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Contamination of Cashew Nut with Myco-toxigenic Fungi

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Forty cashew nut samples randomly collected from Riyadh, kingdom of Saudi Arabia was surveyed using the direct plating technique. The mycological investigation revealed that seventeen fungal species belonging to 11 genera were isolated. *Aspergillus niger* was generally the most distributed and the most frequent fungus. Mycotoxin production ability of *Aspergillus flavus* and *A. niger* was evaluated using HPLC analysis. Most of *A. flavus* isolates were toxigenic and capable of producing maltoryzine (3-10ppb) and sterigmatocystin (325-525 ppb) in the culture media. Meanwhile, 40% were aflatrem (2ppb) producers. On the other hand, 80% of the tested *A. niger* isolates were capable of producing oxalic acids in the culture media. Oxalic acid production ability was ranged from 200 to 625 mg/ml. Mycotoxigenic *A. flavus* and *A. niger* are the most potent contaminants of the food and should be eradicated to minimize the risk of toxin contamination of commoditized nuts.

Key words: Sterigmatocystin, Oxalic acid, Aspergillii, HPLC, Aflatrem.

Cashew nut (*Anacardium occidentale L*) provides high nutritional value for fresh or roasted consuming and its high level of vitamin C is also well known. It has industrial uses in cosmetology, resins, varnishes, desserts, pastry, and others[1,2].

Due to its richness in fat, protein, carbohydrate, vitamins and essential amino acids; it is vulnerable to pre and/or post-harvest molds attack[3,4]. Inappropriate marketing and storage conditions; may lead to extra mycoflora contamination[5,6].

Aspergillus spp., *Penicillium* spp., *Rhizopus* spp., *Mucor* spp. and *Syncephalastrum* sp. Are the most frequent species recovered from non-disinfected cashew nuts[7,8].

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However; the mycotoxigenic Aspergillus, Penicillium and Fusarium are responsible for secretion of different metabolic toxic compounds[9,10] and could be considered the most serious fungal genera contaminate cashew nuts[11,12].

The contamination by mycotoxigenic fungi as well as mycotoxic compounds could affect nut quality[13,14] and harm both human and animal consumers[15,16].

The present study aimed to investigate the natural occurrence of myco-contaminants in cashew nut that imported to Saudi Arabia for food purposes. The evaluation of toxin-producing abilities of isolated Aspergillii was undertaken.

MATERIALS AND METHODS

Sampling and fungi

The cashew samples were collected from 40 different locations in Riyadh city, Kingdom of

Saudi Arabia to be used for fungal detection and isolation. Cashew seed samples were randomly seeded onto Petri dishes containing PDA, in quadruples. The plates were incubated at 27±2°C and examined daily for 7 days, after which the developing colonies were counted. Developing fungi were purified and maintained in slanted PDA. Fungal identification was carried out based on morphological and microscopic characteristics in the Mycological Center, Assiut University, Egypt.

Mycotoxigenicity survey

Aflatrem

A. flavus isolates were cultured in the aflatrem medium[17] and statically incubated, under light or dark conditions at 30°C. Culture filtrates of *A. flavus* were separated after 7 days of incubation. One-gram aliquots was homogenized in 10 ml of a 2:1 mixture of chloroform: methanol, vortexed several times and centrifuged at 2900×g for 10 min at room temperature. The supernatants were removed and the solvent allowed evaporating over night. The samples were re-suspended by adding 500 micro liters of acetonitrile: water (9:1) per each, then vortexed and centrifuged at 150×g at room temperature for 20 min. The collected supernatants were used in the quantifying of aflatrem by HPLC[18].

Maltoryzine

A. flavus were grown in Czapek-Dox broth medium containing malt sprout extract[19]. The production of maltoryzine in the culture media was examined using HPLC[20].

Sterigmatocystin

The production of sterigmatocystin from tested *A. flavus* isolates were analyzed and determined using HPLC[21]. The isolates were grown for 7 days on Kafer medium[20] at 37°C under static conditions. The mycelium was separated by filtration and the sterigmatocystin was extracted with 50 ml acetone for 30 min, followed by 50 ml chloroform by further 30 min. The organic phase was separated, filtered through anhydrous sodium sulfate and evaporated in a fume hood in a water boiling bath. The residue was re-suspended in 500µL HPLC grade methanol and filtered through C-18 columns prior to analysis.

