Laboratory Evaluation of Some Chemical and Biological Insecticides Against The Fourth Instar Larvae and Adults of *Culex Pipiens* Linn. (Diptera: Culicidae).

Ali S. Al-Sarar and Z. Al-Shahrany
Plant Protection Department, College of Food Science and Agriculture, King Saud University, Riyadh, Saudi Arabia.

ABSTRACT

Four synthetic pyrethroids (deltamethrin, alpha-cypermethrin, lambda-cyhalothrin, cyphenothrin) and the three insecticides, temephos, pyriproxyfen as well as biological insecticide *Bacillus thuringiensis* were tested for their activity against the fourth instar larvae of *Culex pipiens* Linn. In addition, the four synthetic pyrethriods were tested for their activity against *C. pipiens* adults. LC_{50} values for the abovementioned insecticides against the 4th instar larvae were 0.00072, 0.0009, 0.0008, 0.034, 0.0059, 0.000079 and 0.012 ppm, respectively. The respective values for the tested insecticides against adults of *C. pipiens*, using the bottle bioassay method, were 0.391, 1.043, 0.5 and 2.617 µg/bottle, in respect. Results of this study could be used for monitoring the appearance of resistance in feral populations at different localities of Saudi Arabia.

Key words: *Culex pipiens*; insecticides; bioassay; resistance.

INTRODUCTION

Mosquitoes are dangerous insects; they annoy people and are vectors to many serious diseases that infect both human and other animals such as filariasis, encephalitis, dengue fever and malaria (Eldridge *et al.* 2000; Turell *et al.*, 2001). The most important problem in mosquitoes control is the development of resistance to many groups of insecticides that are currently used. Pyrethroid resistance in the *Culex pipiens* Linn. has been recorded worldwide, as reported by Cheikh *et al.*, 1998; Kasai *et al.*, 1998; Paul *et al.*, 2004; Xu *et al.*, 2005. Resistance to organophosphorous compounds has been reported also by Cheikh and Pasteur, 1993; Dorta *et al.*, 1993; DeSilva *et al.* 1997; Gonzalez *et al.*, 1999; Thomas *et al.*, *2000;* Baruah, 2004. *Bacillus thuringiensis* and *B. sphaericus* have been successfully used to control mosquito larvae allover the world (Baruah and Das, 1994; Zahiri *et al.*, 2004); however many workers indicated on the incidence of resistance to these biological insecticides in mosquitoes (Wirth *et al.*, 2005). Insect growth regulators (IGRs), i.e. methoprene, pyriproxyfen were used to control mosquitoes that have different modes of action from those of pyrethroids , organophosphates and *Bacillus* *thuringiensis* (Chowdhury *et al.*, 1999). However many populations of mosquitoes developed, to a great extent, resistance to this class of chemicals (Cornel *et al.*, 2003). The most effective way to overcome the problem of insect pest resistance to any insecticide is to stop using the same compound for many generations and replacing it with a different chemical structure having varied mode of action. This work was performed to test the efficacy of the aforementioned insecticide classes (pyrethroids, organophosphates, IGRs and *B.t.*) against the susceptible strain of *C. pipiens*. The obtained results from this work could be used as a guide to follow up the incidence of resistance in *C. pipiens* to any group of these insecticides in different locations in Saudi Arabia.

MATERIALS AND METHODS

Insecticides:

Delta–endo toxin (99.9% purity) *Bacillus thuringinsis* var. *israelensis*, pyriproxyfen (Sumilarv® WDG 0.5%) , cyphenothrin (Jokilat® 5% EC) from Sumitomo Chem Ltd, Japan; temephos (an experimental formulation) from WHO, deltamethrin (2.5% EC) from Agric Chem. Industries, Jordan, alpha-cypermethrin (10% EC) from Modern Insecticides, India, lambda-cyhalothrin (5% EC) from an Indian Company were used.

