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Mycological Contamination Assessment of Poultry Feeds in Algeria

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Abstract: *Toxigenic fungi are regularly found in poultry feeds. The deterioration of food by fungi results in economic losses. Additionally, the production of toxins, which can adversely affect animal health and pose harmful effects on humans if transmitted through milk, meat and eggs. This study aims to isolate and identify pathogenic fungi from raw materials and commercially prepared poultry feed. The isolation of fungi was conducted using the dilution plate method, and fungal isolates were identified at the genus/species level based on macroscopic and microscopic characteristics.*

Molds isolated included Penicillium, Aspergillus, Fusarium, Rhizopus, Alternaria, Talaromyces, and Mucor with Aspergillus, Penicillium and Fusarium being the most frequently occurring genera. Fungal counts ranged from 1×10^3 to 17×10^3 CFU/g. The highest fungal contamination among primary products was 16×10^3 CFU/g in barley, followed by 11×10^3 CFU/g in bran, and then 6×10^3 , 4×10^3 CFU/g in both maize and soybean, respectively. Among the commercially prepared poultry feed, the highest fungal contamination was 17×10^3 CFU/g in chicken food, followed by 9×10^3 CFU/g in broiler food, and the lowest fungal contamination values was 1×10^3 CFU/g for finisher food. The moisture content of poultry feeds samples ranged from 14.70 % to 18.36%. It is evident that poultry feed is highly susceptible to fungal contamination, requiring specific storage and handling conditions to reduce fungal infections and protect the health of humans and animals.

Key words: *Contamination, Isolation, Pathogenic fungi, Poultry feeds.*

INTRODUCTION

Numerous fungi commonly contaminate poultry feeds, which can happen before harvest, during transportation or during storage. Like other microorganisms, molds will assimilate and utilize the most readily available nutrients in the animal food they grow upon and spoilage may result in the loss of 5 to 100% of the nutrients in the feed [1]. In addition to lowering the feed's nutritional content and quality, these fungi also produce mycotoxins, which are extremely dangerous for both human and animal health [2]. Human exposure to mycotoxins can occur via ingestion, inhalation, or contact, possibly due to contamination within the food supply chain [3, 4]. These toxins, which are secondary metabolites, can lead to various health issues, including immune suppression, gastrointestinal disturbances, and reproductive disorders [5, 6, 7].

More than 500 mycotoxins have been reported, most of which are under regulation or testing, while new mycotoxins are often discovered [2].

High levels of mycotoxin and fungi contamination in animal feeds have been reported in a number of publications worldwide [8, 9, 10, 11]. More than 100.000 fungal species are considered as natural contaminants of food and agricultural products [12]. The majority of the pathogenic species isolated from raw materials and commercially prepared poultry feed belong to the genera *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* [13, 14, 15].

The aim of this study was to isolate and identify pathogenic fungi present in raw materials and commercially prepared poultry feed from important production region of Algeria, preceded by determination the relative moisture content in poultry feeds samples.

MATERIAL AND METHODS

SAMPLES COLLECTION

Poultry feeds samples were collected from Eastern Poultry Group (ONEB), located in the Setif region in eastern Algeria, in 2018. Six commercially prepared poultry feeds samples including laying hens, chicken,

broiler, starter, grower pullet and finisher, and four primary products including barley, bran, soybeans and maize. Commercially prepared feed are collected from storehouses (stored in bags) and primary products are collected from silos.

DETERMINATION OF MOISTURE CONTENT

The moisture content of poultry samples was determined according to the method [16] as follows: 1 gram of ration samples was accurately weighted and dried to remove available moisture for 4 h, in the oven at 130 °C, then reweighed.

Moisture content percentage was calculated by using the formula as follows:

Moisture content % = $\text{Moister content (\%)} = \frac{C-B}{C} X\ 100$

Where:

C = the weight of the sample before drying.

