RESEARCH ARTICLE

**Short-term metabolic resistance inductive effect of different agrochemical groups on *Anopheles gambiae* mosquitoes** [version 2; peer review: 1 approved, 1 approved with reservations]

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**Abstract**

**Background:** In order to assess the impact of the different groups of agricultural pesticides used in Côte d'Ivoire on the increase of mosquitoes resistance to insecticides, the expression profiles of 7 P450 cytochromes and one GSTE2 of *Anopheles gambiae* involved in mosquito resistance to insecticides were studied. The goal of this study was to determine the effect of short exposure of mosquito larvae to different groups of agricultural pesticides on mosquito resistance.

**Methods:** Three groups of pesticides were selected: (i) agricultural insecticide solutions, (ii) non-insecticide pesticide solutions (a mixture of herbicides and fungicides), and (iii) a mixture of the first two. A fourth non-pesticide solution was used as a control. Four groups of each stage II larvae (strain Kisumu, male and female) were exposed to 20% concentrated solution for 24 hours. Susceptibility tests for dichlorodiphenyltrichloroethane (DDT) and Deltamethrin were carried out on adults aged 2-5 days. Quantitative reverse transcription polymerase chain reaction (RT-qPCR) was performed to quantify the expression of eight metabolic genes involved in mosquito resistance to insecticides.

**Results:** Susceptibility to DDT showed a similar increase in the time required to knock down 50% of mosquitoes (kdt₅₀) in 1 colonies exposed to insecticides and non-insecticides compared to the control colony. As for deltamethrin, kdt₅₀ was higher in the colonies exposed to insecticides and the pesticide mixture compared to the colony.
exposed to non-insecticides. Of all the genes studied in all colonies, except for CYP6P1 induced only in the colony consisting of the pesticide mixture, no genes were induced.

**Conclusions:** This study confirmed that induction is influenced by the duration, the concentration of the solution and the type of xenobiotic used as an inducer. The overexpression of CYP6P1 confirmed the inductive effect that a short exposure of mosquito larvae to agricultural pesticides could have.

**Keywords**
Induction gene, Anopheles gambiae, cytochromes 450 and GSTE2, agricultural pesticides, Côte d'Ivoire
Introduction
Vector control through the use of insecticides is one of the strategies put in place by the World Health Organisation (WHO) to fight malaria transmission. Despite the huge decrease in malaria cases and deaths between 2000 and 2015 following the implementation of this strategy (World Health Organization, 2015). Malaria in sub-Saharan Africa and is often linked to human activities and climatic conditions that contribute to the proliferation of larval deposits. Rice and vegetable fields create the most favorable biotopes for the proliferation of various malaria vector species in different major geographic regions (Dossou-Yovo et al., 1998). The profound and permanent eco-system changes for irrigated rice fields establishment of favor the proliferation of Anopheles and impact malaria transmission (Koudou et al., 2007; Robert et al., 1991). One major consideration with two implications will further influence malaria transmission. Indeed, the food security which is one of the current priorities that the Food and Agriculture Organization of the United Nations (FAO) faces with the challenge of climate change will incite agricultural overproduction to meet the expectations of the world’s future population. This overproduction suggests an expansion of rice growing areas, which as mentioned above, remain niches for the proliferation of malaria vectors, and an intensification of use of agricultural pesticides (Osta et al., 2012). Intensification of use of agricultural pesticides can contribute to the development of insecticide resistance in the mosquitoes breeding in cultivation areas as molecules used are the same as those used for vector control. Field and laboratory studies have shown the involvement of pesticides used in agriculture in the development of mutational and metabolic resistance in mosquitoes (Fodjo et al., 2018; Guy Bertrand, 2019; Nkya et al., 2014). In Côte d’Ivoire, data on metabolic resistance in mosquitoes is scarce. Resistance studies are most heavily based on the kdr and ace1 mutation genes and the use of synergists (Camara et al., 2018; Zoh et al., 2018) whereas an increase in the kdr gene frequency does not necessarily lead to the development of resistance (Sadia-Kacou et al., 2017). Use of synergists has been described by Chouaibou et al. (2014) as a simple and initial method to detect metabolic resistance in field population of mosquitoes. This method has been used to investigate the involvement of metabolic resistance in the Tiassale mosquito population from Côte d’Ivoire (Chouaibou et al., 2014); however, synergists do not specify the gene responsible for resistance but rather give the families of genes involved (Moores et al., 2009). Data on the metabolic resistance is a prerequisite for the effective implementation of control interventions (Adolfi et al., 2019). Furthermore, identifying the source of metabolic resistance is of the utmost importance to mitigate development and spread of this resistance. Côte d’Ivoire is one of the 10 African countries that import the most agricultural pesticides (Guy Bertrand, 2019). Recent studies have categorized their use according to crop type, the country, and their roles in increasing insecticide resistance in malaria vectors (Fodjo et al., 2018). However, the studies carried out by these researchers do not described the insecticide resistance selective effect of pesticide use in Côte d’Ivoire. In the current study, we investigate the short term metabolic resistance inductive effect of non-insecticide- and insecticide-pesticides used in agriculture in Côte d’Ivoire. Seven P450 cytochromes and one Glutathion-S-Transferase known to be associated with mosquito resistance to insecticides were evaluated and their expression was characterized on An. gambiae Kisumu mosquito colonies at the larval stage. The study aimed to provide a better understanding of resistance and resistance source and provide data useful for planning and implementing a suitable vector control strategy.

Methods
Choice of agricultural pesticides and mosquito induction
The parental strain of mosquito used in this study was Anopheles gambiae originating from Kisumu, which is susceptible to insecticides used in public health. The 2–6 day old parental strain was fed with blood. The larvae from this laying were divided into four colonies of which three were exposed and one non exposed to serve as negative control. Among the exposed groups, one was exposed to insecticides, one to non-insecticides and the last one to a mixture of insecticides+non-insecticides. The choice of these pesticides was based on surveys of farmers in Côte d’Ivoire to identify the pesticides commonly used by farmers (Chouaibou et al., 2016). From this survey, two types of agrochemicals are used: agricultural insecticides (pyrethroids, organophosphates and carbamates) and non-insecticide products (herbicides and fungicides) (Table 1). Mosquitoes were reared (in september 2020) in Centre Suisse de Recherches Scientifiques laboratory in 27 ± 2°C, 80 ± 10 % relative humidity and photoperiod 12h/12 h light/dark.

