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Susceptibility profile of *Aedes aegypti* from Santiago Island, Cabo Verde, to insecticides

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ABSTRACT

In 2009, Cabo Verde diagnosed the first dengue cases, with 21,137 cases reported and *Aedes aegypti* was identified as the vector. Since the outbreak, chemical insecticides and source reduction were used to control the mosquito population. This study aimed to assess the susceptibility of *A. aegypti* populations from Santiago, Cabo Verde to insecticides and identify the mechanisms of resistance. Samples of *A. aegypti* eggs were obtained at two different time periods (2012 and 2014), using ovitraps in different locations in Santiago Island to establish the parental population. F_1 larvae were exposed to different concentrations of insecticides (*Bacillus thuringiensis* var *israelensis* (Bti), diflubenzuron and temephos) to estimate the lethal concentrations (LC_{90}) and calculate the respective rate of resistance (RR_{90}). Semi-field tests using temephos-ABATE® were performed to evaluate the persistence of the product. Bottle tests using female mosquitoes were carried out to determine the susceptibility to the adulticides malathion, cypermethrin and deltamethrin. Biochemical and molecular tests were performed to investigate the presence of metabolic resistance mechanisms, associated with the enzymes glutathione S-transferases (GSTs), esterases and mixed-function oxidases (MFO) and to detect mutations or alterations in the sodium channel and acetylcholinesterase genes. *A. aegypti* mosquitoes from Santiago exhibited resistance to deltamethrin, cypermethrin (mortality < 80%) and temephos (RR_{90} = 4.4) but susceptibility to malathion (mortality ≥ 98%), Bti and diflubenzuron. The low level of resistance to temephos did not affect the effectiveness of Abate®. The enzymatic analysis conducted in 2012 revealed slight changes in the activities of GST (25%), MFO (18%), α-esterase (19%) and β-esterase (17%), but no significant changes in 2014. Target site resistance mutations were not detected. Our results suggest that the *A. aegypti* population from Santiago is resistant to two major insecticides used for vector control, deltamethrin and temephos. To our knowledge, this is the first report of temephos resistance in an African *A. aegypti* population. The low level of temephos resistance was maintained from 2012–2014, which suggested the imposition of selective pressure, although it was not possible to identify the resistance mechanisms involved. These data show that the potential failures in the local mosquito control program are not associated with insecticide resistance.

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1. Background

Aedes aegypti (Linnaeus, 1762) and *Aedes albopictus* (Skuse, 1894) are considered the primary vectors of Dengue and other viruses, such as Chikungunya and Yellow fever. Dengue is currently considered the most important arbovirus for Public Health and has been reported in several countries as large epidemics with high rates of morbidity and mortality (WHO, 2011). It is estimated that over 50% of the world population lives in areas that are at risk of transmission of at least one of the four serotypes of Dengue virus (DENV) and in the presence of one or more vector species (Kyle and Harris, 2008).

On the African continent, there has been an expansion of dengue in recent years (Amarasinghe et al., 2011; Franco et al., 2010). In the Republic of Cabo Verde, a country comprising 10 archipelago islands located along the West African coast, *A. aegypti* was first described in 1931 on the island of São Vicente and was subsequently described on other islands (Alves et al., 2010; Ribeiro et al., 1980).

The first and only outbreak registered in Cabo Verde occurred in 2009 (WHO, 2009), with more than 21,000 cases notified, particularly on the islands of Fogo and Santiago, the latter of which contained approximately 70% of the cases (Cabo Verde, 2011). Analyses of samples from patients during the epidemic revealed the involvement of DENV-3 (Franco et al., 2010). In subsequent years, a small number of cases have also been registered in this country (Cabo Verde, 2011).

Vector control is considered critical for minimizing DENV transmission because of the lack of vaccines or specific treatments (Kyle and Harris, 2008). The use of chemical insecticides, particularly those that belong to the classes of organophosphates and pyrethroids, are important tools for vector control (Bisset, 2002). In Cabo Verde, control strategies are performed in an integrated manner to affect the main vector species of malaria and dengue, *Anopheles arabiensis* and *A. aegypti*, respectively, through the use of chemical insecticides (temephos and deltamethrin), adulterated diesel and the mosquito fish *Gambusia* sp. (Cabo Verde, 2012b).

Over the last 15 years, *A. aegypti* populations resistant to these insecticides have been registered in many countries due to the intensive use of these compounds. Resistance poses a serious threat to many vector control programs, which reflects the few different classes of insecticides that are currently available to control culicids (Hemingway et al., 2004).

