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Relationship between kdr L1014F genotypes and phenotypic-resistance to pyrethroids and DDT insecticides in *Anopheles gambiae s.l*.

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ABSTRACT: We investigated the impact of the *kdr* genotypes on the survival rate of mosquitoes exposed to insecticides in the main malaria vectors *Anopheles coluzzii* and *An. gambiae s.s.*. The genotype-phenotype interaction was investigated following two experimental designs; the first one consisted to determine the survival rate of well-characterized adult mosquito strains sharing different kdr genotypes but same genetic background to various insecticides, whereas the second one consisted to expose wild mosquitoes to the same insecticides. Two to five days old adult females were exposed to DDT (4%), deltamethrin (0.05%), and permethrin (0.75%) following WHO protocols. Alive and dead specimens were kept separately to screen the kdr mutations 1014F. The correlation between the kdr genotype and the survival rate to insecticides was investigated in *An. coluzzii* and *An. gambiae s.s.* using a logistic regression model. In the laboratory strains, after exposure to DDT and permethrin, the survival rate was significantly higher in F/F individuals comparing to L/F and L/L individuals ($p<0.05$). A perfect correlation was observed between the survival rate and the genotype in *An. gambiae s.s.*. The survival chance in this population was multiplied by 1.9 [1.2; 2.8] for L/F and 3.2 [2.1; 4.7] for F/F individuals after exposure to DDT; and 3.7 [1.8; 7.3] for L/F and 9 [4.8; 17.0] for F/F individuals after exposure to permethrin. In the wild population of *An. coluzzii*, the survival rate correlated with the genotype after exposure to permethrin and was significantly higher in F/F individuals comparing to L/F and L/L individuals ($p<0.05$). In L/F and F/F individuals, the survival chance was respectively multiplied by 2.7 [1.4; 5.8] and 3.2 [1.4; 6.9] after exposure to DDT; 2.1 [1.0; 4.1] and 4.1 [2.3; 8.7] after exposure to permethrin; and 2.5 [1.1; 5.3] and 3.9 [1.9; 8.0] after exposure to deltamethrin.
Overall, the mosquito survival rates were significantly higher in wild population comparing to laboratory strains after exposure to pyrethroid insecticides. These results suggest that additional mechanisms such as metabolic resistance might contribute to a large extend to phenotypic resistance in malaria vectors.

KEYWORDS: kdr mutation, kdr genotype, phenotypic-resistance, *An. gambiae s.l*.

1 INTRODUCTION

The control of insects of medical importance is primarily based on the application of insecticides. Today, the number of insecticides available for malaria vector control is limited to four classes (Pyrethroids, Organophosphates, Carbamates and DDT). Ongoing strategies of malaria vector control rely on the use of Long Lasting Insecticidal-treated Nets (LLINs) and Indoor Residual Spraying (IRS) [1]. LLINs and IRS are highly depending to pyrethroid insecticides.

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The widespread use of DDT and more recently pyrethroids for vector control has increased selection to pyrethroids resistance mechanisms in malaria vectors ([2]-[5]). In Africa, the situation of pyrethroids resistance is worrying ([4],[6]-[7]) as it may severely affect the efficacy of vector control interventions as reported recently in Benin ([8]-[9]) and Senegal [10]. Pyrethroids and DDT target the voltage gate sodium channel site. Knock-down resistance (kdr) or target-side mutation is one of the two major forms of resistance to DDT and pyrethroid insecticides. Two alternative substitutions of the Leucine 1014 residue can lead to target site resistance. The Leucine to Phenylalanine substitution at position 1014 (L1014F) was found predominant in West and Central Africa whereas the Leucine to Serine substitution (L1014S) originated from Kenya [11], has recently spread in Central ([12]-[14]) and West African regions ([15]-[17]). Co- occurrence of both mutations in same specimens of *An. gambiae s.l.* was found in Cameroon [12], Equatorial Guinea [18], Gabon [19], Uganda [20], Burkina Faso [16] and Senegal [17].

