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OCCURRENCE OF DEOXYNIVALENOL (DON) TOXIN IN CEREALS AND CEREAL PRODUCTS AND DETECTION BY BIOASSAY IN BASRAH CITY – IRAQ

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Abstract

The aim of this study was to show the occurrence of Deoxynivalenol toxin which is produced naturally contaminated in different cereals and their products. The samples were collected from markets in Basrah city in Iraq. We used microorganisms as a biological tests to detect (DON) toxin in these cereals and their products. DON toxin was detected by thin layer chromatography (TLC). The results showed that 7 out of 76 samples of cereals and their products (9.21 %) contained DON toxin. The positive samples were 5 of 18 wheat samples (27.7%) and 2 of corn from 18 samples (11.11%) DON toxin was not found in all other samples of cereals that were tested including barely , corn ears and other cereal products such as flour , bread and toasted corn. Using microcrograms as abioassay for the detection of DON toxin showed that Bacillus subtilis was more sensitive towards DON toxin compared with E. coli and saccharomyces cerevisiae in all concentration of DON toxin . The minimum inhibitory concentration (MIC) for Bacillus subtilis was 5 ug/ Disk.

Key words : Mycotoxin DON Bioassay Trichothecenes

Introduction

Mycotoxins are naturally secondary metabolites produced by some fungi especially some types of genus Aspergillus , penicillium and Fusarium , they can be found in feeds and foods that may cause diseases or death to animals and human (WHO , 1990) (Bahoot , 2003) (Mohammed et al ; 2020) .

Trichothecenes are an important group of mycotoxins that may contaminted cereals and their products . These toxins are produced by certain Fusarium species that frequently infect corn , wheat , oats , barely , rice and other grains in the field or during storage . (Sobrova , et al , 2010) (Goyarats , et al , 2007) .

Deoxynivalenol (DON) is one of several trichothecenes . It was also known as vomitoxin due to its strong emetics effect after consumption (Peska , 2007) . DON toxin is mainly produced by F. graminearum and F. culmorun .

This toxin is considered as one of most important trichocences . DON toxin belongs to type B of trichothecens (Al-Julafi and Falih , 2001) .

(Malloy and Marr , 1997) . DON contains active carbonial group at carbon atom number 8 , which was 12 , 13 – epoxy -3 , 7 , 15 – trichydroxy – trichothec – 9 – en – 8 – one . The structural formula for DON toxin are shown in Fig No. 1 . It's Molecular formula is C_{15} $H_{20}O_6$. At present time DON and T_2- toxins are considered to be the most dangerous problems facing the world because they influence animal production and food safty (WHO , 1990) (Mohammed et al , 2020

) FAO reported that more than 25% of foods in the world are contaminated with mycotoxins . Many of the known methods used to detect mycotoxins are complex because of the existance of many different agricultural commodities . Now the number of mycotoxins exceeds 200 types . (Nowar and Ntor , 1989) (Mohammed et al , 2020) . The methods used to detect mycotoxins included U.V, HPLC , TLC and biological assay (Van Egmond , 1995) (Ibrahim and Al-Jubory ; 1998) .

The aim of this study was to detect DON toxin in cereals and their products by using a microbiological assay in Basrah city Iraqi . This method was depended because of its low cost and fast performance comparing with other methods .

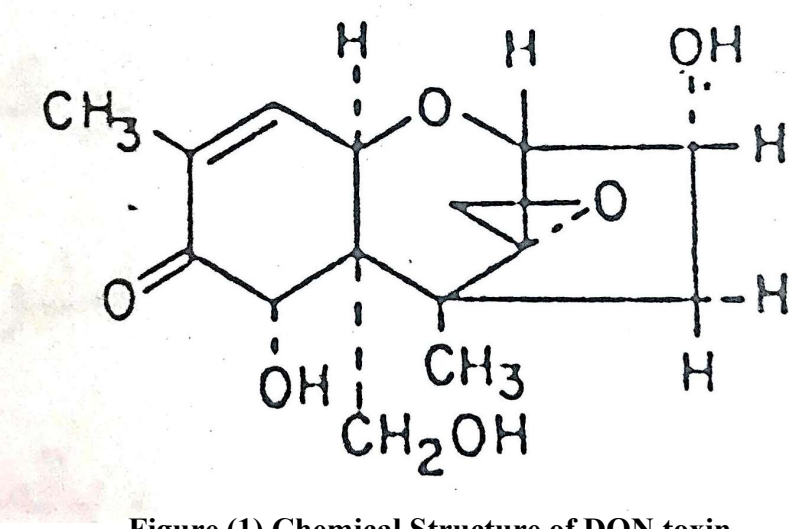

Figure (1) Chemical Structure of DON toxin

Materials and Methods

Cereals samples

Wheat , barely and corn samples were collected from markets in Basrah city Iraq , The weight of each sample was one kg , samples were transferred to laboratory by polyethylene bags . They were 18 samples for each type of cereal cereals products which were purchased from the same markets included bread , flour and toasted corn .

