Assiut J. of Agric. Sci., 40 (1) (159-167)

TOXICITY OF SOME INSECTICIDES AGAINST A LABORATORY STRAIN AND THREE FIELD POPULATIONS OF MOSQUITO, *Culex pipiens* (L)

H. A Ezzeldin

Department of Plant Protection, Faculty of Agriculture, Assiut University, Assiut, Egypt

Abstract: Toxicity of malathion, profenophos, cypermethrin, fenvalerate, methomyl, propoxure, spinosad and abamectin was tested against larvae of laboratory (S) and three field (AM, AU and W) strains of *C. pipiens* (L). Based on LC_{50} values, spinosad was the most toxic compound against the S strain (LC_{50} = 0.0156 ppb), while fenvalerate and cypermethrin were the most effective insecticides against the three field populations. Values of LC_{50} for fenvalerate for AM, AU and W strains were 0.497, 0.315 and 0.868 ppb, respectively, and the corresponding values for cypermethrin were 0.898, 0.367 and 1.21 ppb. The carbamate insecticide, methomyl exhibited the least toxic effect against S, AM and Au strains; while the organophosphorus, malathion was the least toxic compound against W strain. Comparing LC_{50} values of the field strains with those of the laboratory strain (resistance ratio at LC_{50} level), spinosad showed the highest RR value in AM and AU strains (78.82 and 137.25, respectively). Malathion showed the highest RR value in W strain (1744.46). Slope and RR values revealed that all tested field populations were homogenous in their response toward all tested insecticides except for spinosad. The ability to build up resistance against insecticides from different groups was discussed.

Key words: insecticides, laboratory strain, mosquito, field populations.

Introduction

Mosquito species, *Culex pipiens* (L) is considered to be one of the most important diseases vector medical pests. Intensive use of insecticides from different groups for controlling mosquitoes can lead to resistance not only against used insecticides , but also against the new materials through cross-resistance (Golenda and Forgash 1985, Scott 1989, Bisset et al. 1997). The present study has been carried out to investigate resistance and cross-resistance patterns in three field strains from three different areas in Assiut governorate, Egypt, compared with the susceptible laboratory strain. The insecticides tested were conventional such as organophosphorus, carb-

Received on: 31/3/2009 **Accepted for publication on**: 16/5/2009
Referees: Prof.Dr. Taha Y. Helal Prof.Dr. Shehata M. Ibrahim

amates and pyrethroids; in addition to relatively new insecticides such as spinosad and abamectin.

Materials and Methods

Insects:

Collecting and rearing:

A- Susceptible strain:

Susceptible strain used in the present study was brought from the Institute of Veterinary and Medical Insects in Cairo, which reared in the laboratory for 5 years away from any insecticidal pressure.

B- Field populations:

Three field populations of *C. pipiens* (L) larvae were collected from three different areas in Assiut Governorate. The first strain was collected from Arab E-Madabegh area (AM strain), the second was collected from Assiut university field (AU strain) and the third one was collected from Walidia area (W strain). Larvae of all strains were transferred to the laboratory of Plant Protection Department, Faculty of Agriculture, Assiut University and reared on 25± 2°C and 80±5% R.H. in enamel breading trays (40 cm) according to the method of WHO **(Anonymous, 1981)**. Transformed pupae were collected from the aforementioned trays using a wide mouth glass dropper, then pipetted into Petri dishes which placed inside the adult cages (emerging adult cages). Dimensions of emerging adult cages were 33×33×33 cm. The emerged males and females were fed 1% sucrose solution and pigeon blood meals, respectively.

Suitable containers for egg-laying were provided to the cages 48 hours after the females had their blood meal.

Receptacles containing egg rafts were daily collected from the cages, then left undisturbed till hatching. Newly hatched larvae were transferred to the breading trays, each of 2 inches hight of tap water. A maximum of 250 larvae of the same age were placed in each tary. After twenty four hours, the larvae were fed on fresh yeast which sprinkled with water on the surface twice daily. The left non-ingested yeast was carefully removed by medicinal dropper.

All stages of reared colonies were maintained at 25±2°C and 80±5% R.H. Temperature and relative humidity readings were daily measured using a thermograph and hydrograph, respectively.

Insecticides:

Malathion: Diethyl (dimethoxyphosphinothioylthio) succinate. It was supplied as Agrothion,57%E.C(Agrochemica l Co.)

Profenofos: (*RS*)-(*O*-4-bromo-2-chlorophenyl *O*-ethyl *S*-propyl phosphorothioate). It was supplied as Selecron, 72% E.C (Syngenta Co.)

