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Effect the contamination with *Aspergillus flavus* on quantity of some trace elements composition in wheat, rice and corn flour

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Abstract:

This study included effecting Aspergillus flavus fungi on some trace elements (TE) that compositions in three types of flour related to wheat, rice and corn grains . Ability of A.flavus isolates to produce aflatoxin was tested by thin layer chromatography(TLC) ,the positive result was noticed by accruing blue fluorescence under ultra-violate light 360nm with the same retardation factor of stander aflatoxin B1 and B2. Three samples of flour was sterilized by autoclaving then inoculated by A.flavus then digested for measuring concentrations of five TE (copper Cu, Zinc Zn ,Manganese Mn , Lead Pb and chromium Cr) by atomic absorption spectrophotometric ,the results of TE contents of rice flour indicated to significantly decreasing in Cu and Pb minerals ($4058*10^{-4}$ppm and $1135*10^{-4}$ppm to $2029*10^{-4}$ppm and $670.5*10^{-4}$ppm respectively ,but non significantly decreasing Zn element ($1326*10^{-4}$ ppm) in control sample to($1178*10^{-4}$ppm) in contaminated sample, while significantly increasing and non-significantly in Cr and Mn elements where the concentrations were elevated to $329*10^{-4}$ppm and $970.5*10^{-4}$ ppm in contaminated samples compared with control samples ($79*10^{-4}$ppm and $873*10^{-4}$ ppm respectively. Also in wheat flour the results were significantly decreasing in concentrations of Zn ,Pb and Mn ($1455*10^{-4}$ ppm ,$846*10^{-4}$ ppm and $1354*10^{-4}$ ppm to $1178*10^{-4}$ ppm ,$650*10^{-4}$ ppm and $917.5*10^{-4}$ ppm) respectively ,but there were significantly increasing of

*Cu and Cr elements concentrations (3333*10^{-4} ppm and 303*10^{-4}ppm) in contaminated samples compared with control (2029*10^{-4}ppm and 237*10^{-4}ppm) respectively. In corn flour the results showed significantly decreasing in Zn ,Cr and Mn concentrations (3895*10^{-4}ppm ,237*10^{-4}ppm and 2173*10^{-4}ppm) in control samples to (3181*10^{-4}ppm ,158*10^{-4}ppm and 1211*10^{-4}ppm) in contaminated samples respectively ,but there were significantly increasing in Cu and Pb concentrations (1159*10^{-4}ppm and 0 ppm) in control to (2898*10^{-4} ppm and 650*10^{-4}ppm) respectively in contaminated flour samples.*

Key words: *Aspergillus flavus*, contamination, wheat, rice, corn flour

Introduction

Wheat, rice and corn are the seeds of monocot and regarded the first of food groups that wildly consumed, staple food for a large part of the world's human population especially in Asia, also they located in the base of food pyramid as general plane of what to eat each day, where they considered main nutrients for energy protein in visible fat, vitamin B1,vitamin B2, folic acid ,Iron, fiber etc.. (Sheila et al, 2004; Prod STAT and FAO STAT, 2006). These corps also contained enough amounts of microelements or trace elements that essential and very limited quantity requirements of human (Nutrient data ,2004;Cesar ,2005).In biological systems TE are mostly forming metalloproteins and latter are part of enzymatic system have structural functions or use for transporting the protein to their target site in organisms (Cesar ,2005). There are many functions that TE play important role in it ,such as Manganese associated with bone development and with amino acid ,lipids,carbohydrate metabolism, but the toxicity of Mn lead to Parkinson type syndrome (Davis ,1992 ;Rabin et al ,1993 ;Aschner ,2000). Researchers Kanumakata et al. 2000, referred to role of copper elements in development of connective tissue, deficiency of this metal leaded to normocytic, hypochromic

anemia but toxicity of it mostly associated with liver damage. Zinc elements is involving in the activity of about one hundred enzymes such as RNA polymerase, carbonic anhydrase ,deficiency of this metal was common in un developing countries and mainly associated with malnutrition (Hamilton et al ,2002). But these corps are susceptible to fungal attack either in the field or during storage, *Aspergillus flavus* was an important genera associated with contaminated products during storage and transit (Bhat RV.,1988). This fungi was using the compositions of these grains for aflatoxin production and caused several changes in chemical such as carbohydrate , lipid and protein ,so these fungi may reduce nutrient contents of food that infected with it (Fraga ,1985 ;Adebayo et al ,2006). For all these reasons ,this study aimed to know the changes in quantity of some TE that compositions in grain flour of wheat ,rice and corn after infected it *A. flavus* that producing aflatoxin.

Materials and methods

1-Aspergillus flavus isolate:

The fungi isolate was gained from microbiology laboratory of biology department of science collage \university of kufa.

2-Testing ability of A. flavus for aflatoxin production:

These experiments included:

A-Extraction of aflatoxin from *A. flavus* cultures according to Esuneo ,2002 method: one week old of A.flavus culture used for extracting ,the culture was cut by sterile needle and transferred to electric mixer, 20ml of chloroform was added ,then mixed for 10min., mixture was filtered by filter paper(Whatman .1), supernatant was taken to oven for concentration the extract into 1ml only.

