatum* in grain reduced the presence of *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceus* to some extent, particularly at 15°C and high water availabilities (0.95-0.98 aw) [8]. Christensen and Kaufmann [9], working with the fungi associated with seeds, classified them into two groups, i.e., “field fungi,” which contaminate seeds in the field before and during harvest including transit and “storage fungi” which contaminate seeds during transit and storage. Fungi are significant destroyers of foodstuffs and grains during storage, rendering them unfit for human consumption by retarding their nutritive value and often by producing mycotoxins in the foods [10, 11]. Fungi of the genera *Rhizopus*, *Aspergillus*, *Fusarium* and *Penicillium* are commonly present in peanut (*Arachis hypogea* L.) seeds [12] and *A. flavus* is the main species occurring in peanut [13, 14]. A number of fungi that have been associated with cereal grains and soybean seeds include species of the genera *Fusarium*, *Aspergillus* and *Penicillium* [15]. Stored grain is commonly colonized by a range of different fungi, especially by species of *Aspergillus*, *Penicillium* and *Fusarium* and at high water activities (aw), by yeasts [16, 17].

Estimation of fungal contamination of barley grain is important as fungi can proliferate during storage and the 4-5 day malting process [18, 19]. Soil fungi are likely to be a major cause of mortality for buried seed. A few ecological studies have examined the role of these pathogens in natural systems. Invasion of seed by fungi before harvest is governed primarily by plant host-fungus and other biological interactions (e.g. insects), while growth of post-harvest fungi is governed by crop nutrients, abiotic factors (temperature, moisture, light etc) and biotic factors (insects, interference, competition, etc) [20].

Maize (*Zea mays* L.) is one of three major cereal crops that dominate world agriculture [2]. Maize grain is a good substrate for mould infection and production of dangerous mycotoxins which is potentially hazardous to the health of both humans and animals [21]. Maize kernels are processed primarily for livestock feed (78%) and to some extent for human consumption (13%) [22]. Hence, the potential for mycotoxins to be found in foods and animal feeds is high. Effects of mycotoxins in animals include allergic reactions, reproductive failure and unthriftiness, loss of appetite, feed refusal and suppression of the immune system, decreased feed efficiency and mortality.

Numerous investigations have been carried out on cereals seed borne fungi all over the world [23-30]. However for Libya, no published studies, except a few, exist on mycoflora contamination of cereals seeds. The purpose of this research was to isolate and identify the mycoflora of cereals, legumes, oil producing seeds and animal feeds obtained from local markets in Al-Bayda Governote, Libya. This was aimed at providing insight into the presence or absence of fungi with public health implications whose presence could have been introduced as a result of processing or storage. The results might be helpful to both consumers, food processing factories and government regulatory agencies.

MATERIALS AND METHODS

Collection of Seed Samples: Commercial grains from different plant families, Poaceae: wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), fenugreek (*Trigonella foenum-graecum* L.), maize (*Zea mays* L.), sorghum (*Sorghum vulgare* L.) and millet (*Pennisetum glaucum* L.), Leguminosae: pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), kidney bean (*Phaseolus vulgaris* L.), faba bean (*Vicia faba* L.), peanut (*Arachis hypogea* L.), cow pea (*Vigna sinensis* L.), red lentil and green lentil (*Lens culinaris* Medic L.), Rosaceae: almond (*Prunus amygdalus* L.), Juglandaceae: walnut (*Juglans regia* L.), Pedaliaceae: sesame (*Sesamum indicum* L.), Corylaceae: hazelnut (*Corylus avellana* L.) and Anacardiaceae: green almond (*Pistacia vera* L.) were obtained randomly between 2003 and 2006 from local markets in Al-Bayda city, Libya. Two types of animal feed (ration and diet) were also included. Each sample was put in a sterile polyethylene bag which was subsequently sealed. The bags were transferred to the mycological laboratory and kept in a refrigerator at 3-5°C until fungal isolation and identification were completed.