Alpha – cypermethrin: Recemate comprising (R) -)-α-cyano-3-phenoxybenzyl (1S, 3S)-3- (2,2- dichlorovinyl) -2,2-dimethylcyclopropanecarboxylate and (S) -)-α-cyano-3-phenoxybenzyl (1R, 3R)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropancarboxylate. It was supplied as Flectron, 10% E.C (Shell International Chemical Co.)

Fenvalerate: (*S*)-α-cyano-3-phenoxybenzyl (*S*)-2-(4-chlorophenyl)-3-methylbutyrate. It was supplied as Sumicidin, 20% E.C (Sumitomo Chemical Co.)

Methomyl: *S*-methyl (*EZ*)-*N*-(methylcarbamoyloxy)thioacetimidate. It was supplied as Lannate 90% S.P (Dobon di numorz Co., USA)

Propoxur: 2-isopropoxyphenyl methylcarbamate. It was supplied as technical grade (Sumitomo Chemical Co.)

Spinosad:

Mixture of 50–95% (2*R*,3a*S*,5a*R*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*R*)-2-(6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-β-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-14-methyl-1*H*-*as*-indaceno[3,2-*d*]oxacyclododecine-7,15-dione and 50–5% (2*S*,3a*R*,5a*S*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*S*)-2-(6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetrad-eoxy-β-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-hexade-cahydro-4,14dimethyl-1*H*-*as*-indaceno[3,2-*d*]oxacyclodo-decine-7,15-dione. It was supplied as Spinotor, 24% S.C (Dow Agro Sciences Co.)

Abamectin:
Extended von Baeyer nomenclature: mixture of ≥ 80% (10*E*,14*E*,16*E*)-(1*R*,4*S*,5′*S*,6*S*,6′*R*,8*R*,12*S*,13*S*,20*R*,21*R*,24*S*)-6′-[(*S*)-*sec*-butyl]-21,24-dihydroxy-5′,11,13,22-tetramethyl-2-oxo-(3,7,19-trioxatetracyclo[15.6.1.1^{4,8}.0^{20,24}]pentacosa-10,14,16,22-tetraene)-6-spiro-2′-(5′,6′-dihydro-2′*H*-pyran)-12-yl 2,6-dideoxy-4-*O*-(2,6-dideoxy-3-*O*-methyl-α-L-*arabino*-hexopyranosyl)-3-*O*-methyl-α-L-*arabino*-hexopyranoside and ≤ 20% (10*E*,14*E*,16*E*)-(1*R*,4*S*,5′*S*,6*S*,6′*R*,8*R*,12*S*,13*S*,20*R*,21*R*,24*S*)-21,24-dihydroxy-6′-isopropyl-5′,11,13,22-tetramethyl-2-oxo-(3,7,19-trioxatetracyclo[15.6.1.1^{4,8}.0^{20,24}] pentacosa-10,14,16,22-tetraene)-6-spiro-2′-(5′,6′-dihydro-2′*H*-pyran)-12-yl 2,6-dideoxy-4-*O*-(2,6-dideoxy-3-*O*-methyl-α-L-*arabino*-hexopyranosyl)-3-*O*-methyl-α-L-*arabino*-hexopyranoside. It was supplied as Vertemic, 1.8% E.C (Syngenta Co)

Bioassay:

Toxicity of all used insecticides against the four *C. pipiens* (L) strains was tested according to the procedure of World Health Organization WHO, Anonymous

(1981). Six to eight concentrations of each insecticide were prepared in water. At least three replicates were used for each concentration, 20 larvae were added per each replicate. 99 ml of distilled water was placed in a beacker, to which 1 ml of insecticide solution for preparing the required concentration was added. Controls were prepared by adding 1 ml of tap water. All treatments were maintained at 25±2°C for 24 h. till recording the mortality. Mortality percent was corrected using the formula of Abott, 1925. Values of LC_{50}, LC_{90}, slope and confidence limits were calculated according to the method of Finny (1971) with some modification using a computer program.

Resistance ratio values:

Resistance ratio values at LC_{50} level (RR_{50}) were calculated as:

LC_{50} of field strain for the tested insecticide/LC_{50} of S stain for the same insecticide.

While those values at LC_{90} level (RR_{90}) were calculated as:

LC_{90} of field strain for the tested insecticide/LC_{90} of S strain for the same insecticide.

Results and Discussion

Table 1 shows LC_{50}, LC_{90} and slope values of the eight tested insecticides against susceptible laboratory strain (S) of *C. pipiens*. Based on LC_{50} value, spinosad was the most toxic compound (LC_{50} = 0.01565 PPb), while methomyl was the least effective one (LC_{50} = 5.24 PPb).

