Biochemical and molecular resistance mechanisms to DDT and some pyrethroid insecticides in vector of West Nile virus, Culex pipiens

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Abstract

Objectives : Culex pipiens complex act as an important vector 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.

Materials and methods 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 and DDT were used . For comparison of average between two groups T test were used.

Results Our finding showed that there are significant different ($p<0.05$) between the mean activity of α-, ß-esterases and (MFO), in both lambacyhalothrin 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.

Discussion This study showed that the resistance to pyrethroids and DDT in the Cx pipiens is enzymatic, but not targets site insensitivity of sodium channel gene.

Conclusion Findings of this research could provide a clue for logical operations of future chemical control program.

Background

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 mainly is sewage system of cities and due to this life style resistance to the most group of insecticides [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 is spreading around the world [9–14].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 concluded that about DDT, this species is quite resistant [15–16]. 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 [17–18]. 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 [13, 18] .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

Study area

This study was performed in Tehran city (35° 41' 46" N, 51° 25' 23" E), Tehran Province, the capital of Iran (Fig. 1).

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 experiment for biochemical and molecular assays in *Cx. pipiens* 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 resistant were used .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.

Biochemical assays

Forty mosquitoes from three population including : pyrethroid insecticides that have highest resistance ratio (RR) which exposed to insecticide (resistant to Lambdacyhalothrin), resistant to DDT and also lab strains were evaluated for Mixed Function Oxidase (MFO) , Glutathione-S-Transferase (GST),α-and ß-esterases and protein assay. Measurement of enzymes activity generally carried out in accordance with the method which described [20] .At the first adult female of *Cx. pipiens* was individually homogenized along with 200 µl of distilled water in flat-bottomed micro plates which putted on ice. For all biochemical assays, each individually homogenized mosquito was transferred to two wells on a 96 well flat-bottomed micro plate, by a microplate reader (ELX808 Ultra Microplate Reader BIO-TEK ®) after adding buffers and reagents to homogenized according to protocol. Absorbance levels with the use of wavelength specific for each enzyme were measured [20] .Moreover as the correction of errors related to volume of mosquito homogenates due to different protein content from each mosquitoes, absorbance values for mosquito were corrected finally the means of enzyme activities for each mosquitoes strain were compared with the Lab strain by Unpaired t-test, Mann-Whitney test and P-Value<0.05 was considered as significance level.

Molecular study of resistance

In the present research a molecular study also performed on the 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 DDTGgenomic DNA related to each three strains was extracted by Collins method [21]. For amplifying a 521bp fragment of the sodium channel gene by polymerase chain reaction(PCR) which containing the region that kdr mutations occur, primers: Cpp1(5/ CCT GCC ACG GTG GAA CTT C3/) andCpp2 (5/GGA CAA AAG CAA GGC TAA GAA3/) according to thermal cycling conditions: 30 cycles of 94°C for 40 s, 60°C for 50 s and elongation at 72°C for 40 s, followed by final extension at 72°C for 8 minute were used . Bioinformatics soft wares such as Clustal W2 and Blast were used for sequence alignment also for Translation nucleic acids to amino acids ExPASy was used.

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 . The results are summarized in Table.1 which shows the median level of enzymatic activity related to all three populations.

Mixed function oxidase (MFO)

In both DDT and Lambdacyhalothrin resistant populations in comparison to Lab strain, results showed that there are significant difference in MFO activity levels (P<0.05)(Table1)(Fig 2).

Statistical analysis

For comparison of average between two groups T test were used

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) (Table 1) (Fig 2).

