First Report of Three kinds of Mycotoxins Dioxynivalenol, Nivalenol and Fumonisin B2 in Seeds of Seven Wheat Cultivars in Iraq.

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Summary

This study was conducted to detect and quantify three mycotoxins Dioxynivalenol, Nivalenol and Fumonisin B2 in seeds of seven wheat cultivars planted in 17 wheat fields in Basra province, Iraq. This was done by using High Performance Liquid chromatographs analysis. The results revealed that Fumonisin B2 was the predominant mycotoxin, which present in 10 fields. The lowest concentration rate of this mycotoxin was 110 μ g/Kg and the maximum was 11,228 μ g/Kg. Dioxynivalenol as a trichothecene was in the second level detected in 6 fields with a minimum concentration of 8 μ g/Kg and a maximum of 1,060 μ g/Kg. Nivalenol was found only in 4 fields ranging from 272-1900 μ g/Kg. Fumonisin B2 Only three fields showed co-occurrence of two mycotoxins (Fumonisin B2 and Nivalenol) in each, but with various concentration rates. The seven cultivars tested in this study were varied in their reactions to subjected mycotoxins. Adama 99 (A. 99) cultivar showed the highest concentration rate of both Fumonisin B2 and Nivalenol, which present with average percentage of 64% and 58% respectively. While, for Dioxynivalenol, Ebaa 99 (E.99) was on the top, it occurred in 6 fields ranging from 8-1060 μ g/Kg with an average percentage of 43%.

Keywords: Cultivars, Mycotoxins dioxynivalenol, Fumonisin B2, Mycotoxins, Nivalenol, Wheat.

Introduction

According to the statistics of Iraqi Ministry of Agriculture, Wheat (Triticum aestivum L. em. Thell) is the first cultivated harvest in Iraq, which is planted in almost 1,700 million hectares all over the country expressing the priority of this crop for Iraqi people. Also, it is one of the most important sources of human exceedingly food and the grown crop throughout the world (1 and 2). This important food source has been influenced negatively by various fungal pathogens such as Fusarium species. Fusarium head blight (FHB) and Fusarium crown rot (FCR) had damaging impacts on growing wheat causing undeniable yield losses in crop production in the most of cereal-producing countries (1). In addition to the reduction in quantity and quality of wheat production, these pathogenic fungi can be produced various mycotoxins, as the main problematic issue in food safety, in crop grains and other plant tissues, which have harmful impacts on human and animal(3).

For the production of cereal cultivation, the probability of accumulation of mycotoxins in the kernels is the main issue of health hazard (4 and 5). According to (6 and 7), the mycotoxins are the consequences of fungal secondary metabolism, which appeared once development halts or decelerates completely; they probably resulted from adaptation of the fungal growth to stressful conditions, and develop in the wheat seeds before harvesting crop fields. Generally, mycotoxins are differed in their production time, for example, the production of trichothecenes, fumonisins and zearalenone is confined before harvest, whereas ochratoxin A and aflatoxins are generated only after harvest. As stated by (7 and 8) they mentioned that principal toxins created by the diseased species of Fusarium genus are: deoxynivalenol (DON), zearalenone (ZEA), Fumonisins (FB1, FB2 and FB3), Moniliformin (MON), nivalenol (NIV) and derivatives, T2 and HT2-toxin. Commonly, they appeared on the source of grains, animal fodders and feeds . pecifically, Fusarium graminearum Fusarium and pseudograminearum are capable of producing mycotoxins which are the more serious risk of infected cereals and plant straw leading to fatal effect on human and animals (1).

