Journal of Applied Biological Sciences 4 (3): 31-36, 2010
ISSN: 1307-1130, E-ISSN: 2146-0108 www.nobel.gen.tr

Effect of Fungicide, Thiovit® Jet on Several Life History Trait of *Drosophila melanogaster* (Diptera: Drosophilidae)

Sanjib K DAS Sayanti PODDER Sumedha ROY*
Cytogenetics Laboratory, Department of Zoology, University of Burdwan, INDIA

*Corresponding Author
e-mail: roysumedha@rediffmail.com

Received : July 10, 2010
Accepted : October 07, 2010

Abstract

The present work aims to demonstrate the profound effect of a widely used fungicide Thiovit® Jet, on several life history traits in *Drosophila melanogaster.* The fungicide under study has a very wide application in fruit orchards. Freshly laid eggs of wild type *D. melanogaster,* were transferred to different dietary concentrations of the test chemical (20, 30, 40, 50, 100, 200, 400, 800, 1600 and 2000ppm). Larval duration, Pupal duration and Emergence of flies of both treated and control groups were recorded. Results obtained show a significant ($p<0.05$) change in the mentioned parameters in the treated flies in comparison to the control. The maximum noticeable increase in duration has been recorded with 40ppm treatment. Thus the study indicates an effect of a fungicide Thiovit® Jet on the duration of different life stages and thereby suggesting a role of the fungicide on a non-target organism like *Drosophila.*

Key Words: Drosophila, Thiovit, Fungicide

INTRODUCTION

Like other holometabolous insects, *Drosophila* life cycle includes egg, larva, pupa and adult. The life cycle of *Drosophila* melanogaster in ideal condition at 25 °C (77 °F) takes 2 weeks for completion. Females lay approximately 400 eggs (embryos), about five at a time, into rotting fruit or other organic material. The eggs, which are about 0.5millimetres long, eclose after 24 h [1-2]. The resulting first instar larvae after 24 hours moult into second instar larvae which after another 24 hours eclose into third instar larvae. During this time, they feed on the sugar of the fruit and on the microorganisms that decompose the fruit. Total larval duration is of 5-6 days. The third instar larvae encapsulate in the puparium and undergo a five-day-long metamorphosis, after which the adults emerge.

Insect life cycle is controlled by several external (temperature, humidity, food etc) as well as internal factors (role of juvenile hormone, ecdysone etc). Variation in one of the several factor or factors may affect the life cycle. The duration of life cycle may get increased or decreased. Several literatures vividly present the fact that; Drosophila life cycle is mostly affected by temperature [1-2]. Under crowded conditions, developmental time is also seen to increase,[3]while the emerging flies are smaller [3-4]. Chemical induced delay in emergence of adult *Drosophila* has also been reported[5- 6].

Thiovit® Jet is a well-known sulphur containing fungicide used for fungicidal activity all over the world. Several research works have shown that fungicides cause hazardous effects on invertebrates as well as vertebrates. Invertebrates like *C. elegans* [7], *D. melanogaster* [8-12] and vertebrates like humans [13] have been found to be sensitive to various fungicides.

Fungicides are also known to have mutagenic potential in *Drosophila.* Mathew and Al-Doori [9] used mercuric fungicide in the food medium and got a significant increase in sex-linked recessive lethals. Similar results with organo-mercurial fungicide treatment in *Drsosphila melanogaster* have been reported by [14].

Drosophila has been in use in environmental monitoring studies either as a target or as a non-target organism of environmental chemicals. Use of *Drosophila* was recommended by the European Centre for the Validation of Alternative Methods (ECVAM) [15]. Based on all these findings, this paper deals with the effect of a sulphur fungicide on a non-target insect, like *Drosophila melanogaster* who are often exposed to this chemical due to its wide application in fruit orchards.

MATERIAL AND METHODS

Animals for study and their maintenance

The flies and larvae of wild type *Drosophila melanogaster* were cultured on standard food containing agar, corn meal, sucrose and yeast at 22 ±10C in incubator.

Material for treatment

THIOVIT® JET Micro granule Fungicide

Formulation type:	water dispersible granule
Chemical type:	Inorganic
Active Ingredient:	Sulphur
Product Code No:	SAN 7116
CAS No.	7704-34-9

USE: Thiovit® Jet is a registered product of a Syngenta Group Company and its active constituent is 80% elemental sulphur. It is a unique micronised sulphur formulation, wettable with spherical particles that mix easily with water to form a spray with good spreading and sticking properties. It is classified as a fungicide, used for the control of powdery mildew, rust, mites, aphids and thrips in pome and stone fruit, citrus, grapevines, kiwifruit, strawberries, apples and vegetables.

