J. Pest Control & Environ. Sci. Vol 3 No. 1 : 63 - 76 (1991)

Type of inheritance in mosquito Larvae, Culex pipiens resistant to malathion, fenitrothion and tetramethrin insecticides

By

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Abstract

Crosses between malathion, fenitrothion or tetramethrin resistant strains and susceptible strain were done. There were no differences between the two hybrids whether males or females were resistant. It means no sex linked inheritance of resistance to malathion, fenitrothion and tetramethrin. Results of first generation hybrid revealed that inheritance of the insecticide resistance in C. pipiens is due to partially dominant gene(s). Also the character is autosomal or each parent could induce resistance to the hybrid.

Concerning the second generation of the malathion and fenitrothion resistant strains, there was a deflection in the complete LD - P line around 25% kill thus it confirms the responsibility of monofactorial inheritance. While in tetramethrin - resistant strains the observed dose response curve of the progeny of the second generation differs significantly from the curve which might be expected on the basis of monofactorial inheritance. Also deflection of the line at about LC_{50} was not observed. Thus it is concluded that tetramethrin resistance in C. pipiens may be due to more than one gene. The linearity of the second generation curve indicates that this resistance is polyfactorial.

Observed and expected mortality for back-cross were plotted . The deflection in these lines at about 50% kill indicate the segregation of groups of phenotypes at 1:1 ratio (RS : SS). This result confirms the possibility of monogene inheritance to malathion and fenitrothion. Analysis of the observed and expected responses on the bases of monogene inheritance, yielded x^2 values less than that of tabulated. This is another confirmation that inheritance of resistance to malathion and fenitrothion in C. pipiens is monofactorial. But in tetramethrin-resistant strains the observed dose response curve of the progeny of the back-cross differs

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significantly from the expected curve which might be explained on the basis of monofactorial inheritance. The linearity of the observed back-cross curve indicates that this resistance is polyfactorial. Thus it is concluded that tetramethrin resistance in *C. pipiens* may be due to more than one gene.

Introduction

Inheritance of resistance to the insecticides in several insects attracted many investigators. The mode of inheritance for fenthion, parathion and malathion in *C. pipiens* was due to a single gene (Tadano, 1969; Dorval and Brown, 1970; Tadano and sato, 1970). Apperson and Georghiou, 1975 found that the inheritance of resistance to parathion, fenitrothion, fenthion, chlorpyrifos methyl, and methyl parathion in a strain of *Culex tarsalis* was partially dominant and due to more than one gene. Priester and Georghiou, 1979, found that the inheritance of resistance to permethrin in two strains of the southern house mosquito is of polyfactorial origin. Shanahan (1979) suggested that diazinon resistance in *Lucilia curprina* resistant larvae was due to a major gene allele. The inheritance of resistance to chlorpyrifos in *C. quinque-fasciatus* was due to intermediate dominant gene (Curtis and Pasteur, 1981). Also Hemingway, 1982, found that fenitrothion resistance in *Anopheles atroparvus* appears to be dependent on a single najor autosomal, semidominant gene. While Propoxur resistance in the same strain is dependent on more than one gene.

The genetical bases for insecticide resistance is important in understanding the feature of insecticides resistance development and the reversion of this character, an attempt is concerned with genetical type of malathion, fenitrothion and tetramethrin resistance in *Culex pipiens*.

Materials and Methods

Insecticides used:

- Fenitrothion : *O,O* - Dimethyl -*O*- (4- nitro-*m*-tolyl) phosphorothioate. It was supplied as technical grade (90%)
- Malathion : *O,O* - Dimethyl S-(1-2-dicarbethoxyethyl) phosphorodithioate. It was supplied as technical grade (95%)
- Tetramethrin : *N* -(3,4,5,6 tetrahydro phthalimido) - methyl cis-trans chrysanthemate. It was supplied as technical grade (90%)

Strains:

- Susceptible strain of *Culex pipiens*
- Malathion resistant strain (128.2 fold)
- Fenitrothion resistant strain (46.3 fold)
- Tetramethrin resistant strain (71.67 fold)

Genetical technique:

Monofactorial inheritance of resistance can be recognized by charactaristic Mendelian segregation in the F_1 , F_2 and also by 1:1 segregation in appropriate back cross. This technique was used to study the mode of inheritance for resistance to malathion, fenitrothion, and tetramethrin. Resistance levels and genetic studies wnre determined by the dipping method (WHO , 1963.)

