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Contribution of Agricultural Practices on Enzymatic Activity in Anopheles Gambiae Populations from Cotton Areas in Northern Benin

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Abstract— **To have an idea on the contribution of agricultural practices on biochemical characterization of insecticide resistance in Anopheles gambiae populations, Anopheles larvae were collected from cotton field from July to September 2017 and reared until F1 generation.**

Enzymatic mechanisms (Glutathione-S-Transferase, esterase, monooxygenase P450 and total protein) were investigated from the F1 (the parental population after breeding) populations of An. gambiae s.l.

Results from this study showed a significant high level of GST and monooxygenase P450 activities from the wild populations of An. gambiae from Banikoara compared to susceptible Kisumu strain (P>0.05). However, there is no significant difference in the level activity of Esterase (α and β-Naphthyl) from the wild populations of An. gambiae from compared to the susceptible Kisumu strain (P>0,05).

These findings confirmed the contribution of agricultural practices on the selection of enzymatic activity in An. gambiae populations particularly on Glutathione-S-Transferase, and monooxygenase P450. However, the same level of the wild populations in esterase showed compared to the susceptible Kisumu strain showed that there is no clear link between the use of insecticide and enzymatic activity in An. gambiae populations.

Index Terms— **Anopheles gambiae; Enzyme; Cotton; Vegetable farming; Benin..**

I. Introduction

The scaling up of Long Lasting Insecticidal Nets (LLINs), the extent Indoor Residual Spraying (IRS) and treatment with artemisinin-based combination therapy (ACT) are currently the major international strategies to control malaria, particularly in sub-Saharan Africa [1]. Currently only one insecticide class, the pyrethroids, is commonly used to treat LLINs; pyrethroids have the required dual properties of low mammalian toxicity and rapid insecticidal activity [2], and their repellent or contact irritant effects may enhance the personal protection of LLINs. Unfortunately, resistance to pyrethroids is now widespread in African malaria vectors [3].

In fact, the development of pyrethroid resistance in the primary malaria vectors, *An. gambiae* s.l and *An. funestus* [4] is a serious concern. In the last decade, the emergence of resistance in populations of *An. gambiae* to common classes of insecticides used in public health has been reported in many African countries with a high allelic frequency of knock down resistance (Kdr) [5-8].

In Benin, with pyrethroid resistance spreading in *Anopheles gambiae*, the national malaria Control Programme in Benin has introduced a carbamate (bendiocarb) as an alternative to pyrethroids for the Indoor Residual Spraying (IRS) [9]. However, resistance to carbamate particularly to bendiocarb has been documented by Aikpon et *al* [8] three years later after it introducing in areas where IRS strategies were implemented against mosquitoes bites. In fact, a study conducted in Benin, revealed the use of bendiocarb in cotton fields against cotton pests [10].

Benin is still an important producer of cotton in West Africa where 90% of pesticide products are directed against cotton pests [11]. These insecticides are essentially composed of pyrethroids (PYs), organophosphates (OPs) and carbamates which are also the main classes used in public health. The majority of cotton farms observed in northern Benin are located in the upland landscape while the lowland covers the major mosquito breeding sites. According to Akogbeto *et al.* [12] in Benin, insecticide treatments against cotton pests are applied twice a month, for a timeframe of three consecutive months (between July and October) each year. These treatment periods coincide with the rainy season and correspond to the period of high mosquito densities.

While insecticide resistance associated with *knock down resistance (kdr) and Acetylcholinesterase (ace1R)* is well studied at the physiological, behavioural and population level [10; 13- 15] much less is known about the enzymes associated with metabolic resistance.

The present study aimed to provided information on biochemical analysis on *An. gambiae* populations from Cotton field to detect potential increase in mixed function oxidases (MFO), non-specific esterases (NSE) and glutathione S-transferases (GST) activity.

II. Material and Method

A. Study area

Cotton field at Banikoara located in the north-east of Benin (Figure 1) was chosen for investigation. Cotton production is the main activity of the populations in this district. Cotton crop protection represents 90% of the insecticide use in this district for crops protection [10]. The control strategies implemented against cotton pest especially *Helicoverpa armigera* required a regular repeated applications of insecticides during the cotton plant growing cycle. As recommended by the Institut National des Recherches Agronomiques du Benin (INRAB), six consecutive

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treatments are applied at two weeks interval to protect the crop against bollworms, leafworms and sucking pests. These insecticides are essentially composed of pyrethroids (PYs), organophosphates (OPs).

