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OCCURRENCE OF MYCOTOXINS (T_{-2} TOXIN) IN CEREALS AND CEREAL PRODUCTS AND THEIR DETECTION BY BIOASSAY IN BASRAH CITY, IRAQ

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Abstract

This study focuses upon the occurrence of T_{-2} toxin that are produced as a naturally contaminated in wheat, barely and corn and their products which were collected from local markets in Basrah city, Iraq. In addition used of Microorganisms as a biological assay to detect T_{-2} toxin in cereals and cereals products were also studied. Results show that T_{-2} toxin was detected by thin layer chromatography (TLC) was found in 3 out of 76 samples of cereals and cereals products. They were 2 of 18 corn samples (11.11 %) and 1 of corn ears samples (25%). T_{-2} toxin was not found in all other samples of cereals (wheat and barely) and cereal products (flour, bread and toasted corn). Through studying the possibility of using microorganisms as bioassay for detection of T_{-2} toxin, Saccharomyces cerevisiae showed more sensitivity to T_{-2} toxin compared with the *E.coli* and Bacillus subtilis in all concentrations and the detection limit for it was 5 ug/ disk. The type of carbohydrates source in culture media influenced the sensitivity of Saccharomyces cerevisiae to wards T_{-2} toxin.
Keywords : Mycotoxins, Bioassay, T_{-2} toxin, cereals.

Introduction

Mycotoxins are toxic compounds that are naturally produced by certain type of fungi, they are occurring on numerous food–stuff and feeds such as grains and cereals and result in substantial losses to agriculture (Stoloff, 1976). Also containing many agriculture commodity particularly stored products.

Mycotoxins including T_{-2} toxin are considered to be most dangerous problems facing the world because they influence animal production and food safety. FAO reported that more than 25% of foods in the world are contaminated with mycotoxins (Bahoot, 2003). (Konihi and Sugiyoma, 2008) and more than 50% of that percentage (25%) were in Africa. Mycotoxins are ubiquitous, potent, biologically active toxins, which even at low concentrations may cause numerous diseases in animal and humans (Ali–vfhmas *et al*., 1998) and (Mollay and Marr, 1997). T_{-2} toxin is a common trichothecenes type A mycotoxin produced by the *Fusarium* species of Fungi such as *F. sporotrichioides*, *F. Solani* and *F. nivale*. Which can infect grain crops and cereals and its products. (Omurtag and Yazeioglw, 2000) (Yoshizawa *et al.*, 1982) some time T_{-2} toxin may be produced by Trichoderma (Gentey and Cooper, 1983) The structural formula for T_{-2} toxin are shown in fig No. 1 and which has molecular weight 466-5 and Molecular formula $C_{24}H_{34}O_9$.

Mycotoxins detection methods are very complicated due to variety of Agricultural commodity and the huge number of mycotoxins which are now more than 200 types (Nowar and Ntor, 1989) and (Egmond, 1995).

There are many methods to detect the mycotoxins such as U.V., HPLC, TLC and Biological assay. However there are rare published reports on the Detection of T_{-2} toxin by using microorganisms as a biological assay techniques. This technique are a rapid simple and inexpensive in comparison with other tests (Ali–Afhmas *et al*., 1998) (Scott and Bullerm, 1975).

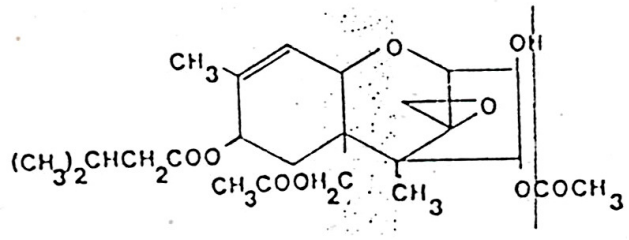

Fig. 1 : Chemical structure of T-2 toxin

The purpose of this study was to detect quantities of T_{-2} toxin contaminating the cereals and cereal products by using a biological assay.

Materials and Methods

Cereals samples

Three types of cereals (wheat, barely and corn) were used, they were collected from local markets in Basrah, each sample weight 1 kg transferred to laboratory by polyethylene bags.

They were 18 samples for each. Cereals products (bread, flour and toasted corn) were also collected from the same markets, they were 6 sample for each flour and bread and toasted corn, weights sample for flour was 500 g and 150–100 g for bread and toasted corn, also four sample of corn ears were collected.

T_{-2} toxin standard

Pure sample of T_{-2} toxin was obtained from Prof. Dr. J. Mirocha, Department of plant diseases, University of Minsota, America (by personal).

The toxin is a white powder and preparation by weight 1mg and dissolve in 1ml from Choroform (Gorst-Allman and Steyn, 1979). The standard solution for toxin was storage at 18 C^o until used.

