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Investigation of fungi contaminating broiler feed in some poultry farms in Syria

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Abstract: Fungi contaminating poultry feed are considered a reason for the decrease in their nutritional value, and a major cause of diseases in poultry. Information about fungi contaminating poultry feed is not available locally. Therefore, this study aimed to investigate fungal contamination of both raw and manufactured feeds used on six different farms dedicated to raising poultry (broiler) in Tartous Governorate - Syria, during the period extending from October 2023 to January 2024; By growing samples of these feeds in the laboratory on Potato Dextrose Agar (PDA) culture, to determine the fungal genera present in those studied feeds. 290 fungal isolates were obtained (169 isolates from raw feed, 121 isolates from manufactured feed) belonging to five genera of filamentous fungi and yeasts, including *Aspergillus spp.* (36.2%), followed by *Yeast spp.* (33.8%), *Penicillium spp.* (4.8%), *Mucor spp.* (3.8%), and the fungus *Trichoderma spp.* (1%), and it was found that *Aspergillus spp.* Fungi were the most common (34.3%, 38.8%) in the raw and manufactured feeds, respectively. The study concluded that the detection of fungi contaminating poultry feed is of great importance, due to their health risks to poultry.

Keywords: Fungal Genera; Raw Feed (Starter); Manufactured feed; Broiler; Farms.

التقصي عن الفطريات الملوثة لأعلاف الفروج في بعض مزارع الدواجن، سورية

المستخلص: تعتبر الفطريات الملوثة لأعلاف الدواجن سبباً في انخفاض قيمتها الغذائية، وسبباً رئيسياً في حدوث الأمراض لدى الدواجن، كما أن المعلومات حول الفطريات الملوثة لأعلاف الدواجن غير متوفرة محلياً، ولذلك هدفت هذه الدراسة الى تقصي التلوث بالفطريات لكل من الأعلاف الخام والأعلاف المصنعة المستخدمة في ست مزارع مختلفة مخصصة لتربية الدواجن (الفروج) في محافظة طرطوس - سورية، خلال الفترة الممتدة من تشرين الأول 2023 وحتى كانون الثاني 2024؛ بزراعة عينات من تلك الأعلاف في المختبر على مستنبت آجار البطاطا (PDA) Potato Dextrose Agar، لتحديد الأجناس الفطرية الموجودة في تلك الأعلاف المدروسة. تم الحصول على 290 عزلة فطرية (169 عزلة من الأعلاف الخام، 121 عزلة من الأعلاف المصنعة) تنتمي الى خمس أجناس من الفطريات الخيطية والخمائر، تشمل الرشاشيات *Aspergillus spp.* (36.2%)، يليه الخميرة *Yeast spp.* (33.8%)، البنسليوم *Penicillium spp.* (4.8%)، فطر *Mucor spp.* (3.8%)، وفطر *Trichoderma spp.* (1%)، وتبين أن فطريات الرشاشيات التابعة للجنس *Aspergillus spp.* كانت الأكثر شيوعاً بنسبة (34.3%، 38.8%) في الأعلاف الخام والمصنعة على التوالي. وخلصت الدراسة إلى أن الكشف عن الفطريات الملوثة لأعلاف الدواجن له أهمية كبيرة، وذلك بسبب خطورتها على صحة الدواجن.

الكلمات المفتاحية: أجناس الفطريات، أعلاف خام (بادئة)، أعلاف مصنعة، الفروج، مزارع.



INTRODUCTION

The infection of fodder crops with fungal pathogens is one of the most important reasons for their production decline in quantity and quality, which causes a decline in the nutritional value of the crop. This applies to the quality of the resulting feed and its economic value, and contamination with mycotoxins remains the greatest threat to human and animal health (Magkos 2003).

Contamination of poultry feed with fungi negatively affects the health and production of poultry, resulting in significant losses. There are more than 100,000 fungal species that are natural living contaminants of food and agricultural products (Abo-Shama, 2015). It has been found that the most common types of fungi in feed belong to the genera *Aspergillus spp*, *Fusarium spp*, *Penicillium spp*, *Rhizopus spp*, and *Mucor spp* (Okoli et al. 2006; Stuper et al. 2015).

It is worth noting the ability of some fungal species, which are belonging to the genus *Aspergillus spp*, to infect animal tissues (Richard, 2007), in addition to the produced mycotoxins by fungi, where about 400 types of different mycotoxins have been identified, which differ greatly in size and structural shapes (Iamanaka et al. 2010).

