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Toxicity of Four Pyrethroid-based Insecticides and Kerosene to a Laboratory Population of *Culex pipiens*

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Abstract: Four commercial pyrethroid-based insecticides, cypermethrin, fenvalerate, Killer and Tyfon super 20 were tested as larvicides against a laboratory strain of *Culex pipiens* and kerosene and Tween 80 1% in kerosene were tested as larvicides and pupicides against the same population. LC_{50} values of tested pyrethroids were 0.00029 ppm (Cypermethrin), 0.026 ppm (fenvalerate), 0.012 ppm (Killer) and 0.014 ppm (Tyfon super 20). The diagnostic concentrations for cypermethrin and Tyfon Super 20 were 0.015 and 0.07 ppm, respectively. LD_{50} values for larvae were 33.42 and 13.11 L ha⁻¹ and for pupae 69.74 and 36.1 L ha⁻¹ for kerosene and T 80 in kerosene, respectively. The results showed that the mixture of kerosene + T 80 could be used as an effective larvicide and pupicide at much lower rates of application compared to kerosene alone, which reduces the environmental pollution to a great extent.

Key words: *Culex pipiens*, pyrethroids, kerosene, pupicide, larvicides, Tween 80

INTRODUCTION

Mosquitoes have long been known to be vectors of human and animal diseases such as West Nile virus, malaria and dengue^[1]. Several methods have been employed to control mosquito populations, but the chemical method (pesticides) is still the main and the most effective one, especially in case of over populations. The subsequent use of a pesticide to control mosquitoes leads to the development of resistance. Mosquitoes have developed resistance to almost all classes of insecticides^[2-7]. The use of petroleum oils, as mosquito larvicides, dates back to 1793^[8]. Recently, refine oils have been evaluated as mosquito larvicides^[9,10].

In this study we report on the toxicity of four commercial pyrethroids and kerosene, used in Saudi Arabia, to a laboratory population of *Culex pipiens* to monitor the appearance of resistance to these pesticides in different field locations; we also have studied the effect of adding the emulsifier Tween 80 on the toxicity of kerosene against larvae and pupae.

MATERIALS AND METHODS

This study was carried out in the Department of Plant Protection, College of Agriculture, King Saud University, in 2004.

Chemicals: α -cypermethrin 10% EC, Tyfon super 20 (s-bioallethrin 1.45% + permethrin 18.85% + piperonyl butoxide 11.4% EC) and Killer (tetramethrin 20% + permethrin 12% + piperonyl butoxide 12% EC) were products of SCIDCO and Fenvalerate 10% EC was a product of Mobeed Company, Saudi Arabia.

Commercial kerosene was purchased from a local source and Tween 80 was a product of Fisher Biotech, New Jersey, USA.

Mosquito colony rearing: A susceptible strain of *Culex pipiens* (kindly provided by Dr. Osama Awad, High Institute of Public Health, Alexandria, Egypt) was reared at 25±2°C in wire-screened cages (45x45x45 cm). Adult females were blood-fed on a pigeon to get egg masses, the colony was provided with 10% sucrose solution to feed on; hatched larvae were fed on finely powdered mouse feed.

Larvicidal activity: Twenty mosquito larvae (late 3rd and early 4th instars) were placed in a 200 mL glass beaker containing 100 mL of distilled water. Solutions of required concentrations were prepared in ethanol. Each concentration was replicated 3 times. Three controls pans that received only the solvent were maintained during the test. Dead larvae were counted 48 h after treatment; larvae that did not move when touched with a thin needle were considered dead.

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Larvicidal and pupicidal activity of kerosene and kerosene-T 80 mixture:

Laboratory tests were conducted in plastic pans (11x17.4x6 cm) filled with one liter of water. Twenty larvae or pupae were placed in each pan before treatment. Area of surface water was estimated as 191 cm², kerosene or 1% T 80 in kerosene was added to surface of water to give the required rates of application showed in Table 3. For example, to apply a rate of 50 L ha⁻¹, 96 µL of the oil was added to each pan; three replicates were used in the case of larvae and two replicates were used in the case of pupae; control pans were run under same conditions. Data was corrected according to Abbott^[11] and probit analysis of data was done according to Finney^[12].

RESULTS AND DISCUSSION

Toxicity of tested pyrethroids: Tested concentrations were selected based on preliminary tests. The highest Fenvalerate tested concentration was 100 fold the highest cypermethrin tested one (0.0008 ppm) (Table 1). Cypermethrin was the most effective pyrethroid with LC₅₀ and LC₉₅ of 0.00029 and 0.002 ppm, respectively (Table 2). The diagnostic concentration of cypermethrin was 0.015 ppm.