Reciprocal cross experiments were done by crossing fourty females resistant to malathion, fenitrothion or tetramethrin to twenty susceptible males and vice versa; fourty susceptible females were crossed to twenty resistant males.

The first generation was inbred to give second generation. The back crosses (BC'S)were carried out as follows:

(R♀ x S♂) F_1 ♀ x S ♂	(BC_1)
(R♀ x S♂) F_1 ♂ x S ♀	(BC_2)
(R♂ x S♀) F_1 ♀ x S ♂	(BC_3)
(R♂ x S♀) F_1 ♂ x S ♀	(BC_4)

The 4th instar larvae for each parent whether it was susceptible (SS) or malathion, fenitrothion-or tetramethrin-resistant (RR), F_1(RS), F_2 and back crosses were treated by dipping method with a wide range of concentrations- LC_{50} values were calculated from regression lines for F_1's hybrid while the complete LD-P lines were drawn for back crosses and F_2 progenies.

The degree of dominance (D) of resistance was determined as described by Priester and Georghiou (1979).

$$D = \frac{2LC_{50}\ (RS) - LC_{50}(RR) - LC_{50}(SS)}{LC_{50}(RR) - LC_{50}(SS)}$$

Where, RR, RS and SS represent the resistant, heterozygote and susceptible populations respectively. LC_{50} values are expressed in terms of their logarithms. The resulting D values will move on a scale from -1 over 0 to + 1, where -1 represents complete recessiveness. O stands for an intermediate genetic expression of gene. The span from 0 to + 1 reflects incomplete (partially) dominance and + 1 complete dominance. The expected F_2 segregation on the base of single factor Mendelian inheritacne was calculated (Georghiou and Garber formula ,1965):

$X(F_2) = a_1$ (S) 0.25 + a_2 (SR) 0.5 + a_3(R) 0.25 where $X(F_2)$ is the expected response of F_2 to a given dose and a_1 , a_2 and a_3 are the observed response of S, SR (hybrid) and R populations to that dose from their respective regression lines. The expected segregation of the back-cross (BC) of the SR to the S population was calculated for each dose by the formula:

X (BC) = a_1 (SR) 0.5 + a_2 (S) 0.5

The observed complete LD-P lines for BC and F_2 progenies were tested for significance by Chi square method of statistical analysis as described by Snedecor and Cochran (1967).

Rusults and Discussion

1- The first generation (F_1):

Concentration series of malathion, fenitrothion and tetramethrin were applied to the 4th instar of susceptible strain ,resistant strain and the first generation (F_1) offspring (♀ S x ♂ R and ♀ R x ♂ S). Percent mortality of larvae was recorded after 24 hours LD-P lines were plotted and statistically analyzed for computing slope and confidence limits values. Table 1 shows the LC_{50}, slope , level of resistance and degree of dominance values. The results showed that the degree of dominance values for the first generation (♂ R x♀ S) was 0.315 while it was 0.395 for (♂S x ♀ R) in malathion resistant strain. Also it was 0.257 and 0.303 in the two crosses, respectively in fenitrothion resistant strain. In tetramethrin resistant strain the degree of dominance for the first generation (♂ R x ♀ S) was 0.151, while it was 0.245 for (♂ S x ♀ R). Comparing these values with those of malathion and fenitrothion it is clear that higher values were obtained for the malathion resistant strain . Generally the inheritance of malathion, fenitrothion and tetramethrin resistance was controlled by partially dominant gene (S). From the recorded results, there were no differences between the two hybrids whether males or females. In malathion resistant strain LC_{50} values were about equal (6.6 - 7.0 mg/ Litre). Also in fenitrothion resistant strain LC_{50} values were about equal (0.56 - 0.58 mg/Litre). The same trend was observed for tetramethrin resistant strain, LC_{50} values were about equal (0.5 - 0.54 mg/ Litre).