Spinosad was more toxic than malathion, α cypermethrin, propoxure, abamectin, fenvalerate, profenophos and methomyl by 4.06, 6.20, 8.31, 14.43, 16.94, 33.07 and 338.14 folds, respectively. The susceptible laboratory strain exhibited relatively high slope values (as expected) toward all tested compounds. Slope values of S strain ranged from about 2 to more than 5 indicating that the laboratory strain is homogenous.

Tables 2, 3 and 4 show the values of LC_{50}, LC_{90}, slope, RR_{50} and RR_{90} for AM, AU and W strains. Values of LC_{50} revealed that the pyrethroid compounds, fenavalerate and α-cypermethrin were the most toxic compounds against the three tested field strains, α-Cypermethrin and abamectin were 0.497, 0.899 and 0.367 and 1.257 PPb for AU strain (Table 3), respectively, and 0.868, 1.21 and 2.08 PPb (Table 3), for the same corresponding compounds. The carbamate methomyl was the least toxic one (Table 2 and 3) against AM and AU strains (LC_{50} values were 5.468 and 7.318 PPb, respectively). While the organophosphate compound, malathion was the least toxic one (Table 4) against W strain (LC_{50} value = 111 PPb). The rest of tested compounds occupied moderate position in the ranking order.

Table(1): Toxicity data of the tested insecticides against the S-strain of *C. pipiens* (L) larvae expressed as LC50, LC90, confidence Limits, slope and (a) values.

	LC50 (PPb)	Confidence Limit		LC90 (PPb)	Confidence Limit		Slope ±*S. E	a ±S. E.
		Lower	Upper		Lower	Upper		
Malathion	0.06363	0.05169	0.07496	0.1640	0.1271	0.2682	3.12±0.57	0.62±1.07
Profenophos	0.5176	0.4685	0.5689	0.9341	0.8003	1.221	4.99±0.77	8.57±2.10
α Cypermethrin	0.09709	0.07643	0.1228	0.4151	0.2772	0.8729	2.03±0.34	0.96±0.68
Fenvalerate	0.2652	0.2188	0.3180	0.7415	0.5521	1.295	2.87±0.50	1.96±1.23
Methomyl	5.292	4.721	5.780	8.931	7.742	11.90	5.64±1.08	16.01±4.05
Propoxur	0.1302	0.1033	0.1596	0.3703	0.2691	0.7165	2.82±0.56	0.97±1.21
Spinosad	0.01565	0.01259	0.1899	0.4763	0.3536	0.8092	2.65±0.43	1.83±0.55
Abamectin	0.2259	0.1849	0.2669	0.5881	0.4605	0.9016	3.09±0.50	2.26±1.22

a = The Y intercept (where the line crosses the Y axis)

Table(2): Toxicity data of the eight tested insecticides against AM strain of *C. pipiens* (L) larvae expressed as LC50, LC90, confidence Limits, slope and (a) values.

	LC50 (PPb)	Confidence Limit		LC90 (PPb)	Confidence Limit		Slope ±S. E	a ±S. E.	RR	
		Lower	Upper		Lower	Upper			RR50	RR90
Malathion	1.321	0.971	1.702	5.86	3.927	12.49	1.98±0.35	1.18±1.14	20.76	35.73
Profenophos	3.984	3.362	4.468	7.799	6.541	1.122	4.39±0.88	10.82±3.2	7.69	8.34
α Cypermethrin	0.8989	0.699	1.076	2.820	2.132	4.800	2.58±0.46	2.62±1.42	9.25	6.79
Es-fenvalerate	0.497	0.428	0.552	0.898	0.775	1.180	4.98±0.90	8.44±2.49	1.87	1.21
Methomyl	5.468	4.214	6.455	13.87	11.00	22.09	3.17±0.36	6.85±2.42	1.03	1.55
Propoxure	4.919	3.0674	5.884	14.79	11.26	26.24	2.68±0.54	4.89±2.08	37.78	39.94
Spinosad	1.221	0.935	1.491	3.781	2.861	6.214	2.61±0.45	3.06±1.43	78.02	7.93
Abamectin	0.9203	0.7091	1.126	3.016	2.186	5.704	2.49±0.47	2.36±1.43	4.07	5.12

Table(3): Toxicity data of the tested insecticides against AU strain of *C. pipiens* (L) larvae expressed as LC50, LC90, confidence Limits, slope and (a) values.