The other major form of resistance termed metabolic resistance results from increased detoxification processes by gene amplification and/or expression [21]. The over-expression of P450 mono-oxygenases has been described from several pyrethroid-resistant populations of *An. gambiae* ([3],[22]) and *An. arabiensis* [23]. In this enzyme family, CYP6M2 is a promising genetic marker for pyrethroid/DDT resistance as it has been demonstrated to metabolize both insecticide classes [24]. A second family of metabolic enzymes, glutathione-s-transferases (GSTs), is thought to play a significant role in DDT and pyrethroids resistance in *An. gambiae* [22]. Recently, Djegbe *et al.* [2] and Djouaka *et al.* [25] reported the presence of CYP6M2, CYP6P3 and GSTe2 in several pyrethroid-resistant populations of *An. gambiae* and *An. funestus* from Benin. In this country, entomological surveys of *An. gambiae s.l.* susceptibility have been carried out in some sentinel sites since 2007 and metabolic resistance was suspected in some *An. gambiae s.l.* populations ([3],[6],[15]). More recently, the presence of this metabolic resistance and both kdr mutations were reported ([2],[6]). However, the impact of kdr, the main pyrethroid-resistance mechanism on the survival rate to insecticides is not well understood. The association between these mutations and the pyrethroid and/or DDT-resistance phenotype in *An. gambiae s.s.* has been shown in several studies using quantitative trait loci (QTL) [26] and the genotype–phenotype association approaches ([9],[27]). However, some authors working on colonized and wild-caught specimens of *An. arabiensis* from Sudan concluded that there was no association between genotype and phenotype [28]. What might partially confound these studies is that it was not yet possible at that period to determine the role of additional resistance mechanisms, such as metabolic resistance. Today, advanced molecular tools are available to screen the metabolic-resistance in resistant mosquitoes. It is therefore possible to infer which resistance mechanisms are having the greatest impact on vector control programs.

In this paper we investigated the association between the presence (yes/no) of kdr genotype and resistance phenotype (resistance/susceptible) and the role of other resistance mechanisms involved in the DDT and pyrethroids insecticides using laboratory and wild strains of *An. gambiae* mosquito. We used two laboratory strains of *An. gambiae* sharing a common genetic background, one susceptible to insecticides (Kisumu) and the second resistant to pyrephroid/DDT (kdrkis) and homozygous for the kdr-L1014F mutation; in order to determine the "weight" of kdr genotypes (L/L, L/F or F/F) in the mosquito survival after insecticide exposure without confounding effect. Finally, we compare the survival rate between kdrkis and wild pyrethroids/DDT-resistant mosquito to show the part of others resistance mechanisms (e.g., metabolic resistance) to provide survival advantage to mosquitoes in contact with insecticides.

2 MATERIALS AND METHODS

2.1 MOSQUITOES STRAINS

2.1.1 LABORATORY STRAIN

Kisumu is a reference laboratory strain originating from the Kisumu region in western Kenya. This strain has been maintained in the laboratory for more than 20 years and is free of any detectable insecticide resistance mechanism (L/L at position 1014). Kdrkis is homozygous resistant for the 1014F allele (F/F at position 1014). This last strain has the same genetic background with Kisumu (strain due to 19 generations of back-crossing between Kisumu and VKPER strains and selection with permethrin). VKPER originates from Kou Valley in Burkina Faso [29] and is homozygote resistant for the 1014F allele. Biochemical assays showed that Kisumu and Kdrkis exhibit similar enzymatic detoxification profiles (Djegbe, pers. comm.). To evaluate the phenotypic expression of heterozygotes individuals (L/F), F1 progeny were produced by mating Kdrkis males (F/F) with Kisumu females (L/L). Each strain was checked for their kdr genotypes before bioassays.

