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RESEARCH PAPER

Studying Toxigenic Fungi and Mycotoxins Detection from Bean (Phaseolus vulgaris L) in Duhok Province/Kurdistan of Iraq.

Lina F. Hussein [1], Asia A. M. Saadullah [2].

[1]Department of Biology, College of Science, Salahaddin University- Erbil, Kurdistan Region, Iraq
[2]Department of Biology, College of Education, Salahaddin University- Erbil, Kurdistan Region, Iraq

A B S T R A C T:
The common bean (*Phaseolus vulgaris* L.) is one of the most widely farmed legumes, with farmers cultivating a variety of cultivar variants in Kurdistan of Iraq. Total of 50 bean samples were tested to establish their mycological contamination and their toxigenic potential under certain conditions, a variety of fungi may develop within bean grains; some of which have the capacity to synthesize mycotoxins. In current study toxigenic fungi were studied in beans (*Phaseolus vulgaris* L.) *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. were the most frequently isolated genera, followed by *Phoma* sp., *Mucor* spp., *Alternaria* spp., *Curvularia* spp., *Rhizopus* spp., *Eurotium* sp. *Chaetomium* spp, Yeasts and *Drechslera* spp. Among 20 Aspergillus 11 strains produced mycotoxins: 30% produced aflatoxins (AFs) ranged between 81-260 ppb; 5% produced ochratoxin A (OTA 70-100 ppb) and 26.6% of *Fusarium* produced Trichothecene T-2/HT-2 (50- 94 ppb). The toxigenic species were *A. flavus*, *A.* parasiticus, *A. ochraceus*, *A. carbonarius A. niger* and *F. sporotrichioides*.

KEY WORDS: Toxigenic fungi, Phaseolus, Beans, Aflatoxins, Ochratoxin A, Trichothecenes.
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1.INTRODUCTION :

Over the last three decades, food contamination by toxigenic molds has gotten a lot of attention. Filamentous and tiny fungus can infest most grains, including wheat, maize, bean, and rice. Some genera can create mycotoxins, which are harmful secondary metabolites that have an impact on food safety (Kuiper-Goodman, 1995).
Common bean (*Phaseolus vulgaris* L.) is a critical vegetable with tall wholesome esteem. Is one of the foremost historically developed crops within the world. It is of asocial, financial and dietary significance in numerous nations, utilized by at slightest 300 million individuals, developed significantly within the creating nations (Rangel *et al*., 2005). A good source of protein is dried beans. carbohydrates and proteins, as well as vital vitamins, minerals, and fibers, and phenol compounds, which are rich in antioxidants.

Contamination of food and rural goods by various types of toxigenic molds could be a real and mostly overlooked problem. Despite decades of extensive research, form pollution continues to be a difficult problem (Gary Munkvold, 2017). FAO estimates that roughly 25% of the world's crops are contaminated by mold and impacted by mycotoxins with the loss that is expected amounting to billions of euros. Molds have been assigned a ranking as a source of harm in the capacity of grains, similar to scary crawly bugs. Inadequate collection of agricultural by-products and agricultural products such as beans, insufficient drying, handling, packaging, temperature, and excessive moisture during storage and shipping all contribute to the growth of fungi. and increase the risk of mycotoxin production, which are secondary metabolites given by toxigenic fungi in contaminated foods. Mycotoxins are released under fungal pressure, and their presence in a network indicates that the

* **Corresponding Author:**
Asia Abdulhamid Abdullah
Email: asia.saadullah@uod.ac
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fungi were last seen lately (Sweeney and Dobson, 1999).

The problem of mycotoxins in food has resulted in increased concern about toxigenic fungal contamination, particularly from the genera *Aspergillus*, *Penicillium*, and *Fusarium*. These fungi have been discovered and identified from a variety of food substrates, including cereals, legumes and their by-products, and are widely distributed in the Duhok environment. Despite this, there are insufficient data on mycotoxins, fungal contamination, and toxigenic fungal potential in Duhok legumes, particularly beans and conditions of storage, this study was conducted to assess ochratoxin A (OTA) pollution and toxigenic contaminants, aflatoxins (AFs), and Trichothecenes 2 (T-2/HT-2) in beans using ELISA approach.

