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RESEARCH ARTICLE

DOES THE INTENSIFICATION OF URBAN AND RURAL AGRICULTURE IN BENIN CONTRIBUTE TO THE EMERGENCE OF ENZYMATIC ACTIVITY IN ANOPHELES GAMBIAESENSULATO?

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Abstract

To explore the contribution of agricultural practices on enzymatic activity in Anopheles gambiae populations, collections of Anopheles gambiaelarve were done in cotton; vegetable farming (famers use for both,insecticides for pest control) and in subsistence farming (no insecticide) from the district of Banikoara, located in Benin. Enzymatic activities such as total protein, monooxygenase P450, Glutathione-S-Transferase and esterasewere investigated on F1 generation females aged 3-5 days. Results from this study showed a significant high level of Glutathione-S-Transferase and monooxygenase P450 activities from the wild populations of An. gambiae particularly in cotton and vegetable farming areas compared to the susceptible Kisumu strain (control) ($P<0.05$). However, there is no significant difference in the level of Esterase (α and β-Naphthyl) activity from the 3 wild populations compared to the control ($P>0.05$). These findings confirmed the contribution of agricultural practices on theemergence of enzymatic activity in Anopheles gambiaes.l particularly on Glutathione-S-Transferase, and monooxygenase P450. However, the same level of the wild populations activity in esterase compared to the control could be explained by the involving of many insecticide resistance mechanisms of An. gambiae associated to the level of enzymatic activity in An. gambiae populations in this locality.

Introduction:-

The United Nations Population Fund (UNFPA) estimated that Africa's population is the fastest growing in the world [1]. It is expected to increase by roughly 50% over the next 18 years, growing from 1.2 billion people today to over 1.8 billion in 2035 [1]. Population growth has resulted in increased demand for food supply. A solution to the problem has been the development of agriculture. Therefore, in order to meet food security and poverty alleviation of growing population in sub-Sahara Africa, peri-urban and urban agriculture is rapidly becoming a major economic

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activity in a number of towns. In general, non-used spaces (marshland, road edges, beaches, etc.) are turned into gardens where vegetable and different kinds of flowers are cultivated. Certain towns like Bouaké in Ivory Coast have hectares of rice cultivations. In Cotonou, Benin, peri-urban agriculture consists of belts of market gardens everywhere in and around the city, produce local and exotic vegetables (lettuce, green beans, carrots, cabbage, cucumber, beetroot, etc.) all year round, and adopt frequent manual watering of crops. Moreover, cotton production in many African countries particularly in Benin became a new activity for many people from the centre to the North of the country in order to fight against poverty. Benin government reported in 2019 that cotton production and vegetable farming activities are believed to be directly responsible for the creation of almost 60,000 jobs, thereby addressing urban demand for food and unemployment[2]. The advantages of theses agricultures are considerable. They contribute to the improvement of living conditions by supplying food, income and employment to the populations. The economic and social impact of urban and peri-urban agriculture is, however, limited by a number of factors including pest and disease problems, excessive and improper use of agrochemicals such as hazardous pesticides which have negative effects on consumer's health and environmental quality.

Chabiet al[3]reported that vegetable and cotton pest control by producers frequently consists in intensive foliar sprays of broad-spectrum chemical pesticides. These insecticides are essentially composed of pyrethroids (PYs), organophosphates (OPs) which are also the main classes used in public health[4].Resistance to this insecticide class is now widespread in the main malaria vectors Anopheles gambiaes.l, Anopheles arabiensis and Anopheles funestus[5–7]. Both enhanced detoxification (8-9)and mutations in the gene encoding the voltage-gated sodium channel (9)have been shown to be important resistance mechanisms. The Leucine to Phenylalanine substitution at position 1014 (L1014F) was found predominant in West and central Africa (10) whereas the Leucine to Serine substitution (L1014S), originated from Kenya [11], has now spread in Benin[12], Togo [13] and Nigeria [14].

While insecticide resistance associated with knock downresistance (kdr) and Acetylcholinesterase (ace1R) is wellstudied at the physiological, behavioural and population level much less is known about the enzymes associated with metabolic resistance.