The most important mycotoxins produced by these fungal pathogens as species-specific are trichothecenes including NIV and DON as well as ZEA and MON, while FB1, FB2 and FB3 produced by other Fusarium sp. (9-12) cited by (13). Type B trichothecenes DON and NIV are the main toxins created by F. graminearum and F. culmorum. NIV is mostly considered as highly toxic to humans and livestock compared to DON (14), even though DON might be highly phytotoxic compared to NIV (15). There has been a provisional maximum tolerable daily intake (PMTDI) of 1µg of the mycotoxin DON per kg of body weight, which was established by the Joint FAO/World Health Organization Expert Committee on Food Additives in 2001(16). dangerous effects Because of the of mycotoxins in humans and animals, a lot of countries have been documented guidelines or controlling restrictions for the maximum criteria of mycotoxins in human food and animal feed. The objective of this study was to detect the main kinds of mycotoxins that were produced by Fusarium species from wheat seeds through High Performance Liquid chromatographs analysis (HPLC), in order to test the occurrence and levels of DON, NIV and FB2 mycotoxins in the south of Iraq. Basra province, Iraq.

Materials and Methods

HPLC Sample preparation :During 2017/2018, after the final harvest, symptomatic wheat heads were collected from a 17 naturally diseased winter wheat fields in the south of Iraq, Basra province. A 30-35 infected heads were collected from each wheat fields that were seeded by different cultivars. Wheat heads with partially infection (upper, central and bottommost) as well as a full head collected individually, infection were cautiously pulled off, combined and air-dried. Seeds of each field were sited in polythene bags and stored in the dark room with a controlled temperature of 10°C before being tested for mycotoxin by HPLC analyses .

A total of 17 samples (100 gm. of wheat seeds from each sample) were analyzed for only three most predominant mycotoxins DON, NIV and FB2 because of the limited fund of this study. The 17 samples were sent to the Ministry of Sciences and Technology, Environment and Water Department, Pollution Treatment Centre, Baghdad for mycotoxin analyses by HPLC. The mycotoxin standards (Reference substances) of fumonisins B2 (FB2, 99% purity), nivalinol (NIV, 98% purity) Deoxynivalinol (DON, 97% purity) were supplied by the Scientific Equipment Laboratory, which were obtained them from Sigma – Aldrich (Germany).

Extraction and Clean-up : A 5gm of each ground grain sample was extracted with 30ml of the extraction solvent (water: acetonitrile: methanol in a proportion of 5:4:1, v/v) in conical flasks, with unceasing shaking for about an hour. The filtration process of the extraction solvent was done through WhatmanTm No. 4 filter paper. Approximately 30ml of every single extract was transferred to a tube with dimension of 18×85 mm, filtered by activated alumina Self-Designed Silica (SDS) and eluted in transparent tips. In order to obtain dry elute, it was evaporated on rotational evaporator. Re-dissolving of the obtained residue was finalized in 1ml of water: acetonitrile: methanol (5:4:1). For purification, every single extract of the 17 was filtered through a separate WhatmanTm filter and the total amount of solution yielded was used for the HPLC analysis (17).

Identification and calculations: Detection and identification of mycotoxin was done by straightforward comparison of retention times via using absorption spectra in the particular wavelength range. The first absorption spectra was generated via pure standard solution of each mycotoxin that was at that moment compared with those from wheat samples. and DON were determined at a NIV wavelength of 220 nm, while FB2 was determined at a wavelength of 254 nm: 220 nm by using a UV detector. The obtained data were managed by chromatographic ChemStation software (Agilent Corp.). For expressing the quantity of concentration in a tested part (in ppm/5gm of sample), according to (18) the following equation was used: $[C*1 = At.*2 / Ast.*3 \times Cst.*4]$

*1: is the quantity of concentration in a tested part; *2: is the peak area of tested mycotoxin at specific retention time, *3: is the peak area of injected mycotoxin standard (reference substance) at a specific retention time and *4: is the concentration of the injected mycotoxin standard.

Results and Discussion

Mycotoxin HPLC analysis: the retention time was used to tag the peak in the chromatogram. The three determined mycotoxins (DON, FB2 and NIV) were detected at retention time between 8.06-8.70, 7.13-7.94 and 6.32-6.90 min. respectively (Fig. 1, 2, 3, and 4).

