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Original article

# Evidence of multiple mechanisms of alphacypermethrin and deltamethrin resistance in ticks *Rhipicephalus microplus* in Benin, West Africa

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ABSTRACT

Ticks are obligate haematophagous arthropods, causing heavy losses in affected livestock. The objective of this study is to investigate phenotypic and genotypic resistance in *Rhipicephalus microplus* populations from Benin. Engorged female adult ticks were collected from cattle in two districts of Benin. Bioassays, biochemical and molecular tests were carried out on these ticks to determine the phenotypic, enzymatic and genetic status of resistance. Results of bioassays showed high resistance factors (RF > 41). The molecular tests showing the presence of the domain II mutation and absence of the domain III mutation in the voltage-gated sodium channel gene. Biochemical tests showed increased activity of esterases, multifunction oxidases and glutathione transferases in resistant samples. Genotyping the samples showed high levels of heterozygous genotypes (73.36% and 63.30%) as compared to homozygous susceptible and resistant genotypes (23.3% and 10%) respective at Samiondji and Betecoucou. A correlation between phenotype resistance and presence of the domain II mutation at the voltage gated sodium channel gene was observed suggesting that this could be associated with resistance. Target site mutation and metabolic detoxification are mechanisms of resistance to pyrethroids in *R. microplus* tick populations from Benin.

## 1. Introduction

The resistance of ticks to acaricides has become a priority research area in the world in recent years, following the decline in susceptibility of ticks, particularly the *R microplus* species to synthetic acaricides (Yessinou et al., 2016). Unfortunately, this resistance is widespread in all countries of the world where the tick *R. microplus* is present, such as Brazil (Mendes et al., 2011), India (Kumar et al., 2015), Australia (Guerrero et al., 2012), South Africa (Lovis et al., 2012), Benin (Adehan et al., 2016), Cote d'Ivoire, Burkina-Faso and Mali (Adakal et al., 2013). In this condition, some breeders are used indigenous control methods or to overuse acaricides for tick control. There are three mechanisms by which ticks develop tolerance the toxic effects of acaricides and they are known for their resistance to the chemical. Knowledge of these mechanisms is essential for the development of control strategies. The resistance to pyrethroids in *R. microplus* results mainly from point mutations in the gene encoding the sodium channels (Guerrero et al., 2012) and gives the ticks the knock-down resistance (kdr). These point mutations at the level of the genes coding for target proteins lead to structural modifications which decrease the affinity of ticks with acaricides (Domingues et al., 2012). Several types of mutations have been identified, one in domain III in ticks from North America, Australia and South Africa (Morgan et al., 2009; Lovis et al., 2012). This mutation is detected at position 2134 (transmembrane IIIS6) of the sodium channel gene and involves the substitution of a thymine (T) by an adenine (A), resulting in the replacement of a phenylalanine by an isoleucine at susceptible and resistant strain, respectively (He et al., 1999). The second mutation was detected at position C190A in domain II S4-5 linker of the sodium channel gene in Australia, Mexico, the United States and Brazil. This mutation results in the substitution of a cytosine in the susceptible strain to an adenine in the resistant strain. This mutation involves the replacement the leucine by isoleucine that was correlated to pyrethroid resistance (Domingues et al., 2012; Stone et al., 2014). Among these mechanisms, there is also the biochemical resistance which manifests by the detoxification or the reduction of the absorption of the acaricides. There are three mains families of enzymes

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are involved in this type of resistance. These are the esterases, cytochrome P450 monooxygenases and glutathione-S-transferases which are involved in the resistance of *R. microplus* to acaricides (Guerrero et al., 2012). The involvement of esterases in pyrethroid resistance has been previously demonstrated in an *R. microplus* ticks (Kumar et al., 2013; Nandi et al., 2015). The overproduction of glutathione *S*-transferase (GST) in *R. microplus* has been associated with resistance to pyrethroid (Enayati et al., 2010; Bellgard et al., 2012; Ghosh et al., 2015). Pyrethroid resistance was characterized from *R. microplus* and cytochrome P450 monooxygenases enzymes were involved in resistance (Ziapour et al., 2016). If these mechanisms of resistance are detected, evaluation of the state of resistance of ticks to acaricides and development of resistance control strategy can be easy. In the present study, the *R. microplus* population was characterized using bioassay, biochemical and molecular methods to assess the resistance status for deltamethrin and alpha cypermethrin with an aim to develop strategy to control against ticks.