The present study aimed to provided information onhow the intensification of urban and rural agriculture in Benin could be a cause of the emergence of enzymatic activityin Anopheles gambiae populations from Cotton and vegetable farming areas to detect potential increase in mixed function oxidases (MFO), non-specific esterases (NSE) and glutathione S-transferases (GST) activity**.**

Material And Method:-

Study areas

To have an idea about the contribution of agriculture on the emergence of enzymatic activity in Anopheles gambiaes.l., 3 different collection points of agriculture from the district of Banikoara(Fig.1) were chosen. In fact, the choice of this district is justified by the fact that this district is the largest producer of cotton and vegetable in the country. The 3 collection points are:

1. (A) Cotton field at Banikoara (Arbonga) located in the north-east of Benin (2°59 E, 11°31 N), where six treatments of insecticides (pyrethroids (PYs) and organophosphates (OPs) are applied at two weeks interval againstagainst bollworms, leafworms and sucking pests.
2. (B) Vegetable farming field (Weterou; 2°29 E, 09°21 N) where less insecticides are used for pest control
3. (C) subsistence farming field (Ginguiri;2°18 E, 07°12 N) where farmers don't use pesticides for pest control

This district is characterized by a Sudanian climate with one rainy season (middle of May to October) and one dry season (November-May) with annual mean rainfall about 1,300 mm

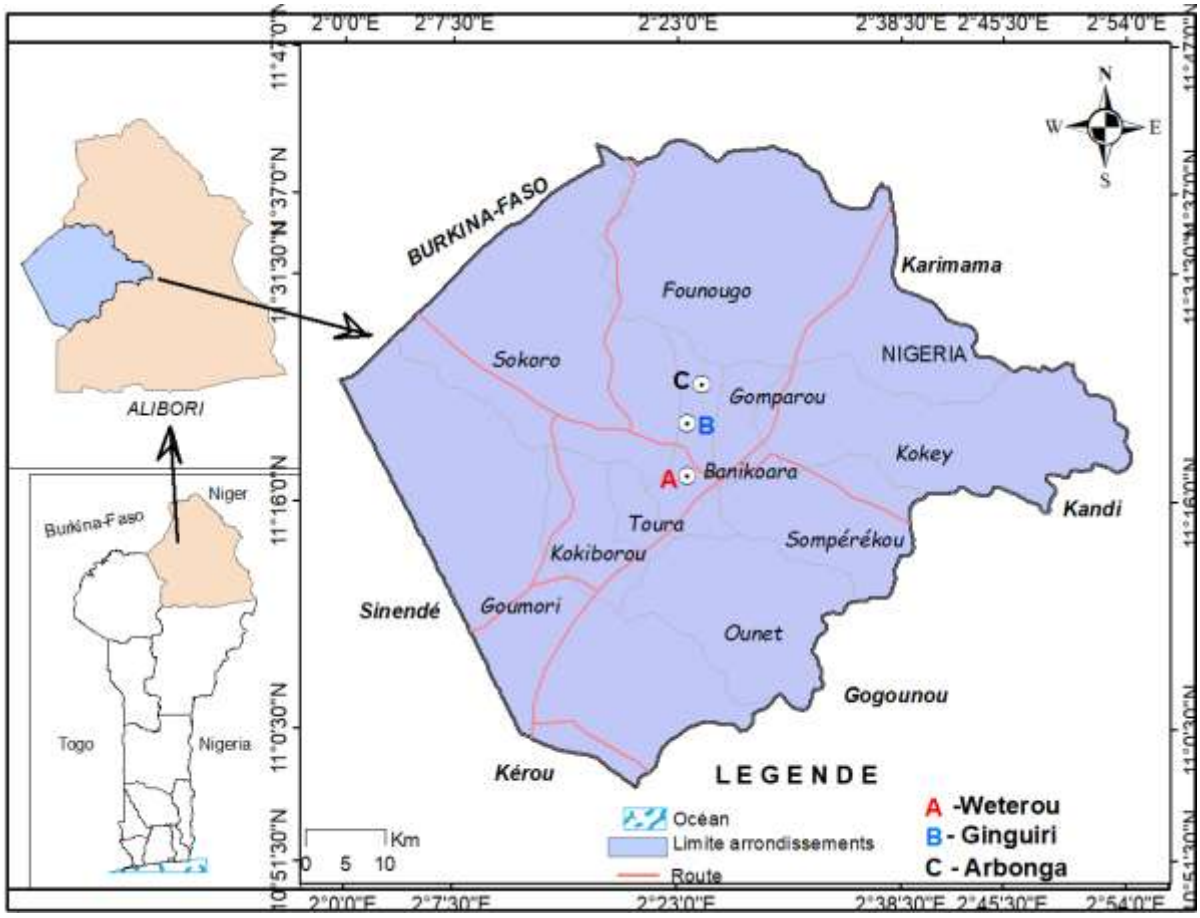


Fig 1:- Map of the study site.