Preparation of stock solution
A starting solution called stock solution was prepared according to the environmental doses of each chemical group recommended by the manufacturers and then combined together by group (e.g. insecticides group, non-insecticides group, and mixture group of insecticides+non-insecticides). For each group of pesticides, the environmental doses according to the manufacture is presented in Table 1.

Determination of lethal dose of 20% of population (ld20)
The determination of ld20 for each colony was done from the mother solution made of a combination of individual chemicals at environmental dose from each group of pesticides. For each colony, 100 Kisumu mosquito larvae were separated into 4 batches of 25 stage II larvae in plastic cups each containing 100 ml of water and cat food (Friskies®). This sample size was chosen because it is the one used in the susceptibility tests on adults. Several doses were used to expose the larvae. These were 0.01 µl, 0.02 µl, 0.05 µl, 0.07 µl for insecticides, 100 µl,
250 µl, 500 µl, 750 µl and 1000 µl for non-insecticides and 1 µl, 3 µl, 5 µl and 7 µl for the mixture group. Each exposure per dose was done in four replicates and the exposure time of the larvae was 24 hours. The \( \text{dl}_{20} \) were obtained by counting live larvae, and the Polo Plus 1.0 (LeOra Software, 2002–2014) was used to estimate the exact concentration to be used later. In this study, \( \text{ld}_{20} \) was chosen in order to minimise the effects of the artificial selection that could occur if the amount of xenobiotic used is high. The AAT Bioquest LD50 Calculator is a free to use alternative software.

**Larvae exposure**
After determining the different \( \text{ld}_{20} \), approximately 1000 stage 2nd instar larvae were exposed per colony for determination of the inductive effect. The inductive effect was evaluated by exposing 2nd instar larvae for 24 hours to a lethal dose of 20% (\( \text{ld}_{20} \)) of each pesticide group. After the 24 hours of exposure, the larvae were rinsed and then transferred to clean tap water. Then, they were fed with ground cat food and left to pupae. The pupae were transferred to the cages dedicated to each colony for breeding. The same selection process was carried out in parallel for all strains except the control colony.

**Bioassays**
Susceptibility tests in tubes were carried out with deltamethrin (0.05%) and dichlorodiphenyltrichloroethane (DDT) (4%) in order to evaluate the level of phenotypic resistance of each selected colony. The protocol was conducted according to standard WHO bioassay protocol (World Health Organization, 2016). Females mosquitoes of each colony were exposed to impregnated paper for one hour. Controls included batches of mosquitoes from each site exposed to untreated papers. During the exposure time, the number knocked-down was recorded at different time intervals 5, 10, 15, 20, 30, 40, 50, and 60 minutes in order to calculate the time required to knock-down 20% and 50% of the mosquitoes (\( \text{kdt}_{20} \) and \( \text{kdt}_{50} \)) and after 60 min exposure mosquitoes were transferred into holding tubes and provided with cotton wools soaked with a 10% sucrose solution. Mortalities were recorded 24 hours post-exposure.

**RNA extraction**
The total RNA of the adult mosquitoes was extracted from four replicates per colony. Each replica contained 10 female control mosquitoes aged 2–5 days and non exposed to insecticides. The extraction kit was that of MagnaMedics with magnetic-bead (MagnaMedics GmbH, Aachen, Germany). It was carried out as follows: mosquitoes were crushed in 200 µl TE buffer. Then 150 µl lysis buffer was added to break up their cells. The mixture was vortexed for 30 seconds, incubated for ten minutes and centrifuged for 2 minutes at 16,000 (g) gravity. The supernatant was transferred to a new Eppendorf tube in which 20 µl magnetic beads and 440 µl binding buffer for the

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**Table 1. Groups, trading name, active substance, class, concentration of substance present in the different pesticides used in this study.**

| Groups               | Trading name                  | Active substance | Class          | Concentration of substance | Environmental dose   |
|----------------------|-------------------------------|------------------|----------------|-----------------------------|----------------------|
| **Insecticides**     | Furadan®                      | Carbofuran       | Carbamates     | 50 g / kg                   | 1 kg / 15 l of water |
|                      | K-optimal®                    | Acetamipride     | Neonicotinoids | 15 g / l                    | 40 ml / 15 l of water |
|                      |                                | Lambda-cyhalothrin | Pyrethroids     | 20 g / l                   |                      |
|                      | Legumax®                      | Deltamethrin     | Pyrethroids     | 12 g / l                    | 50 ml / 15 l of water |
|                      | Verso 480®                    | Chlorpyrifos ethyl | Organophosphates | 480 g / l              | 15 ml / 10 l of water |
| **Non-insecticides** | Banko Plus®                   | Chlorothalonil   | Organochlóres  | 550 g / l                   | 80 ml / 15 l of water |
|                      |                                | Carbendazine     | Carbamates     | 100 g / l                   |                      |
|                      | Garil®                        | Propanil         | Amides         | 360 g / l                   | 80 ml / 15 l of water |
|                      |                                | Trichlopyr      | Pyridines      | 72 g / l                    |                      |
|                      | Glyphader®                    | Glyphosate       | Amino-phosphonates | 360 g / l              | 80 ml / 15 l of water |
emissary effect were added. This new mixture was incubated for ten minutes. The supernatant was removed and 200 µl wash buffer 1 was added. Two hundred microliters of wash buffer 2 were added after vortexing the previous solution and removing the supernatant. The supernatant from the solution made with wash buffer 2 was removed after vortexing and 180 µL elution buffer was added to the new solution. This new solution was vortexed and incubated for 10 min at 50°C. The supernatant was collected in a new tube and stored for analysis.

