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MONITORING OF RESISTANCE IN FOUR FIELD STRAINS OF CULEX PIPIENS USING BIOASSAY AND AGAR GEL ELECTROPHORESIS TECHNIQUES.

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ABSTRACT

A survey of the susceptibility level in larvae of Culex pipiens conducted at four areas representing the Alexandria governorate, namely Abou-Ker, El-Amria, Moharram-Bey and Sidi-Bishr. The standard bioassey technique of the WHO was used in larvae treatments to follow the development of resistance phenomenon. The susceptibility levels for ten insecticides representing the main groups of insecticides are fluctuated around increasing or decreasing with no sing of resistance. The susceptibility level showed that two out of the four localities revealed tolerance to malathion and the most tolerant population was Sidi-Bisher followed with Abou-Ker. Likewise tolerance to fenitrothion, carbaryl and methomyl was observed in two strains (Moharram-Bey and Sidi-Bisher). Sidi-Bisher population is considered the most tolerant population either for organophosphates or carbamates.

A Test for the determination of the esterase responsible to organophosphate resistance, in a single mosquito using agar gel electrophoresis technique showed a close correlation between the frequency of this esterase and level of tolerance in the tested strains.

INTRODUCTION

Bioassay methods are useful in monitoring resistance for many species of insect pests. Many investigators developed and used techniques based on biochemical determination of esterase in monitoring resistance for organophosphate or carbamate insecticides. Georghiou & Saito, 1983 and Moustafa & Abdel-Rahman, 1988 described a simple methods to detect insect resistance to organophosphate insecticides by examining insect esterase activity. Devonshir et al. 1988 used the immunological technique to detect insecticide resistance in Myzus persicase. The levels of resistance to organophosphate, carbamate and synthetic pyrethroid insecticides were reported in Anopheles albimanus and C. pipiens (Georghiou et al (1975)and El-Tarabolsy (1984)

The aim of this course of work is to evaluate the susceptibility of Culex pipiens field strains to certain insecticides from the three major groups: organophosphates (Op's), carbamates and synthetic pyrethroids comparing with the susceptible (laboratory) strain. The relationship between the activity of esterases and C. pipiens tolerance or resistance is also recorded in two collections.

MATERIALS AND METHODS

Insects

larvae of Culex pipiens, were collected from four regions in Alexandria Governorate (Abou-Ker, Sidi-Bishr, Moharram Bey and El-Amria). A susceptible (laboratory) strain was supplied by the high Institute of Health, Alexandria, Egypt, and considered the reference strain in toxicity and biochemical studies. The collected colonies were maintained at a constant temperature, 27 ± 1°C, through out the course of this work. The larvae were reared until pupation and adult

emergence took place for maintaining the stock culture. Fourth instar larvae of the first generation was used for bioassay and adults for the biochemical studies.

Insecticides.

1)**Synthetic pyrethroids.**

- Cypermethrin (CCN 52), 20% E.C. was from Sumitomo Chemical Co. Japan.
- Deltamethrin (RUP 962), 2.5% E.C was obtained from Shell Co.
- Permethrin (Kail) 50% E.C. was obtained from I.C.I Co. England.
- Tetramethrin (Tetramethrin) 25% E.C. was obtained from Sumitomo Chemical.Co. (Japan).

2)**Organophosphate**

- Chlorpyrifos (Dursban), 48 % E.C. was obtained from Dow Chemical Co.
- Diazinon (Diazinon) 25 % E.C was obtained from Ciba Geigy Ltd. Co. Switzerland
- Fenitrothion (Sumithion) 50% E.C. was obtained from Sumitomo Chemical Co. Ltd. (Japan)
- Malathion (Malathion) 57% E.C was obtained from Cyanamid Co.(U.S.A).

3) **Carbamates.**

- Carbaryl (Sevin), 85% W.P was obtained from Union Carbide Co.(U.S.A)
- Methomyl (Lannate) 90% S.P was supplied from Du Pont Company.