	LC50 (PPb)	Confidence Limit		LC90 (ppb)	Confidence Limit		Slope ±S. E	a ±S. E.	RR	
		Lower	Upper		Lower	Upper			RR50	RR90
Malathion	1.67	1.28	2.013	4.39	3.38	7.24	3.05±0.58	4.81±1.94	26.24	26.76
Profenophos	4.68	4.19	5.18	8.39	7.100	11.60	5.05±0.90	13.53±3.33	9.04	8.98
α Cypermethrin	0.3674	0.2752	0.455	1.148	0.8168	2.465	2.59±0.56	1.64±1.48	3.78	2.76
Es-fenvalerate	0.315	0.251	0.381	0.8234	0.5838	2.302	3.07±0.82	2.67±2.07	1.18	1.11
Methomyl	7.318	6.02	8.723	20.25	14.84	39.74	2.90±0.59	6.20±2.29	1.38	2.26
Propoxure	2.175	1.657	2.648	7.637	5.379	15.79	2.35±0.46	2.84±1.58	16.70	20.62
Spinosad	2.148	1.653	2.629	7.131	5.147	13.67	2.46±0.47	3.19±1.59	137.25	14.97
Abamectin	1.257	0.961	1.539	4.016	3.002	6.806	2.54±0.44	2.87±1.42	5.56	6.82

Table 4: Toxicity data of the tested insecticides against W strain of *C. pipiens* (L) larvae expressed as LC50, LC90, confidence Limits, slope and (a) values.

	LC50 (ppb)	Confidence Limit		LC90 (ppb)	Confidence Limit		Slope ±S. E	a ±S. E.	RR	
		Lower	Upper		Lower	Upper			RR50	RR90
Malathion	111	84	137	492	333	102	1.98±0.34	4.97±1.73	1744.46	3000
Profenophos	11.00	8.10	13.60	44.00	29.00	92.00	2.08±0.35	3.37±1.42	21.25	47.1
α Cypermethrin	1.21	0.97	1.46	3.55	2.66	6.02	2.74±0.46	3.43±1.45	12.46	8.55
Es-fenvalerate	0.868	0.66	0.106	2.86	2.08	5.39	2.47±0.47	2.27±1.44	3.27	3.85
Methomyl	15.20	12.30	17.90	37.50	29.9	56.70	3.28±0.58	8.70±2.46	2.87	4.19
Propoxure	10.40	8.80	11.80	20.20	16.73	29.42	4.46±0.87	12.9±3.55	79.87	54.55
Spinosad	3.77	3.33	4.24	7.30	6.06	10.22	4.46±0.74	10.95±2.65	240.89	15.312
Abamectin	2.08	1.79	2.41	4.69	3.72	7.05	3.63±0.58	7.04±1.92	9.21	7.97

Concerning resistance ratio values at LC_{50} (RR_{50}), spinosad showed the highest RR value in AM and AU strains (78.02 and 137, respectively). While malathion showed very high RR_{50} value in W strain (1744.46). Each of fenvalerate and methomyl showed the least RR_{50} value compared with the other values. Both AM and AU strains showed RR_{50} values less than 10 fold against profenophos, α-cypermethrin, fenvalerate, methomyl and abamectin.. Comparing RR_{50} of W strain (Table 4) with the same values in AM (Table 2) and AU (Table 3), W strain had higher RR_{50} value for each tested compound compared with the same corresponding values in Tables 2 and 3. Comparing RR_{50} and RR_{90} values revealed no big differences between these two values for the same tested compound except spinosad. In AM, AU and W strains, spinosad showed that RR_{50} values were 78.02, 137.25 and 240 fold, respectively. While it showed that RR_{90} values were 7.93, 14.97 and 15.32 fold, respectively, for the same corresponding strains. These results indicated that all field populations of mosquito larvae were homogenous in their response toward all tested insecticides except spinosad. These data showed that, probably, the three field tested populations were heterogenous in response to spinosad and present a potential ability to develop higher resistance level to that insecticide (Liu *et al.*, 2004).

It's known that when a strain is selected with an insecticide, resistance extends to other compounds of the same class of insecticides or to compounds with similar mode of action. Acetylcholinesterase (AChE) is a common target for organophosphates and carbamates. Cross – resistance to organophosphates and carbamates can arise from AChE insensitivity. This resistance mechanism has occurred in several mosquito species (Ayad and Georghiou, 1975; Hemingway, 1982 and Hemingway *et al.*, 1985), but it seemed not to be the case in the present study. The mechanism(s) of resistance responsible for the organophosphate, malathion does not confer cross resistance to the carbamate, methomyl. The RR_{50} values for malathion in AM (Table 2), AU (Table 3) and W (Table 4) strains were 20.76, 26.24 and 1744.46, respectively. While the same corresponding values for the carbamate, methomyl were only 1.03, 1.38 and 2.87. These results suggest that the metabolic enzyme(s) in the field mosquito strains may play the major role in conferring resistance against organophosphates rather than the insensitivity of the target site, AChE.