Mosquito collection

Larvae of An. gambiaes.l. were collected at the three sites (Fig.1) and reared at CREC (Centre de RechercheEntomologique de Cotonou) insectary for emergence. Emerging adult females mosquitoes (F0) aged 6-8 days were reared for emergence (F1) where adults 3-5 days were kept at -80 degrees for biochemical analysis.

Biochemical analysis

100 adult females of the wild populations of An. gambiaes.l. from the 3 points (A;B andC) of the study site (Fig. 1) kept at -80 degrees were subjected to biochemical based on the methods decribed by Penillaal[15] 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

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.

Monooxygenase (Cytochrome p450)

Cytochrome P450 activity was determined using the heme-peroxidase assay according to the protocol described by Davidetal[16]. Following the protocol described by Penilla etal [15] , this assay detects the elevation in the amount of heme, which is then converted into equivalent units of cytochrome P450. In addition to the protocol described by

Davidetal [16], Eighty ml of 0.625 M potassium phosphate buffer (pH = 7.2) were added to 20 ml of mosquito homogenate together with 200 ml TetramethylBenzidine solution (0.011 g of 3,3',5,5' TetramethylBenzidine in 5 ml of 70% methanol +15 ml sodium acetate buffer 0.25 M pH = 5.0); 25 ml of 3% hydrogen peroxide were then added and the mixture was incubated for 30 min at room temperature base on the protocol described byNamountougou etal [17] . The absorbance was read at 630 nm and values calculated from a standard curve of cytochrome C following the protocol described by David et al [16].

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 **optical density** value by microplate reader at 450 nm.

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

Data analysis

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

Results:-

Results from this study showed a significant high level of monooxygenase P450 (Fig. 2) and Glutathione-S-Transferase (Fig. 3) activities from the wild populations of An. gambiae from Banikoara particularly in cotton and vegetable farming areas compared to the susceptible Kisumu strain ($P<0.05$). However, there is no significant difference in the level of Esterase activity (α and β-Naphthyl) (Fig. 4) from the wild populations of An. gambiae from the 3 collection points of the study site compared to the susceptible Kisumu strain ($P>0.05$).

