THE DETECTION OF GENOTOXIC ACTIVITY OF THE DELTAMETHRIN AND PERMETHRIN BY SOMATIC MUTATION AND RECOMBINATION TEST WITH *DROSOPHILA MELANOGASTER*

F. Azize BUDAK
Kırıkkale Üniversitesi Fen-Edebiyat Fakültesi Biyoloji Bölümü Kırıkkale/ TÜRKİYE

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ABSTRACT

In this study, different doses of deltamethrin from pyretyroide insecticides and permethrine from organophosphate insecticides have been analysied for their genotoxic effects by somatic mutation and recombination test (SMART) in Drosophila melanogaster. Lethal doses of chemicals used were determined. Trans-heterozygous larvae for two genetic markers mwh and flr^3 were evaluated for 25 ppm, 50 ppm and 75 ppm concentrations of the insecticides. A possitive correlation was observed among total mutations, the number of wings having mutations and percentage survival. Mutations observed were classified according to their size and type per wing and the data evaluated by statistical analysis. Deltamethrin was found more toxic and mutagenic than permethrine.

Key Words: *Drosophila melanogaster, somatic mutation and recombination test, insecticides, genotoxic effect*

DROSOPHİLA MELANOGASTER'İN SOMATİK MUTASYON VE REKOMBİNASYON TESTİ İLE DELTAMETRİN VE PERMETRİNİN GENOTOKSİK AKTİVİTESİNİN BULUNMASI

ÖZET

Bu çalışmada pretiroid insektisitlerden deltametrin ve organofosfatlardan permetrinin Drosophila melanogaster'de genotoksik etkileri Somatik Mutasyon ve Rekombinasyon testi ile analiz edildi. Kullanılan kimyasalların letal dozları belirlendi. İşaret genleri taşıyan mwh ve flr^3 transheterozigot larvaları insektisitlerin 25 ppm, 50 ppm and 75 ppm konsantrasyonları için değerlendirildi. Toplam mutasyon, mutasyon gözlenen kanat sayısı ve yaşama yüzdesi arasında pozitif korelasyon gözlendi. Gözlenen mutasyonlar kanat başına mutasyon oluşturan hücre sayısına ve tipine göre sınıflandırıldı ve veriler istatistiksel analiz ile değerlendirildi. İki pestisit arasından deltametrinin permetrinden daha toksik ve mutajenik olduğu gözlendi.

Anahtar Kelimeler: *Drosophila melanogaster, somatik mutasyon ve rekombinasyon test, insektisit, genotoksik etki*

1. INTRODUCTION

Insecticides are commonly used in agricultural fields. Pesticides are a group of diverse chemical agents used to control insects, weeds or other pests (Hrelia et al., 1993). Firstly arsenic and sulphur were used as pesticides. Then vegetal matters such as nicotine, pretyroide and to classified as carbomate compounds have been used. Mutagenic effects of these chemicals have been known. There are negative effects to use pesticides in ecosistem. Because pesticides do not affect only target organism but they also affect another organisms in ecosistem. Their intended effects, many of these biologically active compounds affect non-target organisms including man himself (Murphy, 1986) Since mutagenity data are part of the weight-of-evidence approach for classifying potantial human carsinogens (Carere and Benigni, 1990; Auletta et al., 1993) pesticides studies have been devoted to the investigate of the genotoxic effect of pesticides and potantial risk to humans.

