STUDY ON ISOZYMES FROM AEDES AEGYPTI STRAINS OF SOME *RESIDENTIAL AREAS* IN VIETNAM

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Abstract. Isozymes from 4 wild- caught collections of Aedes aegypti mosquito were compared by using polyacrylamide gel electrophoresis. These collections were collected from 4 residential areas of Vietnam (North, South, Centre and Central highland area). This study revealed that there is an obvious relation between the insecticide resistance and the expression of isozyme esterase in the mosquito. Some differences of isozymes between larvae and adults were also found.

Key words: Dengue fever, insecticide resistance, strain, *Aedes aegypti*, PAGE, isozyme.

1. Introduction

Dengue/ Dengue hemorrhagic fever (DF/DHF) occurs through out the year but particularly in summer time (usually from June to July). It easily becomes epidemic disease and tends to increase in Southern region and Central highland of Vietnam recently. One reason for this situation is the insecticide resistance in dengue virus vectors in which *Aedes aegypti* is an important one. The mosquito has resisted to some insecticides such as DDT, hexachloride benzen (organochlorine group) and even pyrethroid group which has been considered as a powerful group is now losing its effect in some areas of Southern region and Central highland of Vietnam [1, 2, 7].

There were some domestic and foreign researches [1, 2, 4, 7] studying on the expression of isozyme complex in different *Aedes aegypti* strains in order to explain the resistance ability in mosquito. However, some opinions are controversial.

The objective of this research is to find out the isozyme marker for insecticide resistance by means of study on the electrophoretic expression of several isozymes: Esterase (EST), Octanol dehydrogenase (ODH), Malate dehydrogenase (MDH), Acid phosphatase (ACP), Alkaline phosphatase (ALP). From this study, people can have a more appropriate measure to choose insecticides for each residential area.

Therefore, we have done these works:

- Analyzing the electrophoretic results on polyacrylamide gel of 5 isozymes from different geographical *Aedes aegypti* strains in Vietnam.

- Comparing the electrophoretic expression of esterase from larvae and adults by technique of polyacrylamide gel electrophoresis (PAGE) of isozymes therefore we can find out the best isozyme marker to insecticide resistance of mosquitoes.

2. Materials and methods

Both larvae and adults of *Aedes aegypti* strains are caught in these following different residential areas of Vietnam:

- Northern region: Thinh Liet village - Thanh Tri district (TT), Hanoi; Ly Thai To precinct, Hoan Kiem district (HK), Hanoi.

- Central region: Song Cau (SC) town, Phu Yen province; Tuy Hoa town (TH), Phu Yen; Dong Ha town (DH), Quang Tri province; Trieu Do village - Trieu Phong district (TP), Quang Tri province.

- Southern region: Ben Tre town (BT), Ben Tre province; Dong Xoai town (DX), Binh Phuoc province; Binh Chanh village- Can Gio district (CG), HCM city ; Binh Tay precinct – II district, Thu Duc (TD), HCM.

- Central highland: Thang Loi precinct - Buon Ma Thuot city (BMT), Dac Lac province; Plei Can town - Ngoc Hoi district (NH), Kontum province; Kon Tum town (KT), Kon Tum; Buon Txay town- Krongana district (Kro), Dac Lac province.

The mosquito is isolated by method developed by the Institute of Malaria, Parasitology and Entomology of Hanoi then raised for new larvae and adults (F1) and for F3 descendents, which then can be used in experiments. Five isozymes chosen for analysis on polyacrylamide gel are Esterase (EST), Octanol dehydrogenase (ODH), Malate dehydrogenase (MDH), Acid phosphatase (ACP), Alkaline phosphatase (ALP).

Mosquito samples (adults of F1 or F3, larvae) are crushed with buffer in ice into homogenized state. They are then coolly centrifuged. The supernatant is collected for using in electrophoresis.

Main chemical reagents used in each specific enzyme detection are:

- Esterase (EST): α - naphtyl acetate and β - naphtyl acetate as substrates, Fast Blue RR as color- producer.