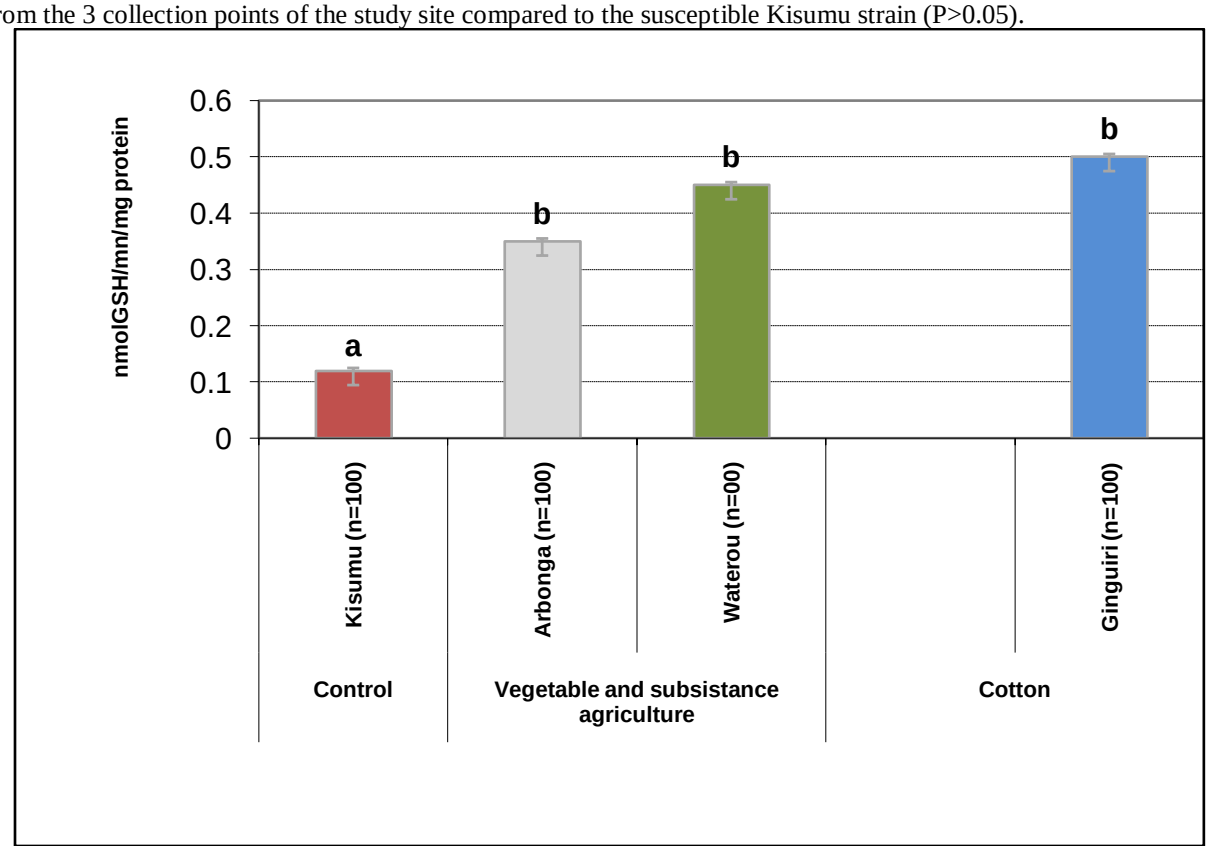


Fig 2:- Mixed Function Oxidases activity of Anopheles gambiae populations from the study sites compared to the control.