The concentration of Thiovit recommended for treating above mentioned diseases, range between 20g-40g per 10L of spray (2000ppm to 4000ppm). It is recommended that it be applied at intervals between 10 days and 2-3 weeks or as necessary. However, in general practice, the concentrations used are much higher and the rates of applications are much more frequent than those recommended

Treatment of animals

Thiovit® 80 (containing 80% w/w sulphur) from Syngenta Group Company was used in the present study. Different concentrations (20, 30, 40, 50, 100, 200, 400, 800, 1600 and 2000ppm) of this chemical were dissolved in water and mixed with *Drosophila* food medium. 25eggs were introduced to different concentrations of thiovit-containing food along with control. The eggs were allowed to hatch and grow on them throughout their development. All the treatments were carried out in duplicate.

Life Cycle study

The life cycle of *Drosophila melanogaster* in control and in different treatment groups were studied. Constant numbers of eggs (25) were introduced in each vial and 4 vials were taken for each group including control. Thereafter the day of pupation followed by emergence of adults and the number of flies that have emerged in control and different treatment groups were recorded till all the flies emerged. From the obtained data, variation in larval duration, pupal duration and the emergence of fly if any between control and different treatment groups have been analyzed.

Statistical analysis of the data

Student's t test was carried out for the analysis of the data, $p<0.05$ was considered significant.

RESULTS

Effects of Thiovit® Jet on larval duration of flies

Effect of different concentrations (20, 30, 40, 50, 100, 200, 400, 800, 1600 and 2000 ppm) of fungicide, Thiovit® Jet is observed on the mean larval duration and compared with the control set. In this study larval duration is considered as the total time taken from the hatching of the eggs (day 1) till the onset of pupation by the insect. A highly significant ($p<0.001$) increase in all the larval duration is noted in the insects treated with 40ppm (19.8 ±0.2118 days) Thiovit® Jet when compared with the control (8.32 ± 0.1724 days).

Similarly, treatments with 20, 30, 50, 100, 200, 400, 800, 1600 and 2000 ppm showed values 9.66 ± 0.2242, 12.6 ± 0.1106, 15.76 ± 0.2596, 10.98 ± 0.1124, 12.06 ± 0.2202, 10.28 ± 0.2587, 12.20 ± 0.0903, 9.06 ± 0.1601 and 9.80 ± 0.1807 days respectively which are found to be significantly ($p<0.05$) higher when compared to the control (Fig. 1).

When the different treatment groups were compared among themselves significant variation ($p<0.05$) in the larval period is observed. The maximum value of larval duration is obtained on treatment with 40 ppm Thiovit® Jet. When the treatment group 400 ppm is compared with 2000 ppm no significant observable variation was noted among them, though the values are found to be significantly higher than the control.

Effects of Thiovit® Jet on pupal duration of flies

In the present study the pupal duration is considered as the time taken by the insect from formation of the pupal case till the emergence of the adult fly. Insects from different treatment groups treated with different concentration of Thiovit® Jet when compared with the control set showed small variations. The 40ppm treatment category showed a significant ($p<0.001$) increase in pupal duration (6.12 ± 0.0464 days) when compared with the control (5.3 ± 0.0654 days). A similar increase in the pupal duration (6.54 ± 0.115 days) is obtained at much higher ($p<0.001$) concentration of 1600ppm when compared to control. Interestingly, a decrease in pupal duration is noted with 200, 400 and 800 ppm doses of Thiovit® Jet treatment, the values being 5.12 ± 0.0464, 4.54 ± 0.0711, 5.08 ± 0.0387 days respectively (Fig. 2).

A lower concentration of 20 and 30ppm and a very high concentration of 2000ppm could not significantly elicit any change in pupal duration when compared to control. When the treatment groups were compared among themselves mostly the variation is found to be significant ($p<0.05$). 30ppm treated category when compared to 100 and 2000 ppm groups were found to be not significantly different. Similarly comparisons between 200ppm and 800 ppm treatment groups were found to be not significantly different. The values of 100 ppm treatment category and 2000 ppm treatment category were also not significantly different. The maximum effective change in duration was noted in 40 and 1600 ppm treatment.

