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## Bioefficacy of *Eucalyptus citriodora* essential oil in the control of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) in chicken farms in Côte d'Ivoire

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### Abstract

*Alphitobius diaperinus* is the most dangerous species in livestock buildings. It can transmit fatal diseases to poultry resulting in heavy losses. This study aims to control *A. diaperinus* using the essential oil of *Eucalyptus citriodora*. For this purpose, a split-plot design with 4 factors and 4 replications was set up in 2 buildings of 72m<sup>2</sup> (12m×6m). In each building, 2 rows of 4 blocks of 3 m<sup>2</sup> (2m×1.5m) were installed. The blocks, labelled D0 to D3, correspond to the doses used. A non-destructive sampling of the litter was carried out. Four (4) samples were taken at 15-day intervals (1st to 45th day) following the rearing cycle. For each block considered, the litter was sampled. The size of the sampling squares was 25 × 40 cm or 0.1 m<sup>2</sup>. Three (3) treatments at 15-day intervals (1st, 15th and 30th day) were carried out. At the end of the rearing cycle, the vital organs and blood of the chickens in each block were collected for analysis. The results showed that *E. citriodora* reduced the population of *A. diaperinus* by 60 to 90%. Analyses of variance revealed a significant difference ( $p < 0.05$ ) between the reduction rates of the different doses and the control. Biochemical, hematological and internal organ tests of the chickens revealed no abnormalities. The essential oil of *E. citriodora* could be used safely in poultry building to effectively control the population of *A. diaperinus*. To be effective, the user should have a good knowledge of the ethology and development cycle of *Alphitobius diaperinus*.

**Keywords:** Poultry house, *Alphitobius diaperinus*, control, biopesticide, *Eucalyptus citriodora*

### 1. Introduction

In Côte d'Ivoire, agriculture is an important economic pillar. It contributes 34% to GDP and 66% to export earnings [1]. Livestock, which is a sub-sector of agriculture, is a fast-growing activity, contributing 4.5% to agricultural GDP and 2% to total GDP [1]. It contributes to food security [2]. Poultry farming, which constitutes an important part of livestock production, is the main outlet for agricultural products and agro-industrial by-products [3].

However, one of the crucial problems in poultry production is the parasitic pressure caused by poor litter management, which leads to the proliferation of certain pests. Litter is a dynamic ecosystem that changes as poultry develops [4]. It is likely to harbor a variety of insect pests and other organisms whose life cycle may be largely dependent on management practices [5]. *Alphitobius diaperinus* is one of the most dangerous insects for chickens among these organisms. This species was introduced into temperate animal production systems through international trade in infested cereal feeds [6]. *Alphitobius diaperinus* has been shown to be dangerous to chickens through its ability to act as an intermediate host and transmit bacteria, viruses, poultry cestodes, avian tapeworms and even the Turkey Coronavirus called Turkey Coronavirus (TCV) chickens [7-8]. It is responsible for the transmission of a number of fatal diseases to chickens such as Gumboro, Newcastle and Marek's disease causing heavy losses. In addition to this damage, there is structural damage. To reduce the pressure of these diseases on flocks of chickens on poultry farms, there is a need to control and manage the population of *Alphitobius diaperinus* on these farms. As poultry is intended for human consumption, the use of synthetic insecticides in these chicken farms to control this beetle could be a source of contamination for both animals and humans. Thus, in this study, the objective is to control *A. diaperinus*' populations using biopesticides, *Eucalyptus citriodora* essential oil, as an alternative to synthetic insecticide treatments.

## 2. Materials and Methods

### 2.1. Study site

#### 2.1.1. Location

This study was carried out on a farm located in the Azaguié sub-prefecture (Figure 1). It is 25 km from Abidjan on the Abidjan-Agboville axis and covers an area of 630 km<sup>2</sup>. It is bounded to the north by the departments of Agboville and Adzopé, to the south by the Abidjan district, to the east by the Alépé department and to the west by the Tiassalé department. The Aqualand farm is located near the Institut des Nouvelles Techniques Agricoles (INTA) in Azaguié, between latitude 5°40' N, longitude 4°6' W and an average altitude of 60 m.

