Role of Para sodium channel gene in insecticide susceptibility of the white fly *Bemisia tabaci* (Homoptera, Aleyrodidae) in some Egyptian Governorates

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Abstract

A survey of resistance to pyrethoids was conducted in 2014 for *Bemisia tabaci* from seven Egyptian Governorates, compared with lambda cyhalothrin- resistant strain and a lab-susceptible strain. Dakhlyia and Beheira populations exhibited the highest levels of resistance to lambda- cyhalothrin and cypermethrin, but Fayuom and kalubyia populations exhibited low levels of resistance. Estimate of realized heritability (H^2) of resistance for lambda cyhalothrin resistant-strain related that resistance buildup after 11 generations. Synergistic on resistant strain and biochemical effects in seven populations revealed that CarE, esterases and cytochrome P450 were involved in the resistance to pyrethroids. Para sodium channel gene was detected in all samples, PCR product yielded shared the sequening with 94% to *B. tabaci* DQ 205200. Nucleotide variations were detected among the collected samples which may be one of the reasons for the insensitivity of Para sodium channel to pyrethroids application in some Govenorates.

Key words: Para sodium channel- Insecticides- susceptibility- whitefly-resistance.

1. Introduction

The sweet potato whitefly, Bemisia tabaci (Gennadius) (Homoptera: Aleyrodidae) is a serious insect pest on a wide range of agricultural and horticultural crops due to its ability to transmit virus diseases and cause substantial direct and cosmetic feeding damage. Control measures still rely heavily on the application of insecticides including significant reliance on pyrethroids, a class of insecticides commonly used in crop protection, animal health and the control of insects endangering human health. Pyrethroids are selectively toxic to invertebrates at low concentrations and account for around 20% of all insecticides used worldwide **(McCaffery & Nauen, 2006)**. However, many species have developed resistance to pyrethroids resulting in insect management problems and increased economic loss to agricultural producers **(McCaffery & Nauen, 2006)**. The primary insecticidal action of pyrethroids results from their disruption of normal sodium channel activation and inactivation kinetics, leading to repetitive neural discharge, convulsive activity, and eventually paralysis and death **(Narahashi, 2000)**. One type of resistance to pyrethroids is termed knockdown resistance, or kdr, which results from reduced nerve sensitivity to pyrethroids and DDT **(Soderlund & Bloomquist, 1990)**. Although first reported in 1951 in the house fly Musca domestica Linnaeus (Diptera: Muscidae) **(Busvine, 1951)**, our understanding of the molecular basis of kdr was limited until the recent cloning of sodium channel genes (homologous to the fruit fly Para gene) from several species with kdr-type resistance **(Park & Taylor, 1997)**. Later, a highly enhanced pyrethroid resistance trait (up to 500-fold) called super-kdr was found in the same insect **(Sawicki, 1978)**. Genetic mapping studies showed close linkage between the kdr and super-kdr traits and the para-type sodium channel gene locus in housefly **(Williamson *et al*., 1993)**, tobacco budworm, Heliothis virescens **(Taylor et al., 1993)** and German cockroach, Blattella germanica **(Dong and Scott, 1994)**. Subsequent studies identified two mutations correlated with these two resistant phenotypes. A leucine to phenylalanine replacement (L1014F) within Trans membrane segment 6 of domain II (IIS6) was associated with moderate (10–30-fold) kdr resistance **(Williamson *et al*., 1996; Dong, 1997)**. In contrast, the housefly super-kdr mutation, M918T, has been identified in only one other insect, the horn fly, where it is again found in combination with the L1014F in a more highly resistant strain **(Guerrero *et al*., 1997)**. A second super-kdr-like mutation has been reported for the diamondback moth, involving threonine to isoleucine change (T929I) at the start of Trans membrane segment IIS5 **(Schuler *et al*., 1998)**. T929I was also found in permethrin-resistant human head lice (Pediculus humanus capitis) populations **(Lee *et al*., 2000)**. In the present study, the level of resistance to lambda-cyhalothrin was estimated in population of B. tabaci subsequent insecticide selection was carried out to build up resistance and this work illustrates the value

of molecular biology in revealing an important and previously uncharacterized resistance in a major crop pest.

2. Material and Methods

2.1. White fly stains:

2.1.1.Laboratory strain:

It was collected from the field and reared in laboratory for 30 generations of central Agricultural pesticides laboratory (CAPL), Agricultural Research centre (ARC) and maintained without insecticide selection pressure for many years in the lab.

2.1.2.Resistant strains:

It derived from the lab. strain and selected with lambda- cyhalothrin for 18 generations as shown in Table (2).

These strains were reared on cotton plant (*Gossypium hirsutun*) and placed under conditios of 27±2°c, 55±5% R.H.**(coudriet *et.al*., 1985)**.

2.1.3.Field- strains:

Adults of *B. tabaci* were collected in the early morning hours from different sites in governorate, insects were collected from seven Governorates as shown in Table (1) **(Faghaly *et. Al*. 2014)** Adults were transported to the lab in a cool box and used to toxicological tests. Samples were taken and stored at 20°c for molecular analysis.

Table (1):- Origin of the collected *B. tabaci* strains from some Egyptian Governorates.

Governorates	Collection sites	Strain symbol
Beheira	Etay El-Barud	B_1
	Wadi El-Natrun	B_2
Gharbia	Zefta	G_1
	Kotoor	G_2
Kalyubia	Qalyoub	K_1
	Banha	K_2
Dakahlia	MitGhamr	D_1
	Bilqas	D_2
Menoufia	Berket El-Sabaa	Mn_1
	Ashmoun	Mn_2
Fayuom	Sinnuris	F_1
	Atsa	F_2
	Ibshway	F_3
Minya	El-Idwa	M_1
	Samalut	M_2
	Mallwi	M_3

2.2-The tested insecticides:-

Two pyrethroids formulations were used:-

Lambda- Cyhalothrin (Karat, 20% EC).

Alpha- Cypermethrin (Fastac, 15%EC).

2.3- Bioassay:-

The bioassay method for obtaining concentration-mortality lines, was used as described by **prabhaker *et al*. (1985)** with some modifications. Attached leaves of cotton plants were dipped for 5 sec. in 100 ml of the desired concentration of each insecticide and allow drying. Twenty adults of *B.tabaci* were exposed to the treated leaves confined in small cages (by an aspirator).

