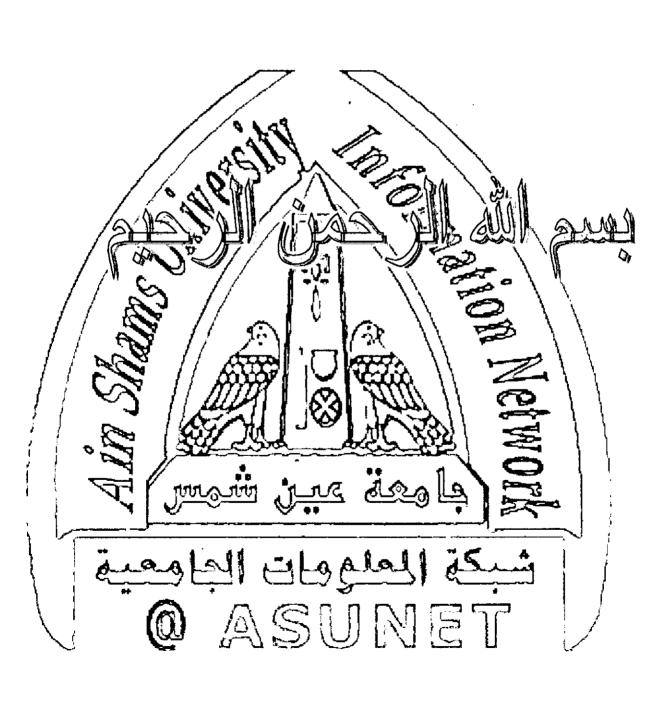


شبكة المعلومات الجامعية





شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الالكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها على هذه الأفلام قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأفلام بعيدا عن الغبار المنافلام بعيدا عن الغبار المنافلام بعيدا عن الغبار المنافلات المنافلة من ٢٠-٠٤% منوية ورطوبة نسبية من ٢٠-٤٠ المنافلة من ٢٥-١٥ المنافلة من ٢٥-١٥ المنافلة المن

















STUDIES ON MYCOTOXINS CONTAMINATING FOOD

A Thesis

For the degree of Ph.D. in Botany - Microbiology

By

Salwa Salama Ahmed Awad Gabal

M.Sc. (Microbiology)

Submitted To

Botany Department, Faculty of Science
Benha University

Supervised by

Prof. Dr.

Hussein Yousry Olama

Prof. of Botany Faculty of Science Benha University Prof. Dr.

Saeb Abd El-Monem Hafez

Prof. of Nutrition Institute of Food Techology Centre of Agricultural Research

Dr. Talat Ibrahim El-Sayed

Assist. Prof. of Microbiology Faculty of Science, Benha University

Dr. Mahmoud Moustafa Amer

Assist. Prof. of Microbiology Faculty of Science, Benha University

2006

6 / 4 ac





الله و من الله و المنافقة المنافقة الله و المنافقة الله و المنافقة الله و المنافقة المنافقة الله و المنافقة ال

Le Mich Mily Le Mily of Le Mily of Lily

صدق الله العظيم سورة هود (الآية رقم ۸۸)

Acknowledgement

First and Forever, ultimate thanks to **ALLAH** The most beneficent and merciful and without his aid this work could not be done.

I would like to express my sincere gratitude and appreciation to Late Prof. Dr. Hussein Yousry. Olama, Professor of Botany, Faculty of Science, Benha University, for his valuable criticism, reading the manuscript, supervision and valuable help.

I am greatly indebted to Prof. Dr. Saeb Abd El-Monem Hafez Professor of Nutrition, Institute Food Technology, Agricultural Research Center, Giza for his closed supervision, encouragement, guidance, valuable advice, his unlimited help during preparation and constant encouragement.

I also wish to express my great thanks to Dr. Talaat Ibrahim El-Sayed, Assistant Professor of Microbiology, Botany Department, Faculty of Science, Benha University, for supervising the work, his careful guidance, continuous constructive criticism and valuable discussions during the course of this investigation and for the revision of the manuscript. I will always be indebted to him for his constant encouragement.

I also wish to express my great thanks to Dr. Mahmoud Moustafa Amer, Assistant Professor of Microbiology, Botany Department, Faculty of Science, Benha University, for his valuable criticism, reading the manuscript, supervision, valuable help and constant encouragement.

Finally, I would like to express my thanks to every one who had participated some way or another in the conduction of this study.