Quantitative real-time PCR (qRT-PCR)
The Bio-Rad CFX 96™ device (Bio-Rad technologies, Hercules, California, USA) allowed to do the quantitative real-time reverse-transcription PCR (qRT-PCR). The qRT-PCR was used to quantify the expression of eight genes (CYP6P3, CYP6M2, CYP9K1, CYP6P4, CYP6Z1, GSTE2, CYP6P1 and CYP4G16) involved in mosquito resistance to insecticides using RPS7 for normalization in each test. A total of four triplex detoxification assays (Detox (A)-Detox (D)) were designed using the three fluorophores, FAM (green), HEX (yellow) and Atto647N (red) of the TaqMan probe. Details of primers and probes are in Table 2. With RPS7, CYP6P3 and CYP6M2 compiled Detox (A), CYP9K1 and CYP6P4 Detox (B), CYP6Z1 and GSTE2 Detox (C) and CYP6P1 and CYP4G16 Detox (D). Reactions were performed in a total reaction volume of 10 µL. The heat cycle parameters were: 50°C for 15 min, 95°C for 3 min, and 40 cycles of 95°C for 3 s and 60°C for 30 s. The

| Reagent | Oligo_Name | Sequence | Assay | Green: FAM, Yellow: HEX, Red: Atto647N | 10µM working stock needed for a total 10 µl reaction |
|---------|------------|----------|-------|----------------------------------------|-----------------------------------------------------|
| Primer  | RPS7_Fj    | CCACCATCGAACCAAAGTGTGA | S7-Detox (A-D) | 0.1                                   |
| Primer  | RPS7_R     | TGCTGCAAAACTCGCTATTC | S7-Detox (A-D) | 0.2                                   |
| Probe   | RPS7_P1    | CGGTGAGTACGGTGAATCCA  | S7-Detox (A-D) | 0.25                                  |
| Primer  | CYP6P3_Fj  | ACAATGATGATGAAAACCTCT | P3-Detox (A) | 0.4                                   |
| Primer  | CYP6P3_R   | GATACATTGTTGTGGCAGCC  | P3-Detox (A) | 0.5                                   |
| Probe   | CYP6P3_P1  | ACGCGATCCGCTTGGCACT  | P3-Detox (A) | 0.35                                  |
| Primer  | CYP6M2_F2  | CTGGCGTTGAAATCCAGAGGT | M2-Detox (A) | 0.6                                   |
| Primer  | CYP6M2_Rj  | GATACTTTGCGCAGTGATTTAAG | M2-Detox (A) | 0.4                                   |
| Probe   | CYP6M2_P   | AGAGAAATCCTGCAAAAGCAACAGGAGA | M2-Detox (A) | 0.25                                  |
| Primer  | CYP9K1_Fj  | CCAGACCGGTGAGTGATGATAC | K1-Detox (B) | 0.2                                   |
| Primer  | CYP9K1_R   | CGTGTGCTGCTTGCAGTCAC | K1-Detox (B) | 0.4                                   |
| Probe   | CYP9K1_P   | CAATCTTCTGATGGAATCCGCG | K1-Detox (B) | 0.3                                   |
| Primer  | CYP6P4_Fj  | CTTGGACAGGTATCAATTGAACCCAC | P4-Detox (B) | 0.4                                   |
| Primer  | CYP6P4_R   | GCACGGTCGTGTAATACGCATC | P4-Detox (B) | 0.5                                   |
| Probe   | CYP6P4_P   | CGATCGAGTACTTCTGCGCGCG | P4-Detox (B) | 0.3                                   |
| Primer  | CYP6Z1_Fj  | CCCGCAACTGATGTTCTGTGTTGAGAC | Z1-Detox (C) | 0.1                                   |
| Primer  | CYP6Z1_R   | TCCGTCGACGGTGTATTGA | Z1-Detox (C) | 0.6                                   |
| Probe   | CYP6Z1_P1  | TGGATGAGTGCATGTTGTTGGGA | Z1-Detox (C) | 0.25                                  |
| Primer  | GSTE2_Fj   | CCGGAAATT TGAGCTAACC | GSTE2-Detox (C) | 0.1                                   |
| Primer  | GSTE2_R    | GCTTACCGGGGTATTCTGCG | GSTE2-Detox (C) | 0.4                                   |
| Probe   | GSTE2_P    | CGTCTGACGGGACTGAGGCACG | GSTE2-Detox (C) | 0.3                                   |
| Primer  | CYP6P1_Fj  | ACAGGTTGTAAGCAGAACCAC | P1-Detox (D) | 0.1                                   |
| Primer  | CYP6P1_R   | GGTGTGAACTCTGTTCCGCAAGA | P1-Detox (D) | 0.5                                   |
| Probe   | CYP6P1_P   | CCGCTGAAACGACGCTGCG | P1-Detox (D) | 0.3                                   |
| Primer  | CYP4G16_Fj | GTCAAGAAGATTCGTCGGACAC | G16-Detox (D) | 0.2                                   |
| Primer  | CYP4G16_R  | TCTTCAAGGTGCTTGAGTGC | G16-Detox (D) | 0.2                                   |
| Probe   | CYP4G16_P1 | CTGCAGCGGCCGATCATTTTGAAGC | G16-Detox (D) | 0.3                                   |
samples were amplified in two technical replicates for analysis of gene expression for each population. Each series always included one control. The real-time PCR system was used to calculate Ct values for each reaction, which were then used to calculate fold changes using the Pfaffl method (Pfaffl et al., 2002).

Data analysis
The induction effect of the respective pesticides were assessed in term of knockdown effect in adult mosquitoes by comparing the time necessary for 20% and 50% of the population to be knocked-down (kdt<sub>20</sub> and kdt<sub>50</sub> respectively). The kdt<sub>20</sub> and kdt<sub>50</sub> were calculated using the PoloPlus software compared between exposed and non-exposed colony (control). The calculation of the 95% confidence intervals (CIs) and the significance was calculated with the Pfaffl method (Pfaffl et al., 2002). This method was implemented in REST 2009 v2.013. It allows the entry of Ct values for the unexposed colony (control) and exposed strains for each gene with confidence intervals (CI) and 95% P values. The normaliser Ct values for the RPS7 gene and each target gene for each sample are jointly re-assigned to the exposed and unexposed strains and fold changes are calculated based on mean values after 2000 interactions. For correlation analysis between expression levels, these were calculated as relative quantification (RQ) units (RQ = 2<sup>-ΔCt</sup>). The significance level was set at α = 0.05.