Insecticide resistance results from a mutational event that cause a heritable change in the susceptibility profile of a given population, thereby changing the physiological, behavioral or morphological traits of that population, which are selected as a consequence of the prolonged and extensive use of a chemical compound over time. Among the mechanisms associated with resistance to chemical insecticides, the major resistance mechanisms include the rapid detoxification of these compounds and the modification of the targets of the binding proteins to insecticides in the central nervous system of the insect (Hemingway et al., 2004; Hemingway and Ranson, 2000). Metabolic resistance is characterized by the involvement of family enzymes, such as glutathione S-transferases (GSTs), cytochrome P450 oxidases and esterases (α, β, and nonspecific) (Hemingway et al., 2004). In mosquitoes resistant to chemical insecticides, these enzymes can be overexpressed and/or undergo modifications, thereby increasing the capacity for detoxification (Braga and Valle, 2007; Hemingway et al., 2004; Hemingway and Ranson, 2000; Russell et al., 2011). The target site type resistance involves a set of target molecules for different insecticide compounds, such as the neuronal receptor γ-aminobutyric acid (GABA), the acetylcholinesterase enzyme (AChE) and the voltage-dependent sodium channel (Nav) (Asih et al., 2012; Ffrench-Constant et al., 2000; Hemingway and Ranson, 2005; Wondji et al., 2011). Mutations in the genes encoding these molecules lead to structural changes in the proteins, which compromises or prevents the binding of the insecticide to the target (Alout et al., 2011; Hemingway and Ranson, 2005; Kliot and Ghanim, 2012).

The aim of the present study was to evaluate the susceptibility of the *A. aegypti* population from Santiago Island, Cabo Verde, to different chemical and biological insecticides, locally used or with potential for use, to subsidize the current actions of the vector control program in this country.

2. Methods

2.1. Aedes aegypti *sampling and rearing*

This study was conducted in the Island of Santiago in Cabo Verde (15°06′14.21″, 23°38′33.28″). The eggs were collected using oviposition traps (OVT) made of disposable plastic bottles adapted from the previously described model of Santos et al. (2012). From March to June 2012, 107 OVT were installed, and the majority of these traps were set up in the City of Praia, the capital of the country, in peridomestic urban areas from seven of the nine counties of Santiago Island (Calheta São Miguel, Praia, Ribeira Grande, Santa Catarina, Santa Cruz, São Domingos and Tarrafal) (Fig. 1). Approximately 2000 eggs were collected from the OVT. A second sampling using 241 OVT installed in the four main counties of Santiago Island (Praia, Santa Catarina, Santa Cruz and Tarrafal) was conducted from January to February 2014. At that time, 1016 eggs were collected, with 410 eggs from Praia and 606 eggs from the other counties (Santa Catarina, Tarrafal and Santa Cruz). Each OVT remained in the field for 5 days.

The eggs were sent to the Entomology Laboratory of the Aggeu Magalhães Research Center/FIOCRUZ-PE, Brazil, to establish and maintain the adult population of *A. aegypti* from Santiago island, according to the previously described procedures of Carvalho-Leandro (Carvalho-Leandro et al., 2012). In 2012, a single sub-population was established, and in 2014, two sub-populations were established, representing Praia, CV-Praia2014 and the different biomes of Santiago island, with the counties of Santa Catarina, Tarrafal and Santa Cruz, CV2014.

Species identification of the adults was performed according to (Huang, 2004). The parental populations and the following generations were maintained under controlled temperature (25 ± 2 °C), relative humidity (58% to 86%) and photoperiod (12/12 h light/dark) conditions. The larvae were fed cat food (autoclaved), whereas the adults were fed a 10% sucrose solution ad libitum, and in addition the females were fed chicken blood (*Gallus gallus*).

2.2. Larval bioassays

The larvae of the F_1 and F_2 generations of CV2012 and the F_1 generation of CV-Praia2014 and CV2014 were exposed to multiple increasing concentrations of technical grade temephos organophosphate (Lot: SZE9105X and SZB9105XV, Sigma) (0.014–0.050 mg/L) (WHO, 1981), a lyophilized standard of *Bacillus thuringiensis* serovar *israelensis* (Bti) IPS82 (0.004 to 0.05 mg/L) (de Barjac and Larget-Thiéry, 1984) and technical grade diflubenzuron (Lot:5ZE7800X, Sigma), an insect growth regulator (IGR) inhibitor of chitin synthesis, (0.4–3.5 μg/L) (Martins et al., 2008). The test with the first two larvicides was performed using homogeneous groups of 20 third-instar larvae (L_3); each concentration was tested in triplicate, and the mortality was registered after 24 h of exposure. For diflubenzuron, groups of 10 larvae (L_3) were used, and eight replicates were tested for each concentration. The bioassay was completed when the mortality of all aquatic forms