Oxalic acid

Tested isolates of *A. niger* were cultivated on Czapek-Dox broth medium to investigate its abilities to produce oxalic acid. The oxalic acid was separated using a CLC-C825 CM caption exchange column; mobile phase, 90% H_2O and 10% CH_3OH; flow rate, 1 ml/min and temperature 35°C[22]. The concentration of oxalic acid secreted by *A. niger* was determined using HPLC analysis.

Statistical analysis

ANOVA and correlation analysis of the fungal isolation frequency were performed using SPSS 16.0 software package. The same software was used in the cluster analysis by the unweighted pair-group method based on arithmetic mean (UPGMA).

RESULTS

The mycological examination of the cashew samples revealed the presence of 17 fungal species that belonged to 11 genera. However, *Alternaria alternate, Aspergillus flavus, A. f.* var. *columinaris, A. niger, A. terreus, Bartalinia robillardoides, Chaetomum globosum, Chaetomum spp., Fusarium semitectum, Penicillium aurantiogrisum, P. Chrysogenum, P. paxilli, Pestallotia pezizoides, Rhizopus stolonifer, Sclerotium complanatum, Scepulariopsis sphaerospora, Syncephalastrum racenosum* were isolated. Relative distribution of isolated fungi over samples revealed that *A. niger* was the most predominant and the highest distributed species while, *P. pezizoides* was the least one (Fig. 1).

Table 1. ANOVA of the isolation frequencies of fungi isolated from cashew samples

Source	df	M S	F	Sig.	R.C*
Samples	39	3.502	1.958	0.000	3.87
Fungi	16	80.241	44.857	0.000	88.58
Samples x Fungi	624	6.842	3.825	0.000	7.55
Error	2040	1.789			

* Relative contribution (%)

Table 2. Comparable frequencies of fungi isolated from cashew nuts collected from different location in Riyadh city

Fungal isolates		Sample numbers																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Trans.	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
2	%	0.00	0.00	0.00	0.00	0.00	27.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	19.64	5.56	0.00	0.00	0.00
	Trans.	0.71	0.71	0.71	0.71	0.71	4.75	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	3.48	1.72	0.71	0.71	0.71
3	%	0.00	0.00	0.00	0.00	0.00	0.00	37.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Trans.	0.71	0.71	0.71	0.71	0.71	0.71	5.51	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
4	%	37.50	36.68	34.17	12.50	48.35	47.03	39.58	65.83	77.50	25.00	10.00	25.00	27.50	12.50	42.86	0.00	30.56	45.00	50.35	12.50
	Trans.	4.64	4.65	5.05	2.31	6.22	6.12	5.58	8.09	8.73	3.04	2.12	3.04	4.07	2.31	4.98	0.71	4.27	5.10	6.96	2.31
5	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Trans.	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	3.91	0.71	0.71	0.71	0.71	0.71	0.71	0.71
6	%	0.00	0.00	30.83	0.00	0.00	0.00	16.68	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Trans.	0.71	0.71	5.00	0.71	0.71	0.71	3.17	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
7	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Trans.	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
8	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Trans.	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
9	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.11	0.00	0.00	0.00
	Trans.	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	2.66	0.71	0.71	0.71
10	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.28	0.00
	Trans.	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	3.55	0.71
11	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.00	22.50	0.00	0.00	0.00	47.50	0.00	23.82	26.79	0.00	0.00	13.20	0.00
	Trans.	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
12	%	0.00	0.00	30.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Trans.	0.71	0.71	5.00	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
13	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Trans.	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
14	%	37.50	13.33	4.17	12.50	51.65	0.00	6.25	9.17	0.00	0.00	15.00	0.00	0.00	12.50	8.25	3.57	2.78	7.50	0.00	12.50
	Trans.	4.64	2.94	1.57	2.31	7.00	0.71	1.79	2.52	0.71	0.71	2.47	0.71	0.71	2.31	1.98	1.49	1.38	2.30	0.71	2.31
15	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	21.18	0.00
	Trans.	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	4.17	0.71
16	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Trans.	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
17	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Trans.	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	7.70	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71