Determination of Moisture Content of Seeds: Twenty grams of each seed sample were milled and dried in an oven at 105°C for 24 h, then cooled in desiccators and re-weighed 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 Whatman No. 1 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

Blotter Test: The blotter test [31, 32] was used to isolate the fungal pathogens associated with the sample seeds as well as to determine the viability of seeds after the storage periods. Modified Potato Dextrose Agar (PDA) media, Malt Extract Agar (MEA) media, Yeast Extract Dextrose Chloramphenicol Agar (YEDC) media, Sabrand's Dextrose Agar (SDA) media were used. Sterile Whatman filter papers were placed in sterile 9 cm petri dishes and moistened with sterile distilled water to provide moist condition. Ten grains of each plant species were placed in each petri dish lined with the filter papers. Seeds of plant species were replicated four times and incubated at room temperature for seven days. Daily observations were made to count the number of germinated seeds and fungal colonies that appeared. The resulting fungi were isolated and pure cultures were prepared to identify them.

Agar Plate Technique: The grains were external sterilized by 4% sodium hypochlorite to 1 minute then washed by sterilized distilled water and placed in petri dishes on Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Yeast Extract Agar (YEA) and Sabrand's Dextrose Agar (SDA) and spaced properly. They were incubated for a fixed period of time (7 days) at 25°C. The growing fungi on contaminated grains were photographed using camera and were isolated on plate agar media (PDA, MEA, YEA and SDA). The isolated fungi were identified using light microscope after slides were stained by Lactophenol (20g of Phenol in a mixture of 20g of Lactic Acid, 40g of Glycerol and 20 ml of water) by Anon. [33], Henselov and Hudecov [34] and Gwary *et al.* [35].

Identification of Fungi: The taxonomic identification of fungi (based on morphological macro- and microscopic

characteristics) was carried out according to Booth [36, 37], Raper and Fennell [38], Pitt [39, 40], Moubasher [41], Samson [42] and Summerell [43].

Statistical Analysis: Analysis of variance (ANOVA) of collected data and tests of significance were carried out using SAS statistical package [44] and Duncan's test was used to adjudge the differences between treatment means.

RESULTS AND DISCUSSION

Ten fungal genera were isolated, which were related to four fungal classes namely Oomycetes, Zygomycetes, Ascomycetes and Deuteromycetes. Among these fungal genera, 20 species and two strains of *Saccharomyces cerevisiae* were isolated and identified. These species were *Aspergillus flavus*, *A. ochraceus*, *A. terreus*, *A. niger*, *A. candidus*, *A. clavatus*, *A. fumigatus*, *Penicillium chrysogenum*, *P. canescens*, *P. waksmanii*, *Fusarium oxysporum*, *F. graminearum*, *F. sporotrichioides*, *Rhizopus stolonifer*, *Mucor piriformis*, *Alternaria tenuissima*, *Rhizoctonia solani*, *Pythium ultimum*, *Phyllactinia rigida* and *Saccharomyces cerevisiae* (Table 1). The findings are in agreement with the outcome of El-Maghraby and El-Maraghy [45], who isolated *Aspergillus*, *Penicillium* and *Fusarium* from feedstuffs collected from different farms in the Beida Governorate, Libya.

Among the cereals, the percentages (%) of contamination of sorghum grain was the highest (about 45%) and it differed significantly ($P < 0.05$) from other grains such as maize (29.09%), wheat (12.44%), barley (11.17%) as shown in Table 2. This result is related to that of Fakhrunnisa [46] who isolated seed-borne mycoflora from 27 samples of sorghum, 19 samples of wheat and 14 samples of barley in Pakistan. Seed-borne mycoflora previously reported from other parts of the world include *Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Cladosporium* sp., *Fusarium moniliforme*, *F. oxysporum*, *F. pallidoroseum*, *Drechslera tetramera*, *Nigrospora* sp., *Phoma* sp. and *Rhizopus* sp. [47, 48].

Percentage fungal contamination of peanut seeds were the highest amongst the legumes (37.94%), followed by pea (37.50%) and lentil was with least infection (6%) (Table 3). Freshly harvested peanut seeds have also been reported to be contaminated with moulds and the dominant genera comprised of *Aspergillus*, *Penicillium* and *Fusarium* [49, 50, 51].

