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# Evaluation of the Toxicity of Glyphosate and its Impact on the Tolerance of *Ancistrotermes guineensis* to Chlorpyrifos-ethyl and the Methanolic Extract of *Tithonia diversifolia*

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**Abstract** **Original Research Article**

The massive use of glyphosate as a means of weed control is increasingly observed in cocoa plantations in Ivory Coast. Added to this is the ineffectiveness of the treatments. These observations led us to evaluate the level of resistance of *A. guineensis* to Chlorpyrifos-ethyl and *T. diversifolia* extract after exposure to glyphosate. The toxicity of glyphosate on *A. guineensis* workers was evaluated by direct toxicity. For the evaluation of the impact of glyphosate on the tolerance of A. guineensis workers, 50 workers taken at random from 75 workers previously exposed to glyphosate for 4, 6, 12 and 24 hours at concentrations 1.9, 2.14 and 3.56 g/l, were used for the bioassays with Chlorpyrifos-ethyl (22.4 mg/l) and *T. diversifolia* extract (10 mg/l). The study revealed no toxicity of glyphosate on *A. guineensis* workers. On the other hand, it showed that the exposure of workers of *A.guineensis* to glyphosate could modify their sensitivity to Chlorpyrifos-ethyl. This modification begins 4 hours after contact with glyphosate and at the lowest concentration. Tolerance of *A. guineensis* workers to Chlorpyrifos-ethyl is linked to the duration of exposure to glyphosate. After 24 hours of Chlorpyrifos-ethyl treatment, the mortality of unexposed workers and those exposed (4 and 24 hours) to glyphosate (1.9g/l) was respectively 60.00±3.65, 23.00± 1.9 and 15.00±1.15. Unlike Chlorpyrifos-ethyl, the study revealed no impact of glyphosate on the sensitivity of *A. guineensis* workers to the *T. diversifolia* extract. After 24 bioassays with *T. diversifolia*, the mortality of unexposed workers and those exposed (4 and 24 hours) to glyphosate (1.9g/l) was respectively 30.40±1.67, 30.40±1 .69 and 29.20±2.28. Given the cases of resistance observed with chemical products, the fight against termites must be done with natural plant extracts.

**Keywords:** Exposure, Glyphosate, Tolerance, *Tithonia diversifolia*, Chorpyrifos-ethyl.



## 1. INTRODUCTION

The Controlling crop pests represents an important operation in crop production today in Ivory Coast (Ochou *et al.*, 2012). This control requires extensive use of chemical phytosanitary products (Ano *et al.*, 2018). During the application of these products, most of the quantities added reach the ground, either directly or indirectly by leaching from the foliage of the treated plants by rain. The soil therefore occupies a central position in regulating the fate of pesticides in the environment. It will have a dual role of storage and purification (Barriuso *et al.*, 1996).

In the soil, pesticides are affected by different processes (physical, chemical and biological). These processes condition the degradation, the transfer of pesticides to other compartments of the environment, that is to say water, plants, the atmosphere and consequently their potential impact on exposed living beings, in particular macrofauna including termites.

In the gas and liquid phase, the pesticide is degraded by microorganisms and transferred to groundwater, while in the solid phase it remains stored in the soil (Mamy *et al.*, 2011). Among the pesticides available on the market, glyphosate is the most widely used worldwide. Its consumption in France in 1999 was 7,500 tonnes (Aamand and Jacobsen, 2001). In the United States, glyphosate consumption fluctuates between 17,000-22,000 tonnes per year (Cox, 2000). In Ivory Coast, glyphosate is widely used in cocoa plantations (Ano *et al.*, 2018, Siapo *et al.*, 2018a). The

presence of glyphosate and its metabolite in significant quantities in surface water and in the soil in particular, could have consequences on the organisms that live there. Indeed, according to Rodolphe (2011), glyphosate, widely used to control weeds, causes changes in the cellular behavior of mosquito larvae and adults. This modification allows them to develop a certain form of resistance and therefore tolerance to insecticides such as permethrin and imidacloprid (Rodolphe, 2011). In Côte d'Ivoire, termite damage and ineffective treatment in rural areas are increasingly observed (Tahiri and Mangué, 2007 ; Akpesse *et al*., 2019). The persistence of damage and the ineffectiveness of treatments in rural areas requires the assessment of the level of resistance of termites to pesticides.

