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Malathion exposure alters the level of major soluble proteins in the larvae of *Aedes aegypti*

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Abstract

Mosquito born diseases like dengue fever, Chikungunya, malaria, yellow fever, Zika, etc are posing an enormous risk to human population world wide. Various strategies are being employed to control the mosquito including the use of synthetic insecticides. Continuous application of synthetic insecticides results in development of resistance by mosquitoes and environmental health hazards. When the fourth instar larvae of *Aedes aegypti* were treated with the LC₁₀ concentration of malathion, there was an increase in the protein concentration after 24 hours. On SDS-PAGE there was an increase in the intensity of major protein bands (74 kDa, 78 kDa, and 34 kDa) compared to the control. The major protein the last instar larvae are storage protein like hexamerins and range in molecular weight from 66-85 kDa. The study of alteration in storage protein on exposure to malathion will be helpful in understanding their role in the toxicity and resistance to the insecticide.

Keywords: *Aedes aegypti*, malathion, storage proteins

1. Introduction

Mosquitoes are vectors of many diseases including malaria, filariasis, dengue fever, yellow fever etc. The presence of *Aedes* mosquito is reported in areas where more than half of the world's population is inhabited. Among the vector-borne diseases, Zika, Dengue, Chikungunya, and yellow fever are the major disease spread by *Aedes* mosquito. In the beginning, the major *Aedes* vectors namely *Aedes aegypti* and *Aedes albopictus* was found only in Africa and Asia respectively, but today they are distributed worldwide. As a result, the global incidence of dengue has increased 30-fold over the past 30 years [1]. Due to globalization, the spread of *Aedes aegypti* and *Aedes albopictus* has occurred across Africa, the Middle East and Europe, which continues even today [2]. A hike in competition for food between larvae of *Aedes* species was observed due to the increased invasion of *Aedes albopictus* mosquito [3, 4]. *Aedes* mosquitoes are popular in crowded areas where there is no proper water management and sewage treatment. Global warming due to increase in green house gases is estimated to range between 1 °C and 3.5 °C by 2100 AD [5]. Temperature, humidity, precipitation, wind and duration of day light all contribute towards mosquito ecology, survival, behavior and disease transmission rates [2]. Warmer water temperature may speed up the maturation of mosquito larvae leading to shorter life cycle [6]. Thus there is an increasing threat posed by mosquitoes negatively impacting on human health and economy. More than 3.9 billion people in over 129 countries are at risk from dengue, with an estimated 96 million symptomatic cases and an estimated 40,000 deaths every year. In recent decades, the global incidence of dengue has grown dramatically and in each year, there are 100-400 million infections estimated [7].

As effective vaccines are not available for arboviral diseases like dengue fever, preventing the spread by mosquitoes is the only way to reduce the incidence of the disease. Thus mosquito control is of paramount importance to reduce disease burden. There are many methods available for the control of mosquitoes including the use of insecticides, insect growth regulators botanicals, etc. Toxicity of these agents on non-target organisms may hamper the normal fauna also. Also the development of resistance by mosquito is hindering the success of the mosquito control using conventional insecticides.

Among the conventional insecticides, organophosphate insecticides like malathion are widely used to control agricultural pests and mosquitoes [8]. The toxicity of malathion is due to its metabolic product malaxon which inhibits acetylcholinesterase leading to hyperexcitability, restlessness, convulsions, paralysis and death [9]. Due to long-term use of conventional pesticides, mosquitoes tend to develop resistance against these pesticides. For instance, *Culex quinquefasciatus* resistant to malathion was reported from Cuba after continuous application for seven years [10] and *Ae. aegypti* with resistance to malathion was also reported which showed cross resistance to pyrethroids, mainly deltamethrin [11]. Insecticide susceptibility to malathion of both *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) larvae was determined in Thailand from July 2003 to April 2004 and found that *Aedes aegypti* (L.) was susceptible to malathion and had low levels of resistance to malathion with a Resistance ratio (RR) 95 ranging from 2.2 to 6.6 [12]. The development of resistance against pesticides in mosquitoes, especially in *Culex quinquefasciatus* may be due to overproduction of esterase B, synthesis of insensitive acetyl cholinesterase, [9] or due to increase in cytochrome P450 [13]. Examining the protein profile changes accompanying the exposure to insecticides will be useful in assessing qualitative and quantitative changes in proteins which in turn may help identify proteins associated with exposure to insecticides. Changes in proteins on treatment with insecticide in mosquitoes larvae is not explored to a great extent. Storage protein represents a major protein in the larvae of mosquito. Identifying and examining the role of such proteins will be helpful in designing better strategies for the control of mosquitoes. In this study we examined the protein profile changes accompanying the treatment of *Aedes aegypti* mosquito larvae with malathion.

