Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2017, Volume 5, Issue 7
ISSN: 2321–3647(online)

Response of Single and Double Mutant Forms of *Drosophila melanogaster* Under Stress of Alphamethrin

P.N. Saxena[1*], Devika Srivastava[1], Sanjay Kumar[2]
1.Dept. of Zoology, School of Life Science, Khandari Campus, Dr. Bhimrao Ambedkar University, Agra- 282001, India
2.Dept. of Zoology, Acharya Narendra Dev College, University of Delhi, New Delhi- 110019, India

ABSTRACT

Alphamethrin is an important type II pyrethroid, a cyano-derivative that exhibits toxic response in the mutants of *Drosophila melanogaster* which has been adjudged on the basis of LC_{50} values. The concentrations to assess LC_{50} value of alphamethrin were the same for double mutants (*sepia ebony* and *sepia vestigial*) and *single* mutants (*ebony*, *sepia*, *vestigial*). Double mutant (*sepia vestigial*) have been adjudged most resistant whereas single mutant (*sepia*), the most susceptible. The observed difference in LC_{50} among single and double mutants are due to some genetic consequences which include single chromosome with mutation on one arm, two chromosome with mutation on both the arms, distance of mutation site from centromere, nucleotide bases, pigmentation, wing pattern and rate of mutation, effect of pesticide toxicity on cell survival and metabolism.

Keywords : Alphamethrin, LC_{50}, sepia, ebony, vestigial

*Corresponding Author Email: dr_pnsaxena@gmail.com
Received 08 July 2017, Accepted 15 July 2017

Please cite this article as: Saxena PN *et al*., Response of Single and Double Mutant Forms of Drosophila melanogaster Under Stress of Alphamethrin. American Journal of Pharmacy & Health Research 2017.

INTRODUCTION

Alphamethrin, a cyno-derivative is a synthetic pyrethroid. Pyrethroid insecticides are very special chemical class of active ingredients which are found in many of the latest insecticides found on store and used by many professionals who manages pest in the society. The name pyrethroid means "pyrethrum-like" which is produced by chrysanthemum flowers and refers to the origin of this class of pesticides. Pyrethroids are found in many commercial products used to control insects, including household insecticides, shampoos and pet sprays. Pyrethroids extensively used not only as an ectoparasiticide in animals, but also in agricultural crop production and public health programmes which has been selected as a synthesized chemical in the present investigation.

Drosophila melanogaster has clear morphology, easy rearing under the laboratory conditions, with short life cycle and cosmopolitan distribution. They exhibit short generation time (about 10 days at room temperature) so several generations can be studied within a few weeks with high fecundity and were easy to grow in laboratory with easily identifiable morphology. *Drosophila melanogaster* sequenced genome of 139.5 million base pairs has been annotated and contains appropriate 15016 genes. More than 60% of the genome appears to be functional non-protein coding DNA involved in gene expression control. Besides also has four pairs of chromosomes(X/Y pair of chromosome and three autosomes) (kumar & saxena 2017)[1].

Drosophila melanogaster mutants have been selected for the comparison of mortality rate of alphamethrin (type II pyrethroid). Five mutant forms of *Drosophila melanogaster* have been considered which include - *sepia(se), ebony(eb), vestigial(vg), sepia ebony(se;eb)* and *sepia vestigial(se;vg).*

MATERIALS AND METHOD

Rearing and Maintenance of *Drosophila melanogaster* mutants

Five mutants of *Drosophila melanogaster* were selected for experimentation. These include *ebony (e), vestigial (vg), sepia (se), sepia ebony (se;eb)* and *sepia vestigial (se;vg).*

Food Ingredients

The following ingredients were used for the preparation of one unit food-

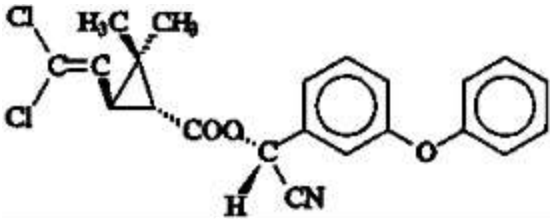
Distilled water: 360.00 ml, agar agar: 2.00 gm, corn flour: 17.00 gm, sugar: 12.00 gm, yeast: 3.00 gm, nepazine:1.00 gm, propionic acid: 1.00 gm and 70% alcohol : 1.00 ml.