At least seven concentrations of each insecticide were tested and three cages were used as replicates for each test, the treated leaves with the insect adults were kept in constant lab conditions

2.4- Selection pressure:

The population of resistant strain was selected with $LC_{25,}$ LC_{50} for lambda Cyhalothrin formulation. The level of developing resistance was determined at generations 1, 3, 6, 9, 12, 15 and 18. Resistance ratio (RR) was determined by dividing the LC_{50} of the R-strain by the LC_{50} of lab-strain.

2.5. Estimation of realized heritability:-

Realized heritability (h2) was estimated by using the method described by **Tabashnik (1992)** as follows:

$$h^2 = \frac{\text{Response of Selection ®}}{\text{Selection differential (S)}}$$

Response to selection (R) was estimated as follows:

$$R = \frac{(\text{Log final } LC_{50} - \text{Log initial } LC_{50})}{n}$$

Where the final $LC_{50}$is the LC_{50} of population after n generations of selection and initial LC_{50} is for the parental population before selection.

The selection differential (S) was estimated as follows:

S = ip.

n: number of generations.

Where i is the intensity of selection and is calculated according to **Falconer(1989)** and $^{\delta}p$ is the phenotypic standard deviation, calculated as :

$^{\delta}p = [1/2(\text{initial slope} + \text{final slope})]^{-1}$

Or $(\text{mean slope})^{-1}$

To estimate either a change in R, S, and h^2 during the selection pressure, each parameter was calculated for the first and second half of the experiment (12 generations in each half).

The response to selection (R) can be estimated as follows:

$R = h^2S$

The number of generations required for a tenfold increase in LC_{50} was calculated as follows:

$G = R^{-1} = (h^2S)^{-1}$

Effect of heritability on projected rate of resistance increase at constant slope value was assessed by drawing a graph between percent mortality and generations. We used three values of h^2 (one value was calculated from F1 to F24 and other two values were assumed theoretically and same procedure was adopted for effect of slope on projected rate of resistance evolution at calculated constant value of h^2.

2.6- Synergistic Effects:

The Synergists used in this study were:-S,S,S tributyl phosphoro trithioate (DEF 72%EC), an inhibitor for both CarE and glutathione-S-Tranferase (GST).

Piperonyl buytoxide (PB 50% EC), an inhibitor of P_{450}. The resistance were evaluate in R-strains by mixing with 50 ppm, 25 ppm and 10ppm of each synergist with LC_{50} of the G_{i8} for R-resistant, the toxicity was compared as:-

$$\text{Synergistic ratio(SR)} = \frac{LC_{50} \text{ of insecticide alone}}{LC_{50} \text{ of insecticide + Synergist}}$$

2.7- Biochemical Assays:

Insects were homogeniged in distilled water (50 mg/1ml) Homogenates were ccntrifuged at 8000 r.p.m for 15 min at 5ºc in a refrigerated centrifuge the deposits were discarded and the supernatants were kept in a deep freezer till use

2.7.2- CarE assay:-

CarE activity was measured according to methods described by **Simpson *et al.* (1964)** using methyl butyrate (MeB)as substrate, the reaction mixture contained 200 µL of the enzyme solution , 0.5 mL Phosphate buffer (0.067 M, pH7) and 05 mL AChBr (3mM).

The test tubes were incubated at 37ºC for exactly (30 min). One mL of alkaline hydroxylamine (equal volume of 2M hydroxylamine choloride and 3.5M NaOH) and 0.5 mL HCI (one part concentrated HCL: Two pasts distilled water) were added to the test tubes. The mixture was shaken thoroughly and allowed to stand for 2 min. Then, 0.5 mL ferric chloride solution (0.9M) dissolved in HCl (0.1M) was added and Mixed well. The decrease in MeB resulting from hydrolysis by CarE was read at 515nm using double beam UV/visible spectrophotometer (Milton Roy spectronic 1201 UV-Visible, USA)

2.7. 3- GST Assay:-

GST activity was determined based on the technique of **Habig *et al.* (1974)** using 1-chloro-2, 4-dinitrobenzene (2, 4-CDNB) as a substrate. The reaction mixture comprised of 10 µL reduced glutathione (GSH) (10 mM) in sodium phosphate buffer (100 mM, pH 6.5) and 10 µL of the enzyme solution. The reaction was initiated by adding 10 µL of 2,4-CDNB (6 mM in methanol) resulting in a final volume of 30 µL. The plates were immediately transferred to absorbance micro plate reader (Biotech Instruments, Inc., Winooski, VT, USA). The reactions were allowed to continue for 5 min and absorbance readings were taken at 340 nm automatically once per min against blanks (wells containing all reaction components except the enzyme solution). The increase in absorbance was linear throughout the 5 min reading interval. An extinction coefficient of 9.6 $mM^{-1}cm^{-1}$ was used to calculate the amount of 2, 4-CDNB conjugated

2.7.4- MOs assay:-

MOs activity was detected through the transformation of *p*-nitro anisole to *p*-nitro phenol through *O*-demethylation via the enzyme *p*-nitro anisole-*O*-demethylase based on the methods of **Hansen and Hodgson (1971)** with slight modifications. The standard incubation mixture contained 1 mL sodium phosphate buffer (0.1M, pH 7.6), 1.5 mL enzyme solution, 0.2 mLNADPH (final concentration 1 mM), 0.2 mL glucose-6-phosphate(final concentration 1 mM) and 50 µg glucose-6-phosphate dehydrogenase. The reaction was initiated by the addition of *p*-nitro anisole in 10µL acetone to give a final concentration of 0.8 mM and was incubated for 30 min at 37°C. The incubation period was terminated by the addition of 1mLHCl (1N), and *p*-nitro phenol was extracted with $CHCl_3$ and NaOH (0.5 N). The absorbance of NaOH solution was measured at 405 nm. An extinction coefficient of 14.28 $mM^{-1}cm^{-1}$was used to calculate the concentration of 4-nitrophenol.

2.7.5- Total protein:-

Total protein content was determined according to **Bradford (1976)**.