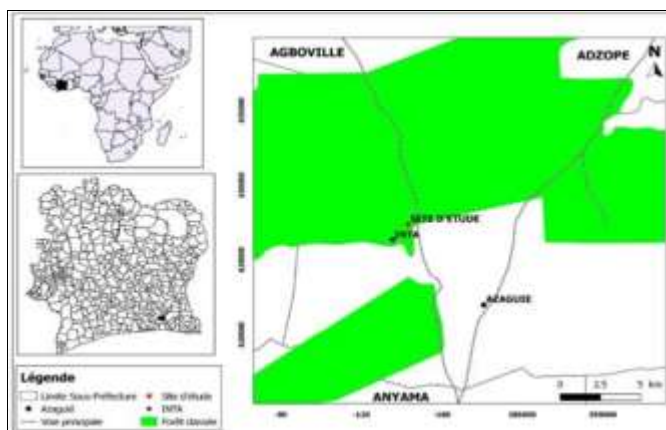


Fig 1: Location of the Aqualand experimental farm (Côte d'Ivoire)

#### 2.1.1. Experimental climatic conditions

During this study, the average temperature, humidity and pressure in the rearing buildings were  $26.69 \pm 0.51$  °C,  $85.89 \pm 3.23\%$  and  $1011.66 \pm 0.69$  hpa respectively.

### 2.2. Materials

#### 2.2.1. Animal biological material

The animal consisted of *Alphitobius diaperinus*, all other litter insect species and poultry.

#### 2.2.2. Choice of biological plant

The plant material consisted of essential oil extracted from the leaves of medicinal plants obtained from *Eucalyptus citriodora* (Myrtaceae). These leaves were harvested in Bingerville, a town located 18 km from Abidjan.

The *Eucalyptus citriodora* essential oil was chosen from among 4 plants with insecticidal effects. This choice was justified by the fact that this plant presented the best insecticidal potential on adult beetles in the laboratory and did not record any toxic effect on treatments carried out on female rats and chickens by the oral route at the single dose of 2000 mg/kg.

### 2.3. Technical equipment

The sampling equipment consisted of a decimeter for delimiting the sampling squares, trowels and shovels for sampling the litter, gloves, sorting trays, pillboxes and entomological forceps for handling the insects.

In the laboratory, the biopesticide preparation equipment consists of a Clevenger-type hydrodistiller used to extract the biopesticides, test tubes, acetone and micropipettes (0-100 µl and 100-1000 µl), dry tubes EDTA (Ethylenediamine tetra acetic acid) tubes for blood collection, a Hitachi model 704 automatic analyser for biochemical analysis and a Sysmex model XN-550 automatic analyser for haematological

analysis. The biopesticide application equipment consists of three (3) hand-held sprayers of 1.5 litres each.

### 2.4. Methods

The *Eucalyptus citriodora* essential oil previously tested in the laboratory on *Alphitobius diaperinus*' adult individuals in petri dishes showed high efficacy among 4 biopesticides (*Mentha viridis*, *Cymbopogon citratus*, *Eucalyptus citriodora*, *Ocimum gratissimum*). Prior to the control trials in chicken farms, in a natural environment, acute oral toxicity tests were first carried out on female rats and then on chickens and proved the non-toxicity of the essential oil of *E. citriodora*.

#### 2.4.1. Effect of *Eucalyptus citriodora* essential oil on *Alphitobius diaperinus*' populations

##### 2.4.1.1. Experimental set-up

The experimental design is a split-plot with 4 factors and 4 replicates. The trials were carried out in two traditional livestock buildings with manual watering and a surface area of 72 m<sup>2</sup> (12 × 6 m). In order to reduce the microbial population, the buildings were cleaned. Five (5) days after cleaning, they were disinfected with cresyl. The walls and posts were sprayed to a height of 1 meter as well as cracks and joints that could provide a refuge for living organisms. After disinfection, a 14-day sanitary vacuum was observed before the chicks were introduced into the buildings. Using netting and black tarpaulin, the blocks were formed. In each farms, measuring 12 m in length and 6 m in width, 2 rows of 4 blocks of 3 m<sup>2</sup> (2 × 1.5 m) were installed (Figure 2). Litter was spread evenly over the blocks. The blocks, labelled D0 to D3, corresponding to the different doses used. Two buildings were used for the study. In each building, two replicates were made per dose, i.e. 4 replicates per dose for the two test buildings. Feeders and drinkers were placed in the blocks to feed the chickens. Chicks (34-46 g) fresh from the hatchery were preheated to 37 °C for 7 days in a special building. After heating, 20 chicks were introduced into each block. The chicks were been fed regularly and monitored by a technician until the end of the breeding cycle. Three treatments were carried out per dose in each building at the start-up stage, the growth stage and the finishing stage. The temperature, relative humidity and atmospheric pressure in the buildings were measured 3 times a day (6 h, 12 h and 18 h) using an IHM thermo-hygrometer and a barometer and then averaged.

