

The effect of diazinon on egg fertility and development in *Drosophila melanogaster*

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Abstract: This study investigated the effects of diazinon, an organic phosphoric insecticide, on some developmental features of *Drosophila melanogaster*. The aim was to determine if any developmental inhibition occurs when diazinon is applied and if so, to determine which developmental stages are affected. Therefore, diazinon was applied to *Drosophila melanogaster* eggs, larvae, and adults, and its effects were observed. During the experiments 3 concentrations with values lower than LC₅₀ were utilized (6×10^{-5} , 6×10^{-6} , 6×10^{-7} ppm). Diazinon solution was applied to *Drosophila melanogaster* by means of nutrition, adding it to the culture medium. The results show that development from egg to third instar larva was significantly inhibited in the F₁ and F₂ generations. The development of larva to adult was only inhibited in the F₂ generation. The application of diazinon to females did not cause any change in the rate of egg laying. The overall results show that diazinon's toxicity was strongest during the early stages of development; however, it also affected the F₂ generation.

Key words: Diazinon, egg and larvae development, *Drosophila*, pesticide, toxicology

Diazinonun *Drosophila melanogaster*'de gelişim ve yumurta verimi üzerine etkisi

Özet: Bu çalışmada organik fosforlu bir insektisit olan diazinonun, *Drosophila melanogaster*'in bazı gelişimsel özellikleri üzerine etkisi incelenmiştir. Çalışmanın amacı, diazinonun *Drosophila melanogaster*'in farklı gelişim dönemleri üzerine uygulanması sonucu gelişimsel aksama olup olmadığını gözlemektir. Eğer gelişimsel aksaklık varsa, hangi gelişimsel dönemin daha duyarlı olduğunu belirlemektir. Bu amaçla yumurta, larva ve ergin dönemlerinde diazinon uygulanmış ve etkileri gözlenmiştir. Deneyle LC₅₀ değeri altında üç konsantrasyon kullanılmıştır (6×10^{-5} , 6×10^{-6} , 6×10^{-7} ppm). Diazinon solüsyonu *Drosophila melanogaster*'e beslenme yoluyla, besi yerine ilave edilerek uygulanmıştır. Sonuçlar, yumurtaların üçüncü instar larvalara gelişiminde, F₁ ve F₂ neslinde ciddi aksamalar olduğunu göstermiştir. Larvaların ergin bireylere gelişimi ise sadece F₂ neslinde aksamıştır. Ergin dişilere diazinon uygulaması ile yumurta bırakma oranında değişiklik olmadığı gözlenmiştir. Tüm sonuçlar dikkate alındığında, toksik etkinin gelişimin erken safhalarında daha etkili olduğu ve F₂ neslinde de devam ettiği ortaya çıkmıştır.

Anahtar sözcükler: Diazinon, yumurta ve larva gelişimi, *Drosophila*, pestisid, toksikoloji

Introduction

Organic phosphoric pesticides are widely used in agriculture and for controlling harmful domestic insects. Most people living in and around agricultural areas, particularly agricultural laborers and their families, are directly exposed to these pesticides. The general population is also indirectly exposed to pesticides from residue on food or while applying them to houses (1).

Diazinon is an organic phosphoric insecticide (O, O-diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl phosphorothioate). It causes continuous stimulation in the organism by inhibiting acetylcholine esterase and results in the paralysis of the nervous system of insects. Once applied to products and plants, it mixes with ground water, is washed away by surface water, and diffuses into large volumes of water. Thus, it can affect many organisms apart from the target (2). Because of their alkylating features such compounds are potential mutagens (3). Alkylating agents can alkylate all the bases in DNA, causing mutation (4-8).

Although many studies on the toxic effects of diazinon have been published, there is little information about its effects during the early embryonic stages (9,10). Based on research conducted in humans and animals, organic phosphoric pesticides affect reproductive functions in males and, in particular, the quality of semen and the hormone balance (11). Any effect during the early life stage affects development and growth, and consequently threatens the health of the population (12). Thus, we should evaluate the risk to which strains and populations that are not targeted are exposed. The present study investigated the toxic effects of diazinon on egg laying and developmental features in *Drosophila melanogaster* at different developmental stages.

Materials and methods

The *Drosophila melanogaster* Oregon-R strain was used. The cultures used were kept in a refrigerated incubator at 25 ± 1 °C and 40%-60% relative humidity under dark conditions. The culture medium was standard *Drosophila* medium (SDM) containing corn meal, molasses, yeast, agar, water, and propionic acid (13).