### Results

#### Lethal dose for 20%

The lethal dose necessary to induce 20% of mortality in the population were 0.05 µl /100 ml for insecticides, 900 µl /100 ml for non-insecticides and 5 µl /100 ml for the mixture (Sadia et al., 2021). These concentrations were used to assess the induction effect of the respective pesticide groups in adult mosquitoes. The mortality rate obtained 24 hours after exposure to deltamethrin and DDT was 100% in all colonies tested and control colony.

#### Knock down effect on adult mosquitoes

Exposure of mosquito larvae to a lethal dose of 20% resulted in a slight increase in the time required to knock down 20% and 50% of the adult mosquitoes in all colonies exposed to deltamethrin. In the colony exposed to the insecticides, the time required to knock down 20% (kdt<sub>20</sub>) and 50% (kdt<sub>50</sub>) of the mosquitoes at the adult stage was 13.147 and 18.187 minutes respectively. In the one exposed to non-insecticides, the kdt<sub>20</sub> was 10.561 min and the kdt<sub>50</sub> was 14.858 min. When exposed to deltamethrin in the mixture colony, the kdt<sub>20</sub> was 16.399 min and the kdt<sub>50</sub> was 18.501 min. In the control colony, the kdt<sub>20</sub> was 6.578 min and the kdt<sub>50</sub> was 10.463 min (Table 3).

Concerning the exposure to the adult stage with DDT, in the control colony the kdt<sub>20</sub> was 15.393 min and the kdt<sub>50</sub> 21.665 min. In the colony exposed to insecticides the kdt<sub>20</sub> was 16.803 min and the kdt<sub>50</sub> 23.348 min. In the colony exposed to non-insecticides, the kdt<sub>20</sub> and kdt<sub>50</sub> were 18.771 minutes and 23.902 minutes respectively. The kdt<sub>20</sub> was 16.925 and the kdt<sub>50</sub> was 22.534 min in the mixture colony (Table 4).

The kdt<sub>50</sub> was similar in the colonies exposed to the insecticides and in the one exposed to the mixture when exposed to deltamethrin. On the contrary, when exposed to DDT, it was significantly similar in insecticides and non-insecticides colonies compared to the control colony (Figure 1).

#### Expression of the different genes studied

RT-qPCR analyses assessed the expression of several different genes in all exposed colonies compared to the control colony (Table 5). The RNAs of each colony included 10 mosquitoes per pool and four replicates per colony. From the eight genes studied, only the CYP6P1 gene in the mixture insecticides+non-insecticides was significantly increased in expression (α = 0.05). The fold change of this gene was 3.144 suggesting that exposure of An. gambiae larvae to the pesticide mixture allowed induction of the CYP6P1 gene. Concerning the other genes, no significant increase was observed in the exposed colonies compared to the control colony. (α = 0.05)

#### Discussion

In Côte d’Ivoire, it has been found several times that agriculture contributes to the development of resistance in mosquitoes (Camara et al., 2018; Fodjo et al., 2018; Mouhamadou et al., 2019; Zoh et al., 2018). The aim of this study was to assess the expression of selected cytochromes involved in insecticide resistance in susceptible mosquito larvae when they are exposed to different groups of agricultural pesticides used in Côte d’Ivoire at larval stage.

The bioassay results showed a slight increase variation of kdt<sub>50</sub> to deltamethrin in colonies exposed to insecticides and

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### Table 3. Different times (minutes) needed to knock down 20% and 50% (kdt<sub>20</sub> and kdt<sub>50</sub>) of mosquitoes exposed to different groups of agricultural pesticides (insecticides, non-insecticides and a mixture of all compounds). P value > 0.05 in all colonies.

| Control | Insecticides | None-insecticides | Mixture |
|---------|--------------|-------------------|---------|
| Delta   | Delta        | Delta             | Delta   |
| kdt<sub>20</sub> | 6.578        | 13.147            | 10.561  | 16.399  |
| kdt<sub>50</sub> | 10.463       | 18.187            | 14.858  | 18.501  |

### Table 4. Different times (minutes) needed to knock down 20% and 50% (kdt<sub>20</sub> and kdt<sub>50</sub>) of mosquitoes exposed to different groups of agricultural pesticides (insecticides, non-insecticides and a mixture of all compounds). P value > 0.05 in all colonies. DDT=dichlorodiphenyltrichloroethane.

| Control | Insecticides | None-insecticides | Mixture |
|---------|--------------|-------------------|---------|
| DDT     | DDT          | DDT               | DDT     |
| kdt<sub>20</sub> | 15.393       | 16.803            | 18.771  | 16.925  |
| kdt<sub>50</sub> | 21.665       | 23.348            | 23.902  | 22.534  |
Figure 1. Number of mosquitoes knocked down as a function of time. The letters a, b and c represent the insecticides, non-insecticides and mixture colonies exposed to deltamethrin, respectively. The letters d, e and f represent the insecticides, non-insecticides and mixture colonies exposed to DDT (dichlorodiphenyltrichloroethane), respectively. The blue colour represents the control colony and the red colour represents the different colonies exposed to pesticides.