## 2. Material and methods

### 2.1. Study area

#### 2.1.1. Tick collection

In this study, 1200 Engorged females of *R. microplus* were harvested from 80 animals (15 ticks per animal). This collection was carried out on four breeds of bovine (Lagunaire, Borgou, Azawak and crossbreeds) of Samiondji (7°25 North–2°22 East) and Betecoucou farm (7°45N–2°20E) (Fig. 1). The ticks were then transported to the Biotechnology Unit of Animal Production and Health (URBPSA) of the Polytechnic School of Abomey-Calavi. The identification of *R. microplus* was carried out using a binocular microscope (Olympus® SZ51), with the key developed by Walker et al. (2003). Ticks were put into Petri dishes and were kept in an incubator. The eggs were collected two weeks later and transferred to glass tubes sealed with hydrophobic cotton to allow larval hatching under the conditions described by Ibelli et al. (2012) (27–28 °C and 85–90% relative humidity). The resistance test was carried out at the URBPSA and the biochemical test at the Center of Research Entomology of Cotonou with larvae that were between 14 and 21 days of age.

### 2.2. Resistance tests

The larval packet test (LPT) standardized and recommended by FAO (2004) for bioassay was done to test for phenotypic resistance to the pyrethroids (alphacypermethrin and deltamethrin), on the ticks larval collected from two districts.

### 2.3. DNA extraction

The extraction of DNA from the larvae of *R. microplus* was carried out on 30 individuals per study area at the ratio of 15 live larvae and 15 larvae dead after the LPT. The extraction technique consists of finely grinding the tissues of the larvae in 100 µl of Cetyl Trimethyl Ammonium Bromide (CTAB) 2%, with a grinding piston. The ground product was heated in a water bath at 65 °C for 5 min, in order to free the samples of a maximum amount of protein which can interfere in the subsequent steps. A volume of 100 µl of chloroform was added to the grounded product and the supernatant was recovered after centrifugation at 12.000 rpm' for 8 min. Nucleic acids were precipitated using 100 µl of isopropanol after 15 min of centrifugation at 12.000 rpm'. The deposit was dried at speed bac for 4–5 min before being take again in 20 µl of distilled water and let at room temperature overnight. The DNA solution was stored at −20 °C.

### 2.4. Mutation T2134A

The presence of mutations of the sodium channel gene was evaluated on tick larvae *R. microplus*. Primers were designed from an alignment of partial sodium channel gene sequences of *R. microplus* sequences (GenBank: AF134216) to amplify the transmembrane segment S6 of the domain III. The following primers were used (Inqaba Biotec™, South Africa): FG-221 TTATCTTCGGCTCCTTCT Wild type-specific sense, FG-222 TTATCTTCGGCTCCTTCA Resistant-specific sense and FG-424 TCATTGAAATTGTCGATAATAACAC as described by He et al. (1999) and reported by Guerrero et al. (2001). The FG-221 and FG424 primers produce a 68 bp product for the susceptible allele and primers FG-222 and FG424 yield amplicons of 68 bp size for the resistant allele. The amplifications were carried out in a volume of 25 µl of PCR product. The reaction mixture of the PCR consisted of 1 × buffer, 2 mM $MgCl_2$, 0.2 mM NTP, 2 µM each primer, 1.5 U Taq polymerase, 2 µl genomic DNA (Eurogentec) and 11.2 µl of distilled water. The thermal cycler used was Mastercycler® nexus®. The conditions of the PCR reaction were: an initial denaturation at 94 °C for 5 min followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 2 min and a final extension at 72 °C for 10 min. The electrophoresis was carried out on 1.5% agarose gel (Promega, USA) with the amplified DNA and visualized under UV light.