Table (1) : Resistance level and degree of dominance for malathion, fenitrothion and tetramethrin resistant strains and F_1 generation of *C. pipiens*.

Insecticides	Strain or Cross	LC_{50}	Confidence limits of LC_{50} Values		Degree of dominance	level of resistance	Slope
Malathion	Susceptible	0.078	0.074	0.082	---	---	5.26
	Resistant	10.00	9.600	10.40	---	128.2	7.30
	F_1 (♂ R x ♀S)	6.60	6.34	6.87	0.315	84.6	7.08
	F_1 (♀ R x ♂ S)	7.00	6.73	7.28	0.395	89.7	7.29
Fenitrothion	Susceptible	0.019	0.018	0.020	---	---	4.19
	Resistant	0.88	0.84	0.92	---	46.3	6.57
	F_1 (♂R x ♀ S)	0.56	0.53	0.59	0.257	29.47	5.46
	F_1 (♀ R x ♂ S)	0.58	0.55	0.61	0.303	30.53	5.34
Tetramethrin	Susceptible	0.012	0.011	0.013	---	---	3.73
	Resistant	0.86	0.81	0.91	---	71.67	5.47
	F_1 (♂ R x ♀ S)	0.50	0.467	0.534	0.151	41.57	4.28
	F_1 (♀R x ♂ S)	0.54	0.503	0.579	0.245	45.00	4.55

It means that no sex linked inheritance of resistance to malathion, fenitrothion and tetramethrin. In other words the response in this case resembled the resistant type indicating autosomal character. Also the straight lines character for reciprocal crosses are withn the lines for susceptible and resistant strains.

The results of first generation hybrids revealed that the inheritance of malathion, fenitrothion and tetramethrin in C. pipiens are due to partially dominant gene (s). Also the character is autosomal or each parent can induce resistance to the hybrid. The degree of dominance greatly varied according to the insecticide used and the species in concern. Several investigators have reported the genetic resistance. Tadano (1969) proved that inheritance of resistance to malathion and fenthion in a colony of C. pipiens pallens was monofactorial. These factors were incompletely dominant. While Dorval and Brown (1970) found that the inheritance of fenthion resistance in C. pipiens fatigans was mainly due to a single slightly recessive gene. Tadano and Sato (1970) concluded that inheritance of parathion resistance in larvae of C. pipiens pallens strain was monofactorial incomplete dominant. Also Cochran (1973) found that the inheritance of pyrethrins resistance in Blattella germanica (L.) is due to simple autosomal incomplete dominant trait. Apperson and Georghiou (1975), found that inheritance of resistance to fenitrothion, fenthion, chlorpyrifos methyl and parathion in a strain of Culex tarsalis was partially dominant. Herath & Davidson (1981) found that malathion resistance in Anopheles stephensi was due to single intermediate dominant gene.

2- The second generation (F_2):

Fourth instar larvae of the second generation were dipped in different concentrations of malathion, fenitrothion and tetramethrin. Complete LD-P lines for F_2 are shown in Figs 1 & 2, and 3 no straight lines were obtained for malathion and fenitrothion resistant strains but showed general tendency for deflection. This indicates that the F_2 population segregates into classes of phenotype and consequently that resistance is due to a major gene. Steady of perecent kill values observed around 25 percent kill, from the well known Mendelian laws, it is a confirmation for monofactorial inheritance responsibilty for resistance phenomenon. In order to lest this possibility, the observed results were subsequently analyzed for goodness of fitting to monofactorial inheritance by the chi^2 method. It is noticed that the observed mortalities are generally in agreement with the expected value on the bases of a major single factor.

These findings confirm the monofactorial inheritance for malathion and fenitrothion. However in tetramethrin resistant strain the observed dose response curve of the progeny of the second generation differs significantly from the curve which is expected on the basis of monofactorial inheritance. Also deflection in these lines at about LC_{25} was not observed. Thus it is suggested that tetramethrin resistance may be due to more than one gene. The linearity of the second generation curve indicats that this resistance is expected as polyfactorial.