Suseptibility tests

The susceptibility tests were carried out according to the method described by the WHO Expert Committee on Insecticides (1963) with slight modifications. Every concentration was replicated three times. Early fourth-instar larvae were placed in plastic cups (180 ml. capacity) containing 100 ml of tap water.

The tested insecticides were dissolved in acetone and prepared in the form of aqueous solutions containing the required concentration. Each cup received twenty five

larvae and left under laboratory conditions. The percentage mortalities were recorded twenty four hours after exposure and compared with those of controls under the same conditions. LC_{50} values were estimated from the lines fitted on logarithemic probability paper. The slopes and confidence limits were determined statistically as discribed by Litchfield and Wilcoxon (1949).

Determination of esterase activity.

The method is based on qualitative biochemical determination of esterase activity as recorded by Pasteur et al (1981), with minor modifications to suit our laboratory conditions. One gm agar and 0.5 gm polyvinylpyrrolidon (k-90) were dissolved in 100 ml boric buffer (pH 8)and heated. This hot solution is enough to fill 2 glass plates of 17 X 20 Cm dimentions. Single adult of *C. pipiens* was crushed with a glass rod on glass plate in 10 ul distilled water. A strip of 8X5 mm filter paper was immersed in the resultant adult homogenate. About 50 strips of filter paper were placed on an agar plate. After 30 min. conditioning in refrigerator at 4-5°C. The agar plate was placed on the agar gel electrophoresis device for 4 hours. The strips were removed and the plate was sprayed with 1% acetone solution of the B or - naphthyl acetate. The plate was incubated at 37°C for 30 min and few drops of 0.4% aqueous solution of fast blue salt B were added. The determination included susceptible and field strains at the same time. Individuals whose reaction colour is purplish are considered tolerant or resistant while individuals whose reaction colour is not apparent are considered as susceptible.

RESULTS AND DISCUSSION

Susceptibility levels of Culex pipiens larvae from different regions in Alexandria to Certain insecticides.

The susceptibility levels of four synthetic pyrethroid, four Organophosphate and two carbamate insecticides to larvae of Culex pipiens, from the four regions in Alexandria were studied. Fourth larval instar was bioassayed according to the WHO method (1963). Regression lines were statistically analyzed by Litchfield and Wilcoxon method (1949) and LC_{50} values were deduced as ug/Litre with their confidence limits and slope values.

1) Abou-Ker strain:

Toxicity of cypermethrin, deltamethrin, permethrin tetramethrin, chlorpyrifos, diazinon, fenitrothion, malathion, carbaryl and methomyl were determined by dipping method to the fourth instar larvae of susceptible and Abou-Ker field strain of C. pipiens. LC_{50} values were calculated from Ld-p line. The obtained results were statistically analyzed and recorded in Table (1). Ratio of LC_{50} values for Abou-Ker starin to susceptible one was calculated based on (F/S) ratio. The results showed that the Abou-Ker strain had slight tolerance to malathion (2.1 fold), while slightly change from susceptibility was detected for fenitrothion, deltamethrin, chlorpyrifos, carbaryl and permethrin, with tolerance ratios of 1.85, 1.65, 1.47, 1.35 and 1.17 fold, respectively. On the other hand cypermethrin, tetramethrin, diazinon and methomyl showed more efficiency to this strain with F/S ratios of 0.88, 0.88, 0.85 and 0.65 fold, respectively.

2) Sidi-Bishr strain:

Susceptibility levels of sidi-Bishr strain to the same tested insecticides were

Table (1): **Susceptibility of fourth instar larvae of Culex pipiens to certain insecticides.**