2. Materials and Methods

2.1 Malathion

JKP Killers 50% EC was diluted to get the desired concentration.

2.2 Culturing of mosquito larvae

Eggs of *Ae. aegypti*, obtained from Bio-control Research Laboratory of Pest Control India Ltd, Bengaluru were hatched and reared in the laboratory. Larvae were fed with yeast granules. Third and fourth instar larvae were used for experiments.

2.3 Determination of LC₅₀

Third or fourth instar larvae were treated with different concentrations of malathion as per the WHO protocol. A

control was also kept without malathion. Larvae were fed with yeast granules and mortality was recorded after 24 hours and LC₅₀ was calculated from a plot of log concentration of malathion verses percentage mortality.

2.4 Treatment of larvae with sub-lethal concentration of Malathion and estimation of protein concentration and separation of proteins by SDS-PAGE

Eighty numbers of fourth instar larvae were exposed to LC₁₀ (0.03µg/ml) of malathion for 24 hours. A control without malathion was also done. Equal weight of larvae from test and control were collected after 24 hrs and stored at -20 °C. Both control and test larvae were homogenized in bicarbonate buffer (pH 9.0) and the homogenates were centrifuged at 9390xg at 4 °C for 10 minutes. The supernatant was divided in to two equal halves and one half used for protein estimation by modified Lowry's method [14] and other half was treated with TCA to precipitate protein and washed with acetone to remove lipids. Same quantity of protein from test and control of the lipid free fraction was used to load on to SDS-PAGE [15].

2.5 Statistical analysis

Statistical analysis was done using R program.

2.6 Determination of molecular weight

After SDS-PAGE the gels were photographed in a Gel Documentation system (Biorad). Molecular weights of the protein bands were calculated using the mobility of proteins from a plot of log molecular weight versus relative mobility of the standards.

3. Results and Discussion

3.1 Toxicity of Malathion to *Aedes aegypti* mosquito larvae

The third instar larvae when exposed to different concentrations of malathion, the highest death rate was observed for 0.18µg/ml (70%) followed by 0.12µg/ml (60%), 0.06µg/ml (50%), 0.03µg/ml (30%) (Table1). As the concentration increased, percentage of mortality also increased, indicating that the effect is concentration dependent. LC₅₀ value obtained was 0.06µg/ml.

The fourth instar larvae were also exposed to different concentrations of malathion. The highest death rate was observed for 0.18µg/ml (50%) followed by 0.12µg/ml (25%), 0.06µg/ml (15%), 0.03µg/ml (10%) (Table1). Here also it is observed that the concentration is directly proportional to the percentage of mortality. As the concentration increased the percentage of mortality also increased indicating that the effect is concentration dependent. The LC₅₀ value was found to be 0.18µg/ml.

Table 1: Effect of Malathion on mortality of 3rd and 4th instar *Ae. aegypti* larvae.

Sl. No.	Concentration of Malathion (µg/ml)	Percentage Mortality ± SE	
		3 rd instar larvae	4 th instar larvae
1	0.00	0.00	0.00
2	0.03	20±10	10±0
3	0.06	50±0	17.5±2.5
4	0.12	60 ±0	27.5±2.5
5	0.18	70 ±0	50±0

When *Culex pipiens* L. fourth instar larvae were treated with malathion, the LC₅₀ value was found to be 0.0027-0.0043 ppm [16]. In *Aedes aegypti* LC₅₀ value for different strains

varies between 1.18 and 3.49 µg/bottle in 2008 and it was raised to 2.31 and 3.07 µg/bottle respectively in 2010 [17]. In a study conducted in Brazil during 2017 to 2018, malathion

application at the local DD 20 µg/bottle resulted in 73 (55.3%) populations throughout the country were resistant. At the same time no population was resistant, and only 10 (7.6%) populations in eight states exhibited decreased susceptibility (mortality ratios between 90 and 98%) on exposure to the WHO DD (50 µg/bottle). Thus gradual development of resistance on prolonged use of insecticides is becoming a great threat to mosquito control programs.

3.2 Effect of Malathion on protein profile of 4th instar larvae of *Ae. aegypti*

When the fourth instar larvae were exposed to LC₁₀ (0.03µg/ml) of malathion, there was a significant increase in concentration of soluble proteins compared to control (Table 2). In the case of membrane protein, on exposure to LC₁₀ concentration of malathion, there was no significant change in protein concentration compared to control (data not shown). An increase in haemolymph protein concentration was observed by Resmitha and Vadakkadath Meethal when *Spodoptera mauritia* larvae are exposed to the insect growth regulator, pyriproxifen or methoxyfenozide [18, 19].