Indeed, cocoa producers in Ivory Coast, although using phytosanitary products to protect their plantation against pests, complain about termite damage in their cocoa plantation. To understand the persistence of termite attacks in cocoa plantations in Côte d'Ivoire, we set ourselves the objective in this study of evaluating the impact of glyphosate on the sensitivity of *Ancistrotermes guineensis* workers to Chlorpyrifos-ethyl and with methanolic extract of *Tithonia diversifolia*.

## 2. MATERIAL AND METHODS

### 2.1. Material

#### 2.1.1. Biological material

The biological material consists of the leaves of *T. diversifolia* and the workers of *Ancistrotermes guineensis*. *T. diversifolia* is a common plant in Ivory Coast. The aqueous and methanolic extract of the leaves of *T. diversifolia* on the termite *A. guineensis* have been reported in several studies in Ivory Coast (Diby *et al.*, 2015, Siapo *et al.*, 2018 b). The leaves used for the preparation of the methanolic extract come from the commune of Bingerville (Abidjan-Côte d'Ivoire). *A. guineensis* workers were used for direct toxicity test in the laboratory. These workers come from the National Floristic Center (CNF).

#### 2.1.2. Chemical Material

The synthetic chemicals used consist of:

- The insecticide Pyriforce whose active ingredient is Chlorpyrifos-ethyl which belongs to the organophosphate family,
- The Kalach 360 SL herbicide whose active ingredient is glyphosate which belongs to the chemical family of phosphonoglycines.
- Ethyl alcohol 70% and distilled water were used.

Pyriforce and Kalach, in liquid form, were supplied respectively by the Solevo and Callivoire groups. They belong to classes II and III respectively according to the World Health Organization (WHO) classification. They are therefore slightly and moderately dangerous respectively, according to the WHO.

### 2.2. Methods

#### 2.2.1. Preparation of Chlorpyrifos-ethyl and glyphosate

The Pyriforce dosed at 480 g/l of Chlorpyrifos-ethyl in liquid form was used. Indeed, the Pyriforce is commonly used by growers in Ivory Coast to combat termites. Three (3) concentrations were prepared after dilution with distilled water. For this preparation, one milliliter (1 ml) of the stock solution was diluted in 1 liter of distilled water to obtain a new stock solution with a concentration of 0.48 g/l. One milliliter (1 ml) of this new solution was diluted in 100, 50 and 20 ml of distilled water to obtain concentrations 4.8 respectively; 9.4 and 22.9 mg/l.

The Kalach used is dosed at 360 g/l of glyphosate. Two (2) lower concentrations (1.9 and 2.14 g/l) and one higher concentration (3.56 g/l) were prepared, in order to see the impact of underdosing and overdosing. overdose of glyphosate on the sensitivity of *A. guineensis* workers to insecticides. The concentrations 1.9, 2.14 and 3.56 g/l are obtained respectively after dilutions of 0.8, 0.9 and 1.5 ml of the stock solution in 150 ml of distilled water. The concentrations were used for the direct workers toxicity test of *A. guineensis*.

#### 2.2.3. Preparation of *Tithonia diversifolia* extract

This preparation is carried out in accordance with the methods described by Kaushik and Vir (2000). Thus, the fresh leaves of *Tithonia diversifolia* are harvested between 6 and 7 a.m. in the town of Bingerville. They are dried in the open air away from direct sunlight for 2 weeks. The dry leaves are then pulverized with an electric mill. Thirty grams (30g) of powder are dissolved in 1 90% methanol. The mixture obtained is stirred for 48 hours at room temperature (25°C) using a magnetic stirrer of the IKAMAG RCT type (Staufen, Germany). The mixture is filtered 3 times on cotton and on Whatman No. 4 paper. The collected solution is evaporated by rotary evaporation in a rotavapor to obtain a total methanolic extract which is dried under vacuum.