B. Mosquito collection

Larvae of *An. gambiae s.s.* were collected at the two sites (Figure 1) and reared at CREC (Centre de Recherche Entomologique de Cotonou) insectary for emergence. Emerging adult females mosquitoes (F_0) aged 6-8 days were fed with guinea pig blood and reared for emergence (F1) where adults 3-5 days were kept at -80 degrees for biochemical analysis.

C. Biochemical analysis

60 adult females of the wild populations of *An. gambiae s.s.* from the study site (Figure 1) were kept at -80 degrees and were subjected to biochemical based on the methods decribed by Penilla al [16] to compare the levels of activity of mixed function oxidases (MFO), non-specific esterases (NSE) using α-naphtyl acetate as a substrate and glutathione S-transferases (GST) to the laboratory Kisumu susceptible reference strain.

Individual mosquitoes were homogenized in 200 µl ml distilled water. Each of 10 ml of the homogenate was used for monooxygenase, glutathion S-transferase and protein assay. The other twenty µl ml of homogenate was used for esterases assay.

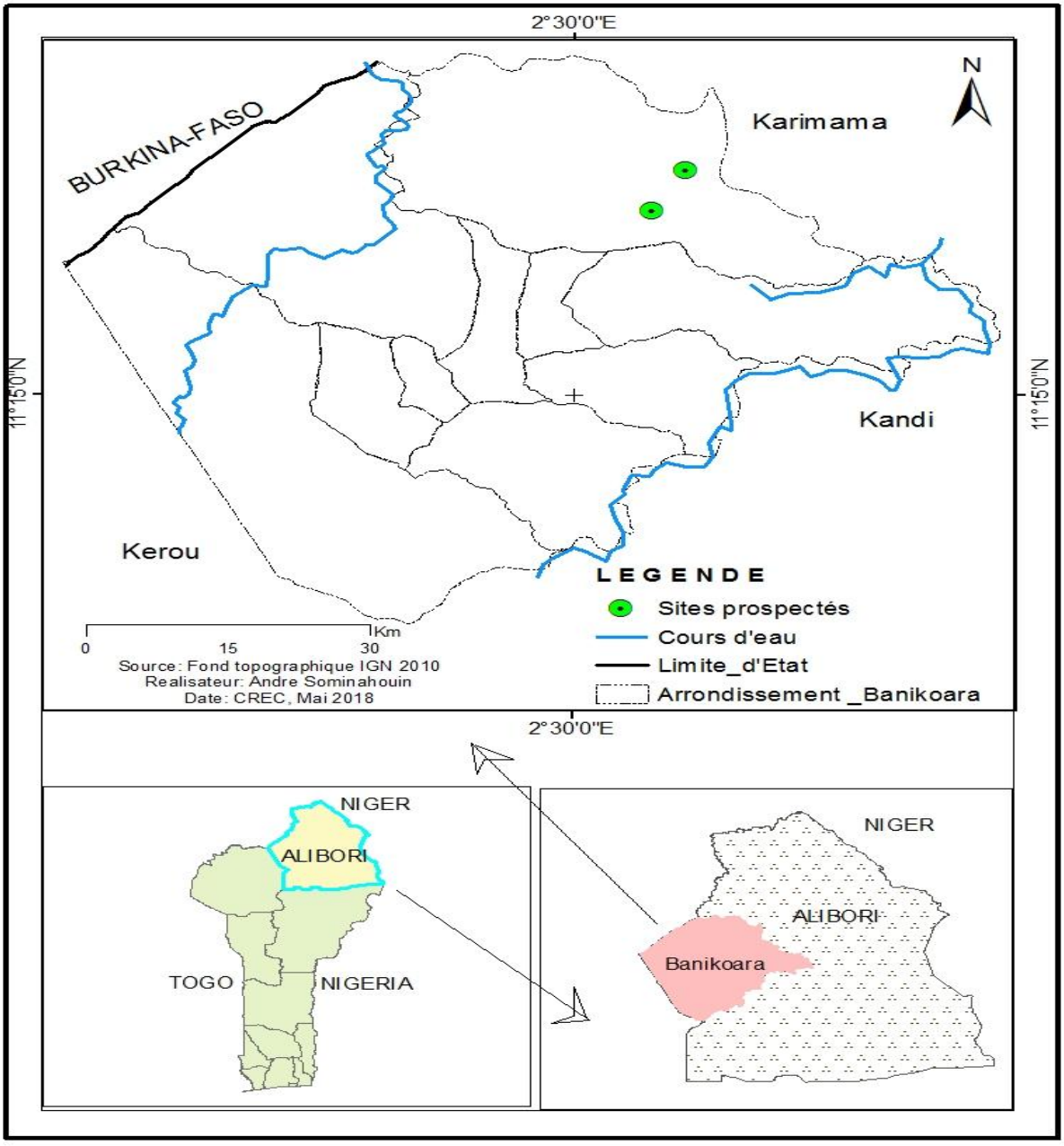


Figure 1: Map of Benin showing the study site

D. Glutathione -S-transferase (GST) assay

10 µl of each homogenate was transferred to a microplate well followed by 200 µl of the GSH/CDNB working solution which was prepared by adding 0.060g of glutathione solution(GSH) in 20 ml of Phospahte sodium buffer 0.1M and 0.013gr (in 1 ml of methanol) 1-chloro-2,4-dinitrobenzene (CDNB). The plates were read after 5 mins with the ELISA plate reader at a wave length of 340 nM. GST activity was expressed as: mMoles/ min / mg protein

