

Fungal populations and mycotoxins in silage in Assiut and Sohag governorates in Egypt, with a special reference to characteristic *Aspergilli* toxins

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Abstract

Forty silage samples were collected from Assiut and Sohag governorates in Egypt to measure the presence of fungal population in silage. Forty-three species and 2 species varieties belonging to 17 genera were isolated using glucose Czapek's and Sabouraud's dextrose agar media at 28 °C. The most prevalent genera were *Aspergillus* (57.5 and 100% of the samples), *Penicillium* (100 and 55%) on the two mentioned media, respectively. Also, *Fusarium oxysporum* and *Gibberella fujikurori* were recovered in moderate incidences. Mycotoxin profiles were also determined in these samples: Aflatoxins showed the highest incidence rates of occurrence, it occurred in 22.5% of all samples analyzed. Other mycotoxins were detected from all samples (T_2 toxins and sterigmatocystin at incidence of 7.5 and 5%, respectively). The screening of the characteristics mycotoxins of different isolates of *Aspergillus* isolated from silage samples was tested. The results clarified that some mycotoxins (aflatoxins – aspergillic acid – beta nitro propionic acid – cyclopiazonic acid – kojic acid and sterigmatocystin) were produced by some isolates of *A. flavus*. Some isolates of *A. fumigatus* could produce gliotoxin and verrucoligen. All of *A. niger* isolates tested were able to produce kojic acid. One isolate of *A. ochraceous* formed ochratoxin A and other isolate produced penicillic acid. Concerning *A. terreus* isolates, the results showed that 5 isolates were able to produce citrinin and 4 isolates had ability to produce patulin. *A. versicolor* isolates showed the ability to produce ochratoxin A.

Key words: *Aspergilli* toxins, silage mycoflora, silage mycotoxins

Introduction

A central issue in the field of feed quality and storage is the problem of mold spoilage. Fungal growth reduces nutritional value and may result in the production of mycotoxins and allergenic spores [4]. Most mold species that regularly have been isolated from silage belong to the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Mucor*, *Absidia*, *Monoascus*, *Scopulariopsis* and *Trichoderma* [13, 28].

Mycotoxins are highly toxic secondary products of the metabolism of some fungi mainly belonging to *Aspergillus*, *Penicillium*, *Fusarium*

and other genera, especially when foods are considered [3, 13, 35, 41]. Toxic syndromes caused by mycotoxin ingestion by humans and animals are indicated as mycotoxicosis [14]. It has been estimated that at least 300 fungal metabolites are potentially toxic to human and animal, and it can be realistically assumed that other mycotoxins are likely to be discovered. It is reported that as much as 25% of the world's cereals are contaminated with known mycotoxins [11]. Mycotoxins can be present in processed food made from mycotoxins-contaminated materials and they can also occur in milk, meat and eggs when the feeding animals ingest these mycotoxin. Mycotoxins may exit in

food products as the parent compound or they may be changed to another toxic metabolite in the animals. Mycotoxins' chemical, biological, and toxicological properties are diverse [14]. Depending on the type and amount of toxins present in the silage, health problems can range from minor digestive upset, small fertility problems and reduced immune function to serious liver and kidney damage and abortions [37].

There is still uncertainty under which conditions mycotoxins are formed in silage. Heavily infested silage does not necessarily contain high quantity and quality of mycotoxins [28]. Mycotoxins affect more than one system simultaneously and, therefore, produce a multiplicity of responses by the affected animal resulting in increased difficulty of attributing the response to a simple body system.

This study was designed to determine the natural occurrence as well as distribution of mold and mycotoxins in 40 local silage samples. The potential of different *Aspergillus* isolates for mycotoxins production was also recorded.

Materials and methods

Collection of samples

Forty samples of silage (500 g each) were collected from farms, mills and retail markets at Assuit and Sohag governorates (20 samples each). The samples were placed in a double sterile polyethylene bags (to minimize the loss of water content and provide sufficient aeration), sealed, transferred immediately to the laboratory, kept in cool place (5 °C) until mycological and mycotoxins analysis. The moisture content of the samples was directly determined by dry weight method.