### 2.3. Evaluation of the toxicity of glyphosate on *Ancistrotermes guineensis*.

To evaluate the toxicity of glyphosate on the termite pest *Ancistrotermes guineensis*, the protocol used is that of Tahiri (2012). The test is carried out at room temperature in the laboratory (28°C). Petri dishes containing 3.5 g of soil were used for the toxicity test. Using a pipette, the concentrations 1.9; 2.14 and 3.56 g/l were tested. Thus, 1 ml of each concentration is poured and mixed with the soil. A control with distilled water was carried out. After storage, the boxes are dried in the open air for one hour. Fifty (50) *A. guineensis* workers are then introduced into these closed devices. Each test is repeated 5 times. Mortality assessment is estimated every 2 hours after treatment for 24 hours. After 24 hours, counting was carried out every day until all the

insects died. The lethal concentration (LC50) is calculated based on mortality after 24 hours of treatment.

### 2.4. Impact of glyphosate on the tolerance of *Ancistrotermes guineensis* to Chlorpyrifos-ethyl and *Tithonia diversifolia* extract

To evaluate whether prior exposure of *Ancistrotermes guineensis* workers to glyphosate could have an impact on their sensitivity to synthetic insecticides and plant extracts, in particular the methanolic extract of *Tithonia diversifolia*, 75 workers from *A. guineensis* were first placed in contact with glyphosate for 4 and 24 hours at different concentrations (1.9, 2.14 and 3.56 g/l) of the herbicide (glyphosate) before the Chlorpyrifos bioassays-ethyl (22.4 mg/l) and the methanolic extract of *T. diversifolia* (10 mg/l).

Pre-exposure was carried out by direct toxicity in Petri dishes containing 3.5 g of soil moistened with 1 ml of each concentration. Fifty (50) *A.guineensis* workers, taken at random from those pre-exposed to glyphosate, are then transferred to Petri dishes containing 3.5 g of soil moistened with 1 ml of the insecticide solution (Chlorpyrifos-ethyl or *T. diversifolia*) to the different concentrations. A control with 90% methanol is carried out. This test was carried out at ambient laboratory temperatures between 27 to 28°C and humidity of 75%.

For each concentration, 5 repetitions were carried out. During this test, the impact of exposure time and different concentrations on the tolerance behavior of A. guineensis was investigated. During this test, the duration of exposure to glyphosate and the concentration of glyphosate were evaluated on *A. guineensis*. The time required to kill half (TL50) of the workers exposed to glyphosate at different times was calculated using XLSTAT software.

### 2.5. Statistical analyzes

Analyzes of variance are statistical tests for comparing means from several samples. One-way analysis of variance (ANOVA) was carried out with Statistica software (version 6.0), then homogeneous means were grouped using Tukey's HSD test.

Lethal concentrations 50 (LC50) and lethal time 50 (LT50) were determined by probit analysis on the basis of termite mortality obtained in 24 hours with XLSTAT software (version 2019). The chosen probability threshold is $P < 0.05$.

## 3. RESULTS

### 3.1. Toxicity of glyphosate on *Ancistrotermes guineensis*

Three increasing concentrations (1.9 g/l, 2.14g/l and 3.56g/l) of glyphosate were tested on *A. guineensis* workers. The mortality of *A. guineensis* workers obtained in the controls (distilled water) is identical to that observed with the different concentrations of glyphosate. The analysis showed that the mortalities recorded with the three concentrations of glyphosate are not statistically different from that recorded with distilled water (control) after 24, 48 and 72, 96 and 120 hours of treatment (Anova, $P > 0.05$) (Table 1).

Glyphosate does not appear to be toxic to *A. guineensis* workers at these different concentrations. Probit analysis performed on the basis of termite mortality during contact toxicity tests, in 24 hours, shows that the concentration of glyphosate necessary to kill 50%, lethal concentration 50 (LC50), of the termite population tested is 42.46 g/l.