Fig1 **Variation in larval duration on treatment with Thiovit in *Drosophila melanogaster***

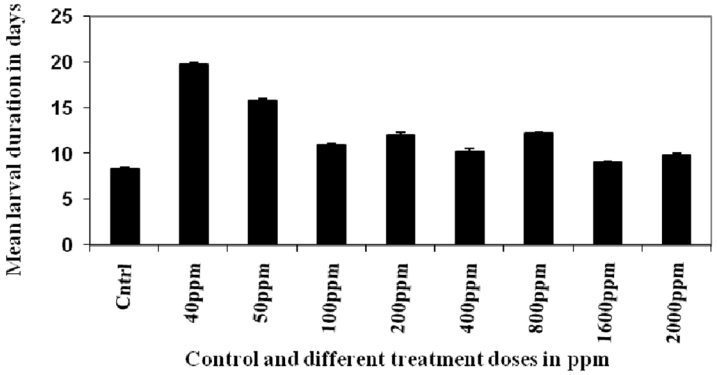


Figure 1: Variation in the larval duration of Drosophila melanogaster due to treatment with different concentrations of fungicide, Thiovit® Jet.

Drosophila melanogaster larvae were reared in Thiovit® Jet containing food with 20, 30, 40, 50, 100, 200, 400, 800, 1600, 2000 ppm of the chemical. A control set of insect was maintained in normal food. The data is expressed as the mean larval duration in days. The data represents Mean ± SE of two pooled determinations. Each pool consisted of 50 larvae. The vertical lines denote the Standard Error.

Effects of Thiovit® Jet on emergence of flies

On day 0 equal numbers of eggs were placed in different culture bottles containing different concentration of fungicide, Thiovit® Jet containing food. The culture bottles were maintained at 22 ± 10C and emergences of flies were noted after the completion of larval and pupal stages. The emergence pattern in treated flies were compared with their normal counterpart and it is observed that, different treatment concentrations were able to elicit significant ($p< 0.05$) changes in the nature of emergence.

Mean emergence time is found to be 28.02 ± 0.2329 days in 40ppm treatment category which is significantly ($p< 0.001$) higher than the control flies whose emergence time is 15.52 ± 0.2199 days, so a distinct delay in emergence is noted. Similarly all the treatment categories (20, 30, 50, 100, 200, 400, 800, 1600 and 2000ppm) were able to elicit a significant ($p< 0.001$) delay in emergence of flies when compared to control. These values are found to be 15.62 ± 0.3905, 18.74 ± 0.1171, 23.76 ± 0.2733, 17.38 ± 0.1124, 19.06 ± 0.2183, 16.8 ± 0.2213, 19.28 ± 0.1108, 17.60 ± 0.1641 and 17.26 ± 0.1891 days respectively (Fig. 3).

When compared among them, the different treatment categories expressed significant ($p<0.001$) variation in the emergence pattern. Though when the value of the 100 ppm treatment category was compared with 1600 ppm flies, the change was non significant. Similarly, comparison of the result of the 2000 ppm treatment category with the 400 ppm and 1600 ppm treatment groups were found to be non significant.

Considering (Fig. 4) which shows the pattern of percentage emergence of flies following exposure to different doses of Thiovit® Jet, is seen that the control flies shows nearly 80% emergence on 16th day, whereas in treatment groups 400, 1600 and 2000 ppm only 54%, 14% and 28% of flied have emerged respectively. More interestingly no flies emerged in treatment groups of 40, 50 and 800 ppm on 16th day. But treatment group 100 and 200 ppm showed 32% and 14% emergence on 17th day and treatment group 50 and 800 ppm showed a low emergence of 2% and 12% on 18th day. Most notably no emergence was seen up till 23rd day in 40 ppm treatment category. Only 4% emergence was observable on 24th day in this group. Though a definite variation in emergence pattern is noted in the entire treatment category when compared to control, yet an interesting observation shows that there is almost 100% emergence in all the treatment categories similar to their control counter part.

Figure 2: Variation in the pupal duration of Drosophila melanogaster due to treatment with different doses of fungicide, Thiovit® Jet

Drosophila melanogaster larvae were reared in Thiovit® Jet containing food with 20, 30, 40, 50, 100, 200, 400, 800, 1600, 2000 ppm of the chemical. A control set of insect was maintained in normal food. These larvae were allowed to pupate and the pupal duration were analysed from them. The data is expressed as the mean pupal duration in days. The data represents Mean ± SE of two pooled determinations. Each pool consisted of 50 larvae. The vertical lines denote the Standard Error.