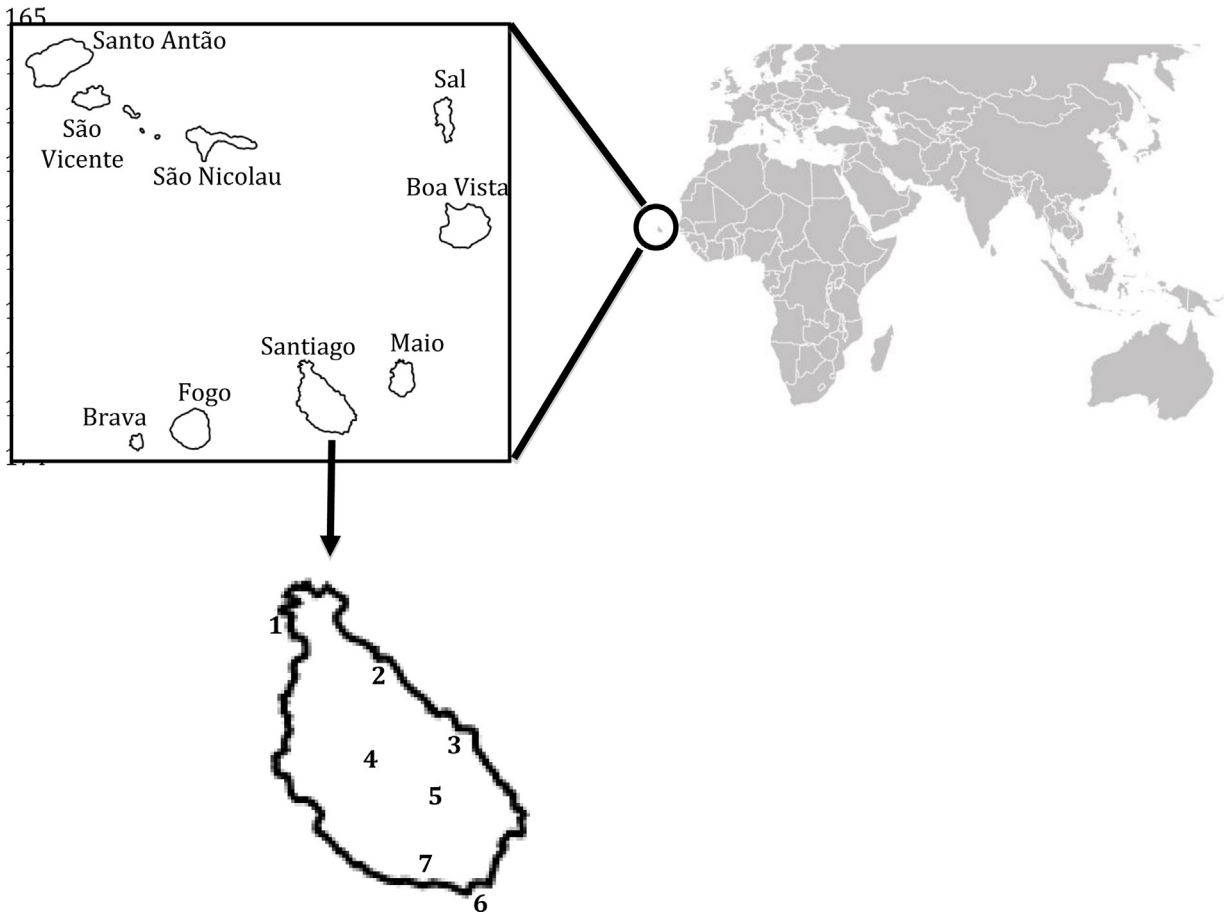

Fig. 1. Map of Cabo Verde and Santiago Island showing the sample sites (counties). (1) Tarrafal; (2) Calheta São Miguel; (3) Santa Cruz; (4) Santa Catarina; (5) São Domingos; (6) Praia and (7) Ribeira Grande. Collections were performed in all sites in 2012, and in 2014 only in 1, 3, 4 and 6.

was obtained or when all of the larvae in the control group reached the adult stage, for a maximum period of 30 days of exposure, as previously described by (Araujo et al., 2013). The bioassays with the three insecticides were repeated three times. The lethal concentration for 90% of the larvae (LC_{90}) for temephos and Bti and the concentration for 90% inhibition of emergence (IE_{90}) for diflubenzuron were estimated using linear regression Log-Probit (Finney, 1971) in the statistical package SPSS 8.0/Windows. The resistance ratios (RR_{90}) were calculated using the LC_{90} of the reference susceptible strains, Rockefeller and RecL, for temephos and Bti, respectively. The population was classified considering the following criteria: susceptible ($RR < 3$); low resistance ($3 \leq RR \leq 5$); moderate resistance ($\geq 5 \leq 10$ RR); and high resistance ($RR > 10$) (Mazzarri and Georghiou, 1995).