Insecticides	Strains						
	Susceptible			Abou-Ker			
	LC_{50} ug/l	(Confid. limits)	Slope	LC_{50} ug/l	(Confid. limits)	Slope	Mean* F/S*
Cypermethrin							
1st collection	1.20	(1.10 - 1.30)	3.56	1.10	(0.96 - 1.25)	2.12	0.88
2nd collection	1.20	(1.13 - 1.27)	4.95	1.00	(0.88 - 1.10)	2.18	
Deltamethrin							
1st collection	0.26	(0.23 - 0.28)	3.32	0.29	(0.26 - 0.32)	2.32	1.65
2nd collection	0.24	(0.22 - 0.26)	4.69	0.52	(0.46 - 0.56)	2.46	
Permethrin							
1st collection	2.10	(1.90 - 2.30)	3.40	2.40	(2.02 - 2.80)	1.90	1.17
2nd collection	2.00	(1.90 - 2.10)	4.83	2.40	(2.10 - 2.70)	2.15	
Tetramethrin							
1st collection	11.00	(10.0 - 12.0)	3.18	11.0	(8.70 - 15.0)	2.31	0.88
2nd collection	12.00	(11.1 - 12.9)	3.78	9.0	(8.00 - 10.0)	2.50	
Chlorpyrifos							
1st collection	5.00	(4.60 - 5.40)	3.69	5.60	(4.90 - 6.30)	2.18	1.47
2nd collection	6.10	(5.70 - 6.60)	4.26	11.00	(9.80 -12.20)	2.60	
Diazinon							
1st collection	12.00	(11.0 -13.00)	3.51	9.60	(8.30 -11.00)	2.06	0.85
2nd collection	11.00	(10.2 -11.80)	4.40	10.00	(9.00 -11.00)	2.78	
Fenitrothion							
1st collection	19.00	(18.0 -20.00)	4.19	23.00	(20.0 -26.0)	2.31	1.85
2nd collection	20.00	(19.0 -21.00)	4.30	49.00	(45.0 -53.0)	3.27	
Malathion							
1st collection	75.00	(70.0 -79.00)	4.50	100.00	(58.0 -117.0)	1.77	2.10
2nd collection	78.00	(73.0 -82.00)	4.92	220.00	(190.0-250.0)	2.37	
Carbaryl							
1st collection	1000	(930 - 1060)	4.56	1100	(850 -1180)	1.95	1.35
2nd collection	1000	(941 - 1060)	4.71	1600	(1440 -1780)	2.63	
Methomyl							
1st collection	50.0	(46.0 - 54.0)	3.61	25.0	(21.0 -29.0)	1.96	0.65
2nd collection	40.0	(37.0 - 43.0)	4.25	32.0	(29.0 -35.0)	2.82	

$$F/S^{*} = \frac{LC_{50} \text{ for field strains}}{LC_{50} \text{ for susceptible strain}}$$

* Mean of F/S in two collections

Table (2): **Susceptibility of fourth instar larvae of Culex pipiens to certain insecticides.**

