Biochemical and Molecular Resistance Mechanisms to DDT and Some Pyrethroid Insecticides in vector of West Nile virus, *Culex pipiens*

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Abstract

Culex pipiens **complex act as an important vectors of several vector-borne diseases such as filariasis, West Nile virus, Japanese encephalitis and bird malaria. This study was designed in order to clarify the molecular and biochemical resistance mechanisms in Cx. pipiens to DDT and some pyrethroid insecticides from Tehran, capital of Iran.Profile activities of α- and ß-esterases, Mixed Function Oxidase (MFO), Glutathione-S Transferase (GST), were tested for Cx .pipiens strain with resistance ratio of 85.75 to Lambdacyhalothrin and also about DDT resistant strain in comparison with Lab strain. In the present research a molecular study also performed on both lambdacyhalothrin and DDT. Resistant strains for detection of the mutation in the sodium channel gene which is associated with kdr insecticide resistance to pyrethroid insecticides and DDT. Finding showed that there are significant different ($p<0.05$) between the mean activity of α- , ß-esterases and (MFO), in both lambdacyhalothrin and DDT resistant strain in comparison with Lab-Strain, but there are no significant difference ($p>0.05$) about (GST) in the both strain in comparison with Lab-Strain. Molecular study for detection of L1014F or L1014S mutation in sodium channel gene showed lack of the mutation responsible for insecticide resistance to pyrethroid and DDT. This study showed that the resistance to insecticides in the Cx pipiens is enzymatic, but not targets site insensitivity of sodium channel gene are responsible for pyrethroid and DDT resistance. Findings of this research could provide a clue for logical operations of future chemical control program**

Introduction

Culex pipiens complex is a worldwide species and among its members, *Cx. pipiens pipiens* and *Cx. quinquefasciatus* are two more important vectors of several vector-borne diseases regarding to different group of pathogens which causing filariasis, West Nile virus, Japanese encephalitis and bird malaria [1- 4] . Culex Genus habitat 5mainly is sewage system of cities and due to this life style resistance to the most group of insecticides in this species it is not excluded [5- 8] . Based on finding of many studies around the world as evaluating resistance status of *Cx.pipiens* to insecticides, result have been indicated that this species has multiple insecticide resistances or at least resistance to one group of insecticides , moreover during recent years resistance to pyrethroids insecticides and DDT in *Cx.pipiens* complex is spreading around the world [9-11].According to previous studies in Tehran related to evaluation of susceptibility and Irritability level to insecticides which performed on *Cx. pipiens* complex ,indicated that resistance ratio in *Cx. pipiens* to different groups of insecticides increased and also it can be stated that about DDT this species is quite resistant . In the most species of mosquitoes, resistance to different groups of insecticides caused by two main mechanisms which known as metabolic resistance due to enzymatic detoxification by enzymes involved in resistance including cytochrome P450 oxidases, esterases and Glutathione-S-Transferases(GST) and also it seems that resistance to pyrethroid compounds caused by increasing level of oxidases and second reason is target site insensitivity due to substitution in nucleotides which about pyrethroid compounds and DDT. Resistance cases this mutation lead to translating leucine to phenylalanine or leucine to Serine in the S6 hydrophobic segment of domain II in the sodium channel gene that known as Knock down resistance (kdr)mutation . In some species from Culex genus the same kdr mutation is present at position 1014 which known as L1014F(TTA to TTT) orL1014S (TTA To TCA) in pyrethroid-resistant cases .The present study was performed for clarifying the molecular and biochemical resistance mechanisms in *Cx. pipiens* to DDT and some pyrethroid insecticides.

Materials and Methods

This study was performed in Tehran city (35° 41′ 46″ N, 51° 25′ 23″ E), Tehran Province, the capital of Iran

Mosquito strains

The wild larval stage strains of *Cx. pipiens* collected from collection site in the study area and for more investigation all wild -caught larval were reared in the insectary of School of Public Health, Tehran University of Medical Sciences under the standard condition. Moreover as comparison a laboratory strain of *Cx. pipiens* were used.

Insecticides

In the following for biochemical and molecular assays in *Cx. pipiens* In the current study four Insecticides including: DDT 4 %, Lambdacyhalothrin 0.05 %, Deltamethrin 0.05 %, Cyfluthrin 0.15 % were used base on World Health Organization(WHO) standard method for evaluating susceptibility status of *Cx. pipiens* [19].

The process for selection of insecticides-resistant population

Criteria for selection of insecticides-resistant population is the highest resistance ratio (RR) about pyrethroids insecticides tested. Selection process carried out on adult by exposing with a time that lead to about 50-70% mortality. in this study due to highest resistance ratio about Lambdacyhalothrin, for attaining a homogenous resistant population, 3 generation after the initial population with more than 80-fold .More over because of wild strain with 1 hours exposure to DDT and during a period of 24 hours after recovery yielded no mortality, both of these population selected for biochemical and molecular studies about resistance.