### 2.5. Mutation C190A

Genomic DNA was purified from larvae of *R. microplus* to identify the C190A mutation. The reaction mixture consists of 1 × buffer, 1.5 mM $MgCl_2$, 0.2 mM NTP, 1 µM each primer, 1.5 U Taq polymerase (Eurogentec), 2 µl genomic DNA and 14.7 µl of distilled water. The primers were used (Inqaba Biotec™, South Africa): FG-447 5′-GAACTT GTGTTTACTTTCTTCGTAGT-3′ Downstream non-diagnostic, DB-011 5′-GGAAAACCATCGGTGCTC-3′ Wild type-specific sense and DB-012 5′-GGAAAACCATCGGTGCTA-3 'Resistant-specific sense. The thermal cycler used of Mastercycler® nexus®. The conditions of the PCR reaction were: 94 °C cycle for 5 min, followed by 40 cycles of 94 °C for 45 s, 60 °C for 30 s and 72 °C for 40 s with a final step of 72 °C for 7 min. The PCR fragments were fractionated on a 1.5% agarose gel (Promega, USA) stained with ethidium bromide and visualized under UV light.

### 2.6. Biochemical analyses

The enzymatic activity of tick larvae *R. microplus* was evaluated according to (Hemingway et al., 1998) protocol. These enzymes were mainly esterases, cytochrome P450 mono-oxygenases, Glutathione-s-transferases (GST) and total protein. As these enzymes degrade rapidly at room temperature, the tick larvae were crushed on ice in 200 ml of distilled water and then the extract was centrifuged at 14.000 rpm' for 2 min. Depending on the activity to be measured, the procedure was as following:

### 2.7. Esterase

For activities esterase, 10 µl of tick larvae in two replicates were placed in each well plate to which was added 90 µl of 1% Triton Phosphate Saline (PBS) Buffer pH 6.5. The mixture thus made, the plate was left for 10 min at room temperature. Then, 100 ml of a solution composed of: 600 µl alpha-Naphthyl acetate 0.3 M (or beta-Naphthyl acetate) and 3 ml of Triton PBS pH 6.5 were added to which 7 ml $H_2O$ were added. The plate was incubated again for 30 min at 25 °C. and then 100 µl of a solution composed of: 10 mg of Fast Garnett Salt (FGBC) dissolved in 12 ml of distilled water were added. The mixture was incubated again for 10 min at ambient temperature with a lid and an end-point reading was made at 550 nm, the crushed diluted to 1/5.

