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FUNGAL AND TRICHOTHECENES (TCT) CONTAMINATION OF POULTRY FEED FROM DUHOK PROVINCE, KURDISTAN OF IRAQ

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ARTICLE

Fungal and Trichothecens (TCTs) Contamination of Poultry Feed From Duhok Province, Kurdistan of Iraq

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Abstract

Fungi are capable of reducing the nutritional value of food for human and animals and producing many mycotoxins. Mycotoxins-contaminated animal feed has adverse effects on productivity and animal health. As well, mycotoxins may be carried over into meat and eggs when poultry are fed with contaminated feed. Data on the occurrence of T-2 and HT-2 toxins in poultry feed from Kurdistan region of Iraq are very limited. T-2 toxin is the most toxic type A trichothecene mycotoxin. It is the secondary metabolite of the *Fusarium* fungi, and is common in grains and animal feed. Toxic effects have been shown both in experimental animals and in livestock. In this study a total of 30 poultry feed samples during October 2019 to June 2020 from Duhok province were analyzed for mycological and mycotoxin examination. Presence of T-2/HT-2 toxins was determined by enzyme-linked immunosorbent assay (ELISA) method. Among the 30 analyzed samples ten samples (33.33%) samples were contaminated with T-2/HT-2 toxins. Concentration ranging between 57 and 549 parts per billion.

Keywords: T-2/HT-2 toxins, Poultry feed, ELISA, Duhok

1. Introduction

Contamination of poultry feed caused by fungal of secondary metabolites – mycotoxins is a major snag in poultry production that causes harmful effects on the performance and healthy, and through poultry meat consumption can causes severity effect to human health. The poultry nutrition includes mostly grain as a source of carbohydrates, and potentially toxigenic fungi are the main contaminant of grains [50] (Fig. 1).

Major of medically and agriculturally concern mycotoxins is Aflatoxins, ochratoxins produced by *Aspergillus*, *Penicillium* and *Fusarium* species is the main producer of fumonisins, zearalenone, and trichothecenes, which invade many crops in field and may grow on feedstuffs and foods including feeding stuffs for poultry during storage under favorable conditions of humidity and temperature [44].

Grain or feed ingredients in their product may encounter healthy of livestock problems or poor performance of animal, reduction of reproduction, suppression of growth, and finally animal death [5]. Trichothecenes are sesquiterpenoid mycotoxin. It is the secondary metabolite produced by fungi from the order Hypocreales, including *Fusarium* fungi, Trichothecenes are the most dispersed and the most diverse ones including 200 analogs T-2 toxin is the most toxic type [6,49], was first isolated from the mould *F. tricinctum* (*Fusarium sporotrichoides*) [9,48]. It is produced primarily by *Fusarium* species *Fusarium acuminatum*, *Fusarium nivale*, *Fusarium oxysporum*, *Fusarium poae*, *F. sporotrichoide*s, and *Fusarium solani*. However, Trichothecenes are also known to be produced by other fungal genera like *Trichoderma*, *Trichotecium*, *Myrothecium*, and *Stachybotrys* [9,22,23,27,48,53]. Nearly 160 trichothecenes have been identified and are classified into 4 groups depending on their chemical structure. The major ones are T- 2 and HT-2 toxins (group A) and nivalenol (NIV) (group B) and is common in grain and animal feed. Toxic effects have been shown both in experimental animals and in livestock.

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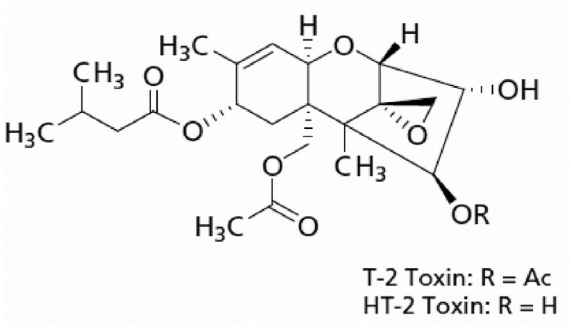

Fig. 1. Chemical structure of T-2 toxin. www.impactjournals.com/oncotarget/.

