Institute of Graduate Studies and Research
Department of Environmental Studies

Assessment of mycotoxins in some stored grains from different localities in Egypt

A thesis submitted in a partial fulfillment of the requirements for the degree of Ph.D.

In

Environmental Studies

Submitted by

Mahmoud Abd El-Azim El-Sadany

B.Sc. of Agricultural Science, 2004
Alexandria University
Master degree in Environmental Studies, 2014
Institute of Graduate Studies and Research - Alexandria University

2018

LIST OF CONTENTS

LIST OF TABLES

Page

LIST OF ABBREVIATIONS

Afla	:	Aflatoxins B_1
FUM	:	Fumonisins B
Cit	:	Citrinin
DON	:	Deoxynivalenol
Ergot	:	Ergotamine
HT-2	:	HT-2 toxin
T-2	:	T-2 toxin
NIV	:	Nivalenol
OTA	:	Ochratoxin A
Sterig	:	Sterigmatocystin
ZEN	:	Zearalenone
SEM	:	Scanning electron microscopy
FTIR	:	Fourier Transform Infrared
ICP-MS	:	Inductively Coupled plasma
ZEN	:	Zeta Potential Cell
CD	:	Circular dichroism spectrometer
Contact Angle	:	Multidimensional Contact Angle Measurement
MCR	:	The MCR rheometer
Wm	:	Amount of each toxin in the test sample
Wa	:	Amount of each toxin corresponding
Vf	:	The final volume of re dissolved elute
Wm	:	Amount of each toxin in the test sample
Wa	:	Amount of each toxin corresponding
Vf	:	The final volume of re dissolved elute
Vi	:	Volume of injected eluate
Vs	:	Volume of test portion passing through the column
Wa	:	Amount of each toxin corresponding
Vf	:	The final volume of re dissolved elute
WHO	:	World Health Organization
$Afla^{Test}$	:	The immunoaffinity columns of aflatoxins B_1

DON[Test]	:	The immunoaffinity columns of deoxynivalenol
OTA[Test]	:	The immunoaffinity columns of Ochratoxin A
FUM[Test]	:	The immunoa columns of fumonisins B_1
ZEN[Test]	:	The immunoa umns of Zearalenone
AP	:	Ammonium p
H1NMR	:	nuclear magnetic resonance spectroscopy
TaS@Z	:	Zein coating of Triticum aestivum
Zinc@Zein	:	The zinc coordinated zein composite
TaS@Z-Z	:	Zinc@Zein was coated on wheat grain
EDAX	:	Energy-dispersive X-ray spectroscopy
SDS-PAGE	:	SDS-polyacrylamide gel electrophoresis

7. Summary

Egypt has one of the highest rates of human and animal consumption of a commercially grains such as (wheat, maize, rice and barley) in the world. A large problem is present in maintaining safe storages of grains for animal and human consumption. So, present study conducted to assess the effect of storage of grains such as (wheat, maize, rice and barley) on mycotoxins content such as Afla; DON; OTA; ZEN and Fum at ambient environmental condition for (3, 6, 9 and 12 months) in Upper, Middle and Lower of Egypt. The mean of the relative humidity and temperature through the previously mentioned period was [(55%, 4-22°C), (37%, 10-35°C), (30%, 19-37°C) and (40%, 10-32°C)], [(63%, 9-18°C), (45%, 12-32°C) (59%, 22-34°C) and (50%, 13-30°C)] and [(55%, 4-22°C), (37%, 10-35°C), (30%, 19-37°C) and (40%, 10-32°C)], respectively.