**Table 1: Mortality rate of *A. guineensis* workers treated with glyphosate**

| | Mortality in % ± Standard deviation | | | | |
|---|---|---|---|---|---|
| **Doses** | 24 Hours | 48 Hours | 72 Hours | 96 Hours | 120 Hours |
| **Control** | 4.00 ± 1.00 a | 6.00 ± 1.00 a | 12.00 ± 1.72 a | 25.20 ± 1.29 a | 44.00 ± 5.48 a |
| **1.9 g/l** | 4.80 ± 2.08 a | 7.20 ± 1.52 a | 12.00 ± 1.00 a | 24.00 ± 2.58 a | 42.00 ± 3.56 a |
| **2.14 g/l** | 5.20 ± 1.52 a | 8.00 ± 1.00 a | 10.00 ± 1.00 a | 26.80 ± 1.72 a | 43.20 ± 2.58 a |
| **3.56 g/l** | 6.00 ± 1.00 a | 6.80 ± 0,58 a | 14.00 ± 1.00 a | 23.40 ± 1.58 a | 45.80 ± 3.95 a |
| **Pvalue** | 0.07 | 0.64 | 0.099 | 0.35 | 0.26 |

*In the same column, the means ± Standard deviation followed by the same letter are not statistically different at the 5% level (ANOVA) according to Tukey's HSD test*.

### 3.2. Impact of glyphosate on the tolerance of *Ancistrotermes guineensis* to Chlorpyrifos-ethyl

#### 3.2.1. Impact of exposure time to glyphosate

Whatever the concentration of glyphosate (1.9, 2.14 and 3.56 g/l) and the exposure time, workers of *A. guineensis* have mortality well below controls (not exposed). This mortality is 2 to 4 times lower. The statistical analysis shows a significant difference between the mortality rate of unexposed workers and those exposed to glyphosate at all treatment times (Anova, $p<0.05$). Whatever the concentration of glyphosate, the more exposure time increases, the less mortality there is. After 24 hours of treatment with Chlorpyrifos-ethyl, the mortality rate of worker of *A. guineensis* exposed for 4 hours is statistically higher than those exposed for 24 hours to glyphosate (Anova, $p<0.05$). After 120 hours of treatment with Chlorpyrifos-ethyl, the mortality rate of workers exposed for 4 hours is statistically higher than that of workers of *A. guineensis* exposed for 24 hours to glyphosate (Anova, $p<0.05$) (Table 2). Glyphosate therefore leads to tolerance of *A. guineensis* workers to Chlorpyrifos-ethyl.

This tolerance increases depending on the exposure time of *A. guineensis* workers to glyphosate.

### 3.2.2. Impact of glyphosate concentration

Regardless of the exposure time of workers to glyphosate (4 and 24 hours), workers of *A. guineensis* mortality is identical between concentrations. The lowest concentration records the same mortality rate as the highest concentration. After 24, 48, 72, 96 and 120 hours of treatment, statistical analysis showed no significant difference between the concentrations (ANOVA, P > 0.05). The tolerance of workers to Chlorpyrifos-ethyl is not dose dependent (Table 2).

**Table 2: Mortality rate of Ancistrotermes guineensis workers using Chlorpyrifos-ethyl (22.9 mg/l) after exposure to glyphosate**