3- Back - cross (BC):

The larvae from the different back-crosses were treated by different concentrations of malathion, fenitrothion and tetramethrin. The complete LD-P lines were plotted from the observed and expected percent mortalities for back-cross (Figs. 4,5 and 6). In malathion and fenitrothion resistant strains the observed drawn lines for the BC gave complete LD-P lines and not straight lines. Also the results clearly indicate a steady state of percent kill around 50 for the back-crosses and showed plateaux around 50% kill. The statistical chi^2 method of analysis showed that, there were no significant differences between expected and observed lines. Thus suggesting the possibility monogene inheritance of resistance to malathion and fenitrothion. While in tetramethrin resistant strain the observed dose response curve of the progeny of the back-cross differs significantly from the expected curve which might be expected on the basis of monofactorial inheritance. Computation of the 95% confidence limits confirms the significance of differences between the observed and expected curves. The linearity of the observed back-cross curve confirms again that this resistace is polyfactorial. Therefore , tetramethrin resistance may be due to more one gene.

It seems that inheritance of malathion, Fenitrothion and tetramethrin resistance in Culex pipiens larvae is proved to be autosmal character or each parent can induce resistance to the hybrid, which is not sex linked inheritance.

Results of F_1 hybrids of resistant strain in this study revealed that the inheritance is due to gene(s) with degree of dominance fitted will with the partially dominant effect.

Analysis of second generations and back-crosses confirmed greatly the monogene inheritance for resistance to malathion and fenitrothion

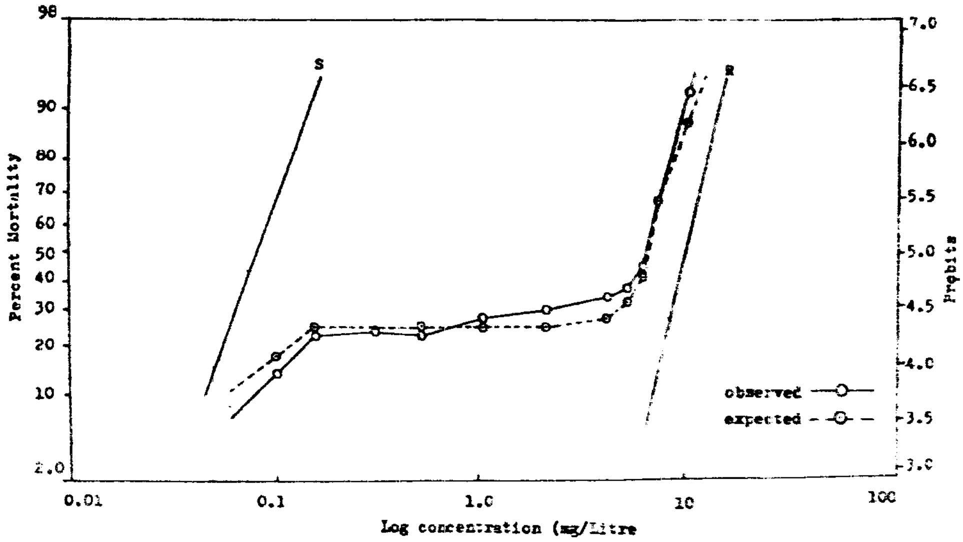

Fig. (1) : Dosage mortality relationships for malathion against resistant, susceptible and second generation progeny of *Culex pipiens*.