Table 1: Fungi which were associated with the examined commercials grains and animal feed and their relative distribution

Family Fungus	Name of plant													
	Poaceae							Fabaceae (Leguminosae)						
	Wheat	Barley	Corn	Sorghum	Fenugreek	Millet	Pea	Chike pea	Kidney beans	Faba bean	Cow pea	Red lentil	Green lentil	Peanut
<i>Aspergillus niger</i>	+	+	+	+			+	+		+	+		+	+
<i>Aspergillus flavus</i>	+	+	+	+										+
<i>Aspergillus ochraceus</i>	+			+			+							
<i>Penicillium canescens</i>	+	+	+											
<i>Rhizopus stolonifer</i>	+	+	+	+	+		+	+			+	+		+
<i>Fusarium oxysporum</i>	+	+												
<i>Saccharomyces cerevisiae</i>	+	+	+	+	+	+	+	+	+		+	+	+	+
<i>Alternaria tenuissima</i>						+								
<i>Penicillium chrysogenum</i>										+	+	+	+	+
<i>Mucor piriformis</i>														+
<i>Saccharomyces cerevisiae</i> 1														+
<i>Pythium ultimum</i>														
<i>Phyllactinia rigida</i>														
<i>Fusarium graminearum</i>														
<i>Aspergillus fumigatus</i>														
<i>Aspergillus terreus</i>														
<i>Aspergillus clavatus</i>														
<i>Rhizoctonia solani</i>														
<i>Penicillium waksmanii</i>														

Family Fungu	Name of plant								
	Rosaceae		Juglandaceae		Pedaliaceae		Corylaceae (Animal feed)		
	Almond		Walnut		Sesamum		Hazelnut	Diet	Ration
<i>Aspergillus niger</i>	+						+	+	+
<i>Aspergillus flavus</i>	+						+		+
<i>Aspergillus ochraceus</i>	+					+	+		+
<i>Penicillium canescens</i>									
<i>Rhizopus stolonifer</i>	+		+			+	+	+	+
<i>Fusarium oxysporum</i>	+						+	+	
<i>Saccharomyces cerevisiae</i>			+		+		+	+	+
<i>Alternaria tenuissima</i>			+				+		
<i>Penicillium chrysogenum</i>	+								+
<i>Mucor piriformis</i>	+								
<i>Saccharomyces cerevisiae</i> 1									
<i>Pythium ultimum</i>	+								
<i>Phyllactinia rigida</i>	+								
<i>Fusarium graminearum</i>			+						
<i>Aspergillus fumigatus</i>							+		
<i>Aspergillus terreus</i>							+		+
<i>Aspergillus clavatus</i>							+		
<i>Rhizoctonia solani</i>							+		
<i>Penicillium waksmanii</i>								+	+

Table 2: Percentages of contaminated cereal samples indicated by different isolation techniques/media

Used technique	Cereals			
	Wheat	Barley	Corn	Sorghum
Blotter test technique	12.00	04.00	17.33	14.33
Agar Plate (PDA)	04.00	10.00	09.67c	37.00b
Agar Plate (SDA)	75.00a	32.00	58.00
Level of significance	n.s.	n.s.	*	n.s.

n.s.: not significant at p < 0.05, *: differ significantly at p < 0.05
a, b, c: Means with different letters are differed significantly at p < 0.05
....: not included in the analysis

Table 3: Percentage of contaminated legume seed samples indicated by different isolation technique

Used technique	Legume seeds		
	Lentil	Pea	Peanut
Blotter test technique	7.00	45.00	35.69
Agar plate (PDA)	5.00	30.00	40.19
Agar plate (MEA)
Agar plate (YEA)
Level of significance	n.s.	n.s.	n.s.

Table 4: Percentage of contaminated oil seeds (nuts) samples indicated by different isolation technique

Used technique	Fatty seeds		
	Walnut	Almond	Hazelnut
Blotter test technique	69.00	48.13	22.78
Agar plate (PDA)	73.33	50.00	10.67
Agar plate (MEA)	36.07	08.00
Agar plate (YEA)
Level of significance	n.s.	n.s.	n.s.

n.s.: not significant at p < 0.05,: not included in the analysis

Table 5: Percentage of contaminated animal feed samples indicated by different isolation technique

Used technique	Animal feeds	
	Diet	Ration
Blotter	21.25	07.50 b
Agar Plate (PDA)	40	62.50 a
Level of significance	n.s.	*

n.s.: not significant at p < 0.05, *: differ significantly at p < 0.05, a, b:
Means with different letters differ significantly at p < 0.05
....: not included in the analysis

Table 6: Isolated fungi from different kinds of legumes using different isolation techniques