Diazinon (99.50% pure) was provided by the Syngenta Anonymous Company of Agricultural Industry and Commerce (Syngenta Limited, İstanbul, Turkey). In order to simulate agricultural conditions the medium used in the experiments was a water solution of diazinon. First, the lethal concentration of diazinon was determined. For this aim a stock dissolution of 1.000 ppm was obtained, which was then diluted to the desired concentrations (14). Different concentrations of diazinon solution were added to the culture medium and in each culture medium 20 females and 20 adult males were mated. After 24 h the numbers of dead and living individuals were counted and the death rate was calculated. Accordingly, the LC_{50} concentration was between 6×10^{-4} ppm and 1×10^{-4} ppm. For the experiments 3 concentrations below the LC_{50} value (6×10^{-5} , 6×10^{-6} , and 6×10^{-7} ppm) were used. The substance was applied to eggs, larvae, and adults by means of nutrition, adding it to the culture medium. SDM alone was used in the control group and the F_2 generation.

To observe egg development, 50 females were transferred to culture medium containing diazinon solution and kept in the culture medium for 5 days. Ten females chosen randomly from these were transferred to petri dishes that did not contain diazinon solution. After 24 h the females were removed from the culture medium and the eggs they had laid were counted under a dissection microscope. To determine how many of the eggs developed, the culture medium was kept in an incubator for 5 days and developed larvae were removed after being counted. The number of eggs laid by females and the number of third instar larvae removed were compared.

In order to observe egg development in the F_2 generation, F_2 generation individuals, obtained from female individuals exposed to diazinon in the F_1 generation, were used. To observe the effect of diazinon exposure in the F_1 generation on later generations, no substance was applied to F_2 generation individuals. F_2 generation individuals completed their development in the culture medium that contained only SDM. Apart from this, the process for the F_2 generation was the same as that for the F_1 generation.

To observe the development of larvae, young third instar larvae of the same age were obtained from a stock culture. Each of 50 larvae was placed into the culture medium of the control group and experimental group. This process was performed 4 times. The individuals that reached maturity were counted twice each day, and then were removed (13,15). The procedure used for larval development in the F₁ generation was the same as that used for the F₂ generation during egg development. The aim here was to determine if the potential effect observed during larval development in the F₁ generation continued in the F₂ generation even though diazinon was not applied.

To observe egg fertility 50 females were placed in culture medium containing diazinon solution for 5 days. Ten females were selected randomly from these and transferred to petri dishes that did not contain diazinon solution. After 24 h the adult females were removed from the petri dishes and their eggs were counted under a dissection microscope.

The process applied for observing egg fecundity in the F₂ generation was the same as that used for the F₁ generation; the only difference was that F₂ generation individuals obtained from females exposed to diazinon for 5 days were transferred to culture medium containing only SDM. Again, after 24 h they were removed and the eggs they laid were counted (16).

The data obtained were analyzed using 2 statistical methods. The z-test was used to evaluate the egg and larval development results. The rates were converted to z-points and differences between the 2 groups were evaluated.

$$z = \frac{(p_1 - p_2)}{\sqrt{\frac{p_1 q_1}{n_1} + \frac{p_2 q_2}{n_2}}}; q_i = 1 - p_i$$

Minitab for Windows v.13.0 was used to perform this calculation. Microsoft Excel and the t-test, which compares the difference between 2 average rates, were used for the statistical evaluation of egg fertility.

To compare the results labels G1, G2, G3, G4, G5, G6, G7, and G8 were given to each experiment group, as follows: G1: 0 ppm, F₁ generation; G2: 6 × 10⁻⁷ ppm, F₁ generation; G3: 6 × 10⁻⁶ ppm, F₁ generation; G4: 6 × 10⁻⁵ ppm, F₁ generation; G5: 0 ppm, F₂ generation; G6: 6 × 10⁻⁷ ppm, F₂ generation; G7: 6 × 10⁻⁶ ppm, F₂ generation; G8: 6 × 10⁻⁵ ppm, F₂ generation.

Results

The LC₅₀ value for diazinon was between 6 × 10⁻⁴ and 1 × 10⁻⁴ ppm. In the experiments concentrations below the LC₅₀ value, namely 6 × 10⁻⁵, 6 × 10⁻⁶, and 6 × 10⁻⁷ ppm, were tested. In the F₁ generation groups to which diazinon was applied the development of egg to larva was inhibited (Table 1). Diazinon decreased

Table 1. Effect of diazinon on egg development.