E. Monooxygenase (Cytochrome p450) assay

10 µl of homogenate were placed in separate of microtitre plate followed by addition of 80 µl 0.625M potassium phosphate buffer (pH 7.2). Ten mg of 3,3,5'5', Tetramethyl Benzidine(TMBZ) in 5 ml methanol were prepared and a 15 ml of 0.25 M sodium acetate buffer(pH 5.0) was prepared. Two hundred µl of the above TMBZ solution was added in to each well followed by 25 µl of 3% hydrogen peroxide. The plate was read after 2 hours at 630 nm

F. Esterase assay

20 µl of homogenated were placed in separate wells of microtitre plate. 200 µl of 0.3 mM Alpha/Beta napthyl acetate were added to each well. Leave the plate at room temperature for 1 min and then added 50 µl of fast garnet. After 30 minutes, enzyme activity was determined as an OD value by microplate reader at 450 nm.

G. Protein assay

The total protein content of individual mosquitoes was determined using the Bio –Rad Protein Assay Kit (Bio -Rad Laboratories) in order to detect the differences in size among individuals that might require correction factors for the enzyme assays

H. Data analysis

Biochemical assay data (enzymatic activity per mg protein, levels of MFO, NSE and GST between Kisumu and field populations ***An. gambiae s.s.***) were compared using Mann-Whitney non-parametric ***U***-test (Statistica software).

III. Results

Results from biochemical assay showed a significantly higher level of GST activity of GST activity from the wild populations of *An. gambiae* from the study site compared to susceptible Kisumu strain (Mann-Whitney test, P>0.05) (Figure 2). The same trend was observed with Monooxygenase (Cytochrome p450). (Figure 3)

