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Comparative Efficacy of Alphamethrin, on Gene Expression of Wingless Lac Z and Engrailed Lac Z, the Transgenic Forms of *Drosophila melanogaster*

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ABSTRACT

Type II Pyrethroid exhibited its potential against transgenic forms of *Drosophila melanogaster*. The intrastrain crosses and respective observations with regard to genetic expression on the imaginal disc have revealed the potentiality of the experimental compound alphamethrin on both transgenic flies, wingless lac Z and engrailed lac Z . The reduction in wing size in both the forms (wingless lac Z and engrailed lac Z) is also an outcome of engrailed gene which acts as a pleotropic gene governing different functions in different parts of *Drosophila.* The differential expression of gene in the two transgenic forms reflect differential interaction in terms of reduced response of pyrethroid with the concerned gene.

Keywords: Pyrethroid, LC_{50}, Imaginal Disc, Lac Z

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INTRODUCTION

Historians have traced the use of pesticides to the time of around 1000 B.C. but the earliest records of insecticides pertain to the burning of "brimstone" (sulphur) as a fumigant. In the past decade pyrethroids have emerged as a major class of highly active insecticides due to their high bio-efficiency and relatively low toxicity in comparison to organochlorine and organophosphate pesticides. The pyrethroids are the synthetic analogues of the natural pyrethrins obtained from *Chrysanthemum cinerariaefolium*. Pyrethrins are naturally-occurring insecticidal esters of chrysanthemic acid (pyrethrins I) and pyrethric acid (pyrethrins II), originally found in the flowers of *Chrysanthemum cinerariaefolium* (Asteraceae) and *C. coccineum* (Todd *et al*., 2003; Davies *et al.*, 2007)[1,2]. Pyrethrin-I, cinerin-I, and jasmolin-I are esters of chrysanthemic acid whereas pyrethrin-II, cinerin-II, and jasmolin-II are esters of pyrethric acid (Essig & Zhao,2001)[3]. pyrethroids are formulated as emulsifiable concentrates, wettable powders, granules, and concentrates for ultra low volume application, being worldwide used in agriculture, in homes and gardens, and for treatment of ectoparasitic diseases. Type II pyrethroids (cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, fenvalerate, fenpropathrin, flucythrinate, flumethrin, fluvalinate, alphamethrin and tralomethrin) are derivatives of pyrethrins that include a cyano group and may elicit sinuous writhing and salivation (Todd *et al.*, 2003)[1]. Pyrethroids stimulate nerve cells to produce repetitive discharges, causing paralysis (known as insect 'knockdown') and possible insect death (Gunasekara, 2004; Davies *et al*., 2007)[4,2]. Insects using *Drosophila melanogaster*, readily respond to toxins such as phytotoxins, metal ions and insecticides in their environment.

D. melanogaster has been selected for the present investigation because of its clear morphology, easy rearing under the laboratory conditions, with short life cycle, and cosmopolitan distribution. *D. melanogaster* sequenced genome of 139.5 million base pairs has been annotated and contains approximately 15016 genes. More than 60% of the genome appears to be functional non-protein-coding DNA involved in gene expression control. It also has only four pair of chromosomes (X/Y pair of chromosome and three autosomes) and possesses abundance of genetics abundance of genetic variability, which is of the advantage that the effect due to any conventional or xenobiotic substance can be documented well because of its link with a particular gene.

In the present study transgenic *Drosophila melanogaster* were used because these forms contain functional genes which have been experimentally introduced by genetic engineering from another species and these forms also called as genetically modified organisms (GMO_S). Corresponding changes whatsoever under the effect of pyrethroids can easily be assessed.

MATERIALS AND METHOD

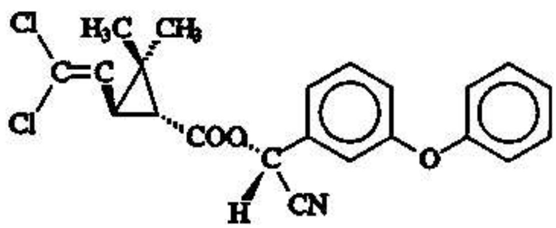
Test Chemical

Alphamethrin, a synthetic pyrethroid, was selected for the present study to investigate the expression of gene in engrailed lac z and wingless lac z mutant of *Drosophila melanogaster* after sublethal exposure to experimental pyrethroid.