Table 5. Expression of genes in the different colonies exposed to the pesticides compared to the control colony. The number with the star is over-expressed with significant difference. CI=confidence interval.

|                 | Insecticides | None-insecticides | Mixture          |
|-----------------|--------------|-------------------|------------------|
|                 | Ratio        | P-value           | 95% CI           | Ratio          | P-value           | 95% CI           | Ratio        | P-value           | 95% CI           |
| CYP6P3          | 0.127        | 0.147             | 0.004-2.069      | 0.169          | 0.186             | 0.003-8.277      | 0.002        | 0.134             | 0.00-1.628       |
| CYP6M2          | 0.000        | 0.011             | 0.00-0.002       | 0.000          | 0.099             | 0.0-11621.9      | 0.000        | 0.003             | 0.00-0.002       |
| CYP9K1          | 0.173        | 0.615             | 0.00-10.435      | 14.296         | 0.736             | 0.008-1003007    | 0.116        | 0.478             | 0.00-6.311       |
| CYP6P4          | 1.350        | 0.745             | 0.082-10.426     | 1.699          | 0.575             | 0.164-693.519    | 0.420        | 0.595             | 0.005-9.153      |
| CYP6Z1          | 1.070        | 0.664             | 0.271-3.583      | 1.417          | 0.451             | 0.377-3.476      | 1.292        | 0.548             | 0.409-3.631      |
| GSTE2           | 0.314        | 0.000             | 0.099-0.746      | 0.906          | 0.853             | 0.269-2.457      | 0.258        | 0.059             | 0.067-1.170      |
| CYP6P1          | 0.966        | 0.855             | 0.528-2.342      | 0.859          | 0.481             | 0.469-1.589      | 3.144 *      | 0.030             | 0.865-13.813     |
| CYP4G16         | 0.654        | 0.218             | 0.316-1.714      | 0.745          | 0.351             | 0.397-1.817      | 0.354        | 0.053             | 0.100-1.069      |
mixture compared to unexposed colony. This could be explained by the short duration of exposure, which was one day, and also by the low dose of agricultural pesticides used, which was 20%. Indeed, Poupardin showed that pre-exposure of Aedes larvae to a sublethal dose of insecticide does not necessarily lead to increased tolerance to the insecticide. This may indicate that the dose or duration of exposure was not sufficient to obtain a significant toxicological effect (Poupardin et al., 2008). In the colony exposed to non-insecticides, a slight increase in kdt50 was observed when adults of this colony were exposed to deltamethrin despite the lack of pyrethrinoids in the solution. Given the susceptibility of the parental colony, this slight variation could be due to the presence of organochlorine in the solution. Indeed, the organochlorines present in the solution could induce an action of the genes responsible for the cross-resistance of mosquitoes to organochlorines as well as pyrethrinoids, resulting in a slight increase in kdt50 and thus a cross-resistance between pyrethrinoids and DDT, even though deltamethrin is not used in the solution. This has been observed in several African countries where DDT resistant mosquitoes could easily be resistant to DDT with deltamethrin as both insecticides have the same sites of action and also have certain genes in common that may allow insecticide degradation even though there are no mutational genes (Orjuela et al., 2019; Tchigossou et al., 2018; Wanjala et al., 2015). Also, the fact that the kdt50 of the colonies exposed with insecticides and with the mixture of pesticides is higher than the one in colony exposed with non-insecticides could suggest that the insecticides and mixture had a higher inductive effect than the non-insecticide colony. Indeed, the inductive effect may also be influenced by the nature and type of xenobiotic used as an inducer (Poupardin et al., 2010).

The real-time qPCR allowed the expression of several different genes to be evaluated in all exposed colonies compared to the control colony. Eight genes (CYP6P3, CYP6M2, CYP9K1, CYP6P4, CYP6Z1, GSTE2, CYP6P1 and CYP4G16) related to insecticide resistance were characterised in this study. GSTE2 confers resistance to dichlorodiphenyltrichloroethan (DDT) and carbamates, CYP3P6 confers resistance to pyrethroids and carbamates, CYP6M2 confers resistance to pyrethroids (Adolfi et al., 2019; Riveron et al., 2014). CYP4G16 catalyses the biosynthesis of epicuticular hydrocarbons leading to cuticular resistance (Balabanidou et al., 2016). CYP6Z1 metabolises benzoicar, pyrethroids and DDT resistance (Chiu et al., 2008; Ibrahim et al., 2016). CYP6P4 is associated with resistance to alpha-cyano and non-alpha-cyano pyrethroids (Ibrahim et al., 2016). CYP9K1 was found over-expressed in the deltamethrin-resistant Bioko population (Haruna et al., 2020; Vontas et al., 2018). CYP6P1 was over-expressed in mosquito populations in the Kou Valley resistant to DDT with permethrin, dieldrin and deltamethrin (Kwiatkowska et al., 2013). Of the eight genes studied, only one was differentially over-expressed and one was under-expressed (Willoughby et al., 2006). The other genes did not differ significantly. CYP6M2 was under-expressed in almost all the selected colonies with the exception of the non-insecticide colony where this gene was not significant. While CYP6M2 is responsible for the resistance of mosquitoes to deltamethrin and DDT. This gene has been over-expressed in some cities in Côte d’Ivoire where agriculture is intensive and mosquitoes are resistant to several classes of insecticides used in public health (Edi et al., 2014; Oumbouke et al., 2020). This result could explain the low level of kdt50 observed in the different exposed colonies. The fact that this gene is under-expressed in these colonies could confirm the fact that the relationship between the capacity of an enzyme to metabolise an insecticide and its induction (induction in genetics is the set of cellular and molecular mechanisms leading to the triggering of the expression of a specific gene) by the insecticide is not always correlated (Willoughby et al., 2006). Indeed, despite the presence of pyrethrinoids in these two colonies, the CYP6M2 gene was under-expressed in these two colonies, whereas this gene was several times over-expressed in mosquito populations under pyrethroid pressure (Djouaka et al., 2008; Mitchell et al., 2012; Stica et al., 2019). This result may suggest that overexpression of this gene is a long process that takes place over several generations.

Our results also show that CYP6P1 was significantly induced in the mixture colony. Despite the involvement of this gene in the resistance of mosquitoes to DDT and pyrethroids, the tdk50 of this colony was not the highest of all selected colonies. This could imply that other genes not studied here may have been involved in increasing kdt50 in the other colonies. The high level of expression of CYP6P1 may indicate that this particular P450 plays a major role in the xenobiotic response during the adult stages in mosquitoes of this colony. The expression pattern observed at the CYP6P1 gene in the colony composed of the pesticide mixture could explain the kdt50 observed at the level of this colony when adults are exposed to deltamethrin. Also the overexpression of the CYP6P1 gene confirms the inductive effect that a short exposure of mosquito larvae to agricultural pesticides could have.

