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RESISTANCE CHARACTERIZATIONS OF BAYGON INSECTICIDE IN *Culex pipiens* LARVAE

By

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ABSTRACT

In the present study the *Culex pipiens* larvae were subjected to continuous laboratory selection with baygon for 15 successive generations. Resistance to baygon was increased gradually throughout the successive generations of selection, but after 12 generations of selection the level of resistance was suddenly increased to 49.5 times higher than the parent larvae. Moreover, the resistance level was found to be 308.7 times more resistant than the parent at the end of selected generation (F_{15}). The slope function values of the regression lines were gradually declined with selection indicating progression of the development of resistance. This *C. pipiens* –resistant strain (F_{15}) was left without propoxur treatment pressure for 18 successive generations. The results showed that the levels of resistance gradually decreased during the successive relaxed generations compared to the normal strain. It reached about one-tenth the level of R-strain after 18 relaxed generations. The obtained results also showed that the pattern of cross - resistance to some insecticides and IGRs in baygon –resistant strain indicating no clear cross-resistance between baygon and all tested insecticides (sumithion 3 fold, daiazinon 1.46 fold, sumicidin 1.7 fold & permethrin 1.22 fold), only dursban insecticide recorded a positive correlation with 4.8 fold. The IGRs results showed also no correlation to the baygon –resistant strain.

INTRODUCTION

The extensive use of the chemical insecticide all over the world for the control of economic insect pests and medically important vectors of many diseases were led to the development of high levels of resistance to these used insecticides. According to World Health Organization (W.H.O.) this phenomenon constitutes a serious threat to the successful control of such pests. Resistance has been reported not only by the new synthetic insecticides but also to the insect growth regulator, chemosterilants and even some of the biological control agents (Sawicki, 1979). Serious attention has been paid to study this phenomenon from various points of view hoping to overcome it as reported by Mouchet, (1964) and Georghiou *et al.*, (1966). Lee *et al.* (1996) reported that the larvae and adults of *Culex tritaeniorhynchus* showed high resistance to all the highest diagnostic dose of the organophosphate and carbamte insecticides with mortality range of 0.0 apprx 36.0% in Pyongtaek and in Musan. Nielsen-Leroux *et al.*, (1997) reported that field *Culex pipiens pipiens* (L) mosquitoes that were collected after a control failure with Spherimos in southern France developed high resistance (gt 10,000fold) to *Bacillus sphaericus* crystal toxin after 8 generations of laboratory selection. Gonzalez *et al.* (1996) studied the resistance change to different insecticides in *Culex quinquesfasciatus* strain select at the laboratory with doses of pyrethroid lambda-cyhalothrin that would cause a larva mortality of 90 %, it was attained an increase of the resistance to this insecticide of 144.5 times compared with the original level, and it was obtained a resistant strain (287x). There was an increase of the levels of resistance to methyl-pyrimifos (2.4 times), propoxur (6 times), DDT (5,2 times), clorpirifos (22 time), cypermethrin (67.5 times), and deltamethrin (20.2 times).

The present study aimed to investigate the resistance of *Culex pipiens* larvae to the baygon insecticides, so that the knowledge of the rate of development and reversion of insecticide resistance, also its cross resistance with other insecticides and the insect growth regulator would be of a valuable information in the research of the *Culex pipiens* larvae control.

MATERIALS AND METHODS

Chemicals used

The chemical insecticides used were the carbamate, baygon (technical grade

97%), the organophosphates, sumithion (technical grade 96.5%);diazinon (technical 92%) and dursban (technical grade 56.4%E.c.), the synthetic pyrethroids ,permethrin (technical 92.6%) and sumicidin (technical 95.5%) and the insect growth regulator, bay sir 8514 (E.c. 6.5%) & IKI (Ec. 5%)

The different concentrations of the insecticides or the insect growth regulators were prepared from the stock solution by dissolving a known amount of each of them in the distilled water. Usually ethyl alcohol was used as a solvent for all the chemicals tested for making the stock solution, except the insect growth regulators, which they were dissolved in acetone. Concentrations were expressed in parts per million (p.p.m); in order to calculate the Lc`s.

