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Effect of Cypermethrin on Some Developmental Stages of *Drosophila melanogaster*

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Abstract This study investigated the effects of cypermethrin, a synthetic pyrethroid, on some developmental features of *Drosophila melanogaster*. Before the experiment the lethal concentration of this insecticide was determined. Cypermethrin solution was applied to *Drosophila melanogaster* by means of nutrition, by adding it to the culture medium. In the control group no such substance was applied. Our aim was to find out whether any developmental toxic effects occur, and, if they do, during which stage, by observing the rates of egg-laying of adult females, the development of eggs, and the development of larvae. The results showed that there was no significant difference in the rates of egg-laying. However, the decrease in the rate of egg development revealed that eggs and early embryonic stages were sensitive to toxic effects. The same toxic effect was not observed in third instar larvae. The toxic effect was observed to be strongest in the early stages of development.

Keywords Drosophila · Cypermethrin · Developmental features

The increasing use of chemicals in agriculture can be destructively noxious for the environment, and thus for the health of human beings (Vélazquez et al. 1990). Pyrethroids, artificial compounds active in pharmacological circumstances, are formed on the basis of a substance called pyrethrum, which is formed naturally. Pyrethroids are representative examples of 25% of the insecticides being applied in agriculture worldwide (Miadoková et al. 1992). Their features of pest specificity and low degree of toxicity for mammals have lately made these synthetic pyrethroid pesticides attractive, leading to their extensive use (Batiste-Alentorn et al. 1987; Institóris et al. 1999). The insecticides belonging to these synthetic pyrethroid groups affect the peripheral and central nervous systems of insects. They initially increase the secretion in neurons and then cause their paralysis. They show these effects in the synapses and ganglions of the nerve cords of insects. As synthetic pyrethroids are characteristically lipophilic, they are stored and segmented in adipose tissues of organisms (Ware 1983).

Some studies have shown the in vivo genotoxicity of cypermethrin even at very low concentrations (Mukhopadhyay et al. 2004). In order to evaluate their potential hazardous side effects on humans, and to eliminate or reduce their undesirable effects, various studies such as toxicological, mutagenic, and carcinogenic evaluations are needed. The genotoxic potential of pesticides has been examined in various studies in recent decades, which have reported *Drosophila melanogaster* to be an instrumental eukaryotic organism in in vivo genotoxicity evaluations (Kaya et al. 2000). Because of the inadequate empirical information in the literature, the effects of cypermethrin on the development of *Drosophila melanogaster* are reported here. Despite the fact that there have been many studies carried out concerning the effects of cypermethrin on *Drosophila melanogaster* using various empirical methods (Mukhopadhyay et al. 2004, 2002, 2006; Rani et al. 1997), the current information about the development of egg and

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larvae, and fertility of *Drosophila melanogaster* is scanty. In this paper various developmental effects of cypermethrin uptake during some developmental stages of *Drosophila melanogaster* are reported.

Materials and Methods

In the tests we carried out, *Drosophila melanogaster* Oregon-R strain, a circle and red-eyed strain not carrying any mutant genes, was used. The cultural medium was set as the Standard Drosophila Medium (SDM) containing corn meal, molasses, yeast, agar, water, and propionic acid (Uysal and Kaya 2004). The strain was provided by Hacettepe University and *Drosophila* strains and experimental groups were kept in an incubator of 25 ± 1°C.

In the experiment, technical grade cypermethrin (99.50% pure, Singenta Limited, Istanbul) was used. In order to imitate the agricultural medium applied, cypermethrin was dissolved in water. At first a stock dissolution of 1,000 ppm was obtained and then it was diluted to the desired insecticide concentration (Batiste-Alentorn et al. 1987). In order to determine the lethal concentration for cypermethrin, it was added to culture medium in different concentrations. Then, for each culture medium 20 female and 20 male adult individuals were mated. After 24 h, the dead and live individuals were counted, and the death rate was calculated. The results showed the LC_{50} concentration to be between 80 ppm and 110 ppm. The pesticide was applied to the experimental organisms by means of nutrition. For this aim, three concentrations below the value of LC_{50}, i.e., 10, 20, and 40 ppm, were mixed into the medium cultures of larvae and adult individuals. Only SDM was used in the control group.

To determine fertility, 50 female individuals were transferred to each culture medium containing pesticide solution. These female individuals were kept in the culture medium for 5 days. Ten female individuals chosen randomly were transferred to petri dishes not containing pesticide solution. After incubation for a day, the individuals were removed, and eggs were counted. After the count, the development of mentioned eggs was also observed. The development of eggs was observed until the stage of third instar larvae, and the number of third instar larvae was recorded. The number of eggs left by the adult female individuals and the number of developed instar larvae were also compared.