They were six samples for each type in addition to four samples of corn ears . The weight of flour samples were 500 g , 150 to 100 g for each bread and toasted corn .

Deoxynicelnol toxin Standard

Pure DON toxin was obtained by proffessor Dr. J. Mirocha / Department of plant diseases in Minsota university in American (by personal) .

DON toxin in a white powder and it was prepared by weighting 1 mg of DON dissolved in 1 mL of chloroform (Grost- Allman and Steyn , 1979) . The stock solution was stored at – 18 C until the usage .

TLC technique

Merck pro-coated silica gel plates (20 x 20 cm and 0.25 mm) for thickness were used . The plates were activated by putting them in oven at 110 C for one hour before usage .

The standard solution of DON toxin and Extractions of contaminated cereals and their products were spotted on baseline 2 cm from the bottom of the plate with a graduated 5 uL pipette . Then the plates were developed 16 cm in the appropriate solvent system in a tank lined with filter-paper (Grost – Allman and Steyn , 1979) .

Analytical – reagents grade were used throughout .

Detection of DON toxin

The silica gel plates were tested by using the spray reagents Alminum chloride 20% (prepared by Ethyl alcohol) .

The plates were put in an oven at 110 C for 10 min and the spots were tested by U.V Some spotes were blue (positive) which means that they contained DON toxin (Ichinoe et al , 1983). Other were negative and had no DON Toxin .

Culture media

All the media that were used in this study were from oxoid company . They were yeast – extract –peptone glucose agar for the activation and growth of sacchormyces cerevisiae Nutrient agar media for Bacillus subtilis while macconekey agar media was used for E.coli.

Microbial isolates

Pure culture for B. subtilis and E.coli were obtained from the center of murin science Basrah university Iraq . The isolate of saccharomyces was purchased from local market and activated on Yeast – Extract peptone – glucose at 25 C for 18h and cultured at PDA media (pitt and Hocking 1997) .

Extraction of DON toxin from contaminated cereals and their products

The detection of DON toxin in contaminated cereals and their products were made according to the methods described by (Chang et al , 1984) and modified by (Abbas et al , 1985) as follow : 50 g of contaminated milled sample was taken and extracted by blending in awaring blender by adding 200 ml of acetonitril . and water (84 : 16) for 3 min at high speed. The filterate passed throughout filter paper (Whattman No. 4)

10 ml from filtrate was collected and passed on activated chorcol-alumina column as shown in Fig. No. 2 .

The filtrate was collected in flask and evaporated to dryness . The Residne was disolved in amount of acetone and transfered into vial and it was evaporated to dryness . The residue was dissolved in 2 ml of mixture of methanol and water (77 : 23) .

Then they were mixed well for 1 min . The detection for DON toxin was done by TLC and some reagents according to (Ichinoe et al , 1983) .