| Bioassay Hours (H) | Exposure time | Mortality (%) ±Standard Deviation | | |
|---|---|---|---|---|
| | | 1.9 g/l | 2.14 g/l | 3.56 g/l |
| 24 H | Unexposed | 60.00± 3.65 c | 60.00± 3.65 c | 60.00±3.65 c |
| | Exposed 4 H | 23.00±1.9 b | 24.00±1,15 b | 26.40±2.51 b |
| | Exposed 24 H | 15.00±1.15 a | 16.00±1.15 a | 16.00±1.72 a |
| 48 H | Unexposed | 75.00±6.19 f | 75.00±6.19 f | 75.00±6.19 f |
| | Exposed 4 H | 60.40±6.19 e | 58.60±1,91 e | 62,00±2.51 e |
| | Exposed 24 H | 19.60±1.91 d | 24.00± 6.32 c | 19.00±1.72 c |
| 72 H | Unexposed | 93.40±7.89 i | 93.40±7.89 i | 93.40±7.89 i |
| | Exposed 4 H | 75.40± 5.50 h | 73.60±5.50 h | 78.00±3.65 h |
| | Exposed 24 H | 33.00±2.58 g | 33.60± 2.51 g | 30.00±3.65 g |
| 96 H | Unexposed | 100.00±0.00 l | 100.00±0.00 l | 100.00±0.00 l |
| | Exposed 4 H | 84.00±7.72 k | 82.60± 7.72 k | 82.00± 5.62 k |
| | Exposed 24 H | 61.00±5.00 j | 59.60±4.43 j | 49.00±10.52 j |
| 120 H | Unexposed | 100.00±0.00 m | 100.00±0.00 m | 100.00±0.00 m |
| | Exposed 4 H | 100.00±0.00 m | 100.00±0.00 m | 100.00±0.00 m |
| | Exposed 24 H | 86.60± 3.00 n | 87.00±10.24 n | 80.60± 7.18 n |

*On the same line, the means ± Standard deviation followed by the same letter are not statistically different at the 5% threshold according to Tukey's HSD test.*
*In the same column, the means ± Standard deviation followed by the same letter are not statistically different at the 5% threshold according to Tukey's HSD test.* H: Hours

### 3.2.3. Impact of glyphosate on bioassay time

Regardless of the concentration of glyphosate, the time required to kill 50% (LT50) of workers *A. guineensis* with Chlorpyrifos-ethyl increases with the duration of exposure to glyphosate. For all exposure concentrations, the lowest LT50 was recorded in workers exposed for 4 hours to glyphosate and the highest LT50 in workers exposed for 24 hours. By comparing the LT50 of workers of *A. guineensis* not exposed to glyphosate to that of workers exposed for 24 hours to glyphosate whatever the exposure concentration, the LT50 is approximately 5 times higher (Table 3).

**Table 3: Time that kills 50% (LT50) of *Ancistrotermes guineensis* workers with Chlorpyrifos-ethyl after exposure to glyphosate**

| Concentrations of glyphosate | Workers exposed to glyphosate | | Workers not exposed to glyphosate | RTL50 |
|---|---|---|---|---|
| | Exposure time | TL50 (H) | TL50 (H) | |
| 1.9g/l | Exposed 4 H | 46.36 | 18.27 | 2.57 |
| | Exposed 24 H | 80.95 | 18.27 | 4.43 |
| 2.14 g/ l | Exposed 4 H | 47.76 | 18.27 | 2.61 |
| | Exposed 24 H | 80.51 | 18.27 | 4.4 |
| 3.56 g/l | Exposed 4 H | 43.8 | 18.27 | 2.39 |
| | Exposed 24 H | 91.29 | 18.27 | 4.99 |

*RTL50: Ratio of the TL50 of workers exposed for 24 hours to glyphosate by TL50 of unexposed workers; TE : Exposure time to glyphosate; TL50: Lethal Time which kills 50% of Ancistrotermes guineensis workers ; H : Hours*

### 3.3. Study of the impact of glyphosate on the tolerance of *Ancistrotermes guineensis* to *Tithonia diversifolia*

### 3.3.1. Impact of exposure time to glyphosate

Whatever the exposure time of workers of *A. guineensis* to the different concentrations of glyphosate (1.9; 2.14 and 3.56 g/l), the mortality of workers from *A. guineensis* to Tithonia diversifolia does not vary statistically (Anova, p >0.05). By comparing the mortality observed in workers not exposed to glyphosate to that obtained in workers exposed to glyphosate, no difference was observed (Table 4).

### 3.3.2. Impact of glyphosate concentration on the tolerance of *Ancistrotermes guineensis*

Regardless of the exposure time of workers to glyphosate (4, 6, 12 and 24 hours), workers mortality remains the same regardless of the concentrations. The lowest concentration records the same mortality rate as the highest concentration, after 24, 48, 72, 96 and 120 hours of treatment. For the exposure times to glyphosate, the statistical analysis showed no significant difference between the concentrations (ANOVA, p>0.05) (Table 4).