Fig 2 **Variation in pupal duration on treatment with thiovit in *Drosophila melanogaster***

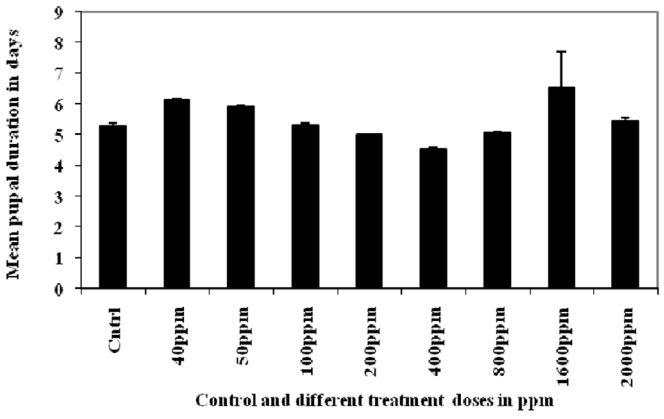


Fig 3

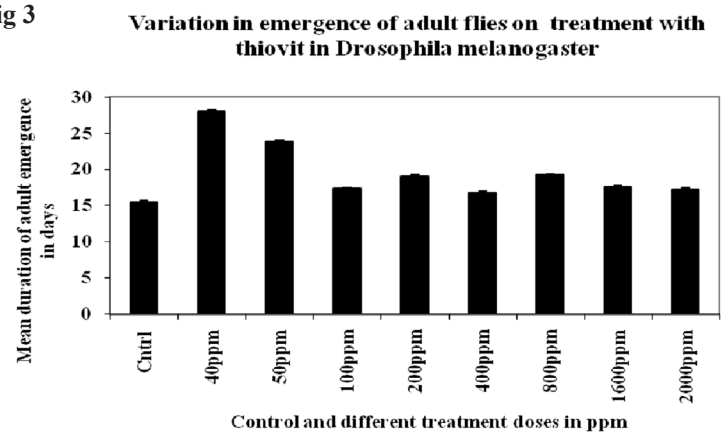


Figure 3: Variation in the emergence of adult fly, Drosophila melanogaster due to treatment with different doses of fungicide, Thiovit® Jet.

Drosophila melanogaster larvae were reared in Thiovit® Jet containing food with 20, 30, 40, 50, 100, 200, 400, 800, 1600, 2000 ppm of the chemical. These larvae are allowed to pupate and the pupae are subsequently allowed to emerge as adult flies. A control set of insect was maintained in normal food. The data is expressed as the mean time taken up to emergence in days. The data represents Mean ± SE of two pooled determinations. Each pool consisted of 50 larvae. The vertical lines denote the Standard Error

DISCUSSION

Larval Duration:

The result shows a significant increase in the larval duration with different doses of treatment with fungicide, thiovit. Although, all the concentration of treatment were able to induce significant increase in larval duration but the maximum duration of larval period is achieved on treatment with 40ppm. A nearly dose dependent change is noted with 20ppm, 30ppm, 40ppm treatment when compared to control. The above-mentioned subsequent doses were able to elicit significant increase in larval duration. The nature of response seems to be a bit biphasic where subsequent increase of concentration like 50,100,200,400, 800, 1600, 2000ppm were able to maintain a higher larval duration than the control but a significant fall in the value is observed when compared with 40ppm dose. This can be explained by the fact that any treatment initially can elicit a dose dependent response but soon after, saturation may be reached which inhibits any further increase in the response.

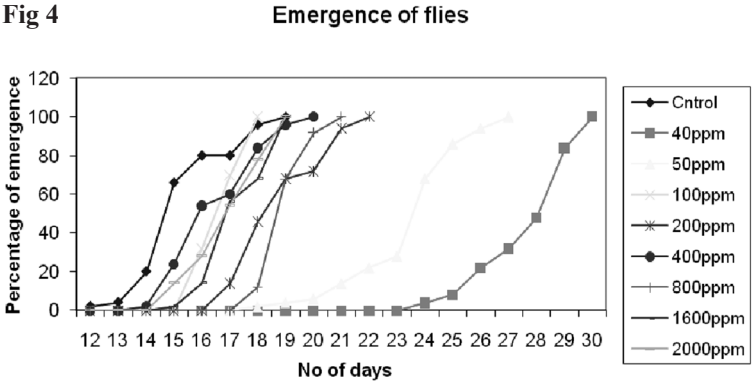
Thiovit the fungicide is seen to produce a definite effect on larval period of *Drosophila melanogaster.* The treatment schedule of different doses of thiovit and the observation makes it clear that, the insects cultured in the food containing thiovit, take more time than their control counterpart. This may be due certain hindrance caused by the exogenous chemical, which slows down the larval developmental process, thereby increasing the total larval duration. Similar to the finding of [16], the chemical in the culture medium might be producing the somatic effect thereby increasing the developmental time. Nazir et al. [5,6] working with *Drosophila* found that, the chemical chloropyrifos and captafol were able to elicit similar lengthening in the development process of *Drosophila melanogaster* respectively.