2.MATERIALS AND METHODS

2.1. MATERIALS

2.1.1 Samples collection and Mycoflora identification:

Fifty samples of beans (*Phaseolus vulguris* L.), collected randomly at diverse time during one year (June 2020-June 2021) from general stores and road markets at Duhok Governorates. A 1,000 g minimum sample size was used. Tested Bean were partitioned into two bunches; the primary bunch was treated with sodium hypochlorite arrangement (1%) for two min (disinfected), whereas the second bunch was (untreated) on-disinfected. All tested beans were washed by sterilizing water for three minutes, then dried between two sterilized channel papers and plated on potato dextrose agar (PDA) and Dichloran Rose Bengal chloramphenicol (DRBC) agar. Ten beans/dish and three dishes were utilized as replication for each of the treatments. At 28°C, all plates were incubated for 5-7 days. All fungus development was exchanged and filtered single spore procedures onto PDA medium containing anti-microbial (chloramphenicol) to suppress bacterial growth (Klich, 2002).

Creating molds were refined on MEA medium (5-7 days incubation), then diagnosed at college of science, mycology research laboratory based on morphological and microscopic characteristics utilizing the accessible of writing concurring to Raper and Fennell (1965), Domsch et al. 1980, Klich, 2002 and Pitt 2009 for *Aspergillus*, Booth (1977) and Nelson et al. (1983) for *Fusarium*, and Singh et al. (1991) for *Aspergillus*, *Penicillium* and *Fusarium*, see Maren and Johan (1988). On medium, the fungi's cultural features in terms of growth, colony character, texture, and sporulation were noted.

After seven days of incubation, measurements are taken. Lactophenol microscopic mounts with or without cotton blue are available. Microscopical slides were studied with oil immersion to identify the ornamentations of the stipe and conidia. Mycotoxins were extracted from fungal cultures, and the production of aflatoxins (AF), Ochratoxin A, and T2 by arbitrarily selected confines of *Aspergillus* section Flavi and section circumdati, as well as fusarium species, were screened using the Bragulat et al. (2001) used a centrally inoculating yeast extract sucrose (YES) plate method followed by a 7-day incubation at 25° C. At a halfway point between the edge and the middle of the forming colonies, agar plugs (0.5 cm in diameter) were evacuated from the colony's borders. In a tiny vial, the three plugs were combined with 1 ml methanol, forcefully shaken, and kept at room temperature for 1 hour before being blended again and the extricates separated. Via millipore channel (0.22um) width (Millex GP Channel Unit Carrigtwohill Co. Ireland). The following formula was used to calculate the isolation frequency of detected species from samples.

$$\text{Isolation frequency FR \%} = \frac{\text{The number of samples on which a fungus has been found}}{\text{Total number of samples}} \text{X100}$$

2.1.2 Mycotoxins analysis

For analyses of mycotoxins the chemical connected immunosorbent test (ELISA) was used to examine the quantitative levels of AFs, OTA, and T-2/HT-2. The mycotoxins test was carried out in accordance with the information provided by the company (Veratox Aflatoxins, OTA, T2 quantitative Test, Neogen Corporation, USA). The number of mycotoxins produced by confinement was calculated using the standard bend obtained by mycotoxins measurements and expressed in parts per billion (ppb).

2.2 Analytical statistics

The data were evaluated using a one-way analysis of variance (ANOVA) followed by multiple mean comparisons and expressed as mean standard error (mean S.E). The data from this study was transformed to arcsine and analyzed using the SAS program, with Duncan's

multiple range test (SAS Institute Inc., Gary) was used to compare means.

3.RESULTS AND DISCUSSION

3.1. Extraction Yield

In Duhok province, the common bean is a widely cultivated legume. Agricultural products' temperature and moisture during storage and harvest allow fungi to grow, resulting in mycotoxins contamination, as well as their by-products, including beans, may be exposed to a high occurrence of Common beans contain biological contaminant., posing a food safety challenge because they can have an impact on the bean at various stages of the manufacturing process.

Their presence can have a negative impact and harm customers' health by having a direct harmful effect or by causing nutritional deficiencies, as well as lowering crop yields, which has an economic impact. The research literature on prevalence of biological contaminants in the common bean (*Phaseolus vulgaris* L.) was examined in order to determine the main concerns to food safety posed by fungal contamination and the possibility for mycotoxins.

Fungus contamination was investigated in 50 samples of beans gathered in Duhok province. A total of seventeen species from 13 genera were isolated. Table 1 and Figure 1 show the frequency of their occurrence.

