



بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



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

في درجة حرارة من ١٥-٢٥ مئوية ورطوبة نسبية من ٢٠-٤٠%

To be Kept away from Dust in Dry Cool place of
15-25- c and relative humidity 20-40%

بعض الوثائق
الأصلية تالفه

بالرسالة صفحات
لم ترد بالأصل

OK on Disk

MOLECULAR STUDIES ON THE EFFECT OF SOME NATURAL BIOLOGICAL PESTICIDES IN EXPERIMENTAL ANIMALS

Thesis

**Submitted for the Partial Fulfillment of the
Requirements for the Degree of Ph.D of Science in
Biochemistry**

By

**Fatma Mohamed Hamam Mohamed
(B.Sc & M.Sc. Biochemistry)**

Supervised by

Abdel E H J

Prof.Dr.

**Abdel-Halim Abdel-Hady Mostafa
Professor of Biochemistry
Faculty of Science
Ain Shams University**

M.S. Salama
Prof.Dr.

**Mohamed Sayed Salama
Assistant Professor of
Molecular Biology
Department of Entomology
Faculty of science
Ain Shams University**

**Dr. Nadia Youssef Sadek Morcos
Lecture of Biochemistry
Faculty of science
Ain Shams University**

Nadia

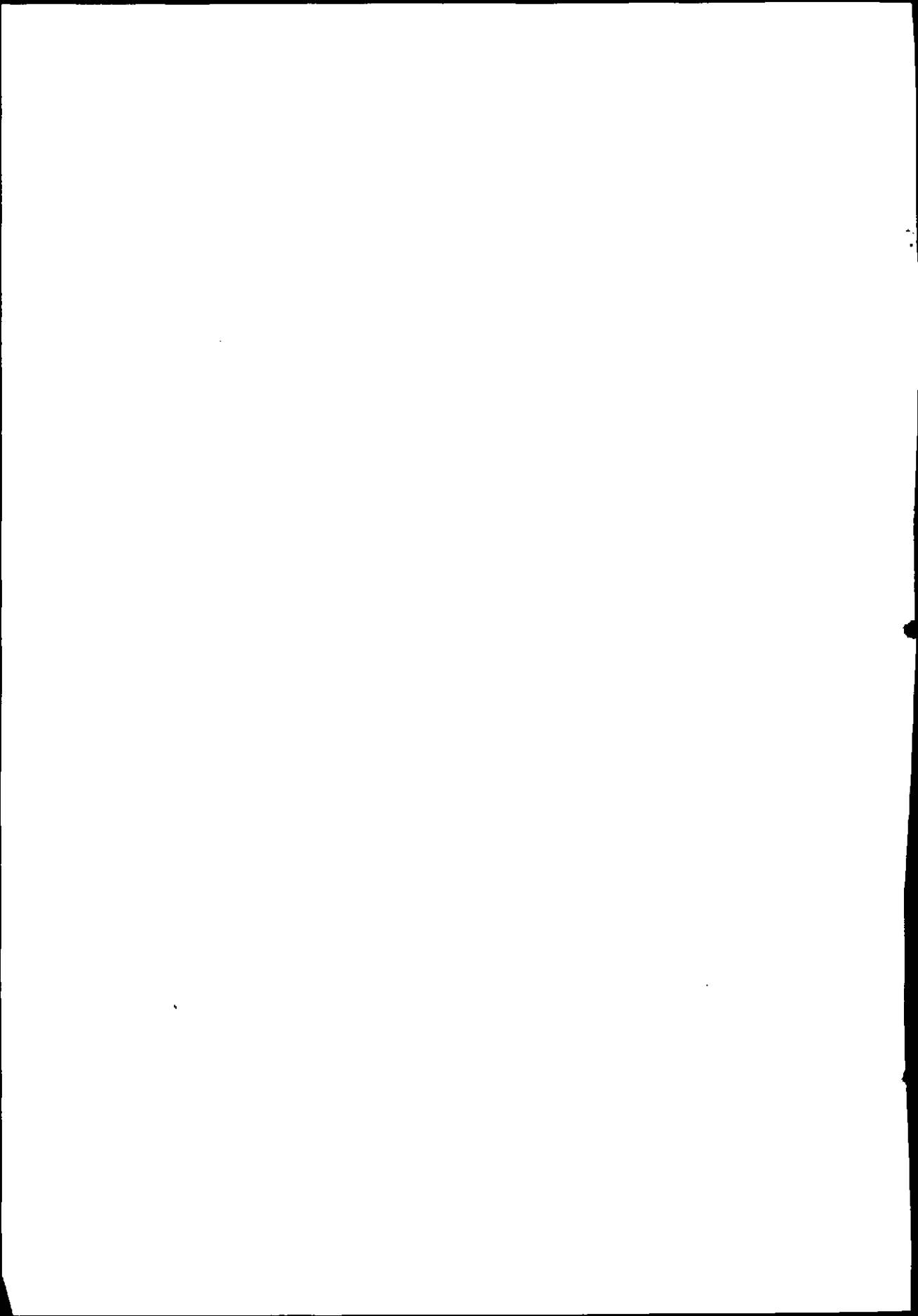
**Dr.El Hussein Nagib El
KHatib
Researcher in Mammalian
Toxicology Department
Central Agriculture Pesticides
Laboratory**