2.1.2 WILD MOSQUITO POPULATION

An. gambiae larvae mosquito were collected from four different sites in Benin (Cotonou, Tori-Bossito, Bohicon and Malanville) in the framework of a WHO/TDR project on "Insecticide Resistance Mechanisms in Benin" ([2],[15]). All larvae were brought back to the laboratory of Centre de Recherche Entomologique de Cotonou (CREC) for rearing. Emerging adult female mosquito (F_0) were used for insecticide susceptibility tests and molecular assays.

2.2 INSECTICIDE SUSCEPTIBILITY TEST

Insecticide susceptibility tests were carried out using the WHO standard protocol [1]. Two to five days old and non blood-fed adult females *An. gambiae* were tested. Batches of 20–25 mosquitoes were exposed to test papers impregnated with 0.75% permethrin, 0.05% deltamethrin and 4% DDT. Impregnated papers were obtained from the WHO reference centre at the Vector Control Research Unit, University Sains Malaysia (Penang, Malaysia). For each test session, about 100 mosquitoes were used. Controls included batches of mosquitoes from each strain exposed to untreated papers. The knockdown (KD) effect of each insecticide was recorded every 10 minutes over the one-hour exposure period. Mosquitoes were then transferred to a recovery tube and provided with 10% sugar solution. Final mortality was recorded 24 hours post-exposure. Dead and alive mosquitoes were then stored individually in codified tubes with desiccant and preserved at -20°C until laboratory processing.

2.3 MOLECULAR IDENTIFICATION AND KDR GENOTYPING

Only field collected mosquitoes were used for molecular assays. For each insecticide, equal number of alive and dead mosquitoes was subjected to DNA extraction according to the bioassay. Specimens were identified to species by RFLP-PCR ([30]-[31]), and the genotype at the kdr locus was determined using HOLA (Hot Oligonucleotide Ligation Assay) technique according to protocol described by Lynd et al. [32].

2.4 DATA ANALYSIS

Correlation between survival rates to DDT, permethrin, deltamethrin and kdr phenotype was investigated using a logistic regression model with the statistical software package R 2.4. Fisher's exact test was used to assess the relationship between survival rates and kdr genotypes in the presence of DDT, permethrin and deltamethrin. The level of significance was set at $p<0.05$.

The parameters used in the model were as follow:

$$Prob(survival = alive|kdr) = \frac{1}{1 + \exp[-(\beta_0 + \beta_1 \times 1_{RS} + \beta_2 \times 1_{RR})]}$$

« Survival rate" is a dichotomic variable expressed as dead (=0) and alive (=1).

"Genotype" is a categorical variable expressed as L/L = 0, L/F =1 and F/F = 2.

β_0, β_1 and β_2 = model parameters.

3 RESULTS

3.1 BIOASSAY AND SPECIES IDENTIFICATION

Respectively 1,728 and 1,391 laboratory strain and wild caught mosquitoes were exposed to Public Health insecticides. The number of alive and dead mosquitoes after exposure period was recorded (**tables 1 and 2)**. Overall, 1,391 *Anopheles gambiae s.l.* wild populations' mosquitoes were successfully genotyped, among which 1,039 mosquitoes were *An. coluzzii*, 305 were *An. gambiae s.s.* and 47 mosquitoes were *An. arabiensis* (**table 1**). Data from all sites were pooled according to insecticide and the correlation between genotype and phenotype was determined. Because of the low number of *An. arabiensis* mosquitoes observed, statistical analyses were performed only with *An. gambiae s.s. and An. coluzzii*.

