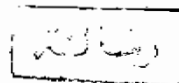


**BIOCHEMICAL PARAMETERS AS CORRELATED WITH
THE MUTAGENIC EFFECT OF PESTICIDES ON
DROSOPHILA MELANOGASTER**

By



SALWA MOHAMMED MAHMOUD KASSEM

632195
S. M
A thesis submitted in partial fulfillment
of
the requirements for the degree of
DOCTOR OF PHILOSOPHY

In

51840

AGRICULTURAL SCIENCE
PESTICIDES

Department of Plant Protection
Faculty of Agriculture
Ain Shams University

1995



Approval sheet

"Biochemical paramters as correlated with the mutagenic effects of
pesticides on Drosophila melanogaster"

By

Salwa Mohammed Mahmoud Kassem

B.SC.Agric.(Entomology), Ain Shams Univ ,1979

M.SC.Agric.(Pseticide), Ain Shams Univ, 1986

This thesis for Ph. D.Degree has been approved by;

Dr. A. E. Salama *A - E. Salama*

prof. of Pesticides Fac. of Agric. Tanta University.

Dr M. A .Rashed

M. A. Rashed

Prof .of Genetics, Faculty of Agric. An Shams University.

Dr.A.A.Selim

C. A. Selim
Sat - 11 - 2 - 1995

Prof .of Pesticide Chemistry and Toxicology

Ain Shams Univ.

(Supervisor)

Date of examination: 11/ 2 / 1995.



**"BIOCHEMICAL PARAMETERS AS CORRELATED WITH
THE MUTAGENIC EFFECTS OF PESTICIDES ON
DROSOPHILA MELANOGASTER"**

By

Salwa Mohammed Mahmoud Kassem

B.Sc. Agric. (Entomology), Ain Shams Univ. 1979

M.Sc. Agric. (Insecticides). Ain Shams Univ. 1986

Under the supervision of:

Dr. A.A. Selim

Prof. of Pesticide Chemistry and Toxicology,
Plant Protection Dept., Faculty
of Agriculture, Ain Shams University, Cairo,
Egypt.

Dr. A.A.M. Awad

Prof. of Genetics, Faculty of Agriculture,
Ain Shams University, Cairo, Egypt .

Dr. A.De Hondt

Prof. of Genetics, Lab of Cell. Biology. National
Research Center, Giza, Egypt.

ABSTRACT

The present study aimed at investigating the response of parent and F_1 generations of *Drosophila melanogaster* to diazinon and dursban, at LC50 and LC5.

- Medium lethal concentration was estimated to be 0.447 & 0.237 ppm for diazinon and dursban, respectively while the slope was 2.7 & 4.91.

- In male sterility test both insecticides exerted direct effect on spermatozoa and spermatids, and indirect effect on other spermatogenesis stages with dursban.
- Dursban induced direct mutagenic effect as dominant female sterility at LC50 represented by 0.8% female sterility.
- A significant increase in sex-linked recessive lethals occurred with both insecticides.
- The effect of both insecticides was studied on A Ch E, Bu Ch E, A Ph, AL Ph, GO, GOT, GPT and LDH and total protein. Both pesticides inhibited all but GPT in both parent and F₁ generations, dursban was more effective.
- Correlation coefficients were calculated between enzyme activities and each of male sterility, dominant female sterility and sex-linked recessive lethals, and were found indicative in certain cases.
- Electrophoregrams revealed different isozymes for esterase and indophenole oxidase. The effect of dursban was greater with dursban on esterase of males and indophenole oxidase of female.

KEY WORDS

Drosophila melanogaster, diazinon, dursban, mutagenicity and enzyme activity.

ACKNOWLEDGMENT

The author wishes to express her sincere gratitude to Dr. A.A. Selim, Dept. Prof of Pesticide chemistry and toxicology and Ex. head of plant Protection Dept., Fac. Agriculture, Ain Shams Univ., For suggesting the problem, scheme of work, supervision, criticism and revising the manuscript. Thanks are also due to Prof. Dr. H.A. De Hondt, Lab. of cell Biology, National Research Center and Prof. Dr. A.A.M. Awad, Dept. of Genetics, Fac. Agriculture, Ain Shams University for supervising this work and for their constructive guidance and help during the progress of the study.

The author wishes also to express her deepest appreciation to Prof. Dr. A.Z. El-Abidin Salam, Ex. head Dept. of Genetics, Fac. Agriculture, Ain Shams University, and to Dr. Mari, Traize, Lab. of cell Biology, National Research Centre, for their untiring guidance, never ceasing encouragement and continued interest.