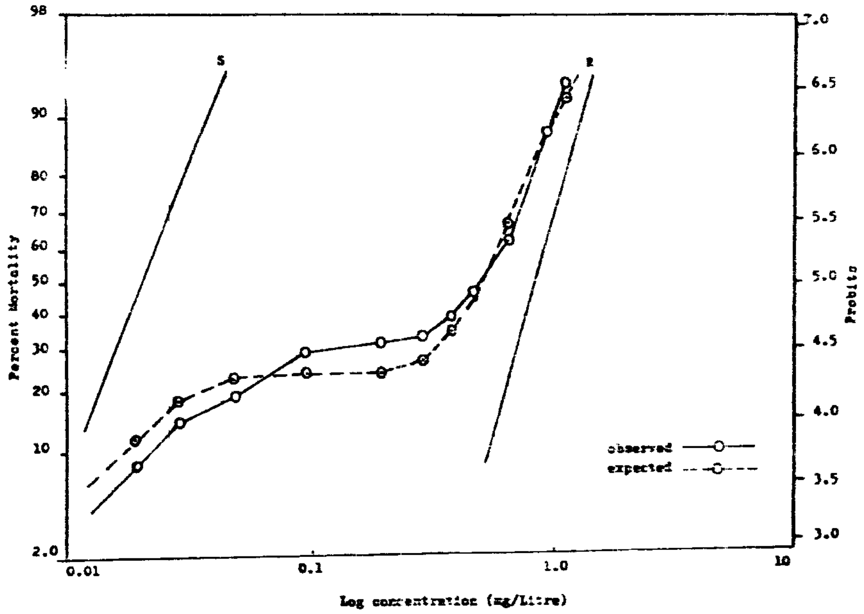

Fig. (2): Dosage mortality relationships for fenitrothion against resistant, susceptible and second generation progeny of *Culex pipiens*.

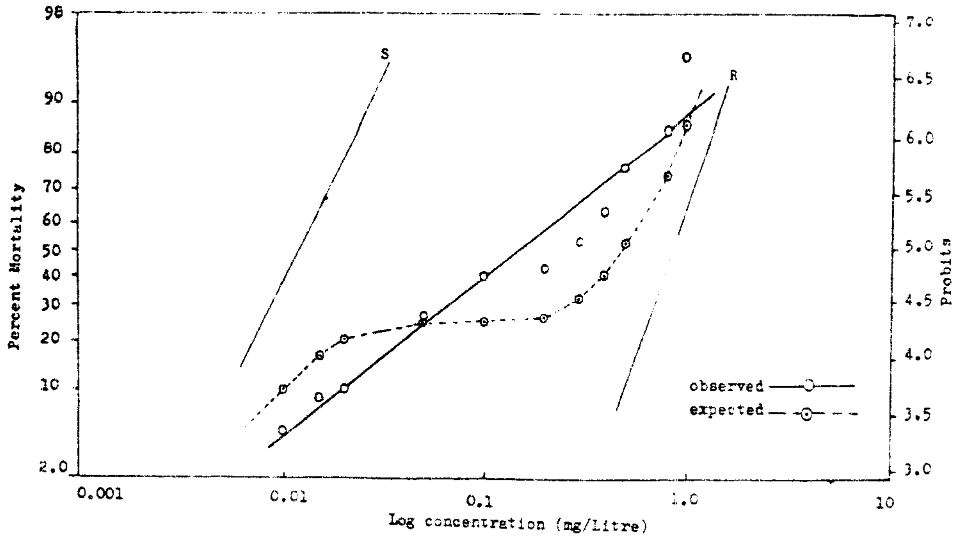

Fig. (3): Dosage mortality relationships for tetramethrin against resistant, susceptible and second generation progeny of Culex pipiens.