B-Detection of aflatoxin presence by thin layer chromatography TLC carried out according to Sobolev and

Dorner,2002 method: Activation TLC in oven 120c^0,1h. then put the spots of sample extract and stander aflatoxin on one strait line (about 2 cm between spots) , then transfferd TLC to mobile phase system that consist of chloroform and methanol(98:2) ,after the ended of retardation TLC was taken out and dried ,then put under UV light to calculate Rf of spots and compared between them.

3-Preparing the samples of flour:

The samples of wheat.rice and corn grains were gained from local markets of Al-Najaf al ashraf city and grinded by mill to prepare flour, then sterilization by autoclaving in 121c^0 and 1 bar pressure for 15 min.,then put the samples in clean disposable cups with covered in three replications for each types of flour to inoculate it and three other as control (without inoculation).

4-Preparation of inoculum and contamination samples:

7 days old cultures of A.flavus used a source of conidia the suspension was filtered and center fuged twice for 5 min. and re suspended in sterile distilled water (Railey et al,1997). About 50g of each type of flour samples were uniformly inoculated with diluted spors solution for three replications , the inoculated samples were transferred to incubate at 30c^0 for 15 days.

5-Trace elements determination: included two stages

A-Digest samples: 0.2 g of each samples were taken and put in conical flask(250 ml) and added mixing of perchloric acid $HclO_4$ and sulphric acid H_2SO_4 with 1:1 ratio and leaved for one night to complete digestion ,then transferred for tow hours on digestion apparatus(figur1) .

B-Atomic absorption spectrophotometric (figure 2): For determination of selected TE (copper Cu,Zinc Zn,Maganese

Mn),and tow heavy metal (Lead Pb and Chromium Cr) Jorhem L.,2000 method was fallowed.

Figure1 showed samples after digestion

Figure2 showed (A.A -6300-DAIGEN, SHIMAZU-JAPAN)

Results and discussion:

The testing of TLC was showed that *A.flavus* isolate could produce aflatoxin through seeing blue fluorescence under ultra-violate light 360nm having the same RF of stander aflatoxin B1 and B2 (figure 3), this result agreed with another study referred to ability of 75% of *A.flavus* isolates to produce aflatoxin B1 (Yu et al ,2004).

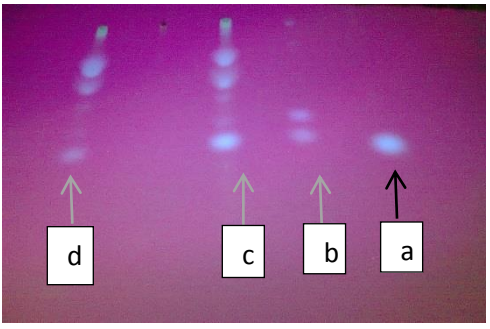

Figure 3 showed TLC under uv light a-stander aflatoxin B1, b-stander aflatoxin B2, c and d- samples

Tables 1, 2 and 3 showed inter action of *A. flavus* inoculation with micro minerals concentrations composition in wheat , rice and corn flour samples . the TE contents of control and

inoculated samples were found tend to increased and decreased in three types of flour such as (Cu ,Mn and Pb) ,but it tend to decreased in Zinc metal concentration and increased of Chromium concentration after inoculated samples with A. flavus. These results lined with Ehsan et al (2009) who showed that various minerals like Ca,P,Pb,Mg,Fe,Zn and Se were decreased while Na,Mn,Cu and Cd increased after inoculated the almond seeds with *A. flavus.* The decreased of Zinc metal in all inoculated samples might be illustrated the *A. flavus* dependence on substrate compositions of flour of microelements and used it for aflatoxin production (Fraga ,1985;Ade bayo et al ,2006), but the height chromium metal concentration might be related to absorption it from contaminated water by fungi and used this element for forming cellulase degradable enzyme that usage for degrading plant cells.

Table 1 microelements concentrations compositions in rice flour samples

microelements	Control $*10^{-4}$ppm	Inoculated $*10^{-4}$ ppm	±Sd
Cu	4058	2029*	±1159
Zn	1326	1178	±285
Mn	873	970.5	±62.5
Pb	1135	670.5*	±72.5
Cr	79	329*	±118

Table 2 microelements concentrations compositions in wheat flour samples

microelements	Control $*10^{-4}$ppm	Inoculated $*10^{-4}$ ppm	±Sd
Cu	2029	3333.5*	±1304.5
Zn	1455	1178.5*	±18.5
Mn	1354	917.5*	±62.5
Pb	846	650*	±196
Cr	237	303*	±66

Table 3 microelements concentrations compositions in corn flour samples

microelements	Control $*10^{-4}$ppm	Inoculated $*10^{-4}$ ppm	±Sd

Cu	1159	2898.5*	±1739.5
Zn	3895	3181.5*	±520.5
Mn	2173	1211.5*	±142.5
Pb	0	650*	±237
Cr	237	158*	±26

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