Test Chemical

Molecular formula : $C_{22}H_{19}Cl_2NO_3$

Molecular weight : 416.3

Structural formula :



(A) (1R, *trans*) (α R)

Single Mutants

The *ebony* (chromosome III mutant): Mutations in *Drosophila melanogaster* causes two abnormalities, one black adult cuticle and the other light pupal case relative to the wild type, is a recessive trait and show poor phototaxis, ineffective in complete mating in light, and unable to incorporate beta-alanine into their pupal case, a defect related to tyrosine metabolism (Wittkopp, 2002)[2].

The *Sepia* (chromosome III mutant): Mutations in *Drosophila melanogaster* cause change in eye color. It is not a sex-linked trait, but a recessive allele influencing eye color, with "wild type" being dominant. In fact, "*sepia*" is probably an allele that causes the scarlet pigment not to be present in the fly eye, and the scarlet allele causes the brown pigment to be absent. Hence, bright red, not the brown-red wild type flies have been observed (Stanic, 2005)[3].

The *vestigial* (chromosome II mutant): Mutations in *Drosophila melanogaster* cause deletions of parts of the wings. The *vestigial* (*vg*) locus of *Drosophila melanogaster* is involved in wing margin development. *Vg* may induce expression of genes that are required for specific morphological changes and *vg* was first identified as a key "selector" gene that specifies wing identity during *Drosophila* developments (Williams *et al*., 1991)[4].

Double Mutants

The *sepia ebony* (chromosome III mutant): Mutations in *Drosophila melanogaster* cause black eyes with the advancement of age. Red-brown eyes at emergence darken to *sepia* and ultimately to black as the fly ages with shiny black body color (Srivastava & Saxena 2017)[15].

Sepia vestigial (chromosome II and III mutant): Mutations in *Drosophila melanogaster* cause vestigial wings, *sepia* eyes, is multichromosomal mutant. *Vestigial* is a novel nuclear protein with no known homologs, except for an N-terminal domain resembling that of Paired. *Vestigial* expression is evident in thoracic and abdominal segments, in the embryonic primordial of the wing and haltere discs, in descrete cells, in the ventral nerve cord (Srivastava & Saxena 2017)[15].

Determination of LC_{50} of *Drosophila* mutants (Table 1)

Different concentration i.e. 4, 2, 1, 0.5, 0.025, 0.125, 0.625 (µl/100ml food) of test compound were mixed with food and the prepared food was poured in different culture bottle which were already marked with respective concentrations. Bottles were kept in BOD for one day. The easy counting was done on next day with etherized flies with diethyl ether. The bottles were then plugged with sterile cotton. A control batch of 10 flies was also run, given same amount of dilutant, the distilled water. The survival percentages of flies at each concentration were recorded after 48 hrs. The data so obtained were analyzed statistically by log dose/ probit regression line method (Finney, 1971)[5].

Table 1: LC_{50} values of *Drosophila* mutants under stress of the Alphamethrin

Strains8:00-8:30	LC_{50} (in µl/100 ml food)
Ebony 8:30-9:45	0.01514
Sepia 9:45-10:30	0.00053
Vestigial 10:30-10:45	0.077
Sepia Ebony 10:45-12:30	0.0019
Sepia Vestigial 12:30-1:30	0.195

RESULTS AND DISCUSSION

The observations on the mutants of *Drosophila melanogaster* have been made in order to assess the effect of alphamethrin, a synthetic type II pyrethroid. The survival data have been statistical analysed using centring constants, variation,'t' test, F test, chi(λ)-test and correlation *vide infra*.

Comparison of toxicity evaluation of Alphamethrin on mutants of *Drosophila melanogaster*

Toxicity evaluation of alphamethrin and their comparison on single and double mutants of *Drosophila melanogaster* have been assessed on the basis of LC_{50} value, variance, fiducial limit in single mutants- *ebony (eb), sepia (se) and vestigial (vg)* and Double mutants- *sepia ebony (se;eb)* and *sepia vestigial(se;vg)* (Table 2 - Table 5).

The Double mutant *sepia vestigial (se;vg)* has been found to be most resistant among all mutants following alphamethrin treatment.