LIST OF CONTENTS

	Subject	Page
IN	TRODUCTION	1
	REVIEW OF LITERATURE	5
	1.1. Seed borne fungi of groundnut	5 .
	1.2. Seed borne fungi of corn	5
	1.3. Definition of aflatoxins	7
	1.4. Natural occurrence of aflatoxins (B ₁ , B ₂ , G ₁ and G ₂)	8
	1.5. Structure and properties of aflatoxins $(B_1, B_2, G_1 \text{ and } G_2) \dots$	14
	1.6. Types of aflatoxins and character of flatoxins B ₁	16
•	1.7. Toxicological effects of aflatoxin (B ₁ , B ₂ , G ₁ and G ₂) ;	16
	1.7.1. Toxicological effect of aflatoxin B ₁ could be divided	
	into the following two parts	32
	1.7.1.1. Carcinogenisty of aflatoxin B ₁	32
	1.7.1.2. Acute toxicity of aflatoxin	41
	1.8. Character of AFT B ₁ LD ₅₀ of rats	42
•	1.9. Effect of acute and chronic toxicity on rats	42
	1.10. Effect of amount of protein in diets on rats	44
	1.11. Definition and effect of lipid peroxidation	44
	1.12. Safety of antioxidant vitamins and β-carotene	45
	1.13. Vitamins E, C and other cartenoids as antioxidants	46
	1.14. Interaction among vitamin C, E and β-carotene	47
	1.15. Antioxidant activity of ascorbic acid and α -tocopherol in	
	vitro studies	48
	1.16. Roles of vitamins on aflatoxins contaminated diet	49
	1.17. Effect of vitamin E on aflatoxin	51
	1.18. Effect of aflatoxin B ₁ on enzymes activities	52

	1.19. Role of vitamin E in diets of rats	54
	1.20. Effect of vitamin E on blood ALT, AST, GSH-Px activates	55
	1.21. Biochemical changes of aflatoxicosis	56
	1.22. Histopathology of aflatoxicosis	57
2.	MATERIALS AND METHODS	62
	METHODS	62
	2.1. Materials	62
	2.1.1. Peanuts and corn	62
	2.1.2. Aflatoxins standards	62
	2.1.3. Thin layer chromatography (TLC) plates	62
	2.1.4. Media	62
	2.1.5. Aflatoxins production	63
	2.1.6. Animals and diagnostic kits	63
	2.2. Methods	64
	2.2.1. Isolation of molds from tested foodstuff	64
	2.2.2. Identification of isolated molds	64
	2.2.3. Maintenance of isolated molds	65
	2.2.4. Aflatoxin extraction	65
	2.2.4.1. From in vitro (fungal cultures)	65
	2.2.5. Separation of aflatoxins	66
	2.2.5.1. Thin layer chromatography (TLC) technology	66
	2.2.5.2. Confirmation test	66
	2.2.6. Detection of aflatoxins	66
	2.2.6.1. Calculation of aflatoxins	67
	2.2.7. Toxicological studies of aflatoxins B ₁	68
	2.2.7.1. Animals (Experimental animals) for first	
	experiment	68
	2.2.7.2. Experimental design for first experiment	68

2.2.7.3. Animais (expe	rimental animals) for second	
experiment		9
2.2.7.4. Experimental	design for aflatoxin B ₁ for	
second experime	ent 69	9
2.2.8 Biochemical analysis	72	2
2.2.8.1. Blood sampling		2
2.2.8.2. Organs		2
2.2.8.3. Determination o	f serum total protein 72	2
2.2.8.4. Determination o	f total cholesterol	2
2.2.8.5. Determination o	f urea 73	3
2.2.8.6. Determination o	f total bilirubin 73	3
2.2.8.7. Determination o	f creatinine 73	3
2.2.8.8. Determination	of serum transaminases (AST	
and ALT)	74	4
2.2.9. Histopathological techni	que 74	4
2.2.10. Statistical analysis	75	5
3. RESULTS AND DISCUSSION		5
3.1. Fungi isolated from seeds samp	ole of peanut	5
3.2. Fungi isolated from grains sam	ple of corn 77	7
3.3. Screening of aflatoxin production	on by various isolates of fungi,	
isolated from peanut and corn	grown in liquid medium 77	7
3.4. Biochemical observation	78	3
3.4.1. Effect on activaties of	plasma enzymes (ALT, AST,	
ALP)		9
3.5. Biochemical analysis of blood	d of rats which injected with	
aflatoxin B ₁		9
3.5.1. Change in total protein .)
3.5.2. Change in total cholester)

-List of	Contents
----------	----------

#4

3.5.3. Change in bilirubin and urea	80
3.5.4. Change in creatinine and plasma enzymes(ALT & AST)	80
3.6. Histopathological changes	100
3.6.1. Rats liver	100
3.6.2. Rats kidney	102
3.6.3. Rats heart	104
3.6.4. Rats spleen	104
3.6.5. Rats lung	104
3.6.5. Rats brain	105
3.7. Discussion of histopathology	105
3.7.1. Effect on body weight and some organs of rats	105
3.7.2. The control group (-ve AFT)	107
3.7.3. Group of AFB ₁ only	107
3.7.4. Effect of supplementation the contaminated diet with	
vitamins on rats	107
4. SUMMARY	109
F DEFEDENCES	111
6 ARARIC SUMMARY	1