Table 1: Frequency % of fungal genera on DRBC+PDA medium

Number	**Frequency % of fungal genera on DRBC and PDA medium**	
	Fungal genera	**Frequency %**
1	*Aspergillus parasiticus*	20.33
2	*A. flavus*	18.83
3	*A. niger*	18.5
4	*Fusarium sporotrichioides*	15.06
5	*A. carbonarius*	13.83
6	*A. fumigatus*	11
7	*Penicillium citrinum*	7.67
8	*Mucor circinelloids*	7
9	*A. foetidus*	6.17
10	*Rhizopus stolonifer*	6
11	*Alternaria alternata*	4.5
12	*A. nomius*	3.67
13	*A. ochraceus*	3.17
14	*Curvularia* sp.	3.17
15	*Phoma* sp.	2.83
16	*Eurotium amstelodami*	1.67
17	*Chaetomium* sp.	1.33
18	*P. expansum*	1.17
19	Yeasts nonidentified	1.06
20	*P.glabrum*	1
21	*Fusarium oxysporum*	1
22	*Drechslera* sp.	1

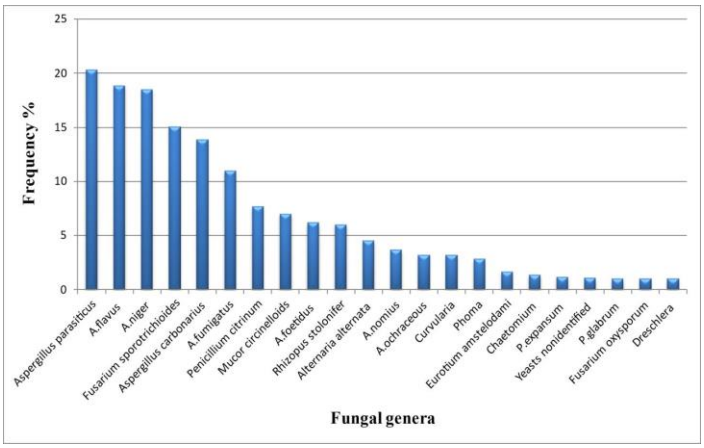

Figure 1: Frequency % of fungal genera on DRBC and PDA medium

Most of the recovered fungi were previously reported from beans in many parts of the world. Three species were isolated with high frequency namely: *Aspergillus parasiticus* (20.33), *A. flavus* (18.83) and *A. niger* (18.5), followed by *A. carbonarius* and *A. ochraceous* and *A. fumigatus* (13.83, 11,

3.17 %). Eight species of *Aspergillus* were found, with the most diversity of all of the retrieved taxa. When *A. parasiticus* and *A. flavus* grow on incorrectly stored grains, aflatoxins, one of the most dangerous carcinogens, are produced. *A. nomius*, in addition to black Aspergilli and Aspergilli in section Flavi, was found in this investigation on beans. *A. nomius* has been found in figs in California and has been reported to be aflatoxigenic (Bayman et al., 2002). Acute aflatoxicosis or, in severe cases, liver failure can result from ingesting greater quantities of aflatoxins (Fung et al., 2020).

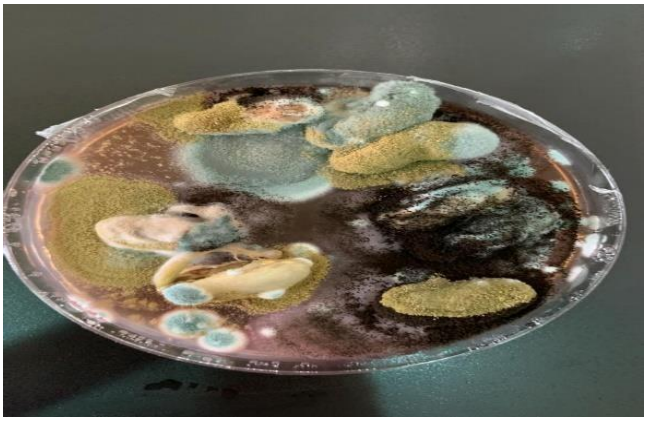

Figure 2.a: Growth of A. flavus and *A. parasiticus* on Phaseolus bean on PDA medium.

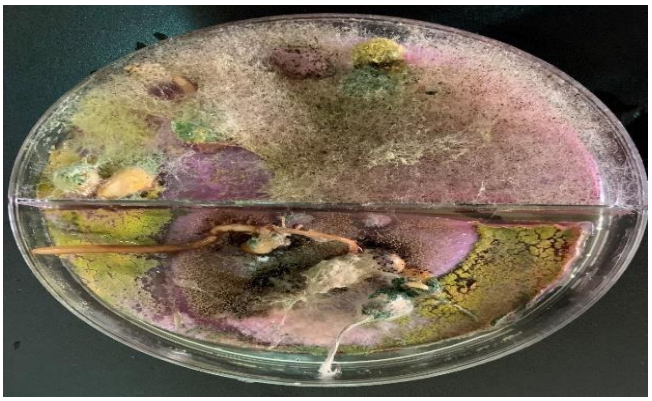

Figure 2.b: Growth of *A. flavus* and *A. parasiticus* Phaseolus bean on DRBC medium.