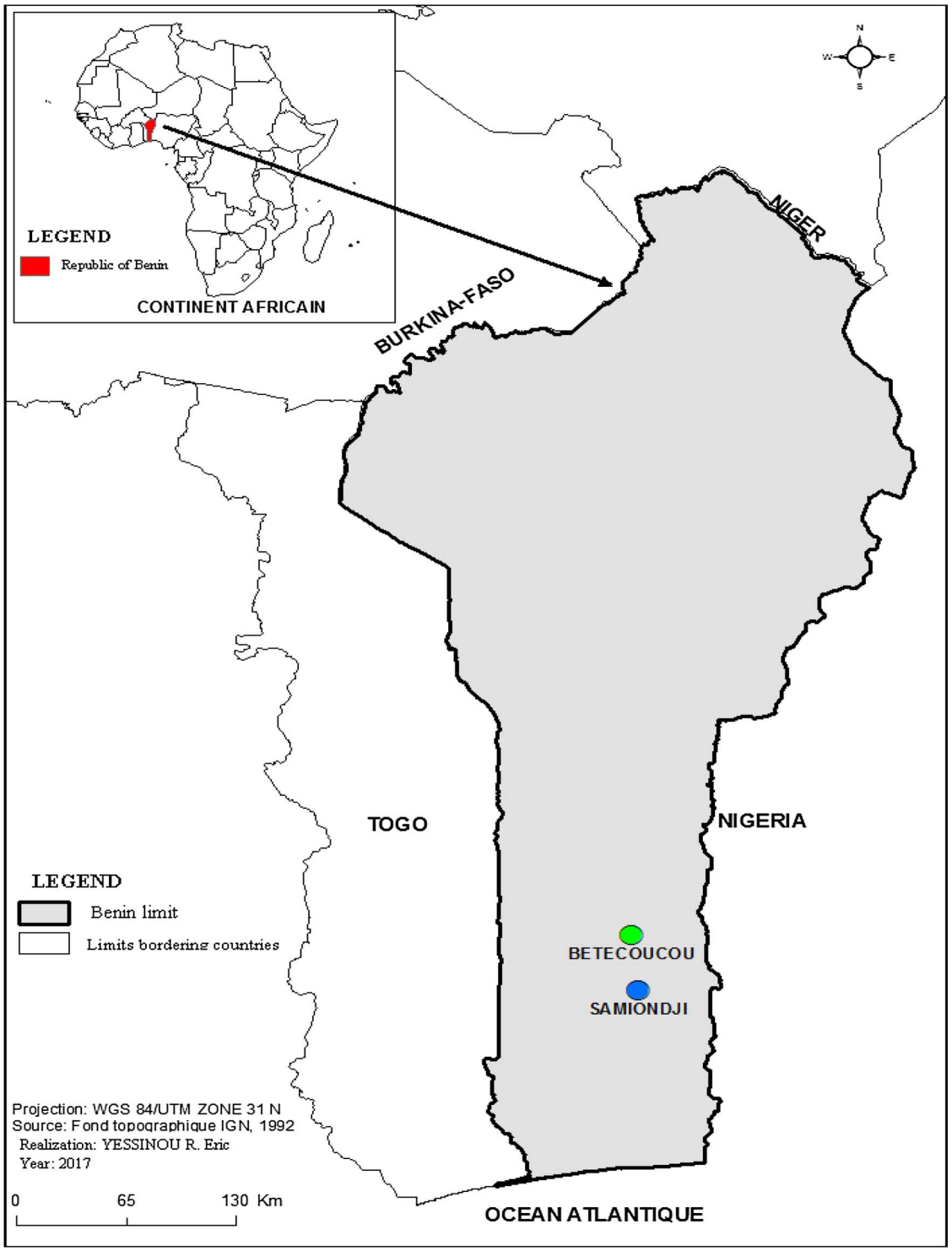


**Fig. 1.** The map of Benin showing the districts where *R. microplus* ticks were collected.

### 2.8. Oxydases (Cytochrome P450)

Cytochrome P450 activity was determined using the heme-peroxidase assay according to William and Janet (1997) The assay detects the elevation in the amount of heme, which was then converted into equivalent units of cytochrome P450. After 20 µl of tick larvae were mixed into two replicates in each well, 80 µl of Potassium Phosphate ($KHPO_4$) buffer 0.0625 M pH 7.2 was added. Then 200 µl of a solution: 0.012 g 3, 3 ', 5, 5 ' Tetramethyl Benzidine (TMBZ) dissolved in 6 ml of methanol and 12 ml of Buffer Sodium Acetate ($NaC_2H_3O_2$) 0.25 M pH 5.0 then 25 ml of a solution of 3% hydrogen peroxide in each well. The plate was incubated for 1 h and a final point reading was performed at 630 nm every 10 min.

### 2.9. Glutathione-S-transferases (GST)

To measure glutathione-*S*-transferases (GST) activity in ticks, 200 ml of GSH/CDNB working solution (100 ml of an extemporaneous solution of 0.6% weight/volume reduced glutathione in 0.1 M sodium phosphate buffer pH = 6.5 + 0.013 g of 1-chloro-2, 4-dinitrobenzene

diluted in 1 ml of 70% methanol) was added to each replicate of tick larvae homogenate. The reaction was read at 340 nm immediately as a kinetic assay for 5 min. An extinction coefficient of 5.76 $mM^{-1}$ (corrected for a path length of 0.6 cm) was used to convert absorbance values to moles of product. GST specific activity was reported as the rate of formation of GSH produced in mmol $min^{-1}$ $mg^{-1}$ protein.

### 2.10. Total protein

In each well of the plate, 10 µl of crushed tick larvae extracts in two replicates were added to which 290 µl of solution compose of: Coomassie and Protein Assay Reagent diluted in half in distilled water (1 vol of Protein Reagent for 1 vol of water) was added. After incubation of the plate for 5 min at ambient temperature, the final point reading was carried out at 590 nm, the crushed diluted to 1/2.