Table 2. Continued

Fungal isolates		Sample numbers																			
		21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1	%	0.00	0.00	0.00	0.00	20.09	45.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	50.00	0.00	0.00	0.00	0.00
	Trans.	0.71	0.71	0.71	0.71	3.99	5.91	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	6.24	0.71	0.71	0.71	0.71
2	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.00	0.00	0.00	0.00	3.57	0.00	13.57	25.00	0.00	0.00
	Trans.	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	3.91	0.71	0.71	0.71	1.49	0.71	2.91	3.04	0.71	0.71
3	%	37.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	28.57	0.00	41.68	59.52
	Trans.	5.21	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	3.82	0.71	6.35	6.85
4	%	50.00	14.58	70.83	0.00	0.00	20.83	33.33	0.00	68.75	7.48	25.00	0.00	25.00	12.50	17.86	0.00	22.14	54.17	6.25	0.00
	Trans.	6.30	3.07	7.48	0.71	0.71	3.58	4.31	0.71	8.05	2.31	3.04	0.71	3.91	2.31	2.65	0.71	3.65	6.53	1.79	0.71
5	%	0.00	0.00	0.00	0.00	22.32	0.00	33.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Trans.	0.71	0.71	0.71	0.71	4.18	0.71	5.18	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
6	%	0.00	37.51	0.00	0.00	25.00	0.00	0.00	0.00	0.00	18.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Trans.	0.71	5.46	0.71	0.71	3.91	0.71	0.71	0.71	0.71	3.39	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
7	%	0.00	0.00	0.00	25.00	0.00	0.00	0.00	0.00	0.00	23.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Trans.	0.71	0.71	0.71	3.91	0.71	0.71	0.71	0.71	0.71	4.38	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
8	%	0.00	47.92	0.00	0.00	0.00	0.00	0.00	0.00	31.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.14	0.00	0.00	0.00
	Trans.	0.71	6.68	0.71	0.71	0.71	0.71	0.71	0.71	4.30	0.71	0.71	0.71	0.71	0.71	0.71	0.71	1.88	0.71	0.71	0.71
9	%	0.00	0.00	0.00	37.50	19.64	0.00	0.00	30.71	0.00	0.00	25.00	0.00	37.50	0.00	0.00	50.00	28.57	0.00	25.00	0.00
	Trans.	0.71	0.71	0.71	4.64	4.05	0.71	0.71	4.24	0.71	0.71	3.91	0.71	5.21	0.71	0.71	6.24	3.82	0.71	4.48	0.71
10	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.71	0.00	0.00	0.00	0.00	37.50	75.00	0.00	0.00	0.00	0.00	0.00	0.00
	Trans.	0.71	0.71	0.71	0.71	0.71	0.71	0.71	3.13	0.71	0.71	0.71	0.71	5.21	8.57	0.71	0.71	0.71	0.71	0.71	0.71
11	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	33.75	0.00	16.68	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Trans.	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	5.16	0.71	2.58	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
12	%	0.00	0.00	0.00	0.00	0.00	0.00	33.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	27.08	40.48
	Trans.	0.71	0.71	0.71	0.71	0.71	0.71	5.18	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	5.24	5.49
13	%	0.00	0.00	0.00	0.00	12.95	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Trans.	0.71	0.71	0.71	0.71	3.30	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
14	%	12.50	0.00	4.18	0.00	0.00	0.00	0.00	3.57	0.00	3.13	0.00	8.33	0.00	12.50	3.57	0.00	0.00	20.88	0.00	0.00
	Trans.	2.31	0.71	1.57	0.71	0.71	0.71	0.71	1.49	0.71	1.43	0.71	1.98	0.71	2.31	1.49	0.71	0.71	4.03	0.71	0.71
15	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Trans.	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
16	%	0.00	0.00	0.00	37.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Trans.	0.71	0.71	0.71	5.51	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
17	%	0.00	0.00	0.00	0.00	0.00	33.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Trans.	0.71	0.71	0.71	0.71	0.71	4.31	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71

Table 3. Correlation among frequencies of fungi isolated from cashew nuts collected from different location in Riyadh city

Fungi	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	1.000	-0.114	-0.102	-0.252	0.150	0.073	-0.064	-0.069	0.375*	-0.082	-0.129	-0.093	0.372*	-0.207	-0.045	-0.045	0.245
2		1.000	-0.046	0.037	-0.116	-0.150	-0.094	-0.042	0.089	-0.122	-0.036	-0.137	-0.066	-0.085	-0.066	-0.066	-0.090
3			1.000	-0.080	-0.104	0.017	-0.084	-0.040	0.106	-0.109	-0.171	0.494**	-0.059	-0.126	-0.059	-0.059	-0.080
4				1.000	-0.089	-0.076	-0.228	0.104	-0.420**	-0.077	0.135	-0.137	-0.220	0.229	0.219	-0.220	-0.059
5					1.000	0.133	-0.064	-0.070	0.039	-0.084	0.206	0.278	0.517**	-0.209	-0.045	-0.045	-0.061
6						1.000	0.191	0.458**	-0.047	-0.108	0.002	0.163	0.371*	-0.135	-0.058	-0.058	-0.079
7							1.000	-0.057	0.145	-0.068	0.258	-0.076	-0.037	-0.101	-0.037	0.646**	-0.050
8								1.000	-0.075	-0.073	0.115	-0.082	-0.040	-0.184	-0.040	-0.040	-0.054
9									1.000	0.152	0.240	0.026	0.254	-0.335*	-0.083	0.314*	-0.113
10										1.000	0.054	-0.099	-0.048	-0.030	0.259	-0.048	-0.065
11											1.000	-0.155	-0.074	-0.112	0.207	-0.074	-0.101
12												1.000	-0.053	-0.194	-0.053	-0.053	-0.073
13													1.000	-0.119	-0.026	-0.026	-0.035
14														1.000	-0.119	-0.119	-0.162
15															1.000	-0.026	-0.035
16																1.000	-0.035
17																	1.000