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). From each *Cx.pipiens* population forty samples of sodium channel gene fragment was sequenced. By using Bioinformatics software such as Clustal W2 and Blast all sequencing results were checked in order to ensure that results are valid. More over as comparing sequencing results of this study with other sequences recorded in Gen Bank by Blast result showed that our sequence is more similar to *Culex pipiens pallens* (Accession number: GU198941.1). Based on our finding. More investigation of kdr region showed that: L1014F substitution due to changing TTA codon to TTT and L1014S substitution (TTA to TCA) causing resistance in the kdr gene was not observed. More over in all three population in the mentioned region, similar *Culex pipiens pallens* (Accession number: GU198941.1) , TTA codon was exist(highlighted in yellow) (Fig 4). According to ExPASy results related to translating nucleic acids to amino acids ,sequences of *Cx.pipiens* sodium channel gene were compared with other similar amino acid sequences of mosquitoes were available in the gene bank. By comparing result of current study with The most similar sequences related to *Cx .pipiens* pallens results revealed that in the region with possibility Kdr mutation at position 1014 in the sodium channel gene due to translation leucine to phenylalanine or serine There is no mutation and this fact ,confirmed lack of Kdr mutation in two *Cx .pipiens* populations. Except a substitution due to changing TTA codon to TTT in the exonI (highlighted in gray) resulted in single amino acid substitution of leucine to phenylalanine in *Culex pipiens pallens* ,all other amino acids were quite similar and also These differences, may be considerable to insecticide resistance In the future about this species (Fig. 4).

Table 1. Quantification of enzymatic activity of MFO, GST , α and β-esterase in three populations (resistant to Lambdacyhalothrin, resistant to DD.T and lab-strian) of *Culex pipiens*

Strains	MFO (EU Cyt.p450/mg)		GST (mM/min/mg)		α-esterase (µM/min/mg)		β-esterase (µM/min/mg)	
	Na	Median b	Na	Median b	Na	Median b	N a	Median b
Lab	40	-0.000019	40	0.06857	40	0.0001	40	0.00034
Resistant to DDT	40	0.000021	40	0.11485	40	0.00061	40	0.00062
Resistant to Lambdacyhalothrin	40	0.000036	40	0.13222	40	0.00073	40	0.00048

a Number of mosquitoes tested.
b Median value for each enzymatic activity

Discussion

In the present investigation the biochemical and molecular basis of resistance in two population, resistant to DDT and pyrethroids insecticides and Lab strain 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 difference ($p > 0.05$)about (GST) in the both populations compared 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 compared with Lab-strain. We cannot definitively ignore the important role of GST in resistance to organochlorines and pyrethroids by detoxification of them [22–23]. One of the reasons which can explained that why this difference was not significant is moderate resistance to DDT in lab strain of Cx pipiens.. There are many reports about metabolic resistance to DDT and pyrethroid insecticides due to increase level of enzymes in medically important vectors of mosquitos, especially in Culex genus. MFO as one of the most influential enzymes alone or with other enzymes responsible for biochemical resistance mentioned as a majority reason for resistance to Pyrethroid insecticides and DDT [24–27]. Moreover in some species more than one enzyme introduced as an enzyme responsible for resistance to DDT and Pyrethroid insecticides. In resistant to DDT and Pyrethroid insecticides populations of Aedes aegypti from Trinidad and Tobago after biochemical surveying for detection of resistance mechanism result showed that elevated levels of α-esterase and MFO enzymes in all Ae. aegypti populations were present also approximately in all populations increased levels of leand GST levels were indicated 27 Activities of esterases, monooxygenases in Anopheles stephensi from Dubai (DUB-R) resistant to permethrin were measured and result showed that level of all enzymes in resistant strain comparing with susceptible strain were significantly higher [28]. In Culex.quinquefasciatus mosquitoes from Benin related to four different localities ,resistant to DDT and permethrin in three areas were detected and also higher levels of α and β and GST activity in field collected compared with susceptible strain [12] .In our study except GST activity which there was no significant difference between two population in compression of Lab strain ,result

