Positive correlation between malathion resistance and fecundity within natural populations of *Drosophila melanogaster*

Burcu KOÇAK MEMMİ, Emel ATLI

Department of Biology, Faculty of Science, Hacettepe University, 06800, Ankara - TURKEY

Abstract: The correlation between malathion resistance and fecundity was investigated in the fruit fly, *Drosophila melanogaster*. Previous studies have shown that insecticide resistance is negatively correlated with reproductive components in insects. To investigate this relationship, the fecundity (egg production) was determined in 2 natural and 2 esterase 6 (Est-6) marker populations (4016 and 4211) and malathion resistance levels (LC_{50}) were determined in the 4 populations of *D. melanogaster*. The resistance levels and Est-6 allozyme frequencies were compared to the results of 2 natural populations from Turkey (Antakya-Centre and Antalya-Serik) estimated in previous studies. In addition, malathion resistance levels (LC_{50}) were determined in 2 mutant populations using the paper contact method and the results were tested with Finney's probit analysis. In the bioassays, 4016 marker stock was more susceptible than the Antakya population and 4211 was more susceptible than the Serik population. The daily mean egg production numbers of females were calculated and compared using variance analysis ($P < 0.05$). The most productive population was found to be Antakya, followed by 4016, Serik, and 4211 populations, in decreasing order. These results indicate that malathion resistance is positively correlated with fecundity of 2 natural and 2 Est-6 marker stocks of *D. melanogaster*.

Key words: *Drosophila melanogaster*, esterase, fecundity, insecticide, organophosphate, resistance, malathion

Drosophila melanogaster doğal populasyonlarında malathiona karşı direnç ve yumurta verimi arasındaki ilişki

Özet: Bu çalışmada, mevye sineği *Drosophila melanogaster*'de malathion direnci ve yumurta verimi arasındaki ilişki araştırılmıştır. Önceki çalışmalar, böceklerde insektisit direncinin üreme öğeleri ile negatif ilişki içinde olduğunu göstermiştir. Bu ilişkiyi araştırmak amacıyla, *D. melanogaster*'in iki doğal ve iki esteraz 6 (Est-6) mutant populasyonu (4016 ve 4211) dişilerinde yumurta verimi ve malathiona karşı direnç düzeyleri belirlenmiştir. Önceki çalışmamızda malathiona karşı direnç düzeyleri (LC_{50}) ve Est-6 izozimleri Antakya Merkez ve Antalya Serik bölgeleri kullanılarak karşılaştırılmıştı. Bu araştırmaya ek olarak, iki mutant populasyonda malathion direnç düzeyleri kağıt temas yöntemi ile belirlendi ve sonuçlar Finney Probit analizi ile değerlendirildi. Denemelerde, 4016 mutant soyu Antalya populasyonundan ve 4211 populasyonu Serik populasyonundan daha hassas bulundu. Günlük ortalama yumurta verimi sayıları hesaplandı ve ortalamalar varyans analizi ile karşılaştırıldı ($P < 0,05$). En verimli populasyon sırasıyla Antakya, 4016, Serik ve 4211 populasyonları olarak belirlendi. Bu sonuçlar, populasyonlarda malathion direncinin artışına paralel olarak yumurta veriminin de arttığını göstermiştir.

Anahtar sözcükler: *Drosophila melanogaster*, esteraz, yumurta verimi, fekundite, insektisit, organofosfat, direnç, malathion

Introduction

Insecticide resistance became a very serious problem throughout the world in the last half of the twentieth century. Over 500 species of insects are now resistant to one or more pesticides (1). As such, unconscious and continued consumption of several insecticides gave acceleration to evolution of resistance in the field populations of many insects. In order to solve the resistance management problems, it is very important to understand the accompanying factors and contribute to improve knowledge on insecticide resistance.

The biochemical basis of insecticide resistance was previously studied in *Drosophila melanogaster*; *Culex quinquefasciatus*; *Culex pipiens;* the blowfly, *Lucilia cuprina*; and the house fly, *Musca domestica*. It was found that the glutathione S-transferase enzymes, oxidases, acetylcholinesterases, and general esterases play an important role in conferring or contributing to insecticide resistance in insects (2). Remarkably, esterase enzymes contribute to developmental changes, tissue distributions, and reproductive fitness in insect populations including *D. melanogaster*. Furthermore, it is known that Est-6 enzyme contributes both to insecticide resistance and reproductive fitness (3-5).