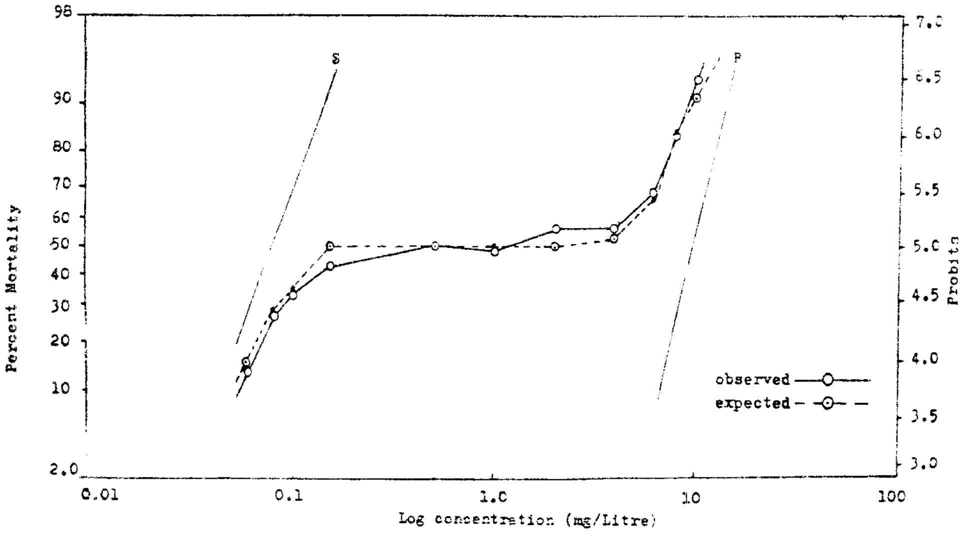

Fig. (4): Dosage mortality relationships for malathion against resistant, susceptible and back-cross progeny of Culex pipiens.

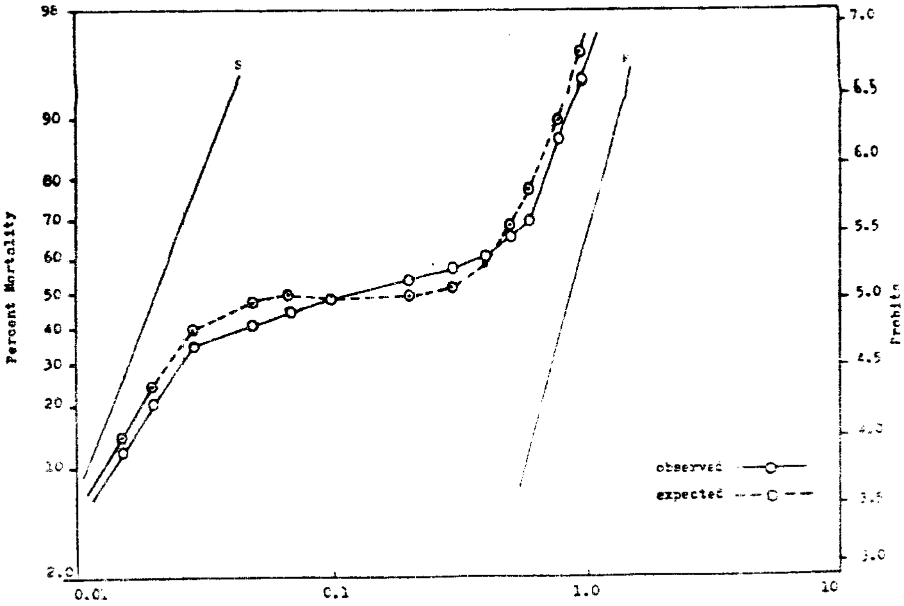

Fig. (5) : Dosage mortality relationships for fenitrothion against resistant, susceptible and back-cross progeny of _Culex pipiens_.

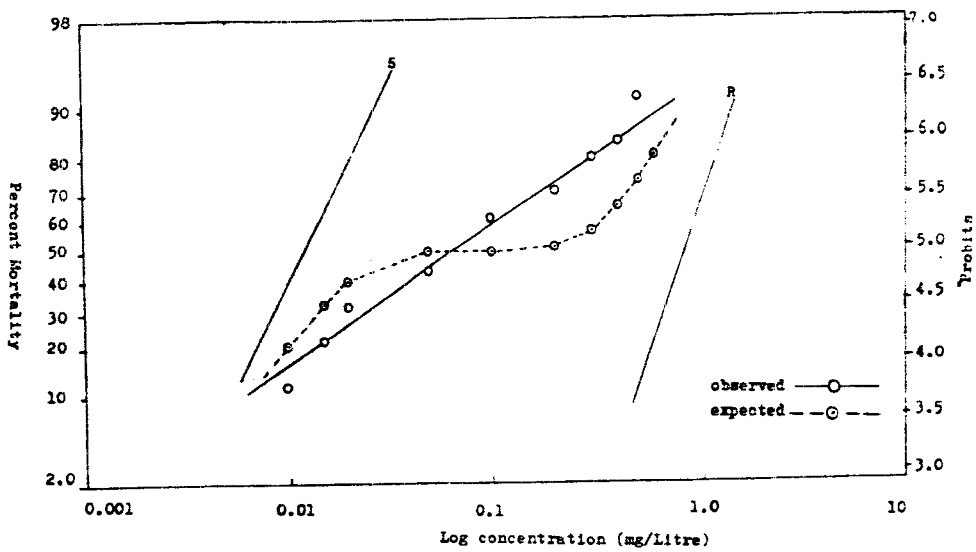

Fig (6) : Dosage mortality relationships for tetramethrin against resistant, susceptible and back-cross progeny of _Culex pipiens_.