2.3. Examination of the residual larvicidal activity of temephos under semi-field conditions

This test was conducted using the sub-population *A. aegypti* CV-Praia2014, in an open area, using a sand granule temephos formulation (Abate®, Lot: GRT-12F-12613, P.A 1%) at a final concentration of 0.1 g/L (the same concentration used in the vector control program of Cabo Verde). The test was performed in fiber water tanks with a 250 L capacity, with 70 L of drinking water treated with temephos at 24 h before larval colonization. For the positive and negative controls, we used Rockefeller larvae in containers treated or not with temephos. The experiments were conducted in triplicate, and the containers were exposed to sun light or protected in the shade to simulate two different environmental conditions. The temperature and relative humidity of the environment were recorded daily, and the pH and water temperature were measured weekly. For all containers, 30% of the water volume was replaced twice a week to simulate the use of water for domestic purposes. The containers were capped with a net to avoid the external colonization of other insects. To verify the residual activity of the product, 50 larvae per container per week were introduced; 24 h after exposition, the surviving larvae were removed and counted to determine the final mortality. The tests were conducted for a period of 3 months.

2.4. Adult bioassays

The adult bioassays were performed using the sub-population CV2012 based on the protocol of Brogdon and McAllister (1998) and da-Cunha et al. (2005), using single doses (diagnostic doses) of pyrethroid, cypermethrin (Lot: S1E8297X, Sigma) (8 μg/μL), deltamethrin (Lot:127K1099, Sigma) (3 μg/μL), and the organophosphate malathion (Lot: SZB9146XV, Sigma) (30 μg/μL), capable of killing 100% Rockefeller females (susceptibility reference). Batches of 15–25 female mosquitoes, fed 10% sucrose without blood supply, were used at two days after the emergency. The female mosquitoes were exposed to the different insecticides in impregnated bottles, and the mortality was recorded every 15 min to a maximum of two hours. Mortality was registered when the mosquitoes were unable to fly in the bottle. Bottles impregnated with the solvent (acetone) were used as negative controls. Each bioassay was performed in triplicate and repeated three different times. For the experiments using pyrethroids, after 2 h, the

mosquitoes were transferred to cages without insecticides to determine the final mortality after 24 h due to the knockdown effect. The susceptibility status was classified using the criteria of Davidson and Zahar (1973): susceptible (≥98% mortality), under verification (<98>80%) or resistant (mortality ≤80%).

2.5. Enzymatic assay

To detect metabolic resistance, the enzymatic activity of glutathione S-transferases (GSTs), esterases α, β and PNPA (*p*-nitrophenyl acetate/sodium phosphate) and mixed function oxidases (MFOs), associated with the detoxification of xenobiotics, was evaluated. In addition, the rate of inhibition of acetylcholinesterase (AchE), the target site of organophosphates, was measured using a previously established protocol (Brasil, 2006). For each experiment, we used 100 adult females of the sub-populations CV2012 and CV-Praia2014, at one day post-emergence, without blood supply and previously stored at −70 °C. Each test was repeated on at least three different dates. The enzymatic profile of each population was classified according to the percentage of individuals with enzymatic activity higher than 99 percent of the activity observed for the Rockefeller strain. The sub-populations were classified as unaltered (≤15%); altered (≥15% ≤50%); or highly altered (>50%) (Montella et al., 2007).

2.6. Screening of mutations in the voltage-gated sodium channel gene (NaV)

Female mosquitoes (F_2) from CV2012, previously phenotyped as susceptible (36 individuals) and resistant (36 individuals) to cypermethrin based on the bottle bioassay, were used in the analyses of the following kdr mutations (knockdown resistance): Leu982Trp, Ile1011Met, Ile1011Val, Ile1014Phe, Val1016Gly, Val1016Ile and Phe1534Cys (Brengues et al., 2003; Martins et al., 2009a,b; Saavedra-Rodriguez et al., 2007). Total DNA was extracted using NaOH precipitation, according to Rudbeck and Dissing (1998). A DNA fragment encompassing the first four codons described above was obtained from PCR using the primers Nav2021Fw: 5′ GACAATGTGGATCGCTTCCCG 3′ and Nav2021Rev: 5′ GCACGGACGCAATCTGGC 3′. These primers were manually designed to amplify a 620 bp fragment from a region between the 20th and 21st exon of the NaV gene. PCR was performed in 25 μl reactions containing: approximately 20 ng of DNA, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 0.4 mM of each primer and 1 unit of Platinum Taq DNA polymerase® (Invitrogen).The PCR conditions were 94 °C for 5 min, followed by 30 cycles at 94 °C for 1 min, 64 °C for 1 min and 72 °C for 1 min, and a final extension temperature of 72 °C. The products were separated on a 1% agarose gel, stained with ethidium bromide and visualized on a UV light trans-illuminator. Each PCR product was quantified on a Nanodrop 2000 (Thermo Fisher Scientific), diluted to approximately 20 ng/μL and submitted to capillary sequencing (ABI 3100 – Applied Biosystems). The electropherograms were edited and analyzed using Codon Code Aligner 4.2.2. The same samples were used to examine the Fen1534Cys using allele-specific PCR, according to Harris et al. (2010).