Insects:

Late 3rd and early 4th *Culex pipiens* larvae and adults of a susceptible strain were maintained at laboratory conditions (27°C and 80% R.H.) for three years.

Larvicidal activity:

Twenty mosquito larvae were placed in a 200 ml glass beaker containing 100 ml distilled water. Stock concentrations dissolved in ethanol were added to obtain the required final concentrations and stirred with a glass rod; three replicates were used for each concentration. Controls received solvent only and dead larvae were counted 48 h after treatment (Al-Sarar *et al.*, 2005).

Adulticidal activity:

Adulticidal activity was carried out as follows: 250 ml glass Weaton bottles were dosed with the test insecticides that dissolved in 1.5-2 ml acetone. Bottles were capped and rolled around to coat the entire inside surface, including the cap. Bottles were then uncapped and acetone was allowed to evaporate while rolling the uncapped bottles; control bottles were treated with acetone only. Adult mosquitoes were added to treated bottles (12-25 mosquitoes per bottle) and bottles were capped again. Dead mosquitoes were counted after 0.5, 1 and 1.5 h and % mortality was calculated for each dose (Brogdon and McAllister, 1998).

Statistical analysis:

Obtained data were analyzed according to Finney (1971) and control mortality, if any, was corrected using Abbott formula (1925).

RESULTS AND DISCUSSION

Larvicidal activity:

Results of larvicidal activity of the test insecticides are presented in Table (1) and illustrated in Fig (1). Temephos showed LC_{50} and LC_{95} values of 0.0059 and 0.016 ppm, respesctively; this result is in a good agreement with Macoris *et al.*, 2005, who reported that the mean LC_{50} of temephos was 0.007 ppm and with Rye *et al.*, 2003, who recorded the value 0.00072 ppm as the LC_{50} of temephos against *C. pipiens*. Mostafa, *et al.*, (1982) mentioned that the LC_{50} of temephos against *C. pipiens* was 0.006 ppm. On the other hand , *Culex* sp have developed resistance to temephos in many areas worldwide *C. quinquefosciatus* was found to be 56 times more resistant to temephos after four generations only (DeSilva *et al.*, 1997). LC_{50} of pyriproxyfen was 0.000079 ppm (Table1), El-Shazly and Refaie (2002) estimated the LC_{50} value of pyriproxyfen to be 0.00042 ppm, while this value was 0.000029 ppm (Kawada *et al.*, 1994). LC_{50} values of pyriproxyfen ranged from 0.000018 – 0.000029 ppm (Schaefer *et al.*, 1988 and Ali *et al.*, 1999). *Bacillus thuringiensis* was effective with LC_{50} value being 0.012 ppm, this result agrees with Klowden *et al.*, (1983) and Bekheit (1984), the LC_{50} values obtained from their work were 0.018 and 0.027 ppm successively. Pyrethroid insecticides had different toxicities against larvae, with deltamethrin and lambda-cyhalothrin being the most effective ones, having very close LC_{50} values (0.0007 and 0.0008 ppm, in sequence), followed by alpha-cypermethrin (LC_{50} = 0.0009 ppm) and cyphenothrin being the least effective (LC_{50} = 0.034 ppm). LC_{50} value of cypermethrin against *C. pipiens* was equal to 0.0012 ppm (Al-Sarar *et al.*, 2005), which is very close to our value of the present study.

Adulticidal activity:

