

Long-Term Effects of the Treated Municipal Wastewater of the City of Riyadh on the Mosquito *Culex pipiens* L.*

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Abstract. Studies of the effect of the secondary treated municipal wastewater (SW) on the longevity of the larval stage of the mosquito *Culex pipiens* L. were started in the year 1987, and continued for more than three years. Two separate aquamedia were used for rearing a susceptible strain of the mosquito. In the first, SW was the sole liquid for the developmental stages of the first group of the insect, and fresh water (FW) was that used for the second. Susceptibility to chlorpyrifos insecticide, of the larvae of the two groups was evaluated periodically, in the form of percent mortalities, LC50 values and slope of toxicity curves.

The results exhibited that longevity of the larval stage of SW-reared mosquitoes was less than those of FW-reared ones with 3.7 and 8.3 days in the years 1987 and 1989, respectively. An increase in the susceptibility of the SW-reared larva to chlorpyrifos was observed. Values of LD50 for the first group of insects ranged from 1.6 to 4.2 ppb; and those for the second were 2.25 to 4.10 ppb. Slopes of the toxicity curves ranged from 1.79 to 2.4 and from 1.7 to 1.9 for the first and second groups, respectively.

Introduction

Chemical pollutants of the secondary treated municipal wastewater (SW) include a variety of organic and inorganic substances. The inorganic constituents are well defined and can easily be detected, whereas the organic pollutants are much more difficult to measure and to identify. Only the volatile compounds can be separated and identified. Chlorination process for the disinfection purposes have shown to produce some organohalides and other low molecular weight compounds [1]. Chlorination of the wastewater had no significant effect on concentrations and types of traces of organic compounds [2]. SW may contain halogenated and non-halogenated aliphatics and aromatics, such as phenols [3] cresols [4 p 35-37]; chlorophenols [5];

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chloroform, trichloroethylene, toluene, xylenes, and dichlorobenzenes [1]. In addition to these organics, SW may include also humic substances, pesticide residues, polycyclic aromatics and surface active agents [4 p 40-41].

The major part of the Riyadh-Sw is utilized in agricultural irrigation, and the other part is discharged to a pond, through an uncovered channel of "Al-Azizia" south of the city, on which Mosquito can survive and flourish.

Mosquito larvae (*C. pipiens* L.) reared on SW could be affected from its pollutants by three ways; (a) the pollutants could be toxic to the larvae, (b) they could be beneficial for their growth and development, and (c) they could affect the insect response to the applied insecticides, used for its control, through the "selection pressure" phenomenon, exerted by the chlorinated hydrocarbon residues presented in the SW media. Hence, the main objective of this study is to investigate the long-term effect of SW-rearing of the mosquito on the longevity of the larval stage, and on the insecticidal response of the larvae to chlorpyrifos insecticide.

Materials and Methods

1. Rearing of the larvae

A colony from the mosquito, *C. pipiens* L., sensitive to pesticides was obtained early in the year 1885 (exactly on 14/4/1985). The colony was reared in our laboratory, on an artificial diet, and completely protected from the effect of the residues of the pesticides or any other chemicals. The sensitivity of the mosquito strain to the insecticide, chlorpyrifos (*O,O*-diethyl- (3,5,6-trichloro-2-pyridyl)- phosphorothioate), was periodically tested. On the year 1987 exactly on 10/9/1987), the colony was divided into two groups the first was reared on SW, while the second was continued its rearing on fresh water (FW). The rearing of each was continued for more than three years, in an environmentally controlled room, maintained on $25 \pm 2^\circ\text{C}$ (see table 1).

Table 1. Characteristics of secondary treated municipal wastewater (SW) and freshwater (FW)*.

Water type	Specifications
SW	pH, 7.26; EC.1.61 d Sm; COD, 89 mg/l; Cations and anions (in mg/l): NH ₃ -N,20.7; PO ₄ -P, 6.9; K, 15.1; Ca, 128; Mg, 28; Na, 140; SO ₄ , 326; Cl, 170; HCO ₃ , 195; and TSS, 1050 mg/l.
FW	pH, 7.8; EC.0.70 d Sm; Cations and anions (in mg/l): K, 0.10; Ca, 3.27; Mg, 1.89; Na, 1.95; SO ₄ , 187; Cl, 66; HCO ₃ , 67 and TSS, 450 mg/l.

* Source: [7]

Rearing of the larvae, in each of the two aquamedia was accomplished in rearing pans, according to the method of WHO [6, p 310-311]. Yeast granules were sprinkled on the surface of each aquamedia every two days, and the water of each was renewed every week. Larval time was calculated starting from hatching time upto pupation. Emerged pupae in each media were collected as they formed and placed in an emergence cage for each of the SW and FW colonies. Emerged-out adults in each cage were maintained in a temperature of $25 \pm 2^\circ\text{C}$, and relative humidity not less than 80% inside the cages. Cotton pads, soaked in glucose solution (100g/l) were kept inside the cages for the adult males to feed on. A bare-backed pigeon was introduced inside each cage every night to provide blood meal for the adult female mosquitoes. An enamel powder, filled with respect water, was placed inside each cage for oviposition, and the two cages were kept under light illumination for 9 hours, and kept dark for the rest of the day.