Three kinds of black Aspergilli were found (*A. foetidus*, *A. carbonarius*, and *A. niger*). In the Kurdistan region, several species were detected in soil and other agricultural items (Abdullah, and Abdullah, 2009; Abdullah and Muhamed, 2011; Saadullah and Abdullah, 2012a, Abdullah and Saadullah, 2013).

Isolates from *A. carbonarius*, *A. ochraceous*, and to a lesser extent *A. niger* demonstrated production of ochratoxin A potential and were isolated often from dried vines naturally polluted with ochratoxin A in the Kurdistan region (Saadullah and Abdullah, 2012a). Because of their ability to create mycotoxins, *A. flavus*, *A. parasitius*, *A. carbonarius*, and to a lesser extent, several species in the genus *Fusarium*, are the most important species contaminating beans (Moretti et al.,1998; and Heperkan et al., 2012a). *A. flavus* and *A.* n*iger* were also the most frequent species on dried beans, according to Pitt and Hocking (2009).

Figure 3.a: Growth of *A. niger* on Phaseolus bean on PDA medium.

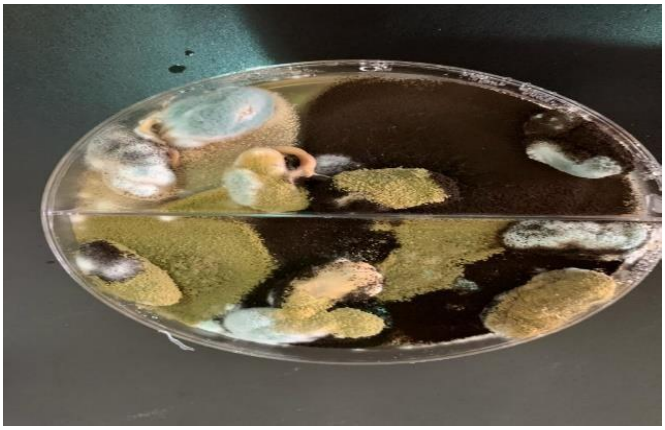

Figure 3.b: Growth of *A. carbonarius and A. niger* on Phaseolus bean on MEA medium.

Aspergillus spp. and *Penicillium* spp. are the most important storage fungus, while *Penicillium* spp. was represented by three species in the number of species isolated from beans. *P. citrinum* was the most frequent species (2.83%), followed by *P. expansum* and *P. glabrum* (1.17 and 1 %) respectively. Same results found by the Exudate Akwa et al. (2020) they isolated from beans in Kenya. There *Penicillium*, resistant species capable of growing at low water activity and tolerating high temperature (Smith and Onion, 1994).

These findings are comparable to those of Ruiz et al. (1996) in Argentina, who found that *Aspergillus* spp. and *Penicillium* spp. had the highest occurrence, with 40 and 15 percent, respectively, for freshly harvested beans. Scussel et al. (1998) looked at bean samples from various Brazilian states and found that these two genera were common (29.7 and 19.3 percent, respectively).

Fusarium sporotrichioides isolated in high frequency in the current study (15.06%) *Fusarium* is a phytopathogenic fungus that is one of the most economically important genera. This observation is concerning because *Fusarium* is a common food contamination that can lead to the development of fumonisins. *Fusarium langsethiae*, *F. Sporotrichioides*, and *F. Poae* are the most common *Fusarium* species. T-2 is rapidly converted in vivo to HT-2, which has comparable deleterious effects to T-2 with minor changes in strength (Schuhmacher-Wolz, U et al., 2016).

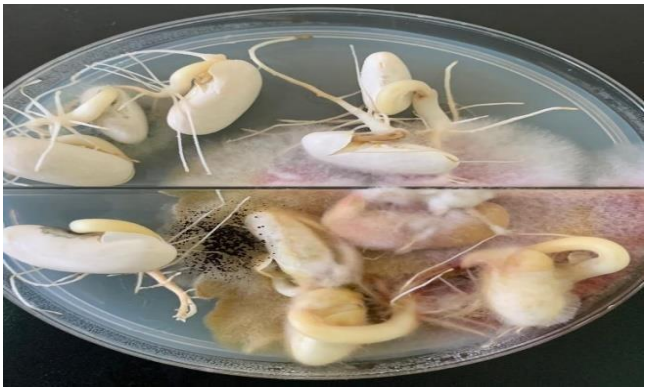

Figure 4.a: *Fusarium* on Phaseolus bean on PDA medium.