The results of mycotoxin HPLC analyses showed that FB2 was the predominant mycotoxin, which presented in 10 (TI1, TJ, TKH, TS, D, TI2, TI3, MSR, HM and QRS) out of the 17 tested wheat fields. The lowest concentration rate of this mycotoxin terminated in positive samples of $110 \,\mu g/Kg$ in the TI1 field and the maximum rate was 11,228 µg/Kg in the MSR fields (Table,1). DON was in the second level as a trichothecene mycotoxin detected in 6 wheat fields (QM, TK, MSP, ML1, N and H), with a minimum concentration rate of 8 µg/Kg in the H field and a maximum concentration rate of $1,060 \,\mu\text{g/Kg}$ in the MSP wheat field (Table,1).



Figure, 1: HPLC chromatogram presented peak detection by absorbance at l = 220 nm and peak spectra for Deoxynivalenol (DON) concentration in wheat seeds in ppm/ 5gm.



Figure, 2: HPLC chromatogram presented peak detection by absorbance at l = 220 nm and peak spectra for Fumonisin B2 (F B2) concentration in wheat seeds in ppm/ 5gm.



Figure, 3: HPLC chromatogram presented peak detection by absorbance at l = 220 nm and peak spectra for Nivalenol (NIV) concentration in wheat seeds in ppm/ 5gm.



Figure, 4: HPLC chromatogram presented peak detection by absorbance at l = 220 nm and peak spectra for Fumonisine B2 and Nivalenol (NIV) concentrations in wheat seeds in ppm/ 5gm.

Table,1: Myco-toxins concentration rates in wheat seeds obtained by HPLC analysis using chromatographic software.

Seed / (Field)	Cultivar	Toxin	St. Con. (ppm)	Peak Area (mV.s) for St.	Peak Area (mV.s) for Sam	Sam. Con. (ppm /5gm)	Sam. Con. (µg/Kg)
QM	В.	DON	10	709.214	5.306	0.07	14
ТК	R.	=	=	=	134.384	1.9	380
MSP	E.99	=	=	=	376.405	5.3	1,060
ML1	AG 3	=	=	=	330.746	4.6	920
N	Res. 22	=	=	=	202.054	2.8	560
Н	AG 3	=	=	=	2.776	0.04	8
TI1	B.	FB2	10	118.942	6.513	0.55	110
TJ	A.99	=	=	=	193.508	16.27	3,254
ТКН	B.	=	=	=	51.847	4.35	870
TS	A.99	=	=	=	136.939	11.5	2,300
D	AG 3	=	=	=	153.778	12.92	2,584
TI2	AG 3	=	=	=	46.766	3.9	780
TI3	AG 3	=	=	=	67.021	5.63	1,126
MSR	A.99	=	=	=	667.779	56.14	11,228
HM	A.99	=	=	=	578.903	48.6	9,720
QRS	Res. 22	=	=	=	338.145	28.4	5,680
TI1	В.	NIV	10	819.380	43.534	1.36	272
MSR	A.99	=	=	=	304.118	9.5	1,900
LM2	AGRI. S	=	=	=	97.841	3	600
TJ	A.99	=	=	=	71.322	2.23	446

1ppm = 1000 ug/kg.; The obtained concentration (ppm /5gm) then multiply by 1000 and divided by 5.

The mycotoxin NIV was found only in 4 samples belonging to 4 wheat fields (TI1, MSR, ML2 and TJ), which present ranging from 272-1900 μ g/Kg in TI1 and MSR wheat fields respectively (Table,1). Based on 17 examined samples, the incidence was 58.82% for FB2, 35.29% for DON and 23.52% forNIV. Only three wheat fields (TI1, TJ and MSR) showed two mycotoxins (FB2 and NIV) in each, but with various concentration rates.