Fungal groups	Technique	Oomycetes		Zygomycetes						Ascomycetes					Deuteromycetes			Number of detected fungi by			
		Ph.r	Fy.u.	M.p.	Rh.z	A.c.	A.fl	A.fu	A.n	A.o.	A.t	P.c.	Rh.s	S.c.	S.c.1	Alt.t	F.g.	F.o.	Technique	Total	
Cowpea	BLO										*								1	3	
	PDA							*			*		*							3	
Chickpea	BLO							*											1	3	
	PDA				*			*					*							3	
Green lentil	BLO							*				*							3	4	
	PDA							*			*		*							3	
Faba bean	BLO							*					*						1	4	
	PDA				*			*			*		*							4	
Kidney bean	BLO																		0	1	
	PDA													*						1	
Red lentil	BLO				*														1	3	
	PDA				*						*		*							3	
Pea	BLO				*			*											2	4	
	PDA				*			*	*				*							4	
Peanut	BLO				*		*	*			*		*							4	8
	PDA				*		*	*			*		*	*						6	
	MEA			*			*	*			*		*							3	
	YEA			*	*		*	*			*		*							5	
Total fungi isolated		0	0	2	9	0	3	0	13	1	0	9	0	9	1	0	0	0	48		
Number of contaminated seeds kind		0	0	1	5	0	1	0	6	1	0	5	0	8	1	0	0	0		9	

The percentages of fungal infection in oil seed samples were highest in walnut seed (71.17%), followed by almond seed (48.07%) and the lowest in hazelnut seed (15.48%) as shown in Table 4. These results are in agreement with those reported by Abdel-Gawad and Zohri [52] that found a wide range of moulds representing several genera and species from 5 seed samples, but the highest fungal count was also recorded in walnut. Percentages of contamination in animal feeds were 35% in ration seed and 30.63% in diet (Table 5). The results in this study are similar to Krnjaja [53] that animal feeds are contaminated with *Fusarium* spp., *Aspergillus* spp. and *Penicillium* spp.

Isolation Technique: The number of different seed-borne fungi isolated in various media clearly showed that there were differences in contamination for each kind of fungus on the different media used. The highest percentage of contaminations with *Aspergillus niger* was shown by wheat, barley, maize, sorghum, peanut, chickpea, faba bean, green lentil, peanut, almond, hazelnut and ration in PDA media using Blotter test technique, while the highest percentage of contaminations with *Rhizopus stolonifer* was also shown by sorghum, fenugreek, pea, green lentil, peanut, almond, walnut, sesame, hazelnut and diet on PDA using Blotter test technique. Results differed using other media such as MEA, YEA, SDA and Agar plate technique, as shown in Table 6. This indicates that there are sensitivity differences in the various isolation methods used and also varying with organism and media. On the other hand, the high percentage of contaminations with *Saccharomyces cerevisiae* was shown by sorghum, fenugreek, millet, pea, chickpea, kidney beans, faba bean,

maize, green lentil, peanut, walnut, sesame and hazelnut in PDA media in Agar plate technique as shown in Table 6. The finding of this study was different from that of Embaby and Abdel-Galil [54]. Agar plate (PDA medium) was better for seed testing than Blotter test method and gave higher numbers of colony of fungi with all tested legume seed samples.

Contaminations With Main Genera of Fungi That Affect Human Health: Mycotoxins are secondary metabolites mainly produced by the four genera of fungi -*Fusarium*, *Claviceps*, *Aspergillus* and *Penicillium*, which grow on almost every kind of nourishing medium [55, 56]. *Aspergillus flavus* was isolated from tested seeds of different plants, with the highest contamination in peanut seeds (49.38%) and it differed significantly with other seeds such as hazelnut (19.2%), ration (18.75%), wheat (7.67%), as shown in Fig. 1. Association of varieties of fungi including species of *Aspergillus* causing significant losses in seed quality and nutritional quality of grains have been reported [57]. The study is in agreement with results from similar studies [58, 59, 60]. In these studies, peanut had the highest contamination by *A. flavus*. Vaamonde [61] noted that contamination by *A. flavus* was more in salted peanut (69%) and less in pure peanut samples in Argentina. The highest contamination of *A. niger* was in pea seeds (60%) and it differed significantly with other seeds such as peanut (56.5%), maize (32.44%), sorghum (20%), almond (18.67%), wheat (12%), barley (11.94%) and lentil (5%), as shown in Fig. 2. The findings were closely related to the observation of Ahmad and Singh [62]; El-Kady [63]; Tseng [64]; Tseng and Tu [24]; Saber *et al.* [65] and Ciccarese [66].