Tyfon Super 20 (contains 18.85% permethrin) and Killer (contains 12% permethrin) showed equal toxicities with LC₅₀ and LC₉₅ values of 0.014, 0.012, 0.049 and 0.042 ppm, respectively (Table 2). The diagnostic concentration for Tyfon Super 20 was 0.07 ppm. Fenvalerate was the least toxic compound with LC₅₀ equals to 0.026 ppm and LC₉₅ equals to 0.156 ppm. Kawakami^[7] tested permethrin against *C. pipiens* larvae and found that the LC₅₀ was 0.01 ppm. Kawakami^[7] also mentioned that a field strain of *C. pipiens* developed 14.7 fold resistance to permethrin. Bisset *et al.*^[4] found that *C. pipiens* subjected to lambda-cyhalothrin selection did not develop cross resistant to cypermethrin, this was in contradiction with Xia *et al.*^[13] who reported that *C. pipiens* resistant to lambda-cyhalothrin developed 41 and 28 fold resistance to permethrin and cypermethrin, respectively.

Larvicidal and pupicidal activity of tested oils: Larvae were more susceptible than pupae to the two tested materials. The mixture of kerosene + T 80 was more toxic to both larvae and pupae than kerosene alone was. Kerosene, at the rate of 35 L ha⁻¹, caused only 48.3% larval mortality, while kerosene + T 80, at 18.2 L ha⁻¹, resulted in 85% larval mortality (Table 3). Adding T 80 at 1% to kerosene reduced the LC₅₀ from 33.42 to 13.11 L ha⁻¹ (Table 4), this means that T 80 synergized the effect of kerosene by 2.55 fold. The LD₅₀ values of

Table 1: Mortality % of *Culex pipiens* larvae due to tested insecticides

Conc. (ppm)	Killer M (%)	Tyfon Super (%)	Fenvalerate M (%)	Conc. (ppm)	Cypermethrin M (%)
0.005	NT	8.7	8.7	0.0001	22.8
0.010	47.4	33.4	22.8	0.0002	33.4
0.020	66.6	73.7	28.1	0.0004	49.2
0.030	86	NT	NT	0.0008	87.7
0.040	100	89.5	68.4	-	-
0.080	NT	NT	87.8	-	-

NT = Not Tested

Table 2: Probit analysis for toxicity of tested insecticides against larvae of *Culex pipiens*

Pesticide	LC ₅₀ (95% FL)	LC ₉₅ (95% FL)	Slope±SE
Cypermethrin	0.00029 (0.00027 - 0.00035)	0.002 (0.0013-0.0032)	1.97±0.045
Tyfon Super-20	0.014 (0.012-0.015)	0.049 (0.038-0.061)	2.99±0.064
Killer	0.012 (0.01- 0.014)	0.042 (0.034-0.052)	2.98±0.110
Fenvalerate	0.026 (0.023-0.030)	0.156 (0.112-0.219)	2.11±0.030

Table 3: Toxicity of kerosene (K) and kerosene + T 80 to *Culex pipiens*

Larvae		Pupae					
K	K + T 80	K		K + T 80			
AR	AR	AR	AR	AR	AR		
L ha ⁻¹	M (%)	L ha ⁻¹	M (%)	L ha ⁻¹	M (%)		
25	28.3	13.0	46.7	40	12.8	30	23.1
35	48.3	18.2	85.0	50	20.5	40	66.7
50	86.7	26.0	95.0	75	59.0	50	89.2
75	93.3	-	-	100	74.4	75	100
				125	92.3	-	-

(AR = Application Rate (L ha⁻¹))

Table 4: Probit analysis for toxicity of kerosene (k) and kerosene + T 80 to *Culex pipiens*

Stage	Material	LC ₅₀ (95% FL)	LC ₉₅ (95% FL)	Slope±SE
Larvae	K	33.42 (31.11-35.9)	73.5 (63.6-84.96)	4.81±0.22
	K + T 80	13.11 (12.04-14.29)	24.35 (21.5-27.7)	6.12±0.69
Pupae	K	69.74 (65.8-73.7)	147.4 (130.3-166.7)	5.06±0.15
	K + T 80	36.1 (34.6-37.7)	54.7 (50.5-59.2)	9.12±0.82

kerosene and kerosene + T 80 against pupae were 69.7 and 36.1 L ha⁻¹, respectively. These results indicate that kerosene alone could not control larvae and pupae at reasonable rates of application. To achieve 95% pupal mortality, kerosene should be applied at 147.4 L ha⁻¹ (Table 4), this rate is not economic and could have detrimental effects on aquatic organisms; adding T 80 reduces this rate almost to one third. Golden Bear-1111, a commercial petroleum oil and BVA 2 mosquito larvicide, a new surfactant oil, were effective against larvae of *Culex* at the rate of 14 L ha⁻¹^[10]. In this study the mixture of kerosene+T 80, at the rate of 24.35 L ha⁻¹, caused 95% kill in larvae of *Culex pipiens*. The emulsifier Triton enhanced the effect of Diesel against larvae of *Culex pipiens*^[14] and addition of the surfactant Pyroter

CPI-40^R at 2% to the methylated soy oil enhanced the efficacy of the oil against the 4th stage larvae of *Culex pipiens*^[9]. The results of this study show that the mixture of kerosene + T 80 could be used as an effective mosquito larvicide and pupicide; this mixture reduces the amount of kerosene required to control these stages and subsequently reduces the environmental pollution.

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