The Est-6 locus in *D. melanogaster* encodes the structure of a beta-carboxylesterase. This locus is highly polymorphic in natural populations (6). The Est-6 enzyme is synthesized in the anterior ejaculatory duct of the male and transferred to females during copulation (7).

Insecticide resistance has developed in natural populations of *D. melanogaster* although they are not usually direct targets of pesticide applications. Several research groups have defined the relationship among insecticide resistance, Est-6 allozymes, Est-6 activity levels, and fitness components in several organisms including *D. melanogaster*. For example, Est-6 activity levels were found to be negatively correlated with the number of eggs laid in *D. melanogaster* (4). However, elevated esterase activity levels were found to be positively correlated to insecticide resistance mechanisms against organophosphates in *Aedes aegypti* (8).

In our research, we aimed to investigate the effects of insecticide resistance on fecundity in field

populations whose Est-6 allozyme patterns were established previously. The mean egg production numbers were determined in the natural populations (Antakya-Centre and Antalya-Serik) from Turkey and in the heterozygous $Est-6^{FS}$ and null $Est-6^{0}$ marker stocks (4016 and 4211, respectively) of *D. melanogaster*. In order to determine the resistance levels, malathion, representing the organophosphate (OP) insecticide group, was used. The resistance levels (LC_{50}) were determined in the 2 Est-6 marker stocks of *D. melanogaster* (4016 and 4211). The resistance levels and mean egg production numbers of the marker stocks were compared to those of the natural populations. The results were discussed and related to Est-6 allozyme frequencies.

Materials and methods

The organism and environmental conditions

Two natural (Antakya and Serik) and 2 marker populations (4016 and 4211) were used. The natural populations were collected from Turkey during the summer of 2007. The marker stocks were obtained from the Bloomington Stock Center. The 4016 marker stock is a heterozygous variant of Est-6 and Est C. This stock has a multiply inverted chromosome (TM6) and the inversions prevent recombination with normal chromosomes (Est-6[S] Est-C[F] Fdh[S] Lap-D[F] Acph-1[A] Tpi[6]/TM6, Est-6[F] Est-C[S] Fdh[F] Lap-D[S] Acph-1[B] Tpi[4]) and 4211 (Est-6[0] Est-C[n]). Homozygosity for either chromosome is lethal. Consequently, neither Est-6 nor Est-C allele should be homozygous in adults of 4016. The 4211 marker stock is a null variant of Est-6 and Est C. The flies were kept in a *Drosophila* culture room (Hacettepe University, Ankara, Turkey) at 25 ± 1 °C and relative humidity of 50%-60% and in 12 hr light, 12 h dark periods on a standard *Drosophila* medium (9).

The malathion bioassays

Malathion (CAS No: 121-75-5) [S-(1,2-dicarboethoxyethyl) O,O-dimethyldithiophosphate)] is one of the most frequently used OP insecticides because of its low acute toxicity as compared to other OP insecticides. The malathion resistance levels (LC_{50}) were found by using the filter paper contact method in the 4 populations of *D. melanogaster* (10). Technical grade malathion was used in the bioassay

experiments. In the natural populations, the treatment doses were 1.6384, 2.040, 2.56, 3.2, and 4 µg/cm^2. In the marker populations, the treatment doses were 0.131, 0.513, 1.003, 1.254, and 1.568 µg/cm^2. The doses were prepared using the serial dilution method (0.8-fold serial dilution). The flies (3-5 days old) were placed in empty glass vials, 10 flies in each vial with appropriate filter paper. The experiments consisted of 5 doses and 3 broods. The bioassays were replicated 5 times. After 24 h, flies unable to move were counted as dead. The malathion resistance levels of the natural populations were calculated according to Memmi (11).

Determination of mean fecundity

In order to determine the mean fecundity of the populations, virgin females were used. One female and 3 males of the same age (3 days old) were crossed in empty glass culture bottles. Then spoons containing standard *Drosophila* medium were placed in these culture bottles immediately. These spoons were changed every 24 h and the eggs were counted for a period of 10 days. It is generally accepted that egg production in the first 10 days of adult life is a good reference for the whole adult life egg production of this organism (12-14).

Statistical methods

The malathion resistance levels (LC$_{50}$) were tested with Finney's probit analysis (15). The mean egg numbers of each population were calculated. The statistical analysis of the differences of daily egg numbers between groups was performed with the analysis of variance (ANOVA) test (P < 0.05) using SPSS 10.0. The binary comparisons of the populations' egg numbers were analyzed using Games-Howell, Dunnett C, and LSD. The mean differences were significant at the 0.05 level. The significance of the Est-6 allele frequency differences of the populations was statistically analyzed using Pearson's chi-square test. The correlations between the matrices of mean fecundity numbers and the matrices of resistance levels (LC$_{50}$) were compared using Mantel's correlation test (16) and the XLSTAT program.