Results of the test compounds against C. *pipiens* adults are shown in Table (2) and Fig (2). The bottle bioassay technique was used because it has many advantages: a) it is quick and results can be obtained in 2-3 h; b) can be prepared simply at any place, at any time for any insecticide; c) results are reproducible without the need of large numbers of mosquitoes and replications; and d) results can be correlated with other tests for determining what mechanism is responsible for resistance. The used insecticides could be arranged dessendingly as follows: deltamethrin> lambda-cyhalothrin> alpha-cypermethrin> cyphenothrin. In other words, deltamethrin proved to be the most effective insecticide and cyphenothrin was the least effective one. The LD_{95} values for these insecticides were 9.216, 18.21, 22.214 and 112.926 µg/bottle, respectively. This method determines also the contact toxicity of any insecticide without any need to anethesize insects as in case of the topical application method, which also requires a microapplicator. Ansari and Rezdan (2001) mentioned that good results were obtained by treating curtains with10 µg/cm^2. Cement and wooden surfaces treated with deltamethrin at 5 µg/cm^2 resulted in 100% mortality against *C. quer quifosciatus* (Das and Kalyanasanderson, 1984). The LD_{50} of alpha-cypermethrin against *C. pipiens* was determined as 2.5 µg/cm^2 (Tran *et al.*, 2000), which is not in accordance with the value of the present study (1.043 µg/bottle). Obtained data from this study could be used to follow up the incidence of resistance in *C. pipiens* for wild type strains at different locations in Saudi Arabia by comparing the LC_{50} and LC_{95} values of laboratory strain with those of field strains.

REFERENCES

Abbott, S. W. (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.

Ali, A.; Chowdhury, M. A.; Hossain, M. I.; Mahmud-Ul-Ameen; Habiba, D. B. and Aslam, A. F. (1999). Laboratory evaluation of selected larvicides and insect growth regulators against field –collected *Culex quinquefasciatas* larvae from urban Dhaka, Bangladesh. J. Am. Mosq. Control Assoc., 15(1): 43-47.

Al-Sarar, A. S.; Hussein, H. I.; Al-Rajhy, D. and Al-Mohaimeed, S. (2005). Susceptibility of *Culex pipiens* from different localities in Riyadh city to insecticides used to control mosquitoes in Saudi Arabia. J. Pest Cont. Environ. Sci.,13: 79-88.

Ansari, M. A. and Razdan, R. K. (2001). Concurrent control of mosquitoes and domestic pests by use of deltamethrin-treated curtains in the

New Delhi Municipal Committee, India. J. Am. Mosq. Control Assoc., 17:131-136.

Baruah, I and Das, S. C. (1994). Laboratory and field evaluation of *Bacillus thuringiensis* and *B. sphaericus* against mosquito larvae. J. Commun. Dis., 26(2): 82-87.

Baruah, K. (2004). Laboratory bio-assay of temephos and fenthion against some vector species of public health importance. J. Commun. Dis., 36(2):100-104.

Bekheit, S. S. (1984). Laboratory trails with *Bacllus thuringiensis* serotype H-14 controlling mosquito larvae. Egyptian Society of Parasitology, 14(1): 71-76.

Brogdon,W. G. and McAllister, J. C. (1998). Simplification of adult mosquito bioassays through use of time mortality determinations in glass bottles. J. Am Mosq.Control Assoc.,14:159-164.

Cheikh, H. B.; Ali-Haouas, B.; Marquine, M. and Pasteur, N. (1998). Resistance to organophosphorous and pyrethroid insecticides in *Culex pipiens* (Diptera: Culicidae) from Tunisia. J. Med. Entomol. 35: 251-260.

Cheikh, H. B. and Pasteur, N. (1993). Resistance to temephos, an organophosphorous insecticide, in *Culex pipiens* from Tunisia, North Africa. J. Am Mosq. Control Assoic., 9(3): 335-337.

Cornel, A. J.; Stanich, M. A; McAbrr, R. D. and Mulligan, F. S. (2002). High level methoprene resistance in the mosquito *Ochlerotatus nigromaculis* (Ludlow) in central California. Pest Manag. Sci., 58: 791-798.

Das, P. K. and Kalyanasanderson, M. (1984). Evaluation of K-otrine a synthetic pyrethroid for insecticidal efficacy against mosquito vectors. Indian J. Med. Res., 80:74-77.

DeSilva, D.; Hemingway, J.; Ranson, H. and Vaughan, A. (1997). Resistance to insecticides in insect vectors of disease: estα3, a novel amplified esterase associated with amplified estβ1 from insecticide resistant strains of the mosquito *Culex quinquefasiatus*. Expermental Parasitol., 57:253-259.