Table 4. Aflatrem, maltoryzine and sterigmatocystin productivity of *A. flavus*

A. flavus isolates	Mycotoxins (ppm)		
	Aflatrem	Maltoryzine	Sterigmatocystin
A. f. 1	00.00	3.00	450.00
A. f. 2	00.00	00.00	00.00
A. f. 3	2.00	10.00	525.00
A. f. 4	2.00	9.00	325.00
A. f. 5	00.00	00.00	00.00

Table 5. Production of oxalic acid by *A. niger* isolates

A. niger isolates	Oxalic acid (mg/ml)
A. n. 1	625.00
A. n. 2	200.00
A. n. 3	425.00
A. n. 4	00.00
A. n. 5	550.00

The analysis of variance of the fungal isolation frequency (Table 1) revealed that fungus (F), sample (S) and the (F x S) interactions were all highly significant sources of variation in the fungal isolation frequencies. The fungus was the first in importance as a source of variation in isolation frequency, while F x S interaction was the second important topic.

The significance of fungus × sample interaction indicates that the isolation frequencies are different according to the samples source. For example, an equal isolation frequencies of *A. niger* and *R. stolonifer* from samples No. 1 and No. 4 was shown, but the later was significantly less frequent in sample No. 2 and No. 3 than the former. Although, *A. niger* and *A. terreus* showed an equal isolation frequencies from sample No. 27, *A. terreus* was isolated from sample No. 25 but *A. niger* not found. While, *A. niger* showed varied isolation frequencies from samples No. 31 & 38, *A. flavus* was isolated in an equal frequency, from both samples with highly significant difference. On the other hand *F. semitectum* was significantly more frequent in sample No. 39 than the *A. niger* and the *vice versa* in sample No. 17 (Table 2).

Correlation analysis showed that some of the isolated fungi were positively and/or negatively correlated with the others. Highly significant positive correlation was found among

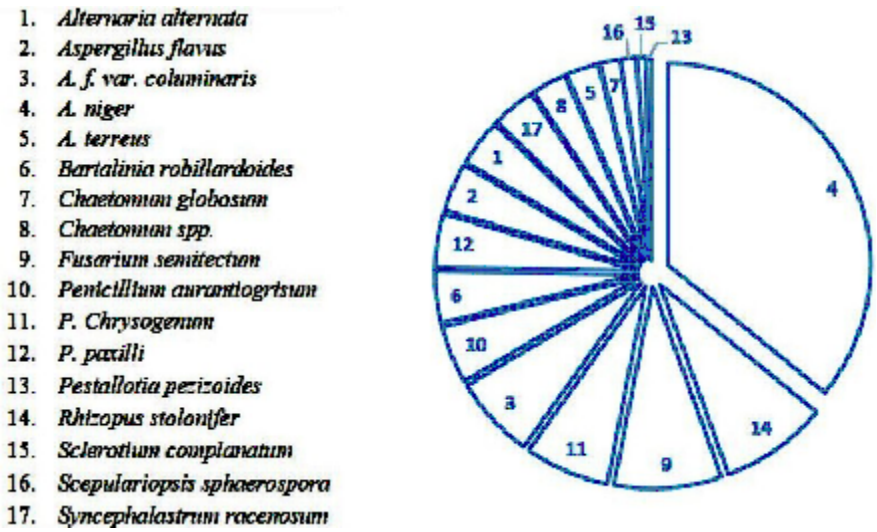

Fig. 1. Frequencies' of fungal species isolated from cashew samples

some of the fungal species such as: among *Aspergillus* f.var. *columinaris* and *Penicillium paxilli* as well as among *A. terreus* and *P. pezizoides.* Meanwhile, highly significant negative correlation was among *A. niger* and *F. semitectum*. Although, *F. semitectum* exhibited significant negative correlation with *R. stolonifer* it was positively correlated with *A. alternate* (Table 3).