### 2.11. Data analysis

Graph Pad Prism 4 software and POLO PC software were used to compare the mean and calculate $LC_{50}$ and $LC_{95}$ as well as slopes. Biochemical assay data (enzymatic activity per mg of protein) of wild specimens of *R. microplus* were compared with to the IVRI-I susceptible strain (Castro-Janer et al., 2010; Ghosh et al., 2015; Kumar et al., 2015; Shyma et al., 2012). Resistance factors (RF) for different isolates were worked out between $LC_{50}$ of field isolates and $LC_{50}$ of reference susceptible IVRI-I line of *R. microplus*. Resistant factor (RF) = $LC_{50}$ value of field ticks/$LC_{50}$ values of susceptible ticks. As per the calculated value of resistance factor (RF), the resistance status in the field population of *R. microplus* was classified as susceptible (RF < 1.4), level I resistance (1.5 < RF < 10.0), level II resistance (10.1 < RF < 25.0), level III resistance (26 < RF < 40), and level IV resistance (RF > 41) (Shyma et al., 2015)

## 3. Results

### 3.1. Tick resistance to acaricides

The lethal doses ($LC_{50}$ and $LC_{95}$) as well as the confidence intervals (IC) obtained on the *R. microplus* strains harvested from Samiondji and Betecoucou with respect to alphacypermethrin and deltamethrin were presented in Table 1. The resistance tests carried out with alphacypermethrin showed resistance factors (RF = 58.70 at Samiondji and RF = 62.25 at Betecoucou), the resistance factors observed with the deltamethrin on the Samiondji and Betecoucou farm were respectively of RF = 170. 52 and RF = 123.15. The resistance factors obtained on the two districts were considerably greater than 41 for all the acaricides tested. The slopes obtained on the strain of these two districts were small compared to the sensitive strain, evidence of emergence of *R. microplus* resistance to synthetic acaricide. Overall, *R. microplus* was resistant to the compounds tested: alphacypermethrin and deltamethrin.

**Table 1**
Slopes and ($LC_{50}$; $LC_{95}$) with 95% confidence limit of alphacypermethrin and deltamethrin obtained by LPT for susceptible IVRI-I line of *R. microplus*.

| Echantillon | Slope ± SE | $LC_{50}$ (95% IC) | $LC_{95}$(95% IC) | RF |
|---|---|---|---|---|
| **Alphacypermethrin** | | | | |
| Samiondji | 1.44 ± 0.08 | 1.93 (1.55–2.53) | 26.81(15.58–57.72) | 62.25 |
| Betecoucou | 1.62 ± 0.8 | 1.82 (1.48–2.33) | 18.82 (11.60–37.47) | 58.70 |
| **Deltamethrin** | | | | |
| Samiondji | 1.46 ± 0.08 | 3.24 (2.29–5.39) | 43.15 (19.16–172.74) | 170.52 |
| Betecoucou | 1.43 ± 0.09 | 2.34 (1.93–2.96) | 32.92 (20.20–63.65) | 123.15 |

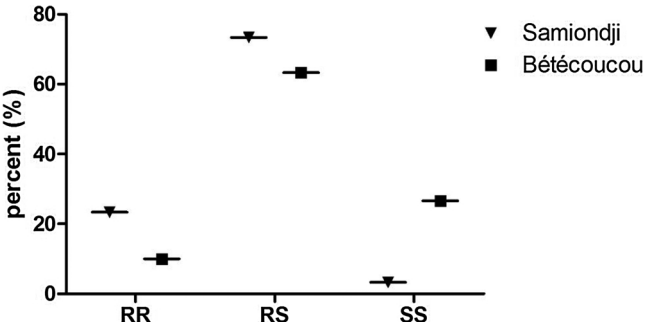


**Fig. 2.** Distribution of genotypes in the tick population *R. microplus*.

### 3.2. Mutations T2134A and C190A identified on the tick R. microplus

The PCR performed on *R. microplus* larvae only amplified the wild-type allele for the T2134A mutation with the FG-221 and FG-424 primers (AF134216 GenBank) but the primer pair FG-222/FG-424 (GenBank AF134216) representing the resistant allele could not be amplified despite the resistance of these ticks to pyrethroids. The absence of the T2134A mutation does not exclude the presence of other mutations in the sodium channel gene which may be the cause of tick resistance *R. microplus* to the acaricides tested. The samples from both districts displayed the C190A mutation. The resistant genotypes were (23.3% and 10%) as compared to homozygous susceptible (SS) and heterozygous genotypes (RS) were (73.36% and 63.30%) respective at Samiondji and Betecoucou. Tick larvae of susceptible *R. microplus* homozygous susceptible (SS) were also noted in Samiondji (3.33%) and Betecoucou (26.66%) (Fig. 2). Among both survivors and nonsurvivors, all genotypes (RR, RS and SS) were found. However, the homozygous resistant genotype (RR) was only found among survivors and the heterozygote genotype (RS) was the most prevalent genotype after LPT (Fig. 3).