CONTENTS

	Page
I. INTRODUCTION	1
II. REVIEW OF LITERATURE	3
a) Mutagenicity of organophosphorus (OP).....	
insecticides in <i>Drosophila melanogaster</i>	3
b) Mutations influencing male fertility	
in <i>Drosophila melanogaster</i>	6
c) Dominant female sterility assay (DFS)	14
d) Mutagenicity of organophosphorus compounds	18
using the six-linked recessive lethals.....	
in <i>Drosophila melanogaster</i>	
e) Mutagenicity of organophosphorus (OP)	
insecticides in other organisms	23
f) Determination of enzyme activity	
Esterases, Acid phosphatases, Alkaline phosphatase,	
Glucose oxidase and Lactate dehydrogenase	25
g) Total protein.....	31
h) Detection of mutagenicity through esterase.....	33
and indophenol oxidase isozyme variations	
III. MATERIALS AND METHODS	40
a) Materials	40
1. <i>Drosophila</i> stocks [Oregon - K (O-K), Muller-5	40
(M-5), and Homozygous enzyme strain (HES)]	
2. Insecticides (Diazinon and Dursban)	41
b) Methods :.....	41

	Page
1. Preparation of the insecticidal stock.....	41.
solutions and determination of LC50 and LC5	42.
2. Sterile male test	42.
3. Dominant sterile female test	46.
4. Sex-linked recessive lethals	46.
5. Sterile female test.....	47.
6. Enzymatic determinations of esterases, acid and.....	
alkaline phosphatases, glucose oxidase,.....	47.
transaminases (GOT* & GPT) and lactate dehydrogenase..	
7. Determination of total protein	61.
8. Isozyme determination:	63.
(sample preparation, buffer solution, gel.....	
preparation, application of samples,.....	
isozyme visualization)	
1. Esterase isozymes	64.
2. Indophenol oxidase	65.
IV. RESULTS AND DISCUSSION.....	66.
1. Male sterility test:	66.
a. Spontaneous male sterility test	66.
b. Induction of male sterility test	66.
by diazinon and dursban	
2. Female sterility test in treated parents	75.

When mention (6) throughout the whole text =

GOT = Aspartate amino transferase.

GPT = Alanin amino transferase.

	Page
3. Dominant female sterility (DFS)	75.
a. Spontaneous dominant female sterility (DFS)	75.
b. Induction of dominant female sterility test.....	76.
by diazinon and dursban	
4. Sex-linked recessive lethals (SLRL)	81
a. Spontaneous SLRL	81
b. Induction of SLRL by diazinon and dursban	82
5. Enzymatic activities:	88
a. Effect of diazinon and dursban on acetylcholine...esterase.....	89.
b. Effect of diazinon and dursban on Butyrylcholine ..esterase.....	92.
c. Effect of diazinon and dursban on acid phosphatase.....	95.
d. Effect of diazinon and dursban on alkaline.....phosphatase.....	99.
e. Effect of diazinon and dursban on glucose oxidase.....	99.
f. Effect of diazinon and dursban on transaminases... (GOT and GPT)	105
g. Effect of diazinon and dursban on lactate.....dehydrogenase	111
6. Effect of diazinon and dursban on total protein.....	115
7. Isozyme electrophoresis: Esterase and indophenol...oxidase isozyme.....	118
V. SUMMARY	123
VI. REFERENCES	132
VII. APPENDIX	150
X. ARABIC SUMMARY	

12. Glucose oxidase mean activity of.....	103
homozygous enzyme strain <i>Drosophila melanogaster</i>	
13. Glutamate-oxaloacetate transaminase mean activity of.....	106
homozygous enzyme strain <i>Drosophila melanogaster</i>	
14. Glutamate-pyruvate transaminase mean activity of.....	109
homozygous enzyme strain <i>Drosophila melanogaster</i>	
15. Lactate dehydrogenase mean activity of	113
homozygous enzyme strain <i>Drosophila melanogaster</i>	
16. Total protein mean of homozygous	116
enzyme strain <i>Drosophila melanogaster</i>	
17. Acetylcholine esterase activity of.....	150
homozygous enzyme strain <i>Drosophila melanogaster</i>	
18. Butyrylcholine esterase activity of.....	151
homozygous enzyme strain <i>Drosophila melanogaster</i>	
19. Acid phosphatase activity of homozygous	152
enzyme strain <i>Drosophila melanogaster</i>	
20. Alkaline phosphatase activity of.....	153
homozygous enzyme strain <i>Drosophila melanogaster</i>	
21. Glucose oxidase activity of homozygous	154
enzyme strain <i>Drosophila melanogaster</i>	
22. Glutamate-oxaloacetate transaminase activity of.....	155
homozygous enzyme strain <i>Drosophila melanogaster</i>	
23. Glutamate-pyruvate transaminase activity of.....	156
homozygous enzyme strain <i>Drosophila melanogaster</i>	