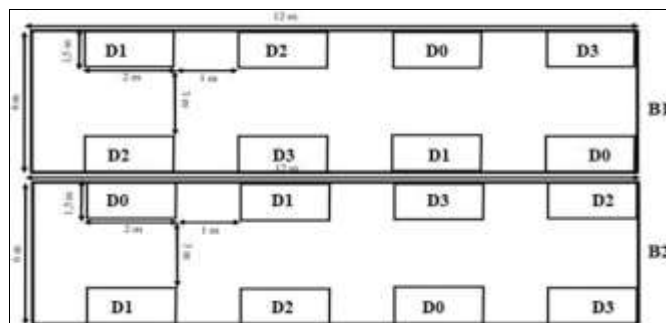


Fig 2: Treatment setup

D0: Control, D1: Dose 1, D2: Dose 2, D3: Dose 3

B1: Chickens breeding building 1; B2: Chickens breeding building 2

##### 2.4.1.2. Collection of insects within the different blocks

To evaluate the efficacy of *Eucalyptus citriodora* essential oil against avian insects, non-destructive sampling of the litter was carried out. Prior to treatment, litter was collected from

each block. The insects were counted and then returned to the litter. Four (4) samples were taken at 15-day intervals before and after each treatment according to the rearing cycle. At the start-up stage, 2 samples were taken (1st and 15th day), one sample at the growth stage (30th day). The fourth sample was taken at the finishing stage (45 days) before chickens left for the slaughter. For each block considered, the litter was sampled according to the 3 zones: near wall zone (NWZ); watering area (WA) and feeding area (FA). The size of the sampling squares was 25 × 40 cm or 0.1 m<sup>2</sup>.

#### 2.4.1.3. Mode of application of the treatments

Four (4) doses were applied to each building. One dose was assigned to each block. The doses applied were as follows:

1. Control application (untreated): D0;
2. Single dose application (19.7 l/ml): D1;
3. Single + half dose application (29.55 l/ml): D2;
4. Double dose application (39.4 l/ml): D3;

Three (3) treatments at 15 day intervals (1st, 15th and 30th day) were carried out. The first application was made after the bedding was in place. Three (3) hand-held sprayers labelled D1 (19.7 l/ml), D2, (29.55 l/ml) and D3 (39.4 l/ml) were used to apply the different doses of *E. citriodora* essential oil. During treatment, the spray head was brought as close as possible to the bedding. Walls and tarpaulins were sprayed at least 1 m high.

#### 2.4.2. Evaluation of the efficacy of *Eucalyptus citriodora* essential oil

The efficacy of the essential oil was evaluated by counting the insects collected from each block before and after each treatment. Based on the number of insects collected, the percentage reduction was determined according to the following formula:

$$\text{Reduction rate (\%)} = \frac{\text{Insects number before treatment} - \text{Insects number after treatment}}{\text{Insects number before treatment}} \times 100$$

#### 2.4.3. Determination of the effect of *Eucalyptus citriodora* essential oil on chickens

The chickens were weighed weekly. According to the treatment doses, the masses were mentioned on a card. At the finishing stage, 3 chickens in each block were sacrificed. For each individual, blood was collected in EDTA (Ethylenediamine tetra acetic acid) and dry tubes. The chickens were then dissected and the vital organs (kidney,

heart, liver, spleen and lung) were removed, rinsed in 10% NaCl and weighed. The blood samples collected in the tubes were used for biochemical and hematological analysis.