### 3.3. Biochemical analysis

#### 3.3.1. Esterase activities

Enzymatic activities of esterases are elevated in the field populations when compared with those of the most susceptible field population in the two districts. But, this activity was lower on the Betecoucou farm (0.20 ± 0.02) than on the Samiondji farm (0.23 ± 0.01). There was a significant difference in tick enzymatic activity between the control strain (IVRI-I) versus that of the two districts (P < 0.001) (Fig. 4).

#### 3.3.2. Cytochrome P450 monooxygenases activities

Populations of *R. microplus* were expressed oxidase activities comparable with the susceptible strain IVRI-I (P < 0.001). The average oxidase expressed by the larvae of *R. microplus* was 0.71 ± 0.03 at Samiondji and 0.57 ± 0.02 at Betecoucou (Fig. 5).

#### 3.3.3. Glutathione-S-Transferases (GST) activities

GST expression was noted in the population of *R. microplus* from the various study areas. The average GST of the tick larvae examined in Samiondji and Betecoucou were 0.77 ± 0.04 and 0.57 ± 0.05 respectively. However, there were high GST activities in Samiondji and Betecoucou ticks larvae compared to the susceptible strain (IVRI-I) (P < 0.001) (Fig. 6).

## 4. Discussion

*R. microplus* on the districts of Samiondji and Betecoucou was controlled by several chemicals including alpha-cypermethrin and deltamethrin. After the larval packet test (LPT), the highest slopes were

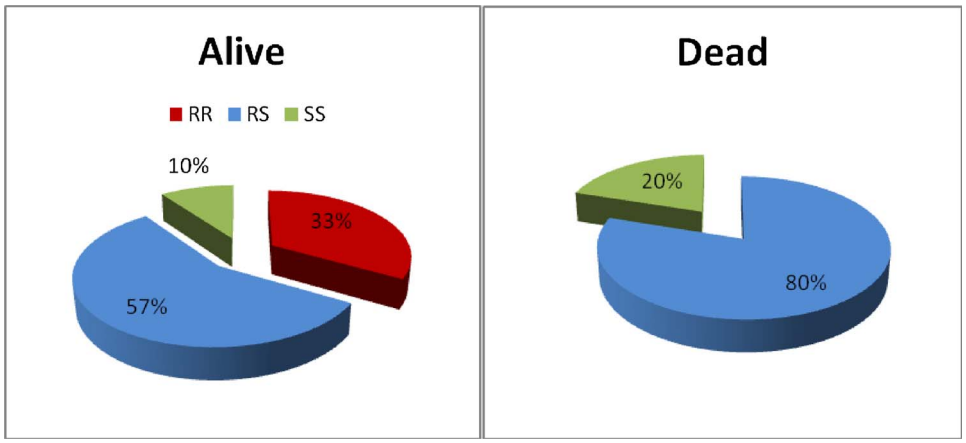


**Fig. 3.** Kdr genotypes distribution among live and dead *R. microplus* individuals after LPT to deltamethrin and alphacypermethrin.