**Conclusion**

This study, which aimed to evaluate the expression level of certain cytochromes following the short term exposure of larvae to different groups of pesticides, confirmed that induction is influenced by the duration and concentration of exposure and the type of xenobiotic used as an inducer. With the exception of CYP6P1, which was induced in the strain composed of the pesticide mixture, all other genes studied were not induced suggesting that short-term exposure can still induce metabolic resistance. Other genes not studied in this study could contribute to induction. Therefore, it would be good to do a general mapping of all the genes involved in resistance by sequencing the exposed mosquitoes. Also, it would be ideal to see the effect of these agricultural pesticides on mosquitoes over several generations of selections.

**Data availability**

**Underlying data**

Zenodo: Short-term metabolic resistance inductive effect of different agrochemical groups on Anopheles gambiae mosquitoes. https://zenodo.org/record/4791753#.YK0W6thKtUk (Sadia et al., 2021).
This project contains the following underlying data:
- susceptibility test and qRT-PCR.xlsx (The different tabs present the susceptibility tests carried out with DDT and deltamethrin in control, insecticide, none-insecticide and mixture colonies respectively. Following the susceptibility test tabs is the metabolic gene tab with detoxes A, B, C and D.)

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

Acknowledgments
We thank Centre Suisse de Recherches Scientifiques, Côte d’Ivoire laboratory staff.

References

Adolfi A, Poulton B, Anthousi A, et al.: Functional Genetic Validation of Key Genes Conferring Insecticide Resistance in the Major African Malaria Vector, Anopheles Gambiae. Proc Natl Acad Sci U S A. 2019; 116(1): 25764-72.

Babahaniou V, Kompouraiki A, MacLean M, et al.: Cytochrome P450 Associated with Insecticide Resistance Catalyzes Cuticular Hydrocarbon Production in Anopheles Gambiae. Proc Natl Acad Sci U S A. 2016; 113(33): 9268-73.

Balaba et al.: Use and Misuse and Their Impact in the Environment. Neglected Public Health Threats for Workers and Population. PubMed Abstract

Chouaibou M, Fodjo BK, Tia E, et al.: Mapping Insecticide Resistance in Anopheles Gambiae s.l. in Côte d’Ivoire. Parasit Vectors. 2017; 11(1): 19.

Chiu TL, Wen Z, Rupasinge SG, et al.: Anopheles Gambiae s.s. Populations. BMC Genomics. 2009; 10(2): 6855-60.

Chouaibou MS, Fodjo BK, Toure B, et al.: Influence of the Agrochemicals Used for Rice and Vegetable Cultivation on Insecticide Resistance in Malaria Vectors in Southern Côte d’Ivoire. Malar J. 2016; 15(1): 426.

Djouakrou A, Cissokho B, Cissoko O, et al.: Anopheles Gambiae s.s. Populations. Acta Trop. Elsevier B.V.; 2014; 130(1): 108–11.

Djouakrou A, Cissokho B, Cissoko O, et al.: Anopheles Gambiae s.s. Populations. Acta Trop. Elsevier B.V.; 2014; 130(1): 108–11.

Djouakrou A, Cissokho B, Cissoko O, et al.: Anopheles Gambiae s.s. Populations. Acta Trop. Elsevier B.V.; 2014; 130(1): 108–11.

Djouakrou A, Cissokho B, Cissoko O, et al.: Anopheles Gambiae s.s. Populations. Acta Trop. Elsevier B.V.; 2014; 130(1): 108–11.

Djouakrou A, Cissokho B, Cissoko O, et al.: Anopheles Gambiae s.s. Populations. Acta Trop. Elsevier B.V.; 2014; 130(1): 108–11.

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Djouakrou A, Cissokho B, Cissoko O, et al.: Anopheles Gambiae s.s. Populations. Acta Trop. Elsevier B.V.; 2014; 130(1): 108–11.

Djouakrou A, Cissokho B, Cissoko O, et al.: Anopheles Gambiae s.s. Populations. Acta Trop. Elsevier B.V.; 2014; 130(1): 108–11.

Djouakrou A, Cissokho B, Cissoko O, et al.: Anopheles Gambiae s.s. Populations. Acta Trop. Elsevier B.V.; 2014; 130(1): 108–11.

Djouakrou A, Cissokho B, Cissoko O, et al.: Anopheles Gambiae s.s. Populations. Acta Trop. Elsevier B.V.; 2014; 130(1): 108–11.

Djouakrou A, Cissokho B, Cissoko O, et al.: Anopheles Gambiae s.s. Populations. Acta Trop. Elsevier B.V.; 2014; 130(1): 108–11.

Djouakrou A, Cissokho B, Cissoko O, et al.: Anopheles Gambiae s.s. Populations. Acta Trop. Elsevier B.V.; 2014; 130(1): 108–11.
DDT Resistance in an Anopheles Funestus Population from Benin. *Parasit Vectors*. 2018; *11*(1): 602.

Vontas J, Grigoraki L, Morgan J, et al.: Rapid Selection of a Pyrethroid Metabolic Enzyme CYP9K1 by Operational Malaria Control Activities. *Proc Natl Acad Sci U S A*. 2018; *115*(18): 4619–24.

Wanjala CL, Mbugi JP, Ototo E, et al.: Pyrethroid and DDT Resistance and Organophosphate Susceptibility among Anopheles Spp. Mosquitoes, Western Kenya. *Emerg Infect Dis*. 2015; *21*(12): 2778–81.

Willoughby L, Chung H, Lumb C, et al.: A Comparison of Drosophila Melanogaster Detoxification Gene Induction Responses for Six Insecticides, Caffeine and Phenobarbital. *Insect Biochem Mol Biol*. 2006; *36*(12): 934–42.

World Health Organization: Global Malaria Programme. Eliminating Malaria. Geneva: World Health Organization. 2015; 243.