Generation	ppm	Total number of eggs	Total number of larvae	Rate of development	Z value
F ₁	Control (G1)	1584	1206	76.13	(G1-G2) 15.88**
	6 × 10 ⁻⁷ (G2)	1245	597	47.95	(G2-G3) -7.49** (G2-G4) -0.94 n.s.
	6 × 10 ⁻⁶ (G3)	1344	852	63.39	(G1-G3) 7.52** (G3-G4) 6.87**
	6 × 10 ⁻⁵ (G4)	1167	582	49.87	(G1-G4) 14.48**
F ₂	Control (G5)	1542	1231	79.83	(G5-G6) 5.89**
	6 × 10 ⁻⁷ (G6)	872	600	68.80	(G6-G7) -1.33 n.s. (G6-G8) 11.92**
	6 × 10 ⁻⁶ (G7)	949	680	71.65	(G5-G7) 4.58** (G7-G8) 13.86**
	6 × 10 ⁻⁵ (G8)	1430	635	44.40	(G5-G8) 21.28**

** P < 0.01 n.s.: not significant

the rate of egg development at all concentrations. Comparison of the experimental groups (apart from G2-G4 to G6-G7 experiment groups) showed there was an overall effect that was more pronounced at higher diazinon concentrations. This shows that the eggs laid in the culture medium containing diazinon were inhibited at every stage of development, before they could develop into third instar larvae. It was interesting that the effect continued in the F₂ generation even though the eggs developed in culture medium that did not contain the pesticide solution. This indicated that the effect of diazinon on the F₁ generation continued in the F₂ generation. Based on these results, diazinon inhibited egg development in the F₁ and F₂ generations.

Data on larval development are given in Table 2 (rate of development refers to the ratio between the number of larvae and the number of eggs). Diazinon did not inhibit the development of larva to adult in the F₁ generation. Comparison of the experimental groups to the control groups, and the experimental groups to each other, showed that the differences were not statistically significant (Table 2). In the F₂ generation, however, larval development was inhibited. Moreover, when the experimental groups were compared to each other there was a significant difference between all of them. This shows that the toxic effect of diazinon on the organisms in the F₁

generation appeared in the following generation. The same result was observed for egg development, as the toxic effect continued in the F₂ generation. These 2 results indicate that diazinon, despite being tested below the lethal concentration (LC₅₀), remained in the body and its toxic effect appeared in the following generation, or even if it was metabolized its metabolites exerted a toxic effect on a later generation.

The effect of diazinon on egg fertility was also examined. Diazinon did not negatively affect egg fertility. As shown in Table 3, there was no significant difference between the experimental and control groups, indicating that diazinon had no effect on daily egg fertility in either generation.

Discussion

The present study's results show that diazinon inhibited the development of eggs at all concentrations in the F₁ and F₂ generations (Table 1). F₁ and F₂ generation eggs left in the culture medium that contained diazinon solution were inhibited at all developmental stages, before becoming third instar larvae. The results of many other studies support these findings. A study on the effects of 3 different pesticides (diazinon, dinoseb, and esfenvalerate) on Chinook salmon (*Oncorhynchus tshawytschya*) eyed eggs and alevins showed that metabolic disorders and

Table 2. Effect of diazinon on the development of larvae.

Generation	ppm	Total number of eggs	Total number of larvae	Rate of development	Z value
F ₁	Control (G1)	200	159	79.50	(G1-G2) 0.45 n.s.
	6 × 10 ⁻⁷ (G2)	200	159	79.50	(G2-G3) -0.30 n.s.
	6 × 10 ⁻⁶ (G3)	200	182	91.00	(G2-G4) -1.46 n.s.
	6 × 10 ⁻⁵ (G4)	200	194	97.00	(G1-G3) 0.17 n.s. (G3-G4) -1.20 n.s. (G1-G4) -1.00 n.s.
F ₂	Control (G5)	200	160	80.00	(G5-G6) 0.12 n.s.
	6 × 10 ⁻⁷ (G6)	200	159	79.50	(G6-G7) -3.29** (G6-G8) 2.85**
	6 × 10 ⁻⁶ (G7)	200	182	91.00	(G5-G7) -3.16** (G7-G8) 6.17**
	6 × 10 ⁻⁵ (G8)	200	134	67.00	(G5-G8) 2.98**

** P < 0.01 n.s.: not significant

Table 3. The effect of diazinon on egg fertility.

Generation	Group	N	Average	Standard deviation	t	Sd.	P	
F ₁	Total number of eggs	Control Group	12	109.33	43.47	-0,021	46	0.983
		Experimental Group	36	109.69	53.56			
F ₂	Total number of eggs	Control Group	12	120.67	80.15	1.191	46	0.240
		Experimental Group	36	98.14	47.05			

P > 0.05 The difference between groups is not significant.

developmental defects occurred during early life stages (17). When diazinon and chlorpyrifos were applied to adults and juveniles of the earthworm *Aporrectodea caliginosa*, it was observed that growth and cocoon production of juvenile individuals were sensitive to these pesticides (18). In a similar study the effect of α -cypermethrin on *Rana arvalis* eggs and larvae was observed, and metamorphosis was inhibited in individuals exposed to the insecticide during the early stages of life. This study also reported that hatchability decreased and growth regressed (19). Another study applied beta-cyfluthrin to *Drosophila melanogaster* and reported a decrease in hatchability (20). Moreover, another study reported that there was a decrease in the body weight of *Fundulus heteroclitus* larvae exposed to diazinon during the egg phase (21).