about other enzymes activity level was in parallel to three mentioned studies about resistant to DDT and Pyrethroid insecticides in mosquitoes [12, 28]. Molecular study for detection probable mutations at position 1014 in sodium channel gene showed lack of the mutation responsible and also by translation nucleic to amino acids result indicated that mutation in two Cx .pipiens population due to translation leucine to phenylalanine or serine was not exist and with emphasis to this finding we can describe there are no Kdr mutation in our field collected populations of Cx .pipiens. Based on some studies about resistance to DDT and Pyrethroid insecticides. High insecticide resistance correlated with mutations at position 1014 in sodium channel gene Which is known as the kdr mutation indicated in mosquitoes [29–30]. In six out of seven Pyrethroid insecticides populations of Anopheles gambiae, mutations in sodium channel gene reported as responsible for survival to pyrethroid exposure (Martinez-Torres et al., 1998). The results of Chen et al. study (2010) showed that high L1014F mutation frequency in six populations of Cx. pipens pallens caused high prevalence of pyrethroid resistance in Eastern China [13]. In another study two alternative kdr alleles, both resulting in a L1014F substitution were detected in some pyrethroid insecticides and DDT resistant strains of Cx. quinquefasciatus [14]. Multiple insecticide resistance mechanisms in Anopheles gambiae and Cx. quinquefasciatus in have been reported ,also in permethrin and DDT resistant populations of An. gambiae and Cx. quinquefasciatus this resistance to insecticides attributed to presence of target site insensitivity due to kdr mutation [12]. Finally according to our finding there are any evidence for molecular bases of resistance to pyrethroid insecticides and DDT due to kdr mutation in Cx.pipiens. It seems that biochemical mechanism through enzymes activity may be lead to this highly level resistance to insecticides in Cx.pipiens.

Declarations

Conflict of Interest:

The authors declare that there is no conflict of interest.