2.8- Statistical analysis:-

The percentage mortality of treated larvae was corrected against the of the control using Abbott's formula **(Abbott, 1925)**. Then the corrected mortality was subjected to probit analysis **(Finney, 1971)**. Data of the biochemical assays were analyzed using one-way analysis of variance (ANOVA). When the NAOVA statistics were significant (P>0.05), the means were compared by Duncan's multiple rang test. All the analyses were computed by IBM® SPSS® statistics 21.0 (IBM Corp, Armonk, NY, USA)

2.9- Extraction of gDNA and RNA, cDNA Synthesis cloning and sequencing:-

Total RNA was extracted from 10 *B.tabaci* adults using Tri-reagent as described by **Vontas, J.G. *et.al* (2001)**. First strand cDNA synthesis was carried out, with an olig (dt) adaptor primer [5'GACTCGAGTCGAC-

ATCGA. (dt) 17 3'], using superscript III (Invitrogen).

gDNA (Total nucleic acid) was extracted from adults by placing them in a 0.5 ml tube and grinding with a pestle in 50 µL of ice-cold lysis buffer (100mM of NaCL and 10mM of tris-HCL, pH 8.0) containing 0.4 mg/ml of proteinase K..Extracts were incubated at 55ºc for Lh and at 85ºC for 5 min prior to a 5min centrifugation (10000g) to pellet debris.

The supernatant was used as the source for the polymerase chain reaction (PCR). Primers F1(5'GCCAAATCCTGGCCAACT) and R_4(5'GAAATTACTCAGCAACAAC-GC) from the known mRNA sequence of *B.tabaci* Para sodium channel gene **(Morin *et. al.* 2002)** were used to amplify a 370 bp fragment of the *B.tabaci* Para sodium channel gene. Primer F1 and R.Int and (5'CTTTCCGCACCTCTGATGGGC) were used to amplify a 369bp product (condon 905-952 form S_4) Amplifications from approximately 40ng cDNA or gDNA were performed in 1×Taq reaction buffer (promeg, UK) with a final concentration of 2 mM MgCl2 and 0.5µM each primer, with cycling conditions 94ºC for 5 min, 30 cycles of (55ºC for 30S, 72ºC for 45S and 95ºC for 15S), followed by 72ºC 5min . The PCR product was purified using QiaQuik columns (Qiagen) and either sequenced directly using primers given above , and sevsral randomly chosen colonies. Sequence data analyzed .

3. Results

3.1. Bioassay

3.1.1. Resistance development

Data presented in Table (2) showed changes in the response of *B.tabaci* adult towards selection pressure of lambda-cyhalothrin's LC_{50} value increased by stability till 413.13 ppm in the 18th generation and resistance ratio (RR) reached to 77.08 fold.

Table 2. Lambda -cyhalothrin in *B.tabaci* during selection for 18 generations

Generations	Slope±EC	LC50 in ppm	RR
Lab-strain	1.12±0.44	5.36 (1.77-13.53)*	-
Perant	1.39 ± 0.43	6.86 (3.34-11.39)*	1.28
G1	2.93 ± 0.53	13.18 (9.33-17.04)*	2.46
G3	1.76 ± 0.47	18.05 (10.63 – 27.40)*	3.37
G6	0.72 ± 0.28	59.87 (16.02–221.83)*	11.17
G9	0.85 ± 0.37	(116.05 (1.72 – 26.06)*	21.65
G12	1.31 ± 0.41	144.69 (73.52 – 248.27)*	26.99
G15	0.88 ± 0.16	235.62 (139.19 – 337.96)*	43.96
G18	1.79 ± 0.49	413.16 (269.79 – 1035.8)*	77.08

$$\text{RR(Resistance Ratio)} = \frac{LC_{50} \text{ of the field strain}}{LC_{50} \text{ of the lab-strain}} \quad \text{*F.L.95\%}$$

3.2- Realized heritability (h2):-

The present data in fig.1 was showed that the selection for resistance to Lambda- cyhalothrin against *B.tabaci* support the ability to develop resistance to this insecticide in the field, where the spray with recommended dose, buildup of resistance after only 4 generations.

3.3- Synergism of R-strain:-

The results in Table (3) showed that DEF enhanced the toxicity of lambda-cyhalothrin against R_1 strain which exhibited SR= 16.82 and the level of resistance decreased to 4.48 fold by 10ppm, but for 10ppm PB exhibited SR= 10.59 and the level of resistance decreased to 19.5 fold, this finding indicated major contribution by esterase then Cytochrom P_{450} to lambda Cyhalothrin resistance *B.tabaci*

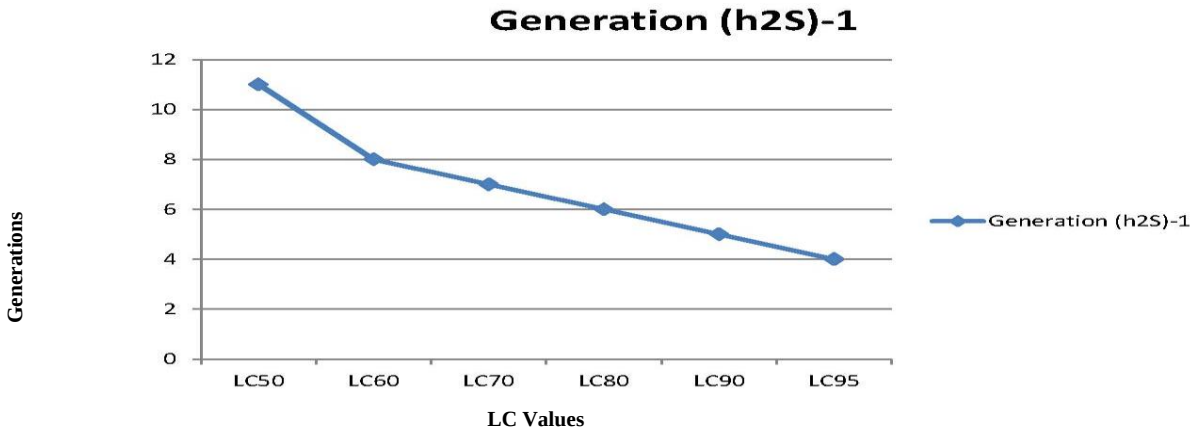

Fig (1):- Estimation of realized heritability for Lambda –cyhalothrin in *B.tabaci*

Table 3. Synergism of Lambda-Cyhalothrin by DEF and PB in the adults of *B.tabaci*, G_{i8} form R- strain.