Insecticides	Strains								
	Moharram-Bey			Sidi-Bisher			El-Amria		
	LC_{50} (Conf.limits) ug/l	Slope	Mean**F/S*	LC_{50} (Conf.limits) ug/l	Slope	Mean**F/S*	LC_{50} (Conf.limits) ug/l	Slope	Mean**F/S
Cypermethrin									
1st collection	1.60(1.30-1.80)	2.19	1.06	1.50(1.27-1.76)	2.26	1.34	1.00(0.85-1.10)	2.02	0.92
2nd collection	1.00(0.80-1.12)	2.40		1.70(1.50-1.90)	3.01		1.20(1.05-1.25)	2.15	
Deltamethrin									
1st collection	0.24(0.20-0.28)	1.93	0.91	0.30(0.26-0.35)	1.79	1.41	3.00(0.25-0.35)	2.02	1.05
2nd collection	0.22(0.19-0.25)	2.08		0.40(0.35-0.45)	2.14		0.23(0.20-0.26)	0.24	
Permethrin									
1st collection	2.80(1.70-2.30)	1.84	0.98	2.30(1.90-2.70)	1.92	1.25	2.50(2.10-3.00)	1.72	1.15
2nd collection	2.00(1.70-2.30)	2.01		2.80(2.40-3.20)	2.27		2.20(1.90-2.50)	1.89	
Tetramethrin									
1st collection	11.00(9.40-12.30)	2.02	0.96	13.00(11.00-15.00)	2.12	1.84	12.00(10.2-13.8)	2.28	1.05
2nd collection	11.00(9.80-12.30)	2.47		30.0(27.00-33.00)	2.78		12.00(10.7-13.5)	2.50	
Chlorpyrifos									
1st collection	5.20(4.60-5.90)	2.28	1.54	5.00(4.00-5.60)	2.39	1.12	6.90(6.00-7.90)	2.26	1.25
2nd collection	12.50(10.0-13.3)	2.74		7.60(6.80-8.40)	2.71		6.80(6.10-7.60)	2.59	
Diazinon									
1st collection	9.00(7.90-10.20)	2.20	1.12	12.00(10.5-13.7)	2.12	1.18	16.0(13.7-18.5)	2.13	1.33
2nd collection	17.00(15.4-18.7)	2.90		15.00(13.0-17.0)	2.49		15.0(13.5-16.6)	2.71	
Fenitrothion									
1st collection	20.00(17.80-22.40)	2.86	1.15	25.00(22.00-28.00)	2.59	2.06	25.0(22.2-27.7)	2.96	1.41
2nd collection	25.00(23.00-27.00)	3.52		56.00(49.00-64.00)	2.08		30.0(27.0-33.0)	2.18	
Malathion									
1st collection	90.00(81.00-99.00)	3.05	1.63	120.0(104.0-138.0)	2.28	2.41	85.0(74-96)	2.17	1.57
2nd collection	160.00(148.0-172.0)	3.65		250.0(220.0-280.0)	2.52		155.0(138-174)	2.58	
Carbaryl									
1st collection	950 (850-1060)	5.58	1.08	1100 (950-1250)	2.09	1.15	700.0(615-795)	2.86	0.95
2nd collection	1200 (1085-1325)	2.86		1200 (1076-1338)	2.62		1200.0(1100-1300)	3.32	
Methomyl									
1st collection	62.00(53.00-71.30)	2.27	1.39	50.00(43.0-57.0)	2.18	1.13	65.0(56.0-75.0)	2.17	1.09
2nd collection	62.00(55.00-69.00)	2.48		50.00(44.0-56.0)	2.62		35.0(31.0-39.9)	2.18	

*F/S = $\frac{LC_{50} \text{ field strains}}{LC_{50} \text{ for susceptible strain}}$

**Mean of F/S in two collection.

also evaluated as well as in Abou-Ker strain. LC_{50} values were calculated from Ld-p lines for susceptible strain and for Sidi-Bishr strain. The results showed that this strain had slight tolerance to malathion and fenitrothion. While tetramethrin, deltamethrin, cypermethrin, permethrin, diazinon, carbaryl methomyl and chlorpyrifos slightly changed from susceptibility. The tolerance ratios were 2.41, 2.06, 1.84, 1.41, 1.34, 1.25, 1.13, 1.18, 1.15 and 1.12 fold, respectively (Table 2).

3) **Moharram-Bey strain:**

Permethrin, tetramethrin and deltamethrin showed more efficiency to this Moharram-Bey strain comparing with the susceptible one, with F/S ratios of 0.98, 0.96 and 0.91 fold, respectively, while the strain had slight tolerance to the rest of tested insecticides (Table 2)

4) **El-Amria Strain:**

The results showed that none of the tested insecticides induced resistance or tolerance in this El-Amria strain. Cypermethrin and carbaryl were more efficient to this strain than to the susceptible one, while the strain had slight changing from susceptibility to malathion, diazinon, chlorpyrifos, permethrin, methomyl, deltamethrin and tetramethrin.

Cypermethrin, tetramethin diazinon and methomyl showed more efficiency to Abou-Ker strain comparing with the other insecticides, while Moharram-Bey strain showed slight sensitivity to permethrin, deltamethrin and tetramethrin. Cypermethrin and carbaryl showed efficiency in El-Amria strain.