Acknowledgements

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Figures

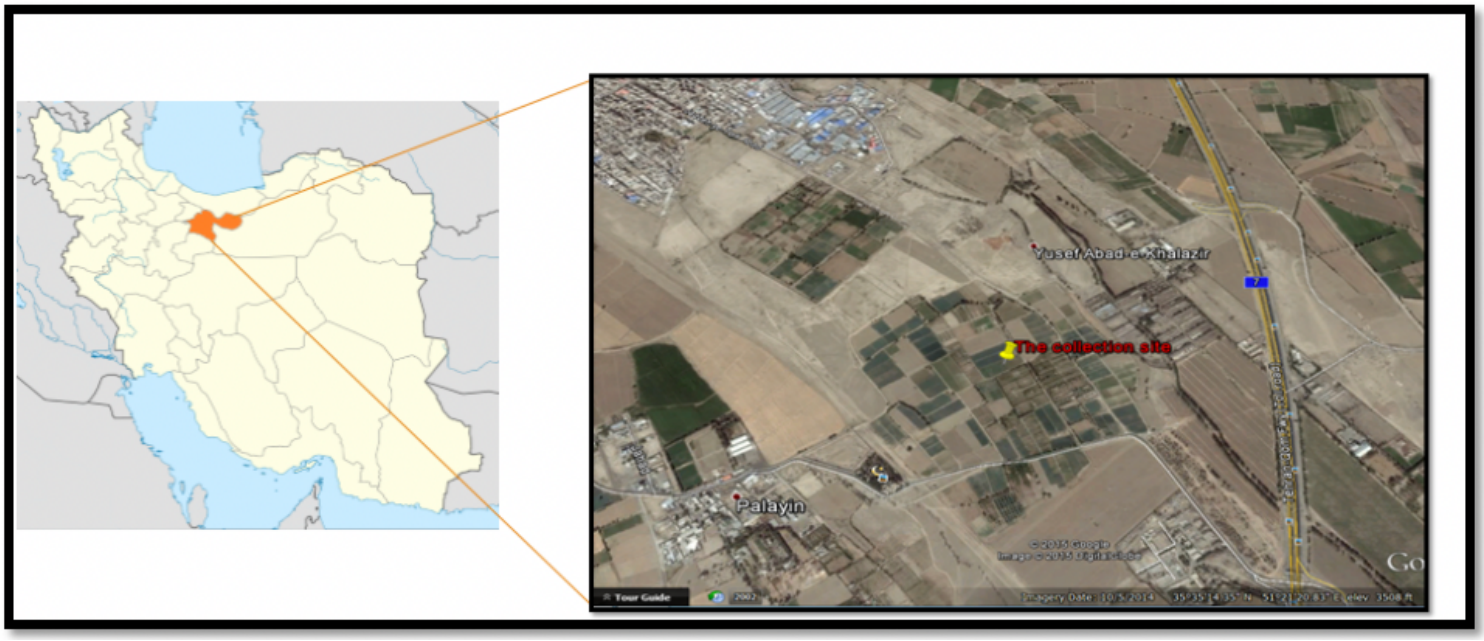

Figure 1

Map of study area in Tehran city and the collection site, Iran

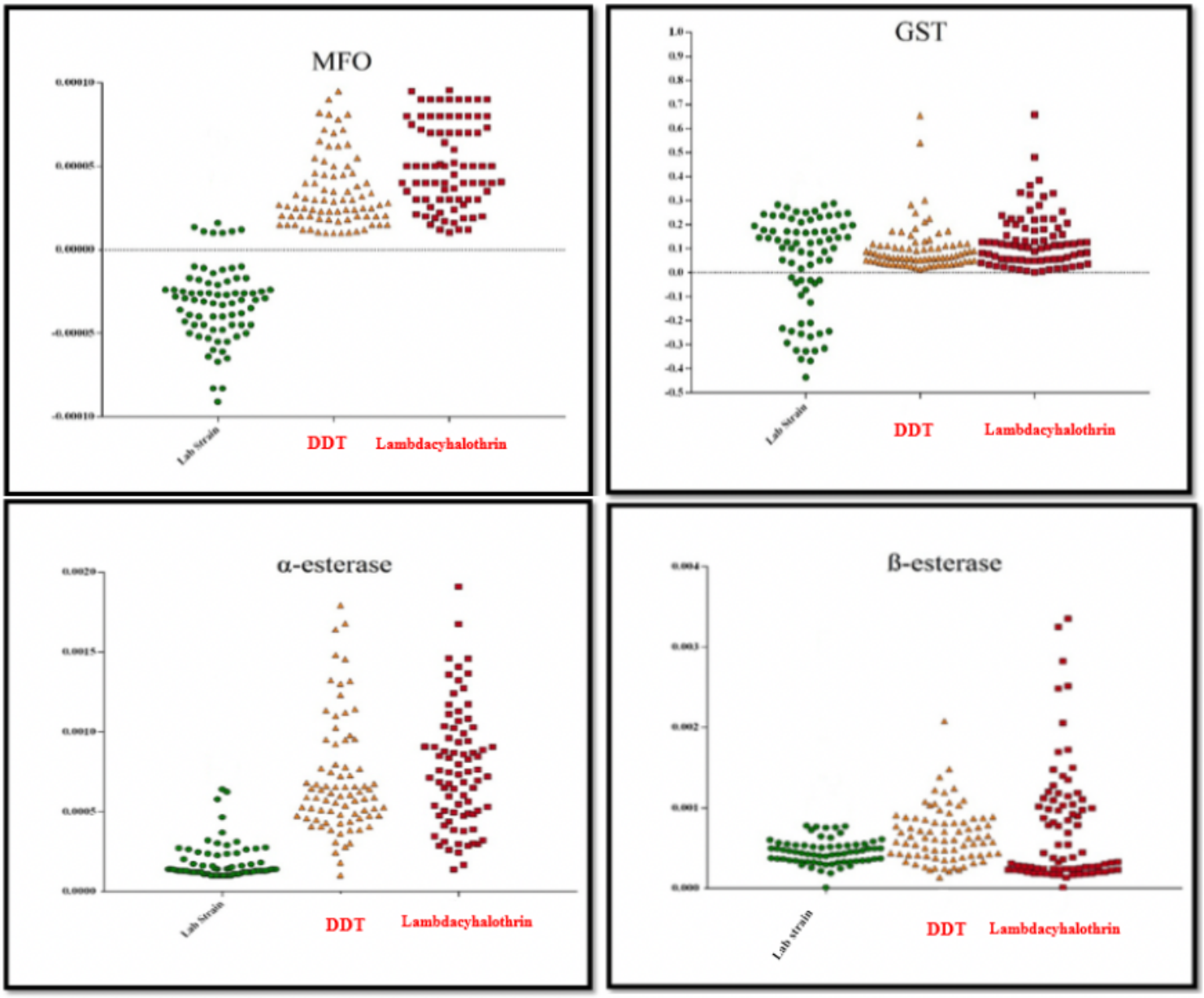

Figure 2

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

