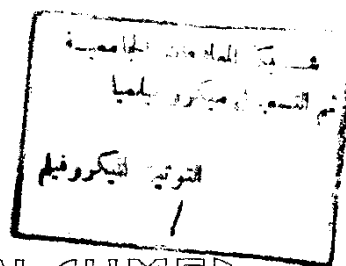


THE PARALLEL RESPONSE OF ENZYME LOCI IN DROSOPHILA AND YEAST TO THE ENVIRONMENTAL STRESSES OF POLLUTANTS

BY

EKRAM SALAH EL-DIN AHMED



A thesis submitted in partial fulfillment

of

the requirement for the degree of

DOCTOR OF PHILOSOPHY

in

Agricultural Science

(Genetics)

631.523
F.S.



45116

Department of Genetics
Faculty of Agriculture
Ain Shams University
1994

Approval sheet

**THE PARALLEL RESPONSE OF ENZYME
LOCI IN DROSOPHILA AND YEAST TO
THE ENVIRONMENTAL STRESSES OF
POLLUTANTS**

BY

EKRAM SALAH EL-DIN AHMED

B. Sc. Genetics, Fac. of Agric., Ain Shams University (1976)

M.Sc. Genetics, Fac. of Agric., Ain Shams University (1989)

This thesis for Ph. D. degree has been approved by:

Prof. Dr. *Hamed Abdel Maksood Nafei* *H.M.N.*

Prof. of Genetics, Faculty of Agriculture, Cairo
University

Prof. Dr. *Samir Abdel Aziz Ibrahim* *S.A. Ibrahim*

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

Prof. Dr. *A. L. El-Abidin Salam* (Supervisor) *A.L.E.S.* (117)

Prof. and Head Department of Genetics, Fac. of Agric.
Ain Shams University.

Date of examination:- 27\ 8\ 1994



THE PARALLEL RESPONSE OF ENZYME LOCI IN DROSOPHILA AND YEAST TO THE ENVIRONMENTAL STRESSES OF POLLUTANTS

BY

EKRAM SALAH EL-DIN AHMED

B. Sc. Genetics, Fac. of Agric., Ain Shams University (1976)

M.Sc. Genetics, Fac. of Agric., Ain Shams University (1989)

Under the supervision of:

Prof. Dr. *A. L. El-Abidin Salam*

Prof. and Head Department of Genetics, Fac. of Agric.
Ain Shams University.

Prof. Dr. *H. A. de Hondt*

Prof. of Genetics, Department of Cell Biology,
National Research Center.

ABSTRACT

The possible mutagenicity of the two pesticides, nudrin and meothrin were evaluated using two living systems: *Drosophila melanogaster* and *Saccharomyces cerevisiae*. The results indicate

that, the two pesticides showed a mutagenic effect on the genetic background of each of cholinesterase (ChE) and aliesterase (AliE) in *Drosophila* and yeast. Meanwhile, nudrin and meothrin induced gene conversion, reversion and mitotic crossing over in *S. cerevisiae* strain D7.

The results obtained in the present study indicate that the two pesticides proved to be mutagenic in two eukaryotic systems; *Drosophila* and yeast.

Key words: Mutagenic activity, pesticides, Enzymes, *Drosophila*, Yeast.

ACKNOWLEDGMENT

The writer wishes to express her sincere gratitude to Professor Dr. *A. Z. El-Abidin Salam*, head of the Department of Genetics, Faculty of Agriculture, Ain Shams University, and Professor Dr. *H. A. de-Hondt* Department of Cell Biology, National Research Center for supervising this work and for their constructive criticism during the progress of the study.

Thanks are also due to Dr. *Mary Therase Ibrahim*, Assistant Professor of Genetics, and Dr. *Samia Soussa*, Lecturer of Genetics, Department of Cell Biology, National Research Center for their help offered during the progress of this study.

Thanks also to Dr. *Thorayia Fahim* Lecturer of Entomology, Faculty of Science, Ain Shams University for her help throughout the experiments of this study.

Finally, the writer acknowledges the guidance by the staff members of the Genetics Department, Faculty of Agriculture, Ain Shams University, and the members of the Cell Biology Department, National Research Center.

Contents

	page
1- Introduction.....	1
2- Review of Literature.....	4
2.1- Mutagenic potentiality of pesticides.....	4
2.1.1- Mutagenicity tests using <i>Drosophila melanogaster</i>	4
2.1.1.1- Sex linked recessive lethals (SLRL).....	4
2.1.1.2- Enzyme activities.....	5
2.1.1.3- Detection of mutagenicity by isozyme variations.....	7
2.1.2- Mutagenicity tests using yeast.....	8
2.1.2.1- Pesticide induced mitotic crossing over, conversion and reverse mutation.....	9
2.1.2.2- Protein variation in yeast.....	10
3- Materials and Methods.....	11
3.1- Stocks.....	11
3.1.1- Drosophila stocks.....	11
3.1.2- Yeast stock.....	12
3.2- Pesticides.....	14
3.3- Drosophila experimental design.....	14
3.4- Yeast experimental design.....	23
4- Results and Discussion.....	34

4.1- Drosophila experiments.....	35
4.1.1- Induction of sex linked recessive lethals.....	35
4.1.2- Mutagenic effect of pesticides on the enzyme activities.....	40
4.1.3- Electrophoretic variation.....	50
4.2- Yeast experiments.....	57
4.2.1- Mutagenic effect of pesticides in <i>S. cerevisiae</i>	57
4.2.2- Protein variation in <i>S. cerevisiae</i>	64
4.2.3- Enzyme activities in <i>S. cerevisiae</i>	67
5- Summary and conclusions	75
6- References.....	79
Arabic Summary	