B = the weight of the sample after drying

MYCOLOGICAL ANALYSIS OF POULTRY FEED SAMPLES

The isolation and identification of fungi from poultry feed samples were carried out using the dilution method [17]. Ten grams of each sample was added to vial containing 90 ml of 0.1% Peptone Water (w/v), was thoroughly shake to mix the solution, and kept for approximately 30 min at room temperature. Serial dilutions to 10^{-3} were prepared. 0.1ml of 10^{-2} and 10^{-3} dilutions was spread on the surface of solid media PDA (Potato Dextrose Agar) supplemented with 0.05mg.ml-^{1} chloramphenicol. Plates were incubated at 28± 2 °C for 7 days**.** Pure culture of the distinct colonies (depending on morphology) was achieved by subculturing of the isolates on PDA plates. The fungal isolates were identified to the genus/species level based on macroscopic and microscopic characteristics [18].

TOTAL FUNGAL COUNT

The isolation frequency (Fr) and relative density (RD) of the fungi isolated were calculated by using the formula 1 and 2 respectively [13].

$$\text{Fr (\%)} = \frac{\text{number of samples withgenus species}}{\text{total number of samples}} X\ 100 \quad (1),$$

$$\text{RD (\%)} = \frac{\text{number of genus or species}}{\text{total number of isolated funhi}} X\ 100 \quad (2)$$

Statistical analysis was performed using IBM SPSS Statistics 25. Differences among samples were evaluated by one-way ANOVA followed by Tukey's test ($p < 0.05$). Graphs were generated using GraphPad Prism and Microsoft Excel.

RESULTS

MOISTURE CONTENT ANALYSIS

Analysis of moisture content in raw materials and commercially prepared poultry feeds revealed significant differences among the samples (ANOVA, $p = 0.005$), with moisture levels ranging from 14.70% to 18.36%. Raw materials generally exhibited higher moisture compared to commercially prepared poultry feeds (Figure 01). For the raw material products, the highest moisture content was recorded with bran 18.36 ± 0.91 % followed by maize seeds, barley and soybean, 17.90 ± 0.66 %, 15.47 ± 0.57% and 14.93 ± 0.15 % respectively. In commercially prepared poultry feed samples, the highest moisture content was 15.43 ± 0.45 % in laying hens, followed by chicken, starter, finisher 15.00 ± 3.29 %, broiler 14.8 ± 0.51 % and grower pullet 14.7 ± 0.32 % (Figure 01 b, a).

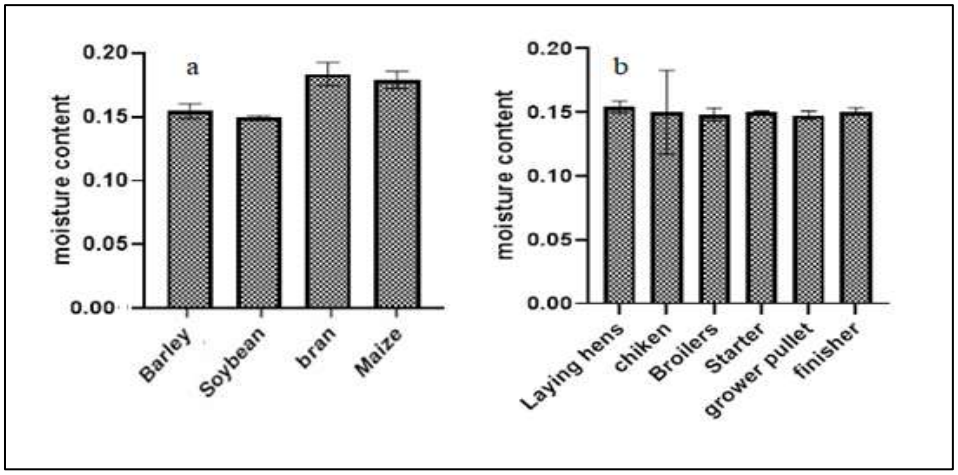

Figure 01: Mean of moisture contents (MC) in raw material products (a) and commercially prepared poultry feed (b). Error bars represent standard deviation (SD) of three replicates (n=3).