and fenitrothion organophosphorus insecticides. These results are in agreement with Moustafa et al.,(1982) who found that autosomal single partially dominant gene was responsible for the inheritance of resistance to azinphos-methyl and sulprofos in the Egyptian cotton leafworm Spodoptera littoralis. The data has shown also that inheritance of resistance to tetramethrin (synthetic pyrethroids) may be a polyfactorial type. This result agreed with that of Priester and Georghiou (1979), who concluded that permethrin resistance in the C.P. quinquefasciatue is of autosomal polyfactorial origin.

Many investigators supported the previous results. Shanahan (1979) found that inheritance of diazinon resistance in Lucilia cuprina is due to a major allele. Curtis and pasteur (1981) found that inheritance of resistance to chlorpyrifos in C. quinquefasciatus from different regions was due to single intermediate dominant gene. While Beeman and Schmidt (1982) found that inheritance of resistance to malathion in Plodia intrepunctella, was controlled by a single autosomal gene or by closely linked set of genes. Hemingway (1982), found that fenitrothion resistance in Anopheles atroparvus from Spain appears to be dependent on a single major autosomal, semidominant gene. While the propoxur resistance in the same population is dependent on more than one gene. In housefly Shono, et al., (1982) found that resistance to malathion was almost due to completely dominant gene. Georghiou and Saito (1983), found that inheritance of malathion resistance in C.P. fatigans was due to monofactorial by an autosomal gene of partial dominant effect.

References

Apperson, S.C. and G.P. Georghiou 1975. Inheritance of resistance to organophosphorus insecticides in Culex tarsalis.Cop.Boll. Wld. Hlth. Org. 52: 97-100 .

Beeman, R.W. and B.A. Schmidt 1982. Biochemical and genetic aspects of malathion-specific resistance in the Indianmeal moth (Lepido -ptera:Pyralidae). J. Econ. Entomol. 75: 945 - 949.

Cochran, D.G. 1973. Inheritance and linkage of pyrethrins resistance in the German cockroach. J. Econ. Entomol. 66: 27-30.

Croft, B. A., A.W.A. Brown and S.A. Hoying 1976.Organoghosphours-resistance and its inheritance in the predaceous mite Amblyseius fallacis J. Econ Entomol. 69: 64-68.

Curtis, C.F. and N. Pasteur 1981. Organophosphate resistance in vector populations of the complex of Culex pipiens L.(Diptera:Culicidae). Bull. ent. Res. 71: 153 - 161.

Dorval, C. and A.W.A. Brown, 1970. Inheritance of resistance to fenthion in Culex pipiens fatigans Wied. Bull. Wld. H lth. Org. 34:727-734.

Georghiou, G.P. and M.J. Garber 1965. Studies on the inheritance of carbamate-resistance in the housefly (Musca domestica L.). Bull. Wld. Health. Org. 32: 181 - 196.

Georghiou, G.P. and T. Saito. 1983. Pest Resistance to Pesticides. Plenum Publ. Corp. New York PP. 809.

Hemingway, J. 1982. Genetics of organophosphate and carbamate resistance in Anopheles atroparvus (Diptera: Culicidae). J. Econ. Entomol. 75: 1055 - 1058.

Herath, P.R.J. and G. Davidson 1981. Studies on the nature of malathion resistance in a population of Anopheles stephensi from southern. Iran. Mosq. News. 531 - 535.

Inoue, K. 1979. The change of susceptibility of mite population to dicofol and genetic analysis of dicofol-resistance in the citrus red mite, Panonvchus citri. Journal of Pesticide Science. 4: 337-344.

Katez, A.J.; W.J. Collins and R.V. Skavaril 1973. Resistance in the German cockroach (Orthoptera: Blattellidae): the inheritance of diazinon resistance and cross resistance. Journal of Medical Entomology. 10: 599 - 604. Review of Applied Entomol., 68: 494.