Dorta, D. M.; Vasuki, V. and Rajavel, A. (1993). Evaluation of organophosphorous and synthetic pyrethroid insecticides against six vector mosquito species. Rev. Saude Publica, 27(6): 391-397.

Eldridge, B. F.; Scott, T. W.; Day, J. F. and Tabachnick, W. J. (2000). Abrovirus diseases, in: Eldridge, B. F. and Edman, J. D. (Eds.), Medical Entomology, A Textbook on public health and veterinary problems caused by arthropods. Kluwer Acadimic Publishers, Dordrecht, pp. 415-460.

El-Shazly, M. M. and Refaie, B. M. (2002). Larvicidal effect of the juvenile hormone mimic pyriproxyfen on *Culex pipeins*. J. Am. Mosq. Control Assoc.,18(4): 321-328.

Finney, D. N. (1971). Probit analysis. 3[rd] Ed., Combridge Univ. Press. London, pp: 318.

Gonzalez, T.; Bisset, J. A.; Diaz, C.; Rodriguez, M. M. and Brandolini, M. B. (1999). Insecticide resistance in a *Culex qunquefasciatus* strain from Rio de Janeiro, Brazil. Mem. Inst. Oswaldo Cruz, Rio de Janeiro, 94(1): 121-122.

Kasai, S.; Weerashinghe, I. S. and Shono, T. (1998). P450 monoozygenases are an important mechanism of permethrin resistance in *Culix quinquefasiatus* Say larvae. Arch. Insect Biochem. Physiol., 37: 47-56.

Kawada, H.; Kohama, T. and Abe, Y. (1994). Larviciding activity of a water-soluble granular formulation of the insect growth regulator, pyriproxyfen against *Culex* mosquito. Jpn. J. Eniron. Entomol. Zool., 6: 86-77.

Klowden, M. J.; Held, G. A. and Bulla, A. (1983). Toxicity of *Bacillus thuringiensis* subsp. *ilsraelensis* to adult *Aedes aegypti* mosquitoes. Appl. Environ. Microbiol., 46(2): 312-315.

Macoris, M. L. G.; Andrighetti, M. T. M.; Nalon, K. C. R.; Garbeloto, V. C. and Junior, A. L. C. (2005). Standerdization of bioassays for monitoring resistance to insecticides in *Aedes aegypti*. Dengue Bull., 29:176-182.

Mostafa, A. A. (1982). Evaluation of certain insecticides from organophosphate and carbmate against *Culex pipiens* (L). Unpublished M. Sc. Thesis in pesticide Ain Shams University. Cairo. Egypt.

Paul, A.; Harrington, L. C.; Zhang, L. and Scott, J. G. (2004). Insecticide resistance in *Culex pipiens* from New York. J. Am. Mosq. Control Assoc., 21: 305-309.

Rye, D.; David J. and Jean-Claude M. (2003). Factors influencing the toxicity of xenobiotics against larval mosquitoes. C. R. Biologies, 326: 317-327.

Schaefer, C. H.; Miura, T.; Dupars, E. F.; Mullia, J. F. S. and Wilder, W. H. (1998): Efficacy, non-target effect and chemical persistence of S-31183a promising new mosquito (Diptera: Culicidae) control agents. J. Econ. Entomol., 65: 1066-1071.

Thomas, T. G.; Sharma, S. K.; Prakash, A.; Sokhey, J. and Sharma, B. R. (2000). Insecticide susceptibility status of *Culex* *tritaeniorhynchus* giles, vector of Japanese encephalitis in Delhi. Jpn. J. Infect .Djs., 53(1): 11-14.