3. Results

3.1. Identification of Aedes species

More than 3000 eggs were collected in the ovitraps in 2012 and 2014, and the reared adults were identified through morphological analysis. All samples were identified as *A. aegypti aegypti.* None of the individuals analyzed belonged to the subspecies *A. aegypti formosus* (Huang, 2004), and no other colonizing species of ovitraps, such as *A. albopictus*, were detected.

3.2. Analysis of susceptibility to larvicides and IGR:

The sub-populations CV2012 and CV-Praia2014 displayed temephos resistance with values of RR_{90} = 4.4 and RR_{90} = 3.3, respectively (Tables 1). Considering the previously established criteria, these sub-populations showed a low level of temephos resistance. However, the sub-population CV2014 was susceptible to this same compound. For Bti and diflubenzuron, all sub-populations were considered susceptible (Table 1).

3.3. Residual activity of Abate® temephos

In the semi-field test, the persistence of temephos in containers exposed to the sun light was nine weeks, which eliminated 100% of the larvae. In the 10th week, the residual activity declined to 70%, and in the following week, the activity declined to 53% (Table 3). Under shadow conditions, the persistence of temephos was 12 weeks, which eliminated 100% of the larvae. The persistence was lower in containers exposed to the sun than to the shade.

3.4. Analysis of susceptibility to adulticides

After the exposure of *A. aegypti* females to pyrethroid, the knockdown effect was observed for deltamethrin and cypermethrin. Less than 75% mortality was observed for both insecticides, which confirmed the resistance to pyrethroid (Table 2). However, the CV2012 sub-population was sensitive to the organophosphate malathion.

3.5. Characterization of resistance mechanisms

The enzymatic analysis conducted with samples from 2012 revealed discrete changes in the activity of GST (25%), mixed-function oxidases (18%), α-esterase (19%) and β esterase (17%). In contrast, no significant changes in the activities of these enzymes were detected in the samples collected in 2014 (Table 4). No alterations in the *AchE* enzyme were observed in the sub-populations studied. The evaluation of the activity of detoxification enzymes two years later showed that this metabolic resistance mechanism was not selected.

The analysis of NaV gene sequences from 72 individuals revealed that the *A. aegypti* population from Cabo Verde was monomorphic for all studied sites, regardless of the phenotype of the individuals. The only exception was at codon 982, where a synonym mutation (TTG/TTA) in 13 individuals, four susceptible and nine resistant individuals, was observed. No variation was observed at codon 1534. Both susceptible and resistant individuals displayed the wild-type genotype (Phe/Phe). These results showed that the target site mechanism of resistance is likely not present in population CV2012. However, it is important to note that we analyzed a low number of individuals in the present study.

4. Discussion

The assessment of insecticide susceptibility status is important for vector control interventions and enables the prevention or management of resistance (Montella et al., 2007). The results from bioassays with temephos indicated a low level of resistance to this insecticide in *A. aegypti* sub-populations from Praia, Santiago Island, Cabo Verde. The level of resistance remained constant from 2012 to 2014 in the sub-population CV-Praia2014, suggesting that there has been no evolution of resistance. This result was directly confirmed using a semi-field test, which showed the efficiency of

Table 1
Temephos and *Bacillus thuringiensis* serovar *israelensis* (Bti) lethal concentrations (LC_{90} and LC_{50}) and diflubenzuron emergence inhibition rates for *Aedes aegypti* larvae from Santiago Island, Cabo Verde (CV). The resistance ratios (RR_{90}) are also indicated.