Our results revealed that the mean concentration values of Aflatoxins in wheat, maize, rice and barley in Upper, Middle and Lower of Egypt were (14.2 - 20.8 - 9.3 and 3.5µg/Kg), (14.2 - 22.6 - 8.4 and 0µg/Kg) and (13-17.6 -7.4 and 2.8µg/Kg), respectively, while Dontoxin were (72.2 - 50 - 0 and 39.6 µg/Kg), (55.2 - 44 - 0 and 32.4 µg/Kg) and (42 - 0 - 33.8 and 24.4 µg/Kg), respectively. Ochratoxin were (3.58 – 5.8 - 0 and 0 µg/Kg), (4.7 - 0 - 0 and 3.3 µg/Kg) and (0 - 0 - 4.5 and 3.1 µg/Kg), respectively. Zearalenone were (0 - 0 - 3.3 and 5.4 µg/Kg), (0 - 0 - 3.8 and 4.9 µg/Kg) and (0 -1.5 - 2.92 and 4.7 µg/Kg), respectively. While Fumonisinsis were (0 - 0 - 3.3 and 5.4 µg/Kg), (0 – 6.9 - 0 and 0 µg/Kg) and (0 -5.3 - 0 and 0 µg/Kg), respectively. The concentration of Afla; DON; OTA; ZEN and Fum was increase during storage period at natural or ambient environmental condition, highest level during the storage periods detected in Lower of Egypt, while the lowest level detected in Upper of Egypt and moderate level values of mycotoxin detected in middle of Egypt.

The occurrence of Afla; DON; OTA; ZEN and Fum in wheat, maize, rice and barley was investigated. The concentration values of Afla; DON; OTA; ZEN and Fum in wheat, maize, rice and barley, the maximum Afla detected was 21 µg/Kg in maize and the minimum was 1 µg/Kg in wheat, with means of 11 µg/Kg and 7.5 µg/Kg. highest contaminated found in maize and lowest in rice. The maximum of DON detected was 220 µg/Kg in wheat and the minimum was 95µg/Kg in maize, with means of 206.6 µg/Kg and 98.3µg/Kg, respectively. The highest contaminated found in wheat and the lowest in rice, while OTA detected was 7 µg/Kg in rice with minimum was 1µg/Kg in maize and barley, with means of 4, 3 and 2.3 µg/Kg, respectively. The highest contamination found in rice and the lowest in maize and barley and without contamination detected in wheat, in another side the ZEN detected was 75 µg/Kg in rice and the minimum was 50 µg/Kg in maize, with means of 74 µg/Kg and 63.6µg/Kg, respectively. The highest contamination found in maize and the lowest in rice, and not detected contamination in barley, but Fum detected was 325µg/Kg in maize and the minimum one was 315µg/Kg in rice, with means of 320µg/Kg and 320.5µg/Kg, respectively. The highest contamination found in maize and the lowest one was in rice, and not detected contamination in wheat and barley.

Also, it was found maximum value of *Pseudomonadaceae* detected was (7×10^6) cfu/g in wheat and the minimum one was (5×10^3) cfu/g in rice, with means of (5.3×10^6) cfu/g and (6.3×10^5) cfu/g, respectively. The highest contamination wheat and the lowest one in maize, with value of *faecal coliform* detected being (8×10^3) cfu/g in wheat and the minimum (28×10^2) cfu/g

in rice, with means of (5.2×10^3) cfu/g and (31.3×10^2) cfu/g. The highest found in barley and the lowest in wheat and without contamination detected in maize, While *Salmonella* detected was (5×10^4) cfu/g in barley and the minimum (1×10^3) cfu/g in maize and wheat, with means (4×10^4) cfu/g, (3.3×10^2) cfu/g and (3.3×10^2) cfu/g, respectively. The highest level was found to be in rice and the lowest in maize and wheat. On the other side, the *Bacillus cereus* detected was (4×10^4) cfu/g in barley and the minimum (15×10^2) cfu/g in maize, with means of (2.5×10^4) cfu/g and (17×10^2) cfu/g, respectively. The highest level was found to be in wheat and the lowest in barley, and no detected contamination in rice. The *Staphylococcus* was detected (4×10^3 cfu/g) in rice and the minimum (7×10^2 cfu/g) in maize, with means of (3×10^3) cfu/g and (6.3×10^2) cfu/g, respectively. The highest found to be in wheat and the lowest in rice.

The current study showed that, total bacterial count contamination in wheat, maize, rice and barley samples in a range of 3 – 20 ($\times10^3$), 5 – 20 ($\times10^4$), 5 – 14 ($\times10^3$) and 5 – 12 ($\times10^6$) cfu/g, with an average value of 11.5 ($\times10^3$), 12.3 ($\times10^4$), 6.8 ($\times10^3$) and 6.9 ($\times10^6$) cfu/g, respectively. Total bacterial count contamination in wheat, maize, rice and barley samples was in a range of 13 – 32($\times10^3$), 17 – 24($\times10^4$), 16– 20($\times10^5$) and 58 – 72($\times10^5$) cfu/g, with an average value of 20.6 ($\times10^3$), 12.8($\times10^4$), 17.7 ($\times10^5$) and 64.4 ($\times10^5$) cfu/g, respectively.