2. Larvicidal activity measurements

Larval susceptibility to chlorpyrifos of each of SW-reared (SW-RC) and FW-reared (FW-RC) colonies was evaluated several times during the rearing time. Tests were accomplished according to the method of WHO [6, p 310-311], in which, technical grade (98-99% a.i.) chlorpyrifos, was used to prepare a concentration stock (in ppb range) in ethanol (96%). The tests were carried out in 250 cc-capacity jars, each contained 200 cc of distilled water, in which the proper concentration of the insecticide was dissolved. The insecticidal treatments involved the concentration range of 0.5-8.0 ppb of chlorpyrifos, in addition to a control and a blank treatments. The blank treatment contained an amount of ethanol equal to that added as a solvent of the highest concentration of the insecticide. Replications were three; and each with 20 larvae at the early fourth instar. Dead and alive larvae in each jar were counted, at the end of the 24-hr. incubation time, from which percent mortalities and LC50 values were estimated.

Results and Discussion

1. Larvicidal Activity of SW

Numbers of dead larvae in each of the two aquamedia (SW and FW) were counted periodically during the whole time of the test. The percent mortality of the mosquito larvae of either SW or FW aquamedia, at any time of the test, and for any larval stage, did not exceed 1%. Hence, no larvicidal activity for the SW was observed, inspite of its contents of organic and inorganic pollutants (Table 1). However SW media caused a reasonable improvement in the mosquito larval growth and development.

2. Effect on Larval Duration

Results of the larval duration of the mosquito, during 1987, 1988, 1989 and 1990 years are presented in Table 2. The means of the larval time, during the first year,

were 12.9 ± 1.3 and 16.6 ± 2.2 days, for the SW and FW reared insects respectively. Similar trend was also observed with the other three years, since the longevity of the SW-reared larval time was always shorter than that of the FW-reared one. Comparing to the FW aquamedia, SW decreased the larval-time of the mosquito with 3.7 days for the first year, 5.4 days for the second; 8.4 days for the third and 7.4 days for the first- half of the fourth year. Hence, SW improved the larval growth, decreased each of the larval-time and the generation-time, and increased number of generations, when compared to the FW. Actually, the production of larvae from SW was rapidly increased and every two or three generations, a lot of them was destroyed to maintain reasonable larval populations for the generation propagation. In each of the SW and FW colonies of the insect, number of the egg-masses, hatching, and fecundity time were not measured. However, in spite of the presence of high contents of the total soluble solids (TSS), N, P, and K in the SW (Table 1), their presence proved to be beneficial for the mosquito growth and propagation.

Table 2. Effect of sewage water (SW) on the longevity (days) of *Culex pipiens* larvae.

Period of time	SW	FW	Difference
	Mean \pm SD	Mean \pm SD	
First year			
1 st.	12.8 ± 1.1	16.6 ± 1.9	-3.8
2 nd.	12.8 ± 1.3	17.2 ± 2.7	-4.4
3 rd.	13.0 ± 1.9	16.2 ± 2.2	-3.2
4 th.	13.0 ± 1.4	16.2 ± 2.5	-3.2
Overall Mean \pm SD	12.9 ± 1.3	16.6 ± 2.2	-3.7
Second year			
5 th.	17.6 ± 2.5	19.8 ± 1.9	-2.2
6 th.	14.0 ± 0.7	20.6 ± 2.3	-6.6
7 th.	16.2 ± 1.8	20.6 ± 3.0	-4.4
8 th.	17.0 ± 3.8	24.4 ± 1.5	-7.4
Overall Mean \pm SD	16.0 ± 2.6	21.4 ± 2.8	-5.4
Third year			
9 th.	17.8 ± 2.2	24.4 ± 3.5	-6.6
10 th.	15.0 ± 1.2	23.6 ± 3.2	-8.6
11 th.	15.2 ± 1.5	24.4 ± 2.5	-9.2
12 th.	15.4 ± 2.2	24.8 ± 1.8	-9.4
Overall Mean \pm SD	15.9 ± 2.0	24.3 ± 2.7	-8.4
Fourth year			
13 th.	16.8 ± 2.5	23.0 ± 2.1	-6.2
14 th.	14.6 ± 1.1	23.2 ± 2.9	-8.6
Overall Mean \pm SD	15.7 ± 2.2	23.1 ± 2.4	-7.4

3. Effect of SW-rearing media on the larvicidal activity of chlorpyrifos

This test was first conducted on a *C. pipiens* L. strain, sensitive to the insecticides; and its LC50 value for chlorpyrifos insecticide, on the first date of its rearing in our laboratory was 5.2 pb (on 14/10/1985). The mosquito colony was divided into two groups on 10/9/1987, and started rearing on either SW or FW aquamedia since then. Chlorpyrifos was used as an indicator of the sensitivity of each of these two colonies, by drawing *ld-p* lines, and evaluating the values of LC50 and slope of each line.