MYCOLOGICAL ANALYSIS OF POULTRY FEED SAMPLES

The mycological analysis showed that the fungi content amount in CFU ranged from 1×10^3 CFU/g to 17×10^3 CFU/g (Table 1). The greatest fungal contamination for raw material products was 16×10^3CFU/g in barley, followed by11×10^3 CFU/g in bran, 6×10^3 CFU/g in maize and 4×10^3 CFU/g in soybean. The greatest fungal contamination for commercially prepared poultry feed was 17×10^3 CFU/g in chicken food followed by 9×10^3 CFU/g in broiler, 8×10^3 CFU/g in laying hens food and 5×10^3 CFU/g in grower pullet. The lowest values for fungal contamination were 4×10^3CFU/g and 1×10^3CFU/g for both of starter and finisher food respectively.

Table 1. Total fungal counts of poultry feed samples (CFU/g).

Product	Sample	Total fungal count (CFU/g*)
raw material products	Barley	16×10^3
	Bran	11×10^3
	Maize	6×10^3
	Soybean	4×10^3
commercially prepared poultry feed	Chicken	17×10^3
	Broiler	9×10^3
	Laying hens food	8×10^3
	Grower pullet	5×10^3
	Starter	4×10^3
	Finisher food	1×10^3

*Colony forming units per g of sample

In point of fungal species view several fungi species were isolated with different relative density percentage (RD %). including, *Alternaria*, *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus* and *Talaromyces*. For Raw material, *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus* and *Talaromyces* were isolated. The relative density percentage (RD %), in barley sample, was 6.25% for *Penicillium* and *Talaromyces*, 62.5% and 25% for *Aspergillus* and *Rhizopus*, respectively. In soybean sample, it was 25% for *Rhizopus* and *Penicillium* and 50% for *Aspergillus*. In bran samples, each of *Penicillium*, *Aspergillus* and *Fusarium* had relative densities of 45.45%, 27.27% and 18.18% respectively, followed by *Rhizopus* 9.09%. In maize samples, *Aspergillus* had the most relative density of 50%, followed by *Fusarium* and *Mucor,* with 33.33% and 16.67%, in that order (Table 2).

Table 2. Relative densities percentage (RD %) of different genera of fungi isolated from raw material samples.

Fungal isolates	Samples			
	Barley	Soybean	Bran	Maize
Penicillium spp.	6.25%	25%	45.45%	0
Aspergillus spp.	62.5%	50%	27.27%	50%
Fusarium sp.	0	0	18.18%	33.33%
Mucor sp.	0	0	0	16.67%
Rhizopus sp.	25%	25%	9.09%	0
Talaromyces sp.	6.25%	0	0	0

For commercially prepared poultry feed, *Aspergillus*, *Fusarium*, *Penicillium* , *Rhizopus* and *Alternaria* were isolated. For the relative density of *Aspergillus*, *Penicillium* and *Rhizopus* in laying hens food was 62.5%, 25% and 12.5%, respectively. *Aspergillus* spp. had the most relative abundance of 76.47% in chicken, followed by *Penicillium* 17.64%, and *Rhizopus* 5.88%. In broiler food, RD % of *Aspergillus* and *Penicillium* was 44.40% and 33.30%, respectively, followed by *Fusarium* and *Rhizopus,* with 11.10%. *Penicillium* spp. had the most relative density of 50% in starter food followed by *Aspergillus* and *Fusarium,* with 25%. In grower pullet food RD % of each of *Penicillium* and *Aspergillus* was 60% and 40%respectively (Table 3).

Table 3. Relative Densities percentage (RD %) of different genera of fungi isolated from prepared poultry feeds samples.

Fungal isolates	Samples					
	Laying hens	Chicken	Broiler	Starter	Grower pullet	Finisher
Penicillium spp.	25%	17.64%	33.30%	50%	60%	0
Aspergillus spp.	62.5%	76.47%	44.40%	25%	40%	0
Fusarium spp.	0	0	11.10%	25%	0	0
Rhizopus sp.	12.5%	5.88%	11.10%	0	0	0
Alternaria sp.	0	0	0	0	0	100%

In the fungi isolated frequency percentage point of view, different percentage were recorded from 90% to 25%. For the raw material, the higher percent frequency in barley and soybean was 98% for *Aspergillus* followed by *Penicillium* and *Rhizopus* 75%, for *Talaromyces*, the frequency percentage in barley was 25%. In bran, *Aspergillus* had the most isolation frequency of 98% followed by *Penicillium*, *Rhizopus* and *Fusarium,* with 75%, 75% and 50%, respectively. Higher frequency for maize was observed by *Aspergillus* and *Fusarium* genera, with 98% and 50%, respectively, followed by 25% for *Mucor* (Figure 02).