El-Khatib

**Biochemistry Department
Faculty of science
Ain Shams University**

1999

o.v.



ACKNOWLEDGMENT

*AT first, I thank **GOD** for performing this research work.*

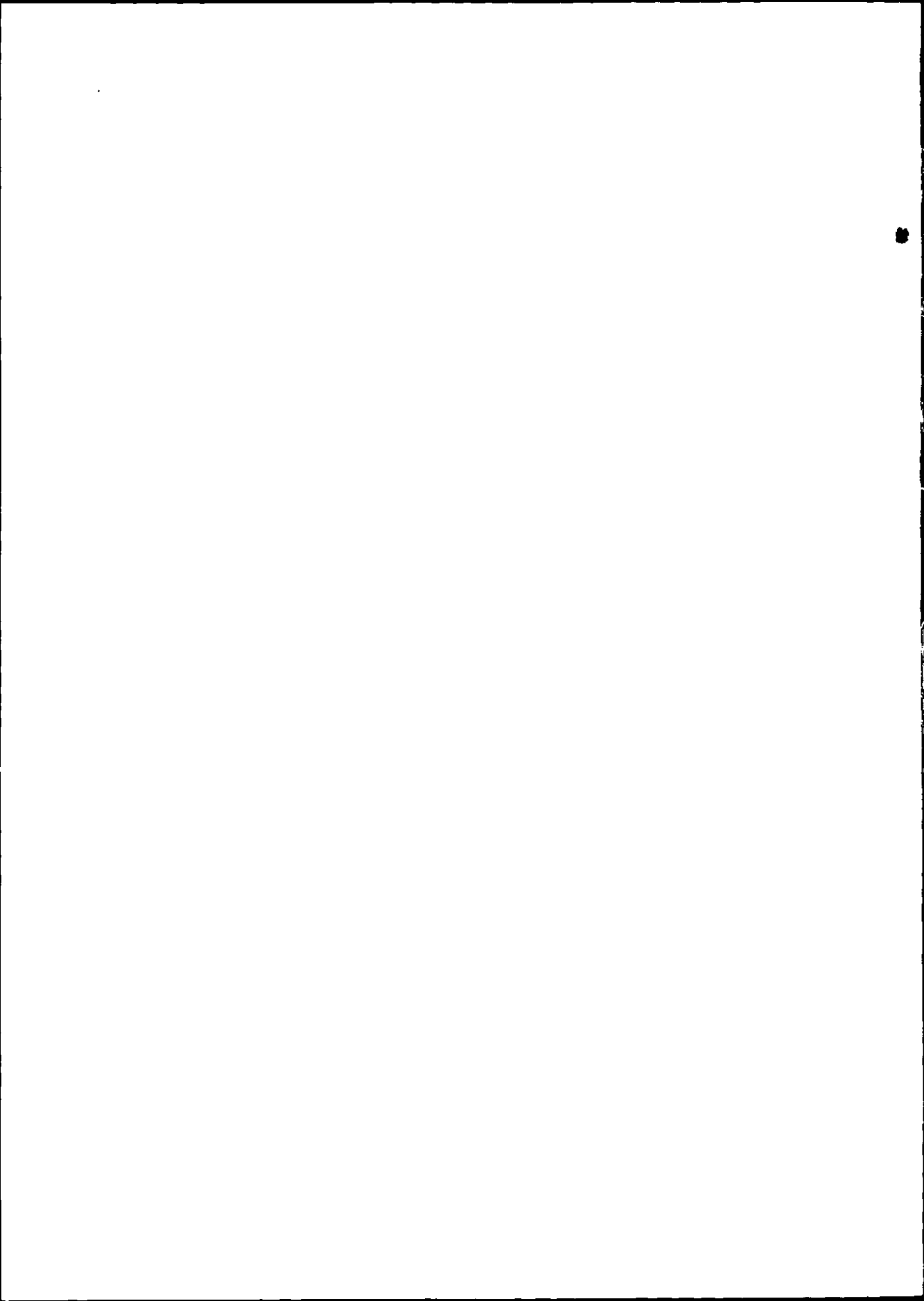
*I would like to offer deep thanks and gratitude to **Prof. Dr. Abdel-Halim Abdel-Hady Mostafa**, Professor of Biochemistry, Faculty of Science, Ain Shams University, for his kind supervision and valuable advise that are greatly appreciated and whose support made this work possible.*

