Original Article

Occurrence of Toxinogenic Fungi in Livestock Feeds in Eastern Algeria

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DOI: 10.47750/pnr.2023.14.S02.293

Abstract

Background: The ubiquitous nature of molds, their ability to colonize diverse substrates, and the lack of effective control measures have contributed to the high incidences of molds and mycotoxin contamination in foods and feeds. These dangerous secondary metabolites enter the food chain in the field, during storage, or later.

Material and Methods: A total of 40 livestock feed samples were randomly collected from ten farms in eastern Algeria. The ten types of analyzed feed were: wheat bran, dried bread, alfalfa, straw, oat hay, barley, date scraps, grass silage, corn silage, and concentrate.

Results and Discussion: Results show that all the samples were contaminated with different species of fungi. A total of 248 fungal strains were isolated. In terms of frequency, the genus Aspergillus was the most frequent fungi recovered from 32 samples (80%) between all feeds studied, followed by the genus Penicillium recovered from 31 samples (77.5%).

Conclusion: As a preventive measure, appropriate agricultural practices could provide unfavorable conditions for the development of fungi and toxinogenesis. However, the installation of a mycotoxins-monitoring program may be necessary to protect animals' and consumers' health.

Keywords: Animal Health; Monitoring Program; Mycotoxins; Public Health; Toxinogenic Molds

INTRODUCTION

Besides the beneficial effects of molds, these microorganisms can damage foods, feeds, and other agricultural commodities, leading to postharvest losses [1]. It not only brings about great economic losses but also represents a high risk for human and animal health through the synthesis of mycotoxin [2]. 30 to 40 % of existing molds can elaborate mycotoxins under favorable conditions [3]. The majority of the toxic species belong to Five kinds of fungi, called toxigenic. These are the genera: Aspergillus, Penicillium, Fusarium, Alternaria, and Claviceps. Animal feed products are at risk of mold damage through pre- or post-harvesting stages of agriculture. Food products with high fungal contamination and higher humidity rates are susceptible to early spoilage if inappropriately stocked [4]. Unfortunately, there are no direct measures for the prevention of infection of animal feeds with fungi. Fungal identification is very important to provide information about which mycotoxins could be present [5]. Because of the practical absence of data about the fungi involved in the deterioration and the production of mycotoxins in the most important livestock feeds, it is important to identify the species of fungi in feeds with special emphasis on mycotoxigenic species, which pose a potential risk to human and animal health [6]. Therefore, this study aimed to isolate and identify the most important species of fungi, in cow feeds in an extensive region of the main cattle breeding area in the east of Algeria, to determine the frequency of mycoflora and their relative density, and to compare the mycoflora at different husbandries as well as different seasons.

MATERIAL AND METHODS

Feed Samples Collection

A total of 40 livestock feed samples were randomly taken, respecting sampling precautions. Samples were collected from two pilot farms, and nine traditional farms, located in regions with different climates in Algeria (Table 1). For some samples, the collection was carried out in two periods (cold period: from October to March, hot period: from April to September), but for others in a single period during the year. Ten different feed types were analyzed (wheat bran, dried bread, alfalfa, straw, oat hay, barley, date scraps, grass silage, corn silage, and concentrate). All the samples were transported to the laboratory of Mohamed Khider University of Biskra within 24 hours, stored at 4°C, and protected from light until their mycological analysis. During the collection process, no sample showed visible signs of mold contamination.

Table 1: Cow feeds sample collection.

Farm location	Sampling period	Type of feeds
Batna1 (Tf)	Cold	Straw, wheat bran, pasture
Batna2 (Tf)	Cold	Oat hay, wheat bran, dried bread, pasture,
Batna3 (Tf)	Cold	Oat hay, wheat bran, pasture
Batna4 (Tf)	Cold	Straw, barley, pasture
Batna1, 2, 3 et 4 (Tf)	Hot	Pasture and straw
Constantine Kadri (Pf)	Hot	Concentrate and straw
Constantine Kadri (Pf)	Hod	Oat hay, alfalfa, concentrate, and pasture
Constantine Kadri (Pf)	Cold	Oat hay, alfalfa, concentrate, and grazing
Constantine Baaraouia (Pf)	Cold	Grass silage, concentrate, and straw
Constantine Baaraouia (Pf)	Hot	Grass silage, concentrate, Oat hay, and pasture
Constantine Baaraouia (Pf)	Cold	Grass silage, concentrate, Oat hay, and pasture
Biskra1 (Tf)	Hot	Grass silage, wheat bran, Oat hay, concentrated, date scraps
Biskra2 (Tf)	Cold	Corn silage, date scarps, and wheat bran
Guelma (Tf)	Hot	Pasture and straw
M'sila (Tf)	Hot	Grass silage, wheat bran, alfalfa, and pasture
Constantine Melha (Tf)	Hot	Pasture and straw

Tf: Traditional Farms, Pf: Pilot farms.