Table 1: Number of alive and dead laboratory strain of An. gambiae s.s. after exposure to insecticides

Insecticides	Laboratory strain of *An. gambiae s.s.*				
	Phenotypes	Genotypes			
		L/L	L/F	F/F	**Total**
4% DDT	Alive	44	85	142	**271**
	Dead	146	108	50	**304**
	Total	190	193	192	**575**
0.75% Permethrin	Alive	12	36	101	**149**
	Dead	194	131	90	**415**
	Total	206	167	191	**564**
0.05% Deltamethrin	Alive	13	18	75	**106**
	Dead	175	178	130	**483**
	Total	188	196	205	**589**

Table 2: Number of alive and dead An. gambiae s.s., An. coluzzii and An. arabiensis mosquitoes after exposure to insecticides and kdr genotypes

Insecticides	Phenotypes	*Anopheles coluzzii*				*Anopheles gambiae s.s.*				*An. arabiensis*			
		Genotypes											
		L/L	L/F	F/F	Total	L/L	L/F	F/F	Total	L/L	L/F	F/F	Total
4% DDT	Alive	27	26	27	80	14	4	8	26	1	1	1	3
	Dead	51	18	16	85	8	4	1	13	9	4	0	13
	Total	**78**	**44**	**43**	**165**	**22**	**8**	**9**	**39**	**10**	**5**	**1**	**16**
0.75% Permethrin	Alive	14	69	150	233	4	10	55	69	0	0	4	4
	Dead	40	95	95	230	5	35	40	80	11	1	0	12
	Total	**54**	**164**	**245**	**463**	**9**	**45**	**95**	**149**	**11**	**1**	**4**	**16**
0.05% Deltamethrin	Alive	12	53	141	206	7	9	43	59	2	1	3	6
	Dead	36	63	106	205	6	16	36	58	5	4	0	9
	Total	**48**	**116**	**247**	**411**	**13**	**25**	**79**	**117**	**7**	**5**	**3**	**15**

3.2 RELATIONSHIP BETWEEN SURVIVAL RATE AND GENOTYPE

In the laboratory strains, after exposure to DDT and permethrin, a perfect correlation was observed between the survival rate and genotype. The survival rate was significantly higher in F/F in comparison with L/F and L/L individuals ($p<0.05$). In contrast, no correlation was found between the survival rate and the kdr genotypes after exposure to deltamethrin ($p>0.05$). With this insecticide, only F/F individuals showed a high survival rate (**Figure 1**).

The wild population of *An. coluzzii* demonstrated a significant correlation between the survival rate and genotypes after exposure to permethrin. The survival rate decreased significantly from F/F individuals, L/F individuals and L/L individuals ($p<0.05$). After mosquitoes exposure to DDT and deltamethrin in this same species, only F/F individuals showed high survival rate, whereas L/F and L/L mosquitoes showed similar trend in survival rates (**Figure 2**). Overall, the survival rates were significantly higher in wild mosquito population than the laboratory strain after exposure to pyrethroids insecticides ($p=0.00$) (**table 3**).

Table 3: Comparison of survival rates between laboratory strains and wild population of An. gambiae s.s..

Insecticides	Genotypes			
	Laboratoy strain	Wild strain	Odd-ratio	*P-value*
4% DDT	L/L	L/L	**3.0**	0.008
	L/F	L/F	1.2	0.177
	F/F	F/F	1.3	0.388
0.75% Permethrin	L/L	L/L	**11.3**	0.000
	L/F	L/F	1.0	0.001
	F/F	F/F	1.2	0.117
0.05% Deltamethrin	L/L	L/L	**11.6**	0.000
	L/F	L/F	5.0	0.000
	F/F	F/F	1.6	0.000