Test insect

The larvae of *C. pipiens* were obtained from Miet El-Attar village, Qualyubia Governorate. The larvae were successfully reared and maintained at the Entomology Department, Faculty of Science, Zagazig University, Benha branch. The field-collected larvae were reared without being exposed to any insecticides or the insect growth regulators to raise their susceptibility and it was referred as the original normal susceptible strain. This strain is also considered as the base line of the selected pressured resistant strain with which the results of selections were compared. Assessment of toxicity was based on the mortality of the tested larvae. The early and late third larval instars of *C. pipiens* were exposed for 24 hours to different concentrations of baygon and/or the other tested toxicants in distilled water. Another groups of larvae were left without any treatments and served as control. Four replicates of the desired concentrations of each compound were prepared, in 250 ml glass beaker. Each beaker received 25 larvae of the early and late third larval instars according to the experiment carried out. The percentages of mortality were plotted against the tested concentrations and the LC_{50} values were determined graphically. Mortality percentages were corrected by Abbott's formula (1925) if the mortality in control exceeds 10%, other wise the test must be repeated. The mortality lines were drawing by plotting the probit mortality data against the log of the concentrations used by using a logarithmic paper. The lines were fitted by eye and the median lethal concentrations (LC_{50}) were determined graphically. The slope function of the mortality lines was taken as a criterion of the degree of homogeneity of the population in its response to toxicant, according to Hoskins and Gordon (1956). The numerical value of the slope function was calculated as follows:

$$\textbf{Slope function} = \frac{LC_{84}/LC_{50}+LC_{50}/LC_{16}}{2}$$

Selection for resistance was carried out on generation by treating the third instar larvae with insecticide-water mixture. Large numbers of larvae were employed and the selection pressure was always sufficient to cause 75% mortality or more. According to the response of the treated larvae to selection, a higher concentration of the toxicant was sometimes used in subsequent generation. Later further selection was carried out for many generations until a high level of resistance, which maintained after relaxation of pressure with insecticide for three generations, is the evidence of stability of resistance. The resistant strain was compared with the normal strain to determine the resistance level, or resistance ratio by the following equation:

$$\textbf{R.R.} = \frac{LC_{50} \text{ of the resistant strain}}{LC_{50} \text{ of the normal strain}}$$

The selected strain after achieving certain levels of resistance was left without insecticide pressure. Relaxation was carried out on a sub- colony of the selected generations and the reversion of resistance in *C. pipiens* larvae was measured after three generation of relaxation.

The selected strain after attaining high levels of resistance was tested for cross-resistance to the other types of insecticides. The resistance levels were estimated. In comparisons of the relative resist abilities as called resistance ratios (LC_{50} of resistant strain/LC_{50} of susceptible strain), differences of 5-fold or more were considered as indicating positive correlation (true resistance differences); those between 1 and 4-fold (tolerance differences) as indicating no correlation and any differences less than one as representing a probable negative correlation.

The statistical analysis

The statistical analysis was performed using t-test.

RESULTS

Results of the susceptibility level of the original normal susceptible strain to baygon and that subjected to baygon-selection pressure from F_1 to F_{15} are shown in the table (1) and graphically illustrated in figure (1). The obtained data indicated that the LC_{50} was increased to 0.69ppm indicating a slight development of resistance. The level of resistance of the third generation is increased to 3.5 times more than the normal strain (parent), and the slope function of the LD-p line is markedly declined after selection indicating progression of the development of resistance. Moreover complete death was occurred at the concentration higher than 80 p.p.m in the fifteen-generation, indicating progression in the development of resistance to about 308.7 times higher than the original individuals. The population is considered in the state of actual resistance.