On the bginning of the rearing, values of LC50 for the SW and FW mosquito colonies were similar, and equal to 5.2 ppb (on 14/10/1985). Such value was dropped for SW-colony to 4.2 ppb on 26/10/1987; to 3.9 ppb on 3/4/1988 and finally to 1.6 ppb on 27/11/1989 (Fig. 1). Hence, during two years (1987-1989) of rearing on SW aquamedia, LC50 values of chlorpyrifos were decreased from 4.2 ppb to 1.6 ppb, which means that, the SW-rearing media of the larvae, increased their sensitivity to the insecticide. In the meanwhile, slope values of the *ld-p* lines for these three dates were increased from 1.79, to 1.9 and finally to 2.4 for the three dates of the 87, 88, and 1989 respectively.

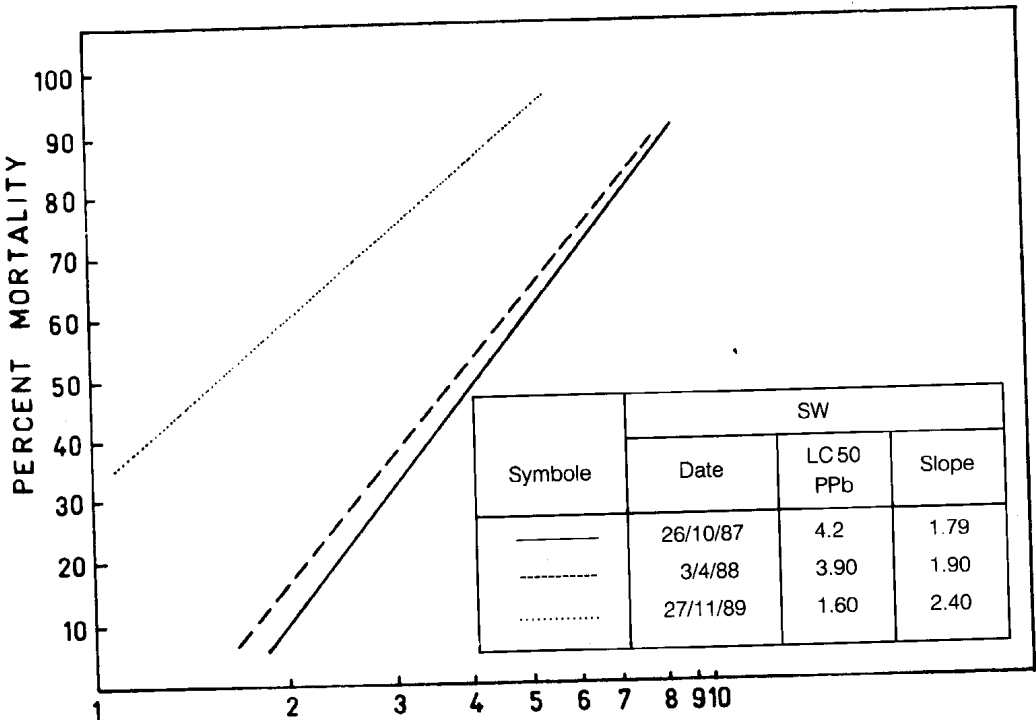


Fig. 1. *Ld-p* lines of chlorpyrifos on SW-reared colony of mosquito.

Figure 2 shows the sensitivity of the FW-colony to chlorpyrifos. The values of LC50 in this cases was decreased from 4.10 ppb on 13/10/1987 to 3.25 ppb on 26/10/1988 and finally to 2.25 ppb on 27/11/1989. Hence, FW-rearing of the mosquito, decreased their LC50 values for chlorpyrifos from 4.10 ppb to 2.25 ppb. but, the slope values of the *ld-p* lines were increased from 1.70, to 1.80 and finally to 1.90 for the three dates of the 87, 88, and 1989 respectively.