Pupal Duration: Mean pupal duration is seen to be affected by different concentration of fungicide (thiovit treatment). On comparison with control, different treatment categories showed significant variation. Maximum increase in pupal duration is observed in 40 and 1600 ppm treatment category. On close observation the response to thiovit treatment is seem to be biphasic. On comparison with control the first three low doses (20, 30, 40ppm) were seen to manifest a dose dependent increase in mean pupal duration. Further higher concentration of thiovit failed to elicit any further consecutive increase in value. An interesting observation shows a decrease in pupal duration in three consecutive treatment groups 200,400 and 800ppm from the control value and subsequent high peak in the 1600 treatment category. *Drosophila* is known to have drug metabolizing enzymes similar to those of mammals [12]. Thus fungicide insult

Figure 4: Variation in percentage emergence of Drosophila melanogaster due to treatment with fungicide, Thiovit® Jet.

Drosophila melanogaster larvae were reared in Thiovit® Jet containing food with 20, 30, 40, 50, 100, 200, 400, 800, 1600, 2000 ppm of the chemical. These larvae are allowed to pupate and the pupae are subsequently allowed to emerge as adult flies. A control set of insect was maintained in normal food. The data is expressed as percentage of emergence on specific days. Data represents the percentage of flies emerged on a particular day. All the groups for study consisted of 100 larvae each.

Fig 4



may activate these enzymes as has been suggested by Devonshire and Field (1999) [17who postulated that an increase in the amount of detoxificant enzymes help to develop resistance, thus decrease sensitivity of the applied insecticides or pesticides. In the present study the doses 200, 400 and 800ppm may have triggered the detoxificant enzymes, thereby balancing the effect and bringing down the pupal duration towards the control value. A further increase in the treatment concentration, 1600ppm shows a different picture, which might be due to the failure of the detoxificant enzymes to nullify the effects produced by such a high concentration of thiovit.

Discussion on emergence of flies

A definite increase in the total development process of *Drosophila* is seen. A clear delay in the emergence of treated flies was observed when compared with the control counterpart. The 40ppm concentration was able to elicit maximum delay in emergence followed by 50ppm dose. A definite stair case nature of expression is noted when results of control, 30ppm and 40ppm doses are compared. The maximum delay was found with 40ppm treatment, a fall value was noted in 50ppm category. The 100, 200, 400, 800, 1600, 2000ppm categories were more or less maintaining a constant level, which is significantly higher than the control counter part, yet was much lower than the 40ppm treatment group. This results shows definite effect of the chemical on the development process of *Drosophila melanogaster,* these effects were very much at per with the effect shown by other chemicals like, captan, captafol, folpet, chloropyrifos and cypermethrin on *Drosophila* development and life processes [5,6,18]. Similarly different doses of nuvan and dimecron were seen to affect the emergences of flies, *Drosophila* melanogaster and interestingly very high concentration of nuvan was able to produce no emergence because of larval death [19]. In this study almost 100 percent emergence was observed signifying no larval death due to the treatment.

CONCLUSION

The present work clearly demonstrates a well-defined effect of a fungicide on several life history traits of a non-target organism, *Drosophila melanogaster*. The significant variation in larval and pupal duration in the treated insects clarifies the effect of Thiovit on the life cycle of this insect, thereby forwarding the idea of impact of chemicals used as fungicide or pesticide on several non-target organisms like *D. melanogaster*. The present study deals with the effect of the chemical on a single generation of the insect, hence cannot forward a justified theory unequivocally on the impact of a continuous exposure to this fungicide generation after generation. A further study with three or more generation may convincingly propound a theory on the degree of impact of continuous treatment in a non–target organism.

Acknowledgements

The authors are grateful to Calcutta University *Drosophila* stock centre for providing wild type *Drosophila melanogaster*. Thanks are due to Prof. Jagat Roy of Benaras Hindu University and Prof. Rabindra Nath Chatterjee of Calcutta University for their valuable suggestions. Also we are thankful to the University of Burdwan for laboratory facilities.