*I would also like to express my heartfelt respect and gratitude to **Prof. Dr. Mohamed Sayed Salama**, Professor of Molecular Biology, Faculty of Science, Ain Shams University, for his technical guidance, continual support and encouragement that brought this work to parturition.*

*My sincere thanks and gratitude are very cardially offered to **Dr. Nadia Morcos**, Lecturer of Biochemistry, Faculty of Science, Ain Shams University for her patient guidance through every part of this research and her encouragement and advice when I faced problems.*

*Gratefull appreciation is also due to **Dr. El Hussein Nageb El-Khatib**, Researcher of Mammalian Toxicology, Central Agriculture Pesticides Laboratory, for his valuable advice, valuable facilities and encouragement during the preparation of this work.*

Finally my special thanks are to my colleagues and friends at Central Agricultural Pesticides Laboratory for their unending support and encouragement.



ABBREVIATIONS

A.I.	: Active ingredient
aF	: Acetric fragment
AP-PCR	: Arbitrary Primer- Polymerase Chain Reaction
ATPase	: Adenosine 5- triphosphatase
AVM	: Avermectin
<i>Bam</i> HI	: <i>Bacillus amyloliquefaciens</i>
Bp	: Base pair
B.t	: <i>Bacillus thuringiensis</i>
b.wt.	: Body weight
CAPL	: Central Agriculture Pesticides Laboratory
CFU	: Colony form units
CNS	: Central nervous system
Cry Protein	: Crystal protein
DDC	: Diethyldithiocarbamate
Dmin	: Double minute
DHAVM	: Dihydroavermectin
DNA	: Deoxyribonucleic acid
Dnase	: Deoxyribonuclease
D NTPs	: Deoxynucleotide 5- triphosphate
D.W	: Distilled water
<i>Eco</i> RI	: <i>Escherichia coli</i>
EDTA	: Ethylenediaminetetra acetic acid
EMS	: Ethyle methane sulfonate
F	: Fragment
FCS	: Faetal calf serum
GABA	: Gamma aminobutyric acid
<i>Hind</i> III	: <i>Haemophilus influenzae</i>
HPLC	: High Performance Liquid Chromatography
ICP	: Insecticidal crystal proteins
I/M	: Intramuscular
I/P	: Intraperitoneal
IU	: International unit