Insecticide	Population	N° Larvae	LC_{50} (mg/L)[a] (C.I)[b]	LC_{90} (mg/L)[a] (C.I)[b]	RR_{90}[c]	Status
Temephos	$Rockefeller_{2012}$	1440	0.0058 (0.0048–0.0069)	0.0086 (0.0074–0.0102)	1.0	
	$Rockefeller_{2014}$	1800	0.0072 (0.0069–0.0083)	0.0102 (0.0099–0.0130)	1.0	
	CV2012	2520	0.026 (0.025–0.027)	0.038 (0.036–0.040)	4.4	Resistant
	CV-Praia2014	2400	0.024 (0.022–0.028)	0.034 (0.030–0.030)	3.3	Resistant
	CV2014	1800	0.015 (0.012–0.019)	0.025 (0.019–0035)	2.5	Susceptible
–						
Bti	RecLab	1020	0.016 (0.012–0.020)	0.030 (0.026–0.039)	1.0	
	CV2012	1620	0.011 (0.010–0.012)	0.023 (0.020–0.027)	0.8	Susceptible
–						
Diflubenzuron	Rockefeller	1630	0.39 (0.29–0.45)[d]	0.94 (0.87–1.03)[d]	1.0	
	CV2012	1600	1.34 (1.25–1.43)[d]	2.08 (1.96–2.25)[d]	2.2[d]	Susceptible

Rockefeller = *A. aegypti* standard susceptibility strain to chemical insecticides; RecLab = *A. aegypti* susceptibility laboratory strain to chemical insecticides from Pernambuco/Brazil; CV2012 = *A. aegypti* population from Santiago Island, Cabo Verde, collected in 2012; CV-Praia2014 = *A. aegypti* population from Praia, Cabo Verde, collected in 2014; CV2014 = *A. aegypti* population from Santiago Island, Cabo Verde, collected in 2014.

[a] Lethal concentration for 50% and 90%.

[b] C.I = confidence intervals.

[c] Resistance ratio for LC_{90}.

[d] Emergency inhibition rate for 50% and 90% of the exposed individuals to diflubenzuron, and the confidence intervals and resistance ratios are also indicated.

Table 2
Mortality (%) of *Aedes aegypti* females from Santiago Island, Cabo Verde, exposed to a diagnostic dose of deltamethrin, cypermethrin and malathion in impregnated bottle assays.

Adulticide (concentration/bottle)	Population	N° Females	Average mortality/time[c]						Status
			15 min	30 min	45 min	1 h	2 h	24 h[d]	
Deltamethrin (3 μg/μL)[a]	Rockefeller	148	84%	100%	--	--	--	100%	
	CV2012	191	84%	100%	--	--	--	72%	Resistant
–									
Cypermethrin (8 μg/μL)[a]	Rockefeller	149	95%	100%	--	--	--	100%	
	CV2012	163	13%	79%	88%	95%	100%	75%	Resistant
–									
Malathion (30 μg/μL)[b]	Rockefeller	156	27%	95%	100%	--	--	--	
	CV2012	197	24%	99%	100%	--	--	--	Susceptible

[a] Pyrethroid.

[b] Organophosphate; Rockefeller = *Aedes aegypti* standard susceptibility strain to chemical insecticides; population of *Aedes aegypti* from Santiago Island, Cabo Verde, collected in 2012.

[c] Mortality for pyrethroids and organophosphate (Malathion) evaluated at different times after insecticide exposure.

[d] Mortality recovered at 24 h, without contact with the pyrethroids.

Table 3
Residual activity of temephos (Abate®, sand granules) in tank water treated with 0.1 g/L of the product under simulated field conditions, against *Aedes aegypti* larvae from the Cabo Verde population, collected in 2014.

Experimental condition	Average larval mortality/week												
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th
Temephos containers-sun	93	96	100	100	100	100	100	100	100	70	53	–	–
Temephos containers–shade	96	99	100	100	100	100	100	100	100	99.3	100	99	98
Temephos positive control-sun	100	100	100	100	100	100	100	100	100	100	100	100	100
Temephos positive control-shade	100	100	100	100	100	100	100	100	100	100	100	100	100
Negative control-sun	0	0	0	0	0	0	0	0	0	0	8	–	–
Negative control-shade	0	0	0	0	0	0	0	0	0	0	0	6	0

Abate® containing 1% temephos.

the larvicidal commercial product at the same concentration used for the anti-vectorial program in Cabo Verde for a 3 month period. The lower persistence of Abate® observed in the present study, in containers exposed to the sun, was associated not with resistance mechanisms but rather with the photo-degradation mechanism of the compound. This finding suggests the need for using integrated control measures, considering the specific environmental conditions observed in Cabo Verde, particularly due to the intense solar radiation.