Estimation of fungi

The dilution plate method by Johnson and Curl [17] was employed for isolation of fungi. Glucose-Czapek's supplemented with chloramphenicol (20 µg/ml) and rose Bengal (30 ppm) as bacteriostatic agent and Sabouraud's dextrose agar supplemented with chloramphenicol (20 µg/ml) and cycloheximide (0.5 g/l) for isolation of saprophytic, pathogenic and cycloheximide resistant fungi were used.

Ten plates for each sample (five plates for each medium) were used. The plates were incubated at 28 °C for 1–2 weeks and the developing fungi were counted and identified according to macro and microscopic characteristics [23, 29, 31, 35].

Extraction of mycotoxins from silage samples

Fifty gram of each sample were homogenized for 5 min in a high-speed blender (16,000 rpm) with 100 ml chloroform. The chloroform extract was decanted off and the content re-extracted by another 100 ml chloroform. The chloroform extracts were combined, washed with an equal volume of distilled water, dried over anhydrous sodium sulfate, filtered then concentrated under vacuum and the dry material was transferred to a dram vial with a small amount of chloroform, which was evaporated to near dryness and cleaned up [44].

Detection of mycotoxins

Thin layer chromatographic technique of the clean extract was done on precoated silica gel Plate type 60 F₂₅₄ (Merck) for the presence of mycotoxins according to standard procedures described by Roberts and Patterson [33] and Samson et al. [35].

Verification of different recorded mycotoxins

A simple confirmation methods of recorded mycotoxins on precoated silica gel plates were described by Dos Santos [12], Larg, [20], Samson et al. [35] and Wyllie and Morehouse [43]. The TLC plates commonly used are normal phase silica gel plates. For some acidic metabolites like cyclopiazonic acid, citrinin, luteoskyrin etc... it can be useful to impregnate the plate with oxalic acid. This is simply done by dipping the plate in an 8% solution of oxalic acid in water or methanol followed by air drying.

TLC – conditions [35]

After application the TLC-plate, any suitable TLC-procedure can be performed. We have found the following solvents (saturated conditions) very useful:

TEF: Toluene/Ethyl Acetate/Formic acid (90%)
5:4:1

CAP: Chloroform/Acetone/Iso propanol 85:15:20

CM: Chloroform/Methanol 97:3.

After elution and air drying in a dark fume hood, the TLC-plates are examined in visible light (VIS), long wave UV-light (UV-366) and short wave UV-light (UV-254). Some metabolites are treated with ½ minute in UV-254 followed by UV-366.

The following spray reagents are useful for visualizing and verification of secondary metabolites:

- Sp1: 0.5% *p*-anisaldehyde in ethanol/acetic acid/conc. sulphuric acid 17:2:1 (most metabolites).
- Sp2: 50% sulphuric acid in water (e.g. aflatoxin – B₁ & B₂; verruculogen; viridicatin; cyclopi-azonic; streigmatocystin; T2 toxin).
- Sp3: FeCl₃ in butanol and heating for 5 min. at 130 °C (e.g. Aspergillilic acid; kojic acid; penicillilic acid; citrinin; verruculogen).
- Sp4: 20% AlCl₃ in 60% ethanol and heating for 5 min. at 130 °C (e.g. penitrem A; trichothecenes B; streigmatocystin; gliotoxin; T2 toxin).
- Sp5: NH₃ vapors in 1–3 min (mycophenolic acid; xanhomegnin; viomellin, penicillilic acid; ochratoxin A; kojic acid; citrinin; patulin).

Culture of Aspergillus isolates for toxins production

Selective 45 isolates of *Aspergillus* species collected from the current study were examined. The tested isolates were represented by six species as the following: *A. flavus* (15 isolates), *A. fumigatus* (12 isolates), *A. niger* (4 isolates), *A. ochraceus* (2 isolates), *A. terreus* (9 isolates), and *A. versicolor* (3 isolates) as in Table 3. Inoculate were prepared from 7-days old culture of each isolates on PDA slope as spore suspensions in 0.2% (v/v) aqueous tween 80. Isolates were inoculated into 250 ml Erlenmeyer flasks each containing 50 ml Czapek's liquid medium supplemented with 0.2% yeast extract and 1.0% peptone and incubated at 28 °C for 10 days as static culture (PYCZ).