It has been implicated in several outbreaks of human mycotoxicoses. Toxic effects in poultry include inhibition of protein, DNA, and RNA synthesis, cytotoxicity, immunomodulation, cell lesions in the digestive tract, organs and skin, neural disturbances and low performance in poultry production (decreased weight gain, egg production, and hatchability). The Toxic effects of T-2 in poultry include inhibition of protein, DNA, and RNA synthesis, cytotoxicity, immunomodulation, cell lesions in the digestive tract, organs and skin, neural disturbances and low performance in poultry production (decreased weight gain, egg production, and hatchability) [39,47,52]. This research work was aimed to isolate fungi from poultry feed ingredients; and the presence of sum of T-2/HT2 toxins in poultry feed in Duhok province/Kurdistan region of Iraq by using ELISA test.

2. Materials and methods

2.1. Sample collection

A total of 30 feed samples (500g each), including poultry feeds and feed stuffs served for poultry collected from different farms and different feed depots as well as from Veterinary Service Centres in Duhok government areas the feeds were treated as ready-to-serve. Samples collected at nine different points of each barrel: Three points of the upper third, three points of the middle third, and three points of the lower third. Each sample stored in paper sacks packaging at room temperature (about 25 °C). The samples taken for the mycological analysis in the present study had no additives or preservatives that might interfere with fungal growth and immediately analyzed upon arrival or they were stored for 2–3 days.

2.2. Sample preparation

The dilute plate technique was used for isolation and enumeration of fungi [4]. Ten grams of each representative sample was blended with 90 ml of autoclaved distilled water on a horizontal position and shake for 30mins to form uniform suspension. For each feed sample, five dilutions 10^{-1}, 10^{-2}, 10^{-3}were made from each dilution [41]., then 0.1 ml of the dilution 10^{-2} and 10^{-3} was inoculated by spread plate technique to achieve uniform distribution of the spores then aseptically cultured in triplicate on each of these four different media: potato dextrose agar (PDA), Dichloran rose bengal chloramphenicol (DRBC), Dichloran glycerol (DG18) and Malt extract agar(MEA) and incubated at 25 and 37 °C for 5–7 days with intermittent observation of the fungal growth.

2.3. Mycobiota identification and determination

Isolates from growing colonies were sub cultured on appropriate media. For fungi suspected to belong to the genus *Fusarium* were sub cultured onto plates with Malt Extract Agar (MEA). For identification of species in the genera *Aspergillus* and *Penicillium*, pure colonies were grown on these media according to Klich [24] and Samson et al. (2000). The media are as follows: Czapek Yeast Extract Agar incubated for 7 day at 25 °C (CYA25), and 37 °C (CYA37), Czapek Yeast Extract Agar with 20% (CY20S) and Malt Extract Agar was incubated for 7 day at 25 °C (MEA).

For each culture, four plates were used, two of CYA and one for each of CY20S and MEA. Each plate was inoculated at the center and incubated for seven days. One CYA was incubated at 37 °C. The rest were incubated at 25 °C. Based on macroscopic and microscopic characteristics of pure cultures of obtained isolates from analysed poultry feed samples fungi genera were identified according to keys and descriptions provided by ofWatanabe [51]., Pitt and Hocking (1997), [24]; Samson et al., 2000, [45]. *Fusarium* spp. according to [8,37], Leslie and Summerell (2006)., and other fungi according to [41,45]. The measurements were taken after seven days incubation. Microscopic examination of the isolate was done using wet mount and slide culture technique [41,45]. The ornamentations of the conidia and conidiophores were examined by oil immersion.

The Isolation Frequency (Fr) of detected species in samples was calculated according to Gonzalez [19] as follows:

$$\text{Isolation frequency FR \%} = \frac{\textbf{Number of samples on which a fungus appeared}}{\textbf{Total Number of Samples}} \times 100$$

2.4. Preparation of samples for ELISA technique

The Content of T-2/HT-2 toxins were determined quantitatively by the enzyme linked immunosorbent assay method (ELISA) technique in Central Veterinary Laboratory/Veterinary Directorate in Duhok governorate. All samples were analysed in duplicate with Quantitative T-2/HT-2 Toxins test kit (Veratox T-2/HT-2 quantitative Test, Neogen Europe). Range of quantification for T-2/HT-2 toxins test kit was between 25 and 250 ppb. The extractions procedures were according to manufacturer's protocol. In brief, 5 g of ground sample was extracted with 25 mL of 70% methanol for T2-toxin in sealed vials. After shaking 2 min, mixture was filtered and the filtrate was directly tested with ELISA kit as manufacturer describes.

