Insecticide Resistance Development in *Culex pipiens* Population under Selection Pressures with Temephos

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Abstract: A collection of *Culex pipiens* from Boussalem, Northwestern Tunisia, with a low level of temephos resistance (14.90 at LD95) was selected to higher resistance with temephos in the laboratory. After 6 generations of pressure, the temephos resistance ratio increased to 103.95 at LD95. Synergism tests showed that temephos resistance was not associated with monooxygenase and esterases or (GST); however, evidence of insensitive acetylcholinesterase was found. It is evident that this important vector species, *Culex pipiens*, has the potential to develop resistance to temephos.

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Keywords: *Culex pipiens*, selection pressures, temephos resistance, organophosphate, esterase, acetylcholinesterase.

1. Introduction:

The mosquito *Culex pipiens* occurs in tropical and temperate zones. Their nuisance biting and disease that vehicle (Kolberg, 1994; OMS, 2005; Turell et al., 2001; CDC, 2002; Pelah et al., 2002; Krida et al., 2011; Hoogstraal et al., 1979; Meegan et al., 1980; Darwish & Hoogstraal, 1981; Moutailler et al., 2008; Harb et al., 1993; Krida et al., 1998; Vinogradova, 2000; Abdel-Hamid et al., 2009; Abdel-Hamid et al., 2011; Wasfi et al., 2014) pushed humans to fight actively in many countries using insecticides. In recent decades, *Culex pipiens* has developed resistance to a wide variety of insecticides (Ben Cheikh et al., 1998; Chandre et al., 1998; Bisset & Soca, 1998; Yebakima et al., 2004; Liu et al., 2004, Paul et al., 2005; Cui et al., 2006; Ben Cheikh et al., 2008; Anes, 2013; Marcombe et al., 2014).

Only three loci are responsible for major resistance, *Est- 2*, *Est-3*, and *Ace-1*. *Est- 2* and *Est-3* super locus *Ester*, encode esterase A and B that trap insecticides. In the case of resistance, these esterases are produced in excess by a process of amplification. The *Ace-1* gene codes for the target of insecticides, acetylcholinesterase1 (AChE1). In the case of resistance, this target is mutated, which reduces its affinity for organophosphorus insecticides (OP) (Bourguet et al., 1997; Lenormand et al., 1998a; Weill et al., 2003; 2004).

Resistance to OP in *Culex pipiens* is an excellent model for studying adaptation to a new environment. The objective of this study was to establish a temephos-resistant colony to develop research programs that will study the evolution of resistance responses in *Culex pipiens* under laboratory conditions.

2. Materials and Methods:

2.1. Mosquito Strains

Culex pipiens were collected as larvae and pupae in the Governorate of Jandouba (Boussalem), Northern Tunisia, in 2004. The field collected strain of *Culex pipiens* was reared in the insectarium for further tests. A susceptible laboratory strain of *Culex pipiens* (S-LAB) was used to compare the susceptibility status of the field strains.

2.2. Insecticides

Two technical grade insecticides were used for selection and bioassay: the organophosphates temephos (9l‰; American Cyanamid, Princeton, NJ), and the carbamate propoxur (997o; Mobay). Two synergists were used to help detect detoxification enzymes involved in resistance: S, S, S {ributyl phosphorothioate (DEF), an esterase inhibitor, and piperonyl butoxide (pb), an inhibitor of mixed function oxidases.

2.3. Selection Procedures

The collected strain was preceded for selection pressure to temephos. This strain was selected for 6 generations by exposing late third or early fourth instars to the concentrations which produced 50–75 % mortality (Paeporn et al., 2004).

2.4. Bioassay Procedures and Data Analysis

The late third or early fourth instar larvae of all groups were used for bioassay. The procedures were recommended by the WHO (1963). The results were analyzed for the median lethal concentration (LD50) and

LD95 by probit analysis using a Basic program (Raymond, 1985).

2.5. Esterase's Detection

Esterase phenotypes were established by starch electrophoresis (TME 7.4 buffer system) as described by Pasteur et al. (1981, 1988) using homogenates of thorax and abdomen.

3. Results and Discussion:

Culex pipiens was placed under selection pressure and each generation was tested for susceptibility to temephos (Table 1). Resistance rates (RR) were variable from one generation to the next, sometimes it increased, sometimes it decreased. Thus, in the natural population of Boussalem the RR at LD50 was 21.45 then it steadily increased until the 3rd generation of selection to reach 177.06 then decreased significantly to 16.29 at the 5th generation but at the 6th generation it rose again and reached a value of 119.64. The reason for this variation is unknown but effects related to uncontrolled environmental parameters or inter-strain genetic variability is probably to be involved. A similar study on rapid and instability development of physiological resistance was reported for *Anopheles stephensi* (Verma and Rahman, 1986).

The values for slopes of regression lines varied from 0.87 to 3.85. The highest value was obtained from the F1 generation (the lowest resistance rates) and the smallest value was from the F4 generation (Highest resistance rate). In fact, selection pressure favored an increased frequency of resistant alleles (Brown & Pal, 1971). The linearity of concentration-mortality curves was accepted ($P<0.05$) only for Bou.nat (Table 1). In the presence of the synergist PB, linearity of concentration-mortality curves was rejected ($P<0.05$) in Bou.tem6.T.PB. In the presence of the synergist DEF, the linearity of concentration-mortality curves was rejected ($P<0.05$) for Bou.tem6.T.DEF.