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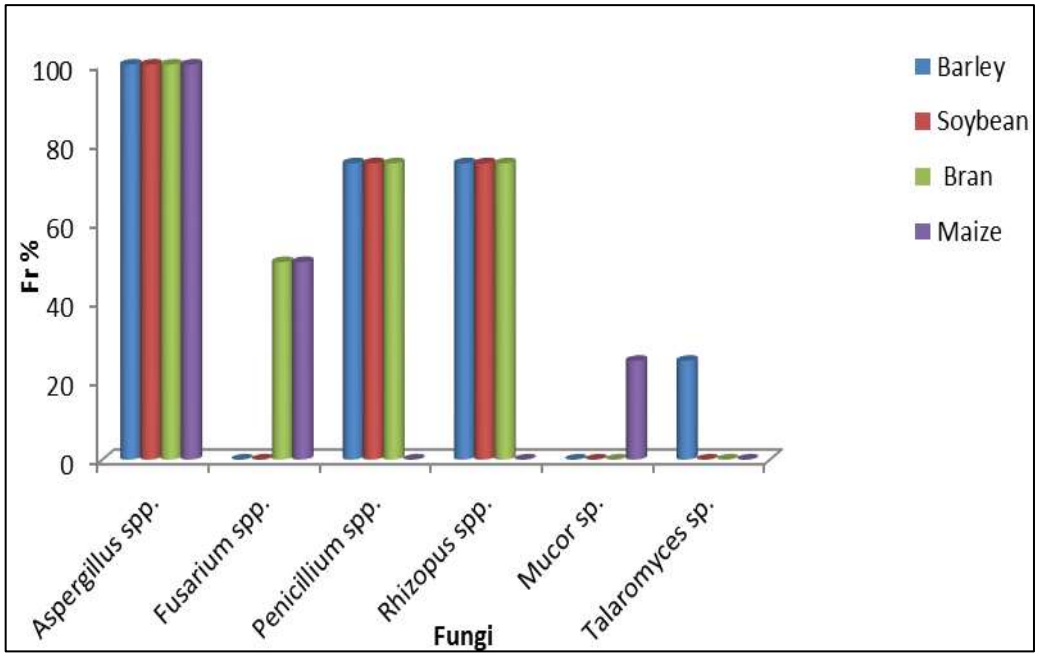

Figure 02: Frequency (Fr %) of fungi isolated from raw material samples.

For commercially prepared poultry feeds samples. *Penicillium* and *Aspergillus* had most isolation frequency in laying hens and chicken with 83.33%, followed by 50% for *Rhizopus*. In each of broiler and starter samples, *Penicillium* and *Aspergillus* had most isolation frequency with 83.33%, followed by 33.33% for *Fusarium*. However, in finisher a predominance of *Alternaria* was observed by 16.67% (Figure 03).