Regarding cultivars, the seven cultivars examined in this study were varied in their reactions to subjected three mycotoxins. The Adana 99 (A. 99) cultivar showed the highest concentration rate of both FB2 and NIV mycotoxins, which present with average percentage of 64% and 58% respectively (Fig.5). The FB2 was ranging from (2,300-11,228 μ g/Kg) and the NIV ranged between 272-1900 μ g/Kg, which are both higher than the concentration rate of DON that detected in other cultivars.

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Figure, 5: illustrated the cultivar types that showed concentration rates of mycotoxins (1) FB2 and (2) NIV as percentages in the 17 selected wheat fields in the south of Iraq/ Basra province.

Whereas, for DON mycotoxin, the Ebaa 99 (E.99) cultivar was on the top, which occurred in 6 fields ranging from 8-1060 µg/Kg with an percentage 43% average of (Fig.6). Importantly, the co-occurrence of examined mycotoxins were discovered together in several cultivars, such as the Barcelona (B.), Adana (A.99), Abu Ghraib 3 (AG 3) and Research 22 (Res.22) cultivars. The cooccurrence of three mycotoxins was discovered only in the (B.) cultivar but with almost low concentration rate 14, 110 and 272 µg/Kg for DON, FB2 and NIV respectively, whereas the (A.99), (AG 3) and (Res.22) cultivars were showed co-occurrence of two mycotoxins in each. In contrast to the Rasheed (R.) cultivar, which displayed only DON with 380 μ g/Kg and the AGRI Saaten (AGRI S.) cultivar showed only NIV with 600 μ g/Kg



Figure, 6: illustrated the cultivar types that showed concentration rates of mycotoxins (DON) as percentage in the 17 selected wheat fields in the south of Iraq/ Basra province.

Fungi belonging to the genus Fusarium produce several Mycotoxins as secondary metabolites, which are concerned due to their occurrence worldwide as natural contaminants in various commodities of plant sources, particularly in cereal grains (19). Due to the toxicity and existence of these mycotoxins, they have been emphasized by guidance or legal regulations in numerous countries, for example, in 2003, the FAO have performed a survey in 100 countries around the world and found that about 87% of their population possessed detailed guidelines for mycotoxins in human food and animal feed. However, these regulations or guidance of permitted limits are varied from country to country(20).

On the subject of fumonisin mycotoxin, the European Commission has been issued the maximum tolerable levels for the quantity of both types of fumonisins (B1 and B2), ranging from 200 - 4000 μ g/kg for processed cereal grains (as baby food for children) and unprocessed maize respectively cited in (19). The results of this study was clearly indicated the risky levels of all examined mycotoxins, the concentration rate of FB2 mycotoxin in the majority of examined samples was higher than the permitted levels established by the European Commission. As 9 out of 10 terminated samples present the rate of FB2 between 780-11,228 μ g/kg, except one sample

(in the TI1 wheat field) showed FB2 in concentration rate at $110 \ \mu g/kg$.

Concerning DON as one of the type-B trichothecene determined in this study, according to the European Commission, the maximum legalized levels in human food ranged from 200-1250 µg/kg for processed cereal grains (as baby foods) and for unprocessed cereals, respectively (19). However, the maximum permitted level of DON in cereals and pasta, if consumed directly by human, is 750 µg/kg, while in bread, biscuits and breakfast cereals is set at a range of 500 µg/kg (21). Our results showed that 4 samples present DON in less than permitted levels, ranging from 8-560 µg/Kg, except 2 samples in the MSP and ML1 wheat fields that showed DON in a rate of 1,060 and 920 µg/Kg, respectively. An Iraqi study conducted by (22) investigated wheat straw collected from animal farms using ELISA methods reported that the majority of tested contaminated with samples was DON mycotoxin at a range of 722 μ g/Kg. Whereas, our results found that the range of DON in examined wheat seeds was 490.34 µg/Kg, which means less than the average level indicated in the straw wheat but it still at the unsafe concentrations.