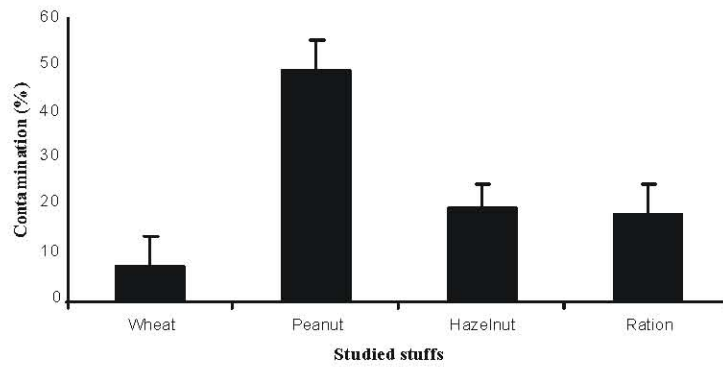


Fig. 1: Ranking the studied stuff according to their contamination with *Aspergillus flavus*.

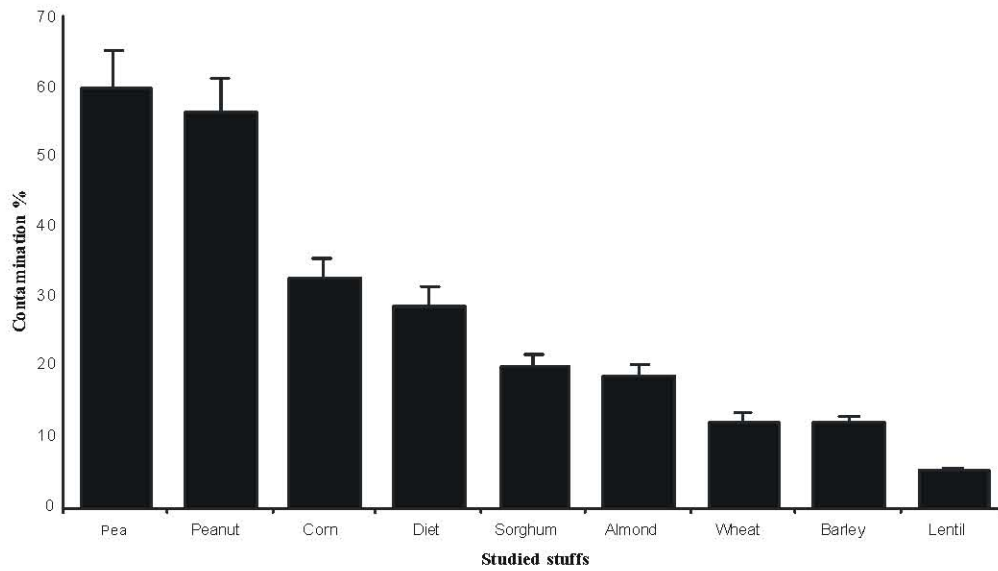


Fig. 2: Ranking the studied stuff according to their contamination with *Aspergillus niger*.

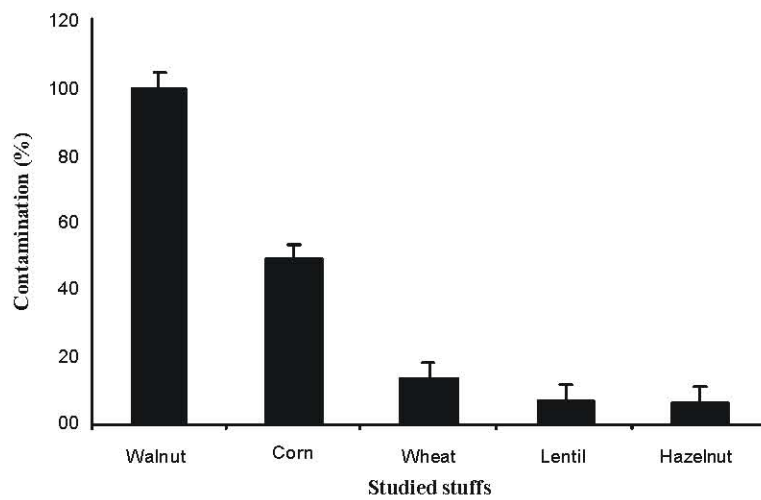


Fig. 3: Ranking the studied stuff according to their contamination with *Saccharomyces cerevisiae*