2.5. Statistical analysis

The data obtained from this study were converted to arcsine and analysed using SAS program and means were compared using Duncan's multiple range test [46].

3. Results and discussion

The contamination of animal feed with fungi and production of mycotoxins is one of the major important threats to human and animal [30]. In the present study, all the samples analysed were contaminated with different kind of fungi. The frequency percent were obtained from different poultry feed samples on the four-culture media Dichloran Rose Bengal Chloramphenicol Agar (DRBC), MEA, PDA to enumerate total cultural fungi, Dichloran 18% Glycerol Agar (DG18) for xerophilic fungi was presented in Table 1 and Table 2.

The genera of fungi isolated from poultry feed by dilution method on PDA, MEA, DRBC glycerol medium and their frequency of occurrence is presented in Tables 1 and 2, Figs. 2–4. A total of 20 genera in addition to Yeasts and non –sporulating mycelia were identified. Five genera were isolated with highest frequencies (listed in decreasing order) *Aspergillus*, *Penicillium*, *Cladosporium*, *Rhizopus* and Fusarium from PDA, MEA while on DRBC

Table 1. Frequency % of fungal genera on PDA and MEA media.

S.N.	frequency % of fungal genera on PDA and MEA media	Frequency %
	Fungal	
1	*Trichoderma*	9
2	*Rhizopus*	15
3	*Alternaria*	9
4	*Cladosporium*	17
5	*Stachybotrys*	10
6	*Phoma*	10
7	*Aureobasidium*	5
8	*Aspergillus*	30
9	*Penicillium*	25
10	*Eurotium*	6
11	*Emericella*	7
12	*Neosartorya fisheri*	5
13	*Mycelia sterilia*	15
14	*Chrysosporium*	3
15	*Rhizomucor*	7
16	*Absidia*	8
17	*Fusarium*	13
18	*Neoscitilidium*	5
19	*Curvularia*	4
20	*Scopulariopsis*	3
21	*Yeasts*	15
22	*Monilia*	5

Table 2. Frequency % of fungal genera on DRBC and DG18 media.

S. N	Frequency % of fungal genera on DRBC+18 GLYCEROL	Frequency %
	Fungal genera	
1	***Mortierella***	**5**
2	***Rhizopus***	**15**
3	***Alternaria***	**4**
4	***Ulocladium***	**10**
5	***Stachybotrys***	**6**
6	***Chetomium***	**10**
7	***Byssochlamys***	**6**
8	***Aspergillus***	**20**
9	***Penicillium***	**17**
10	***Eurotium***	**10**
11	***Emericella***	**11**
12	***Gymnoascus***	**4**
13	***Mycelia sterilia***	**12**
14	*Myrothecium*	**10**
15	***Mucor***	**12**
16	***Absidia***	**10**
17	***Fusarium***	**16**
18	***Neosartorya***	**5**
19	***Curvularia***	**3**
20	***Gymnoascus***	**5**
21	***Epicoccum***	**4**
22	***Yeast***	**5**