Insecticide	LC_{50} (PPm)	Slope ± S.E	S.R	RR	RSR
Lambd Cyhalothrin	413.16	1.79 ± 0.49	-	77.08	
+50 ppm DEF	0.43	1.07 ± 0.11	960.84	0.08	179.26
+25ppm DEF	2.92	0.73 ± 0.06	141.49	0.54	26.40
+loppm DEF	24.57	1.17 ± 0.11	16.82	4.38	17.60
+50ppm PB	0.35	0.18 ± 0.05	1180.46	0.07	220.23
+25ppm PB	3.27	0.63 ± 0.07	126.35	0.61	23.57
+10ppm PB	39.0	1.07 ± 0.11	10.59	19.5	3.95

[Synergistic Ratio=. (SR) $\frac{LC_{50} \text{ of lambda-Cyhalothrin alone}}{LC_{50} \text{ of lambda-Cyhalothrin + Synergist}}$

(RSR) Relative synergism= (RR of unsynergized / LC50 of synergized treatment).

3.4-Adult susceptibility of whitefly, B. tabaci (Genn) of Field populations:-

3.4.1- Effect of two pyerthroid insecticides lambda-cyhalothrin and alpha-cypermethrin.

Adult susceptibility of whitefly, *B. tabaci* (Genn) of field populations collected from seven Governorates through the year 2014 and to two synthetic pyrethroid compounds. The results were presented in Tables (4 & 5).

The data concerning the effect of the used insecticide on adult of *B.tabaci* indicated that when the adults that exposed to the tested insecticides, high mortality was achieved 24 hours after treatment especially for lambda-cyhalothrin which was more efficiency than alpha-cypermethrin. LC_{50}'s of lambda-cyhalothrin were 0.05, 0.28, 0.47, 0.72, 2.07, 7.39 and 11.11 ppm for Kalyubia, Fayuom, Minya, Menoufya, Gharbia, Dakahlia and Beheira Governorates, respectively.

The synthetic pyrethroid alpha-cypermethrin was less effective than lambda Cyhalothrin, the LC_{50}'s values were 9.14, 10.58, 16.89, 18.40, 31.79, 54.91 and 83.25 ppm for Fayoum, Kalyubia, Minya, Menoufia, Gharbia, Dakahlia and Beheir Governorate populations and with slope values 1.10 ± 0.11, 1.48 ± 0.19, 1.09±0.14, 1.31 ± 0.11, 0.89 ± 0.13, 1.36 ± 0.17 and 1.44 ± 0.21 respectively.

Data in Tables (4,5) indicated that the resistance spectrum of Beheira Governorate field population was the most resistante to the two synthetic pyrethroid insecticides.The highest levels of resistance was clearly manifested for alpha-cypermethrin than Lambda-cyhalotrhin. Also, confidence limits of LC_{90} refereed to that the resistance to this insecticide (alpha-cypermethrin) will increase from using to another while refereed to the same resistance for lambda-cyhalotrhin in Menoufia Governorate field population

Table 4. Susceptibility of Laboratory and Field strains of adult Whitefly, *B. tabaci* to lambda-cyhalotrhin insecticide.

Governorate	LC_{50} in ppm (F.L)	Slope	LC_{90} in ppm (F.L)	X^2
Kalyubia	10.58 (7.96 – 13.73)	1.48 ± 0.19	77.35 (50.01 – 153.81)	2.66
Dakahlia	54.91 (40.51 – 72.61)	1.36 ± 0.17	477.41 (404.96 – 952.15)	4.67
Menoufia	18.40(10.56-29.70)	1.31± 0.11	175.03(117.08-508.84)	14.49
Gharbia	31.79 (22.82 – 47.45)	0.88 ± 0.13	887.02 (359.74 – 4591.46)	7.86
Minya	19.86 (12.21 – 23.4)	1.09 ± 0.14	247.46 (134.77 – 666.74)	3.95
Fayoum	9.14 (6.85 – 12.28)	1.15 ±0.11	131.45 (77.68 – 279.06)	3.39
Beheira	83.25 (57.19 – 114.12)	1.44 ± 0.21	638.44 (397.95 – 1388.84)	6.57
Lab. S	2.35(0.98- 5.13)	1.44±0.13	14.56(10.23-30.32)	0.88

F.L.: Fiducial limit

Table 5. Susceptibility of Laboratory and Field strains of adult Whitefly, *B. tabaci* to alpha-cypermethrin insecticide.

Governorate	LC_{50} in ppm (F.L)	Slope	LC_{90} in ppm (F.L)	X^2
Kalyubia	10.58 (7.96 – 13.73)	1.48 ± 0.19	77.35 (50.01 – 153.81)	2.66
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F.L.: Fiducial limit

3.5- Biochemical assay:-

The data in Table (6) showed that the enzymatic activity of CarE, GST, and P450 was assayed in laboratory and field stains collected from that different localities Table 6.

The insects collected from Fayoum , Kalyubia and Minya showed lower enzyme activity than that of lab-stain Fig.(2). while, the enzyme activity showed significantly higher values in insects collected from Dakahlia, Beheira and Gharbia ($P \leq 0.05$), it's still lower than resistant-strain.