One study showed that the most common species in poultry feed were *Aspergillus spp* at 44.5%, followed by *Penicillium spp* at 7.22%, and *Fusarium spp* at 6.7% (Saleemi et al. 2010). In Nigeria, a study was conducted to evaluate the level of fungal contamination in poultry feed during the rainy season (September - April), and the results showed that the most common fungal genus is *Aspergillus spp*, followed by *Fusarium spp* (Anifowose et al. 2021).

It was also found in Iran that the most frequent fungal genera are *Fusarium spp* (90%) and *Aspergillus spp* (70%) in raw and manufactured feeds, respectively (Ghaemmaghami et al. 2018), while another study in Iraq showed the isolation of fourteen different genera of molds in poultry feed, the most common contaminated fungi were *Aspergillus spp* (88.8%), followed by *Penicillium spp* (62.2%), *Mucor spp* (62.2 %), *Rhizopus spp*, and *Scopulariopsis spp* (Shareef, 2010); The fungus *Penicillium spp* had the highest contamination rate of 74.58% (44 isolates), followed by the fungus *Aspergillus spp* with 20.34% (12 isolates), then the fungus *Trichoderma spp* with 3.39% (2 isolates), and finally, the fungus *Rhizopus spp* with 1.70% (one isolate) in six samples of poultry feed in Algeria (Najiha, 2019).

Based on previous studies, the presence of fungi in poultry feed may cause diseases in poultry, or increase poultry mortality rates because of mycotoxins in these contaminated feeds, and therefore they can be considered a threat to the health of poultry and humans; this requires periodic scrutiny of the content of poultry feeds for these dangerous contaminants, and since there are no documented local studies to date on the diagnosis of fungi contaminating ready-made poultry feeds, this research was therefore carried out to investigate and detect the fungal species contaminated with ready-made feeds used in some broiler farms.

MATERIALS AND METHODS

Sample collection: Identical quantities were randomly taken from the stock of raw feed bags, and after mixing them, one final sample with weight (500 g) was taken and kept in a transparent plastic bag with the sample card. Another sample was taken in the same way from the used manufactured feed bags in each of the studied farms. Thus, 12 samples of broiler feed were collected (6 raw feed samples, 6 manufactured feed samples), from 6 designated farms for raising broilers in Tartous Governorate, then they were transported to the laboratory in the Faculty of Agricultural Engineering at Tishreen University and stored in the refrigerator at 7°C until use.

Examination of samples: Laboratory tests of feed samples were conducted in the laboratories of

the Plant Protection Department at the Faculty of Agricultural Engineering at Tishreen University according to the following:

Fungal isolation: The process of fungal isolation was carried out on a general solid nutrient medium, which is Potato Dextrose Agar (PDA), where the contents of each sample were remixed individually, in succession, in the isolation chamber, and only (1 g) of it was taken to Erlenmeyer containing (9 ml) of sterile distilled water. The resulting suspension mixture was mixed well with a glass rod for five minutes to obtain a concentration of 10^{-1} , then the dilution was performed again by taking (1 ml) of the solution and adding (9 ml) of sterile distilled water to it to obtain a concentration of 10^{-2} , and in the same way to obtain a concentration of 10^{-3} . For each treatment (concentration), three glass Petri dishes (replicates) with a diameter of 9 cm were allocated and each replicate contained the nutrient culture medium at a thickness of 2 mm, where 1 ml of each concentration was put on the surface of the nutrient culture medium for each replicate; Also, three Petri dishes were allocated for treating the control in the same way, where 1 ml of sterile distilled water was put on the surface of the culture in each of them.

Incubation and monitoring: All replicates of the treatments were incubated in the dark at a temperature of $25 \pm 2^{\circ}\text{C}$ and monitored for 10 days until the fungal colonies appeared and differentiated. Then, they were examined to identify and determine the fungal genera, and their number was calculated using a set of macroscopic and microscopic morphological features at the genus level according to standard methods (Pitt and Hocking 2009; Samson et al. 2010; Bennett, 2010).

The frequency percentage was calculated through the following equation: Percentage frequency % = Number of Isolates of one Species / Total Number of Isolates of all Fungi X 100 (Saleemi et al. 2010).

Data analysis: The SPSS v25 program was used to analyze the data. The Independent Samples T-Test was conducted to verify the significance of the significant differences between the average results of the studied elements for the raw and manufactured feed samples.

RESULTS

The results of fungal isolation for 12 samples of broiler feed, taken from the targeted farms in the study, showed the growth of fungal colonies and yeasts. Based on the results, the minimum value, the highest value, and the average number of fungal colonies of the isolated fungi were calculated.