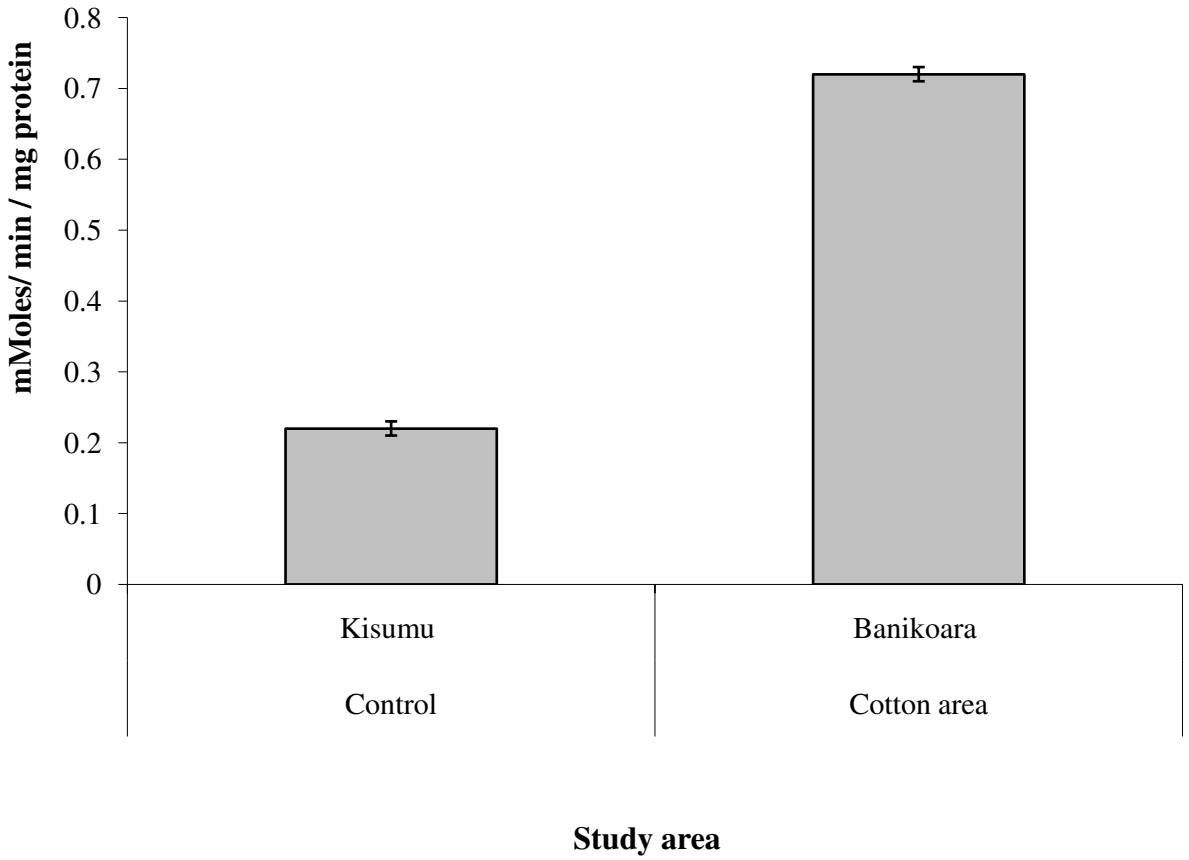


Figure 2 : Gluthation activity of *Anopheles gambiae* populations from the study areas