Finally, it is especially clear in Egypt that the weather is very suitable for the growth of fungi which are able to produce the toxin. Furthermore; it is an area which is very suitable for mycotoxins production. Aflatoxin production was found to be highly wide spread in grain rather than others mycotoxin such as DON; OTA; ZEN and Fum. It can be concluded that the production of mycotoxin in grain is independent of the level of fungal contamination of grain before production of toxins that can be observed with total fungal count.

The present study designed a trial or attempt for elimination, prevention or fortification of grains during storage for risk reduction due to contamination with mycotoxins and bacterial count growth . The effect of coated wheat seeds with zinc coordinated zein (Zinc@Zein) on the amount of both fungal and bacterial contents. Grain moisture is the key for microbial contamination to occur; hence we envisage coating the grain with edible hydrophobic moisture barrier “zein”. On the other hand Zinc is also an anti-microbial agent both as ions and in oxide forms (at Nano scale) that can complement grain protection .The zein film is also proved to support the activity of the loaded active ingredients, hence 0.2% was fixed as the optimum coating condition for further experiments. Further the thickness of the coating is measured to be around 700 to 900 nm with the cross-section. Although this is not even around the grain, especially at the furrows in grain surface the thickness is slightly more, the EDAX mapping the spot EDAX also show significant increase in the zinc content in TaS@Z-Z, compared to the control.

The surface of the coated grain is smooth compared to the grain without coating. Hence the profilometer shows marginal reduction in the roughness in the profile of the zein coated grain (45) compared to the control grain (54). However with Zinc@Zein coating, TaS@Z-Z shows marginal increase in roughness to 49.5. The characteristic band at 19 and 22 kDa, there was slight increase in the molecular weight of the Zinc@Zein at both 19 and 22 kDa region, which may be attributed to confirm that the interaction of zinc on zein in Zinc@Zein didn’t fragment the protein. Spectra shown the typical negative maxima of α-helix structure at 207 and 222 nm, the α-helix of zein diminished with the addition of the zinc ion. Hence complement the coating life. By

comparison between them shows, in Zinc@Zein there is significant control in viscosity than zein alone. In grain coating, it was mean the higher viscosity to increases and improve the degree of coating and affects the quantity and quality of the matter that adheres to the substrate.

Interestingly the contact angle of [illegible]c@Zein is found more than zein alone, in contrast to earlier non-sticky plasticizer that redu[illegible]act angle. This increase in the contact angle is attributed to the unwrapping of the hydro[illegible]omain.

The amount of zein and zinc required for coating 1 Kg of grain was estimated. It was found that approximately ~2-3 g of zein would be sufficient for coating 1 kg of grain, since in 0.2% zein coating procedure standardized, it is estimated that a maximum of 70 mg of zinc could be fixed on the grain of 1 kg. This is ~5 times the zinc that is present in the native grain; this zinc amount fortified within the WHO limit .The treatment with TaS@Z 0.2% -Z showed 4.5 times increase in the availability compared to the control. Hence it is assumed that this coating may assist in mineral release with the stimuli from the gastric enzyme. The coated samples TaS@Z and TaS@Z-Z showed less moisture compared to control, which may be due to the hydrophobic zein moisture barrier. The result for effect of TaS@Z-Z on amount of aflatoxin infection content with different culture concentration values of aflatoxin in wheat seeds sample show proximally 65% reduction in the percentage compared to the control and TaS@Z. Anti-microbial effect of the coating with the seed borne bacterial pathogen *Pseudomonas syringae*, showed 80% reduction in the pathogen colony compared to the control and TaS@Z.

The above coating technique can be a viable strategy for the long term grain storage as the zein can avoid moisture flux and the zinc ions can provide anti-microbial property. Here the zinc is spread on the solid grain surface with Nano scale film, which increases the zinc surface density, hence more control for pathogens at less zinc concentration. Hence this technology could help maintain phyto-sanitary quality in seed industry. The cost of grain preservation by this method may be nil, as there is a bonus of nutrient fortification.