Kb	: Kilo base
KDa	: Kilo Dalton
Kg	: Kilogram
LD ₅₀	: Lethal median dose
Min	: Minute
NCE	: Normochromatic erythrocyte
NEL	: No effect level
PCR	: Polymerase chain reaction
Ppm	: Part per million
Qr	: Quadri radial
R	: Ring
RAPD	: Random amplified polymorphic DNA
RE	: Restriction enzyme
RFLP	: Restriction fragment polymorphism
Rnase	: Ribonuclease
Sb	: Chromosome break
S/C	: Subcutaneous
Sd	: Chromosome deletion
SDS	: Sodium dodecyl sulfate
Sg	: Chromosome gap
Ss.p	: Subspecices
TA	: Thymidine adenine
TAE	: Tris acetic acid EDTA
Taq. Pol	: Taq DNA polymerase
tb	: Chromatid break
td	: Chromatid deletion
tg	: Chromatid gap
TK	: Thymidine Kinase
TLC	: Thin layer chromatography
Tris	: Tris (hydromethyle) aminomethane
<i>Xba</i> I	: <i>Xanthomanus badrii</i>
<i>Xho</i> I	: <i>Xanthomanus</i>

CONTENTS

AIM OF WORK -----	PAGE 1
INTRODUCTION -----	2
REVIEW OF LITERATURE	
1-Avermectin -----	6
2-Dipel 2x (<i>Bacillus thuringiensis</i>)-----	30
MATERIALS AND METHODS	
I- Cytogenetic studies -----	53
II- Molecular genetics -----	60
STATISTICAL ANALYSIS -----	71
RESULTS	
1- Avermectin-----	72
I-Cytogenetic studies-----	73
2- Dipel 2x (<i>Bacillus thuringiensis</i>) -----	92
II-Molecular genetics	
A- RAPD-PCR of genomic DNA-----	118
1- Avermectin-----	118
2- Dipel 2x (<i>Bacillus thuringiensis</i>) -----	160
B-Restriction endonuclease analysis of genomic DNA	200
1- Avermectin-----	200
2- Dipel 2x (<i>Bacillus thuringiensis</i>) -----	231

DISCUSSION -----	258
I- Cytogenetic studies-----	260
II- Molecular genetics -----	269
SUMMARY -----	290
REFERENCES -----	296
ARABIC SUMMARY	

LIST OF TABLES

Table No.	Table Title	Page No.
1-a	Tested pesticide data (Avermectin)	53
1-b	Tested pesticide data (Dipel 2x)	54
2	Types of chromatid and chromosome aberrations.	58
3	Oligonucleotid sequence of primers and their G+C contents .	65
4	Restriction endonucleases used	68
5	Oral LD ₅₀ of Avermectin in rats .	72
6	Chromosomal aberrations induced by Avermectin single oral dose (1 day)	75
7	Chromosomal aberrations induced by Avermectin repeated doses (7 days)	76
8	Chromosomal aberrations induced by Avermectin repeated doses (14 days)	77
9	Chromosomal aberrations induced by Avermectin repeated doses (28 days)	78
10-a	The statistically analysis of chromosomal aberrations (Z-Test) at different oral doses and different time intervals of Avermectin treatment .	79
10-b,c	The statistical analysis (F-Test) of changes in chromosomal aberrations in relation with dose levels and periods of Avermectin treatment.	80
11	Effect of Avermectin treatment (single dose) on induction of micronuclei in Normochromatic erythrocyte (1 day).	81
12	Effect of Avermectin treatment (repeated doses) on induction of micronuclei in Normochromatic erythrocyte (7 days).	82

Table No.	Table Title	Page No.
13	Effect of Avermectin treatment (repeated doses) on induction of micronuclei in Normochromatic erythrocyte (14 days).	83
14	Effect of Avermectin treatment (repeated doses) on induction of micronuclei in Normochromatic erythrocyte (28 days).	84
15	The statistical analysis of the micronucleus cells at different oral doses and different time intervals of Avermectin treatment.	85
16	Oral LD ₅₀ of Dipel 2x in rats.	92
17	Chromosomal aberrations induced by Dipel 2x repeated doses (7 days)	94
18	Chromosomal aberrations induced by Dipel 2x repeated doses (14 days)	95
19	Chromosomal aberrations induced by Dipel 2x repeated doses (28 days)	96
20-a	The statistical analysis of chromosomal aberrations (Z-Test) at different oral doses and different time intervals of Dipel 2x treatment.	97
20-b,c	The statistical analysis (F-Test) of changes in chromosomal aberrations in relation with dose levels and periods of Dipel 2x treatment.	98
21	Effect of Dipel 2x treatment repeated doses on induction of micronuclei in Normochromatic erythrocyte (7 days).	99