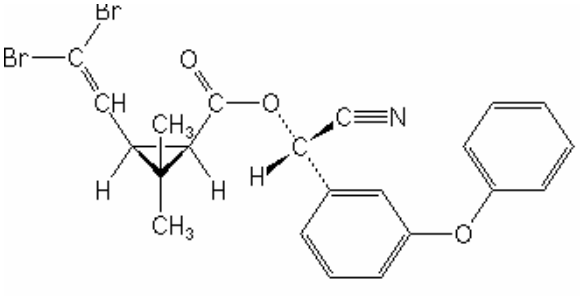
Chemicals have been tested used to various methods. One of method of these used commonly methods is Somatic Mutation and Recombination Test (SMART) (Graf et al., 1984, Graf et al., 1989, Watabane et al., 1996, Ekebaş et al., 1999). Somatic spots were grouped into three categories: (i) small single spots (ii) large single spots and (iii) twin spots. Spots comprising; 1 or 2 mwh or flr^3 cells were scored as small single spots; and 3 or more cells were scored as large single spots. Spots consisting of neighboring mwh and flr^3 cells were counted as twin spots. As cell genetics show, wing spot test assays detect several genetic endopoints. Genetic changes induced in somatic cells of the wing's imaginal discs lead to the formation of mutant clones on the wing blade. Single spots are produced by somatic point mutation, deletion, etc. and mitotic recombination occurring between the 2 markers. Twin spots are produced exclusively by mitotic recombination the proximal marker flr and the centromere of chromosome 3. To determine the frequency of spots detected by mwh clones on the marker-heterozgous wings (mwh single spots plus mwh clones of twin spots) are compared with the frequency of mwh clones on the balancer-heterozygous wings. The difference in mwh clone frequency is a direct measure of the proportion of recombination (Graf et al., 1984, Frei, et al., 1992). The SMART in *Drosophila melanogaster* was designed to detect genotoxic activity in a rapid and simple way in somatic cells.

In this study, deltamethrin and permethrin have been tested for genotoxic effect. The aim of this study, is to compare the genotoxic effects of deltamethrin and permethrin by SMART method in *Drosophila melanogaster.*

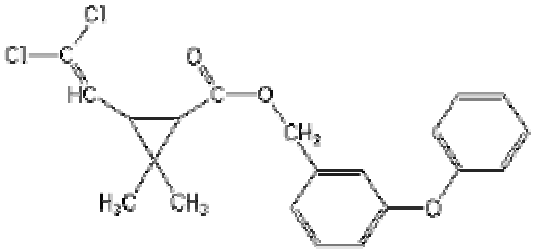
2. MATERIALS AND METHODS

2.1. Chemicals: Two insecticides (deltamethrin as a pyrethroid and permethrin as a organophosphate) were used to test their genotoxicity. These chemicals, were dissolved in distilled water to obtain the required concentrtions. The structure formulae of chemicals tested are shown below .

(http://www.hclrss.demon.co.uk)



Deltamethrin



Permethrin

2.2. Strains: Two *Drosophila* strains were used in this study. The parental flies; the multiple wing hairs strain with genotypes mwh/mwh and the flr^3 /In (3LR), TM3 Bd^s strain (Lindsley and Zimm, 1992). They were obtained from Professor F.E. Würger (University of Zurich, Switzerland). F_1 larvae, from the cross of mwh/mwh females and flr^3 /TM male introduced into vials at 25 ^{0}C.

2.3. Experimental procedure: Eggs from the crosses between mwh virgin females and flr males were collected during 4-h periods in culture bottles containing Standard medium. after 72 hours trans-heterozygous larvae for two genetic markers *mwh* and *flr^3* were washed and selected for the treatment. For the chronic feeding study, small plastic vials were prepared with 1.5 g dry Drosophila Instant Medium (Carolina Biological Supply Company Burlington, NC,USA) and 5ml of the respective test solutions. Instant medium was prepared with distilled water for the negative controls. 100 larvae were embedded into this medium. The larvae were fed with different concentrations of the test compounds. Feeding ended with pupation of the surviving larvae. The experiments were repeated 3 times. All experiments were performed at 25±1^0 C and at a relative humidity of approximately 65%. Concentrations of the insecticides were: 25ppm, 50 ppm and 75 ppm.

2.4. Preparation and microscopic analyses of wings: Experiments were performed to Graf and et al., 1984. After metamorphosis, all surviving flies were scored irrespective of sex, and classified according to the presecense/ absence of the phenotype, and then stored in a 70% ethanol solution. For observation of mutant spots, the wings were removed and mounted on slides using Faure's solution (gum arabic 30g, glycerol 20 ml, chloral hydrate 50g, water 50ml). Dorsal and ventral surfaces of the wings are analyzed under a compound microscope at 400X magnification for the occurrence of single spots.