Table (6):-The enzymatic activity of CarE, GST, $P_{450\ in}$ laboratory and field stains

Governorate	GST	P450	CarE
Susceptible	22.06	80.6	134
Kalyubia	20.63	90	150.6
Fayoum	18.19	101	889
Minya	17.53	119	949
Menoufia	28.08	119	1360
Garbia	28.32	156	1360
Dakahlia	29.00	165	1381
Beheira	29.25	166	1463
Resistant	32.89	170	2211

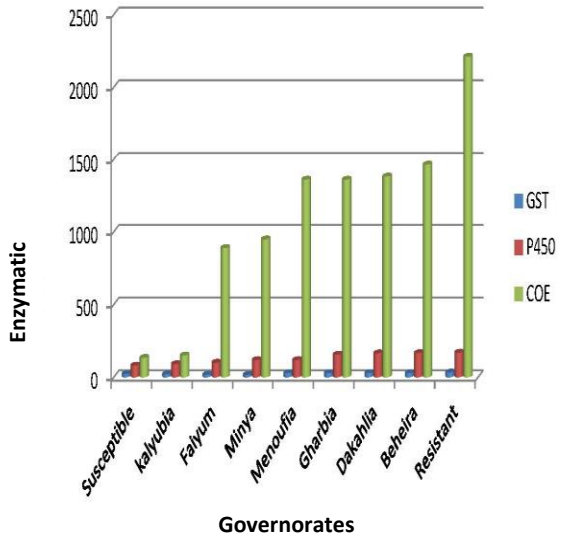

Figure 2 Enzymatic activities of CarE, GST, and P450 in Laboratory and Field strains of *B. tabaci*

3.6- Characterization of voltage gated sodium channel gene of B.tabaci and its position in a molecular phylogeny.

The biochemical and synergistic data resulted an additional pyerthroid resistance mechanisms in the treatment strains perviouly, prompting us to sequence regions of the Para sodium channel gene know to change, which, confers target site insensitivity. A440 bp fragment was amplified from cDNA from the whole strains which were divided into two groups.

First group, the strains collected from [(G_1, F_1, F_2, F_3, K_1, K_2, Mn, M_2 and M_3) Table (1)],table (7) shared the same sequence with lab-strain (Fig.3).

The lab-strain shared the sequencing with 35 % identically to *B.tabaci* s-Q1 sequence on the Gen bank (accession number, DQ 2052209) and shared sequence with 95% to *B. tabaci* SUD-s sequence on the Gen bank (accession number, AJ 440727) Table 7 & Fig.3

The second group collected from [(D_1, D_2, G_2, B_1, B_2, Mn_2 and $M_{1)}$ Table (1)] shared the same sequence with lambda Cyhalothrin resistant- strain Fig (3).

The R-strain shared the sequencing with 94% to *B.tabaci* r1-B1 (accession number, DQ 205200).The data in Table(7&8) and Fig(3) the results were showed differences in sequence two groups, that variants may represent alleles of the same gene. The phylogenetic analysis placed nine fragments in one identical monophyletic group (G_1, F_1, F_2, F_3, K_1, K_2, Mn_1, M_2, M_3 and lab-stain), and the other fragments together with R-strain (Fig.4).

Table (7):- Comparison between nucleotide in resistant lab-strain and Gen Bank alleles of *B.tabaci*

Allele	61	73-74	164	200	206-207	213	241	248	257	277	342	357	383	395-397	399	426	437
r1-B1	A	AC	T	C	TC	T	A	A	T	A	T	G	C	CCA	T	G	A
r1-B2	A	AC	T	C	TC	T	A	A	T	A	T	G	C	CCA	T	G	A
r1-Q1	A	AC	C	T	CT	T	T	*	T	A	C	A	T	CCA	C	A	C
r2 Q1	T	GT	C	T	CT	T	T	*	T	A	C	A	T	CCA	C	A	C
r1-Q2	T	GT	C	T	CT	T	T	*	T	A	C	A	T	CCA	C	A	C
Resistant	A	AC	T	C	TC	T	A	A	T	A	T	A	C	CCA	T	G	A
Lab-Strain	T	AC	C	T	AC	C	G	T	T	G	T	T	G	CAA	A	T	A
S-B1	T	AC	T	C	TC	T	A	A	T	A	T	G	C	CCA	T	G	A
S-Q1	T	AC	C	C	CT	T	A	*	C	G	T	G	C	CCA	T	G	C
S-Q2	T	AC	C	C	CT	T	A	*	C	G	T	G	C	CCA	T	G	C
S-Q3	T	AC	C	C	CT	T	A	*	C	G	T	G	C	CCA	T	G	C
S-Q4	T	AC	C	C	CT	G	A	*	T	A	C	G	C	***	T	G	C