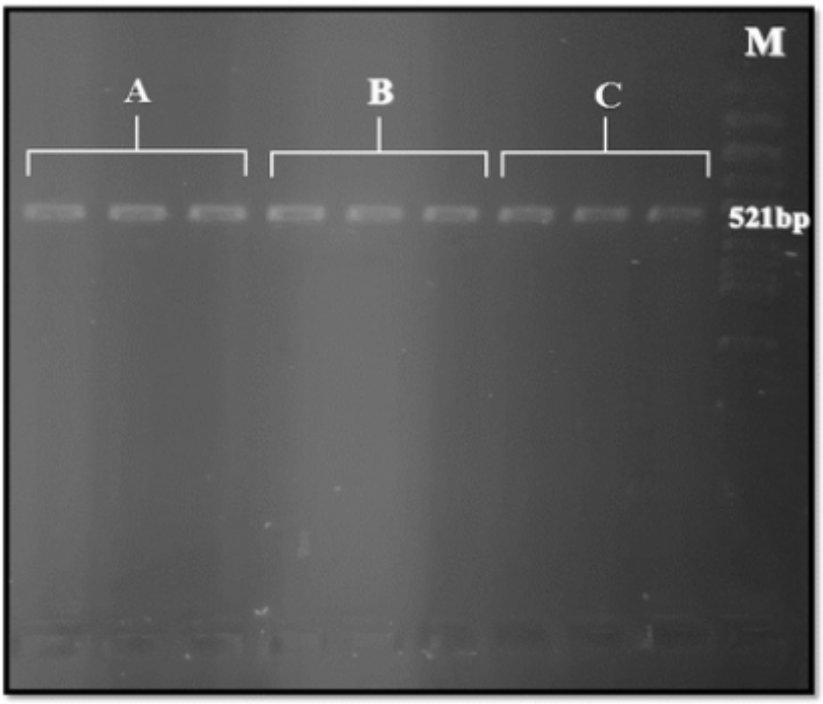

Figure 3

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

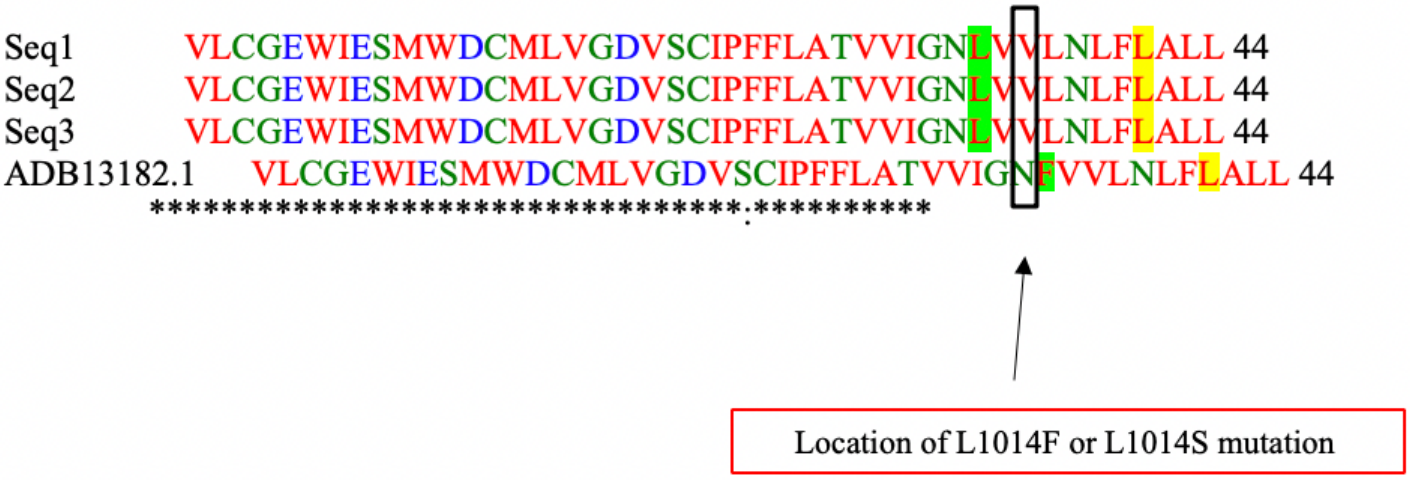

Figure 4

Comparison of translated sodium channel gene region amino acids sequence of this study (Seq1, Seq2, Seq3) with other similar registered genes in gene bank Culex pipiens pallens (S)