The difference in susceptibility to temephos for the two subpopulations examined in 2014 indicates differences in selective pressure, which was much higher in Praia than in other locations. These results are justified because the city of Praia had the largest number of reported dengue cases in the 2009 epidemic and presents environmental conditions that make this area vulnerable to other potential outbreaks (Cabo Verde, 2013). In this case, a larger amount of insecticide must have been used in this area compared with other counties.

In Cabo Verde, temephos has been used to indistinctly treat potential mosquito breeding sites as an integrated vector management strategy. To control malaria, an endemic disease in this country that has caused major epidemics in the past, the use of temephos to target *Anopheles* spp. (malaria vectors) was conducted prior to the dengue outbreak, and the use of this chemical has intensified since 2009 (Cabo Verde, 2012b).

Table 4
Activity of esterases (α, β and PNPA), glutathione S-transferases (GSTs), mixed function oxidases (MFOs) and acetylcholinesterases (AChEs) in the population of *Aedes aegypti* from Santiago island, Cabo Verde; CV2012 = Population of *Aedes aegypti* from Santiago Island collected in 2012.

Enzyme	Population	N° Females	p99[a]	%> p99[b]	Classification[c]
GTS (mmol/′/mg ptn)	Rockefeller	173	2.23	–	
	CV2012	158	–	25%	Altered
	CV-Praia2014	158	–	0	Unaltered
	CV2014	115	–	0	Unaltered
–					
β-Esterase (nmol/mg ptn)	Rockefeller	156	129.74	–	
	CV2012	157	–	17%	Altered
	CV-Praia2014	113	–	0	Unaltered
	CV2014	74	–	0	Unaltered
–					
α-Esterase (nmol/mg ptn)	Rockefeller	157	72.17	–	
	CV2012	159	–	19%	Altered
	CV-Praia2014	113	–	0	Unaltered
	CV2014	112	–	0	Unaltered
–					
PNPA-Esterase (Abs/′/mg ptn)	Rockefeller	80	8.31	–	
	CV2012	95	–	7%	Unaltered
	CV-Praia2014	56	–	0	Unaltered
	CV2014	20	–	0	Unaltered
–					
MFO (nmoles cit/mg ptn)	Rockefeller	161	11.72	–	
	CV2012	158	k	18%	Altered
	CV-Praia2014	101	–	0	Altered
	CV2014	116	–	0	Unaltered
–					
AChE (% inhibition)	Rockefeller	136	98.74	–	
	CV2012	69	–	6%	Unaltered

[a] 99 percentile of Rockefeller.
[b] Percentage of *A. aegypti* from Santiago Island with 99 percentile higher than the 99 percentile for Rockefeller.
[c] Classification of the enzymatic pattern of the population based on the percentage of individuals with enzyme activity higher than the 99 percentile of Rockefeller: unaltered (≤15%); altered (≥15% ≤50%) and highly altered (>50%). Rockefeller = *A. aegypti* standard susceptibility strain to chemical insecticides; CV2012 = *A. aegypti* population from Santiago Island, Cabo Verde, collected in 2012; CV-Praia2014 = *A. aegypti* population from Praia, Cabo Verde, collected in 2014; CV2014 = *A. aegypti* population from Santiago Island, Cabo Verde, collected in 2014.

The low level of temephos resistance observed in *A. aegypti* from Santiago Island is rare and might represent an emerging problem, which indicates that the continued and exclusive use of this chemical could lead to higher levels of resistance, which could eventually compromise the efficacy of this compound and lead to failures of larvicidal coverage in local anti-vectorial control programs. Most studies detect resistance in the field when the product is no longer effective. This was not the case in the present study, and these results are a warning sign to prevent the progress of resistance by introducing other control strategies to complement temephos use, which suggests that the use of this chemical should be routinely monitored.

According to Montella et al. (2007), RR > 10 compromises the efficiency of the product in the field. Temephos resistance is been detected worldwide, primarily in Latin America and Asia (Dhang et al., 2008; Mulyatno et al., 2012). In Africa, there are no records of *A. aegypti* populations resistant to temephos, which does not rule out the possibility that there might be such a population, as there are few studies on dengue vectors compared with *Anopheles* species, which are malaria vectors (Dia et al., 2012; Kamgang et al., 2011). Thus, this study is the first record of temephos resistance in African *A. aegypti* populations. This fact has important implications for vector control on this continent, as the archipelago of Cabo Verde is located in an strategic geographic region linking three continents (America, Africa and Europe), which enables the flow of people (and mosquitoes) between these continents. Moreover, the national air company in Cabo Verde, TACV (Transportes Aéreos de Cabo Verde) has implemented new routes linking Northeastern Brazil in America (where temephos resistance is largely spread) to this country (Beserra et al., 2007; Lima et al., 2006; Llinas et al., 2010). Thus, Cabo Verde could be a potential source of colonization to introduce temephos resistant mosquitoes in Africa.