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

Molecular weight : 416.3

Structural formula :



(A) (1R, *trans*) (α R)

Determination of LC_{50} of Alphamethrin

For the determination of LC_{50} values of alphamethrin same procedure was followed for engrailed and wingless lac z mutant flies of *Drosophila melanogaster*. The flies were used for experimentation after 5-6 generations after acclimatization. The mortality and survival number of flies at each concentration were recorded after 48 hours and were analysed statistically by log dose/probit regression line method (Finney, 1971)[5].

Experimental Protocol for Staning Imaginal Discs (by Grace Panganiban)

1. Third instar larvae were washed in PBS (phosphate buffer solution) for few minutes and dissected in PBS.
2. The tissues were then fixed in 40% of paraformaldehyde for 20 minutes.
3. The larvae were washed four times to remove fixation
4. X-gal was added to staining solution and incubated tissue in this solution for a few hours.
5. Staining solution was removed and rinsed discs once with PBS and re-suspend them in 50% glycerol/PBS. The tissue was left for a few hours in glycerol solution.
6. The imaginal discs in the cube was mounted under a cover slip and sealed cover slip with nail polish for permanent storage.

RESULTS AND DISCUSSION

Assessment of LC_{50} of Alphamethrin in Transgenic Forms

Regression line was drawn on the basis of two variables, log dose and empirical probit on a graph paper and empirical probit on a graph paper and used to determine the expected probit on a graph paper and used to determine the expected probit necessary for LC_{50} determination. The test doses have been converted to nl (nano litre) and then to their logarithms.

The LC_{50} value of alphamethrin after 48 hrs has been calculated as 0.131µl and 0.057µl/100ml food for wingless and engrailed lac Z transgenic flies respectively. Relative resistance has been calculated as 2.29. One tenth of calculated LC_{50} value i.e. 0.0131µl/100ml food has been selected as sub-lethal concentration for wingless lac z flies respectively.

Assessment of Wing Size in Transgenic Forms (Table-1)

Table 1: Wing size (mm) in transgenic *Drosophila melanogaster* after treatment with alphamethrin

S.No.	Experimental sets	Control Mean±S.Em.	Treated Mean±S.Em.	% Decrease
1.	Wingless Lac Z	5.54±0.0832	5.14±0.0929*	0.18
2.	Engrailed Lac Z	5.52±0.20	4.84±0.16*	12.31

Wingless Lac Z:

The size of wing of *Drosophila melanogaster* has been observed decreased after treatment with alphamethrin, ranges from 4.99 to 5.31 with an average of 5.14±0.0929 as compared to control which ranges from 5.42 to 5.50 with an average of 5.54±0.0832. 0.18 percent reduction in the size of wing compared to control has been observed.

Engrailed Lac Z:

The size of wing of *Drosophila melanogaster* has been observed decreased after treatment, ranges from 4.63 to 4.80 with an average of 4.84±0.16 as compared to control which ranges from 4.91 to 5.22 with an average of 5.52±0.20.12.3 percent reduction in the size of wing compared to control has been observed.

Assessment of Gene Expression of Tranagenic Forms (Figure 1- Figure 4)