Previous research results support the results of the present study. The reason why few of the eggs laid in the present study become mature might have been the damage caused by the insecticide's toxicity. In contrast, the effect of malathion on *Pimpla turinellae* egg hatching was studied and a significant difference was not observed between the treatment and control groups (22). The effect of carbaryl on various life phases of *Rana sphenoccephala* was investigated. Carbaryl was tested on eggs, embryos, and tadpoles, and its effect on metamorphosis was examined. No effect was observed on hatchability, but there was (though not very clear) a negative effect on the mass of metamorphosis. Furthermore, it inhibited growth, but did not affect the rate of development (23). Nevertheless, we observed that diazinon inhibited the development of eggs in both generations. The present study also examined the effect of diazinon on third instar larvae. Though not observed in the F₁

generation, there was a toxic effect on larval development in the F₂ generation. This shows that when larvae of the F₁ generation were exposed to the insecticide toxic effects appeared in the F₂ generation (Table 2). Egg development was also inhibited even though F₂ generation eggs did not come into contact with diazinon (Table 1).

Another study reported that diazinon accumulated in organic fats and survived there for more than 1 year. Diazinon was also shown to transfer from the fat it was attached to into the water milieu by way of infiltration or erosion of the fat (24). As diazinon is an alkylating agent, it is possible that point mutations and/or chromosome rearrangement can occur after exposure to it and, as such, fewer eggs laid by females exposed to the insecticide (as compared to the controls) produced viable larvae. In addition, the developmental stage between egg and third instar larva may be more sensitive than the developmental stage after third instar larva. This is due to the toxic effect during egg development that was observed in both generations.

In a study that supports the present findings the toxic effect of 4 pesticides (carbaryl, carbofuran, malathion, and phosphamidon) on the developmental stages of *Cyprinus carpio* eggs, larvae, and tadpoles was tested, and younger embryonic phases (before gastrulation) were observed to be more sensitive to the pesticides. Moreover, morphologic deformations, such as abnormal larval development and larval death, were observed (25). Similar malformations were also observed in the present study, but are not shown in the Table. Diazinon was reported to negatively affect survival, growth, and activity in the larvae of *Bufo melanostictus*, a common species of frog (26).

In the present study diazinon did not have a toxic effect on either generation, in terms of egg fertility (Table 3). In another study it was reported that *Drosophila melanogaster* females regulated the number of eggs laid when fed different nutrients. This regulation of the number of eggs was suggested to occur in order not to endanger insect generation (27). A decrease in egg fertility was not observed in the present study, indicating that there was no toxic effect. In another study malathion given at the level of 0.001 ppm increased the number of eggs laid by adult female individuals of *Pimpla turinellae* L., as compared to the control group (22). The reproduction potential of insects was affected by a series of behaviors and physiologic events that occurred with the coordinative function of the nervous and endocrine system. It was difficult to find any definitive comments about the molecular reason for the positive effects of insecticides on insect reproduction potential (22). On the other hand, it was suggested that insecticides at low sublethal doses, by stimulating the neuroendocrine system, could cause over-secretion of juvenile hormones (ecdysone), which might be the reason for the increase in the number of eggs (22).

In another study that supports the present results an organophosphate compound (chlorpyrifos) inhibited the hatchability of transgenic *Drosophila melanogaster* at high concentration (3000 ppm). At the lowest concentration (0.005 ppm), however, no difference was observed in daily egg fecundity or fertility, but there was a decrease in reproductive performance (15). It was reported that environmental conditions had a significant effect on life span and fecundity. It was also reported that the effect to which the organism was exposed in the larval stage affected fecundity. In another study the effect of diazinon on

various life phases of *Daphnia pulex* was examined and it was reported that first and second stages were more sensitive, and the effect decreased in subsequent stages (28). Triazophos was applied to *Drosophila melanogaster* by injection and caused a remarkable increase in sterility (3). Additionally, cybil, a synthetic pyrethroid, was observed to affect the reproductive success and fecundity of *Drosophila melanogaster* in F₁ and F₂ generations (29). When beta-cyfluthrin was applied to *Drosophila melanogaster*, decreases in hatchability, the number of eggs laid, and fecundity were observed (20).

In conclusion, in the short term diazinon did not to cause a decrease in fecundity, but did decrease egg growth in both generations. Moreover, the toxic effect was more pronounced in the egg and third instar larval stages than in the later third instar larval stages. Above all, it was clear that development was inhibited between the egg and third instar larval stages, and the toxic effect continued in the F₂ generation. Consequently, when organisms that are not targeted are exposed to diazinon during their reproduction stages, some developmental inhibition could occur and, hence, their population demography could change. Research on the effect of diazinon on the developmental stages of non-targeted organisms can be useful, in terms of endangered species.

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