Penicillium spp. was also isolated from the tested seeds samples. It was observed that the highest contamination of *Penicillium spp.* was in walnut seeds (about 100%) and it differed significantly with other seeds such as maize (48.67%), wheat (13.67%), lentil (7%), hazelnut (6.34%), as shown on Figure 3. The study corroborates the report of Gürses [67] who collected 24 walnuts and found *Aspergillus* and *Penicillium* species in all the samples. Sing and Shukla [68] remarked on fungal infection and mycotoxin contamination in fresh and stored kernels of walnut collected from different localities of Uttaranchal (India). He emphasized that fresh samples carried a combination of field and storage fungi, mainly species of *Penicillium*, *Aspergillus* and *Alternaria*.

Saccharomyces cerevisiae was isolated from the tested seeds samples. Walnut seeds had the highest contaminated samples (98%). This differed significantly from other seeds such as almond (70%), sorghum (68%), peanut (31%), hazelnut (11%), pea (10%), as shown in Figure 8. Sing and Shukla [68] found more or less similar results in their study.

The frequencies of growth of *Rhizopus stolonifer*, *Aspergillus niger* and *Penicillium canescens* on barley and wheat are in agreement with those obtained by Sagir and Yildiz [69]. Sorghum grain, pea seed, walnut seed and ration seed were with the highest level of contamination because of moisture content of seeds or may be due to mechanical injury during harvesting and unfavourable storage conditions which affected the enzymes metabolism. Grain crops harbour a microbial community (e.g., filamentous fungi, yeast, bacteria), which is influenced by cultivar, climate and agronomic practices [70]. Growth of fungi and bacteria typically occurs for only a short period during initial stages of fermentation of plant material, after which lactic acid production and alcoholic fermentation pre-dominate [71]. The ability of soil borne pathogens to parasitize seeds is influenced by the structure and condition of the seeds as well as by cultural and climatic factors [72]. Moisture can influence the distribution and spread of many pathogens affecting their development, longevity, germination and infectiveness of fungal spores [73]. Pathogenic and spoilage organisms may be inhibited by production of organic acids, hydrogen peroxide, carbon dioxide and/or antimicrobial substances and also by lowering the pH [74]. Crist and Friese [75] studied five shrub-steppe species in Wyoming and found that fungal pathogens were responsible for up to 35% of seed mortality.

Robin and Chiou [76] showed a negative linear relationship between logarithms of internal fungal populations and logarithms of free glutamic acid content of peanut kernels. Determination of free glutamic acid content in peanut kernels in the initial stages of infection appears to have potential as an index to estimate fungal contamination. Horn [77] suggested that soil is a source of primary inoculum for *Aspergillus flavus* and *A. parasiticus* that produce highly carcinogenic aflatoxins in peanuts. Aflatoxigenic fungi commonly invade peanut seeds during maturation and the highest concentrations of aflatoxins are found in damaged seed. The testa protects the highly nutritious seed contents, but it is clear from observation and experiments that at least some seeds of some species are susceptible to attack by microorganisms. Small seeds may be more vulnerable to pathogens [75].

The highest levels of *A. flavus* and *A. parasiticus* infection and aflatoxin contamination are associated with seed damage [77-82]. Gürses [67] showed that aflatoxin B1 may be found at high levels in hazelnuts, walnuts, peanuts and almonds-except roasted chickpeas. This is a major public health concern and it requires investigations into the reasons for these high levels and means of minimizing or eliminating them. On the other hand, this situation may be a result of adverse pre-harvest conditions of temperature and humidity in the field and improper post-harvest handling and storage. Therefore, the prevention of contamination with toxigenic fungi of foods during harvest, processing and storage is the best way to control aflatoxin formation. In addition, the mold growth and toxin formation may significantly be limited by packaging, removing of the damaged and moldy fruits and mechanical drying prior to storage.

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