Daaboub et al., J Genet Disor Genet Rep 2017, 6:3
DOI: 10.4172/2327-5790.1000161

Journal of Genetic Disorders & Genetic Reports

Research Article

A SCITECHNOL JOURNAL

Chlorpyrifos Resistance Characteristics of *Culex pipiens* (Diptera: Culicidae) from Northern Tunisia

Jaber Daaboub[1,2], Ahmed Tabbabi[1]*, Ali Lamari[1], Mohamed Feriani[1], Chokri Boubaker[1] and Hassen Ben Cheikh[1]

Abstract

We collected four field populations of *Culex pipiens* larvae from Northern Tunisia to study their chlorpyrifos resistance characteristics. Assays were performed using ethanol solutions of chlorpyrifos and data were analyzed using a log-probit program. All samples were resistant to chlorpyrifos (RR>1, p<0.05) and a large variation in the tolerance to this insecticide was observed. The sample 3 was the most resistant with a level higher than 10,000-fold (RR50=43,174). The level of resistance was lower, not exceeding 5-fold in samples 1. CYP450, EST and/or GST enzymes accounted for only a small part of the observed resistances. Synergists tests confirmed biochemical study where four esterases were detected in studied samples with low and average frequencies (0.03-0.68). Moreover, the mortality due to propoxur was significantly correlated with the LC50 of chlorpyrifos (P<0.01) indicated an insensitive AChE 1.

Keywords

Culex pipiens; Chlorpyrifos; Resistance characteristics; Esterases; Acetylcholinesterase 1; Northern Tunisia

Introduction

In addition to their nuisance in urban areas, *Culex pipiens* is the suspected vector in the transmission of West Nile Virus in Tunisia. In order to be able to use insecticides in vector control, the target species must be effectively sensitive to these products under field conditions. Laboratory tests have been commonly recorded resistance to insecticides in many vector populations throughout the world. These resistances can be due to the detoxification of the product by enzymes or to a mutation at the targeted site: the sodium channel for DDT and pyrethroids (kdr) or acetylcholinesterase (AChE 1) for organophosphates (OP) and carbamates [1-9]. Because of their lack of accumulation in the organism, OPs including chlorpyrifos have been used on a large scale since 1935 as insecticides in place of organochlorines. The previous studies realized on *Culex pipiens* populations of some Tunisian areas showed that these mosquitoes have developed high chlorpyrifos resistance levels [10,11] hence the aim of this study was to evaluate chlorpyrifos resistance status of *Culex pipiens* from Northern Tunisia.

***Corresponding author:** Tabbabi A, Laboratory of Genetics, Faculty of Medicine of Monastir, Monastir University, Monastir-5019, Tunisia, Tel: +216-97 085 424; E-mail: tabbabiahmed@gmail.com

Received: May 22, 2017 **Accepted:** June 13, 2017 **Published:** June 20, 2017

Materials and Methods

Mosquitoes

Four field populations of *Culex pipiens* larvae were collected from Northern Tunisia (Figure 1). We stored some adults mosquitoes for biochemical study. We used S-Lab as a susceptible strain without any resistance genes [12], and two resistant strains (SA2, SA5) selected for A2-B2 and A5-B5 esterases, respectively [13].

Bioassays

Assays were performed as described by Raymond et al. [28], using ethanol solutions of chlorpyrifos (99.5% [AI]), brought from laboratory Dr Ehrenstorfer, Germany, and propoxur (99.9% [AI], Bayer AG, Leverkusen , Germany). Chlorpyrifos bioassays included 5-9 concentrations providing between 0 and 100% mortality and 3-5 replicates per concentration on sets of 20 early 4th instars in a total volume of 100ml of water containing 1 ml of ethanol solution of the tested insecticide. The effect on chlorpyrifos resistance of 2 synergists, the DEF (98% [AI], Chem Service, England), and the PBO (94% [AI], Laboratory Dr Ehrenstorfer, Germany) , was studied by exposing larvae to a standard sublethal doses of 0.08 mg/liter for DEF , and 2.5 mg/liter for Pb, 4h before the addition of the insecticide. The DEF is known to inhibit esterases (EST) and/or glutathione-S-transferases (GST) while PBO inhibits cytochrome P450-dependent monooxygenases (CYP450). Propoxur bioassays included one dose (1mg/liter) and five replicates. This concentration kills all susceptible mosquitoes.