Extraction of Aspergillus toxins

After incubation, the control of each flask (medium + mycelium) was homogenized for 5 min in a

high-speed blender with 100 ml chloroform. The extracts procedure was repeated three times. The chloroform extracts were combined, washed, dried, filtered, and concentrated to near dryness, cleaned and mycotoxins detected as previously described [6, 12, 13, 26, 35].

Results and discussions

The results in Table 1 clearly show that silage samples were highly contaminated with molds representing several genera and species. The total viable counts of mold in all silage samples were 131,600 and 9920 colonies/gm dry silage on each of glucose – Czapek's and Sabouraud's dextrose agar media, respectively.

The levels of the different fungal population encountered in the current study were almost in agreement to those observed in corn silage and corn grains by Alexandrov [2], Gonzalez et al. [15], Krustov and Khristov [18] and Mor and Singh [22].

Forty-three species and 2 species varieties belonging to 17 genera were identified from the silage samples on both glucose – Czapek's and Sabouraud's dextrose agar media at 25 °C (Table 1). The previous finding can be correlated with the results obtained by Adebajo et al. [1] could isolate 31 fungal species belonging to 11 genera from corn samples.

By examination of silage samples for the presence of fungi, it has been found that *Aspergillus*, *Penicillium* and *Mucor* were the most prevalent genera on the two isolation media used. These results were similar to a great extent to results obtained by Krustov and Khristov [18]. They studied microflora in corn silage and reported that the genera *Mucor*, *Aspergillus* and *Penicillium* were the most prevalent. Same findings were obtained by Adebajo et al. [1], Alexandrov [2], Oudeelinkerink et al. [28] and Schneeweis et al. [36]. They demonstrated that the majority of molds isolated from corn grains and silage are related to genera *Aspergillus*, *Penicillium*, *Mucor* and *Fusarium*.

Aspergillus was the most dominant genus encountering in 57.5 and 100% of samples, constituting 8.8 and 72.3% of total fungi on Sabouraud's and glucose-Czapek's agar media, respectively (Table 1). It was represented by 9 species and 2 varieties of which *A. flavus*, *A. flavus*

Table 1. Total counts (TC per mg), number of cases of isolation (NCI, out of 40 samples) and occurrence remarks (OR) of fungal genera and species recovered from silage samples at 28 °C