Results

The levels of malathion resistance were calculated previously in Antakya and Serik natural populations.

According to these results, Antakya was quite resistant (♀ LC$_{50}$: 3.850; ♂ LC$_{50}$: 2.512) and Serik (♀ LC$_{50}$: 1.050; ♂ LC$_{50}$: 0.704) was very susceptible (11). In the current research, we determined the malathion resistance levels of the marker stocks 4016 (♀ LC$_{50}$: 1.240; ♂ LC$_{50}$: 0.996) and 4211 (♀ LC$_{50}$: 0.385; ♂ LC$_{50}$: 0.251) and compared them with our previous results. With respect to these results, the Antakya natural population was more resistant than 4016, and Serik was more resistant than 4211 (Table 1).

In order to determine the mean fecundity of populations, the eggs laid in the first 10 days after crossing were counted. As seen in Table 2, the egg production differences between Antakya and Serik, Antakya and 4211, and 4016 and 4211 were statistically significant (P < 0.05). The most productive population was Antakya, followed in decreasing order by 4016, Serik, and 4211 populations (Figure 1).

In the 2 natural *D. melanogaster* populations from Antakya and Serik, the Est-6^F and Est-6^S allel frequencies [Est-6 *Fast* (FF), Est-6 *Slow* (SS) and Est-6 heterozygous (FS)] were determined previously using as a reference the marker stock 4016 with starch gel electrophoresis (11). The Est-6 allele frequencies, the mean egg production numbers, and resistance levels were compared.

As shown in Table 3, Est-6 *Fast* allele frequencies of Antakya males and females were higher than the Est-6 *Slow* allele frequencies. Moreover, Serik males had higher Est-6 *Slow* frequency than did females and Serik females had higher Est-6 *Fast* frequency than did males. The differences between Serik females and males were significant but the differences between Antakya females and males were nonsignificant.

In order to determine the correlation between fecundity and resistance levels of all populations, differences between them were converted to matrices and analyzed with Mantel's test (16). The correlation levels of the matrices of daily mean egg numbers and resistance levels of the populations are shown in Figure 2 (P = 0.001). The estimated correlation coefficient was high (r = 0.810), leading us to conclude that mean daily number of eggs and resistance levels of the populations were positively correlated (Figure 3).

Table 1. The malathion resistance levels (μg/cm^2) of the natural populations Antakya and Serik (Memmi, 2009) and the marker populations 4016 and 4211.

Population	LC$_{50}$ (95% CL)	LC$_{95}$ (95% CL)	Slope (±SE)	X^2
Antakya ♀	3.850 (3.189-6.286)	12.970 (7.311-108.138)	3.118 ± 0.926	3.311
Antakya ♂	2.512 (1.850-3.309)	13.922 (6.874-525.372)	2.212 ± 0.768	0.340
Serik ♀	1.050 (0.215-1.423)	2.392 (2.018-3.859)	4.598 ± 1.644	1.318
Serik ♂	0.704 (0.00-0.00)	1.595 (0.00-0.00)	4.62 ± 3.550	0.828
4016 ♀	1.240 (1.046-1.340)	1.969 (1.740-2.771)	8.200 ± 2.100	0.475
4016 ♂	0.996 (0.826-1.075)	1.438 (1.307-1.882)	10.330 ± 2.788	2.868
4211 ♀	0.385 (0.173-1.325)	22.156 (4.447-598.508)	0.934 ± 0.175	3.249
4211 ♂	0.251 (0.089-0.797)	12.587 (2.59-1081.50)	0.751 ± 0.161	0.001

CL: Confidence level, SE: Standard error, df: 1, P < 0.05

Table 2. The daily mean egg production of natural and marker *Drosophila melanogaster* populations.