In another part of the study, third instar larvae of the same age were obtained from the stock culture. For the F_1 generation, 50 larvae were placed into the culture medium of each control and experimental group. The individuals that had become mature were counted twice a day and then were removed. The same process was repeated four times. For the F_2 generation, the individuals that were maintained in culture mediums containing cypermethrin solution of different concentrations in their egg and larva stages were taken out. These larvae were transferred to SDMs in accordance with the initial concentrations. The development of these larvae was also observed as it was in the F_1 generation. Third instar larvae were transferred to culture medium of the experiment and control groups. The number of developing adult individuals and the number of larvae were compared (Uysal and Kaya 2004).

Data concerning fertility were tested by variance analysis. The statistical method used for the other data was the z-test of the comparison of ratios. The ratios were converted to z-points, and the differences between the counts of the two groups were tested. $z = \frac{(p_1 - p_2)}{\sqrt{\frac{p_1 q_1}{n_1}} + \left(\frac{p_2 q_2}{n_2}\right)}$; $q_2 = 1 - p_1$. For the calculations, Minitab for Windows ver 13.0 statistics program was used.

Results and Discussion

LC_{50} concentration was between 80 and 110 ppm. In the experiments three different concentrations below LC_{50}, i.e. 10, 20, and 40 ppm, were tested.

The third instar larvae development of the eggs left in the culture mediums containing cypermethrin solution was, in almost all experimental groups, different from that in the control groups. All experimental groups were compared both in themselves and with the control groups. In the F_1 and F_2 generations the development of eggs to larvae was inhibited. It is interesting that although the F_2 generation was not subjected to pesticide solution the same toxic effect was observed. The difference between the experimental groups of 10 and 40 ppm in the F_1 generation and the difference between the experimental groups of 10 and 20 ppm in the F_2 generation were not significant. Cypermethrin affected the development between egg and third instar larvae stages negatively in both generations (Table 1). There was a significant decrease in the development of the eggs belonging to the experimental group. Although the F_2 generation included Drosophila culture medium, its development percentage of larvae was much lower than that of the control group. This indicates that the toxic effect in the F_1 generation was still present in the F_2 generation. A similar study analyzed the effect of a synthetic pyrethroid called beta-cyfluthrin on the sepia mutant of *Drosophila melanogaster* and concluded that it caused egg hatching to decrease (Nadda et al. 2005). It is claimed that the reason for this may be inappropriate incorporation of the yolk, causing the embryo to fail to complete its phases of development. Additionally, it has been suggested that a decrease in egg hatching may result from antifeedant

Table 1 The effect of cypermethrin on egg development

Generation	Concentration ppm)	Total number of eggs (n)	Total number of larvae	*p* value (ratio of development)	z value
F_1	Control (G1)	1584	1206	0.761364	(G1−G2) 8.78**
	10 (G2)	1098	660	0.601093	(G2−G3) −5.82**
					(G2−G4) −1.36 (n.s.)
	20 (G3)	978	705	0.720859	(G1−G3) 2.26*
					(G3−G4) 4.58**
	40 (G4)	1183	744	0.628910	(G1−G4) 7.50**
F_2	Control (G5)	1542	1231	0.798314	(G5−G6) 4.57**
	10 (G6)	1548	1128	0.728682	(G6−G7) −0.51 (n.s.)
					(G6−G8) 6.63**
	20 (G7)	1331	981	0.737040	(G5−G7) 3.88**
					(G7−G8) 6.91**
	40 (G8)	1233	752	0.609895	(G5−G8) 10.93**

* $p < 0.05$, ** $p < 0.005$ n.s. not significant

and repellant effects of beta-cyfluthrin, leading to weak and nonviable egg laying (Nadda et al. 2005).

In another part of our experiment, the development of third instar larvae into adult individuals was observed. According to the results, in the F_1 generation, the experimental group that received 20 ppm solution (G3) was significantly different from the control group. A comparison between the same group and the G2 group (10 ppm) also showed the difference to be significant. In the F_2 generation, the 20 ppm experimental group did not differ from the control group; however, this group was significantly different from the other experimental groups. The fact that the experimental groups generally do not differ notably from the control group indicates that there was no toxic effect during the development of third instar larvae into adult individuals.