Table (1) shows the results of a study of fungal genera isolated from ready-made broiler feed, taken from designated farms for the production of broiler chickens, where a total of 290 fungal isolates were isolated (169 isolates from raw feed, 121 isolates from manufactured feed) belonging to five Genera of filamentous fungi and yeasts, including *Aspergillus spp*, followed by *Yeast spp*, *Penicillium spp*, *Mucor spp*, and the fungus *Trichoderma spp*, (Figure 1, 2). The T-test for independent samples showed that there were no significant differences ($P > 0.05$) between the means of the fungal genera isolated from the tested samples of raw and processed broiler feed, except for *Trichoderma spp*, and other fungi (unknown), a significant difference was found ($P < 0.05$).

It was also found that the genus *Aspergillus spp* was the most frequently isolated fungus with (34.3% and 38.8%) from raw and manufactured feed, respectively.

In general, the most common isolated fungi from finished feeds were: *Aspergillus spp*. (36.2%), followed by *Yeast spp*. (33.8%), *Penicillium spp*. (4.8%), *Mucor spp*. (3.8%), and the fungus *Trichoderma spp*. (1%), (Table 2).

Table: (1). Descriptive data for isolated fungal genera from ready-made feed samples for broiler.

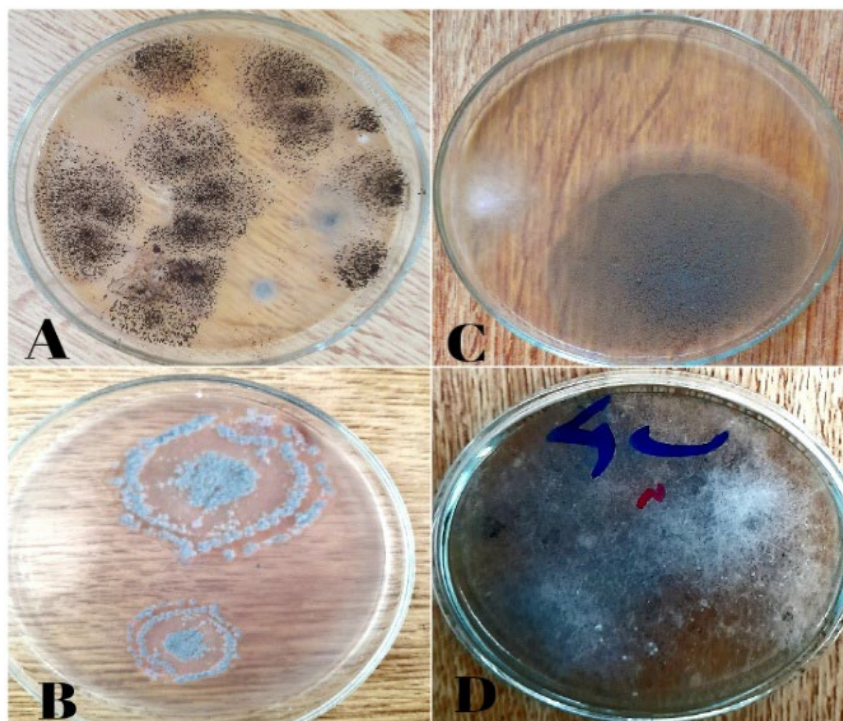
Genus	Raw Feed (n = 6)				Manufactured feed (n = 6)			
	Isolates			Fr%	Isolates			Fr%
	Range	Mean	Range		Range	Mean	Range	
<i>Aspergillus spp.</i>	0 - 50	10 ^a	58	34.3	3- 13	8 ^a	47	38.8
<i>Penicillium spp.</i>	0 - 4	1 ^b	7	4.1	0 - 2	1 ^b	7	5.8
<i>Mucor spp.</i>	0 - 4	1 ^c	7	4.1	0 - 3	1 ^c	4	3.3
<i>Trichoderma spp.</i>	0 - 2	1 ^{d1}	3	1.8	0	0 ^{d2}	0	0
<i>Yeast spp.</i>	0 - 20	9 ^e	54	32.0	0 - 25	7 ^e	44	36.4
other fungi (unknown)	0 - 25	7 ^{f1}	40	23.7	0 - 10	3 ^{f2}	19	15.7
Total			169	100			121	100

^{a, b, /1,2}; Levels of significant differences between the means of fungi for raw and manufactured feeds at the 5% level; n; Number of samples; Fr: Frequency Percentage