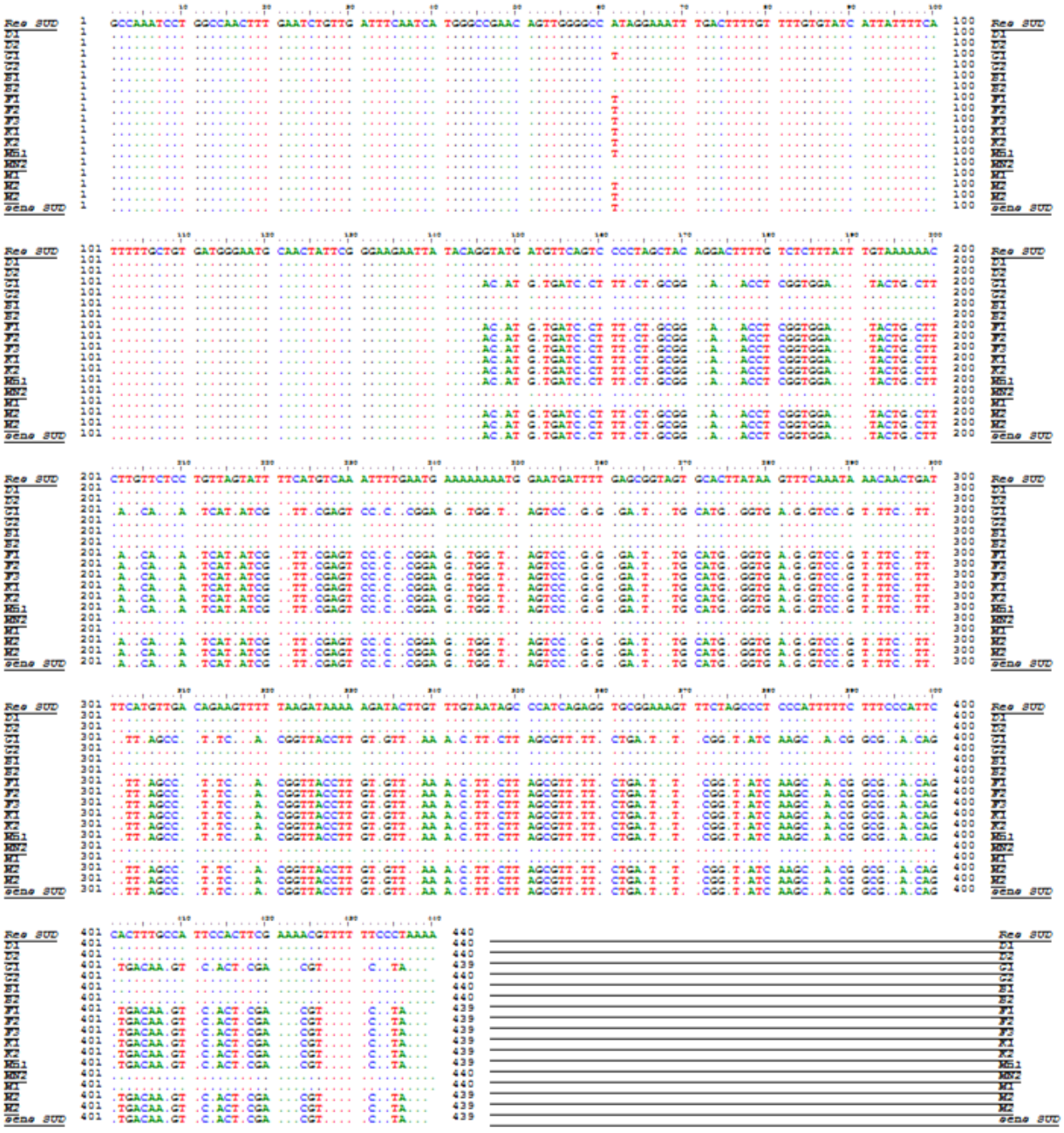

Fig 3. Multiple sequence alignment to neucleotide sequence of laboratory and field strains of *B. tabaci* Para sodium Channel gene isolated fragment and nucleotide variation sites only were shown

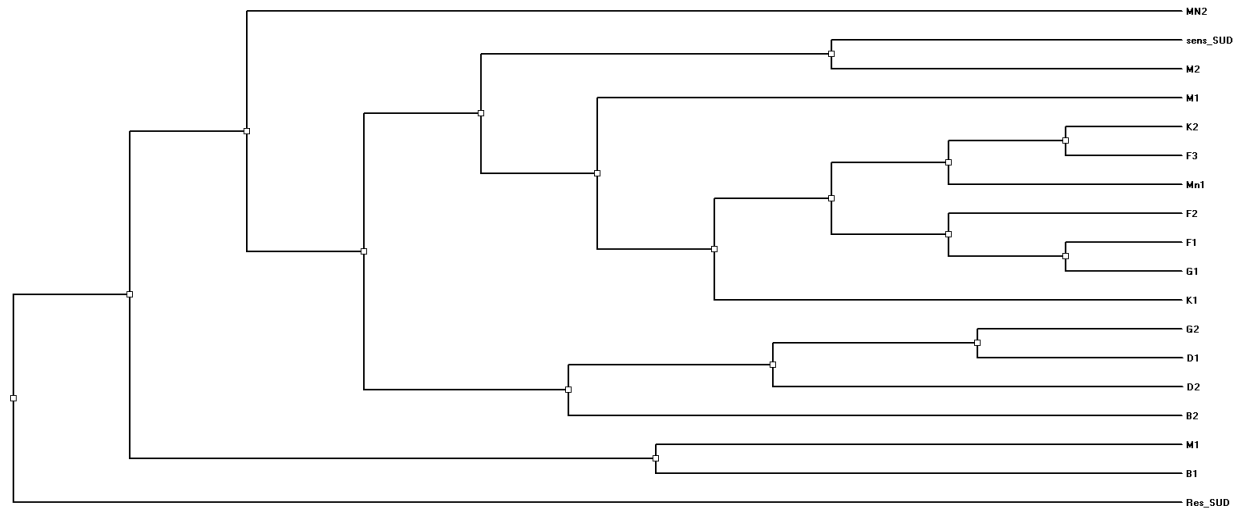

Fig. 4 - Maximum likelihood analysis of resistant and susceptible allele Sequencesof the *B.tabaci* para-sodium channel gene

Analyses were performed by the branch and bound search, with the tree bisection

Table (8):- comparison between amino acid in resistant, lab-strain and the susceptible (SUD-S) strains of *B.tabaci*

Mutation	Amino acid of resistance		Amino acid of lab-strain		Amino acid of Sub-S		Position
L925I	I	ATA	L	TTA	L	TTA	925
D 953 G	G	GGT	D	GAC	D	GAC	953
N 954N	M	ATG	N	AAT	N	AAT	954
V955 M	M	ATG	V	GTT	V	GTT	955
D956F	F	TTC	D	GAT	D	GAT	956
R957 S	S	AGT	R	CGC	R	CGC	957
F958 P	P	CCC	F	TTT	F	TTT	958
P 959 L	L	CTA	P	CCT	P	CCT	959
G 960 A	A	GCT	G	GGC	G	GGC	960
G 961 T	T	ACA	G	GGA	G	GGA	961
E 962 G	G	GGA	E	GAA	E	GAA	962
- 963 -	L	CTT	L	CTA	L	CTA	963
P964L	L	TTG	P	CCT	P	CCT	964
R 965 S	S	TCT	R	CGG	R	CGG	965
W 966 L	L	C TT	W	TGG	W	TGG	966
N 967 Y	Y	TAT	N	AAT	N	AAT	967
F 968 L	L	TTG	F	TTT	F	TTT	968
- 969-	T	ACT	T	ACT	T	ACT	969
- 970-	D	GAC	D	GAC	D	GAC	970
- 971-	F	TTC	F	TTC	F	TTC	971
M 972 L	L	TTG	M	ATG	M	ATG	972
H 973 F	F	TTC	H	CAC	H	CAC	973
- 974-	S	TCC	S	TCA	S	TCA	974
F 975 C	C	TGT	F	TTC	F	TTC	975
	*	TAG	M	ATG	M	ATG	976
I 977 Y	Y	TAT	I	ATC	I	ATC	977
V 978 F	F	TTT	V	GTT	V	GTT	978
F 979 H	H	CAT	F	TTT	F	TTT	979
R 980V	V	GTC	R	CGA	R	CGA	980
V 981 K	K	AAA	V	GTC	V	GTC	981
L 982 F	F	TTT	L	CTC	L	CTC	982
	*	TGA	C	TGC	C	TGC	983
G 984 M	M	ATG	G	GGA	G	GGA	984
E 985 K	K	AAA	E	GAA	E	GAA	985
W 986 K	K	AAA	W	TGG	W	TGG	986
I 987 N	N	AAT	I	ATT	I	ATT	987