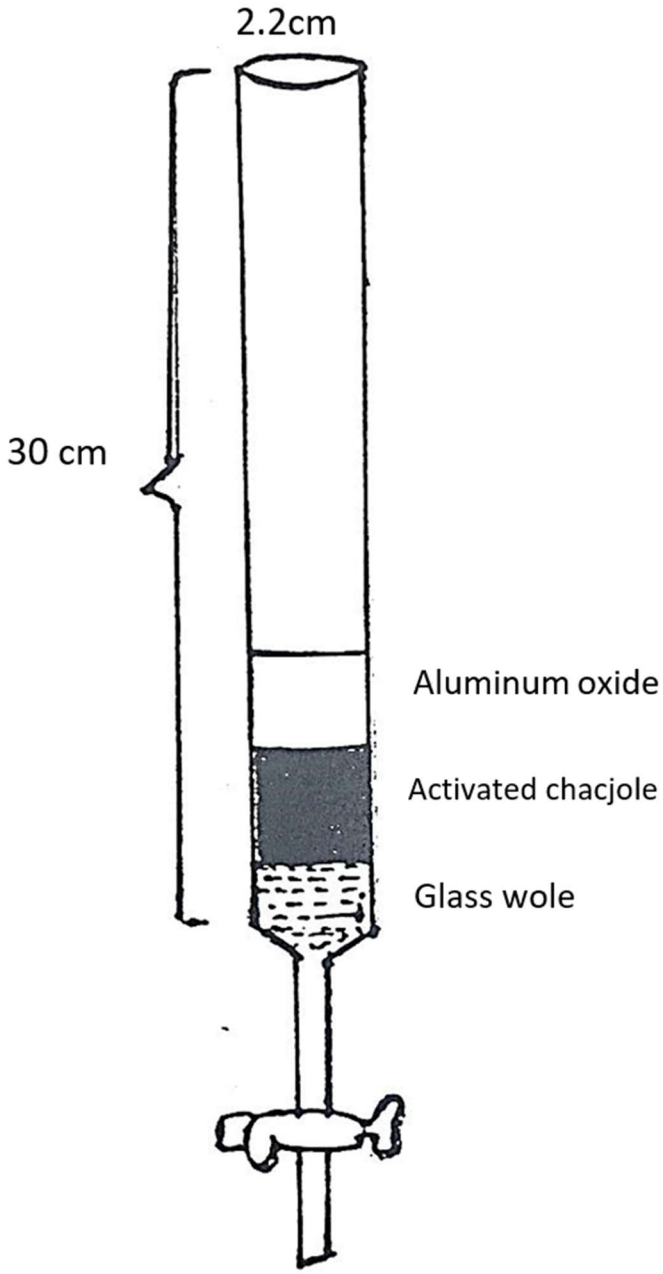

Figure (2) Chromatography column used for purification of DON toxin

Antimicrobial activity of DON toxin

Disk diffusion assay was used to study the antimicrobial activity of standard DON toxin . The disk contained different concentrations of DON toxin and they were placed upon the surface of media which contained the microbial cultures by using streaking methods . The plates were incubated at 37 C for 24 h for Bacillus subtilis and E.coli , while incubation temperature of Sacchoromyces cerevisiae was 25 C for 24 h . The diameter of inhibition zone was measured (mm) that was produced by using different concentrations of DON toxin standard curve was drawn for the more sensitive microbial to DON toxin as shown in Fig. No.3 . (Stott and Bullerman , 1975) (Madhyasta et al , 1994) .

Results and Discussion :

Occurrence of DON toxin in cereals and their products

Thin layer chromatography analysis was done for the detection of DON toxin in all samples . The results showed that the presence of DON toxin in 7 of the 76 samples analyzed (9.21 %) . These results are shown in table No. (1) .

The contaminated samples were 5 out of 18 of wheat (27.77%) and 2 out of 18 of corn (11.11 %) . DON toxin was not detected in all samples of barely and other cereal products such as flour , bread and toasted corn .

DON toxin was not found in all samples of corn ears . The concentration of DON toxin in positive samples ranged from less than 0.10ug/g to 0.80 ug/g.

The highest amount was recorded in corn samples (Table No. 1) . Results obtained from current study showed the occurrence percentage of DON toxin (9.21 %) .

Table (1) Occurrence of DON toxin in cereals and products

Sample	Occurrence positive / total	% contaminated	Rang quantity ug/g
Wheat	5 / 18	27.77	Less than 0.1-0.6
Barely	0 / 18	0.0	—
Corn	2/ 18	11.11	0.60 – 0.80
Flour	0 / 6	0.0	—
Bread	0 / 6	0.0	—
Toasted corn	0 / 6	0.0	—
Corn ears	0 / 4	0.0	—
Total	7 / 76	9.21	Less than 0.10-0.80

— Don toxin not detected .

In agriculture commodities studied . That does not agree with the result reported by (Truckses et al , 1996) which was 40% , and less than findings on (Martins and Martins , 2001) that they found that 80% of wheat samples were contaminated with DON toxin . While (Labgseth and Rundberget , 1999) reported that , from 449 samples of different cereals analyzed in worway to detect DON toxin and other toxins . 17 % and 14 % of barely and wheat samples were positive to DON toxin . The results also do not agree with findings in Saudia Arabia when (Al-Julafi and Al-Falih , 2001) studied the detection of Trichothecenes including DON and T_2 toxin in animal feeds and food stuffs during 1997 – 2000 . They found the high occurrence of DON toxin comparing with other types of trichothecenes . In Koria , Kim ,et al , 1993 , reported that DON toxin was found mainly in the samples of cereals such as barely and corn .

Many studies in last years have been shown the Fusarium mycotoxins have been the largest group of mycotoxins which includes more than 140 types . The Fusarium toxins belong mainly to F.graminearum and F.culmorium due to the high toxicity and their occurrence in different agricultural commodities and their effect on human health . These toxins are abundant in cereals

and their products (Peska , 2007) (Knshiro , 2008) (Yazar and Omurtage , 2008) (Sobrova et at , 2010) (Mohammed et al , 2020) .

Table (2) The effect of DON toxin towards the growth of microorganisms by dis-diffusion assay .