Table: (2). Total frequency of isolated fungal genera from broiler feed

Genus	n= 12	Total Fr %
	Total number of isolates	
<i>Aspergillus spp.</i>	105	36.2
<i>Penicillium spp.</i>	14	4.8
<i>Mucor spp.</i>	11	3.8
<i>Trichoderma spp.</i>	3	1
<i>Yeast spp.</i>	98	33.8
other fungi (unknown)	59	20.3
Total	290	100

n; Total of samples; Fr: Frequency percentage

**Figure: (1).** Isolated fungal genera in Petri dishes (A: *Aspergillus spp.*, B: *Trichoderma spp.*, C: *Penicillium spp.*, D: *Mucor spp.*)

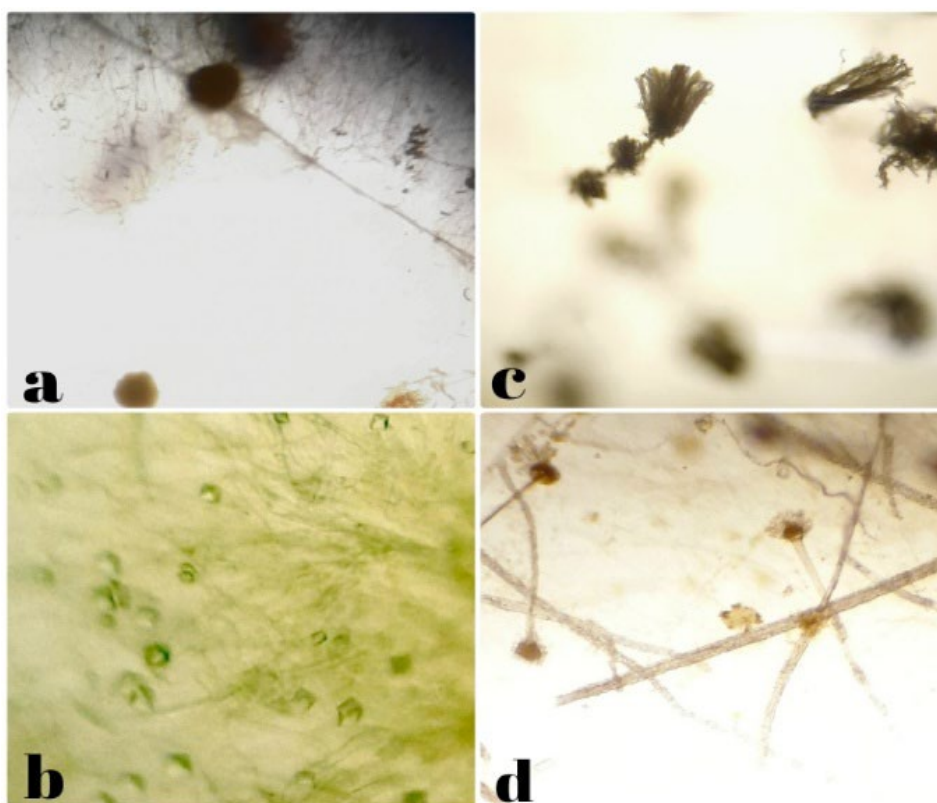


Figure: (2). Isolated fungal genera under the microscope (a: *Aspergillus spp*, b: *Trichoderma spp*, c: *Penicillium spp*, d: *Mucor spp*)

DISCUSSION

Several studies have indicated that *Aspergillus spp* is the most frequent fungus in poultry feed (Anifowose et al. 2021; Shareef, 2010; Saleemi et al. 2010), and this is consistent with the general results of this study; A study (Ghaemmaghmi et al. 2018) showed that *Fusarium spp* was the most frequent fungus, while *Penicillium spp* was the most frequent in a study (Najiha, 2019).

It is worth noting that some studies indicated that the *Aspergillus spp* fungi were the most common in manufactured feeds from raw feeds (Greco et al. 2014; Ghaemmaghmi et al. 2018), and this is consistent with the results of this study; In terms of the number of isolates, the total number of fungal genera isolated from raw feed samples in this study was greater than in manufactured feed samples, and this differs from the study (Ghaemmaghmi et al. 2018).

The reason for the increase in contamination of raw feed may be attributed to poor drying the feed ingredients during agricultural operations and transportation, in addition to inappropriate storage and weather conditions (Ghaemmaghmi et al. 2016), and the use of air in the cooling process can also lead to recontamination of manufactured feed by allowing additional pathogens to reach the feed, in addition to the ability of some fungal species to survive after heat treatment (Ghaemmaghmi et al. 2018; Jones, 2011).

CONCLUSION

The data and results of this study showed that ready-made feed used to feed broilers in some poultry farms is contaminated with different levels of fungal species. It has been noted that heat treatment may reduce fungi, but some species continue to form spores despite being exposed to heat.

This study concluded that detecting contaminated fungi in used feed to feed broilers is of great importance, because of their health risks to poultry, and thus to humans.

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