2.5. Statistical analysis: For evaluation of detected genotoxic effects, frequency of spots per wing in the treated series were compared to negative controls. To evaluate the statistical significance of the results obtained, we followed a multiple decision procedure, based on two alternative hypotheses: (1) the mutation frequency in the treated group is no higher than the mutation frequency in the appropriate control, and (2) the induced mutation frequency in the treated group is no less than m times as high as the observed spontaneous mutation frequency in the

control. The decision procedure was performed as described in the literature (Frei and Wurgler,1988). For statistical calculational binomial test according to Kastenbaum and Bowman was used with 5% significance levels (Kastenbaum and Bowman, 1970).

3. RESULTS

In this study, Somatic Mutation and Recombination Test (SMART) was appllied to test the genotoxicity of deltamethrin as a pyretroid pesticide and permethrin as organophosphate pesticide. 25 ppm, 50 ppm and 75 ppm doses of deltamethrin and permethrin were applied to test their genotoxicity and statistical evaluation of the results were given in Table 1.

Deltamethrin has been shown to be highly toxic at both larval and adult stages than permethrin in *Drosophila melanogaster*. The analysis of the wing spot data from chronic treatments showed that there was no twin spots. Small single spot type of mutations was common than the other type of mutations and the frequency of mutation increased when doses increased for both chemicals. Genotoxicity of deltamethrin was found as positive for small single spot mutation at 75 ppm, but permethrin was found as inconclucive for the same type of mutation at 75 ppm.

The data obtained from application of 25 ppm and 50 ppm concentrations showed nearly the similar results but that of 75 ppm showed a conciderable increase in number of mutations.

4. DISCUSSION

It is known that human and enviromental health are under the threat of hazardous chemical pesticide residues. So it is necessary to know their mutagenic and carsinogenic effect on living things. SMART method is a commonly used method, which was firstly used by Graf et al. (1984) to test the genotoxicity of different chemicals such as different group of pesticides (Osaba et al. 1999, Ekebaş et al., 1999), herbicides (Kaya, et al., 2003), fungicides (Osaba et al., 2002), polycyclic aromatic hydrocarbons (Frölich and Würgler , 1989) e.t.c. used. Graf et al., (1989) were evaluated for its suitability in genotoxicity screening by testing 30 chemicals. They were obtained in two different laboratories, demonstrating that the wing spot test is easily transferable to other laboratories.

There are also many other experiments by using this test. For example, Osaba et al. (2002) tested the genotoxicity of captan, zineb, moneb of fungicides in the wing spot test of *Drosophila melanogaster* . They reported that captan showed negative results to contrast maneb showed positive results.

Frölich and Würgler (1989) were tested improved detectability of polycyclic aromatic hydrocarbons (PAHs) by *Drosophila* wing – spot test. Comparative test with 3 PAHs benzo [a] pyrenre, benz [a] anthracene and 7, 12 dimethylben [a] anthracene demonstrate that with the new strains can be detected as active genotoxic compounds. This is interpereted as indicative of a saturation of cytochrome P-450 dependent activation systems.

Some organophosphate pesticides were tested for their genotoxic activity by Ekebaş

et al. (1999). They reported ordery genotoxicity of these pesticides that dichlorvos, methyl parathion, azomethiphos.

Osaba et al. (1999) tested the pyrethroid allethrin, the methylenedioxyphenolic compound piperonly butoxide, the chlorinated hydrocarbons dieldrin and endrin, the organophosphates dimethoate and malathion. They found negative results for all these chemicals used.

According to the results of this study deltamethrin was more lethal than permethrin so, number of wings examined for mutation was less after deltamethrin applications. Therefore, frequency of mutation was found less (1.23) for deltamethrin. But in reaility deltamethrin is more mutagenic than permethrin. Deltamethrin and permethrin have been firstly tested by this method.