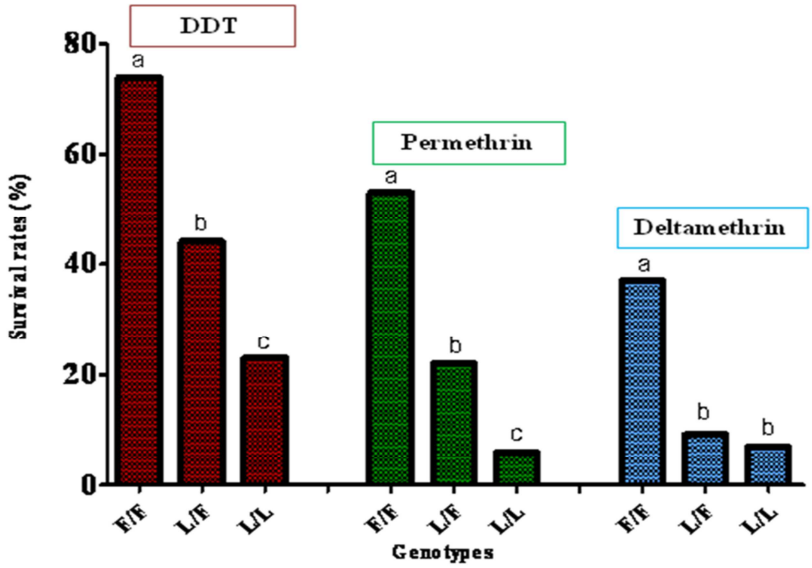

Figure 1: Survival rates to DDT, permethrin and deltamethrin in laboratory strains of An. gambiae s.s.. Histograms with the same letters are not significantly different

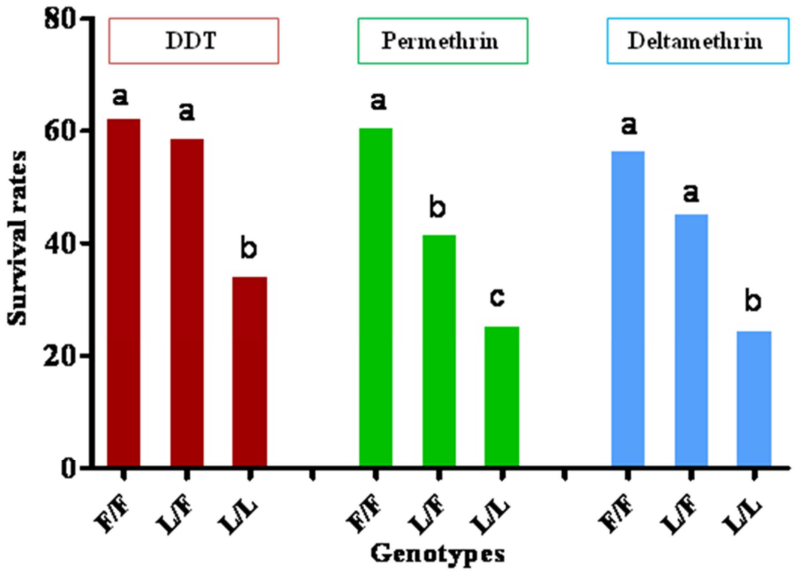

Figure 2 : Survival rates to DDT, permethrin and deltamethrin in wild An. coluzzii population. Histograms with the same letters are not significantly different

3.3 CORRELATION BETWEEN SURVIVAL CHANCE AND GENOTYPE

In the laboratory strains sharing the same genetic background with different resistance genotypes (L/L, L/F and F/F), a significant correlation was found between kdr genotypes and survival chance after exposure to DDT and permethrin. No correlation was found when mosquitoes were exposed to deltamethrin. When exposed to DDT, the survival chance was multiplied by 1.9 [1.2; 2.8] for L/F, and 3.2 [2.1; 4.7] for F/F individuals. With permethrin, this survival chance was multiplied by 3.7 [1.8; 7.3] and 9 [4.8; 17.0] respectively for L/F and F/F individuals. No difference was observed between the survival chance of L/L and L/F individuals when exposed to deltamethrin (p=0.45), but this chance was significantly high in F/F individuals (5.3 [2.3; 9.8], p=0.000) (**Table 4**).

Table 4: Survival chance for each kdr L1014F genotypes in laboratory strains of An. gambiae s.s..