TLC products

Merck pre-coated silica gel plates (20 x 20 cm and thickness 0.25 mm) were used. The plates were activated in oven at 110 C^{o} for 1 hour before used.

Standard solution of T_{-2} toxin and Extractions of contaminated cereals and its products were spotted on a baseline 2 cm from the bottom of the plate with a graduated 5uL pipette, and the plate was then developed 16 cm in the appropriate solvent system in a tank lined with filter-paper (Gorst-Allman and Steyn, 1979). analytical-reagent-grade were used throughout.

Detection of T-2 toxin

The developed plates were examined by using the spray reagents

1. 2,4-dinitrophenyl hydrazine (2,4 DNP).
2. Sulfuric acid 2.5% and the plates were tested under U.V. at 366 nm. (Omurtag and Yaziciglu, 2000).

Culture media

The cultures media were used in this study as following: Yeast extract peptone glucose agar for activation and growth *Saccharomyces cerevisiae* Nutrient agar for activation and growth Bacillus subtilis, Macconkey agar for *E. coli*. All media from oxoids company.

Bacterial isolates

Pure culture for Bacillus subtilis and *E. coli* were obtained for the center of Marine science, University of Basrah, Iraq. While the isolate of *Saccharomyces cerevisiae* was obtained from local market and was activated on yeast-extract-peptone-glucose at 25 C^{o} for 18h. and was cultured on PDA media (pitt and Hocking 1997).

Extraction of contaminated cereal and cereals products

For detection of T-2toxin in contaminated samples of cereals and its products were made according to the methods descried by (Gorst-Allman and Steyn, 1979) as follow : 100g of contaminating milled cereal samples and its products were extracted by blending in awaring blender with 400 ml of methanol-chloroform (1:1), the filtrate was evaporated to dryness, the resultant brown residue was dissolved in 200 ml of 90% methanol and n-hezane (1:1). The methanol layer was evaporated in 200 ml of chloroform and water (1 : 1). The chloroform layer was extracted with saturated sodium hydrogen carbonate solution (3 x 100 ml). The chloroform layer was concentrated and contained T_{-2} toxin.

The dis-diffusion method was used to measure the antimicrobial activity of standard T_{-2} toxin. Discs containing different concentrations of T-2 toxin were placed upon the surface of media which contain the culture of microoganisms by streaking method, then the plates were incubated at 37 C^{o} for 24h for each of *B. subtilis* and *E. coli* and at 25 C^{o} for of saccharomyces. The inhibition zones of microbial growth produced by the different concentration of T_{-2} toxin were measured (mm) and standard curve was drown to the more sensitive microbial to T_{-2} toxin (Scott and Bullerman, 1978); (Madhuasta *et al*., 1994).

Results and Discussion

Natural Occurrence of T_{-2} toxin in cereals and cereal products

Thin – Layer chromatography analysis were used for the detection of T_{-2} toxin in samples. The present study showed the presence of T-2 toxin in 3 of the 76 samples analyzed, giving incidence of 3.94%. The percent and occurrence and relative amounts of T-2 toxin in the agricultural commodities are summarized in Table 1. The contaminating samples were two out 18 of corn (11.11 %) and one out 4 sample of (corn ears) (25%) T_{-2} toxin was not detected in any sample of wheat barely and cereal products such as bread flour and tasted corn. The concentration of total T_{-2} toxin in positive samples ranged from or 0.10ug/g to 0.17ug/g. The highest concentration were recorded in corn samples. Results from the currents study on the occurrence percentage of T-2 toxin (3.94 %) in agricultural commodities agree with findings reported by WHO (1990) which found that the percentage of food contaminates with T_{-2} toxin in the world was less than 10%, and the concentration of T_{-2} toxin in the most food was reached to 0.1ppm. This concentration was agree with finding in this study.

The contaminated percentage in all samples in this study (3.94%) were less than findings on (omurtog and Yazieiogh, 2000) in Turkey during a study of the natural occurrence of T-2 toxin in 30 samples from cereals and cereal products which found the contaminated samples percentage with T_{-2} toxin was 6.7%, and the concentration of toxin in flour corn was 1.90 ppm. In our study T_{-2} toxin was present in the lowest concentration and was detected only in corn ears. This results agree with findings in Saudi Arabia when (Al-Julafi and Al-Falih, 2001) study the detection of Trichothecenes including T-2 toxin in animal feeds and food stuffs during 1997–2000. Similar findings have been reported by other investigators (Schollenberger *et al*., 1999; Kawamura *et al*., 1988). In recent years many studies have shown that fusarium species are contaminated of foods – stuff and feeds, which causes a major agricultural problem and produce an important group of mycotoxins which called trichothecenes and many reports pointed out that some trichothecenes including T_{-2} toxin are closely associated with some alimentary diseases in humans and animals (Al-Julifi and Al-Falih, 2001) and (Omurtag and Yazicioglu, 2000).