The substance applied did not alter the percentage of third instar larvae developing into adult individuals. Comparing the experimental groups with the control group, it was observed that the 20 ppm concentration had a toxic effect only on the F_1 generation (Table 2). Hence we can conclude that cypermethrin had a toxic effect on either between the stages of egg and third instar larvae, or previously during the course of spermatogenesis and oogenesis. In our experiment, while egg development was inhibited due to the insecticides used, the same toxic effect was not observed during larval development. Furthermore, the developmental stage of egg and third instar larvae was more sensitive to toxicity than the after-stages of third instar larvae. A study performed with *Rana arvalis* also yielded results supporting this finding of our experiment. The developmental success of the eggs put in a medium of 1 and 10 ppm alpha-cypermetrin was lower than that of the control group. The same toxic effect has not been observed in tadpole larvae (Greulich and Pflugmacher 2003). A similar result has been observed in a study examining the toxicity of four different pesticides (carbaryl, carbofuran, malathion, and phosphamidon) on the egg, larva, and offspring phases of *Cyprinus carpio*. Earlier embryonic stages (before gastrulation) have been stated to be more sensitive to the pesticides (El-Toukhy and Girgis 1993).

The individuals developing in all concentrations were transferred to the standard culture medium, their egg laying was observed, and lastly the eggs laid in 24 h were counted. The egg fertility of both the F_1 and F_2 generations in all concentrations did not differ significantly. In the fertility part of our research, cypermethrin had no negative effect on egg fertility in either generation (Table 3). In another study supporting this result, the number of eggs laid by female individuals of *Pimpla turinellae* L. was higher than that laid by the control group (Özkan 1995). The reproduction potential of insects is under the effect of a series of behaviors and physiologic events that occur with the coordinative function of the nervous and endocrine system. It is hard to find any definite comments on the molecular reason for the positive effects of insecticides on insect reproduction potential (Özkan 1995). On the one hand, the same study suggested that the insecticides of low sublethal dose, by stimulating the neuroendocrine system, can cause over-secretion of juvenile hormone, and this may be the reason for the increase in the number of eggs (Özkan 1995). On the other hand, the application of cypermethrin on mutant Drosophilas has been observed to cause tissue damage in reproduction organs and increased stress gene expression (HspVO). The same study found that the damage is more remarkable in males than in females

Table 2 The effect of cypermethrin on the liveness-ratio of larvae of *D. melanogaster*

Generation	Concentration (ppm)	Total number of larvae	Total number of adult individuals (n)	Development ratio (*p*)	z value
F_1	Control (G1)	200	162	0.018519	(G1−G2) −0.41 (n.s.)
	10 (G2)	200	158	0.025316	(G2−G3) −1.99*
					(G2−G4) −0.25 (n.s.)
	20 (G3)	200	165	0.072727	(G1−G3) −2.38*
					(G3−G4) 1.77 (n.s.)
	40 (G4)	200	167	0.029940	(G1−G4) −0.68 (n.s.)
F_2	Control (G5)	313	251	0.801917	(G5−G6) 0.97 (n.s.)
	10 (G6)	363	280	0.771350	(G6−G7) −2.74**
					(G6−G8) −0.17 (n.s.)
	20 (G7)	398	338	0.849246	(G5−G7) −1.64 (n.s.)
					(G7−G8) 2.60**
	40 (G8)	376	292	0.776596	(G5−G8) 0.81 (n.s.)

* $p < 0.05$, ** $p < 0.005$ n.s. not significant

Table 3 The effect of cypermethrin on fertility

Generation	Group	n	Average	Standard deviation	t	Sd.	*p*
F_1	Control	12	109.33	43.47	−0.338	46	0.737
	Experimental	36	116.92	73.18			n.s.
F_2	Control	12	120.67	80.15	0.809	46	0.422
	Experimental	36	104.89	49.75			n.s.

n.s. not significant

(Mukhopadhyay et al. 2002). Those researchers have stated in another study that high concentrations of chlorpyrifos increase embryonic mortality by causing a remarkable decrease in the rate of egg hatching in *Drosophila melanogaster*. No toxic effect was observed at much lower concentrations (Nazir et al. 2001). Still another study has concluded that individuals exposed to cadmium in their lives had a clear decrease in egg fertility (Gelegen and Yeşilada 2000). In addition, some fungicides are reported to lower egg fertility, depending on the duration of the effect (Marchal-Segault 1993). Furthermore, triazophos is noted to cause a considerable increase in sterility after application on *Drosophila melanogaster* by means of injection (Vélazquez et al. 1990). It has been also observed that *Drosophila melanogaster* can itself regulate the number of eggs when it feeds on different nutrients. This regulation of egg numbers is suggested to be made in order not to endanger insect generation (Partridge et al. 1987). In another study supporting our results, the effects of spray formulation of cypermethrin on *Euoniticellus intermedrus* (Reiche) were investigated, and it was found that there was no remarkable difference between the experimental and control groups in terms of adult or larval survival, egg production, fertility, or fecundity (Kryger et al. 2006).

This study clearly shows that cypermethrin decreased egg hatching and development even in sublethal concentrations. However, it appeared to have had no negative effect on the percentage of egg laying of females. Furthermore, although in some experimental groups there was inhibition in the development of third instar larvae into adult individuals, larval development was not generally inhibited.

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