Mycological Analysis

Under sterile conditions, the mycoflora of different feeds were analyzed by the direct method described by [7]. Five samples of each studied feed were taken and placed at five different locations in a Petri dish containing 15 to 20 ml of the culture medium, in such a way that they were sufficiently spaced. Several culture media were used in our study (PDA: Potato Dextrose Agar, CYA: Czapek yeast extract agar, MEA: Malt Extract Agar, and Sabouraud). The pH of the culture media was adjusted to 5.6 and sterilization was carried out at 120°C for 20 minutes. An antibiotic (chloramphenicol: 0.25 g/l) was added to the various culture media to inhibit any bacterial proliferation. Petri dishes were then closed with parafilm and incubated at 25°C for three to five days to promote mold growth [8]. After isolation, several transplants of the mold strains were carried out on exposed cultures under the same incubation conditions, until pure strains were obtained. The identification was based on the identification keys of [7] and [9]. The isolation frequency (Fr) and the relative density (Rd), of species, were calculated according to [10], as follows:

$$Fr\ (\%) = (ns/N) \times 100$$

$$Rd\ (\%) = (ni/Ni) \times 100$$

Where:

ns = the number of samples in which a genus/species occurred;

N = the total number of samples;

ni = the number of isolates of a genus/species;

Ni = the total number of fungal isolates obtained

RESULTS AND DISCUSSION

• Fungal Isolation and Identification

Mycological analysis revealed that all the cow feeds samples (n=40) were contaminated by diverse fungi. A total of 248 fungal strains were isolated. Based on macroscopic and microscopic observations, nine fungal genera have been identified, five of them known to be mycotoxigenic [11].

In descending order of predominance, isolated fungi were: Aspergillus, Penicillium, Alternaria, Mucor, Rhizopus, Fusarium, Geotricum, Helminthosporium, and Cladosporium (Table 2).

In terms of frequency, the genus Aspergillus was the most frequent fungi recovered from 32 samples between all feeds studied, followed by the genus Penicillium recovered from 31 samples. In the third and fourth orders were the molds from the genera Alternaria and Rhizopus, recovered from 23 and 22 samples respectively. The following recovered genus was Mucor from 19 samples. In less proportion, other genera were also isolated Fusarium, Helminthosporium, Geotricum, and Cladosporium (Table 2).

Table 2: Frequency (%) and Relative density (%) of the isolated fungi.

	Positive samples	**Frequency (%)**	**Relative density (%)**
Aspergillus	32	80%	27.41%
Penicillium	31	77.5%	24.19%
Alternaria	23	57.5%	13.70%
Mucor	19	47.5%	12.90 %
Rhizopus	22	52.5%	12.09 %
Fusarium	11	27.5%	6.04%
Geotricum	2	5%	1.61 %
Helminthosporium	3	7.5%	1.20 %
Cladosporium	1	2.5%	0.80%
Total	**248**		

In this study, both field and storage fungi were found in analyzed samples (fig. 1, 2). Storage molds like Aspergillus, Penicillium, and Rhizopus are generally capable of developing in water-poor foods during storage, and field molds such as Alternaria, Fusarium, and Cladosporium prefer the medium with a higher water activity (Aw) [12, 13].

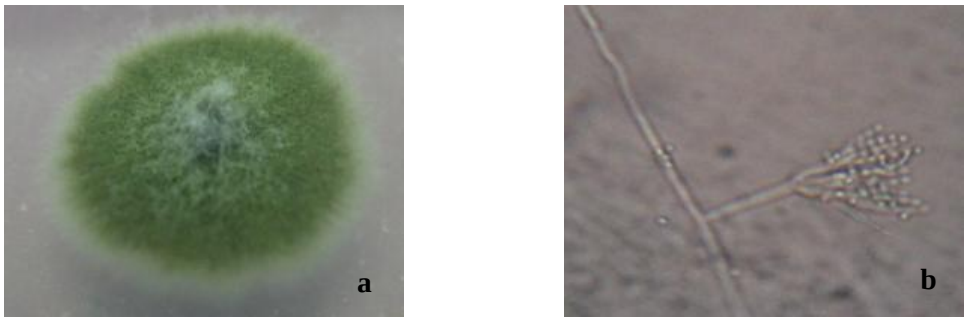

Figure 1. Macroscopic (a) and microscopic (b) appearance (x40) of Penicillium sp1 after 7 days of culture on PDA medium, isolated from a sample of date waste.