The rate of reversion of baygon-resistance are presented in table (2) and graphically illustrated in figure (2). The results clearly indicated that the LC_{50} values were decreased gradually during successive generations of relaxation. The resistance level of F_{18} was recorded 54.80 times more resistance than the parent larvae. The slope functions were almost of low values. In general their values are more or less similar to be 1.83, 1.79, 1.98, 1.80 & 1.84 for F_3, F_6, F_{12}, F_{15} and F_{18}, respectively. However, the F_9 value was 2.05 although insignificant. The rates of relaxation as expressed in relative reversibility to baygon-resistant strain were gradually decreased during the successive relaxed generations compared to the normal strain. It reached to about one tenth after 18 relaxed generations compared to the resistant-strain.

The pattern of cross-resistance of baygon-resistant strain to several insecticides and two insect growth regulators are indicated in tables (3&4)

respectively. Data in table (3) indicated that the relative resistibility of baygon selected and normal strains showed no correlation of baygon resistant to the tested organophosphorous insecticides, hence the resistance levels were only recorded 3 times for sumithion and 1.46 for diazinon indicating that the tolerance difference showed no correlation, whereas 4.8 times for dursban indicating a positive correlation on the basis of p values that were no significant differences in the mortality percent between the normal strain (parent) and resistant strain ($p>0.05$), whereas there was a lower significant difference for dursban ($p<0.05$).

Results of relative susceptibilities of normal and selected-baygon strains to the pyrethroides both the sumicidin and permethrin reveals that baygon-resistant strain is quite susceptible to both of them, where its level of resistance were 1.7 & 1.22 times, respectively, indicating no correlation. There were no significant differences in the mortality percents between the normal strain and the baygon resistant strain to the tested pyrethroides ($p>0.05$).

Data in table (4) reveal that the baygon-resistant strain is quite susceptible to both IKI and bay sir. The levels of resistance after 24, 48 & 72 hours exposure periods for IKI were 1.48, 1.73 & 1.76 times and 1.56, 1.81 & 1.32 times for bay sir indicating no correlation. Also there were no significant differences in the susceptibility levels between the normal strain (parent) and the resistant strain, based on the p values ($p>0.05$).

Table (1): Changes in susceptibility of *C. pipiens* larvae during selection to baygon insecticide from F_1 up to F_{15}.

Generation tested	$Lc_{50's}$ (ppm)	Slope function	Resistance level
N	0.20	4.33	—
F_3	0.69	1.22	3.45
F_6	0.84	2.46	4.20
F_9	1.65	1.85	8.25
F_{12}	9.89	1.62	49.45
F_{15}	61.75	1.49	308.70

N= Normal strain (parent).

Fig.(1):

Resistance levels of *C. pipiens* larvae during selection to baygon(F1-F15)

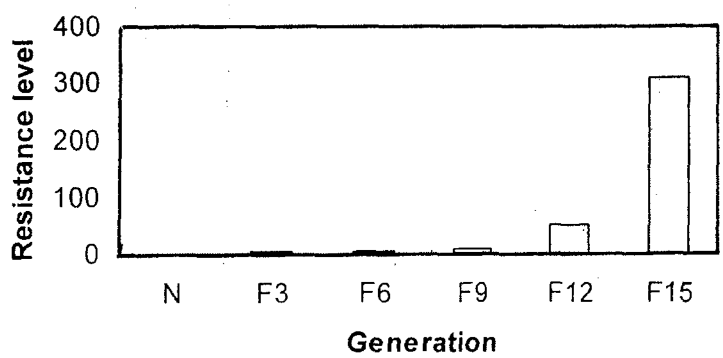

Table (2): Relative reversibility of baygon-resistance in *C. pipiens* larvae after 18 relaxed generations.

Generation tested	Lc_{50}'s (ppm)	Slope function	Resistance level	Relative reversibility
R	61.75	1.49	308.70	1.0
F_3	60.18	1.83	300.90	0.97
F_6	40.70	1.79	203.50	0.66
F_9	30.61	2.05	153.05	0.50
F_{12}	21.18	1.98	105.90	0.34
F_{15}	16.86	1.80	84.30	0.27
F_{18}	10.96	1.84	54.80	0.18
N	0.20	4.33	—	—

R= Baygon-resistant strain after 15 generation of selection.