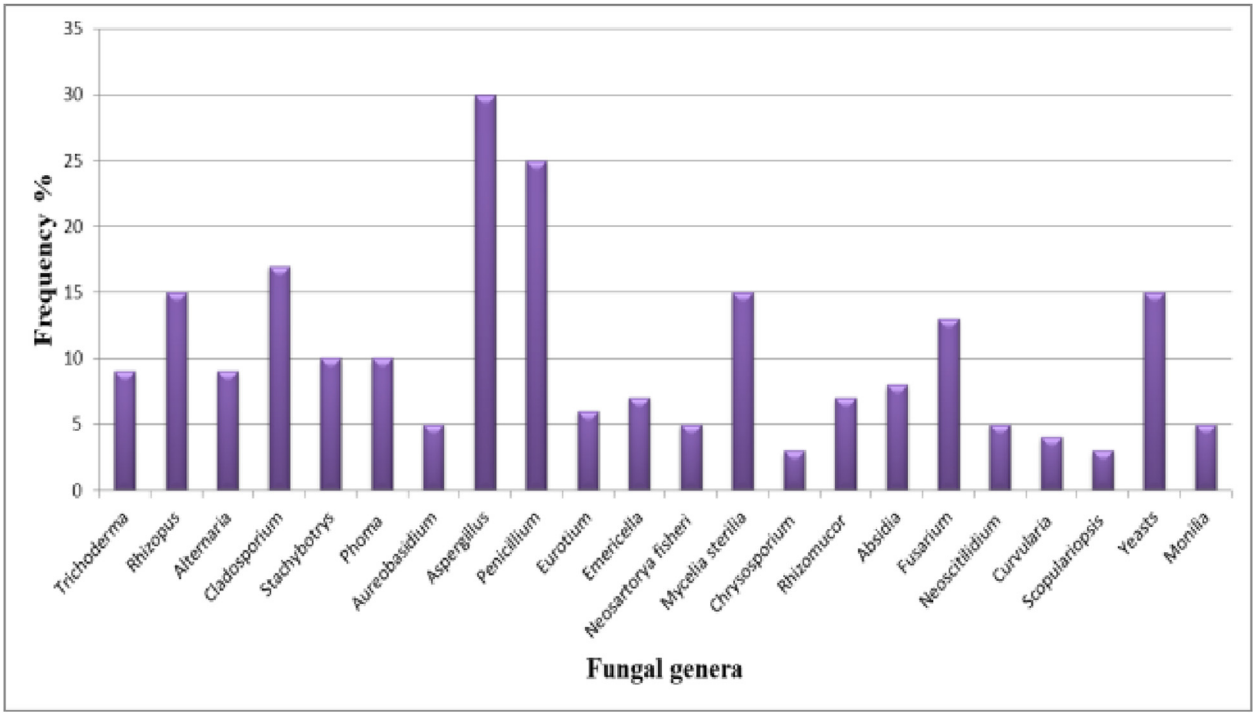

Fig. 2. Percentage of poultry feeds contaminated with viable moulds on DRBC medium.