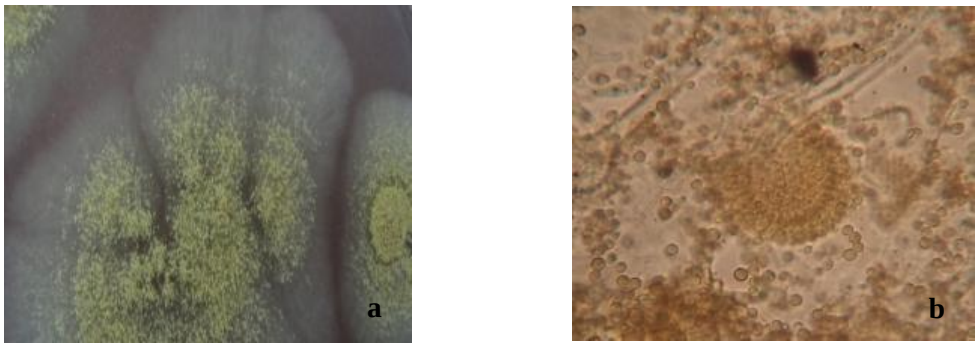

Figure 2. Macroscopic (a) and microscopic (b) appearance (x40) of Aspergillus flavus after 7 days of culture on PDA medium, isolated from a concentrate sample.

The studied region's climate is characterized by high temperatures and high relative humidity, which are environmental conditions favorable to fungal growth and toxinogenesis. Although, there are many papers describing the mycoflora of human foods, only a few data on mycotoxigenic fungi in feedstuff exist. Similar results were found to those reported by [14], who found that Aspergillus species, followed by Penicillium and Fusarium species were the main fungal genera isolated from the cow feeds sampled in Hamadan, Iran. Also, [15] studying beef cattle feeds show that Aspergillus was the most prevalent genus followed by Fusarium and Penicillium spp. The genera of Alternaria, Mucor, Rhizopus, and Cladosporium were also isolated in this study.

In terms of the number of isolates, the main contaminating molds appeared to be from the genus Aspergillus, Penicillium, Alternaria, Mucor, Rhizopus, and Fusarium (Table 3). These microscopic fungi are of great importance because of the potential of contaminating many agricultural commodities resulting in a considerable reduction of nutritional and commercial value [16]. In addition, under favorable conditions of temperature and humidity these microorganisms are likely to produce mycotoxins, which constitute a real risk factor for animal and human health [17]. The likelihood of the transfer of these mycotoxins into animal products, such as meat, eggs, or milk, is considerable [14].

Table 3: The number of isolates by feeds and by genera.

Genera	**Concentrate**	**Straw**	**Oat hay**	**Wheat bran**	**Grass silage**	**Alfalfa**	**Date scrap**	**dried bread**	**Barley**	**Silage of corn**	**N of isolates/genera**
Aspergillus	22	9	5	13	6	5	2	3	2	1	68
Penicillium	12	19	6	7	7	3	4	0	1	1	60
Alternaria	7	8	11	3	1	2	2	0	0	0	34
Mucor	11	5	5	3	5	0	1	0	0	2	32
Rhizopus	6	4	6	4	2	2	3	2	1	0	30
Fusarium	1	4	2	0	5	2	0	0	1	0	15
Geotricum	1	0	2	0	1	0	0	0	0	0	4
Helminthosporium	0	0	0	2	1	0	0	0	0	0	3
Cladosporium	1	0	0	0	1	0	0	0	0	0	2
N of isolates/Feed	**61**	49	37	32	29	14	12	5	5	4	**248**

As shown in Table 3, concentrate has the greatest fungal load with 61 fungal strains among 248 isolated strains, and was contaminated by the greatest variety of fungi. The highest percentage of relative density was shown by Aspergillus species, followed by Penicillium and Mucor species. However, the Helminthosporium genus was never found in concentrate samples examined.

The concentrate is a suitable substrate for the growth of molds, of which the main compound used in its formulation is corn [18]. In Algeria, corn comes mainly from imports where it remains stored for long periods before arriving on local markets (storage in producing farms, ports, etc.). Storage conditions are not always adequate and probably explain the high contamination of the concentrate by Aspergillus and other types of storage molds. This high frequency of contamination by Aspergillus could also be accompanied by the production of aflatoxin B1.

[19] showed that corn kernels are a suitable substrate for fungal infections and the potential production of dangerous mycotoxins. The same study showed that Aspergillus (Aspergillus flavus and Aspergillus parasiticus) is the most dominant genus and that the AFB1 level in the corn samples collected in the cold period (47%) is higher than that found in the samples collected in the hot period (17%).

Wheat bran samples contained Aspergillus at the highest density, followed by Penicillium but at quite lower values compared with concentrate. Particularly, our result corroborates with the study conducted on animal feed cereals marketed in Qatar. The results showed that mixed cereals samples have the highest fungal load, followed by corn, wheat, and wheat bran. In all the tested samples, Aspergillus was the most 217 predominant genus [20].