Over-produced esterases

Esterases of high activity were characterized on homogenates of adult thorax and abdomen by studying esterase activity in the presence of α-and-β-naphtyl acetate after protein separation by starch-gel electrophoresis (TME 7,4 buffer system) as described by Pasteur et al. [14] and were identified by comparing their electrophoretic mobility to that of known over-produced esterases.

Data analysis

Larval mortality was recorded after 24-h exposures, and data were analyzed using a log-probit program of Raymond et al. [15] based on Finney [16]. This program tests the linearity of a dose-mortality response, computes the different lethal doses (LCs) and their confidence interval (CI) at the chosen probability (here P=95%). In this study, the ratios computed were: the resistance ratio (RR) comparing each sample to the reference strain (S-Lab), and the synergism ratio (SR) comparing mortality data observed in presence of the insecticide alone to mortality data observed in presence of the insecticide plus the synergist in each sample. RR and SR are considered significant (P<0.05) when their 95% CI does not include the value 1. To test whether a synergist was more efficient in the field population than in the S-Lab, relative synergism ratios (RSR) were determined. The RSR is equal to the RR for insecticide alone divided by the RR for insecticide plus synergist. A RSR>1 indicates that the synergist has a stronger effect in the field population than in the S-Lab, that is, that the detoxifying mechanism synergized is enhanced in the field population; a RSR<1 shows that the two samples compared are not different as far as the mechanism inhibited by the synergist is concerned [17].

Figure 1: Geographic origin of *Culex pipiens* larvae collected for the study.

Results

Chlorpyrifos resistance

The linearity of the dose/mortality response is accepted ($p>0.05$) for reference strain (S-Lab) and rejected for other studied populations. All samples were resistant to chlorpyrifos ($RR>1$, $p<0.05$) and a large variation in the tolerance to this insecticide was observed (Table 1). The sample 3 was the most resistant (RR50=43,174). The level of resistance was lower, not exceeding 5-fold in samples # 1.

The addition of DEF decreased significantly the tolerance to chlorpyrifos in S-Lab (SR50=1.41, $p<0.05$) (Table 1). It also decreased significantly the resistance ($SR50>1$, $p<0.05$) of 2 among 4 field samples, and the SR was significantly higher than that recorded in S-Lab in the 2 samples (1 and 2). So, the increased detoxification by EST and/or GST was responsible, at least in part, for chlorpyrifos resistance in these samples. This mechanism accounts for only a part of the observed resistances since the RR50 remained significant ($p<0.05$) in the presence of the DEF. The role of the EST (and/or GST) in the chlorpyrifos resistance was relatively important in sample 2 (RR50=54, $p<0.05$, RSR=4.8) while it was minor in the other sample. The addition of DEF to Chlorpyrifos bioassays did not decrease significantly the resistance ($RSR<1$) in samples 3 which manifested the highest chlorpyrifos resistance levels in absence of DEF.

The addition of PBO to chlorpyrifos bioassays significantly increased the tolerance of S-Lab (SR=0.53, $p<0.05$) and decreased the resistance of 3 among 4 field samples (#2, 3, and 4) (Table 1). The recorded SR in these samples was significantly higher than that observed in S-Lab. However, oxidative metabolism accounted for only a small part of the observed resistances because chlorpyrifos resistance ratios remained significant in the presence of the PBO (e.g., chlorpyrifos RR50>10,000-fold in samples # 3).

Cross-resistance chlorpyrifos/propoxur

Mortality caused by propoxur ranged from 0% in samples 3 which showed the highest resistance levels to studied insecticide to 87% in sample 1. The mortality due to propoxur was significantly correlated with the LC50 of chlorpyrifos (P<0.01) indicated an insensitive AChE 1.