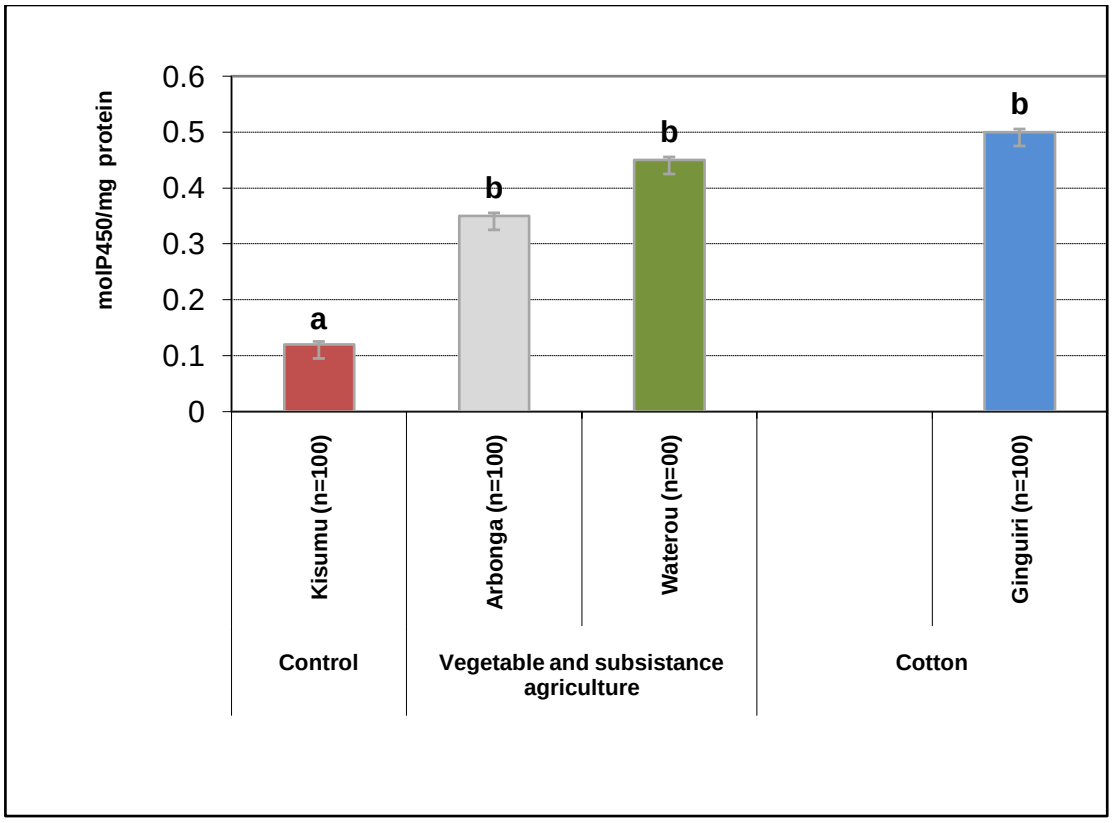
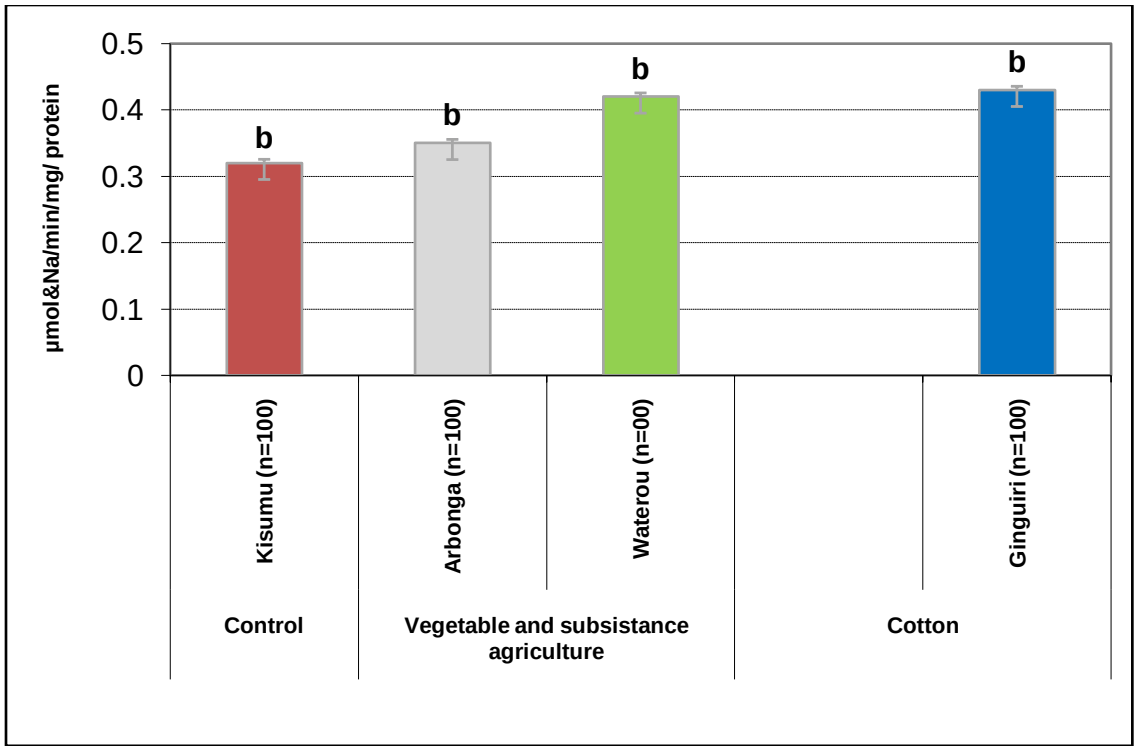


Fig 3:- Gluthation activity of Anopheles gambiae populations from the study areas compared to the control

α esterase.