- Octanol dehydrogenase (ODH): octanol as substrate, coenzyme NAD^+, PMS, NBT (MTT can be used instead of NBT).

- Malate dehydrogenase (MDH): L-malate as substrate, coenzyme $NADP^+$ (or coenzyme NAD^+), PMS and NBT (or MTT).

- Acid phosphatase (ACP): α - naphtyl monophosphate as substrate, Fast Blue RR.

- Alkaline phosphatase (ALP): α - naphtyl monophosphate as substrate, Fast Blue RR.

The polyacrylamide gel is a discontinuous gel which have concentration of 4% and 8% or 4% and 10%, size of 10cm x 8cm x 1.5 mm. The device served for electrophoresis is Hoefer apparatus (Sanfrancisco, USA) or miniProtean apparatus (BioRad). The chemicals were bought from SIGMA Company.

3. Results and discussion

3.1. Electrophoretic properties of esterase

Esterase's electrophoretic result of mosquito in some areas of Central region and Central highland is shown in Fig.1.

In this picture, Kro, KT, NH, BMT samples are *Aedes aegypti* from Central highland, TP, ĐH, TH, SC, from Central region. Although the same amounts of samples were loaded in each well of microtiter-plate, we find out the esterase expressions that are different. It is shown that the activity of esterase from mosquito in Central highland is stronger than that from mosquito in Central region. This result corresponds to direct bioassay results of insecticide on mosquitoes which indicate that the adult mosquito in Central highland has much higher resistance ratio to pyrethroid group than which in Central region (Vu Duc Huong et al. 2003, data not published). Additionally, the fastest esterase bands (EST- 1) in these two groups are different markedly: there are at least 3 bands of isozyme esterase EST-1 from adult mosquitoes in Central highland (BMT, NH, KT) while there are one or two EST-1 from adult mosquitoes in Central region.

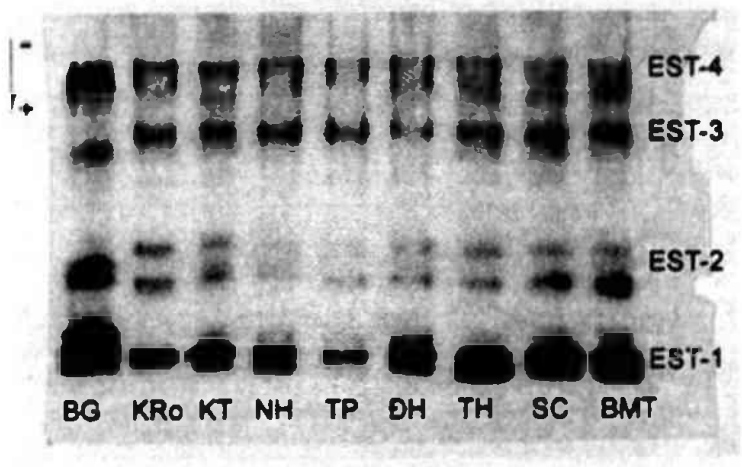

Fig.1: Esterase detection of adult mosquito in Central region and Central highland, in comparison with larva (BG) (on PAG).

In this gel, we also analyzed a larval sample to compare. As can be seen from the picture, all esterase bands of larva are much darker than those of adult mosquito. Fig.2 also makes it clearer.

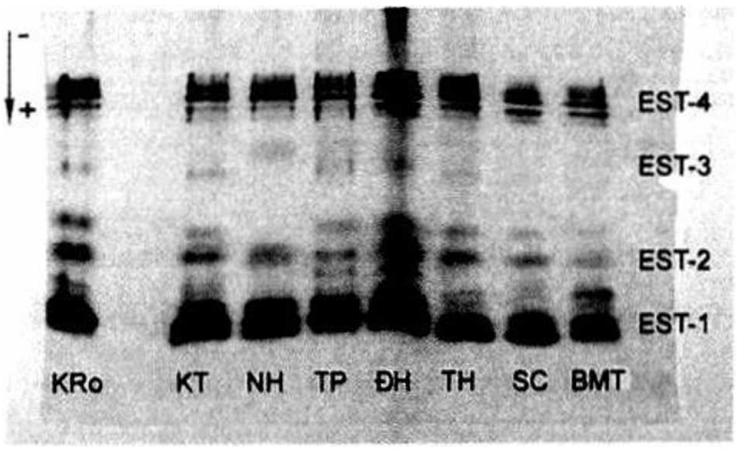

Fig 2: Esterase detection of larva in Central region and Central highland (on PAG).