However, at the end of the experiment, the sensitivity of the SW-colony to chlorpyrifos was greater than that of the FW and this may be due to shorter time of the life cycle, which resulted in greater number of the generations for the former colony than for the latter one. It seems that SW did not apply any chemical pressure (or selection pressure) on the mosquito larvae in spite of its contents of the organic and inorganic pollutants, especially those of haloderivatives of aliphatics and aromatics [3]. SW caused a pronounced improvement in the mosquito-larval growth and development, that lead to shorter time of life cycle, greater numbers of generations, and finally faster levels of sensitivity, when compared to the FW. Such result leads to the question: Is the SW-colony faster than the FW-one, in acquiring the resistance if they were exposed to a pesticide pressure ? In other words, the resistance factor of the SW-reared colony of the mosquito may be greater and faster than that of the FW-reared one, since the *ld-p* line slope was particularly much greater for the SW-reared larvae (2.4) than that of the FW-reared one (1.9).

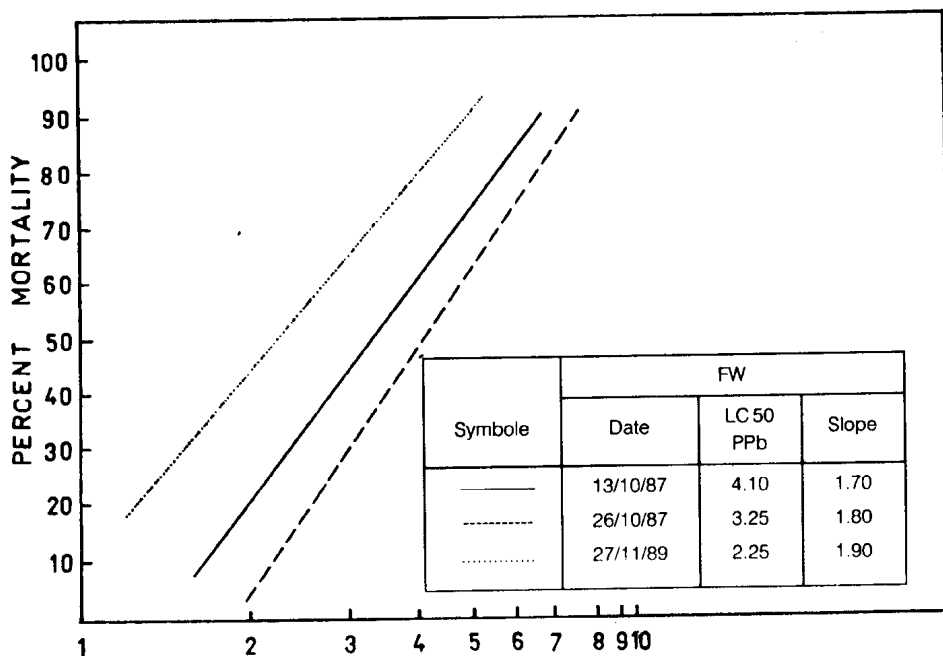


Fig. 2. *Ld-p* lines of chlorpyrifos on FW-reared colony of mosquito.

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التأثير طويل المدى لمياه الصرف الصحي المعالجة لمدينة الرياض على بعوضة الكيولكس

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ملخص البحث. بدىء في دراسة تأثير مياه الصرف الصحي المعالجة لمدينة الرياض على طول العمر اليرقي لبعوضة الكيولكس اعتباراً من عام ١٩٨٧م حيث تم وضع كتل من البيض مأخوذة من سلالة معملية حساسة من الكيولكس في أحواض تربية اليرقات محتوية على مياه الصرف الصحي المعالجة كمعاملة أولى، ووضعت كتل أخرى منها في أحواض ماثلة محتوية على مياه عادية كمعاملة ثانية للمقارنة، وقد استمرت تربية الحشرات في المجموعتين لمدة تزيد على ثلاث سنوات متصلة بجعل مياه الصرف الصحي هي البيئة المائية لتربية حشرات المجموعة الأولى، والمياه العادية هي البيئة المائية لتربية الثانية. كما تم أيضاً اختبار حساسية اليرقات في كلا المجموعتين المذكورتين للتسمم بالمبيد الحشري كلوربيريفوس وذلك بإيجاد النسب المئوية للموت وقيم تركيز القتل النصفية ق ٥٠ وميل خطوط السمية على فترات متباعدة. وقد أظهرت النتائج أن العمر اليرقي للكيولكس في المعاملة الأولى أقل منه في الثانية بمدة تتراوح من ٣,٧ إلى ٤,٨ يوم في عامي ١٩٨٧، ١٩٨٩م على التوالي وأن يرقات المجموعة الأولى أكثر قابلية للتسمم بالمبيد من يرقات المجموعة الثانية حيث تراوحت قيم ق ٥٠ للأولى من ١,٦ إلى ٢,٤ جزء في البليون. بينما تراوحت قيم الثانية من ٢,٢٥ إلى ٤,١٠ جزء في البليون، وتراوحت كذلك قيم ميل الخطوط البيانية للسمية في المعاملة الأولى من ١,٧٩ إلى ٢,٤ بينما تراوحت في الثانية من ١,٧٠ إلى ١,٩٠.