Overproduced esterases

Four esterases were detected in studied samples. The A2-B2 esterases were revealed in 3 samples with a frequency ranged from 0.06 in sample 2 to 0.33 in sample 3. The A4-B4 (and / or A5-B5) esterases were present in all samples with a frequency ranged from 0.03 in sample 1 to 0.68 in sample 3. The B12 and C1 esterases were observed in 2 and 3 samples, respectively.

Discussion

The resistance levels were very high in sample # 3 (RR50>10,000). This could be explained by the massive mosquitoes control using chemical insecticides in Tunisia (Table 2). A strong resistance of Tunisian *Culex pipiens* population to OP chlorpyrifos (410 000-folds) was recorded by Pasteur et al. [18]. The rate of resistance to this insecticide ranged from 800-folds to 4-folds in the world [19-26]. The same situation was recorded in Northern Tunisia despite the small area of study. Liu et al. [27] showed the ability of *Culex quinquefasciatus* to develop resistance to many group of insecticides including chlorpyrifos (OP) and permethrin (pyrethroid). Likewise, Ben Cheikh et al. [11] investigated the resistance of *Culex pipiens* collected between 1990 and 1996 to different OPs insecticides. Resistance to chlorpyrifos was highly variable and reached the highest level (>10,000-folds) recorded worldwide. In contrast, resistance to temephos (OP) was very low not exceeding 10-folds in the samples that showed the highest resistance to chlorpyrifos.

CYP450, EST and/or GST enzymes accounted for only a small part of the observed resistances. Synergists tests confirmed biochemical study where four esterases were detected in studied samples with low and average frequencies (0.03%-0.68%). Moreover, the mortality due to propoxur was significantly correlated with the LC50 of chlorpyrifos (P<0.01) indicated an insensitive AChE 1. These results are consistent with those found in the world: resistances to OPs insecticides can be due to the detoxification of the product by enzymes or /and to a mutation at the targeted site, AChE 1[11,23,28-43].

Conclusion

Other groups of mosquitoes should be used to test their resistance to different chemical insecticides so that the control of vectors will successfully help in the reduction of incidence of vector-borne diseases.

Table 1: Chlorpyrifos resistance characteristics of Tunisian *Culex pipiens* in presence and absence of synergists DEF and PBO.

Population		Chlorpyrifos				Chlorpyrifos +DEF					Chlorpyrifos +PBO		
	LC_{50} in µg/l	Slope	RR_{50}	LC_{50} in µg/l	Slope	RR_{50}	SR_{50}	RSR	LC_{50} in µg/l	Slope	RR_{50}	SR_{50}	RSR
	(a)	± SE	(a)	(a)	± SE	(a)	(a)		(a)	± SE	(a)	(a)	
Susceptible lab strain	0.56	9.0	-	0.17	2.85	-	1.4	-	0.45	1.16	-	0.53	-
	(0.53-0.58)	± 1.04		(0.14-0.20)	± 0.26		(1.08-1.8)		(0.17-1.3)	± 0.43		(0.35-0.79)	
1-Krib	1.2	0.91	2.1	0.29	0.83	1.7	4.1	**1.2**	-	-	-	-	-
	(0.43-3.3)	± 0.17	(1.3-3.3)	(0.15-0.48)	± 0.09	(1.3-2.3)	(2.7-6.1)						
2-Belli	136	0.92	264	9.1	0,72	54.6	14.9	**4.8**	40	0.87	**91.1**	3.3	**2.9**
	(72-257)	± 0.13	(176-344)	(2.9-28)	± 0,14	(35.8-83.2)	(10.0-22.1)		(11-140)	± 0.30	(49.5-167)	(2.0-5.4)	
3-Tazarka	23900	1.38	43173	47400	0.72	281887	0.50	**0.15**	4700	2.42	**10550**	5.0	**4.1**
	(14100-79700)	± 0.29	(26885-69330)	-	± 0.51	(101266-784669)	(0.16-1.50)		(4070-5460)	± 0.21	(7170-15523)	(3.2-8.0)	
4-Sidi khalifa	41	0.84	75.0	203	0.69	1210	0.20	**0.06**	18	0.88	**42.3**	2.2	**1.7**
	(20-84)	± 0.15	(53.7-104)	(108-437)	± 0.13	(915-1600)	(0.14-0.28)		(4.1-86)	± 0.24	(21.6-83.0)	(1.3-3.6)	