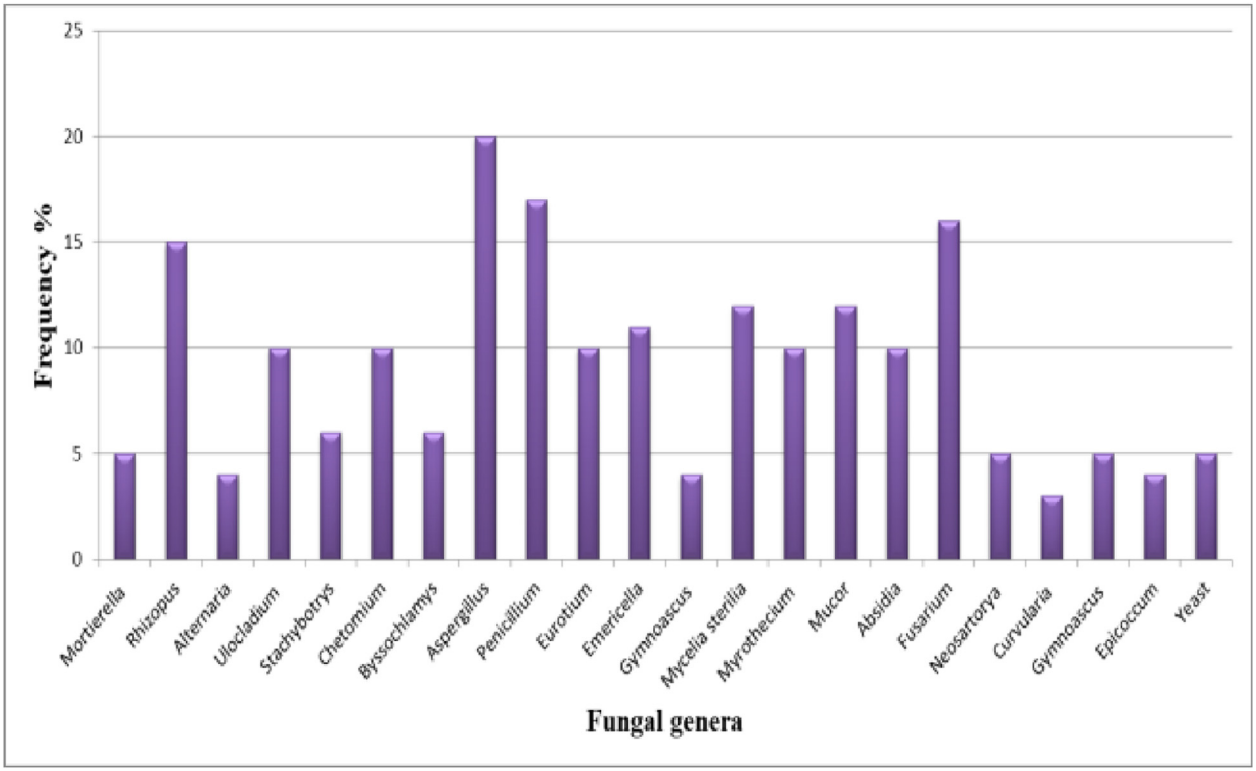

Fig. 3. Percentage of poultry feed contaminated with viable moulds on PDA medium.

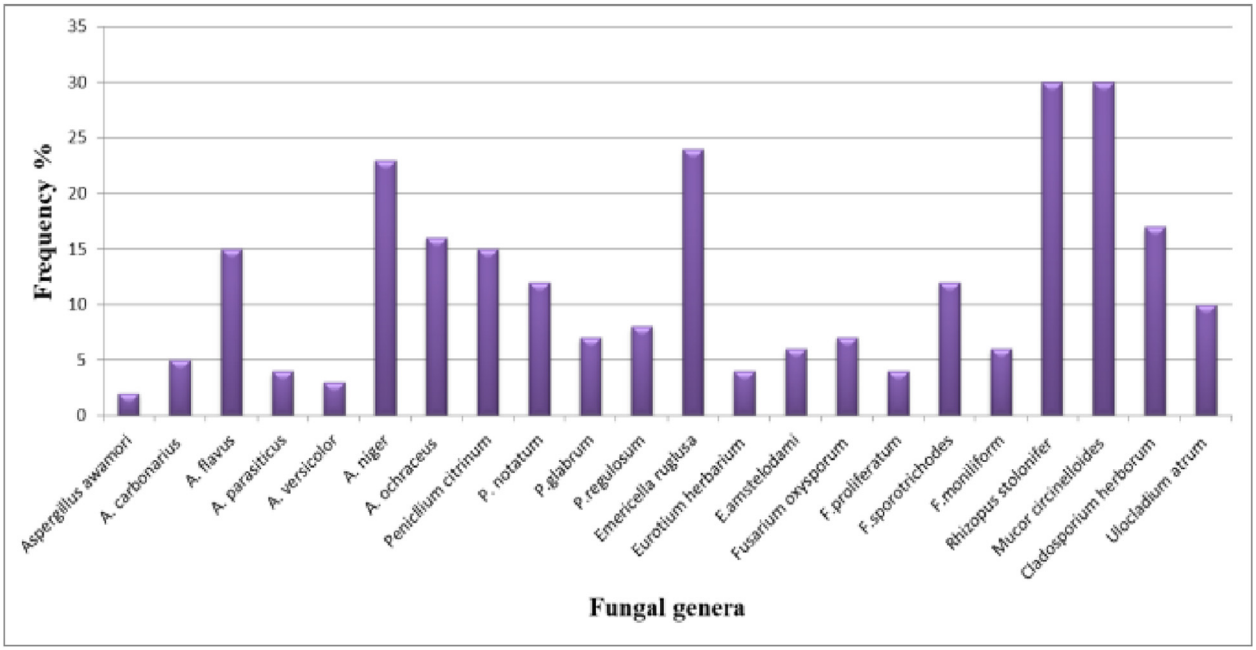

Fig. 4. Percentage of poultry feed contaminated with viable moulds on MEA medium.