Table 2: Toxicity evaluation of Alphamethrin on Ebony mutants of *Drosophila melanogaster*

Experimental individual	**Compound**	**Regression equation**	**LC_{50} (in µI/100 ml food)**	**Variance**	**Fiducial Value**
Ebony	Alphamethrin	5=6+0.78(X-2.46)	0.0154	0.27	m_1=(+)1.70 m_2=(-)0.67

Table 3: Toxicity evaluation of Alphamethrin on Sepia mutants of *Drosophila melanogaster*

Experimental Individual	Compound	Regression Equation	LC_{50} (in µI/100 ml food)	Variance	Fiducial Value
Sepia	Alphamethrin	5=5.40+0.24(X-2.49)	0.00053	1.287	m_1=(+)3.07 m_2=(-)1.99

Table 4: Toxicity evaluation of Alphamethrin on Sepia Ebony mutants of *Drosophila melanogaster*

Experimental individual	Compound	Regression equation	LC_{50} (in µI/100 ml food)	Variance	Fiducial value
Sepia Ebony	Alphamethrin	5=5.23+3.76(X-2.54)	0.0019	0.13	m_1=(+)0.50 m_2=(-)0.004

Table 5: Toxicity evaluation of Alphamethrin on Sepia vestigial mutants of *Drosophila melanogaster*

Experimental individual	Compound	Regression equation	LC_{50} (IN MI/100 ML Food)	Variance	Fiducial Value
Sepia vestigial	Alphamethrin	5=5.49+1.64(X-2.59)	0.195	0.03	m_1=(+)2.36 m_2=(-)2.25

Comparison of survival percentage between single and double mutants after Alphamethrin treatment

Sepia/Sepia ebony

The survival percentage between single/double mutants have been compared by drawing a graph, taking concentration (µl/100 ml food) on X-axis and survival percentage of single mutant *sepia* on Y-axis which has been observed as-10,7,4,1,0,0,0, while for double mutant *sepia ebony* on Y-axis which has been observed as – 10,8,6,2,0,0,0 **(Figure 1)**. Double mutant *Sepia ebony* (chromosome III) has been observed to be most resistant than single mutant *Sepia* (chromosome III).

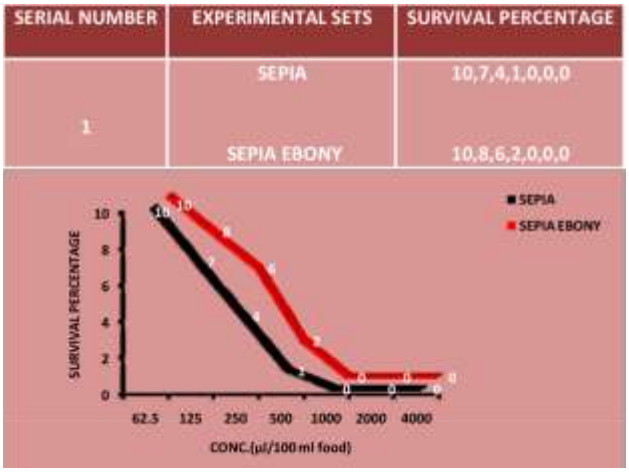


Figure 1: Survival percentage between *Sepia/Sepia ebony* after Alphamethrin treatment

Vestigial/Sepia vestigial

The survival percentage between single/double mutants have been compared by drawing a graph, taken concentration (µl/100 ml food) on X-axis and survival percentage of single mutant *vestigial* on Y-axis which has been observed as-10,6,3,1,0,0,0 , while for double mutant *Sepia vestigial* on Y-axis which has been observed as– 10,7,5,3,1,0,0 **(Figure 2)**.

Double mutant *Sepia vestigial* (chromosomes II and III) has been observed to be most resistant than single mutant *Vestigial* (chromosome II).

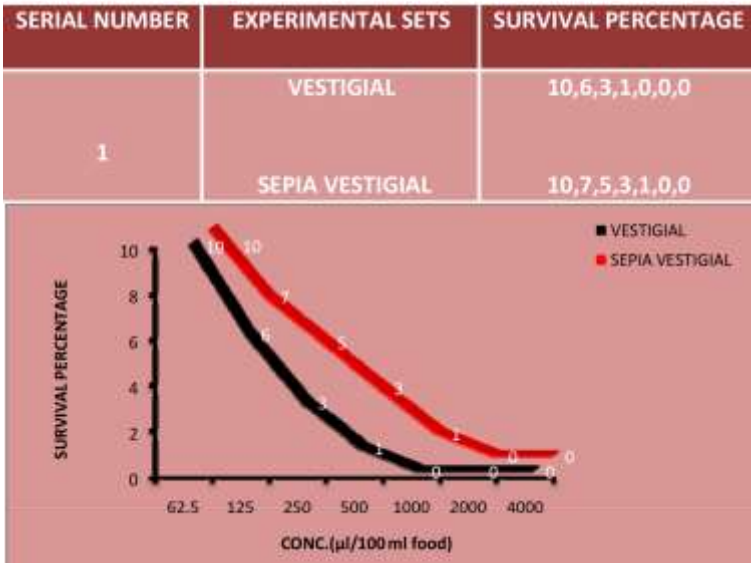


Figure 2: Survival percentage between Vestigial/*Sepia Vestigial* after Alphamethrin treatment