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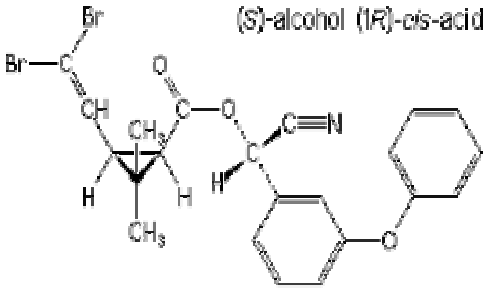

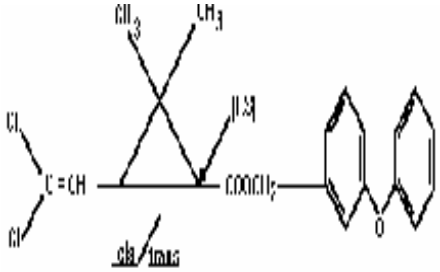

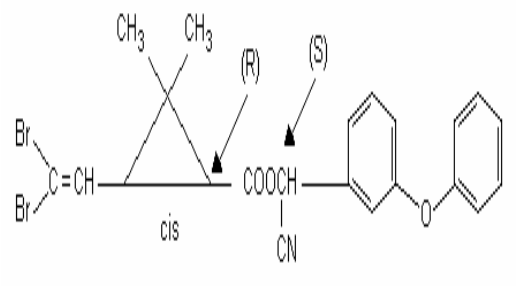

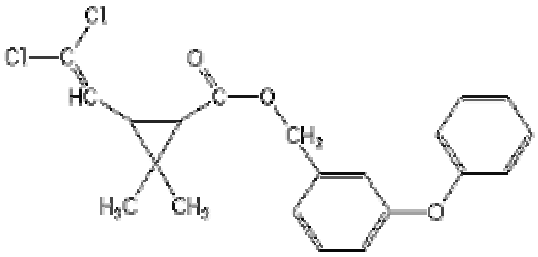

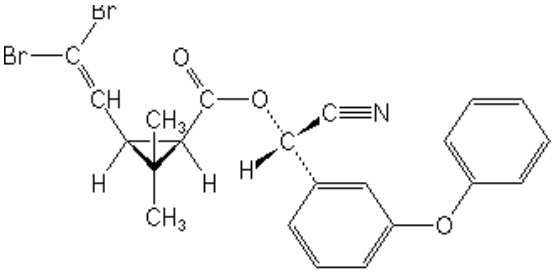

Groups	Concentration (ppm)	Survival (%)	Number of wings	Small single spots (1-2 cells) (m=2)			Large single spots (>2 cells) (m=5)			Twin spots (m=5)			Total mwh spots (m=2)			Total spots (m=2)		
				No.	Fr.	D	No.	Fr.	D	No.	Fr.	D	No.	Fr.	D	No.	Fr.	D
Control group	Distilled water	98	158	16	0.10		5	0.03		2	0.01		16	0.10		23	0.15	
Deltamethrin	25	83	105	78	0.74	i	10	0.09	+	0	0.00	i	18	0.17	i	88	0.89	i
	50	47.3	74	59	0.80	i	3	0.04	i	0	0.00	i	5	0.07	_	62	0.84	i
	75	38.7	40	43	1.08	+	6	0.15	i	0	0.00	i	4	0.10	_	49	1.23	i
Permethrin	25	82.7	131	55	0.42	+	12	0.09	i	1	0.01	i	10	0.08	_	55	0.42	i
	50	52	75	46	0.61	i	6	0.08	i	0	0.00	i	3	0.04	_	52	0.69	i
	75	46.7	82	75	0.91	i	38	0.46	i	0	0.00	i	15	0.18	i	113	1.38	i

Table1: Wing spot test data obtained for deltamethrin and permethrin tested

Fr: frequency, D: statistical diagnosis according to Frei and Würgler, 1988, +: positive, -: negative, i: inconclucive, m: multiplication factors.