This picture is the esterase comparison between larvae of some areas in Central region and in Central highland.

On the side of regional comparison, the difference between the larval EST of these two groups is not as clear as between the adult EST discussed above. Therefore, it can be assumed that EST especially EST- 1 from adult mosquito in Central highland has a close relation with the pyrethroid resistance. The relation between insecticide resistance and isozyme expression in larva may need further research.

On the side of developmental period comparison, as we discussed above, the number of esterase bands and their expression are much higher from larva than from adult mosquito. It may be due to the fact that esterases play a more important role in protecting larva in comparison with adults because larvae always lived in aquatic environment where there are more toxics and insecticide residue than in condition where adult mosquitoes lived.

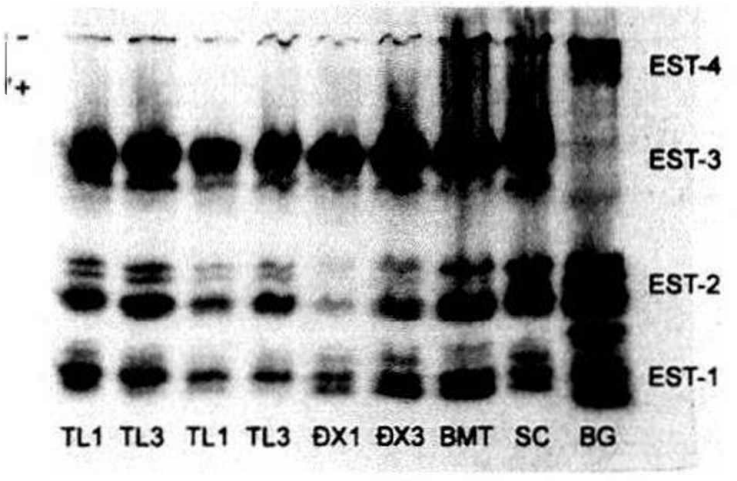

Fig 3: Esterase detection of adult mosquito in Northern, Southern, Central region and Central highland, in comparison with larva (BG) (on PAG).

After analyzing esterase from Thinh Liet (TL- one strain of Northern region), Dong Xoai (DX- Southern region) mosquito of F1 and F3 generation, in comparison with mosquito of Central region (SC) and Central highland (BMT) (Fig.3), we can have some following comments:

- EST- 1 of Central highland mosquitoes are more active (esterase bands expressions are darker) than those of Northern and Southern region samples.

EST-2, 3 of F3 are always more active than F1 (due to bands' color).

When continuously analyzing esterase from larvae in Northern and Southern region mosquito (Fig.4), we again confirm that esterase activity of larva is much higher than that of adult mosquito. We also see that while EST- 4, 2, 1 activity from larvae is stronger than from adults (their bands are heavier), its EST- 3 activity from larvae is markedly weaker than from adult mosquitoes.