N= Normal strain (parent).

$$\text{Relative reversibility} = \frac{LC_{50}\text{ of the reversed strain}}{LC_{50}\text{ of the resistant strain}}$$

fig.(2):

Resistance levels of *C. pipiens* larvae during relaxation of baygon after 18 generation.

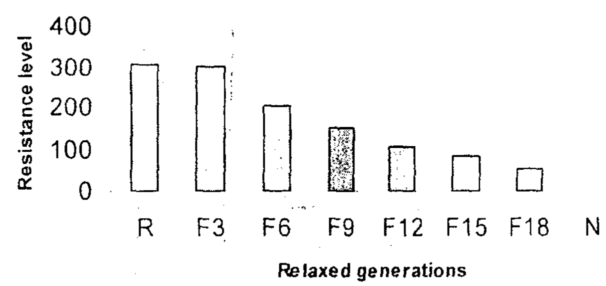

Table (3): Relative susceptibility of normal and baygon selected strain of *C. pipiens* larvae to some insecticides.

Insecticide tested	Lc_{50} s (ppm)of normal strain	Lc_{50} s (ppm)of resistant strain	Slope function of normal strain	Slope function of resistant strain	Resistance level	Tolerance difference
Sumithion	0.015	0.045	2.59	2.24	3.0 fold	No correlation
Diazinon	0.26	0.38	2.16	2.25	1.46 fold	No correlation
Dursban	0.0015	0.0072	2.4	1.33	4.8 fold	Positive correlation
Sumicidin	0.002	0.0034	6.33	4.30	1.7 fold	No correlation
Permethrin	0.0018	0.0022	2.11	2.26	1.22 fold	No correlation

Table (4): Relative susceptibility of normal and baygon selected strain of *C. pipiens* larvae to two insect growth regulators at different time of exposure (24,48&72 hours).

Insect growth regulators used	Exposure Time (hours)	Lc_{50} s (ppm)of normal strain	Lc_{50} s (ppm)of resistant strain	Slope function of normal strain	Slope function of resistant strain	Resistance level	Tolerance difference
IKI	24	1.30	1.93	1.85	2.06	1.48	No correlation
	48	0.30	0.52	2.92	3.42	1.73	
	72	0.13	0.23	3.38	3.25	1.76	
Bay sir	24	1.79	2.80	3.63	3.11	1.56	No correlation
	48	0.26	0.47	3.36	4.76	1.81	
	72	0.19	0.25	3.16	5.20	1.32	

DISCUSSION

It has been established that insecticides resistance is an inherited character, which is an example of evolutionary change. The insecticide acting as a powerful selective agent for concentrating resistant mutants that are present at low frequencies in the original population, with continued selection, the relative frequency of one or a few number of the major genes that contribute directly towards fitness is increased. In the present studies the development of resistance was raised with very low levels at the beginning. With continuous selection as the frequency of the major gene (s) for resistance in surviving population increased, the insects become better organized genetically. Intensive selection pressure provides a more number of survivors enough to maintain genetic variability, Georghiou, (1962). Our findings clearly revealed that the baygon-resistant larvae showed substantial increase in resistance, which reached about 300 fold compared to the original level of the normal strain. Comparison of these data revealed that high resistance to baygon occurred in the *C. pipiens* larvae is most probably cause by the interaction of several genetic factors. The obtained results are also in agreement with the findings of Bakr *et al.* (1989) who induced 3150-fold resistance in *C. pipiens* larvae to carbaryl. Yasutomi and Takahashi (1989) also induced high levels of resistance in *Culex pipiens* larvae to an organophosphorous and a carbamate insecticide. Georghiou *et al.* (1966) investigated the rate of development and other characteristics of baygon-resistance in *C. pipiens fatigans* larvae and found that selective pressure for 35 generations induced more than 25 fold the resistance. On the other hand selective pressure on mosquito adults for 24 generations resulted in only 2.7-fold the resistance. The authors stated that this contradiction might be due to the accidental exclusion of suitable genetic material from the initial population or from subsequent generation rather than to the absence of resistance potential of a particular insecticide in the species as a whole. Hamed *et al.* (1991) whom made a laboratory selection of *C. pipiens* larvae by fenitrothion pressure for 24 generations, which resulted in 25.67, fold increase in resistance to this compound. Also, Bisset *et al.* (1997) subjected *Culex pipiens quinquefaciatus* say, from Cuba to lambda-cyhalothrin selection to evaluate the usefulness of this pyrethroid insecticide for mosquito control. They found high resistance developed after 6 generations of selection, little or no cross-resistance was observed to other pyrethroids (deltamethrin and cypermethrin), to a carbamte (propoxur) and to some organophosphates (chlorpyrifos and pirimiphos-methyl), but high cross-resistance was found to malathion (organophosphates).