Insecticides	Genotypes	*An. gambiae s.s.*		
		Parameters	Odd ratio and CI 95%	*p-value*
4% DDT	L/L	β_0 = - 1.463	1	0.0000
	L/F	β_1 = 0.643	1.902 [1.255 ; 2.882]	0.0020
	F/F	β_2 = 1.161	3. 194 [2.155 ; 4.732]	0.0000
0.75% Permethrin	L/L	β_0 = - 2.843	1	0.0000
	L/F	β_1 = 1.308	3.701 [1.866 ; 7.337]	0.0002
	F/F	β_2 = 2.206	9.077 [4.835 ; 17.043]	0.0000
0.05% Deltamethrin	L/L	β_0 = - 2.671	1	0.0000
	L/F	β_1 = 0.284	1.328 [0.633 ; 2.786]	0.4530
	F/F	β_2 = 1.666	5.291 [2.291 ; 9.846]	0.0000

In the wild population of *An. coluzzii*, a good correlation was observed with all insecticides tested ($p<0.05$). The survival chance was multiplied by 2.7 [1.4; 5.8] and 3.2 [1.4; 6.9] respectively for L/F and F/F individuals after DDT exposure. For the same individuals, this survival chance was multiplied by 2.1 [1.0; 4.1] and 4.1 [2.3; 8.7] respectively after exposure to permethrin. After deltamethrin exposure, the survival chances were 2.5 [1.1; 5.3] and 3.9 [1.9; 8.0] respectively for L/F and F/F individauals. For the wild population of *An. gambiae s.s.*, no difference was observed on the survival chances with all genotypes and all insecticides tested ($p>0.05$) (**tables 5**).

Table 5 : Survival chance according to kdr L1014F genotypes in wild population of An. coluzzii and An. gambiae s.s..

Insecticides	Genotypes	*An. coluzzii*			*An. gambiae s.s*		
		Parameters	Odd ratio and CI 95%	*p-value*	Parameters	Odd ratio and CI 95%	*p-value*
4% DDT	L/L	β_0 = - 0.6360	1	0.0075	β_0 = 0.5596	1	0.207
	L/F	β_1 = 1.0037	2.7283 [1.4690 ; 5.8391]	0.0097	β_1 = - 0.5596	0.5714 [0.1113 ; 2.9329]	0.502
	F/F	β_2 = 1.1592	3. 1873 [1.4690 ; 6.9156]	0.0033	β_2 = 1.5198	4.5713 [0.4803 ; 43.5025]	0.186
0.75% Permethrin	L/L	β_0 = - 1.0498	1	0.0007	β_0 = - 0.2231	1	0.739
	L/F	β_1 = 0.7301	2.0752 [1.0481 ; 4.1089]	0.0361	β_1 = - 1.0296	0.3571 [0.0804 ; 1.5859]	0.176
	F/F	β_2 = 1.5066	4.1513 [2.3300 ; 8.7348]	0.0000	β_2 = 0.5416	1.7187 [0.4339 ; 6.8080]	0.441
0.05% Deltamethrin	L/L	β_0 = - 1.0986	1	0.0009	β_0 = 0.1541	1	0.782
	L/F	β_1 = 0.9258	2.5238 [1.1939 ; 5.3351]	0.0153	β_1 = -0.7295	0.4821 [0.1234 ; 1.8829]	0.294
	F/F	β_2 = 1.3839	3.9904 [1.9809 ; 8.0382]	0.0001	β_2 = 0.0235	1.0238 [0.3155 ; 3.3215]	0.969