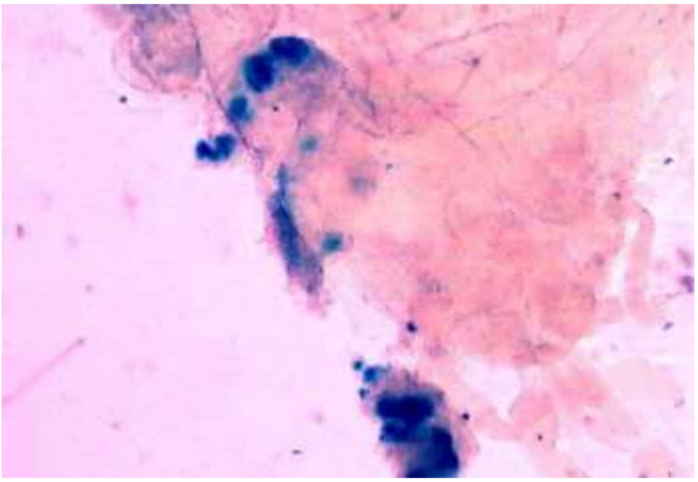


Figure 1: **Imaginal disc of control Wingless lac Z *Drosophila melanogaster***

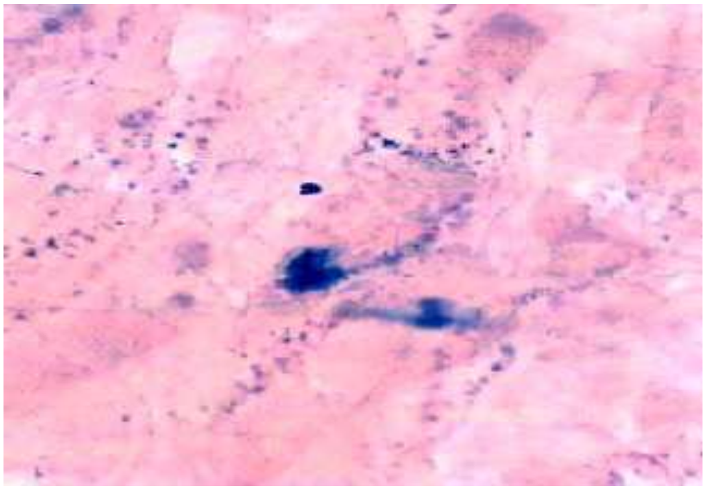


Figure 2: **Imaginal disc of control Engrailed lac Z *Drosophila melanogaster***

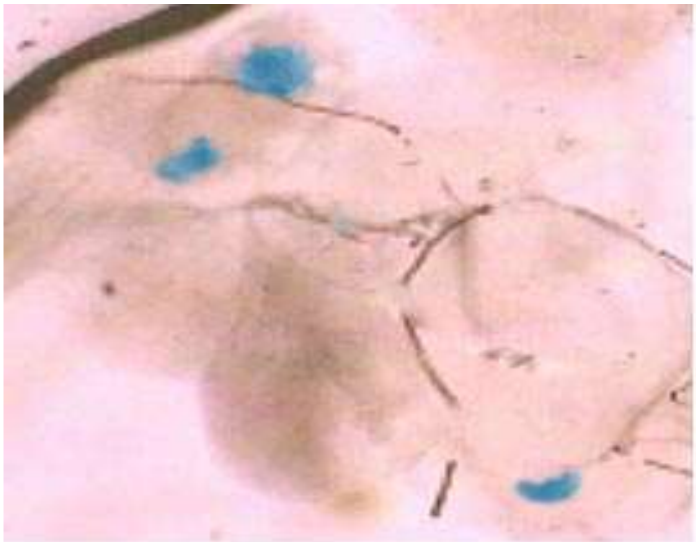


Figure 3: **Imaginal disc of treated Wingless lac Z *Drosophila melanogaster* showing characteristic blue colouration, displayed in stripe pattern in imaginal disc**

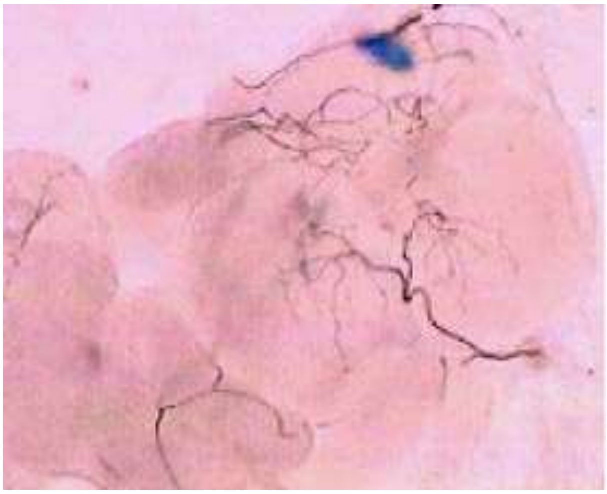


Figure 4: **Imaginal disc of treated Engrailed lac Z *Drosophila melanogaster* showing characteristic blue colouration in the posterior compartment of imaginal disc.**

A characteristics blue colouration, displayed in stripe pattern (wingless lac Z) and in the posterior compartment (engrailed lac Z) of imaginal disc of larvae has been observed.