Group no.	Groups	Number of females	Number of eggs	Daily mean egg production per female ± S.E.	S. D.	Significant differences of the means
1	Antakya	25	1825	7.38 ± 0.46	7.38	1-2*
2	Serik	25	1430	5.72 ± 0.28	4.50	1-4*
3	4016	25	1743	6.97 ± 0.43	6.73	3-4*
4	4211	25	1232	4.93 ± 0.32	4.97	

S.E.: Standard error, S.D.: Standard deviation. * : P < 0.05
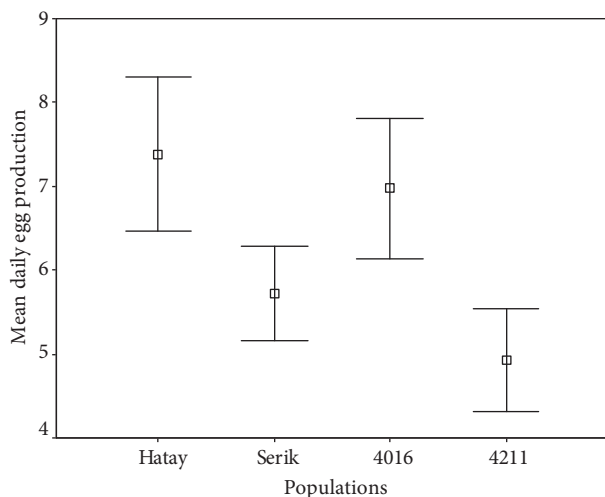

Figure 1. The confidence intervals (95%) of the daily mean egg production in the natural and marker *Drosophila melanogaster* populations.

To determine the correlation between fecundity and Est-6 Fast allele frequency of all populations, differences between them were calculated and converted to the matrices and tested with Mantel's test. The correlation levels of the matrices of mean daily number of eggs and Est-6 Fast allele frequency of the populations are shown in Figure 4 (P = 0.011). The direction and value of the correlation coefficient were found to be highly (r = 0.726) correlated positively (Figure 5).

Discussion

In this study, the correlation between malathion resistance and fecundity (represented by egg laying capacity) was investigated in 4 *Drosophila melanogaster* populations. The resistance levels (LC$_{50}$) and daily egg productions were determined in natural populations from Turkey (Antakya and Serik) and in

Table 3. Est-6 allele frequencies in *Drosophila melanogaster* natural populations and comparison between sexes.

Population	Sex	Allele mobility	Allele frequency	P value[a]
Antakya	♀	S	0.125	0.894
		F	0.875	
	♂	S	0.133	
		F	0.867	
Serik	♀	S	0.250	<0.001
		F	0.750	
	♂	S	0.576	
		F	0.424	

F: *Fast*; S: *Slow,* a: Pearson chi-square test (P < 0.005 significant) (from Memmi, 2009).
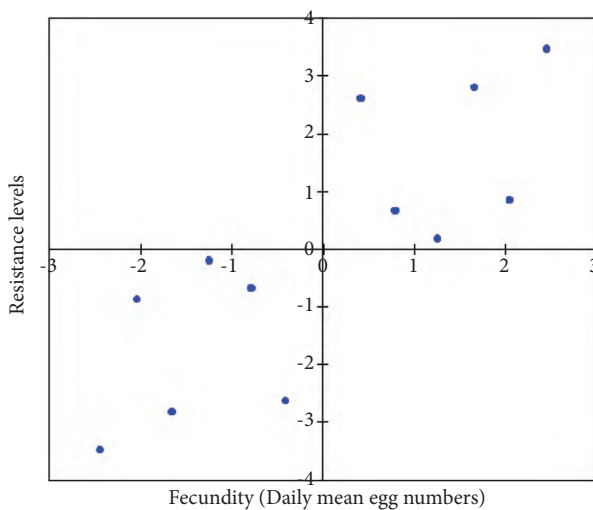
Figure 2. The correlation levels of the matrices of daily mean egg numbers and resistance levels of the populations (P = 0.001).
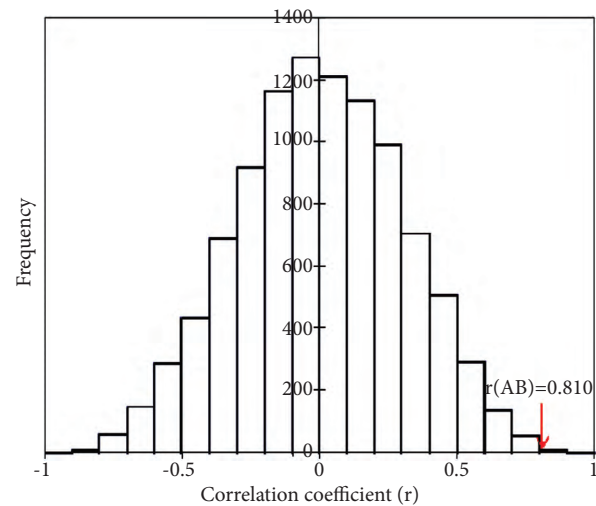

Figure 3. The direction and value of the correlation coefficient obtained from Mantel's test (r = 0.810).

the Est-6 marker stocks (4016 and 4211). Factors which might affect egg production were kept stable in the experiments.