Table 1 : Occurrence of T-2 toxin in cereals and cereal products

Sample	Occurrence no. positive / total	% Contaminated	Rang. Quantity ug/g
Wheat	0 / 18	0	-
Barely	0 / 18	0	-
Corn	2 / 18	11.11	Less than 0.10 – 0.17
Flour	0 / 6	0	-
Bread	0 / 6	0	-
Toasted corn	0 / 6	0	-
Corn ears	1 / 4	25	0.11
Total	3 / 76	3.94	Less than 0.1 – 0.17

- T_{-2} toxin not detected.

In this study T_{-2} toxin was not detected in any samples of cereal products tested similar findings have been reported by other investigators (Omurtag and Yazicioglu, 2001 and Kawamura *et al*., 1988).

The effect of T-2 toxin on microorganisms

The data (Table 2) for inhibition zones of various microorganisms inducted that the concentration of T_{-2} toxin from 1 to 4ug/disk had no effect against all microorganisms which they were tested. When they were increased the concentration of T_{-2} toxin to 5ug/Disk the results indicated that *Saccharomyces cerevisiae* was more sensitive to T_{-2} toxin from the diamater of inhibition zone which was 1 mm and increased to 3.5, 6.5 and 9 mm when the concentration of T_{-2} toxin increased to 10, 20 and 30 ug/Disk respectively, while the same concentrations above had no effect against *Bacillus subtilis* and *E. coli*.

The results obtained during this study showed that saccharomyces cerevisiae was more sensitive to T-2 toxin than B. subtilis and E.coli, the data reported here indicate that a liner relationship clearly exists between the amount of T_{-2} toxin (ug/Disk) and the diamater of inhibition zone (mm) of *Saccharomyces cerevisiae*. A standard curve for T_{-2} toxin inhibition for *Saccharomyces cerevisiae* was established (Figure 2). The lowest amount of T-2 toxin giving a response was 5 ug / Disk. Results are in full agreement with that previous recorded by (Schapper and Khachatourians, 1983 and 1984) Showed that saccharomyces was more sensitive to T_{-2} toxin and reported that high sensitivity of saccharomyces toward T_{-2} toxin because T_{-2} toxin inhibition of protein and DNA synthesis in Eucaryotic cells or damaged of chromosomes.

Table 2 : The effect of T_{-2} toxin towards the growth of Microorganisms by Disk – diffusion assay.

T-2 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	0	0	0
10	2.3	0	3.5
20	5.5	1.7	6.5
30	7.6	2.5	9.6

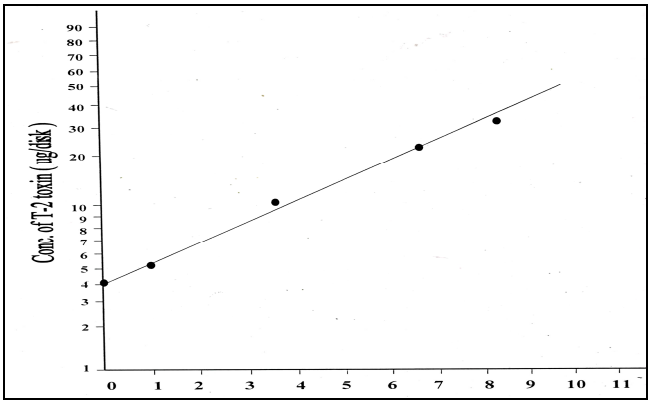

Fig. 2 : Standard curve for the effect of T-2 toxin towards *Saccharomyces cerevisiae*

The effect of carbohydrate source on sensitivity of *Saccharomyces cerevisiae* to T_{-2} toxin.

Results in table 3 indicate that a relationship between the inhibition zone of *Saccharomyces cerevisiae* and type of carbohydrate source in culture media, when the concentration of T_{-2} toxin was constant at (10ug/Disk). The inhibition zone for *Saccharomyces cerevisi*ae was 13.1 mm by using maltose as a source for carbohydrate in media, while the inhibition zone was 6.0 mm at using sucrose and reduced to 4.0 mm at using glucose source for carbohydrate, similar findings have been reported by (Schapper and Khachatourians, 1983, 1984) which they also carried out a study on the effect of Environmental factors on the sensitivity of saccharomyces species to wards T-2 toxin.

Table 3 : The effect of source of carbohydrate on the sensitivity of *Saccharomyces cerevisiae* towards T_{-2} toxin

Source of carbohydrate	The diameter of inhibition zone (mm)
Maltose	13.1
Sucrose	10.1
Glucose	4.0

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