Aspergillus, *Penicillium*, *Fusarium*, *Rhizopus* and *Mucor* isolated with high frequencies.

These moulds include members of the genera *Aspergillus*, *Penicillum*, *Fusarium*, *Cladosporium* and *Alternaria* which could contaminate different agricultural commodities used in the formularization of poultry concluded feed samples like corn, soybean, barley, wheat and others food commodities. These moulds are of great importance because of potential production of mycotoxin. *Aspergillus* species have been known as a soil saprotrophic inhabitant in tropical and subtropical region as well as contaminants of a variety of agricultural commodities but several are important because they produce mycotoxins [24].

Fusarium spp. is widespread in tropical countries, also, in corn in Argentina [17]. Corn is the main component of poultry feed in the samples analysed. Our findings are similar to those studied by Magnoli et al., [32], From 120 samples of poultry feed reported the presence of 15 genera of filamentous fungi. *Fusarium* and *Penicillium* were isolated in 67.5% of the samples and *Aspergillus* in 57.5% of them. Dematiaceous fungi such as *Curvularia*, despite their ubiquitous presence in the environment.

Alternaria represent by one species including *A. alternata*. Species in this genus are including saprophytic fungi and plant pathogenic this is one of the causative agents of black point disease in wheat and reduce the wheat germination [33].

Along with results from the similar studies reported by Heperkan and Alperden [21]; Bragulat et al., [7], and Dalcero et al., [11]; it may be stated that *Aspergillus* (including *Eurotium*), *Penicillium* and *Fusarium* are the typical fungal genera inhabiting poultry feed mixtures.

These results are with Watanabe, T [51]; who found that the most dominant species isolated of poultry feed samples belonged to the genus *Aspergillus*, but not in consistent with Watanabe, T [51]; who found that the most frequent fungi were those from the genus *Penicillium*. Although molds of *Aspergillus* species are more often soil fungi or saprophytes in Figs. 5–7.

Fig. 5. Mixture of fungal growth on PDA medium from poultry feed.

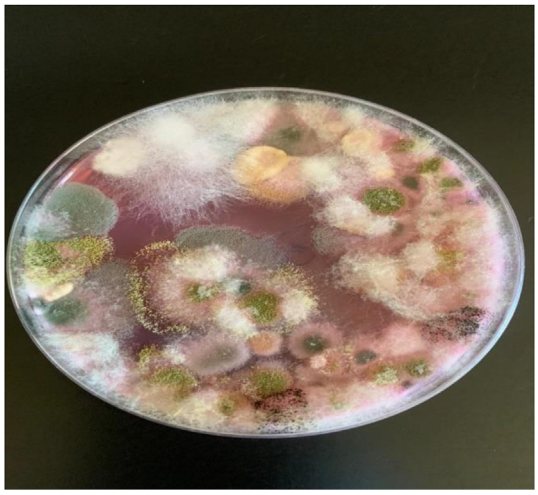

Fig. 6. Mixture of fungal growth on DRBC medium from poultry feed.

Fig. 7. Mixture of fungal growth on MEA medium from poultry feed.

Twenty-two species of fungi were isolated and identified. The frequency occurrence is presented in Table 3. From two culture media; various fungi species were isolated as the natural contaminant of poultry feed in Duhok province.

On the other hand, we found *Aspergillus*, and *Fusarium* species, followed by *Penicillium* sp., as prevalent mycobiota (Table 1) *Aspergillus* represented by 7 species and thus includes the widest diversity among all identified genera. Black aspergilli were represented by three species (*A.carbonarius*, *A. niger* and *A. awamori*). Two species were isolated with high frequency namely: *Apergillus niger* (20%) and *A. flavus* (9%).

Fusarium represent by four species present study from poultry feed and represent as second recovered species *Fusarium proliferatum* with frequency percent 4% followed by *F. sporotrichoides* 12% then

Table 3. Shows the species of fungi identified from thirty samples of poultry feed in all investigated poultry feed samples the most present were fungi from genera.