4 DISCUSSION

Various mechanisms enable *Anopheles* mosquito to resist the action of insecticides, including metabolic resistance, target-site resistance, reduced penetration and behavioral resistance. These mechanisms may allow mosquitoes to resist more than one insecticide (cross-resistance), and *Anopheles* may express more than one resistance mechanism (multiple resistances). Of all these types of resistance, perhaps the most significant in *An. gambiae* populations is knockdown resistance (kdr) [33]. The importance of kdr mutations as a stand-alone mechanism conferring pyrethroids resistance is still subject to debate. In this context, by using both laboratory strain and wild caught population, we demonstrated the contribution of other resistance mechanism (e.g., metabolic resistance) to provide pyrethroids/DDT resistance in *An. gambiae* mosquito from Benin. The two laboratory strains of *An. gambiae s.l.* involved in this study shared the same genetic background but differed from the L1014F kdr genotype. In our laboratory pyrethroid-resistant strain, it seems that kdr was the only resistance mechanism involved. Bioassay using synergists and biochemical tests failed to demonstrate any involvement of metabolic detoxification due to oxidases, esterases or glutation-s-transferases. WHO tube test bioassays showed a high survival advantage in F/F and F/L laboratory strains of *An. gambiae* when exposed to DDT and permethrin,

with a perfect correlation between the kdr L1014F genotype and the survival rate. However, no correlation was found with deltamethrin (type II of pyrethroids). Similar trend were obtained by Matambo with a colony of *An. arabiensis* from the Sennar region of Sudan selected by exposure to DDT [28]. Over the course of this selection, the mortality rate decreased from 90.6% to 12.1% 24hrs post-exposure to 4% DDT. No mortality was observed in the F16 generation after exposure to 0.75% permethrin, while only 24% mortality rate was recorded after exposure to 0.05% concentration of deltamethrin [28]. The relationship between the kdr genotype and the phenotypic resistance in malaria vectors has been extensively reviewed [34]. Martinez-Torres *et al*. [35] have shown that in seven samples from West Africa, the frequency of the L1014F allele correlated strongly with reduced mortality in a permethrin-World Health Organization (WHO) tube test. Also, a clear correlation was shown between inheritance of the kdr L1014S mutation and permethrin resistance ([11],[26]). Simulated field trials of insecticide-treated bed nets (ITNs) in Côte d'Ivoire showed that kdr L1014F had a strong impact on the efficacy of nets treated with pyrethroids (cypermethrin) and etofenprox. Here, L1014F homozygotes showed a survival advantage [36]. The same trend was observed in Burkina Faso, where the protective effect of permethrin-treated plastic sheeting was apparent against susceptible genotypes but not against kdr homozygotes [37]. Similarly in Benin, low mortality of *An. gambiae* L1014F homozygotes was observed after exposure to permethrin-treated nets [38]. In this study, no correlation was found between the survival rate and kdr genotype in wild mosquitoes populations. Furthermore, in wild population of *An. gambiae*, F/L and L/L individuals showed similar survival rate with DDT and no difference was observed between the survival rates after Pyrethroids exposure (**figure 3**). In *An. coluzzii*, F/F and L/F individuals displayed similar survival rates when exposed to DDT and deltamethrin. These results suggested that if kdr L1014F mutations confer a significant effect on vector resistance, they do not fully explain the observed vectors resistance level to insecticides because homozygous susceptible (L/L) and heterozygous (L/F) subjects survived to pyrethroids/DDT exposure, suggesting an involvement of other alternative mechanisms such as metabolic resistance mechanisms. Results obtained in this study agreed with the standpoint suggested by Brooke [39], arguing that kdr may act with certain cofactors that are thus far unidentified. This resistance mechanism could be multigenic and the kdr genotype might not fully explain all the variance in the resistance phenotype. It is possible that besides the L1014F kdr mutation, others mutations in the para-type sodium channel gene might be needed for mosquitoes to survive after exposure to a discriminating concentration of an insecticide. Note that a *de novo* mutation *(N1575Y)* recently emerged within domains III-IV of voltage gate sodium channel in pyrethroid resistant populations of *An. gambiae* from West Africa and seems to occur only in a single long-range haplotype, also bearing the 1014F allele [40]. It has been suggested that the N1575Y mutation may compensates for deleterious fitness effects of 1014F and/or confers additional resistance to pyrethroids insecticides [40].