With respect to NIV, many studies, such as (23)in England, (24) in Japan and (25) in Republic of Korea, have frequently isolated and identified fungi that produce NIV more than those produce DON. Globally, regulatory limits for NIV have not yet been documented. However, as stated by (26 and 27) that NIV is a shared mycotoxin occurred in a number of cereal production regions around the world, particularly in Asia. Many Japanese research works, such as (24 and 28), have reported the co-occurrence of DON and NIV in their local wheat and barley, and consequently both mycotoxins are counted in FHB management approaches (29). Our results put Iraq in a parallel situation like in Japan and Republic of expressly due to Korea. the toxigenic probability of the inhabitant population of fungi. The concentration rates of NIV determined in this study, although the average level (804.5 μ g/Kg) was not higher than 1000 µg/Kg, one out of 4 terminated levels (1900 $\mu g/Kg$) is of pronounced toxicological

importance supposed the greater toxicity of NIV likened with DON .

Outstandingly, all domestic wheat cultivars examined in this study (AG 3, Res. 22, E. 99 and R.) were contaminated with DON. One of them (E.99) had 1,060 µg/Kg, which is very close to the maximum permitted levels issued by European Commission. As reported by [30] that the highest concentration of tolerable DON in raw food for humans is $1250 \mu g / kg$ regulation according to of European Commission. Along with (31-33) escalating Fusarium mycotoxins, consciousness of particularly those produce type-B trichothecenes, such as DON, took place in present years with the reappearance and concern of FHB as the most important risk to food security. In general, the trichothecene group has been related to interminable and deadly intoxication of both humans and animals that consume contaminated food and respectively. Accordingly, these feed mycotoxins considered as immunosuppressant, teratogen and neurotoxin by the World Health Organization (WHO) (34).

Hence, crucial actions such as continuous observing and level determination of all Fusarium mycotoxin in cereal grains. especially in wheat cropping system, food products and commodities should be taken into account of Iraqi researchers in future studies. Association between wheat grains contaminated with mycotoxins and disease severity of FHB has been executed for many years, and positive interactions have been informed for the highest DON concentration (35 and 36). On the other hand, the opposite consequences have also been reported (37 -39).

Another experiment conducted by the same authors of this study (data not published), A.99 cultivar had the highest FHB incidence (22%) and also showed the highest concentrations for both FB2 and NIV mycotoxins (64 and 58) % respectively (Fig. 5). Additionally, the level of DON mycotoxin was at 19% in the AG 3 cultivar (Fig. 6), which had FHB incidence of 21%. Reversely, B. cultivar had 23% of FHB incidence but showed 0%, 7% and 13% of mycotoxin concentration for DON, FB2 and NIV respectively (Fig. 5 and 6). Accordingly, our results in part agree with that of (35 – 36) and later authors (37 - 39) for the positive and negative correlation between FHB occurrence and mycotoxin levels .

Even with the increasing new threat and worry about FCR and FHB diseases in wheat cropping system in Iraq, intensive care and reporting of the incidence of Fusarium toxins in wheat grain were not found or limited to a scarce studies, which focused on what chaff in animal farms. The results of recent study establish the first report of the concentration rate, co-incidence and spatial distribution of FB2 and two type-B trichothecenes (DON and NIV) of great concern from the seeds of main wheat-growing fields in the south of Iraq, Basra province, Iraq.

It can be concluded that the probability of accumulation of mycotoxins in the kernels, those produced by Fusarium especially species, is the main issue of health hazard for animals and humans. The relationship between FHB incidence and mycotoxin concentrations is predominantly associated with the cultivar practiced and particular environmental conditions. FHB concentration in some cultivars connected well with FB2 and NIV occurred in the grains in the recent study, but negative connection was detected for DON. There is a need for comprehensive similar studies to examine more wheat grains produced in the rest of Iraqi provinces detecting mycotoxin types especially those associated with FHB and FCR diseases. Also, the effects of these mycotoxins on grain quantity and quality should be considered to determine their economic losses.