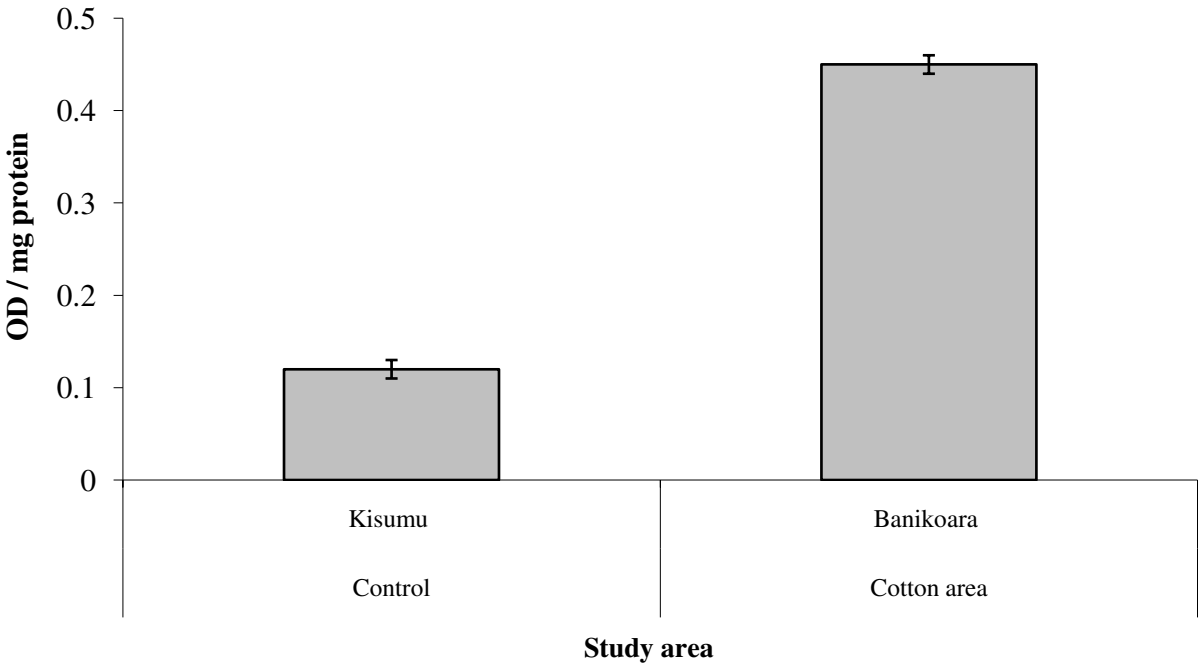
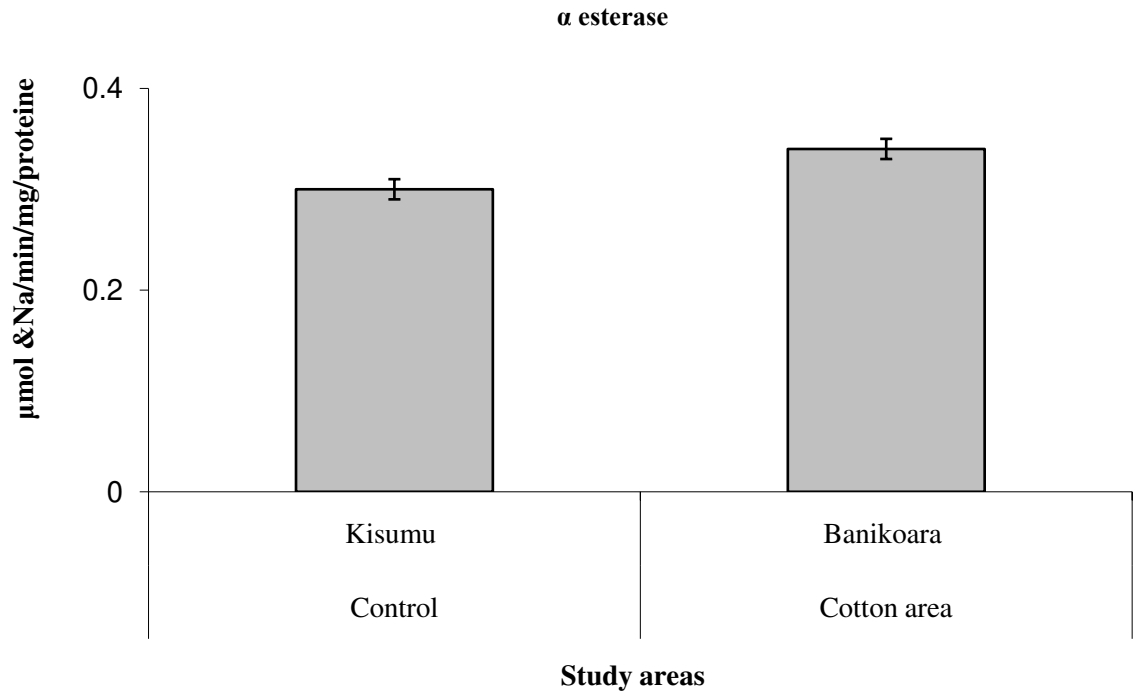


Figure 3: Mixed Function Oxidases activity of *Anopheles gambiae* populations from the study sites

However, there is no significant difference in the level activity of Esterase (α and β-Naphthyl) from the wild populations of *An. gambiae* from the study site compared to the susceptible Kisumu strain (P>0,05)