Reversion of baygon-resistance in *C. pipiens* larvae was obtained during relaxed generations. The results indicated that the baygon-resistant strain was gradually regressed towards its original level of susceptibility. Published results on reversion of carbamate resistance in *C. pipiens* larvae are rare. However, some of the early findings on reversion of insecticide resistance in mosquitoes revealed that resistance of dieldrin in *C. pipiens* was regressed towards the original level of susceptibility as stated by Betzios, (1977), while Georghiou *et al.* (1966) indicated that only a low degree of regression in resistance occurred during ensuing 10 generations when selection with baygon on *C. pipiens* larvae was suspended. The investigators suggested that the respective genes for resistance might be fixed in the population. Crow (1954) attempted to provide a logical explanation for the phenomenon of development and reversion of resistance; he stated that selection for the resistance should ordinarily involve the replacement of susceptible individuals with those carrying the resistance genes. One should then expect that this strain when reared in the absence of insecticidal pressure would revert to susceptibility. Since the susceptible individuals, which have biotic potential would be able to increase their proportion in the population.

The patterns of cross-resistance spectrum to seven compounds representing three groups of insecticides were studied in the baygon-resistant strain of the *Culex pipiens* larvae. In comparisons made in the present study, differences of five-fold or more were considered as indicating positive correlation, those between one and five-fold indicating no correlation, and any difference less than one-fold representing a probable negative correlation. The relative resist abilities of baygon-resistant strain to the organophosphorous in the normal strain revealed positive correlation indicating cross-resistance between baygon resistance and the organophosphorous dursban. In contrast the relative resistabilities of baygon-resistant strain and the susceptible strain revealed no correlation between baygon resistances and the resistance to diazinon (organophosphorous), sumicidin and permethrin (pyrethroides) and the insect growth regulators IKI and bay sir. However, Georghiou *et al.* (1966) demonstrated cross-resistance in *C. pipiens fatigans* strain selected with baygon to the other carbamates, which was high to closely related compounds to the baygon, but was extremely low to remotely carbamates. Also, Ben Cheikh *et al.* (1998) investigated the cross-resistance to the carbamate propoxur, they found that the chlorpyrifos-resistant population were highly resistant to propoxur. The present results are not in harmony with the results published by Baker *et al.* (1989) whom reported cross-resistance of the insect growth regulator dimilin, bay sir and chlorofluzuron with the carbamate carbaryl-resistant strain of *C. pipiens* larvae. However, they found no cross-resistance to the other insect growth regulators tested.

It may be concluded that the pyrethroid insecticides and the insect growth regulators could be used as an alternative to the carbamte baygon against the baygon-resistant strain of *C. pipiens* larvae.

In summary, based on the obtained results, it seems that, a simple control program could be planned against field-strains of *C. pipiens* larvae in their breeding sites in Qualyubia Governorate by using a number of insecticides from different groups and the insect growth regulators specially against the baygon-resistant strain of *Culex pipiens* larvae.