**Table 4: Mortality rate of workers from *Ancistrotermes guineensis* to *Tithonia diversifolia* (10 mg/l) after exposure to glyphosate**

| Bioassay hours (H) | Exposure time | Mortality (%) ±Standard Deviation | | |
|---|---|---|---|---|
| | | 1.9 g/l | 2.14 g/l | 3.56 g/l |
| 24 H | Unexposed | 30.40± 1.67 a | 30.40± 1.67 a | 30.40±1.67 a |
| | Exposés 4 H | 30.40±1.69 a | 30.20±2,00 a | 29.20±1.09 a |
| | Exposés 24 H | 29.20±2.28 a | 28.80±1.09 a | 28.40±2.19 a |
| 48 H | Unexposed | 62.80±4.14 b | 62.80±4.14 b | 62.80±4.14 b |
| | Exposed 4 H | 62.80±4.00 b | 62.40±4,77 b | 62.00±4.23 b |
| | Exposed 24 H | 60.80±6.57 b | 60.40± 7.12 b | 60.00±6.63 b |
| 72 H | Unexposed | 82.00±4.00 c | 82.00±4.00 c | 82.00±4.00 c |
| | Exposed 4 H | 82.00± 4.05 c | 82.00±4.60 c | 81.60±3.84 c |
| | Exposed 24 H | 80.40±4.33 c | 80.00± 8.12 c | 79.00±4.77 c |
| 96 H | Unexposed | 88.40±4.98 e | 88.40±4.98 e | 88.40±4,98 e |
| | Exposed 4 H | 88.40±5.17 e | 88.00± 1.78 e | 87.60± 5,36 e |
| | Exposed 24 H | 80.00±5.09 e | 85.60±5.55 e | 86.00±4,69 e |
| 120 H | Unexposed | 100.00±0.00 d | 100.00±0.00 d | 100.00±0.00 d |
| | Exposed 4 H | 99.20±2.31 d | 98.00±1.78 d | 98.40±1.67 d |
| | Exposed 24 H | 98.00±2.29 d | 98.00±2.00 d | 97.20±1.78 d |
| 144 H | Unexposed | 100.00±0.00 g | 100.00±0.00 g | 100.00±0.00 g |
| | Exposed 4 H | 100.00±0.00 g | 100.00±0.00 g | 99.60±0.89 g |
| | Exposed 24 H | 99.00±2.00 g | 98.40±2.19 g | 98.00±1.41 g |

*On the same line, the means ± Standard deviation followed by the same letter are not statistically different at the 5% threshold according to Tukey's HSD test.*

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H: Hours

### 3.2.3. Impact of glyphosate on bioassay time

The time of exposure of workers to glyphosate therefore has no influence on the mortality of workers from *A. guineensis* to *T. diversifolia*. Comparing the LT50 of unexposed workers to that of workers exposed to glyphosate, whatever the exposure time of *A. guineensis* workers to glyphosate and the concentration of glyphosate, the LT50 is only 1.00 to 1.05 times higher (Table 5).

**Table 5: Time that kills 50% (LT50) of workers from *Ancistrotermes guineensis to Tithonia diversifolia* after exposure to glyphosate**

| Concentrations | Workers exposed to glyphosate | | Workers not exposed to glyphosate | RTL50 |
|---|---|---|---|---|
| 1.9 g/l | TE | TL50 (H) | TL50 (H) | |
| | Exposed 4H | 40.65 | 40.27 | 1 |
| 2.14 g/ l | Exposed 24H | 41.43 | 40.27 | 1.03 |
| | Exposed 4H | 41.07 | 40.27 | 1.03 |
| | Exposed 24H | 41.81 | 40.27 | 1.04 |
| 3.56 g/l | Exposed 4H | 41.36 | 40.27 | 1.03 |
| | Exposed 24H | 42.2 | 40.27 | 1.05 |