Ebony/Sepia ebony

The survival percentage between single/double mutants have been compared by drawing a graph, taken concentration (µl/100 ml food) on X-axis and survival percentage of single mutant *ebony* on Y-axis which has been observed as-10,3,2,1,0,0,0 , while for double mutant *Sepia ebony* on Y-axis which has been observed as– 10,8,6,2,0,0,0.**(Figure 3).** Double mutant *Sepia ebony* (chromosomes III) has been observed to be most resistant than single mutant *Ebony* (chromosome III).

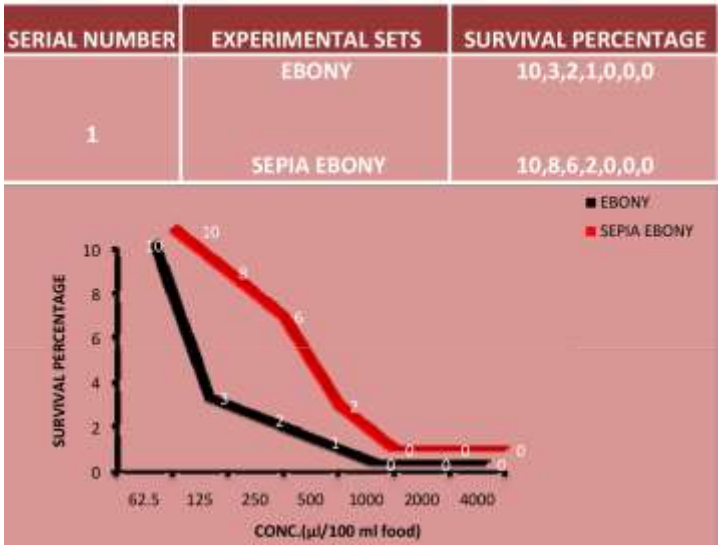


Figure 3: Survival percentage between *Ebony/Sepia ebony* after Alphamethrin treatment

Sepia/Sepia vestigial

The survival percentage between single/double mutants have been compared by drawing a graph, taken concentration (µI/100 ml food) on X-axis and survival percentage of single mutant *sepia* on Y-axis which has been observed as-10,7,4,1,0,0,0 , while for double mutant *Sepia vestigial* on Y-axis which has been observed as– 10,7,5,3,1,0,0.**(Figure 4)**.

Double mutant *Sepia vestigial* (chromosomes II and III) has been observed to be most resistant than single mutant *Sepia* (chromosome III).

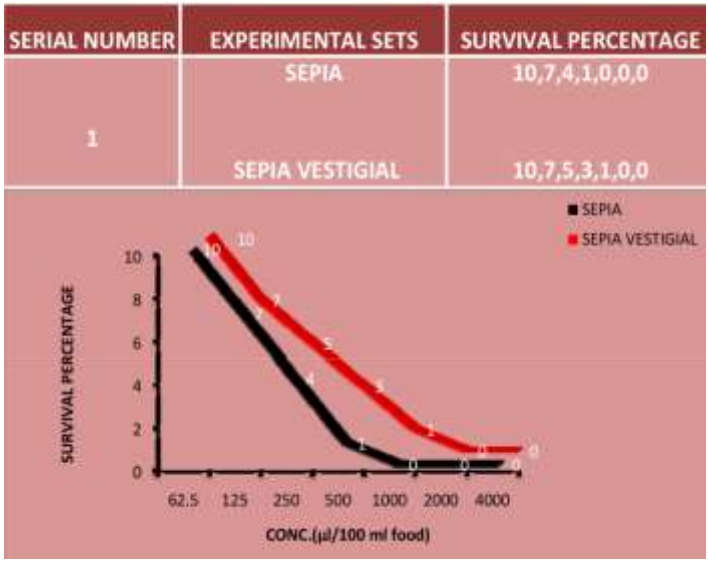


Figure 4: Survival percentage between *Sepia/Sepia Vestigial* after Alphamethrin treatment