Genera and species	Sabouraud's dextrose agar			Glucose –Czapek's agar		
	TC	NCI	OR	TC	NCI	OR
<i>Absida corymbifera</i>	–	–	–	4700	19	M
<i>Acremonium strictum</i>	240	9	L	–	–	–
<i>Aspergillus</i>	880	23	H	95100	40	H
<i>A. candidus</i>	30	1	R	–	–	–
<i>A. flavus</i>	340	10	M	64900	40	H
<i>A. flavus</i> var. <i>columnaris</i>	–	–	–	14100	33	H
<i>A. fumigatus</i>	60	4	R	22700	30	H
<i>A. melles</i>	20	2	R	–	–	–
<i>A. niger</i>	40	4	R	3100	15	M
<i>A. ochraceous</i>	60	4	R	–	–	–
<i>A. sydowii</i>	110	5	L	1200	7	L
<i>A. terreus</i>	190	7	L	3500	12	M
<i>A. terreus</i> var. <i>africans</i>	30	2	R	–	–	–
<i>A. versicolor</i>	–	–	–	200	2	R
<i>Chrysosporium</i>	70	4	R	–	–	–
<i>C. keratinophilum</i>	10	1	R	–	–	–
<i>C. tropicum</i>	60	3	R	–	–	–
<i>Cladosporium cladosporioides</i>	70	5	L	–	–	–
<i>Emericella nidulans</i>	–	–	–	100	1	R
<i>Eupencillium</i>	170	8	L	–	–	–
<i>E. aluticum</i>	140	6	L	–	–	–
<i>E. javanicum</i>	30	2	R	–	–	–
<i>Fusarium oxysporum</i>	–	–	–	4600	12	M
<i>Geotricum candidum</i>	140	12	M	–	–	–
<i>Gibberella fujikuroi</i>	–	–	–	2200	6	L
<i>Mucor</i>	210	11	M	8500	29	H
<i>M. circinelloides</i>	50	2	R	700	5	L
<i>M. hiemalis</i>	160	10	M	7800	29	H
<i>Penicillium</i>	7120	40	H	9500	22	H
<i>P. brevicomactum</i>	30	2	R	300	3	R
<i>P. canescens</i>	450	15	M	–	–	–
<i>P. chrysogenum</i>	1530	28	H	2100	9	L
<i>P. citrinum</i>	290	10	M	500	1	R
<i>P. corylophilum</i>	740	18	M	5000	10	M
<i>P. duclauxii</i>	630	23	H	100	1	R
<i>P. funiculosum</i>	280	17	M	400	3	R
<i>P. glabrum</i>	10	1	R	–	–	–
<i>P. islandicum</i>	220	11	M	–	–	–
<i>P. italicum</i>	30	1	R	–	–	–
<i>P. janczewskii</i>	2280	25	H	100	1	R
<i>P. oxalicum</i>	20	1	R	1000	4	R
<i>P. rubrum</i>	270	15	M	–	–	–
<i>P. variable</i>	340	11	M	–	–	–
<i>Rhizopus stolonifer</i>	10	1	R	100	1	R
<i>Scopulariopsis</i>	90	6	L	5900	12	M
<i>S. brevicaulis</i>	–	–	–	5900	12	M
<i>S. candidus</i>	90	6	L	–	–	–
<i>Syncephalastrum racemosum</i>	190	8	L	900	5	L
<i>Talaromyces</i>	10	1	R	–	–	–
<i>Tricophyton rubrum</i>	30	1	R	–	–	–
Yeast	690	20	H	–	–	–
Gross total count	9920			131600		
No. of genera	17			10		
	41 + 2			24 + 1		
No. of species	var.			var.		

var. *columnaris*, *A. fumigatus*, *A. niger*, and *A. terreus* were the most prevalent species. These results were in agreement with the finding observed by Mor and Singh [22] and Ogundaro [27]. They reported that *A. flavus*, *A. niger*, *A. fumigatus* and *A. terreus* were the most prevalent on cereal grains and other animal feed silage. The remaining *Aspergillus* species showed lowest incidence (Table 1).

The second highest incidence rate was represented by genus *Penicillium*. It was recovered from 55 and 100% of silage samples, constituting 7.2 and 71.8% of total fungi on glucose-Czapek's and Sabouraud's dextrose agar media, respectively. Most of *Penicillium* species were prevalent on Sabouraud's agar medium and with rare incidence or completely absent on glucose medium. From the genus 14 species were identified of which *P. chrysogenum* (70% of samples), *P. duclauxii* (57.5) and *P. janczewskii* (62.5) were the most prevalent. *P. corylophilum* was recovered in moderate frequency of occurrence in both isolation media used. *P. canescens*, *P. citrinum*, *P. funiculosum*, *P. islandicum*, *P. rubrum*, and *P. variable* were recovered in moderate frequency of occurrence. They ranged from 27.5–42.5% of the samples on Sabouraud's dextrose agar medium. The remaining *Penicillium* species were isolated with low or rare frequencies of occurrence (Table 1). Gonzalez et al. [15] and Skrinjar et al. [38] reported that *Penicillium* spp. were the most prevalent in mycopopulation isolated from corn silage. Also, most of these *Penicillium* species were recovered on corn grain or silage by many workers [4, 28, 30].

Mucor was also common genus, recovered from 27.5 and 72.5% of all samples, constituting, 2.1 and 6.45% of total fungi on Sabouraud's and glucose-Czapek's agar media, respectively. Two species were collected of which *M. hiemalis* was the most prevalent species while *M. circinelloides* was less frequent (Table 1). Krustov and Khristov [18] reported that the genus *Mucor* was the most abundant genus in corn silage. This genus was also observed with a variable frequencies and population from corn grain and corn silage by Alexandarov [2], Gonzalez et al. [15] and Oudeelferink et al. [28].