***RTL50**: Ratio of the TL50 of workers exposed for 24 hours to glyphosate by TL50 of unexposed workers; **TE** : Exposure time to glyphosate; **TL50**: Lethal Time which kills 50% of Ancistrotermes guineensis workers; **H** : Hours*

## 4. DISCUSSION

The direct toxicity test showed that glyphosate is not toxic to *A. guineensis* workers at the concentrations used. Similar studies were carried out by Asteraki *et al.*, (1992) to evaluate the toxicity of glyphosate on auxiliaries arthropods such as spiders, beetles, springtails and mites. This study during which the organisms were placed in contact with the herbicide for several days showed that glyphosate was harmless for the different

species. These authors also showed in field conditions that glyphosate has no harmful effects on crabs.

The study investigated the impact of glyphosate on workers of the termite pest A. guineensis at Chlorpyrifos-ethyl and total methanolic extract of *Tithonia diversifolia*. The results showed that the presence of glyphosate in the soil can contribute to the tolerance of *A. guineensis* workers to Chlorpyrifos-ethyl even at sub-lethal doses. Indeed, the mortality of *A. guineensis* workers exposed for 4, 6, 12 and 24 hours to glyphosate is significantly lower than that of unexposed workers, whatever the concentration of glyphosate. The results corroborate those of Rodolphe (2011) who showed that glyphosate causes changes in the cellular behavior of mosquito larvae and adults. He deduces that these modifications lead to the resistance or tolerance of mosquitoes to insecticides, in particular permethrin and imidacloprid. The results obtained in the present study are in agreement with the study of Riaz *et al.,* (2009) which showed that glyphosate could increase the activity of detoxification enzymes known to be involved in resistance to pesticides, notably permethrin, imidacloprid and propoxur in mosquitoes. Boyer (2006) also showed that apart from glyphosate, other herbicides such as Atrazila, the active ingredient atrazine, increases the activity of enzymes in the detoxification of mosquitoes from pesticides. The increase in detoxification enzymes due to atrazine has also been demonstrated in the fall armyworm (Spodoptera frugiperda) (Londono *et al.*, 2004; Yu, 2004).

The results reveal an influence of exposure time to glyphosate on the tolerance of *A. guineensis* workers to Chlorpyrifos-ethyl. Indeed, the study showed that the longer the exposure time to glyphosate, the less mortality was obtained. The results obtained in the present study corroborate those of Riaz *et al*., (2009). Indeed, these authors showed a reduction in the sensitivity of mosquito larvae to permethrin and imidacloprid depending on the duration of exposure to glyphosate.

The results also revealed that the increase in tolerance of *A. guineensis* workers to Chlorpyrifos-ethyl is not dose dependent. Indeed, a greater concentration of glyphosate results in the same tolerance of workers to Chlorpyrifos-ethyl as a small concentration of glyphosate. These results are contrary to those of Riaz *et al*., (2009). Indeed, these authors have shown in mosquito larvae and adults that the increase in the tolerance of larvae to synthetic insecticides is dose dependent. This difference could be explained by the biological nature of the animal material studied. Not all organisms react the same way to a chemical.

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## CONCLUSION

It appears that the mortality rate recorded among workers exposed to different concentrations of glyphosate does not vary significantly from that of workers treated with distilled water. This result shows that glyphosate is not toxic to workers of the cocoa termite pest *A. guineensis*. Exposure of *A. guineensis* workers to glyphosate modifies the tolerance of these workers to Chlorpyrifos-ethyl. In this study, we were also able to show that the increase in tolerance is not related to the dose. This tolerance begins 4 hours after contact with glyphosate and at the lowest concentration. The tolerance of *A. guineensis* workers to Chlorpyrifos-ethyl increases with the duration of exposure to glyphosate. Unlike Chlorpyrifos-ethyl, the study revealed no impact of glyphosate on the sensitivity of *A. guineensis* workers to the total methanolic extract of *Tithonia diversifolia.* Given the impact of chemical insecticides on the environment, human health and cases of resistance observed in arthropods over the decades, the use of natural extracts seems very promising.

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