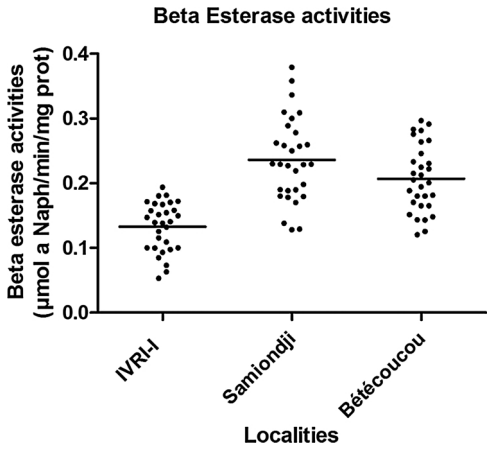


**Fig. 4.** Esterase activities in *R. microplus* populations from study sites.

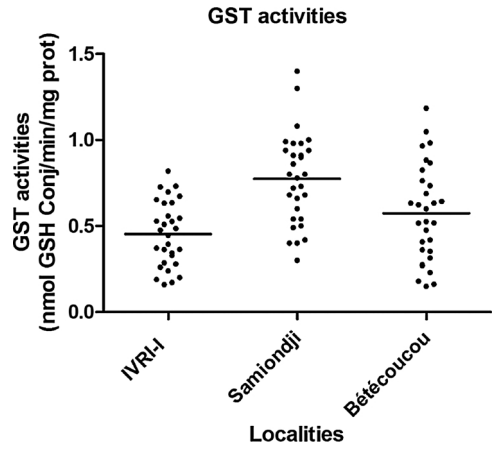


**Fig. 6.** GST activities in *R. microplus* populations from study sites.

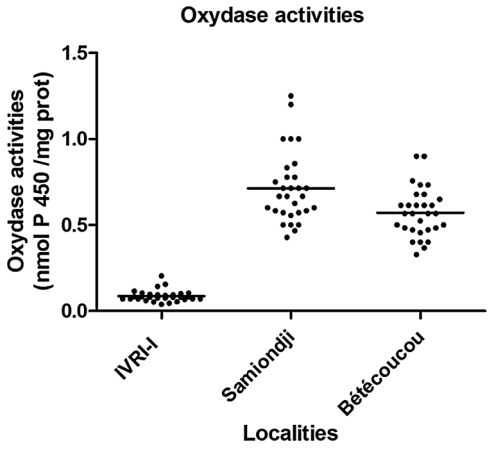


**Fig. 5.** Oxidase activities in *R. microplus* populations from study sites compared with reference strain.

recorded on the susceptible strain with alphacypermethrin and deltamethrin relative to the field strains, thus confirming homogeneity of the populations of *R. microplus* (susceptible strain). The slopes recorded on the larvae of *R. microplus* at Betecoucou were 1.62 ± 0.8 for alphacypermethrin and 1.43 ± 0.09 for deltamethrin. The same observation was made in Samiondji with slopes of 1.44 ± 0.08 and 1.46 ± 0.08 being observed. The results obtained on these two districts were indicated heterogeneity of *R. microplus* population. Resistance factors obtained with alphacypermethrin and deltamethrin were RF = 62.25; RF = 170.52 and RF = 58.70; RF = 123.15 in Samiondji and Betecoucocu respectively. These data revealed a level IV resistance of *R. microplus* ticks to alphacypermethrin and deltamethrin on the Samiondji and Betecoucou districts. The intensive use of acaricide in these study areas was the basis for a high selection of ticks resistant to alphacypermethrin and deltamethrin, due to the presence of a high rate of ticks insensitive. These results were in agreement with the results of the studies carried out in Burkina Faso indicating tick resistance to alphacypermethrin and deltamethrin. (Adakal et al., 2013). The work done in Benin by Adehan et al. (2016)showed resistance of *R. microplus* to pyrethroids, especially alphacypermethrin and deltamethrin. But on Samiondji farm they noted a sensitivity of *R. microplus* to alphacypermethrin, this result is not consistent with our results. Several authors in the world reported that this tick species was resistant to pyrethroids (Lovis et al., 2012; Mendes et al., 2013; Nandi et al., 2015; Sharma et al., 2012; Stone et al., 2014). While the molecular study carried out does not show the domain III mutation the domain II mutation was observed. This could be linked to the origin of these tick populations which is thought to be from Brazil (Madder et al., 2012) where the domain III mutation has been reported to be absent (Andreotti et al., 2011). However, the work on tick resistance to pyrethroids and organophosphates revealed the presence of this mutation in Australia and in the North America (Domingues et al., 2012; Guerrero et al., 2012; Jonsson et al., 2010). Our work confirms the hypothesis that the population of *R. microplus* present in Benin is a Brazilian strain though there is need to investigate this further. Our PCR results showed the presence of C190A mutation of the domain II of the sodium channel gene on both districts. Homozygous resistant (RR) and heterozygous genotypes (RS) were detected in Samiondji and Betecoucou. In the analysis of dead tick larvae after LPT, 80% of the larvae of *R. microplus*