(a), 95% CI ;

RR_{50} : resistance ratio at LC_{50} (RR_{50}=LC_{50} of the population considered/LC_{50} of Slab); SR_{50} : synergism ratio (LC_{50} observed in absence of synergist/LC_{50} observed in presence of synergist); RR and SR considered significant (P<0.05) if their 95%CI did not include the value 1;
RSR : relative synergism ratio (RR for insecticide alone / RR for insecticide plus synergist)

Table 2: Geographic origin of Tunisian *Culex pipiens* populations, breeding site characteristics, and insecticide control.

Code	Locality	Breeding site	Date of collection	Mosquito control (used insecticides)	Agricultural pest control
1	Krib	River	0ct. 2005	Occasional (P)	Yes
2	Belli	River	Aug. 2003	Rare (C,D)	Yes
3	Tazarka	River	May 2005	Very frequent (C, T, Pm, F, P, D)	Yes
4	Sidi khalifa	Water pond	July 2004	None	None

C : Chlorpyrifos ; T : Temephos ; Pm : Pirimiphos methyl ; F : Fenitrithion ; P : Permethrin ; D : Deltamethrin

References

1. Guillemaud T, Rooker S, Pasteur N, Raymond M (1996) Testing the unique amplification event and the worldwide migration hypothesis of insecticide resistance genes with sequence data. Heredity 77: 535-543.
2. Guillemaud T, Makate N, Raymond M, Hirst B, Callaghan A (1997) Esterase gene amplification in Culex pipiens. Insect Mol Biol 6: 319-327.
3. Taylor M, Feyereisen R (1996) Molecular biology and evolution of resistance to toxicants. Molecular Biology and Evolution 13: 719-734.
4. Weill M, Fort P, Berthomieu A, Dubois MP, Pasteur N, et al. (2002) A novel acetylcholinesterase gene in mosquitoes codes for the insecticide target and is non-homologous to the ace gene in Drosophila. Proc Roy Soc Lond: 2007-2016.
5. Corbel V, N'Guessan R, Brengues C, Chandre F, Djogbenou L, et al. (2007) Multiple insecticide resistance mechanisms in Anopheles gambiae and Culex quinquefasciatus from Benin, West Africa. Acta Trop 101: 207-216.
6. Tantely ML, Tortosa P, Alout H, Berticat C, Berthomieu A, et al. (2010) Insecticide resistance in Cx. pipiens quinquefasciatus and Aedes albopictus mosquitoes from La ReunIon Island. Insect Biochem Mol Biol 40: 317-324.
7. Toma L, Menegon M, Romi R, De Matthaeis E, Montanari M, et al. (2011) Status of insecticide resistance in Culex Pipiens field populations from northeastern areas of Italy before the with drawl of OP compounds. Pest Manag Sci 67:100–106.
8. Jones CM, Machin C, Mohammed K, Majambere S, Ali AS, et al. (2012) Insecticide resistance in Culex quinquefasciatus from Zanzibar: implications for vector control programmes. Parasit Vectors 5: 78.
9. Pocquet N, Milesi P, Makoundou P, Unal S, Zumbo B, et al. (2013) Multiple Insecticide Resistances in the Disease Vector Culex quinquefasciatus from Western Indian Ocean. PLoS One 8: e77855.
10. Ben Cheikh H, Marrakchi M, Pasteur N (1995) Mise en évidence d'une très forte résistance au chlopyrifos et à la perméthrine dans les populations de Culex pipiens en Tunisie. Archs Inst Pasteur de Tunis 72: 7-12.
11. Ben Cheikh H, Haouas-Ben Ali Z, Marquine M, Pasteur N (1998) Resistance to organophosphorus and pyrethroid insecticides in Culex pipiens (Diptera: Culicidae) from Tunisia. J Med Entomol 35: 251-260.