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صفات نمو وانحصار المقاومة ليرقات بعوضة *الكيولكس بيبينز* لمبيد البايجون

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استهدف البحث دراسة المقاومة لمبيد البايجون فى بعوضة *الكيولكس بيبينز* ونمو وانحصار المقاومة لهذا المبيد كذلك دراسة عبور المقاومة لتحديد مستويات الحساسية لمبيدات من مجموعات مختلفة على السلالة المقاومة والطبيعية.

أجريت تجارب الاختيار معمليا بتعريض السلالة الحقلية لمبيد البايجون عند مستوى ٧٥٪ إماتة وذلك لعدد من الأجيال المتتابعة مع زيادة التركيزات وإجراء اختبارات الحساسية دوريا لتحديد مستوى المقاومة والتركيز الذى يعطى ٧٥٪ إماتة وقد أمكن بهذه الطريقة الحصول على سلالة مقاومة بدرجة عالية وقد لوحظ ارتفاع مستوى المقاومة تدريجيا فى البداية ولكنها ارتفعت فجأة فى الجيل الثانى عشر إلى ٤٩.٥ مرة عن سلالة الآباء حتى وصلت فى جيل الاختيار النهائى (الجيل الخامس عشر) لحوالى ٣٠٠ مرة تقريبا كما انخفضت قيم دوال الميل تدريجيا بدرجة واضحة مشيرة إلى نمو المقاومة .

تركت السلالة المقاومة لمبيد البايجون بدون أى ضغط انتخابى لدراسة معدل انحسار المقاومة فبعد أن وصلت السلالة المقاومة للمبيد حوالى ٣٠٨.٧ مرة فى الجيل الخامس عشر تركت بدون تأثير للمبيد لعدد ثمانية عشر جيلا وقد أوضحت النتائج أن انخفاض معدلات الانحسار جزئيا بالمقارنة بسلالة الآباء حيث وصلت إلى حوالى العشر بالمقارنة بالسلالة المقاومة . وبمقارنة ذلك لوحظ وجود مقاومة فى يرقات بعوضة الكيولكس بيبينز لمبيد البايجون وربما يرجع ذلك إلى تداخل عوامل وراثية عديدة .

تم دراسة طيف المقاومة لمجموعة من المبيدات المختلفة ومنظمات النمو على السلالة المقاومة لمبيد البايجون وذلك بمقارنة مستوى المقاومة فى هذه السلالة بمستوى المقاومة فى السلالة الطبيعية للمبيدات التى استخدمت فى التجارب فقد أوضحت الدراسة أنه ليس هناك مقاومة ملازمة واضحة للسلالة المقاومة للبايجون حيث لم يلاحظ أى تغيير معنوى فى حساسية اليرقات بين السلالتين مع كل من مبيدات السوميثيون (٣ مرات) ، الديازينون (١،٤٦ مرة) والسوميسيدين (١.٧ مرة) ، والبيرميثرين (١.٢٢ مرة) ، أما مبيد الدورسبان (٤.٨ مرة) فقد أظهر زيادة معنوية طفيفة وتشير النتائج إلى وجود مقاومة ملازمة خفيفة بالنسبة للسلالة المقاومة للبايجون .

وبالنسبة لمنظمات النمو عند اليرقة أوضحت النتائج أن السلالة المقاومة كانت حساسة تماما لها ولا يوجد أى تغيير معنوى فى الحساسية بين السلالتين ويتضح ذلك من انخفاض مستويات المقاومة بعد فترات تعرض ٢٤ ، ٤٨ ، ٧٢ ساعة لـ أى كه أى (IKI) ١،٤٨ مرة ، ١،٧٣ مرة ، ١.٧٦ مرة وباى سير (Bay sir) ١،٥٦ مرة ، ١.٨١ مرة ، ١،٣٢ مرة على التوالى مشيرة إلى عدم وجود مقاومة ملازمة للسلالة المقاومة مع منظمات النمو عند بعوضة الكيولكس بيبينز .