β esterase

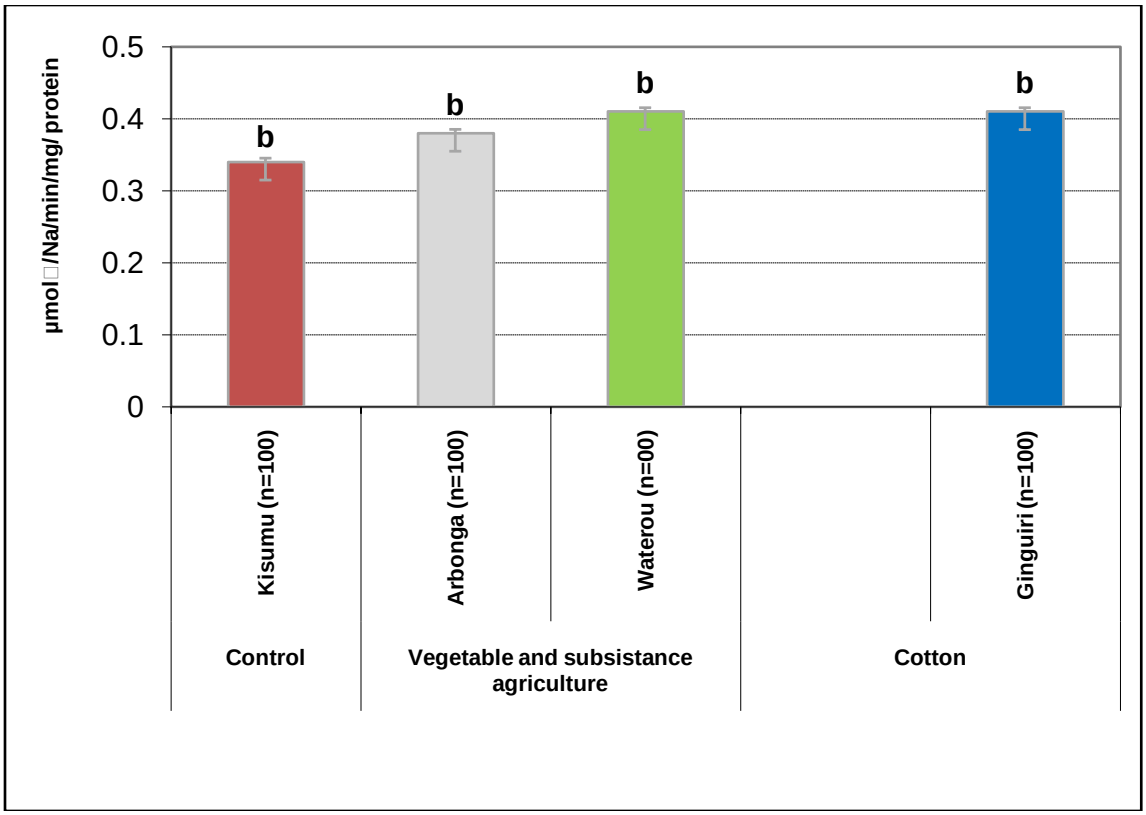


Fig 4:- Esterase activity of Anopheles gambiae populations from the study site compared to the control.

Discussion:-

While insecticide resistance associated with knock down resistance (describe cases of resistance to diphenylethane (e.g. DDT) and pyrethroid insecticides in insects) (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 (Fig. 2-3). In fact, the high level of GST in the wild population of An. gambiae from cotton and vegetable farming areas 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 [18**].** In addition, the high monooxygenase P450 activity in the same wild populations ofAn. gambiaefrom cotton and vegetable farming areas is one of the consequence of the high frequencies of the kdr gene mutation observed in An. gambiae population from this district reported by Assogba et al. [19]. Moreover, Yadouleton et al [20] showed that agricultural practices in cotton and vegetable farming areas seem to have contributed to the emergence of insecticide resistance in Anopheles populations. The authors of this study demonstrated that improper use of insecticides to control vegetable pests in urban areas directly exerted a huge selection pressure on mosquito larval populations in Benin.The high level of GST and P450 activities in the wild populations of An. gambiaes.l.from this localityseemed to be related to insecticide pressure from agricultural practices and vector control programs.

Since these two metabolic genes were found at high level in An. gambiaepopulation at Banikoara compared to the susceptible Kisumu strain and in areas where famers used pesticides for crops control, it would be important to quantify these genes in the future. Moreover, the overexpression of the two enzymes associated to the low activity of esterase seemed to be clear that many insecticide resistance mechanisms of An. gambiaewere associated to the level of enzymatic activity in An. gambiae populations in this locality.

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Competing interests
The authors declare that they have no competing interests.

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