12. Georghiou GP, Meltcalf RL, Gidden FE (1966) Carbamate resistance in mosquitoes. Selection of Culex pipiens fatigans Wied for resistance to Baygon. Bull WHO 35: 691-708.
13. Berticat C, Boquien G, Raymond M, Chevillon C (2002) Insecticide resistance genes induce a mating competition cost in Culex pipiens mosquitoes. Genet Res 83:189-196.
14. Pasteur N, Pasteur G, Catalan J, Bonhomme F, Britton-Davidian J (1988) Practical isozyme genetics. Ellis Horwood Ltd.
15. Raymond M, Prato G, Ratsira D (1993) PROBIT. Analysis of mortality assays displaying quantal response. Praxeme (Licence No. L93019), Saint Georges d'Orques, France.
16. Finney DJ (1971) Probit analysis. Cambridge University Press, Cambridge.
17. Poirié M, Raymond M, Pasteur N (1992) Identification of two distinct amplifications of the esterase B locus in Culex pipiens (L.) mosquitoes from Mediterranean countries. Biochem Genet 30:13-26.
18. Pasteur N, Marquine M, Ben Cheikh H, Bernard C, Bourguet D (1999) A new mechanism conferring unprecedented high resistance to chlorpyrifos in Culex pipiens (Diptera: Culicidae). J Med Entomol 36(6): 794-802.
19. Mouchès C, Magnin M, Bergé JB, Desilvestri M, Beyssat V, et al. (1987) Overproduction of detoxifying esterases in organophosphate resistant Culex mosquitoes and their presence in other insects. Proc Nat Acad Sci USA 84: 2113-2116.
20. Orshan L, Kelbert M, Pener H (2005) Patterns of insecticide resistance in larval Culex pipiens populations in Israel: dynamics and trends. J Vect Ecol 30:289-294.
21. Silvestrini F, Severeni C, Dipardo V, Romi R, Matthaeis ED, et al. (1998) Population structure and dynamics of insecticide resistance genes in Culex pipiens populations from Italy. Heredity 81:342-348.
22. Yebakima A, Marquine M, Rosine J, Yp-tcha MM, Pasteur N (2004) Evolution of resistance under insecticide selection pressure in Culex pipiens quinquefasciatus (Diptera: Culicidae) from Martinique. J Med Entomol 41:718-725.
23. Bisset JA, Rodriguez MM, Diaz C, Soca A (1999) Characterization of resistance to organophosphate insecticides, carbamates, and pyrethroids in Culex quinquefasciatus from the state of Miranda, Venezuela. Rev Cubana Med Trop 51:89-94.
24. Chandre F, Darriet F, Darder M, Cuany A, Doannio JMC, et al. (1998) Pyrethroid resistance in Culex quinquefasciatus from West Africa. Med Vet Entomol 12: 359-366.
25. Cui F, Raymond M, Berthomieu A, Alout H, Weill M, et al. (2006) Recent emergence of insensitive acetylcholinesterase in Chinese populations of the mosquito Culex pipiens (Diptera: Culicidae). J Med Entomol 43: 878-883.
26. Ouedraogo TDA, Baldet T, Skovmand O, Kabre G, Guiguemde TR (2005) Sensibilité de Culex quinquefasciatus aux insecticides à Bobo Dioulasso (Burkina Faso). Bull Soc Pathol Exot 98: 406-410.
27. Liu H, Cupp EW, Micher KM, Guo A, Liu N (2004) Insecti-cide resistance and cross-resistance in Alabama and Florida strains of Culex quinquefasciatus. J Med Entomol 41: 408-413.
28. Raymond M, Heckel DG, Scott JG (1989) Interactions between pesticide genes. Model and experiment. Genetics 123:543-551.