E 988 G	G	GGA	E	GAG	E	GAG	988
S 989 M	M	ATG	S	TCC	S	TCC	989
M 990 I	I	ATT	M	ATG	M	ATG	990
W 991 L	L	TTG	W	TGG	W	TGG	991
D 992 S	S	AGC	D	GAC	D	GAC	992
C 993 G	G	GGT	C	TGT	C	TGT	993
M 994S	S	AGT	M	ATG	M	ATG	994
H 995 A	A	GCA	H	CAT	H	CAT	995
V 996 L	L	CTT	V	GTT	V	GTT	996
G 997 I	I	ATA	G	GGT	G	GGT	997
D 998 P	P	AGT	D	GAT	D	GAT	998
V 999 F	F	TTC	V	GTG	V	GTG	999
S 1000 K	K	AAA	S	TCC	S	TCC	1000
	*	TAA	C	TGT	C	TGT	1001
I 1002 T	T	ACA	I	ATT	I	ATT	1002
P 1003 T	T	ACT	P	CCT	P	CCT	1003
F 1004 D	D	GAT	F	TTT	F	TTT	1004
- 1005-	F	TTC	F	TTT	F	TTT	1005
L 1006 M	M	ATG	L	TTA	L	TTA	1006
A 1007 L	L	TTG	A	GCC	A	GCC	1007
- 1008-	T	ACA	T	ACT	T	ACT	1008
V 1009 E	E	GAA	V	GTC	V	GTC	1009
- 1010-	V	GTT	V	GTT	V	GTT	1010
I 1011 F	F	TTT	I	ATC	I	ATC	1011
G 1012 K	K	AAG	G	GGT	G	GGT	1012
Y 1013 I	I	ATA	Y	TAC	Y	TAC	1013
L 1014 K	K	AAA	L	CTT	L	CTT	1014
V 1015 R	R	AGA	V	GTA	V	GTA	1015
V 1016 Y	Y	TAC	V	GTT	V	GTT	1016
- 1017-	L	TTG	L	TTA	L	TTA	1017
N 1018 F	F	TTT	N	AAT	N	AAT	1018
L 1019 V	V	GTA	L	CTT	L	CTT	1019
F 1020 I	I	ATA	F	TTC	F	TTC	1020
L 1021 A	A	GCC	L	TTA	L	TTA	1021
A 1022 H	H	CAT	A	GCG	A	GCG	1022
L 1023 Q	Q	CAG	L	TTG	L	TTG	1023
L 1024 R	R	AGG	L	TTG	L	TTG	1024
L 1025 C	C	TGC	L	CTG	L	CTG	1025
S 1026 G	G	GGA	S	AGT	S	AGT	1026
N 1027 K	K	AAG	N	AAT	N	AAT	1027
- 1028-	F	TTT	F	TTC	F	TTC	1028
G 1029 L	L	CTA	G	GGA	G	GGA	1029
S 1030 A	A	GCC	S	TCA	S	TCA	1030
S 1031 L	L	CTC	S	TCA	S	TCA	1031
S 1032 P	P	CCA	S	AGC	S	AGC	1032
L 1033 F	F	TTT	L	TTA	L	TTA	1033
S 1034 F	F	TTC	S	TCG	S	TCG	1034
A 1035 F	F	TTT	A	GCG	A	GCG	1035
-1036 -	P	CCC	P	CCA	P	CCA	1036
T 1037 I	I	ATT	T	ACA	T	ACA	1037
A 1038 P	P	CCA	A	GCT	A	GCT	1038
D 1039 L	L	CTT	D	GAC	D	GAC	1039
T 1040 C	C	TGC	T	AAC	T	AAC	1040
V 1041 H	H	CAT	V	GTT	E	GAA	1041
P 1042 S	S	TCC	P	CCA	T	ACA	1042
L 1043 T	T	ACT	L	CTT	N	AAC	1043
R 1044 S	S	TCC	R	CGA	K	AAA	1044

4. Discussion

The results showed that *B.tabaci* biotype B is prevalent when moving towards Upper Egypt, the application of the tested insecticides in the current investigation in Dakahlia and Beheira Governorates *B.tabaci* where the resistance level was high, this harmonic data with **Farghaly *et .al.* (2014)**.

It was evident that, Kalyubia population was the most susceptible one amonge the all populations, followed by Fayoum, ended with Beheira population which had the highest LC_{50} which means that this population was resistant than the others.

But biotype Q is prevalent when moving towards Lower Egypt, where the lower level of resistance was developed. The lower resistance developed by white flies collected from Fayuom and Minya Governorate may be due to lower agricultural development.

According to the exposure to insecticides, through insecticide resistance to lambda-cyhalothrin has been reported in *B .tabaci* in this study, the using continues to this insecticide is effective in reducing population of *B. tabaci* **(Li *et.al.* 2009, salem *et.al.* 2009)**.

In contrast, a strong selection against pyrithroids exhibited high resistance levels (Table 2), where RR=77.08 fold and LC_{50}= 413.16 ppm after selection for the eighteen generations. **Abou-Yousef *et.al.* (2010)** found that resistance in *B.tacaci* for lambda-cyhalothrin was 18.52 fold after 13 generations, and **Fang Zhu (2007)** reported that, selected with permethrin against *Musca domestica* (L.) for 6 generations, resistance level was 1.800 fold.

The present study to estimate of realized heritability showed that resistance level was increased after 11 generations, when spray with LC50.

Estimates h^2 in conjunction with estimate of selection intensity can be used to project rates of resistance development. Prediction based on h^2 must be interpreted cautiously because h^2 of resistance to a particular insecticide can vary between conspecific population as well as within populations as a result of allele frequencies and environmental variation over time, so the predictions made from quantitative genetic theory on the basis of $G=R^{-1}$ gives valuable information to develop strategies for managing pesticide resistance **(Tabashnik, 1992)**. Studying of estimated h^2 provided some information contributing in understanding resistance characteristics in *Aphis Craccivora* which facilitate its resistance management **(Mokbel 2015)**. **El-kady and Devine (2003)** reported that Egypt is considered one of the largest consumers of pyrethroids worldwide, and most of them were used on cotton, with 10 to 12 applications per season were common in the cotton crop.