The Anopheles albimaus, exhibited tolerance to OP's in June 1970, further collections from the same area (El-Salvador)

in February 1971, showed resistance to a variety of insecticides (Georghiou et al. 1975). Moustafa et al., 1977, investigated the levels of r sistance of S. littoralis in Alexandria vicinity from Abees and Maryute for different compounds. The two field strains showed tolerance and cross tolerance to endrin, phosvel, methyl parathion, bromophos, dichlorfos, malathion and carbaryl.

Qualitative determination of esterase activity.

Esterase activity for the adults of susceptible and four field strains of Culex pipiens was determined. The esterase activity was classified into 3 groups in account of the degree of the reaction taking part between enzyme and the substrate. These groups are (A) for no reaction; (A*) for medium intensity of the colour and (A**) for high intensity of the colour, as indicated by Georghiou and Saito (1983). Individuals whose reaction is nil are considered susceptible while individuals whose reaction is purplish are considered resistant. Table(3) presents the esterase activity in Abou-Ker, Sidi-Beshr, Moharram-Bey and El-Amria strains as well as susceptible one of C. pipiens. The results showed that esterase activity in the four field strains was higher than that in the susceptible strain. Sidi-Bishr strain had higher esterase activity (54.20%) followed with Abou-Ker, Moharrm-Bey and El-Amria.

In Conclusion:

The tested strains showed more tolerant to organophosphate and carbamate insecticides comparing with synthetic pyrethroids.

There is a close correlation between the frequency of individuals which had esterase activity and the level of tolerance in the tested strains.

Table (3): **Relation between activity of β-esterase and resistance in *Culex pipiens* adults.**

Strains	No. of insect used	Activity of esterase A^{--}	A^{-}	A	% frequency of high activity
Susceptible	64	0.0	55.0	9.0	0.00
Abou-Ker	48	21.0	22.0	5.0	43.75
Sidi-Beshr	48	26.0	22.0	0.0	54.20
Moharram-Bey	48	18.0	25.0	5.0	37.50
El-Ameria	48	15.0	28.0	5.0	31.25

A = No Activity, A^{-} Low Esterase Activity

A^{--} High Esterase Activity.

The tolerant and slight tolerant in mosquito strains to some insecticides may be explained as a result of the wide application of these insecticides in agricultural areas around Alexandria, or in Alexandria city by the health officers and the inhabitants for insect-control. By-time as a results of the insecticidal pressure, the susceptibility levels of this insect has been changed and the phenomenon of tolerance and slight tolerance to certain insecticides appeared.

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الملخص العربى

رصد المقاومة فى اربع سلالات حقلية من بعوضة الكيولكس بينز باستخدام طريقتى التقييم الحيوى والهجرة الكهربية

تم حصر مستوى الحساسية لبرقات البعوض من النوع كيولكس بينز والتى جمعت من اربع مناطق تمثل محافظة الاسكندرية وهى ابو قير والعامرية ومحرم بك وسيدى بشر واستخدمت طريقة التقييم الحيوى الموصى بها من منظمة الصحة العالمية لمعاملة اليرقات، وذلك لدراسة تطور ظاهرة المقاومة بها .

وقد اظهرت النتائج تذبذب مستويات الحساسية لعشرة مبيدات حشرية ممثلة لمجاميع المبيدات الحشرية الرئيسية ولم تظهر اى سلالات مقاومة لهذه المبيدات، بينما اظهرت سلالتين من الاربعة سلالات المختبرة تحملا لمبيد الملاثيون وكانت اكثر السلالات تحملا هى سلالة سيدى بشر يليها سلالة ابو قير وبالمثل اظهرت سلالتى محرم بك وسيدى بشر تحملا لكل من مبيد الفنتروثيون والكارباريل والميثوميل .

ويمكن القول بان سلالة سيدى بشر كانت اكثر السلالات تحملا لمبيدات الفوسفور العضوية والكارباميات . وتم تقدير نشاط انزيمات الاستبراد المسئولة عن المقاومة لمبيدات الفوسفور العضوية فى اليرقات الفردية باستخدام طريقة الهجرة الكهربية وكانت هناك علاقة ارتباط بين نشاط الاستبريز ومستوى التحمل فى السلالات المختبرة .