Zoh DD, Ahoua Alou LP, Toure M, et al.: The current insecticide resistance status of *Anopheles gambiae* (s.l.) (Culicidae) in rural and urban areas of Bouaké, Côte d’Ivoire. *Parasit Vectors*. 2018; *11*(1): 118.
Open Peer Review

Current Peer Review Status: ✓ ?

Version 2

Reviewer Report 01 December 2021

https://doi.org/10.21956/wellcomeopenres.19153.r46730

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Rousseau Djouaka
International Institute of Tropical Agriculture, Cotonou, Benin

Minor comments:
Overall, I noticed that the comments were addressed in “Answer to reviewer’s comments” but not totally in the manuscript. So, we still have some concerns that need to be remediated before my full approval:
- ‘dl20’ should be changed to ‘DL20’ in the manuscript.
- Authors cited this paper in the manuscript, which should be corrected but this reference is still in the MS.
- The authors agreed to use ‘non-insecticide’ but the word ‘none-insecticide’ is still used in the MS.
- English editing: There’s still a lot of grammatical and typographical errors, words and punctuations misuse as well as several sentences that need to be recast/reviewed. I would recommend the authors conduct a thorough English language editing on the manuscript.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular Entomology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.
Rousseau Djouaka
International Institute of Tropical Agriculture, Cotonou, Benin

Comments:
In this manuscript titled: "Short-term metabolic resistance inductive effect of different agrochemical groups on Anopheles gambiae mosquitoes", Sadia et al. emphasized the contribution of agrochemicals to insecticide resistance selection in Anopheles gambiae. They also highlighted the fact that known resistance-associated mutations like the Kdr and Ace-1 are getting fixed in An. mosquito populations and may not be possible to discriminate insecticide resistance, and that metabolic resistance may be driving insecticide resistance selection in agricultural areas. The authors then assessed the expression level of some selected cytochrome P450 and glutathione S-transferase genes in mosquitoes exposed to agro-insecticides solutions as compared to other agro-pesticides combinations.

This paper is of high public health importance, especially, with the emphasis on the contribution of human activities in the recent cases of malaria resurgence in several sub-Sahara Africa countries. However, in my opinion, the article is not good enough in the present state and some comments must be addressed for clarity and robust communication.

Major comments:
- I was expecting to see the mortality levels at larvae and adult stages after the assessment and not just the knockdown effect in adult mosquitoes. What was the mortality like after 24, 48, 72 hrs of larvae exposure and until adult emergence? Could there have been a greater effect on larvae than the adult with the short exposure of just 24 hours? Could it be that the short insecticide exposure has a knockdown effect and no effect on mortality? Why was the adult mortality not recorded after 24 hrs according to the WHO guidelines? Is there every likelihood that mosquito larvae that survived these insecticide solutions may develop resistance at the adult stage? These and other pertinent questions would have been answered if larvae and adult mortality were monitored and reported. I think this information should be added. At the present state of this manuscript, it may look more like preliminary information. If that was the intention, then the orientation of the title, discussion and conclusion should change. Also, the target-site mutations may have been screened if the exposure was long-term and monitored over generations since it will take a longer period for a mutation to be induced than enzymes being produced. Furthermore, since these targets cannot be assessed in this study, this reason should reflect as one of the study's limitations and be recommended for subsequent studies. It will be good to ascertain if mosquito's exposure to the selected agro-pesticides mixtures can induce known resistant mutations or probably the high kdr/ace-1 mutations reported in agricultural areas are from the migration of resistant mosquitoes.
Also, the justification for choosing 100 larvae for your assessment cannot just be because that is the standard sample size for adult assessment as stated in the manuscript, there should be a more appropriate rationale. What if 20% and even more mosquitoes are lost at larval stages during the experiment? It will be impossible to have the required 125-150 mosquitoes, including the control samples for the adult bioassay. This observation was also noted in the manuscript, how many mosquitoes survived into an adult in each colony? How many female mosquitoes emerged from the survivors? How many female mosquitoes were used for adult bioassay, considering that only 100 larvae were exposed to the chemical solutions? Where are the results of the other doses of agro-pesticides solutions used for the assessment as reported in the methodology? Authors should make sure this information is presented and make the methodology clearer than its present state.

Minor issues:

Introduction:
- “…… Indeed, the food security which is one of the current priorities that the Food and Agriculture Organization of the United Nations (FAO) faces with the challenge of climate change will incite agricultural overproduction to meet the expectations of the world’s future population; this overproduction suggests an expansion of rice growing areas, which as mentioned above, remain niches for the proliferation of malaria vectors, and an intensification of use of agricultural pesticides (Osta et al., 2012), which contribute to the development of insecticide resistance in the mosquitoes breeding in cultivation areas as molecules used are the same as those used for vector control………….”. This statement is too long and should be broken into 2 to 3 sentences.
- “non-insecticides” and “none-insecticides” was used interchangeably throughout the manuscript. Authors should address this and be consistent with “non-insecticide” in the whole document.

Methodology:
- Preparation of mother solution? I guess you were referring to the stock solution, which I think is more appropriate. Please change this to “stock solution”.
- “The determination of d120 for each colony was done from the mother solution”…….. change d120 to LD20 in this sentence and other parts of the manuscript.
- Authors need to be consistent with the use of “non-exposed”. Change “not-exposed” to “non-exposed”.
- If LD20 was selected for this assessment, the authors should justify their decision. This is completely missing in the manuscript and should be addressed.

Results:
- “Lethal dose for 20% The lethal dose necessary to induce 20% of mortality in the population were 0.05 μl /100 ml for insecticides, 900 μl /100 ml for none-insecticides and 5 μl /100 ml for the mixture (Sadia
et al., 2021). These concentrations were used to assess the induction effect of the respective pesticide groups in adult mosquitoes.

It seems the authors cited this paper in the manuscript, if it is a mistake, they should check very well and correct it appropriately.

I was expecting that the authors will check for the significant difference of the kdt20 and kdt50 in the different colonies and establish if the observed differences in kdt are significant. The authors mentioned that some differences observed were significant but the P-value was not reported, which is required for this conclusion. This comment should be addressed so that the bioassay results can communicate better.