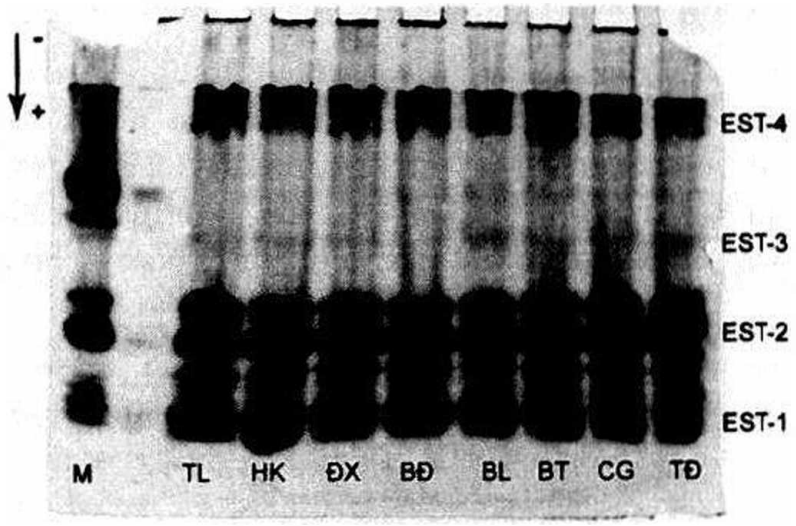

Fig.4: Esterase detection of larvae mosquito in Northern, Southern region, in comparison with adults (M) (on PAG).

After all, it can be thought that isozyme esterase is a good marker for insecticide resistance of the mosquitoes (especially EST- 1) (*and furthermore, for research on two developmental periods of mosquito, esterase marker is also useful (EST- 3, 4)*).

3.2. Electrophoretic properties of Octanol dehydrogenase and Malate dehydrogenase

Isozyme octanol dehydrogenase of *Aedes aegypti* strains in some area of Northern, Southern, Central region and Central highland has been detected but no significant difference was found.

Isozyme malate dehydrogenase of *Aedes aegypti* strains in some area of Central region and Central highland was also detected and the result obtained is

similar to which Saul S.H et al. found in *Aedes triseratus* and *Ae. hendersoni* [8] and no difference can be seen between resistant and sensitive strains.

3.3. Electrophoretic properties of Acid phosphatase and Alkaline phosphatase

No difference in alkaline phosphatase, acid phosphatase between *Aedes aegypti* strains of some areas in Central region and Central highland was found. Thing that can be noted here is that ACP- 2 is more stable than ACP- 1 in separating gel of pH around 8.0, on contrary to in separating gel of pH around 9.0. Besides, no difference in these two enzymes is found between head and thorax part and abdomen part of the mosquito.

4. Conclusion

Base on the analysis of 5 isozymes from *Aedes aegypti* strains in different residential regions of Vietnam, it is deduced that only esterase (especially EST- 1) can be served as a good marker for insecticide resistance of *Aedes aegypti* mosquito. It is assumable due to the capacity of intensive esterase activity of mosquitoes to insecticide degradation, because of the fact that most insecticide has ester linkage [2, 7]. However, other isozyme analysis are also useful in explaining the insecticide resistance of mosquito.

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NGHIÊN CỨU IZOZYM CỦA MỘT SỐ CHỦNG MUỖI AEDES AEGYPTI TẠI MỘT SỐ VÙNG DÂN CƯ CỦA VIỆT NAM

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Sốt Dengue/ hay sốt xuất huyết Dengue là một bệnh xẩy ra quanh năm ở các nước nhiệt đới và á nhiệt đới như Việt Nam và virut gây ra bệnh này được truyền chủ yếu qua muỗi *Aedes aegypti*. Cách tốt nhất để phòng bệnh sốt Dengue là khống chế vectơ truyền bệnh này. Tuy nhiên, việc sử dụng các thuốc diệt côn trùng trong nhiều năm đã dẫn đến sự kháng thuốc ở muỗi. Mục tiêu của nghiên cứu này là phân tích sự tồn tại của các izozym liên quan đến tính kháng thuốc ở các chủng *Aedes aegypti* ở một số vùng dân cư của Việt Nam (miền Bắc, miền Nam, miền Trung và Tây Nguyên).

Các kết quả thu được cho thấy rằng các băng esteraza chạy nhanh nhất (EST-1) có sự khác biệt giữa chủng nhạy thuốc với chủng kháng thuốc trong khi các băng của những izozym khác (octanol dehydrogenaza, malat dehydrogenaza, phosphataza kiềm, phosphataza axit) không thấy có sự khác biệt. Bởi vậy, izozym esteraza có thể được coi là một chỉ thị rõ ràng cho tính kháng thuốc ở muỗi *Aedes aegypti*.