Table 2: Effect of malathion on soluble protein concentration of 4th instar larvae of *Ae. aegypti*

Treatment	Protein concentration (µg/mg tissue) ±SE	p value
Control	0.269 ±0.01	0.04
Test	0.393 ± 0.04	

3.3 Qualitative changes in protein profile on exposure to malation

On SDS-PAGE of soluble proteins, there was an increase in the band intensity of some of the protein bands in the malathion treated compared to control. This is in agreement with increase in protein concentration observed in the treated group. The major protein bands which increased in intensity on treatment with an LC₁₀ concentration of malathion include 78 kDa, 74 kDa and 34 kDa (figure 1). The storage proteins are abundant in haemolymph and the major hexamerins are in the molecular weight range of 66-85 kDa in the larvae of many mosquito species [20]. In this study also there is an increase in intensity of 74 kDa and 78 kDa protein band on treatment with malation in *Ae. aegypti* larvae, probably representing the storage proteins. In *Spodoptera mauritia* upon treatment with methoxyfenozide, an ecdysone mimic, or pyriproxifen, a juvenile hormone mimic, a significant increase in proteins, especially the major protein band of 83 kDa, in the haemolymph was observed [17, 21]. The major protein in the haemolymph of *Spodoptera mauritia* is storage protein, hexamerins. The increase in protein band intensity may due to either an increase in the synthesis of the protein or a decrease in its degradation. The insecticide-responsive proteins may have role in the toxicity or resistance to the insecticide. The identification of insecticide-responsive proteins will be helpful designing strategies for the mosquito control.

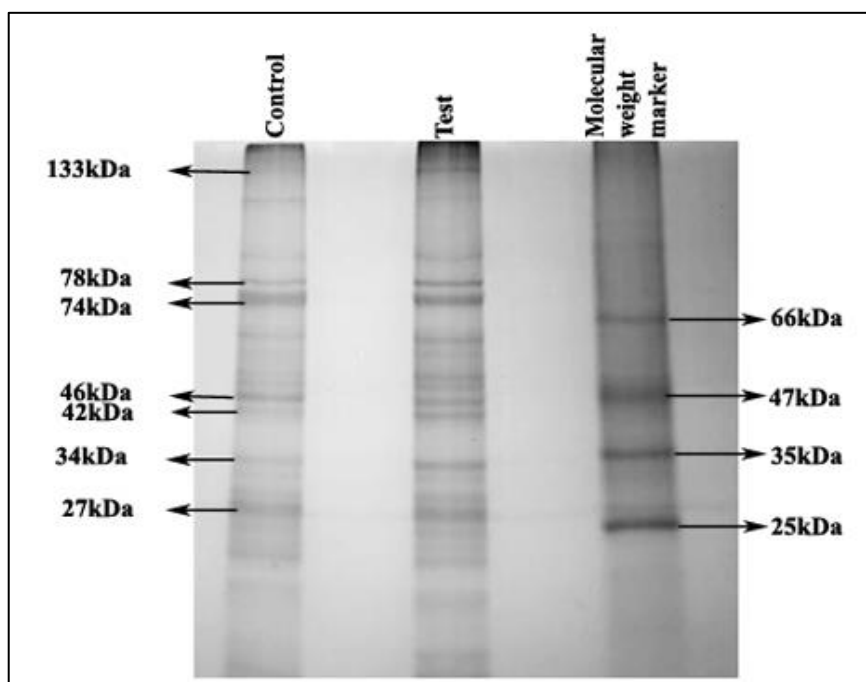


Fig 1: 10% SDS-PAGE of soluble protein of *Aedes aegypti* larvae exposed to LC₁₀ concentration of malathion

4. Conclusions

In the present study we calculated the 24 hr LC₅₀ for malation for *Ae. aegypti* larvae and found to be 0.06µg/ml and 0.18µg/ml for third and fourth instar larvae respectively. On exposure to LC₁₀ concentration of malathion (0.03 µg/ml), there was a significant increase in soluble protein concentration in fourth instar larvae compared to control. Concomitant with this there is an increase in intensity of the some of the major protein bands like 74 kDa and 78 kDa on SDS-PAGE and these abundant proteins may be storage

proteins like hexamerins which range in molecular weight from 66-85kDa in mosquitoes. Identification of these insecticide responsive proteins will be helpful in understanding their role in toxicity or resistance to the insecticide.