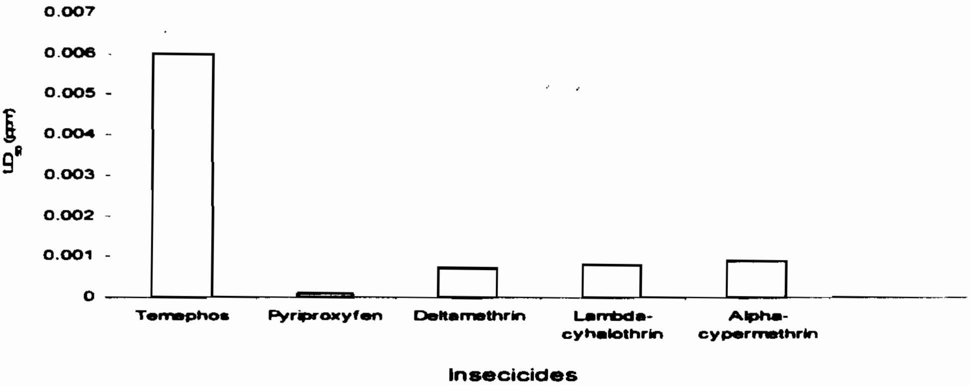
Tran, D. H.; Manh, N.; Ruyen, N.; Cong, L. D.; Co, T.; Thuan, L. K.; Hun, N. Q.; Dihn, P. Z.; Son, D. H.; Schapira, A. and Hii, J. (2000). Comparison of residual effect of bed net impregnated with permethrin, deltamethrin, lambda-cyhalothrin, etofenprox and alpha-cypermethrin in Vietnam, Unpublished Report.

Turrel, M. J.; O'Guinn, M. L.; Dohm, D. J. and Jones, J. W. (2001). Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile Virus. J. Med. Entomol., 38: 130-134.

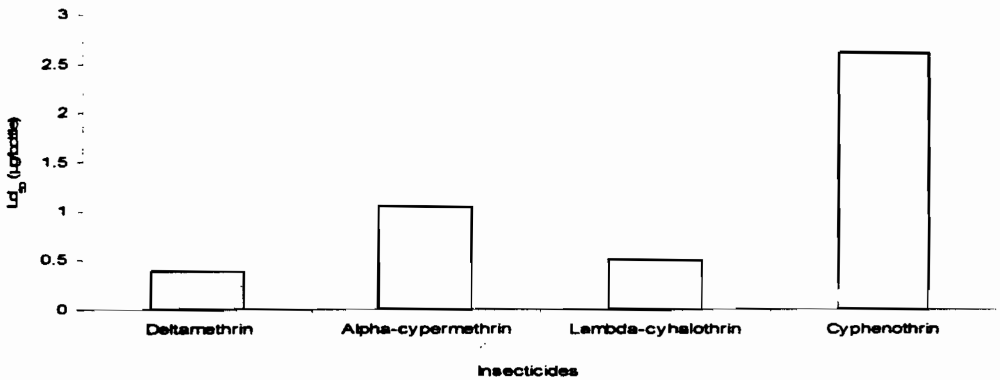
Wirth, M. C.; Jianniro, J. A.; Federici, B.A. and Walton, W. E. (2005). Evaluation of resistance toward *Bacillus sphaericus* or a mixture of *B.* sphaericus+ Cyt1A from *Bacillus thuringiensis* in the mosquito *Culex quinquefasciatus* (Diptera: *culicidae*) J. Invert. Pathol., 88: 154-162.

XU, Q.; Lui, H.; Zhang, L. and Lui, N.(2005). Resistance in the mosquito, *Culex quinquefasciatus*, and possible mechanisms for resistance. Pest Manag. Sci., 61: 1096-1102.

Zahiri, N. A.; Federici, B. A. and Mulla, M. S. (2004). Laboratory and simulated field evaluation of a new recombinant of *Bacillus thuringiensis* ssp. *Israelensis* and *Bacillus sphaericus* against *Culex* mosquito larvae (Diptera: Culicidae). J. Med. Entomol., 41(3): 423-429.