References

Abbott, W.S. 1925 . A method for compating the effectiveness of an insecticide. *J. Econ. Entomol. 18 : 265 – 267.*

Anonymous, 1981 . Instruction for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides – diagnostic test. *WHO/VBC/81.806.*

Ayad, H. and G.P, Georghiou 1975. Resistance to organophosphates and carbamates in *Anopheles albimanus* based on reduced sensitivity of acetylcholinesterase. *J. Econ. Entomol. 68 : 295 – 297.*

Bisset, J.; M. Rodriguez; A. Soca; N. Pasteur and M. Raymond 1997. Cross-resistance to pyrethroid and organophosphorus insecticides in the southern house mosquito (Diptera: culicidae) from cuba. J. *Med. Entomol. 34 : 244 – 246.*

Finney, D. J. 1971 . Probit analysis, 3rd ed. Cambridge University Press, 333 P.

Golenda, C. F. and A.J forgash 1985 . Fenvalerate cross-resistance in a resmethrin-slelcted strain of the house fly (Diptera: Muscidae). *J. Econ. Entomol. 78: 19 – 24.*

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

Hemingway, J.; C.A Malcolm; K.E Kissoon; R.G Boddington; C.F Curtis and N. Hill 1985 . The biochemistry of insecticide resistance in *Anopheles sacharovi*: Comparative studies with a range of insecticide susceptible and resistant *Anopheles* and *Culex* species. *Pestic. Biochem. Physiol. 24: 68 – 76.*

Liu, H., E.W Cupp; K.M Micher; A. Guo and N. Liu 2004 . Insecticide resistance and cross-resistance in Alabama and Florida Strains of *Culex quinquefaciatus. J. Med. Entomol. 41 (3): 408 – 413.*

Scott, J. G. 1989 . Cross-resistance to the biological insecticide abamectin in pyrethroid-resistant house flies. *Pestic. Biochem. Physiol. 34: 27 – 31.*

Assiut J. of Agric. Sci., 40 (1) (159-167)

سمية بعض المبيدات الحشرية على سلاله معملية و ثلاثة مجتمعات حقلية من بعوض الكيولكس بيبنز

حسام عز الدين

قسم وقاية النبات - كلية الزراعة - جامعة أسيوط - مصر

تم اختبار سمية كل من مبيد الملاثيون ، البروفينوفوس ، ألفاسيبرمثرين ، فينفاليرات ، ميثوميل ، بروبوكسر ، سبينوساد وأبامكتين على سلالة المعمل الحساسة وكذلك على ثلاثة مجتمعات حقلية لبعوض الكيولكس بيبنز (سلالة عرب العوامر AM، سلالة جامعة أسيوط AU وسلالة الوليدية W) وبناء على قيم LC_{50} كان مبيد الاسبينوساد هو الأعلى سمية ضد السلالة الحساسة (LC_{50}=0.0156 ppb)، بينما كان فينفاليرات والسيبرمثرين هما الاكثر سمية على الثلاثة مجتمعات الحقليه .وكانت قيم LC_{50} للفينفاليرات لسلالة الـ AU،AM، Wهى 0.497 ، 0.315 ، 0.868 جزء فى البليون على التوالى .و قد كانت هذه القيم0.898 ، 0.367 ، 1.21جزء فى البليون لنفس المجتمعات على التوالى . وكان مركب الكرباميت الميثوميل هو الاقل سميه تجاه السلاله الحساسه ، مجتمع AM،مجتمع AU . وكان المبيد الفوسفورى ملاثيون هو الأقل سميه على مجتمع W . وبمقارنة قيم LC_{50} للسلاله الحساسه S لجميع المبيدات المختبره بالمجتمعات الحقليه (RR_{50}) نجد أن مبيد الاسبينوساد هو صاحب أعلى قيمة للـــ RR فى مجتمع AM، مجتمع AU ،(137.25 ، 78.02على التوالى) والملاثيون اظهر اعلى قيمة RR فى مجتمع W (1744.46) وقد أظهرت قيم الميل وقيم معدل المقاومة ان كل المجتمعات الحقليه للبعوض المختبر كانت متجانسه فى استجابتها تجاه جميع المبيدات المختبره فيما عدا الاسبينوساد.وقد تم مناقشة قابلية مقاومة البعوض لتكوين مقاومة ضد جميع مجموعات المبيدات المختبره.