Results

Selection process

In the present study after detection the highest resistance ratio (RR) about pyrethroids insecticides tested, selection process performed on adult and after 3 rd selection a Lambdacyhalothrin resistant population of Cx.pipiens was achieved with 85.75 -fold resistance ratio at LT50 level.

Biochemical assays

Profile activities of α- and ß-esterases, Mixed Function Oxidase (MFO), Glutathione-S-Transferase (GST), were tested for two Cx .pipiens populations with resistance ratio of 85.75 to Lambdacyhalothrin and resistant to DDT population in comparison with Lab strain and summarized in Table.1 which shows the median level of enzymatic activity related to all three populations.

Mixed function oxidase (MFO)

In the both DDT and Lambdacyhalothrin resistant populations In relation to Lab strain, results showed that there are significant different in MFO activity levels ($P<0.05$)(Fig 1).

Glutathione-S-transferase (GST)

Statistical analysis indicated that, GST activity in the two populations was not significantly different from that of the Lab- strain ($P> 0.05$) (Table 1) (Fig 2).

α and ß-Esterase

In the present study both DDT and Lambdacyhalothrin resistant populations. The median activity levels of α and ß-EST were significantly different from the Lab strain ($P<0.05$) (

Amplification and sequencing of sodium channel gene fragments in *Cx. pipiens*

A 521-bp fragment of the sodium channel gene from all three populations by PCR using primers: Cpp1 (5' CCT GCC ACG GTG GAA CTT C3') andCpp2 (5'GGA CAA AAG CAA GGC TAA GAA3') were amplified (Fig 3).

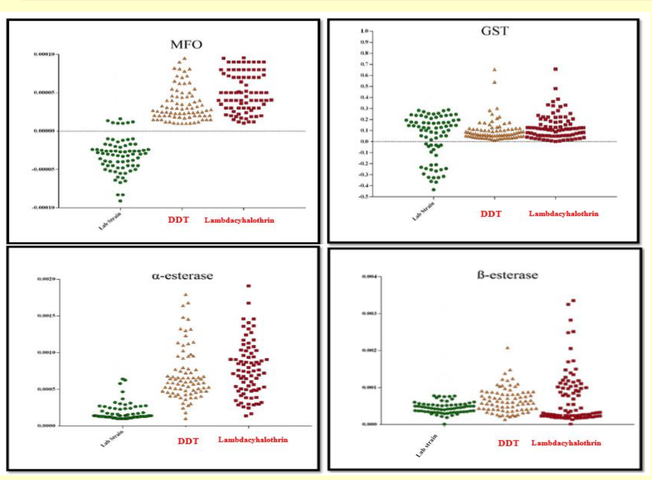

Fig.1. Activity profile of MFO, GST and α and ß-Esterase enzymes in two populations (resistant to Lambdacyhalothrin and resistant to DDT compared with lab-strain of Culex pipiens

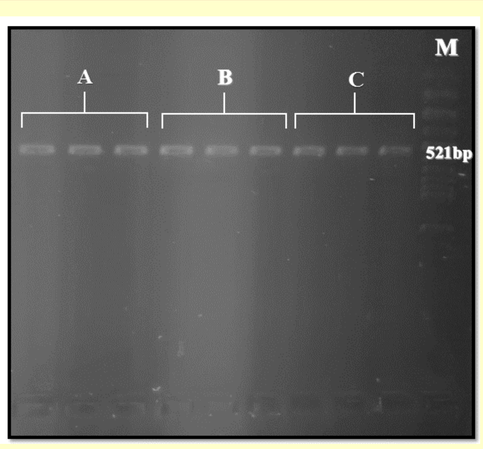

Fig.2. Amplification of sodium channel gene (521bp) in *Cx.pipiens* from three different populations -A:Lab-Strain,B:resistant to Lambdacyhalothrin,C:resistant to DDT.M: 100 bp ladderFig.3 Amplification of sodium channel gene (521bp) in Cx.pipiens from three different populations -A:Lab-Strain,B:resistant to Lambdacyhalothrin,C:resistant to DDT.M: 100 bp ladder

Discussion:

In the present investigation in order to survey the biochemical and molecular basis of resistance in two population which include : resistant to DDT and about pyrethroids insecticides a population with highest resistance ratio in Cx. pipiens from Tehran , a comprehensive study was conducted .Results showed that there are significant different ($p<0.05$) between the mean activity of α and ß-esterase and (MFO), in both lambacyhalothrin and DDT resistant population compared with Lab-Strain .However there is no significant different ($p>0.05$)about (GST) in the both populations compare with Lab-Strain. Molecular study for detection of L1014F or L1014s mutation in sodium channel gene showed lack of the mutation responsible for insecticide resistance which lead to cross-resistance between pyrethroids insecticides and DDT in Cx pipiens populations. Based on our finding ,it seems that metabolic mechanisms due to increased enzyme levels have considerable role in resistance to pyrethroid insecticides and DDT in Cx pipiens populations and the main reason for increased resistance to insecticides are MFO, α and ß esterase . Although there was no significant different ($p>0.05$) about (GST) in the both populations compare with Lab-strain.

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