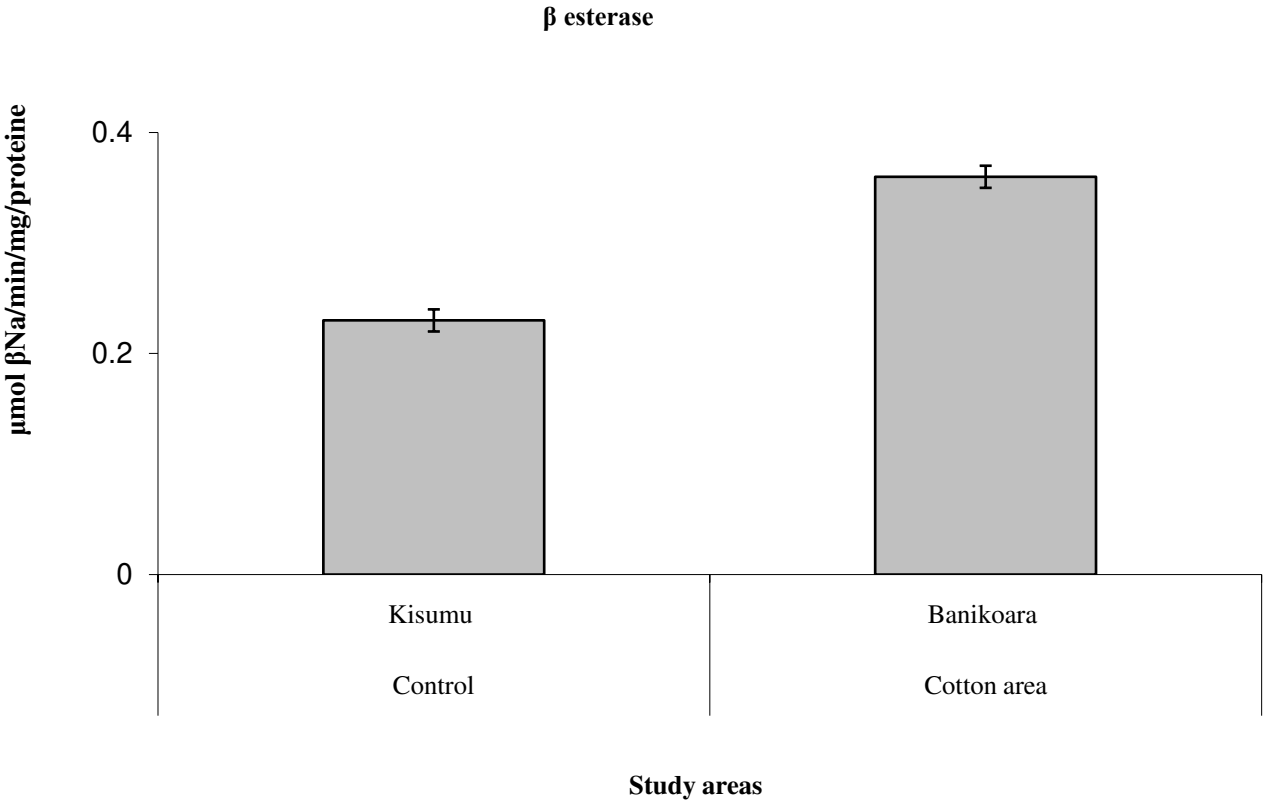


Figure 4: Esterase activity of *Anopheles gambiae* populations from the study site

IV. Discussion.

While insecticide resistance associated with *kdr* is well studied at the physiological, behavioural and population level, much less is known about the enzymes associated with metabolic resistance. One route of metabolic resistance is through up-regulation of detoxification enzymes.

Findings from the present study showed an increase level of GST and monooxygenase P450 activities in the wild populations of An. gambiae compared to the susceptible Kisumu strain. In fact, the high level of GST in the wild population of *An. gambiae* can be explained by the used of DDT during the house-spraying in several districts include the district of Banikoara during the WHO malaria eradication programme in the 1950s [17].

In addition, the high monooxygenase P450 activity in the wild populations of *An. gambiae* is one of the consequence of the high frequencies of the kdr gene mutation observed in An. gambiae population from this district found by Yadouleton et al [10].

Since these two metabolic genes found at high level in *An. gambiae* population at Banikoara compared to the susceptible Kisumu strain, it would be important to quantify these genes in the future using the qPcr technique.

Moreover, the overexpression of the two enzymes seemed to be clear that only kdr mutation can not explain the insecticide resistance found in *An. gambiae* populations at Banikoara, an area where farmers used different families of insecticides for pest control.

V. Conclusion

These findings confirmed the contribution of agricultural practices on the selection of enzymatic activity in *An. gambiae* populations particularly on Glutathione-S-Transferase, and monooxygenase P450. However, the same level of the wild populations in esterase showed compared to the susceptible Kisumu strain showed that there is no clear link between the use of insecticide and enzymatic activity in *An. gambiae* populations.

Competing Interests

The authors declare that they have no competing interests.

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