LIST OF TABLES

Title	Page
: Basal diet composition (standard diet)	70
: Composition of mineral mixture	70
: Composition of vitamin mixture.	71
: Show genera of fungi isolated from grains sample of	82
ground nut (peanut)	
: Show genera of fungi isolated from grains sample of	83
corn (Zea mays)	}
: Screening of aflatoxin production by various	84
isolates of fungi isolated from peanut and corn	
grown in liquid medium (YES).	
: Effect of vitamins supplementation on body and	85
some organs weight of male rats fed on aflatoxins	
contaminated diet.	
: Effect of vitamins supplementation on the activity of	87
plasma alkaline phosphatase (ALP) of male rats fed	w a
on diet contaminated with aflatoxins for one month.	
: Effect of vitamins supplementation on the activity of	89
plasma alanine amino-transferase (ALT) of male	
rats fed on diet contaminated with aflatoxins for	
one month.	
: Effect of vitamins supplementation on the activity of	90
plasma aspartate amino-transferase (AST) of male	,
rats fed on diet contaminated with aflatoxins for	
one month.	
	 : Basal diet composition (standard diet) : Composition of mineral mixture : Composition of vitamin mixture. : Show genera of fungi isolated from grains sample of ground nut (peanut) : Show genera of fungi isolated from grains sample of corn (Zea mays) : Screening of aflatoxin production by various isolates of fungi isolated from peanut and corn grown in liquid medium (YES). : Effect of vitamins supplementation on body and some organs weight of male rats fed on aflatoxins contaminated diet. : Effect of vitamins supplementation on the activity of plasma alkaline phosphatase (ALP) of male rats fed on diet contaminated with aflatoxins for one month. : Effect of vitamins supplementation on the activity of plasma alanine amino-transferase (ALT) of male rats fed on diet contaminated with aflatoxins for one month. : Effect of vitamins supplementation on the activity of plasma aspartate amino-transferase (AST) of male rats fed on diet contaminated with aflatoxins for one month.

Table No.	Title	Page
Table (11)	: Effect of vitamins supplementation on the activity of	91
	blood urea nitrogen (BUN) of male rats fed on diet	
	contaminated with aflatoxins for one month.	مدير
Table (12)	: Effect of vitamins supplementation on the creatinine	92
	level of male rats fed on diet contaminated with	
	aflatoxins for one month.	
Table (13)	: Effect of aflatoxin B ₁ on plasma total cholesterol of	93
	albino male rat injected aflatoxin B ₁ interperitoneal	
	(IP) twice a week for one month.	
Table (14)	: Effect of aflatoxin B ₁ on total protein in albino male	94
	rat injected aflatoxin B ₁ interperitoneal (IP) twice a	
	week for one month.	
Table (15)	: Effect of aflatoxin B ₁ on bilirubin of albino male rat	95
	injected aflatoxin B ₁ inerpreitoneal (IP) twice a	
	week for one month.	
Table (16)	: Effect of aflatoxin B ₁ on urea of albino male rat	96
	injected aflatoxin B ₁ interperitoneal (IP) twice a	
	week for one month.	
Table (17)	: Effect of aflatoxin B ₁ on creatinine of albino male	97
	rat injected aflatoxin B ₁ interperitoneal (IP) twice a	
	week for one month.	
Table (18)	: Effect of aflatoxin B ₁ on (AST) aspartate	98
	aminotransferase of albino male rat injected	e*4
	aflatoxin β ₁ interperitoneal (IP) twice a week for	
	one month.	
Table (19)	: Effect of aflatoxin B ₁ on (ALT), alanine	99
	aminotransferase of albino male rat injected	
	aflatoxin B ₁ interperitoneal (IP) twice a week for	
	one month.	

LIST OF FIGURES

Fig. No.	Title
Fig. (1)	Show genera of fungi isolated from seeds sample of peanut
	(Arachis hypogaea L.).
Fig. (2)	Show genera of fungi isolated from grains sample of corn (Zea
ļ	mays).
Fig. (3)	Effect of vitamins supplementation on body and some organs
	weight of male rats fed on aflatoxins contaminated diet (Mean ±
	S.E).
Fig. (4)	Effect of vitamins supplementation on the activity of plasma
	alkaline phosphatase (ALP) of male rats fed on diet
	contaminated with aflatoxins for one month (Mean ±S.E).
Fig. (5)	Effect of vitamins supplementation on the activity of plasma
	alanine amino-transferase (ALT) of male rats fed on diet
	contaminated with aflatoxins for one month (Mean ±S.E).
Fig. (6)	Effect of vitamins supplementation on the activity of plasma
	aspartate aminotransferase (AST) of male rats fed on diet contaminated with aflatoxins for one month (Mean \pm S.E).
Fig. (7)	Effect of vitamins supplementation on the activity of blood
	urea nitrogen (BUN) of male rats fed on diet contaminated
	with aflatoxins for one month (Mean ±S.E).
Fig. (8)	Effect of vitamins supplementation on the creatinine level of
	male rats fed on diet contaminated with aflatoxins for one
	month (Mean ±S.E).
Fig. (9)	Effect of aflatoxin B ₁ on plasma total cholesterol of albino male
~ *6* (~)	rats injected aflatoxin B_1 interperitoneal (IP) twice a week for
	one month.
	VAAV AAAVAAVA