Table No.	Page
24. Lactate dehydrogenase activity of.....	157
homozygous enzyme strain <i>Drosophila melanogaster</i>	
25. Total protein of homozygous enzyme strain.....	158
<i>Drosophila melanogaster</i>	
26. Variance analysis of acetylcholine esterase activity.....	159
27. Variance analysis of butyrylcholine esterase activity	160
28. Variance analysis of Acid phosphatase activity.....	161
29. Variance analysis of Alkaline phosphatase activity.....	162
30. Variance analysis of Glucose oxidase activity.....	163
31. Variance analysis of Glutamate-oxaloacetate transaminase... activity.....	164
32. Variance analysis of Glutamate-pyruvate transaminase..... activity.....	165
33. Variance analysis of Lactate dehydrogenase activity.....	166
34. Variance analysis of total protein	167
35. Correlation between enzyme activities and each of male or.. female sterility and sex-linked recessive lethality of <i>Drosophila melanogaster</i> at LC50 of diazinon	168
36. Correlation between enzyme activities and each of male or.. female sterility and sex-linked recessive lethality of <i>Drosophila melanogaster</i> at LC50 of dursban	169

LIST OF FIGURES

Fig. No.	Page
1. Toxicity regression line of diazinon to <i>Drosophila melanogaster</i>	44
2. Toxicity regression line of dursban to <i>Drosophila melanogaster</i>	45
3. Effect of <i>Drosophila melanogaster</i> whole homogenate on..... the activity of A Ch E: relationship between time and activity as optical density units	50
4. Percentage of male sterility induced by diazinon and..... dursban in <i>Drosophila melanogaster</i> at LC50 and LC5.....	47.
5. Percentage of dominant female sterility in F ₁ induced..... by diazinon and dursban in <i>Drosophila melanogaster</i> at LC50 and LC5	80.
6. Percentage of sex-linked recessive lethals induced..... by diazinon and dursban in <i>Drosophila melanogaster</i> at LC50 and LC5	87.
7. Histogram represents acetylcholine esterase activity..... of homozygous enzyme strain <i>Drosophila melanogaster</i>	91.
8. Histogram represents butyrylcholine esterase activity..... of homozygous enzyme strain, <i>Drosophila melanogaster</i>	94.
9. Histogram represents acid phosphatase activity..... of homozygous enzyme strain, <i>Drosophila melanogaster</i>	98.
10. Histogram represents alkaline phosphatase activity..... of homozygous enzyme strain <i>Drosophila melanogaster</i>	101

Fig. No.	Page
11. Histogram represents glucose oxidase activity104. of homozygous strain, <i>Drosophila melanogaster</i>	
12. Histogram represents glutamate-oxaloacetate transaminase ..107. activity of homozygous enzyme strain,..... <i>Drosophila melanogaster</i>	
13. Histogram represents glutamate-pyruvate110. transaminase activity of homozygous enzyme strain,..... <i>Drosophila melanogaster</i>	
14. Histogram represents lactate dehydrogenase activity.....114. of homozygous enzyme strain, <i>Drosophila melanogaster</i>	
15. Histogram represents total protein values117. of homozygous enzyme strain, <i>Drosophila melanogaster</i>	
16. Electrophoretic patterns of esterase isozymes in.....119. parents and F ₁ one generations as affected..... by diazinon and dursban at LC50	
17. Electrophoretic patterns of indophenol oxidase.....121.. isozymes in parents and F ₁ generations as affected..... by diazinon and dursban at LC50 concentration	

INTRODUCTION

I. INTRODUCTION

Chemical pesticides are widely used in pest control programmes in agriculture and against man natural enemies in hygiene. Therefore, the extensive use of the tremendous amounts of pesticides raised up the distinguishable problem of environmental pollution.

Several reports indicate that some of these pesticides can produce important side effects, including genetic damage to microorganisms, plants, animals, and mankind (Garrett, et al., 1986). Furthermore, the importance of taking genetic hazards in consideration in occupational health studies has been reported recently by Xamena, et al., (1988).

Organophosphorus pesticides (OP's) which are the most important group of pesticides, are known to react with DNA generally as alkylating agents and, consequently, they are potentially mutagens and/or carcinogens. Some organophosphorus compound were proved to be effective mutagens in a variety of organisms (Waters, et al., 1980). There is a great deal of scanty and inconclusive results, due to the high toxicity of these compounds. This fact implies that more researches are necessary to evaluate adequately the real mutagenic effects of the organophosphorus pesticides present in our environment (Velazquez, et al., 1986).