Correlation between Single and Double Mutants after Alphamethrin Intoxication (Figure 5)

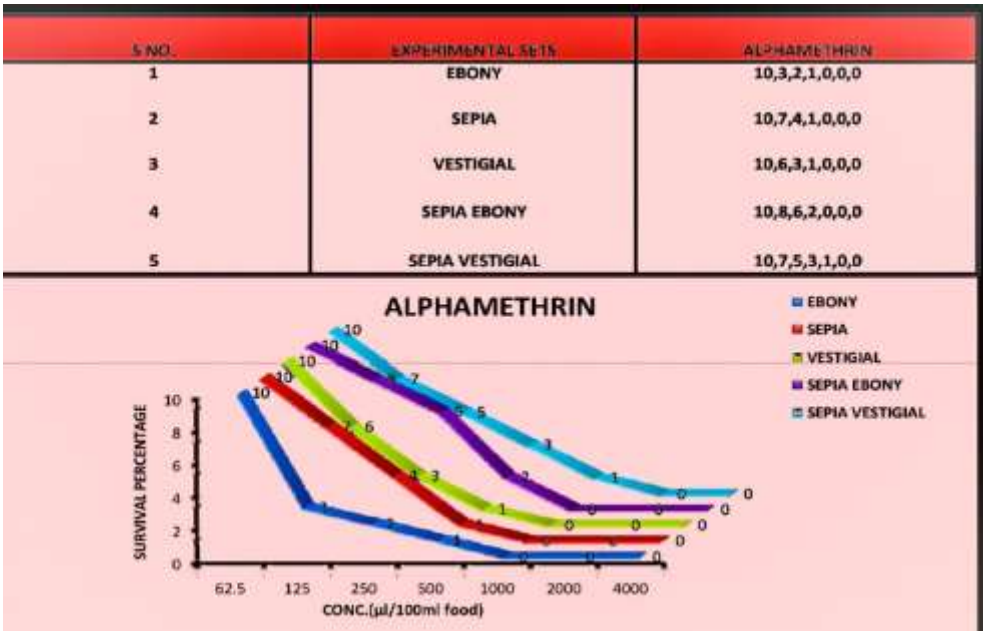


Figure 5: Correlation between Single and Double Mutants after Alphamethrin Intoxication

DISCUSSION

The mutants of *Drosophila melanogaster* treated with various doses of alphamethrin viz., 4, 2, 1, 0.5, 0.025, 0.125, 0.625 (µl/100ml food) resulted in difference in LC_{50} values to the all different doses (table II).

Drosophila melanogaster has four pairs of chromosomes: three autosomes, and one sex chromosome, males do not show meiotic recombiaton, facilitating genetic studies. Recessive lethal "balancer chromosomes" carrying visible genetic markers can be used to keep stocks of lethal alleles in a heterozygous state without recombination due to multiple inversions in the balancer.

Sepia vestigial is resistant in Alphamethrin (type II pyrethroids, synthetic compound), the mutation in *vestigial* gene on chromosome II is the factor of resistance. Recessive mutants survive in population due to heterozygous condition. *Drosophila melanogaster* shows balanced genetic polymorphism, hence are resistant to recessive mutations (Gardner, 1984)[6]. The development of an ability in a strain of an organism to tolerate doses of a toxicant which would prove lethal to the majority of individuals in a normal (susceptible) population exhibited significant genetic variances for susceptibility to the OPs. Toxicity variation in mutants of *Drosophila melanogaster may be* attributed to gene based phenomenon.

The *vg* gene is usually considered to be completely recessive. *Vg* does not have a DNA binding domain but contains two domains important for gene activation (MacKay, 2003)[7]. The results also indicate that an additional large portion of *VG*, outside of these two domains and the SD-binding domain, is dispensable in the execution of these normal *VG* functions. *Vestigial* expression is evident in thoracic and abdominal segments, in the embryonic primordial of the wing and haltere discs, in discrete cells in the ventral nerve cord, and possibly in progenitors of sense organs of the peripheral nervous system (Williams,1991)[4].