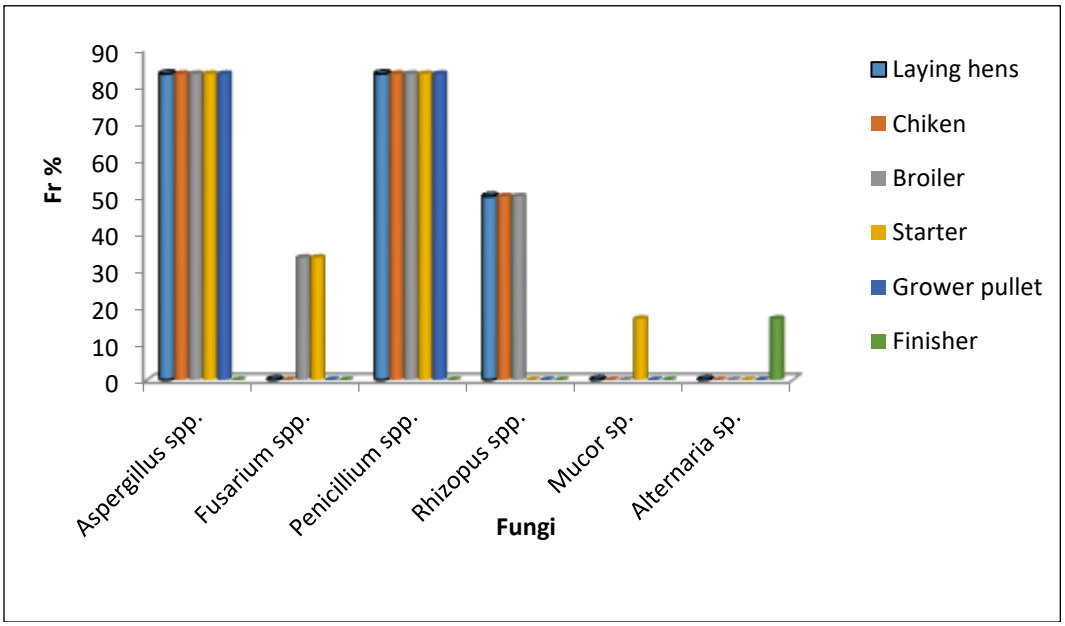

Figure 03: Frequency (Fr %) of fungi isolated from prepared poultry feeds samples**.**

DISCUSSION

The analysis of moisture content in raw materials and commercially prepared poultry feed reveals significant variations. In order to preserve feed quality and avoid spoiling, this moisture content is essential. According to [19], the moisture content shouldn't exceed 11.5% in animal feed. Bran had the highest moisture content (18.36%), followed by maize seed (17.90%), barley (15.40%) and soybean (14.93%). The overall quality of the feed may be impacted by fungal contamination caused by high moisture content in the raw materials [20, 21]. Commercially made chicken diets ranged in moisture content from 14.7% to 15.43%. These results are consistent with the findings reported in [14, 22, 16].

Samples with the highest moisture content exhibited the greatest fungal contamination, because the majority of fungus need 14–15% moisture to grow [23].

Assessment of fungal contamination in poultry feeds showed that all raw materials and commercially prepared poultry feeds were contaminated with fungi. Data on fungi occurring in poultry feeds obtained in Algeria are coincided with those reported in different countries [8, 14, 24, 13].

The contamination values ranged from 1×10^3 to 17×10^3 CFU/g. These results are comparable to those reported in [25, 26]. Our study results demonstrated significant diversity in the presence of fungi in both the raw materials and the final food products. Molds isolated were *Aspergillus Penicillium*, *Fusarium*, *Rhizopus*, *Alternaria*, *Talaromyces* and *Mucor*, which is consistent with earlier reports [13, 27, 14, 26, 28]. [29] found that fungal contamination (*Aspergillus ochraceus* and *Penicillium verrucosum*) was present in 50 feed samples from Bulgaria during two-year research (2006-2007) at a rate of 4×10^3 to 6×10^5 CFU/ g. Moreover, according to [30], the most often isolated fungal taxa were *Aspergillus* and *Rhizopus*, and the average amount of fungal contamination in 45 samples of broiler chicken feed was 7×10^2 CFU/ g.

CONCLUSION

In conclusion, molds, particularly *Penicillium*, *Aspergillus* and *Fusarium*, are major contaminants in poultry feeds and raw materials, posing significant risks to poultry health and food safety. These molds produce various mycotoxins, including aflatoxins, ochratoxin A, rubratoxins, and fuminosins, which are known to have sever detrimental effects on poultry. This paper underscores the critical need for effective monitoring of mold contamination in chicken feed to mitigate the accumulation of mycotoxins. Further research is recommended to explore and implement biological management strategies as a safe and efficient alternative for reducing fungal proliferation and mycotoxins production.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interest.

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