McDonald, P.T. and S.M. Asman 1982. A genetic-sexing strain based on malathion resistance for Culex tarsalis. Mosq. News. 42:531-536.

Moustafa, F.I., A.A. Mahran; Sh. M.I. Kassem and A.A. Nomeir 1982. Inheritance of resistance to azinphosmethyl and sulprofos in the Egyptian cotton leafworm, Spodoptera littoralis (Boisd.) Proc. of the Second Egyptian-Hungarian Conference of Plant Protection, Alex: 524-535.

Plapp, F.W.; L.G. Tate and E. Hodgson 1976. Biochemical genetics of oxidative resistance to diazinon in the house fly.Pesticide

Biochemistry and Physiology. 6: 175 - 182.

Priester, T.M. and G.P. Georghiou 1979. Inheritance of resistance to permethrin in Culex pipiens quinquefasciatus. J. Econ. Entomol. 72: 124 - 127.

Roush, R.T. and F.W. Plapp 1982. Biochemical genetics of resistance to Aryl carbamate insecticides in the predaceous mite, Metaseiulus occidentalis. J. Econ. Entomol. 75: 304-307

Shanahan, G.J. 1979. Genetics of diazinon resistance in larvae of Lucilia cuprina (Wiedemann) (Diptera: Calliphoridae). Bull. Entomol. Research 69: 225-228. (C.F. RAE. 68: 1127).

Shono, T.; M. Horio and M. Tsukamoto 1982. Linkage group analysis of malathion resistance in the housefly. J. UOEH. 4 289-299.

Snedecor, G.W. and W.G. Cochran 1967. Statistical methods. Iowa state College press, Ames, Iowa. U.S.A. 593,pp.

Tadano, T. 1969. Genetical relationships between malathion-resistance and fenthion-resistance in larvae of Culex pipiens pallens Coquillett. Japanese Journal of sanitary zoology. 20: 158-160 (C.F. RAE. 60 : 5).

Tadano, T. and H. Sato 1970. Parathion-resistance in larvae of Culex pipiens pallens Coquillet. Japanese Journal of sanitary zoology. 21: 186 - 188 (C.F. RAE. 61: 1331).

الملخص العربي

نوع وراثة المقاومة فى يرقات بعوض الكيولكس لمبيدات الملاثيون والفنتروثيون والترامثرين

اجريت التلقيحات بين السلالات المقاومة والسلالة الحساسة ولم توجد فروق فى الذرية سواء كانت الذكور هى المقاومة أو الاناث وهذا يعنى ان وراثة المقاومة لمبيدات الملاثيون والفنتروثيون والترامثرين لا ترتبط بالجنس • كما اوضحت نتائج الجيل الاول أن وراثة المقاومة فى باعوضة الكيولكس للمبيدات ترجع الى جين (اوجينات) سائدة سيادة جزيئية • وبدراسة الجيل الثانى والتلقيح الرجعى وجد ان هناك انحناء فى خط الجرعة ـ الاحتمال (Ld. p) حول نسبة الموت ٢٥٪ وكذلك التلقيح الرجعى مع السلالة الحساسة اظهرت انحناء عند نسبة موت ٥٠٪ وهذا يؤكد ان الوراثة ترجع الى جين واحد وذلك بمقارنة الخط الملاحظ والمتوقع اذ لا يوجد فروق معنوية بينهما • بينما اختلف الخط الملاحظ عن المتوقع لمبيد الترامثرين الى جانب ان الانحناء لم يلاحظ عند عمل التلقيح الرجعى مع السلالة الحساسة مما قد يرجح ان وراثة المقاومة لهذا المبيد ترجع الى اكثر من جين او ما يسمى بالوراثة المتعددة وبتحليل خط الارتداد الملاحظ والمتوقع عن طريق مربع كاى وجد ان نتائج التحليل تؤيد وراثة المقاومة على اساس جين واحد لكل من الملاثيون والفنتروثيون كما يؤيد وراثة المقاومة على اساس تعدد الجينات لمبيد الترامثرين •