Absidia corymbiflora, *Fusarium oxysporum*, *Geotrichum candidum* and *Scopulariopsis brevicaulis* were detected as single representatives of genera on one or the two isolation media. They occurred in

moderate or low incidences (Table 2). Almost similar results were reported by Baath et al. [3]. They isolated several fungal species from corn silage and reported that *Fusarium* spp. were the most frequently occurring (more than 85% of all samples). These moulds are nearly similar of those reviewed by Gonzalez et al. [15], L'vova et al. [21] and Pozzi et al. [30].

A total of 960 yeast colonies/40 g dry samples were isolated on Sabouraud's dextrose agar medium at 25 °C. It was recovered from 50% of all examined samples, matching, 6.7% of total fungi. Jespersen et al. [16] could isolate a mixed flora comprising *Candida*, *Saccharomyces*, *Trichosporon*, *Khuyveromyces* and *Debaryomyces* species from raw maize. Oudeelferink et al. [28] reported that yeast population in silage can reach up to 18 CFU/g during the first weeks of ensiling, prolonged storage.

The remaining genera and species were remarked in low or rare frequencies of occurrence on one and/or two isolation media (Table 1). Same species were regarded in corn grain and silage by many researchers all over the world [2, 15, 18, 28, 36].

Natural occurrence of mycotoxins

All 40 silage samples were tested for naturally mycotoxin contaminations and showed that 65% of samples were mycotoxin-free (Table 2). Also, results revealed that 35% of the tested samples (14 out of 40) were contaminated by one of the three following toxins: aflatoxins, sterigmatocystin and T2 toxin. Eight samples were contaminated by aflatoxins B₁ and B₂ while, aflatoxins B₁, B₂, G₁ and G₂ were recovered in the extract of one sample only (No. 35) as in Table 2. Romo and Fernandez [34] recorded that 13% of *A. flavus* group isolated from commercial poultry mixed feeds showed the ability to produce aflatoxin B₁ & B₂. Zohri and Sabah Saber [44] recorded that two out of four types of poultry feedstuffs and one out of three type of their ingredients were polluted by aflatoxins B₁, B₂, G₁ & G₂. CAST [7] recorded that aflatoxin risks in plant, animal and human system.

Two samples (No. 24 and 31) were naturally contaminated by sterigmatocystin. It causes hepatocarcinogen liver and teratogenic effect. Sterigmatocystin is produced by *A. chevalieri*, *A. flavus*, *A. amstelodami*, *A. nidulans*, *A. rubber*,

Table 2. Moisture content and natural occurrence of mycotoxins recorded in the different silage samples

No. of samples	Source of samples	Moisture content	Natural mycotoxin	No. of samples	Source of samples	Moisture content	Natural mycotoxin
1	Farm	11.23	–ve	21	Retail market	10.81	–ve
2	Farm	11.81	Aflatoxin B ₁ & B ₂	22	Retail market	13.01	Aflatoxin B ₁ & B ₂
3	Farm	12.05	Aflatoxin B ₁ & B ₂	23	Retail market	11.02	–ve
4	Farm	9.94	–ve	24	Retail market	11.9	Sterigmatocystin
5	Forage mill	11.19	–ve	25	Retail market	12.71	T2 toxin
6	Forage mill	10.88	–ve	26	Forage mill	9.97	–ve
7	Forage mill	9.02	–ve	27	Retail market	12.13	T2 toxin
8	Forage mill	10.53	–ve	28	Retail market	11.75	T2 toxin
9	Forage mill	9.71	–ve	29	Forage mill	10.14	–ve
10	Forage mill	10.11	–ve	30	Forage mill	11.08	–ve
11	Retail market	10.85	–ve	31	Retail market	10.95	Sterigmatocystin
12	Retail market	12.44	Aflatoxin B ₁ & B ₂	32	Retail market	11.63	Aflatoxin B ₁ & B ₂
13	Retail market	9.83	–ve	33	Retail market	11.96	Aflatoxin B ₁ & B ₂
14	Retail market	11.87	Aflatoxin B ₁ & B ₂	34	Farm	10.15	–ve
15	Retail market	10.00	–ve	35	Farm	12.91	Aflatoxin B ₁ , B ₂ , G ₁ & G ₂
16	Retail market	9.94	–ve	36	Farm	10.17	–ve
17	Forage mill	10.65	–ve	37	Farm	11.07	–ve
18	Forage mill	10.87	–ve	38	Farm	9.92	–ve
19	Retail market	12.11	Aflatoxin B ₁ & B ₂	39	Farm	10.21	–ve
20	Farm	10.39	–ve	40	Retail market	10.23	–ve

A. parasiticus, and *A. versicolor* and *P. leutum*. Table 1 shows the presence of some these species on silage samples tested [13, 35, 43].