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التقرير الاول للكشف عن ثلاثة انواع من السموم الفطرية Fumonisin B2وNivalenol و Dioxynivalenol في سبعة اصناف من بذور الحنطة في العراق محمد حسين مناتي ومهند خلف محمد امين مختبر امراض النبات، قسم علوم الحياة، كلية العلوم، جامعة البصرة E-mail: abo azher70@yahoo.com

الخلاصة

اجريت هذه الدراسة للكشف عن والتقدير الكمي لثلاثة انواع من السموم الفطرية Eumonisian O و Evexynivalenol و Fumonisisn B2 الإستشراب او فصل السوائل عالية الدقة (HPLC). أظهرت النتائج ان12 حقل في محافظة البصرة بأستخدام تقنية الاستشراب او فصل السوائل عالية الدقة (HPLC). أظهرت النتائج ان12 Fumonisisn B2 هو اكثر السموم الفطرية السائدة، حيث تم تسجيله في 10 حقول حنطة بأقل معدل تركيز 110 ميكروغرام / كغم واعلى معدل تركيز قا11. ميكروغرام / كغم واعلى معدل تركيز قال معدل تركيز ما الميكروغرام / كغم واعلى معدل تركيز المعروب ميكروغرام / كغم واعلى معدل تركيز قال المائدة، حيث تم تسجيله في 10 حقول حنطة بأقل معدل تركيز 110 ميكروغرام / كغم واعلى معدل تركيز المعرف ميكروغرام / كغم واعلى معدل تركيز المعرفي ميكروغرام / كغم. أما (Deoxynivalenol) كسم فطري ثلاثي الحلقات فظهر بالمستوى الثاني حيث تم اكتشافه في ستة حقول بأدنى تركيز بلغ 8 ميكروغرام / كغم وأعلى تركيز المولى الاخير معول بأدنى تركيز بلغ 8 ميكروغرام / كغم وأعلى تركيز سمية تراوحت ما بين 272-1900 ميكروغرام / كغم. أما (Dioxynivalenol) كسم فطري ثلاثي الحلقات فظهر بالمستوى الثاني حيث تم اكتيبان بأن حقول بأدني تركيز بلغ 8 ميكروغرام / كغم وأعلى تركيز سمية تراوحت ما بين 272-1900 ميكروغرام / كغم. حيث تبين بأن معدل الاصابة الاجمالي في الحقول ال 17 بهذه السموم الفطرية هو 20% ميكروغرام / كغم. ما كم معدل تركيز بين ألاحمال المواماة الإحمالي في الحقول ال 17 بهذه السموم الفطرية (Sumonisis B2 وعرام / كغم. حيث تبين بأن و20% معدل الاصابة الاجمالي في الحقول ال 17 بهذه السموم الفطرية (Sumonisis B2 وعرام / كغم. حيث تبين بأن معدل الاصابة الاحمالي في الحقول ال 17 بهذه السموم الفطرية وSumonisis B2% وعرام / كم معدل وعرام / كم معدل تركيز بكان من السموم الفرية والاصابة الار وعرام / كم وعرام / كم والا معدل المور المور من السموم الفرية (Sumonisis B2 و 20%) معال ولاك معدل تركيز لكل من (Sumonisis B2 و 20% على ولاك معدلات تراكيز محتلفة. الأمور معنوب قول المعنون المام و 20% و 30% على ولاك محدلات تراكيز تراوحت بين 8 – 100% وعلى الكرو غرام /كم وبمعدل متوسط قدره 30%. والحي الخوي م حيث أظهر الصنف أبار المنف ابا وي العنوا و المام و 30% معدل معدل معدل معنوس موافى 6 حول وبنسب تراكيز تراوحت بين 8 – 100%، معدل مومدل متوسط ق