Figure 4.b: Growth of A. ochraceous on Phaseolus bean on MEA medium.

In addition, the high incidence of *Phoma* (7%) The presence of *Phoma* species in the beans analyzed poses a risk to consumers since some *Phoma* species can produce mycotoxins called cytochalasins (Griften, 1994).

The teleomorphic ascomycetes, namely *Eurotium amstelodami* were detected with percentage frequencies 1.67 %. Furthermore, when *E. amstelodami* isolates from Turkish dried figs were cultured on potato dextrose broth (PDB) medium, they produced Ochratoxin A and Aflatoxin B1 (Senyuva et al., 2008). *Eurotium* species can thrive in areas with limited water activity.

As a result, they're common in dishes with high sugar content (Pitt and Hocking, 1997). Other relatively frequent contaminants were, *Mucor*, *Rhizopus*, *Alternaria*, *Curvularia*, *Chaetomium*, yeasts and *Drechslera* Similar result was found by Romero et al. (2005).

Table 2 shows that from the total of *Aspergillus* strains isolated (20) from beans 30% were aflatoxigenic and 25% were ochratoxigenic. It revealed that the aflatoxins are more than

ochratoxins, while from the total *Fusarium* sp. 26.6% produced T2 this is in line with the study done by (Costa et al., 1998 ,2000, 2002; Cruz et al., 1990).

Table 2: screening selected genera for aflatoxigenic, ochratoxigenic and T-2/HT-2 production abilities

Genera	N	Aflatoxins		Ochratoxins		T2/HT-2	
		N	%	N	%	N	%
Aspergillus sp.	20	6	30	5	25		
Penicillium sp.	10						
Fusarium sp.	8					3	26.6
Others	30						

Table 3: Quantitative production of Aflatoxins and Ochratoxin A and T2 by *Aspergillus* and *Fusarium species in vitro* by ELISA technique.

Fungal sp.	AFs ppb	OTA ppb	T2 ppb
A. flavus 1	260.0		
A. parasiticus 1	200.0		
A. parasiticus 2	145.0		
A. parasiticus 3	100.0		
A. flavus 2	81.0		
A. ochraceus		73	
A. carbonarius		100	
A. niger		70	
Fusarium sporotrichioides 1			50
Fusarium oxysporum 2			94
Fusarium oxysporum 3			58

Table 3 showed the results of screening four isolates for each of *A. flavus*, and *A. parasiticus A. ochraceous*, *A. carbonarius* and *A. niger*, Fusarium isolated from dried beans for their aflatoxigenic, ochratoxigenic and T-2/HT-2 production abilities in culture media as detected by ELISA technique. Two strains of *A. flavus*

showed positive abilities as aflatoxin producers, whereas Three *A. parasiticus* were positive, and *A. ochraceus, A. carbonarius* and *A. niger* to produce OTA. The initial strategy to controlling the incidence of aflatoxins and ochratoxins in beans was to control contamination with aflatoxins and OTA in the field, which is extremely difficult to do because it is predominantly driven by meteorological variables like relative humidity and temperature (Applebaum et al., 1982). The highest levels of these three mycotoxins, on the other hand, have been linked to the post-harvest growth of *Aspergillus* and *Fusarium* molds on improperly preserved foods (Jay, 1992).

The aflatoxin, OTA, and T-2/HT-2 potential of the isolates studied varied significantly. This is consistent with several other studies that show that the ability to produce aflatoxin varies among Aspergillus section flavi isolates (Senyva et al., 2008, Embaby et al., 2012). According to various studies, not all strains of A. flavus may produce aflatoxin, and the ratio of non-aflatoxigenic to aflatoxin-producing isolates varies depending on the isolates' source and location (Schroeder and Bolla 1973; Abdel-Malik et al., 1993; Abdullah and Al-Mousawi, 2009; Abdullah et al., 2009).

4.CONCLUSIONS

In conclusion, based on our results that the contamination of beans by aflatoxigenic strains of *A. flavus* and *A. parasiticus* and by ochratoxigenic strains of *A. carbonarius* as well as by trichothecenes 2 of *Fusarium sporotrichodes* and *F. oxysporum* T-2/HT-2 represent a special hazard to consumers health. Therefore, such contaminated materials should be monitored before use.

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