REFERENCES

[1] Ashburner M., Thompson JN. 1978.The laboratory culture of *Drosophila*. In: The genetics and biology of Drosophila. (Ashburner M, Wright TRF (eds.).. Academic Press, 2A: pp. 1–81.

[2] Ashburner M, Golic KG, Hawley RS.2005. *Drosophila:* A Laboratory Handbook., 2nd ed., Cold Spring Harbor Laboratory Press, pp. 162–4. ISBN 0879697067

[3] Chiang H.C., Hodson A.C. 1950.An analytical study of population growth in *Drosophila melanogaster*. Ecological Monographs 20: 173–206. doi:10.2307/1948580

[4] Bakker K. 1961.An analysis of factors which determine success in competition for food among larvae of *Drosophila melanogaster*. Archives Neerlandaises de Zoologie 14: 200–81.

[5] Nazir A., Mukhopadhyay I., Saxena D.K., Kar Choudhuri D. 2001.Chloropyrifos induced hsp70 expression and effects on reproductive performance in transgenic *Drosophila melanogaster* (hsp70-lacZ) Bg9. Arch.Environ.Contam.Toxicol.41443-449.

[6] Nazir A., Saxena D.K., Kar Choudhuri D. 2003. Induction of hsp70 in transgenic *Drosophila:* biomarker of exposure against phthalimide group of chemicals. Biochim. Biophys. Acta 1621: 218-225.

[7] Jones D., Stringham E.G., Babich S.L. Candido E.P.1996.Transgenic strains of the nematode *C. elegans* in biomonitoring and toxicology: effects of captan and related compounds on the stress response. Toxicology. 109 (2-3): 119-27.

[8] Rahden-Staron I. 2002.The inhibitory effect of the fungicides captan and captafol on eukaryotic topoisomerases in vitro and lack of recombinagenic activity in the wing spot test of *Drosophila melanogaster*. Mutat Res. 518(2): 205-13.

[9] Mathew C., Al-Doori Z. 1976.The mutagenic effect of the mercury fungicide Ceresan M in *Drosophila melanogaster*. Mutat Res. 40(1): 31-6.

[10] Perocco P., Colacci A., Del Ciello C., Grilli S. 1995. Transformation of BALB/c 3T3 cells in vitro by the fungicides captan, captafol and folpet. Jpn J Cancer Res. 86(10): 941-7.

[11] Vargová M., Poláková H., Podstavková S., Sisková A., Dolan L. 1980..Evaluation of the mutagenic effect of the new fungicide trimorphamide. Mutat Res. 78(4): 361-7.

[12] Pai S.B. 1983.Evaluation of mutagenic effect of the fungicide fenaminosulf in *Drosophila melanogaster*. Mutat Res. 119(3): 289-91

[13] Hrelia P., Fimognari C., Maffei F., Vigagni F., Mesirca R., Pozzetti L., Paolini M., Cantelli F,G., 1996.The genetic and non-genetic toxicity of the fungicide Vinclozolin. Mutagenesis 11(5): 445-53.

[14] Gayathri M.V., Krishnamurthy N.B. 1985. Investigations on the mutagenicity of two organomercurial pesticides, Ceresan and Agallol 3, in *Drosophila melanogaster*. Environ Res.36(1): 218-29

[15] Festing M.F.H., Baumans V., Combes D.R., Halder M., Hendricsen F.M., Howard B.R. 1998.Reducing the use of laboratory animals in biomedical research: Problems and possible solutions. ATLA. 26: 283-301.

[16] Lunning K,G., 1966.Drosophila test in pharmacology. Nature 209: 84-6.

[17] Devonshire A.L., Field L.M. 1991: Gene amplification and insecticide resistence. Annu. Rev. Entomol. 36: 1-23

[18] Mukhopadhyay I., Siddique H.R., Bajpai V.K., Saxena D.K., Kar Choudhuri D. 2006.Synthetic Pyrethroid Cypermethrin induced damage in reproductive tissue of *Drosophila melanogaster:* hsp70 as a marker of cellular damage Arch.Environ. Contam.Toxico 51:673-680.

[19] Gupta S.C., Siddique H.R., Saxena D.K., Kar Choudhuri D. 2005.Hazardous effect of organophosphate compound, diclorovos in transgenic *Drosophila melanogaster* (hsp 70-lacZ):Induction of hsp70, anti-oxidant enzymes and inhibition of acetylcholinesterase Biochim. Biophys. Acta 1725: 81-92