The comparison of the malathion resistance levels of the marker stocks 4016 and 4211, obtained in this study, with the resistance levels of Antakya and Serik, previously obtained, showed resistance levels greater for Antakya than for the marker 4016 stock and resistance levels for Serik greater than those for 4211 (Table 1).

A reduction in the egg production parallel with the resistance levels of the 4 populations was detected in our experiments. The most productive population was Antakya, and the others, in decreasing order, were 4016, Serik, and 4211 populations (Table 2, Figure 1). The egg production differences between Antakya and Serik, Antakya and 4211, and 4016 and 4211 were statistically significant (P < 0.05). The Antakya natural population, which was the most resistant, was also the most productive population. Similarly, the least productive population was 4211, which was also the least resistant. The Est-6 heterozygous (Est-6^{FS}) 4016 marker stock's fecundity was significantly higher than that of esterase null mutant marker 4211. These results suggested that there was a positive correlation
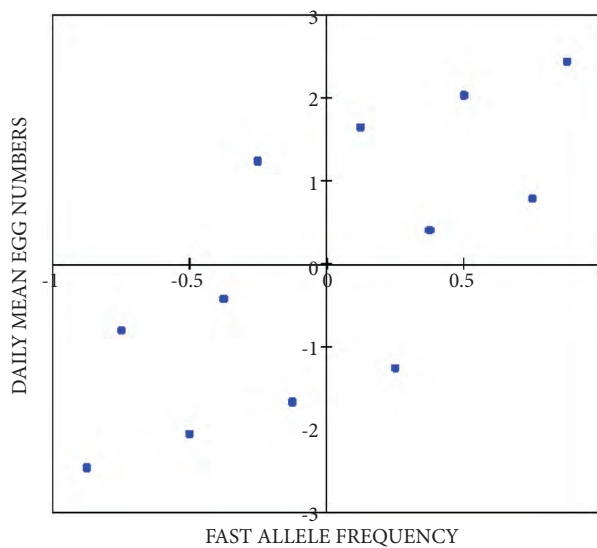
Figure 4. The correlation levels of the matrices of Est-6 fast allel frequency and daily mean egg numbers of the populations (P = 0.011).

Figure 5. The direction and value of the correlation coefficient of Est-6 fast allel frequency and daily mean egg numbers obtained from Mantel's test (r = 0.726).

between malathion resistance and fecundity. Furthermore, differences between the marker stocks seem to be correlated with the presence of the Est-6 enzyme, which has an important role in reproduction (17).

The relationship between resistance and egg production were investigated in many insect groups like the diamondback moth, *Plutella xylostella*; *Musca domestica*; *Culex pipiens*; and *D. melanogaster*. In contrast to our present results on egg laying capacity, it was reported that resistant strains often have some fitness costs like smaller egg size, low viability, and effects on survival rates of offspring (18,19). For example, Chen et al. (18) found a negative correlation between resistance and egg size in the diamondback moth, *P. xylostella*. Furthermore, it was found that survival rate was negatively affected in the resistant lines of *P. xylostella*.

Several authors also have detected significant associations between the fitness components (preadult viability, development time, time to mating, remating frequency, egg production, and fertility) of *D. melanogaster* and the Est-6 activity levels (4,5). It is also reported that Est-6 effects on productivity, egg laying, and egg fertility and wide-spread polymorphism for Est-6 may be adaptively significant
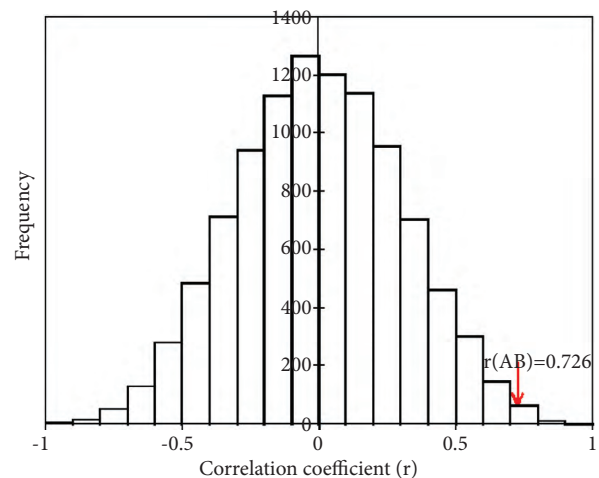
(7). The present results reinforce the involvement of esterase Est-6 in fitness components.