On the other hand, susceptibility of *sepia* in Alphamethrin, mutations on chromosome III probably are due to their presence on right(R) arm, may be considered common factors for susceptibility. Again, absence of PDA synthase enzyme may also be a reason for the susceptibility of *sepia* (Kim, 2013)[8]. Alphamethrin has earlier been reported to be effective insecticide in terms of mortality (Hougard, 2003)[9] and Choudhary (2004)[10] who revealed that fenvalerate (pyrethroid) is toxic as it had a pronounced effect on the rate of development and viability. Further cypermethrin exhibited greater larval and adult mortalities compared to fenvalerate in *Drosophila melanogaster* (Batiste, 1987)[11]. Increase in mortality rates against different doses of lead acetate with a sigmoid curve more appropriately a “dose effect” and post-hatching mortality in *Drosophila melanogaster* by commercial fungicide is already an established fact (Marchal, 1989)[12]; (Haq, 2011)[13]. Again Type I and II pyrethroids produce qualitatively different effects on sodium channel tail currents, divergent actions on intact nerves, and different effects on the excitability (Soderlund and Bloomguist, 1989)[14].

CONCLUSION

Thus it was concluded that toxicity variation in mutants of *Drosophila melanogaster* has been attributed to be gene based phenomenon and the observed difference in LC_{50} among single and double mutants probably are due to same genetic consequences which includes single chromosome with mutation, two chromosome with mutation on both, distance of mutation site from centromere, nucleotide bases, pigmentation, wing pattern and rate of mutation. Since all the mutants are recessive, hence, the heterozygous condition can be considered a tool in assessment of magnitude of **resistance.** ***Sepia vestigial*** is most resistant and ***sepia*** is most susceptible in under alphamethrin stress. It is also summarized that rate of mutation also affect the resistance/susceptibility.

REFERENCES

1. Kumar S, Saxena PN. Comparative Efficacy of Alphamethrin, on Gene Expression of Wingless Lac Z and Engrailed Lac Z, the Transgenic Forms of *Drosophila melanogaster.* Am. J. Pharm Health Res 2017; 5(6): 61-9.
2. Wittkopp PJ, True JR, Carroll SB. Reciprocal functions of the *Drosophila* yellow and ebony proteins in the development and evolution of pigment patterns. Development 2002;129:1849-56.
3. Stanic S, Pavkovic LS. Mating success of wild type and sepia mutants *Drosophila melanogester* in different choice. Riv Biol 2005; 98(3): 513-24.
4. Williams JA, Bell j, carrol SB. Control of *Drosophila* wing and haltere development by the nuclear vestigial gene product. Gene & Dev. 1991; 5(12B): 2481-95.
5. Finney DJ. Probit analysis, 3rd edition, New York ,Cambridge University Press 1971: 30
6. Gardnner EJ, Simmons MJ, Snustad DP. Principles of genetics. 8th edition. Mendalian Genetics 1984; Chapter 2: pp 22.
7. Mackey JO, Kelly HS, Srivastava A , Simmonds A, Williiam JB, Bell JB. An in vivo Analysis of the vestigial Gene in *Drosophila melanogaster* defines the Domains Required for Vg Function. Genetics 2003; 163:1365-73.
8. Kim MS, Korean Red Ginseng Tonic Extends Lifespan in *Drosophila melanogaster.* Biomol Ther. 2013; 21(3): 241-5.
9. Hougard JM, Duchon S, Darriet F, Zaim M, Rogier C, Guillet P. Comparative performances, under laboratory conditions, of seven pyrethroid insecticides used for impregnation of mosquito nets. Bulletin of the world health organization 2003; 81(5): 324-33.
10. Choudhary S. Efficacy of fenvalerate as contact poison against the larval development and viability of mutants form (*sepia*) of *Drosophila melanogaster* (Meigen). Iranian journal of toxicology 2004; 25(1): 117-8.
11. Batiste AM, Xamena N, Valaquez A, Creus A, Marcos R. Studies on the toxicity of cypermethrin and fenvalerate in different strains of *Drosophila melanogaster* Meig. (Insecta, Diptera). Environ Res 1987; 43(1): 117-25.
12. Marchal SD. Toxicity of commercial fungicides in relation to the larvae of *Drosophila melanogaster* Meigen (Diptera, Drosophilidae) abd effect on the sex ratio. J Envision Sci Health 1989; 24(4): 307-24.
13. Haq R, Khan MF, Haq E. Adverse effect of lead acetate on *Drosophila melanogaster.* Journal of Basic and Applied Sciences 2011; **7**(2):157-63.

14. Soderlund DM, Bloomguist JR. Neurotoxic actions of pyrethyroid insecticides. Annu Rev. Entomol. 1989; 34:77-96.
15. Srivastava D, Saxena PN. Response of Drosophila Mutants to Korean Red Ginseng, A Natural Product. International j. Interdis. Sci. 2017; 4(4): 8-18.