Our results showed that increasing in the resistance level of lambda-cyhalothrin-resistant *B.tabaci* populations to follow up it increasing in detoxifying enzymes, this finding is agreement with **(Zhang *et.al.* 2015)**, **Kandil *et.al.* (2008)** reported that piperonyl Butoxide (PB) synergized profenofos toxicity in resistant strain of *B.tabaci* and the role of diethylmaleate (DM) as inhibitor glutathione-S- transferases was clear in the case of profenofos resistant strain, **Fang Zhu (2007)** showed that premethrin resistant-strain of house fly was treated with (PB) an inhibitor of cytochrome P_{450}, the resistance was reduced from 1.800 to 100 fold, **Jiang *et al.* (2011)** indicated that two strains (Changi and Qappal) populations exhibited very high to moderate levels of resistance to cyhalothrin and deltamethrin and synergistic effects of triphenyl phosphate diethylmaleate and piperonyl butoxide on cyhalothrin and carbosulfan in (Changi) population reveated that cylochrome p_{450} were involved in the resistance to cyhalothrin but not carbosulfan, in addition, biochemical (elevated CarE and Cyochrome P_{450}-dependent monoxygenase and GST activities) indicated that the population collected from Upper Egypt Governorates especially Dakahlia and Beheira possess high level of metabolic resistance to pyrethroides, in contrast Lower Egypt *Governorates* especially Fayoum and Minya. The involvement of both oxidative and hydrolytic pathways in pyrethroid detoxification in *B.tabaci* has been previously shown in numerous white fly insecticide resistance studies worldwide and the contribution of that detoxification pathways pyrethroid resistance is comparable to that identified in our study **(Ishaaya *et.al* 1987, Dittrich *et. al.* 1990, Riley *et. al* .2000. Roditakis *et. al.* 2006)**

In many into the frequency of the sodium channel resistant mutations in a global collection of *B.tabaci* Band Q biotype strains, L925I was found in both biotypes while T929V mutation occurred in Q biotype strains **Alon *et.al.* (2006)** and in additions, the examination of the sequence of a region flanking the two mutations in the same study suggests than the L925I mutation occurred independently in Q and B biotypes supporting the evidence of genetic isolation between them **(Alon *et. al* 2006)**. **(Jiang *et .al* 2011)** showed that resistance to pyrethroids is due to a sex linked factor and the sex determination system in *leptinotarsa decemlineata* is XO, this means that the resistant phenotype will therefore be expressed in all males carrying the resistance-conferring allele and all the heterozygous (MW) individuals are females, consequently, more males survived under the selection pressure of pyrethroids and demonstrated that point mutations of S29 LG in the Ache and L1014F in the LdVsccl are responsible for, at least partially the resistance to carbamates and pyrethroics in *leptinotarsa decemlinaeata* in some field populations.

Our results demonstrated that there are many changing in the amino acid, where there are similar between sequence of Para sodium channel gene for the lambda-cyhalothrin-resistant strain and some field populations especially *Upper* Egypt may be due to use randomly for insecticides, and variation in culture crops and the impact of the geographic distance and/ or of the adaptation to the host plant on gene flow, which might dramatically of affect the spread of resistance alleles.

Therefore, our studies and the documented results **(Farghaly 2013, and Jiang *et. al.* 2014)** recommended that rotation between insecticides with different modes of action and without cross-resistance may reduce the development of insecticide resistance.

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الملخص العربي

دور جين قناة الصوديوم في تحديد مقاومة الذبابة البيضاء المجمعة من بعض محافظات مصر لبعض المبيدات

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تم تجميع عينات الذبابة البيضاء من سبع محافظات مختلفة في مصر وهي : القليوبية ، الدقهلية ، المنوفية ، الغربية ، المنيا ، الفيوم ، البحيرة ومقارنتها بسلالتين إحداهما تم عمل ضغط انتخابي لها بمبيد ثياهالوثرين والأخري سلالة معملية حساسة لم تتعرض لأي مبيدات .

أوضحت الدراسة ان عشائر محافظات الدقهلية والبحيرة أظهرت مستوي عالي من المقاومة لمبيدي الثياهالوثرين والسيبرميثرين، بينما عشائر محافظات الفيوم والقليوبية أظهرت مستوي أقل بكثير من نظرائهما .

تم انتخاب السلالة المقاومة لمدة ١٨ جيل معمليلا وقد تبين الآتي :

عند الانتخاب المعملي وجد ان المقاومة تتزايد بنسب ثابتة حتي الوصول الي الجيل ١٨ بقيمة ٤١٣,١٣ جزء في المليون. وبحساب نسب المقاومة تبعا للمعادلة الوروثية (H^2) وجد ان المقاومة في الحقل تظهر بعد الجيل ١١ عند الرش بالتركيز القاتل لنصف العشيرة ، بينما عند استخدام التركيز الموصي به تظهر المقاومة بعد الجيل الرابع .

وقد تم ايضا استخدام المنشطات علي السلالة المقاومة لمبيد لامبادا- ثياهالوثرين .وايضا تم دراسة التأثيرات البيوكيميائية علي السلالات المجمعة من المحافظات المختلفة والسلالة المقاومة و كذلك السلالة المعملية الحساسة والتي اظهرت الآتي :

أن انزيمات CarE , Esrerases , Cytochrome 450 تلعب دورا مهما في المقاومة لمجموعة المبيدات المخلقة بيريثرويد وايضا تم دراسة جين Para sodium channel لكل العينات المجمعة والسلالة المعملية الحساسة والمقاومة ، وقد أظهر PCR تتابع جيني يطابق التتابع الجيني للسلالة الموجودة في بنك الجينات بنسبة ٩٤ %. وايضا قد حدد تباين في النيوكليوتيدات والتي قد تكون احد اسباب المقاومة او الحساسية Para sodium channel تجاه مجموعة البيروثيريدات .