Bioassays using the adulticides produced in 2012 revealed a low level of resistance of *A. aegypti* populations from Santiago Island to deltamethrin and cypermethrin pyrethroids, the former of which is a routinely used insecticide in Cabo Verde to control malaria vectors (Cabo Verde, 2013). The use of insecticides in agriculture, associated with the inadequate management of insecticides in public health programs, has contributed to resistance in disease vectors (WHO, 2004). Thus, the selection pressure exerted to control *A. arabiensis,* the only vector of malaria (Cabo Verde, 2012b) in this country and, indirectly, the use of pyrethroids to control agricultural pests (Cabo Verde, 2012a), might contribute to the resistance to these compounds in *A. aegypti* (Cabo Verde, 2012b). Indeed, Amorim et al. (2013) reported an example of the development of temephos resistance in non-targeted mosquito species in Brazil.

The deltamethrin resistance observed in *A. aegypti* from Cabo Verde might have evolved in a similar way as observed in Brazil, where the loss of insecticide susceptibility in *A. aegypti* occurred in the short period after the introduction of this product (2001–2003) (Montella et al., 2007). This idea could explain the previous results of Dia et al. (2012) for experiments conducted using *A. aegypti* from Cabo Verde in 2009, showing the susceptibility of this population to deltamethrin. However, Dia et al. (2012) did not specify the origin of the mosquito population in the archipelago of Cabo Verde.

In Africa, mechanisms of resistance to DDT and pyrethroids have been demonstrated for *Anopheles* spp. because of the relevance of this species as a malaria vector, but there are few records of pyrethroid resistance in *A. aegypti* populations from this continent (Dia et al., 2012; Kamgang et al., 2011; Konan et al., 2012). The loss of sensitivity to insecticides in *A. aegypti* populations is often associated with metabolic resistance mechanisms that are primarily mediated by changes in the activities of the detoxification enzymes GST and esterase. In general, several studies have indicated the involvement of esterases in temephos resistance (Bisset et al., 2001;

Dhang et al., 2008; Lima et al., 2011; Rodriguez et al., 2002) and GSTs in resistance to DDT and pyrethroids (Brengues et al., 2003; Lumjuan et al., 2005, 2007). In addition, MFOs have been implicated in resistance to pyrethroids (Bariami et al., 2012; Rodriguez et al., 2005). In the present study, the increased activity of these enzymes, which was initially identified in 2012, might reflect the strong selection pressure imposed by the use of temephos and pyrethroids during the dengue outbreak in 2009 and as a prevention measure in subsequent years and the use of pesticides in agriculture and oil derivatives used as larvicides in Cabo Verde (Cabo Verde, 2009; Corbel et al., 2007).

For *A. aegypti* populations from Cabo Verde, the results obtained in 2012 indicated changes in the activities of detoxification enzymes; however, the results obtained in 2014 did not confirm the presence of a metabolic resistance mechanism. Similarly, the analysis in 2012 did not reveal the presence of mutations in the voltage-gated sodium channel gene associated with insecticide resistance. However, it is important to note that the number of individuals analyzed was low, and resistant individuals might indeed exist in Cabo Verde.

On the other hand, the profile of susceptibility to Bti, diflubenzuron and malathion for *A. aegypti* populations from Santiago Island, ensures the safe use of these compounds in anti-vectorial programs in Cabo Verde. Bti-based products are efficient in the larval control of *A. aegypti* worldwide (Becker, 1997; Guidi et al., 2011; Guillet et al., 1990; Lacey, 2007; Williams et al., 2014), including temephos-resistant populations in northeast Brazil, and no Bti-resistant mosquito population has been reported thus far (Araujo et al., 2013).

5. Conclusion

The results of the present study revealed the early emergence of resistance to temephos, cypermethrin and deltamethrin in *A. aegypti* populations from Santiago Island, Cabo Verde, which suggests caution in the future use of these chemicals in the field. However, the proven susceptibility to the other products tested (Bti, diflubenzuron and malathion) provides subsidies for the introduction of new compounds in the local vector control program.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Author contributions

HDRR, MAVMS and CFJA designed the initial project. HDRR, AJFM and LFG collected *Aedes aegypti* samples in Cabo Verde. MHSP, NMS, CFJA and HDRR conducted searches for the kdr mutation. APA, MAVMS, HDRR and DRRAC executed and analyzed the results of the bioassays, semi-field and biochemical tests. HDRR, MHSP and NMS wrote the manuscript. CFJA, MAVMS and LFG revised the manuscript and all authors agreed with the final version.

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