The phenogram (Figure 2) based on average linkage cluster analysis of fungal isolation frequencies (%); showed two distinct groups of the isolated fungi**.** The first group included 12 fungal species (64.7%). *A. flavus*, *A. niger, P. aurantiogrisum, R. stolonifer* and *S. complanatum* were separated in the second group. Different degrees of fungal species association between and/ or within groups could be found.

In respect of mycotoxin analyses; most of the *A. flavus* isolates were toxigenic and capable of producing maltoryzine (3-10ppb) and sterigmatocystin (325-525ppb) in the culture media. Meanwhile, 40% of the tested *A. flavus* isolates were aflatrem (2ppb) producers (Table 4).

Regarding of the oxalic acid production; it was found that 80% of the tested *A. niger* isolates were capable of producing oxalic acid in the culture media. The oxalic acid production ability was ranged from 200 to 625 mg/ml (Table 5).

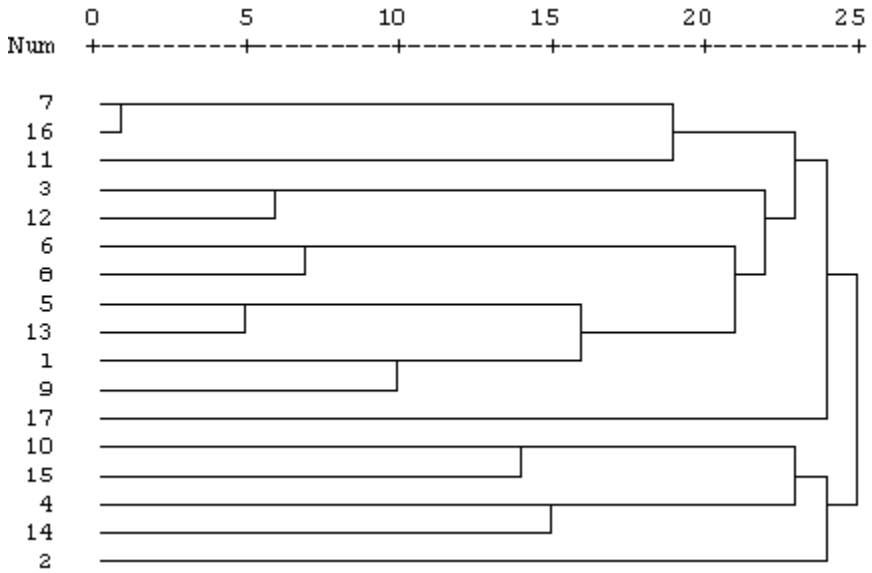

Fig. 2. Phenogram based on average linkage cluster analysis of frequencies of fungi recovered from cashew samples

DISCUSSION

Many fungal contaminants belong to different genera were obtained in this survey with the predominance of *A. niger*. The presence of such fungi could be attributed to the high nutrition value of cashew nut as well as inappropriate marketing and storage conditions[4,6]. This findings was agreed with the reported data[7,8,23].

The analysis of variance exhibited the significance of fungus × sample interaction indicating different isolation frequencies of recovered fungi according to the samples source[24]. Meanwhile, some of the isolated fungi in this study exhibited highly significant positive correlation with each other, implying similar colonization conditions of cashew and/or the possibility of synergism in colonizing the cashew by such fungi[25]. On the other hand, the negative correlation may reflect an antagonism or competitive exclusion between such fungi[10].

The cluster pattern of fungal distribution over samples, suggested that fungi were associated strongly and positively within each group, whereas, they were associated weakly or negatively between groups. Implying the potential existence of sample (environment) related fungi[20,25].

The secretion of different metabolic toxic

compounds by *Aspergillus* spp. in the culture media had frequently been reported[9,10,26]. The production of myco-toxic compounds; aflatrem, maltoryzine and sterigmatocystin by *A. flavus* isolated from nuts as well as the other agricultural products had also frequently been documented[20,25]. The most predominant and the most distributed fungus in the cashew samples; *A. niger* was also capable to produce toxic oxalic acid in the culture media[20,25,27]. The quality of nuts will be subsequently affected by those myco-contaminants[13,14] and they will become risky for human consumers[16,28].

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