List of Tables

	Page
Table (1): Frequencies of spontaneous and nudrin induced sex-linked recessive lethals.....	37
Table (2): Frequencies of spontaneous and meothrin induced sex-linked recessive lethals.....	39
Table (3): Effect of nudrin and meothrin on ChE activity in three categories of <i>D. melanogaster</i>	42
Table (4): Effect of nudrin and meothrin on AliE activity in three categories of <i>D. melanogaster</i>	48
Table (5): Number of genotypes tested, allelic frequencies and proportion of eleven loci in the F ₁ s flies of <i>D. melanogaster</i> for larvae treated by nudrin and control..	53
Table (6): Number of genotypes tested, allelic frequencies and proportion of eleven loci in the F ₁ s flies of <i>D. melanogaster</i> for larvae treated by meothrin and control.....	56
Table (7): Response of <i>S. cerevisiae</i> to treatment with nudrin.....	58
Table (8): Response of <i>S. cerevisiae</i> to treatment with meothrin..	62
Table (9): Effect of nudrin and meothrin on ChE activity in <i>S. cerevisiae</i>	68
Table (10): Effect of nudrin and meothrin on AliE activity in <i>S. cerevisiae</i>	71

List of Figures

	Page
Figure (1): Effect of nudrin and meothrin on ChE activity in three categories of <i>D. melanogaster</i>	44
Figure (2): Effect of nudrin and meothrin on AliE activity in three categories of <i>D. melanogaster</i>	49
Figure (3): Zymogram of α -Gpdh in <i>D. melanogaster</i> (control)....	54
Figure (4): Zymogram of α -Gpdh in <i>D. melanogaster</i> (meothrin variants).....	54
Figure (5): Response of <i>S. cerevisiae</i> to treatment with nudrin...	59
Figure (6): Response of <i>S. cerevisiae</i> to treatment with meothrin.	63
Figure (7): Zymogram of protein banding patterns in <i>S. cerevisiae</i> (control).....	66
Figure (8): Zymogram of protein banding patterns in <i>S. cerevisiae</i> treated with nudrin.....	66
Figure (9): Zymogram of protein banding patterns in <i>S. cerevisiae</i> treated with meothrin.....	66
Figure (10): Histogram representing the ChE activity in <i>S. cerevisiae</i> after treatment with different concentrations of nudrin and meothrin	69
Figure (11): Histogram representing the AliE activity in <i>S. cerevisiae</i> after treatment with different concentrations of nudrin and meothrin	72

Introduction

INTRODUCTION

One of the most important trends of mutation research in recent years is to study the mutagenic effects of chemical compounds in different organisms. The wide use and sometimes abuse of pesticides nowadays should be considered in this respect. Several reports indicate that many chemical pollutants which are widely spread in the environment, such as pesticides and drugs, are mutagenic in various test systems (Tripathi, *et al.*, 1988., Salam *et al.*, 1993). In spite of that, pesticides are still widely used and many new forms are annually produced. This can be attributed to the fact that the application of pesticides is still the most effective tool in pest control programs. Subsequently, pesticides are considered one of the important (components) of modern agriculture. Although mammalian species are the most favourable organisms for testing the mutagenicity of pesticides due to their resemblance to human, a wide spectrum of other living systems is still employed in such tests for the facility in handling them. Many laboratories working on the problem of environmental pollutants consider that *Drosophila melanogaster* and *Saccharomyces cerevisiae* are suitable eukaryotic organisms for testing the mutagenicity of such pollutants. *Drosophila melanogaster* has been used to test the mutagenicity by several workers. Sex-linked recessive lethals test in *Drosophila* provides one of the quickest point mutation tests in eukaryotes. Meanwhile, the structural gene (s) coding for cholinesterase enzyme (ChE) proved to be highly linked to the genetic background of some pesticides resistance (Tobgy, *et*

al., 1976 and Salam and Pinsker 1981). Furthermore, the isozyme polymorphism which was mainly studied by applications of starch gel electrophoresis technique (Ayala, *et al.*, 1972) proved to be a good tool for countering the mutagenic potentiality of such pollutants. The induction of mitotic gene conversions, revertants and mitotic crossing over in the diploid yeast *S. cerevisiae* are strongly correlated with mutagenic effects, and these tests reacts very sensitively with compounds which induce base-pair substitutions as well as frame-shift mutations. This system has revealed the genetic activity of a large number of carcinogens, pesticides and many other chemical mutagens (Siebert and Elsenbrand, 1974).

The aim of the present investigation is to study the potential genetic effects of two pesticides representing two different chemical groups, carbamates and pyrethroids, the carbamate group is represented by nudrin and the pyrethroids group is presented by meothrin, using various methods in the two organisms, i.e., *D. melanogaster* and *S. cerevisiae*

These methods are:

- Sex-linked recessive lethals (SLRL) test.
- Effect of pesticides on enzyme activities using spectrophotometric analysis.

- The potentiality of the two pesticides as mutagens on eleven enzyme loci. These enzymes are Est, Adh, Acph, Ao. Aph, Odh, 6-pgd, α -Gpdh, Mdh, Me and Pgm.
- The induction of mitotic gene conversion, reverse mutation and mitotic crossing over in *S. cerevisiae* strain D7.
- The induction of total protein variation in *S. cerevisiae* strain D7.
- Effect on enzyme activities in yeast using spectrophotometric analysis.

Review of Literature