whose genotype was heterozygous (RS) against 20% homozygous susceptible genotype (SS). Analysis of live larvae after the acaricide test revealed 33% homozygous resistant (RR), 10% homozygous susceptible (SS) and 57% heterozygous genotypes (RS). Resistance to alphacypermethrin and deltamethrin was linked in part to the mutation C190A of the sodium channel gene, which was found in individuals living after the LPT, thus a correlation exists between the phenotypic and genotypic resistance of ticks. These results are consistent with the finding of Stone et al. (2014), who reported that the mutation of domain II (C190A) of the sodium channel gene was associated with the resistance of pyrethroids. The results showed a correlation between the survival of the larvae after the acaricide test and the kdr genotype of ticks (Rosario-Cruz et al., 2009). Studies reported that this mutation correlates with the resistance of arthropods to the synthetic products used for its control (Sangba et al., 2017). The presence of an individual without mutation among living ticks was evidence that other resistance mechanisms were used to escape pyrethrinoids compounds. Enzymatic resistances were found which were manifested by sequestration of the active ingredients of acaricides, or reduced the penetration of acaricide into the organism of ticks (Guerrero et al., 2012). Among these metabolic enzymes were esterases, cytochrome P450 monooxygenases and Glutathione-S-transferases. The biochemical tests carried out on the larvae of *R. microplus* and showed esterase levels of 0.23 ± 0.01 and 0.20 ± 0.00 respectively in Samiondji and Betecoucou against 0.45 ± 0.03 on the sensitive strain $p < 0.001$. Expression of the esterases was noted in the individual ticks of the two districts. The expression of the cytochrome P450 monooxygenases in the sensitive strain was 0.08 ± 0.00 against 0.71 ± 0.03 and 0.57 ± 0.02 $p < 0.001$ respectively in Samiondji and Betecoucou, thus a high activity of the cytochrome P450 monooxygenases was noted on the field strains. As for Glutathione-*S*-Transferases, they were in the order of 0.77 ± 0.04 and 0.57 ± 0.05 $p < 0.001$ in both localities respectively. These results are consistent with Kumar et al. (2013) finding, which showed that the increase in esterase activity in *R. microplus* played a role of detoxification of pyrethroids. The involvement of esterases in resistance to pyrethroids were previously demonstrated in the mosquito *Anopheles gambiae* in Bangui, Central African Republic (Sangba et al., 2017). The increase in esterase activity in *R. microplus* was established as a mechanism of resistance to pyrethroids due to the increased detoxification of acaricides (Aïkpon et al., 2014; Brito et al., 2017; Kumar et al., 2013). Our results are similar to that performed by Singh et al. (2014) who demonstrated the involvement of esterase in the resistance of ticks *R. microplus* to pyrethroids. The high rate of monooxygenase to cytochrome P450 in this study dovetails with the work of (Ghosh et al., 2015). A high activity of cytochrome P450 monooxygenase in all *R. microplus* populations was observed on both districts. Our work is similar to that performed in Iran on *R. annulatus* which revealed, that overexpression of cytochrome P450 monooxygenases induced resistance to pyrethroids (Ziapour et al., 2017). Glutathione S-Transferases (GST) is multifunctional dimeric enzymes that played a role in the detoxification of a wide range of pyrethrinoids compounds (Nandi et al., 2015). The overexpression of glutathione S-transferases (GST) has been implicated in the resistance of *R. microplus* (Ziapour et al., 2017). The work carried out by Enayati et al. (2010) showed that the overexpression of esterases, monooxygenases to cytochrome P450 and glutathione S-transferases (GST) was related to the resistance of ticks to pyrethroids. In this study, the esterase, monooxygenases to cytochrome P450, and glutathione S-transferase (GST) values showed a positive correlation with tick resistance *R. microplus* with alphacypermethrin and deltamethrin. Biochemical tests indicated that enzymes played an important role in the resistance of ticks to pyrethroids, particularly alphacypermethrin and deltamethrin. A correlation showed between the phenotypic, genotypic and enzymatic resistance of *R. microplus* to pyrethroids in Benin, which corroborate with the studies carried out by Kumar et al. (2013).

In conclusion, in Benin, the *R. microplus* populations have developed resistance to deltamethrin and alpha-cypermethrin. This resistance has possibly been mediated by over-expression of esterase, oxidase, GST and mutation in domain II S4-5 linker region of para-sodium channel gene. The results of the study could guide the policy makers to think about designing new control strategy method taking into account the mechanism of molecular resistance of these ticks to the commonly used acaricide.

## Conflict of interest

The authors declare that there is no conflict of interest in this study.

## Acknowledgements

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