Fig(1):Larvicidal activity of the most effective insecticides against *C. pipiens*.



Fig(2): Adulticidal activity of tested insecticides against *C.pipiens*.

Table1. Probit analysis of toxicity of the tested insecticides against *C. pipiens* larvae (susceptible strain).

Insecticide	LD_{50} (ppm)	Confidence Limits	LD_{95} (ppm)	Confidence Limits	Slope ± SE
Temephos	0.0059	0.0051-0.0071	0.016	0.012-0.026	3.752 ± 0.476
B.t.i	0.012	0.0018-0.056	8463.449	7740.68-2212100	0.357± 0.023
Pyriproxyfen	0.000079	0.000055-0.0001	0.0051	0.0016-0.035	0.909 ± 0.121
Deltamethrin	0.00072	0.0005-0.0009	0.0072	0.0044-0.018	1.633 ± 0.277
Cyphenothrin	0.034	0.03-0.115	0.799	0.465-18.08	1.196 ± 0.107
Lambda-cyhalothrin	0.0008	0.0003-0.0011	0.0087	0.0051-0.037	1.596 ± 0.349
Alpha-cypermethrin	0.0009	0.0007-0.0011	0.0047	0.003-0.011	2.338 ± 0.417

Table 2. Probit analysis of toxicity of the tested insecticides against laboratory reared *C. pipiens* adults.

Insecticide	LD_{50} (µg/bottle)	Confidence Limits	LD_{95} (µg/bottle)	Confidence Limits	Slope ± SE
Deltamethrin	0.391	0.248-0.579	9.216	4.495 - 31.262	1.199 ± 0.175
Alpha-cypermethrin	1.043	0.696-1.543	22.214	10.654 - 76.577	1.238±0.176
Lambda-cyhalothrin	0.5	0.31-0.71	18.21	9.81- 49.95	1.054±0.141
Cyphenothrin	2.617	1.69-4.33	112.93	13.3309 - 117.93	1.007±0.188

الملخص العربي

تقييم معملي لبعض المبيدات الحشرية الكيماوية والبيولوجية ضد يرقات العمر الرابع والحشرات الكاملة لبعوضة الكيولكس بيبينز *Culex pipiens* (Diptera: Culicidae)

علي آل سرار و ظفرين الشهراني

قسم وقاية النبات كلية علوم الأغذية و الزراعه- جامعة الملك سعود- الرياض- المملكة العربية السعودية

تم إختبار أربعة مبيدات بيروثريدية (دلتامثرين و ألفاسيبرمثرين ولامداسـيهالوثرين وسـيفونثرين) و ثلاثة مبيدات حشرية أخرى هي تيميفوس و بيربروكسيفينبالاضافة الـى المبيـد الحيـوي باسـيلس ثرونجينيسيس ضد العمر اليرقي الرابع من بعوضة الكيولكس بيبـنز *Culex pipiens*

كما تم إختبار نشاط المبيدات البيروثريدية الأربعه ضد الطور الكامل لـنفس الحـشرة. كانـت قـيم التركيزات القاتلة ل٥٠% من اليرقات للمبيدات المذكورة عاليه هي ٠,٠٠٧٢ ، ٠,٠٠٠٩ ، ٠,٠٠٠٨ ، ٠,٠٣٤ ، ٠,٠٠٥٩ ، ٠,٠٠٠٠٧٩ ، ٠,٠١٢ جزء في المليون. وكانـت قـيم الجرعـات القاتلـة ل٥٠% من الحشرات الكاملة (بطريقة الإختبار داخل زجاجات هويتـون) هـي ٠,٣٩١ ، ١,٠٤٣ ، ٠,٥ ، ٢,٦١٧ ميكوجرام/زجاجة على التوالي.

هذا و يمكن إستخدام نتائج هذه الدراسة في تتبع ظهور صفة المقاومة في الـسلالات الحقليـة Feral strains or wild type strains في مواقع مختلفة من المملكة العربية السعودية.