Recent evidences have also stressed the preeminent role of metabolic resistance as the most important mechanism of resistance in the major Anopheline mosquito vectors [41] with cytochrome P450s especially from the CYP6 and GST families taking the front seat in conferring resistance to the four major insecticides used for public health interventions ([2],[42]-[44]). Interestingly in Benin, some recent studies have shown the presence of four metabolic genes including GSTE2, GSTD3, CYP6P3 and CYP6M2 in *An. coluzzii* collected in the same sentinel sites (Cotonou, Malanville, Bohicon and Tori-Bossito) ([2],[5]). Other major resistance mechanisms exist and decrease the cuticular penetration of insecticides in mosquito species [4]. As the first line of defense against insecticides, a thicker cuticle leads to a slow rate of insecticide absorption and penetration, which reduces the uptake of insecticides. For example, in an *An. funestus* population collected from southern Mozambique, pyrethroids resistance was associated with an increased cuticle thickness [45]. The temporal and spatial of two cuticular proteins in *An. gambiae* revealed the potential function of two proteins (CPLCG3 and CPLCG4) in slowing insecticide penetration [46]. Recently, a functional genomics study revealed that cuticular proteins were associated with deltamethrin resistance in laboratory and field populations of *C. pipiens pallens* [47]. Furthermore, evidence suggests that behavioral resistance also plays a role in reducing the efficacy of insecticide treatment [4]. Genetic changes in mosquito populations may result in decreasing the chance of contacting insecticides through modified feeding and resting activities ([48]-[50]). This suggests that failure in malaria vector control strategies with the field population of mosquito should not only be attributed to the kdr L1014F mutation.

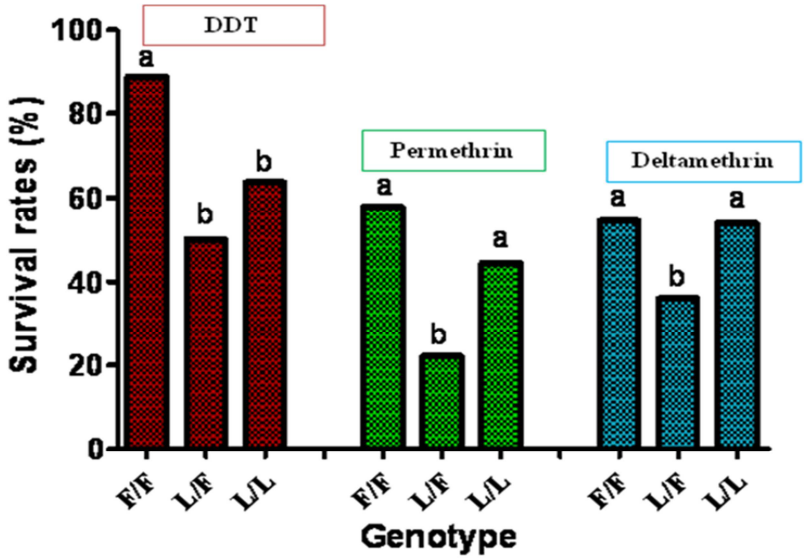

Figure 3: Survival rates to DDT, permethrin and deltamethrin in wild An. gambiae s.s..

Histograms with the same letters are not significantly different.

5 CONCLUSION

Our results revealed that in wild population of malaria vectors, the kdr resistance may act with certain cofactors to be identified. The kdr L1014F mutation alone could not provide survival advantage to pyrethroid insecticides. Suggesting that additional mechanisms such as the metabolic resistance contribute to a large extend to phenotypic resistance in malaria vectors.

AUTHOR'S CONTRIBUTIONS

ID, MA and RD designed the study; ID and FZ collected the mosquitoes, and performed molecular analysis; GD carried out the toxicological test; ID and FZ drafted and wrote the manuscript. All authors read and approved the final manuscript.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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