Three samples (No. 25, 27 and 28) were contaminated by T2 toxin. This toxin was formed as a result of the presence of some species of *Fusarium*, *Stachybotrys*, *Trichoderma*, *Myrothecium* and others [41]. T2 toxin causing hemorrhagic syndrome and have pathological effect on human (emetic, hemorrhage dermal toxin, dermonecrotic, immunologic effect and alimentary tract) and have risks in plant and animal system [7]. USDA [41] recorded that poultry has the highest resistance to the these T2 toxins. Cattle, sheep, and goats have some level of resistance due to their multiple digestive processes.

A determination of the mean moisture contents of the silage sampled gave a range of 9–13%. This is probably explain the high incidence of storage fungi in silage. Ogundaro [27] found that the moisture contents of the grain samples ranged between 16 and 21%. Tables 1 and 2 shows that all the samples which have moisture content ranged from 11.8 to 13.1% were contaminated by *Aspergillus* and *Fusarium* sp. and

contaminated by mycotoxins. The relationship between moisture content, fungal population and mycotoxin production was previously described by Bullerman et al. [5], recorded that storage fungi can grow at moisture level in the range of 13–18%. Toxic species have been observed in most of the genera associated with cereal grains, but particularly with *Aspergillus*, *Penicillium* and *Fusarium*. The species capable to producing the mycotoxins presently of greatest concern. Hence most foods with moisture contents above 13% are susceptible to growth of toxic molds and potential mycotoxin formation. FAO [13] recorded that the maximum acceptable level for storage for 13 different kinds of grains and commodities are ranged from 5 to 13.5. Waniska [42] recorded that aflatoxins is not found at significant levels in the field but does appear on improperly stored high moisture content.

Aspergillus toxins production

From Table 1, results clarified that *Aspergillus* was the most dominant genus on all silage samples. So the *Aspergilli* isolates were chosen for testing their

abilities to produce characteristic mycotoxins using PYCZ liquid medium and the results in Table 3 showed that:-

The 45 *Aspergillus* isolates were extracted and examined for production of different *Aspergillus* toxins. The results in Table 3 revealed that all isolates of *A. flavus* (represented by 15 isolates) were tested. Seven isolates were aflatoxins producers (four isolates produced aflatoxins B₁, B₂, G₁ and G₂ and three isolates produced aflatoxins B₁ and G₁ only). These results were in agreement with the finding observed by Davis et al. [10] and Pitt and Hocking [29]. Davis et al. [10] recorded that the aflatoxins B₁ and G₁ are produced by *A. flavus* on a semisynthetic medium. Nine isolates produced cyclopiazonic acid; while kojic acid was produced by two isolates only. Each of aspergillic acid, beta nitropropionic acid and sterigmatocystin was recorded in the extract of one isolate only.

Twelve isolates of *A. fumigatus* were examined and the results showed that 4 isolates could produce gliotoxin and 6 isolates were verrucologen producers (Table 3). Gliotoxin showed a number of antibiotic effects, also has immunosuppressive capabilities in neurotoxic. Dos Santos et al. [12] examined the activity of 60 isolates of *A. fumigatus* isolated from soils for toxin productions. They recorded that 27 isolates were toxigenic and only (11.1%) of the isolates able to produce gliotoxin. Several fungal species can produce gliotoxin including the pathogenic fungus *A. fumigatus*,

especially at high moisture content above 15% [19, 26, 32]. Nenehed et al. [25] remarked the ability of *A. fumigatus* isolated from maize and commercial feed grains to produce gliotoxins.