29. Ben Cheikh R, Berticat C, Berthomieu A, Pasteur N, et al. (2008) Characterization of a Novel High-Activity Esterase in Tunisian Populations of the Mosquito Culex pipiens. J Econ Entomol 2: 484-491.
30. Liu H, Xu Q, Zhang L, Liu N (2005) Chlorpyrifos resistance in mosquito Culex quinquefasciatus. J Med Entomol 42: 815-820.
31. Whyard S, Downe AFR, Walker VK (1994) Isolation of an esterase conferring insecticide resistance in the mosquito Culex tarsalis. Insect Biochem Mol Biol 24: 819-827.
32. Tomita T, Kono Y, Shimada T (1996) Chromosomal localization of amplified esterase genes in insecticide resistant Culex mosquitoes. Insect Biochem Mol Biol 26: 853-857.
33. Hemingway J, Karunaratne SH (1998) Mosquito carboxylesterases: A review of the molecular biology and biochemistry of a major insecticide resistance in mechanism. Med Vet Entomol 12: 1-12.
34. Hemingway J, Miyamoto J, Herath PRJ (1991) A possible novel link between organophosphorus and DDT insecticide resistance genes in Anopheles supporting evidence from fenitrothion metabolism studies. Pestic Biochem Physiol 39: 49-56.
35. Hemingway J, Hawkes N, Prapanthadara L, Jayawardenal KG, Ranson H (1998) The role of gene splicing, gene amplification and regulation in mosquito insecticide resistance. Phil trans R Soc Lond B Biol Sci 353: 1695-1699.
36. Hemingway J, Hawkes N, McCarroll I, Ranson H (2004) The molecular basis of insecticide resistance in mosquitoes. Insect Biochem Mol Biol 34: 653-665.
37. Rodriguez MM, Bisset J, Fernandez DMD, Lauzan L, Soca A (2001) Detection of insecticide resistance in Aedes aegypti (Diptera: Culicidae) from Cuba and Venezuela. J Med Entomol 38: 623-628.
38. Macoris MLG, Andrighetti MTM, Takaku L, Glasser CM, Garbeloto VC, et al. (2003) Resistance of Aedes aegypti from the state of São Paulo, Brazil, to organophosphates insecticides. Mem Inst Oswaldo Cruz 98: 703-708.
39. Huang HS, Hu NT, Yao YE, Wu CY, Chiang SW, et al. (1998) Molecular Cloning and heterologous expression of a glutathione-s-tranferase involved in insecticide resistance from the diamondback moth Plutella xylostella. Insect Biochem Mol Biol 28: 651-658.
40. Wei SH, Clark AG, Syvanen M (2001) Identification and cloning of a key insecticide-metabolizing glutathione -S- transferase (MdGST-6A) from a hyper insecticide-resistant strain of the house fly Musca domestica. Insect Biochem Mol Biol 31: 1145-1153.
41. Zayed AB, Szumlas DE, Hanafi HA, Fryauff DJ, Mostapha AA, et al. (2006) Use of bioassay and microplate assay to detect and measure insecticide resistance in field populations of Culex pipiens from filariasis endemic areas of Egypt. J Am Mosq Control Assoc 22: 473-482

42. Liu N, Yue X (2000) Insecticide resistance and cross-resistance in the house fly (Diptera: Muscidae). J Econ Entomol 93: 1269-1275.

43. Bisset JA, Rodriguez MM, Diaz C, Soca A (2000) Course of insecticide resistance in Culex quinquefasciatus (Diptera: Culicidae) in a region of La Habana. Rev Cubana Med Trop 52: 180-185.

Author Affiliation

Top

[1]*Laboratory of Genetics, Faculty of Medicine of Monastir, Monastir University, Monastir-5019, Tunisia*

[2]*Department of Hygiene and Environmental Protection, Ministry of Public Health, Bab Saadoun, Tunis-1006, Tunisia*