Discussion:
- The use of “indeed” is excessive in your discussion, you can use other adverbs to emphasize your points.
- “………………….. CYP6M2 was under-expressed in almost all the selected colonies with the exception of the none-insecticide colony where this gene was not significant. While CYP6M2 is responsible for the resistance of mosquitoes to deltamethrin and DDT. This gene has been over-expressed in some cities in Côte d’Ivoire where agriculture is intensive and mosquitoes are resistant to several classes of insecticides used in public health (Edi et al., 2014; Oumbouke et al., 2020). This result could explain the low level of kdt50 observed in the different exposed colonies. The fact that this gene is under-expressed in these colonies could confirm the fact that the relationship between the capacity of an enzyme to metabolise an insecticide and its induction (induction in genetics is the set of cellular and molecular mechanisms leading to the triggering of the expression of a specific gene) by the insecticide is not always correlated (Willoughby et al., 2006)………..”

As much as I agree with the latter fact, there is no assessment in this study that suggests the involvement of CYP6M2 in the observed KD effect, hence no involvement in observed resistance under this circumstance. So, the above justification may not be very appropriate for this discussion. I would suggest that the authors should either remove this statement or recast/review it after addressing this comment.

English editing:
Overall, I observed a lot of grammatical and typographical errors, misuse of words and punctuations as well as several sentences that need to be recast/reviewed. I would recommend that the authors should conduct a thorough English language editing on the manuscript.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly
If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Molecular Entomology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 20 Sep 2021

**Gba Christabelle SADIA**, Nangui Abrogoua University / Centre Suisse de Recherches Scientifiques, Cote d'Ivoire

**RESPONSE TO ROUSSEAU DJOUAKA**

**Major comments**

- **What was the mortality like after 24, 48, 72 hrs of larvae exposure and until adult emergence?**

  The mortality rate obtained 24 hours after exposure to deltamethrin and DDT was 100% in all colonies tested and control colony.

- **Also, the justification for choosing 100 larvae for your assessment cannot just be because that is the standard sample size for adult assessment as stated in the manuscript, there should be a more appropriate rationale.**

  The 100 larvae were used for the determination of the different $dl_{20}$ in each group. After determination of the $dl_{20}$, more larvae were used for induction. About 1000 larvae per colony were used.

**Minor issues**

**Introduction:**

- **This statement is too long and should be broken into 2 to 3 sentences**

  Indeed, the food security which is one of the current priorities that the Food and Agriculture Organization of the United Nations (FAO) faces with the challenge of climate
change will incite agricultural overproduction to meet the expectations of the world's future population. This overproduction suggests an expansion of rice growing areas, which as mentioned above, remain niches for the proliferation of malaria vectors, and an intensification of use of agricultural pesticides (Osta et al., 2012). Intensification of use of agricultural pesticides can contribute to the development of insecticide resistance in the mosquitoes breeding in cultivation areas as molecules used are the same as those used for vector control.

- non-insecticides” and “none-insecticides

I agree to use non-insecticide in this document.

Methodology:

- Preparation of mother solution? I guess you were referring to the stock solution, which I think is more appropriate. Please change this to “stock solution”.

I agree stock solution

- The determination of dl20 for each colony was done from the mother solution”. “........... change dl20 to LD20 in this sentence and other parts of the manuscript.

I agree LD20

- Authors need to be consistent with the use of “non-exposed”. Change “not-exposed” to “non-exposed

I agree non-exposed

- If LD20 was selected for this assessment, the authors should justify their decision. This is completely missing in the manuscript and should be addressed.

In this study, dl20 was chosen in order to minimise the effects of the artificial selection that could occur if the amount of xenobiotic used is high.

Results:

- It seems the authors cited this paper in the manuscript, if it is a mistake, they should check very well and correct it appropriately.

This citation was made by the newspaper. I think this is a mistake.

- P-value

Deltamethrin results
Control vs Insecticides colony :  P = 0.1
Control vs Non-insecticides : P = 0.35
Control vs Mixture: \( P = 0.11 \)

DDT results
Control vs Insecticides: \( P = 0.45 \)
Control vs Non-insecticides: \( P = 0.76 \)
Control vs Mixture: \( P = 0.86 \)

**Discussion:**

we will revisit the CYP6M2 discussion.

**RESPONSE TO THERESIA NKYA**

- **There is a need of justifying the use of 20% solution for the selection experiments.**

In this study, \( \text{dil}_{20} \) was chosen in order to minimise the effects of the artificial selection that could occur if the amount of xenobiotic used is high.

- **Why use only pyrethroids and OC and not other insecticides which are currently widely used for vector control like OP and Carbamates?**

it was hoped to have a response with pyrethrinoids and OCs compared to carbamates and OPs. Indeed, resistance to carbamates and OPs is a process that takes place over several years. Therefore, we used the pyrethrinoids and OP insecticides.

**Competing Interests:** No competing interests were disclosed.

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**Reviewer Report 28 June 2021**

[https://doi.org/10.21956/wellcomeopenres.18615.r44423](https://doi.org/10.21956/wellcomeopenres.18615.r44423)

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**Theresia Estomih Nkya**

1 International Centre of Insect Physiology and Ecology, Nairobi, Kenya
2 Mbeya College of Health and Allied Sciences, University of Dar es Salaam, Dar es Salaam, Tanzania

- Some of the references used are a bit old, there are newer reports from WHO in regards to current malaria status.
- There is a need of justifying the use of 20% solution for the selection experiments.
○ Why use only pyrethroids and OC and not other insecticides which are currently widely used for vector control like OP and Carbamates?

○ How about testing on the new classes of insecticides as they are now being pushed into the market?

**Is the work clearly and accurately presented and does it cite the current literature?**
Partly

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**
Yes

**Are all the source data underlying the results available to ensure full reproducibility?**
Partly

**Are the conclusions drawn adequately supported by the results?**
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** insecticide resistance, vector biology, vector ecology, entomology, larviciding, integrated vector management, operational research

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.