The highest relative density of Penicillium species was related to straw (38.77%) followed by concentrate (19.67%), especially in the cold season. In the straw, 49 fungal strains were isolated. The highest relative density of Alternaria species was related to oat hay, followed by straw. Our results are inconsistent with those conducted by [21]. These authors studied mold flora and mycotoxin contamination in the straw of paddy, maize, and wheat. The results showed that Aspergillus had the highest level of incidence in all types of samples. The lowest relative density was related to Fusarium, Helminthosporium, Geotricum, and Cladosporium species in all samples studied. Moreover, Geotricum genus was not found in any of the straw, wheat bran, alfalfa, date scarps, barley, dried bread, and corn silage, samples analyzed.

In grass silage samples, 29 strains were isolated. The majority of species were related to the genus Penicillium, followed by Aspergillus species. The highest prevalence of Penicillium species found in the present investigation in grass silage is in accordance with those of [22], who analyzed the mycobiota of different types of silages for dairy cows in Spain. Samples of

grass silages were contaminated by the greatest variety of fungi, and the common molds isolated were Penicillium, Mucor, Monascus, and Geotrichum. In a similar study, conducted on fermented feed (corn silage) in Argentina, Penicillium followed by Aspergillus were the most frequent genera [23].

In the current study, Cladosporium genus was found only in concentrate and grass silage samples analyzed. Samples of Alfalfa, date scarp, dried bread, barley, and Silage of corn, were less contaminated with fungal loads, but the genus Aspergillus was dominant in most of these feeds. Similar results were found to those reported by [5] in Irak, who found that the most dominant species isolated from poultry feed samples (corn, wheat, soybean, and barley) belonged to the genus Aspergillus, with a percentage recovery of 91.11%. Nevertheless, our results are inconsistent with those of [3] who found that the most frequent fungi were from the genus Penicillium (35.7%), followed by Aspergillus (20.4%).

The genus of Aspergillus is related to warm or tropical areas and is less frequently encountered in cold areas [24, 25]. Furthermore, the dominance of the genus Aspergillus in the flora contaminating cereals and feeds have been reported in several studies [26-29]. The high frequency and abundance of Aspergillus and Penicillium in our study could be due to inadequate farming practices, poor quality feed, and poor storage conditions [30]. This situation becomes even more complicated when climatic conditions are favorable for the development of mold. In most traditional farms visited, the feed ingredients are not protected against agents of deterioration and infection (insects and mites).

• Seasonal variation in mold contamination

Regarding the seasons, it is worth mentioning that a difference was noticed between fungal contamination during the hot and cold periods. The isolated genera were present in samples from two periods. However, the number of summer isolates obtained was lower than winter ones (57 versus 191) (Table 4).

Table 4: The number of isolates according to the season.

Genera	**Number of isolates**	
	Hot period	**Cold period**
Aspergillus	54	14
Penicillium	48	12
Alternaria	27	7
Mucor	20	12
Rhizopus	22	8
Fusarium	14	1
Geotricum	3	1
Helminthosporium	2	1
Cladosporium	1	1
Total	**191**	**57**

Not surprisingly, we noted an important difference between fungal contamination samples in the cold and hot seasons, because the greatest fungal load, both qualitatively and quantitatively, was felt during the cold season in almost all of the analyzed samples. Aspergillus had a relative density of 15.78% in the cold period and 11.74% in the hot period. The manifest Relative density of Penicillium was 15.78% in cold weather and 8.5% in hot weather. Alternaria, Rhizopus, Fusarium, and Helminthosporium had a higher number of isolates during the cold period, whereas Mucor and Cladosporium had a higher number of isolates in samples from the hot period rather than the cold period.

Our findings agree with the previous reports carried out on cow feeds collected in Iran during the summer and winter seasons [14], where the predominant fungi isolated were Aspergillus species followed by Penicillium, Fusarium, Cladosporium, Alternaria, Rhizopus, and Mucor species. Another study [31] showed that the frequency of contaminated samples collected in winter was significantly higher than that in summer. [32] reported that the levels of contamination were higher in winter and spring than in summer and autumn.

CONCLUSION

Mycotoxicological surveys of livestock feeds are very important, especially in countries, where temperature and relative humidity are favorable for fungal growth. In the present study, 40 livestock samples were tested and both field and storage fungi were found in analyzed samples. A high incidence of mycotoxinogenic fungi including A. flavus and A. ochracius was observed. These results imply that sustainable good practices should be maintained for all feed harvesting, storage, and feeding practices by feed producers and dairy farmers regarding aflatoxin contamination. Although it is impossible to prevent fungal infection and mycotoxin accumulation in cereals, unfavorable conditions for the development of fungi and toxinogenesis could be provided by the implementation of appropriate agricultural practices as preventive measures in the field. On the other hand, it would be interesting to analyze major mycotoxins in animal products, especially aflatoxin M1.

DECLARATIONS OF INTEREST: No conflicts of interest relevant to this article.

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