Thus, our results indicate that malathion resistant natural populations have advantages in fecundity measured by egg laying capacity and consequently that the selection pressure due to insecticide use could also contribute to the selection of the most productive individuals in natural populations of *D. melanogaster*. Because of the importance of the subject we suggest further and more detailed studies at biochemical and molecular levels to determine the associations between the resistance and fitness components.

Acknowledgements

Corresponding author:

Burcu KOÇAK MEMMİ

Department of Biology,

Faculty of Science, Hacettepe University,

06800 Beytepe, Ankara – TURKEY

E-mail: kburcu@hacettepe.edu.tr

References

1. Whalon ME, Mota-Sanchez D, Hollingworth RM et al. The Database of Arthropod Resistance to Pesticides. Michigan State University. http://www.pesticideresistance.org. Arthropod pesticide resistance database, 2003. Accessed 25 Apr 2009.

2. Oakeshott JG, van Papenrecht EA, Boyce TM et al. Evolutionary genetics of *Drosophila* esterases. Genetica 90: 239-268, 1993.

3. Dickenson WJ, Sullivan DT. Gene enzyme systems in *Drosophila*. Springer Verlag. Berlin; 1975.

4. Saad M, Game AY, Healy MJ et al. Associations of esterase 6 allozyme and activity variation with reproductive fitness in *Drosophila melanogaster*. Genetica 94: 43-56, 1994.

5. Oakeshott JG, Saad M, Game AY et al. Causes and consequences of esterase 6 enzyme activity variation in preadult *Drosophila melanogaster*. Heredity 23: 160-169, 1994.

6. Barker JSF, Starmer WT, MacIntyre RJ. Ecological and evolutionary genetics of *Drosophila*. New York and London Plenum Press. New York; 1990.

7. Richmond RC, Gilbert DG, Sheehan KB et al. Esterase 6 and reproduction in *Drosophila melanogaster*. Science 207: 1483-1485, 1980.

8. Flores AE, Grajales JS, Salas IF et al. Mechanisms of insecticide resistance in field populations of *Aedes aegypti* (L.) from Quintana Roo, Southern Mexico. J Am Mos Control Assoc 22: 672-677, 2006.

9. Bozcuk AN. The effects of some genotypes on the longevity of adult *Drosophila*. Exp Geront 13: 279-286, 1978.

10. Miyo T, Oguma Y. Negative correlations between resistance to three organophosphate insecticides and productivity within a natural population of *Drosophila melanogaster* (Diptera: *Drosophilidae*). J Econ Entomol 95: 1229-1238, 2002.

11. Memmi BK. Determination of the levels of resistance against malathion, esterase activities and allozymes in some wild type *Drosophila melanogaster* strains of Turkey, PhD Thesis, Hacettepe University Institute of Pure and Applied Science, 2009.

12. McMillan I, Fitz-Earle M, Butler L. Quantitative genetics of fertility II. Life time egg production of *Drosophila melanogaster*. Genetics 65: 335-369, 1970.

13. Yesilada E, Bozcuk AN. *Drosophila melanogaster*'in yumurta verimi üzerine ABA ve kinetinin etkisi. Turk J Biol 19: 37-44, 1995.

14. Atlı E, Ünlü H. The effects of microwave frequency electromagnetic fields on the fecundity of *Drosophila melanogaster*. Turk J Biol 31: 1-5, 2007.

15. Finney DJ. Probit Analysis. Cambridge University Press, United Kingdom; 1962.

16. Mantel N. The detection of disease clustering and a generalized regression approach. Cancer Res 27: 209-220, 1967.

17. Gilbert DG, Richmond RC. Esterase 6 in *Drosophila melanogaster*: reproductive function of active and null males at low temperature. Proc Natl Acad Sci 79: 2962-296, 1982.

18. Chen XD, Sanada-Morimura S, Yanagi S et al. Genetic relationships between development of insecticide resistance and reduction of egg size as a negative effect on the fitness of the diamondback moth *Plutella xylostella* (Lepidoptera: Yponomeutidae). Appl Entomol Zool 41: 479-486, 2006.

19. Shirley MDF, Richard MS. Genetic basis of a between-environment trade-off involving resistance to cadmium in *Drosophila melanogaster*. Evolution 53: 826-836, 1999.