Verruculogen is the most potent termorgenic mycotoxins known and the metabolism of verruculogen in animals (rats and sheep) have indicated that it is metabolized in the liver to more polar products, which are exerted into the bile. The toxin affects the central nervous system and causing changes in the amino acid neurotransmitter release mechanisms. The toxins are produced by *A. fumigatus* [19] with at least four different termorgenic toxins (fumitermogens A, B, C & D).

Four isolates of *A. niger* were tested and the results revealed that all isolates tested had the ability to produce kojic acid (Table 3). Kojic acid was previously recorded as a neurotoxin for human but also have antimicrobial properties [43]. *A. ochraceus* was represented by two isolates and their extracts proved that only one isolate could produce penicillic acid and the other one had the ability to produce ochratoxin A (Table 3). These toxins were the most common toxins produced by *A. ochraceus* and causing liver and kidney cancer. Penicillic acid was recorded as antimicrobial agents and causing rat's cancer [35, 43].

The extract of only one out of the three isolates tested of *A. versicolor* was able to produce ochratoxin A (OTA) as in Table 3. Many fungal species capable to produce OTA such as *A. ochraceus*, members of *A. glaucus* group, *A. versicolor*,

Table 3. Mycotoxins produced by selected isolates (45) of *Aspergillus* species

Species	No of isolates tested	Characteristic mycotoxins	No. of producing isolates
<i>Aspergillus flavus</i>	15	Aflatoxin B ₁ , B ₂ , G ₁ & G ₂	4
		Aflatoxin B ₁ & G ₁	3
		Aspergillic acid	1
		Beta nitropropionic acid	1
		Cyclopiazonic acid	9
		Kojic acid	2
		Sterigmatocystin	1
		Gliotoxin	4
<i>A. fumigatus</i>	12	Verrucologen	6
<i>A. niger</i>	4	Kojic acid	4
<i>A. ochraceus</i>	2	Ochratoxin A	1
		Penicillic acid	1
<i>A. terreus</i>	9	Citrinin	5
		Patulin	4
<i>A. versicolor</i>	3	Ochratoxin A	1

P. viridicatum, *P. chrysogenum*, *P. verrucosum* var *cyclopium* and *P. verrucosum* var *verrucosum* [8, 38, 39]. Dalcero [9] remarked the production of OTA by members of *A. niger* group using HPLC. Ochratoxin A caused nephrotoxicity, carcinogenic effect on liver and kidney, teratogenic, immunologic and hematopoietic effect [35, 43]. *A. terreus* was represented by nine isolates were tested: five of them were able to produce citrinin and four isolates could to produce patulin (Table 3). Citrinin caused nephrotoxin and immunologic effect, while patulin having immunologic, carcinogenic effect and effect on nervous system [35, 43].

The production of flavutoxin and fumigaclavines by *A. flavus*; fumitoxin; fumitermogen (A&B) and trp-ploquiralins by *A. fumigatus*; malformin by *A. niger*; secalonic acid; viomellin; vioxanthin and xanthomegnin by *A. ochraceus*; citreoviridin by *A. terreus* and sterigmatocystin and nidulotoxin by *A. versicolor* were examined and not detected in the extract of the tested isolates. All these mycotoxins were previously observed by Baath et al. [3], CABI Bioscience [6], CAST [7], FAO [13], Samson et al. [35], Tuomi et al. [40], USDA [41] and Wyllie and Morehouse, [43].

Conclusion

This study recorded that all silage samples tested were highly contaminated by fungi and 35% of them were naturally contaminated by one of three mycotoxins (aflatoxins, sterigmatocystin and T2 toxin). Also, numerous *Aspergillus* species isolated from this substrate were able to produce the characteristic mycotoxins in synthetic medium. The presence of fungal spores in silage under improper storage conditions, the small inoculums can increase rapidly, leading to significant problems [24]. The importance of the presence of these fungal spores is act as a source of many dangerous mycotoxins, which in small amount can be harmful to animal and human health. Once formed, mycotoxins are stable and may remain in feeds long after fungus has died. Hence, silage must be examined for the presence of mycoflora and mycotoxins because of the animals and human health concerns. Also, precautions must be adopted during productions and storage silage to avoid their contamination by mycoflora and mycotoxins.

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