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6. References

1. <https://www.who.int/vector-control>
2. Reiter P. Climate Change and Mosquito-Borne Disease. *Environmental Health Perspectives* 2001;109:1:141-161.
3. Benedict MQ, Levine RS, Hawley WA, Lounibos LP. Spread of the tiger: Global risk of invasion by the mosquito *Aedes albopictus*. *Vector-borne and Zoonotic Diseases* 2007;7(1):76-85.
4. Eskildsen GA, Rovira JR, Smith O, Miller MJ, Bennett KL, McMillan WO *et al.* Maternal invasion history of *Aedes aegypti* and *Aedes albopictus* into the Isthmus of Panama: Implications for the control of emergent viral disease agents. *Plos One* 2018;13(3):e0194874. <https://doi.org/10.1371/journal.pone.0194874>.
5. Intergovernmental panel on climate change (IPCC), WMO/UNEP. A Second Assessment Report of the Intergovernmental panel on climate change 1995, 1-73.
6. Leeson HS. Longevity of *Anopheles maculipennis* race atroparvus, Van Theil, at controlled temperature and humidity after one blood meal. *Bulletin of Entomological Research* 1939;30:103-301.
7. <https://www.who.int/news-room/factsheets/detail/dengue-and-severe-dengue>
8. Gervais JA, Luukinen B, Buhl K, Stone D. Malathion Technical Fact Sheet; National Pesticide Information Center, Oregon State University Extension Services 2009. <http://npic.orst.edu/factsheets/archive/malatech.htm>
9. Cox C, Sumithrin. *Journal of Pesticide Reform* 2003;23:2. <http://www.pesticide.org/get-the-facts/pesticide-factsheets/factsheets/sumithrin>
10. Bisset JA, Rodriguez MM, Diaz C, Ortiz E, Marquetti MC, Hemingway J. The mechanisms of organophosphate and carbamate resistance in *Culex quinquefasciatus* from Cuba. *Bulletin of Entomological Research* 1990;80:245-250.
11. Rodríguez MM, Bisset JA, Díaz C, Soca LA. Cross resistance to pyrethroids in *Aedes aegypti* from Cuba induced by the selection with organophosphate malathion. *Revista Cubana de Medicina Tropical* 2003;55(2):105-11.
12. Ponlawat A, Scott JG, Harrington LC. Insecticide Susceptibility of *Aedes aegypti* and *Aedes albopictus* across Thailand. *Journal of Medical Entomology* 2005;42(5):821D825.
13. Hemingway J, Hawkes NJ, McCarroll L, Ranson H. The molecular basis of insecticide resistance in mosquitoes. *Insect Biochemistry and Molecular Biology* 2004;34(7):653-65.
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. *J Biol. Chem* 1951;193:265-275.
15. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. 1970;227(5259):680-685.
16. Rawash IA, Gaaboub IA, El-Gayar EM, El-Shazli AY. Standard curves for nuvacron, malathion, sevin, DDT and kelthane tested against the mosquito *Culex pipiens* L. and the microcrustacean *Daphnia magna* Straus. *Toxicology* 1975;4(2):133-44.
17. Alvarez LC, Ponce G, Oviedo M, Lopez B, Flores AE. Resistance to malathion and deltamethrin in *Aedes aegypti* (Diptera: Culicidae) from western Venezuela. *Journal of Medical Entomology* 2013;50(5):1031-9. doi: 10.1603/me12254. PMID: 24180108.
18. Resmitha C, Reshma RM, Punnathuparambath B, Meethal KV. The ecdysone mimic, methoxyfenozide, alters the level of major haemolymph proteins in the larvae of *Spodoptera mauritia* Bois. (Lepidoptera: Noctuidae). *Acta Biologica Indica* 2014;3(2):726-730.
19. Resmitha, Meethal KV. The juvenile hormone mimic, pyriproxyfen, increases the level of the major haemolymph protein in the larvae of *Spodoptera mauritia* Bois. Lepidoptera: Noctuidae. *International Journal of Entomology Research* 2017;2:2455-4758.
20. Korochkina SE, Gordadze AV, York JL, Benes H. Mosquito hexamerins: characterization during larval development. *Insect Molecular Biology* 1997;6(1):11-21. <https://doi.org/10.1046/j.1365-2583.1997.00150.x>
21. Vincent L, Prakash S, Reshma RM, Resmitha C, Meethal KV. Storage protein in the haemolymph of 6th instar larvae of *Spodoptera mauritia* Bois. (Lepidoptera: Noctuidae) is increased by the ecdysone mimic, methoxyfenozide. *International Journal of Entomology Research* 2018;3:01-04.