The addition of PB to biossays had no significant effect on temephos dose–mortality responses in S-Lab and Bou.tem6 (95% CI of synergism ratios contained the value 1). The addition of DEF to bioassays did not modify the dose–mortality response to temephos in S-Lab and Bou.tem.6. These results showed that neither esterases (or GST) inhibited by DEF nor P450 cytochrome mediated monooxygenases inhibited by PB played a role in the observed resistance of Bou.tem6. This conclusion was confirmed by the observation that the resistance ratios to temephos did not change significantly in the presence of either synergist. The synergism ratio (SR=1) was equal between Bou.tem6 and S-Lab (Table 2).

Table 1: *Insecticide resistance evolution of Bou.tem larvae under selection pressures with temephos*

Name of population	LD_{50} (a)	LD_{95} (a)	Slope (b)	H (df)	RR_{50} (c)	RR_{95} (c)
S-Lab.T	0.0012 (0.0011-0.0014)	0.0062 (0.0047-0.0094)	2.34± (0.22)	1 (3)	-	-
Bou.nat.T	0.0266 (0.0237-0.0301)	0.0934 (0.0741-0.1283)	3.02± (0.27)	1 (2)	21.45 (17.63-26.10)	14.90 (9.15-24.28)
Bou.tem1.T	0.0596 (0.0402-0.0868)	0.1592 (0.0844-0.3213)	3.85± (0.60)	3.33 (2)	47.90 (31.86-72.01)	25.41 (11.60-55.67)
Bou.tem2.T	0.0723 (0.0191-0.2718)	0.5096 (0.0163-16.8521)	1.94± (0.77)	13.63 (2)	58.13 (29.42-114.84)	81.33 (13.74-481.46)
Bou.tem3.T	0.2203 (0.0013-32.9515)	1.7814 (0.0000-56327470.00)	1.81± (0.88)	10.82 (1)	177.06 (85.28-367.62)	284.29 (29.92-2700.93)
Bou.tem4.T	0.0656 (0.0015-2.8717)	4.8884 (0.0000-2935941.00)	0.87± (0.51)	18.40 (2)	52.76 (22.24-125.14)	780.14 (39.12-15557.85)
Bou.tem5.T	0.0202 (0.0058-0.0738)	0.3792 (0.0188-10.3333)	1.29± (0.24)	3.96 (2)	16.29 (10.44-25.43)	60.51 (19.93-183.76)
Bou.tem6.T	0.1488 (0.0887-0.2586)	0.6513 (0.1578-3.4516)	2.56± (0.47)	3.04 (2)	119.64 (82.08-174.39)	103.95 (36.02-299.93)

Bou: Boussalem; nat: natural population; tem: temephos
(a) In mg/liter, 95% CI in parentheses. (b) Standard errors in parentheses. H: Heterogeneity, (df): testing linearity of the probit mortality/log dose response. (c) RR, resistance ratio (LC50 of the population considered / LC50 of S-Lab); 95% CI in parentheses.

Over produced esterases were investigated in single homogenate using starch gel electrophoresis. A total of 120 mosquitoes were analyzed (20 mosquitoes by selection generation). Starch gel electrophoresis did not disclose any overproduced known esterase in the Jandouba samples. Except for Bou.nat, c*ulex pipiens* of selection temephos showed resistance to Propoxur wich indicates an acetylcholinesterase insensitive. We observed in this study that overproduction of esterases and modifications of AChE are not correlated (only AChE-1 is involved in the resistance of the selected strains to temephos). The only exception is the natural population where there is neither overproduction of esterases nor mutation of AChE. However, Ben Cheik & Pasteur. (1993) and Ben Cheikh et al. (2009) reported the existence of a correlation between the frequency of individuals possessing the Ace-1R allele and those overproduced esterases A and B among the Tunisian populations of *Culex pipiens*.

Table 2: *Responses of Bou.tem strains of Culex pipiens to temephos with and without synergists*

Name of population	LD_{50} (a)	LD_{95} (a)	Slope (b)	H (df)	RR_{50} (c)	RR_{95} (c)	SR_{50} (d)	SR_{95} (d)
S-Lab.T	0.0012 (0.001-0.0014)	0.0062 (0.0047-0.0094)	2.34± (0.22)	1 (3)	-	-	-	-
Bou.tem6.T	0.1488 (0.0887-0.2586)	0.6513 (0.1578-3.4516)	2.56± (0.47)	3.04 (2)	119.64 (82.08-174.39)	103.95 (36.02-299.93)	-	-
S-Lab.T.DEF	0.0003 (0.0002-0.00036)	0.00069 (0.0005-0.0009)	4.99± (0.69)	1 (2)	-	-	3.84 (2.89-5.09)	9.05 (5.07-16.17)
Bou.tem6.T.DEF	0.1586 (0.0993-0.2537)	0.6236 (0.2269-1.7578)	2.76± (0.54)	6.72 (3)	489.90 (278.99-860.24)	901.54 (282.14-2880.75)	0.93 (0.53-1.65)	1.044 (0.26-4.13)
S-Lab.T.PB	0.0021 (0.0017-0.0028)	0.0154 (0.0092-0.0371)	1.94± (0.28)	1 (2)	-	-	0.56 (0.44-0.72)	0.40 (0.21-0.77)
Bou.tem6.T.PB	0.1793 (0.0002-177.071)	1.4614 (0.0000-0.0342)	1.80± (1.31)	15.44 (1)	81.69 (27.69-240.97)	94.89 (1.15-7777.17)	0.83 (0.27-2.51)	0.44 (0.005-39.28)

(a) In mg/liter, 95% CI in parentheses. (b) Standard errors in parentheses. H: Heterogeneity, (df): testing linearity of the probit mortality/log dose response. (c) RR, resistance ratio (LC50 of the population considered / LC50 of S-Lab); 95% CI in parentheses. (d) SR, synergism ratio (LC50 observed without synergist / LC50 observed with synergist); 95 CI in parentheses.

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Conflicts of Interest:

Authors declared no conflicts of interest.

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