S.N	frequency % of fungal species isolated from poultry feed DRBC+18 GLYCEROL medium	Frequency %
	Fungal species	
1	*Aspergillus awamori*	2
2	*A. carbonarius*	7
3	*A. flavus*	9
4	*A. parasiticus*	4
5	*A. versicolor*	3
6	*A. niger*	20
7	*A. ochraceus*	5
8	*Penicllium citrinum*	15
9	*P. notatum*	12
10	*P.glabrum*	7
11	*P.regulosum*	8
12	*Emericella ruglusa*	24
13	*Eurotium herbarium*	4
14	*E.amstelodami*	6
15	*Fusarium oxysporum*	7
16	*F.proliferatum*	4
17	*F.sporotrichodes*	12
18	*F.moniliform*	6
19	*Rhizopus stolonifer*	30
20	*Mucor circinelloides*	30
21	*Cladosporium herborum*	17
22	*Ulocladium atrum*	10

Fusarium oxysoprum 7% while *Fusarium moniliforme* 6%. The result of the present study is on line with many studies around the world.

Four species of *Penicillium* isolated from the present study from poultry feed and represent as third recovered species. *P. citrinium* was the most frequent species (15%).

Followed by *P. notatum* (12.0%) and *P. glabrum* with a value of 7%. *P. regulosum* represents a frequency of 8%. Other isolates of *Penicillium* identified are require usually other molecular test to identify precisely because species are difficult to distinguish from each other and their taxonomy was not fully resolved.

The occurrence of *Aspergillus* spp. And *Fusarium* spp. is widespread in tropical countries, also, in corn in Argentina [10].

Three teleomorphic ascomycetes, namely, *Emericella regulosa* sp. and *Eurotium amestelodami* and *Eurotium herbarium* were detected with percentage frequencies 24%, 4 and 6% respectively. *Scopulariopsis*, *Phoma* and other fungal genera has been recovered from the present study is similar to many references.

Table 4 showed the in vitro levels of T- 2/HT-2 mycotoxin quantities in all samples of poultry feed tested in this study by using ELISA method and expressed in ppb.

Table 4. Shows the in vitro concentrations of T-2/HT-2 toxins poultry feeds using ELISA technique.

No.	Fungal isolate	T-2/HT-2 (ppb)
1	Sample 1	372.3
2	Sample 2	84
3	Sample 3	57
4	Sample 4	85
5	Sample 5	106
6	Sample 6	119
7	Sample 7	133
8	Sample 8	140
9	Sample 9	212
10	Sample 10	16.0

Various analytical methods are available for mycotoxin determination in poultry feed such as PCR technique and HPLC. Although these methods are sensitive, they need an intensive clean-up of the samples and require expensive instruments and reagents.

Thus, Immunological assays such as ELISA used in this study because it's highly sensitive and specific, require minimal sample preparation and allow high rates of sample analysis using T-2/HT-2 standard of known concentration [15].

Among thirty sampled tested ten of them showed positive results and contaminated with T-2/HT-2 mycotoxins. Concentrations of T- 2/TH-2 toxins in the positive samples ranged from 372.3 to 57 ppb these findings are in agreement with data observed by del Pilar Monge *et al*., [31]. The ELISA method detected the highest quantity (372.3) in sample 1, while the lowest was 16.0 in sample 10. Concentrations of T-2 toxin in feed are usually low, and its immunosuppressive effects and secondary infections often make diagnosis difficult.

Prevalence of T2 contamination revealed a significant association between the Iraqi and Iranian manufactures. In poultry, the T-2 toxin has been the causative agent for mouth and intestinal lesions in addition to the impairment of immune responses, destruction of the hematopoietic system, declining egg production, the thinning of egg shells, refusal of feed, weight loss and altered feather patterns, abnormal positioning of the wings, hysteroid seizures or an impaired righting reflex.

4. Conclusions

The present study has provided information about the contaminating several mycoflora of poultry feeds in Duhok province. According to the results of the present study the high toxicity of T-2 and HT- 2 toxins and their occurrence in animal feed need for collecting more data in Duhok province/Kurdistan region of Iraq. Poultry feeds are excellent media for the growth of fungi and so, very high standard of hygiene is necessary to avoid feed contamination. And ensure health and productivity of poultry as well as prevent human foodborne diseases.

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