DON toxin concentration ug/Disk	The diameter of inhibition zone (mm)		
	Bacillus subtilis	E.coli	Saccharomyces cerevisiae
0	0	0	0
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	2.0	0	0
10	3.5	0	0
20	6.0	0	3.9
30	8.0	2.9	5.5

Antimicrobial activity of DON toxin

The effects of DON toxin on microorganisms are shown in table (2) . The inhibition zone of various microorganism indicated that the concentration of DON toxin that ranged from 1ug/Disk to 10 ug/Disk had no effect against sacchoromyces cerevisiae . When the concentration of DON toxin was increased to 20 , 30 ug/Disk the sacchoromyces cerevisae became more sensitive .

The dimeter of inhibition zone was 3.9 and 5.5 mm respectively . The concentration of DON toxin that ranged from 1 to 20 ug / Disk had no effect towards E.coli . The results (Table 2) showed that Bacillus subtilis was more sensitive to DON toxin at concentration 5ug/ Disk . The dimeter of inhibition zone was 2.0 mm and increased to 3.5 , 6.0 , 8.0 mm when the concentration of DON toxin increased to 10 , 20 and 30 ug/Disk respectively .

The amount of DON toxin (5ug / Disk) that inhibited Bacillus subtilus in this study agreed with previous results recorded by (Al-Julifi and Al-Khaliel , 1993) (Boutibonnes , 1980) .

They found that Bacillus was more sensitive towards Ocharotoxin A , but our results do not agree with (Mohammed et al , 2020) and (Ali-vfhmas et al ,1998) when they studied the effect of T-2 toxins against some microorganisms .

The results obtained during this study showed that Baillus subtilis was more sensitive to DON toxin than E.coli and saccharomyces cerevisiau . The data reported here indicate that a liner relationship clearly exists between the amount of DON toxin (ug/Disk) and the diameter of inhibition zone (mm) of Bacillius subtilis . This is shown in (Figure 3) . The minimum inhibitory concentration (MIC) was 5ug/Disk .

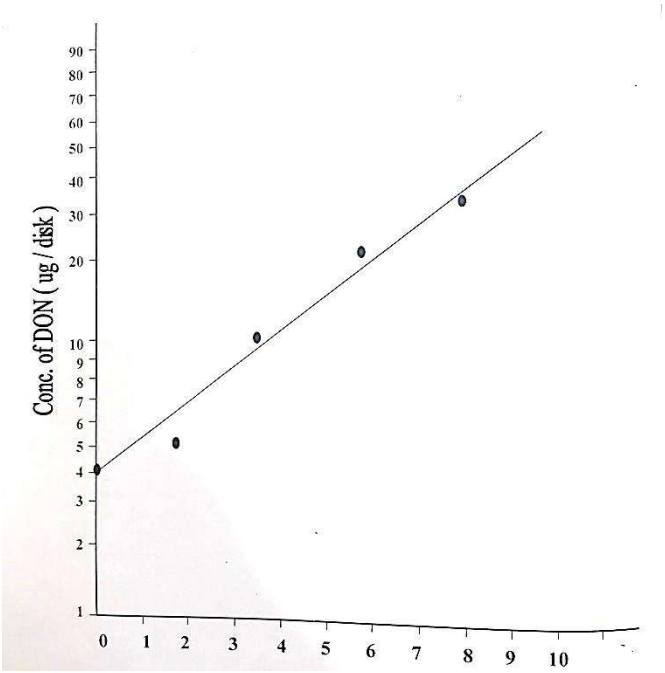

Figure (3) Standard curve for the effect of DON toxin towards Bacillus subtilis

The Effect of incubation temperature on sensitivity of Bacillus subtilis towards DON toxin

Table (3) shows the results of the effect of incubation temperature towards sensitivity of Bacillus subtilis to DON toxin . When the concentration of DON toxin was constant at 10ug/Disk .

It was found that the inhibition zone for Bacillus subtilis was zero at incubation temperature 10 C^o and when incubation temperature was increased to 20 C^o , 30 C^o , 35 C^o , the inhibition zones increased to 3.1 , 7.5 , and 9.7 mm respectively . The study of the effect of some mycotoxins on the sensitivity of some bacteria such as Bacillus was also carried out by some researches who found that mycotoxins might cause a physiological damages , reduce the growth rate and cause inhibition for spore formation in Bacillus .

In addition to that mycotoxins effect in some bacteria Enyme synthesis (Stott and Buller man , 1975) (Boutibonnes , 1980) (Ali-vfhmas et al 1998) (Mohammed et al , 2020) .

Table (3) The effect of incubation temperature on the sensitivity of Bacillus subtilis to *DON toxin .

Incubation temperature C	Inhibition zone (mm)
10	0.0
20	3.1
30	7.5
35	9.7

* The concentration of DON toxin was 10 ug / Disk .

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