DISCUSSION

In Transgenic forms of *Drosophila melanogaster* desired set of genes get incorporated which include wingless lac z and engrailed lac z. The transcription and translation of these genes form a protein, which affect their morphology. Further lac z gene acts as a reporter gene.

Decrease in size of wings compared to control in the present investigation has also been observed. Possibly the pyrethroid affected the transcription rate in the imaginal disc, as a result of which undifferentiated group of cells gets differentiated into a specific part of the adult fly. Thus some proteins, which are required for the morphogenesis of complete wing get reduced, which is reflected in terms of reduction in the length of wing under stress of pyrethroid. Earlier, reduction in wing size has been observed under the stress of insecticides by Bao *et. al.* (2009)[6], Bhat and Baba (1987)[7], Gullien *et. al.* (1995)[9] Birdsal *et. al.* (2000)[8], Dhananjaya and Naik (2009)[10] and pandey *et. al.*(2011)[11],

Expression of gene has been studied on the basis of imaginal disc through enhancers that show how genes are regulated in time and space within multicellular organisms and use enhancers to 'tag' tissues or even individual cells. Transcriptional enhancers are DNA sequences that control expression of nearby genes and direct tissue- specific, positive regulation of the genes. Under the influence of one or more enhancers, a gene can be abundantly expressed in certain tissues of the organisms (i.e. cells in which the activator protein is found) and weakly or unexpressed in other tissues.

Lac z gene fusion are widely employed as reporters to measure transcriptional and translational regulation. For the present study it is advantageous of using lac z reporter system because it provides a sensitive means of detecting and roughly quantitative reporter gene expression in whole larval tissue preparation. Differences in β- galactosidase activity in different regions of imaginal disc, reflects the rate of synthesis of this reporter proteins in this study. Artificial galactosidase substrate x-gal (5-bromo-4-chloro indonyl-D-galactosidase), when hydrolysed by the enzyme, produced their expression in form of vivid dye colouration which has been well observed in the present investigation.

Depending upon the promoter regulating the lac z reporter gene, a characterstic blue colouration was seen in the posterior compartment o imaginal disc of larval tissue that indicates the presence of reporter expression pattern in this compartment, which is characterized by the activity of β-galactosidase in this compartment. Similarly, a characteristic blue colouration was displayed in a stripe pattern in imaginal disc of larval tissue, which once again strengthen the presence of reporter expression pattern in this compartment, on the basis of β- galactosidase activity (*vide supra*).

Decrease transcription rate which affect the RNA polymerase recognizing site on promoter, by modifying their, base sequences thus causing difficulty in recognition by RNA polymerase, and hence decrease in transcription i.e. the rate of transcription (enzymatic protein synthesis) i.e. Galactosidase.

The findings in the present investigation are in affirmation to Phillips and Whittle (1993)[13] who showed wingless expression mediating determination of peripheral nervous system element in late stage of *Drosophila* wing disc development Further, Bryant (1975)[12] also reported the pattern formation in the imaginal disc of *Drosophila melanogaster* besides its fate map, regeneration and duplication.

CONCLUSION

Thus it is concluded that the overall gene expression of the *Drsophila melanoagaster* of both transegenic flies get reduced by alphamethrin which affect on some gene or by repressing those genes which cause the reduction in wing size in both the forms (wingless lac z and engrailed lac z). Alphamethrin exhibit more effect on wingless lac z compared to engrailed lac z , on expression of gene as well as on size of wing. The differential expression of gene in the two transgenic forms reflect differential interaction in terms of reduced response of pyrethroid with the concerned gene.

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