### 2.5. Statistical processing

Statistical processing was carried out using Statistica version 7.1 software. The analysis of variance followed by the Newman-Keuls test at the threshold of 5%, made it possible to determine the homogeneity of the various groups. The daily climatic data were used to calculate the monthly averages of temperature, hygrometry, total rainfall and atmospheric pressure.

## 3. Results and Discussion

### 3.1. Results

#### 3.1.1. Effect of *Eucalyptus citriodora* essential oil on the reduction of *Alphitobius diaperinus*' populations

##### 3.1.1.1. Start-up stage

On the first day of the start-up stage (start-up I), no insects were recorded. Fifteen days after the start of the cycle (start 2), before treatment, the average number of insects per block was 6.66 ± 4.17 (D0), 6.00 ± 2.08 (D1), 9.66 ± 3.71 (D2) and 5.00 ± 1.73 (D3) individuals per block, respectively (Table I). After treatment, blocks treated with single dose (D1), single dose + half (D2) and double dose (D3) recorded 2.66 ± 0.83, 4.66 ± 1.33 and 2.66 ± 0.16 individuals, respectively. The treatments reduced avian insects by 50, 55 and 60% at D1, D2 and D3 respectively. The double dose (D3) was the most effective dose with a 60% reduction in insects. Analyses of variance revealed a significant difference ( $p < 0.5$ ) between the efficacies of different doses used compared to the control.

##### 3.1.1.2. In the growth stage

At the growth stage (30<sup>th</sup> day), the number of insects in the control blocks averaged 47.33 ± 6.38 per block. Prior to treatment, the average number of insects was 27.00 ± 4.35 individuals for the single dose (D1), 19.33 ± 4.09 individuals for the single +half dose (D2) and 23.33 ± 4.63 individuals for the double dose (D3) respectively. All doses used resulted in lower average numbers of insects than the control. The treatments resulted in lower numbers with 8.66 ± 4.51 (D1), 4.66 ± 3.15 (D2) and 2.23 ± 1.66 individuals (D3) respectively. The percentage of insect reduction at this stage of poultry development was 70.37 (D1), 78, 94 (D2) and 91.30% (D3) respectively. Analyses of variance revealed a significant difference ( $F = 49.42$ ;  $p < 0.05$ ) between the efficacy of the different doses compared to the control.

**Table 1:** Average insect numbers as a function of the doses applied

Chickens' stages evolution	Control (D0)	Dose 1 (D1)		Dose 2 (D2)		Dose 3 (D3)	
	Average number of insects in control level	Average number of insects before application	Average number of insects after application	Average number of insects before application	Average number of insects after application	Average number of insects before application	Average number of insects after application
Start-up 1 stage	0	0	0	0	0	0	0
Start-up 2 stage	6.66 ± 4.17	6 ± 2.08	2.66 ± 0.83	9.66 ± 3.71	4.66 ± 1.33	5.33 ± 1.45	2.66 ± 0.16
Growth stage	47.33 ± 6.38	27 ± 4.35	2.66 ± 2.51	19.33 ± 4.09	0.66 ± 1.1	23.33 ± 4.63	0
Finishing stage	65.66 ± 6.93	38.66 ± 2.72	-	28.66 ± 4.80	-	33.66 ± 4.63	-

##### 3.1.1.3. In the finishing stage

At the finishing phase (45<sup>th</sup> day), statistical analyses revealed a significant difference between the efficacy of different doses used to control insects ( $F = 12.74$ ;  $df = 3$ ;  $p < 0.05$ ). The Newman-Keuls test showed a high reduction in the average

number of insects at the double dose (D3) level (33.66 ± 3.84 individuals). The control blocks had a mean number of individuals of 65.33 ± 7.62. All doses of the products used resulted in a lower average number of insects than the control. Analyses of variance revealed a significant difference ( $F =$

7.58;  $df = 3$ ;  $p < 0.05$ ) between the efficacy of the different doses compared to the control. However, the recolonization rate was significant at 15<sup>th</sup>, 30<sup>th</sup> and 45<sup>th</sup> day before treatment. At the growth and finishing stage, the Newman-Keuls test revealed two homogeneous groups. The control constituted the first group. The second homogeneous group was been constituted by treatments D1, D2 and D3 (Figure 3).

**3.1.2. Effect of *Eucalyptus citriodora* essential oil on chickens**

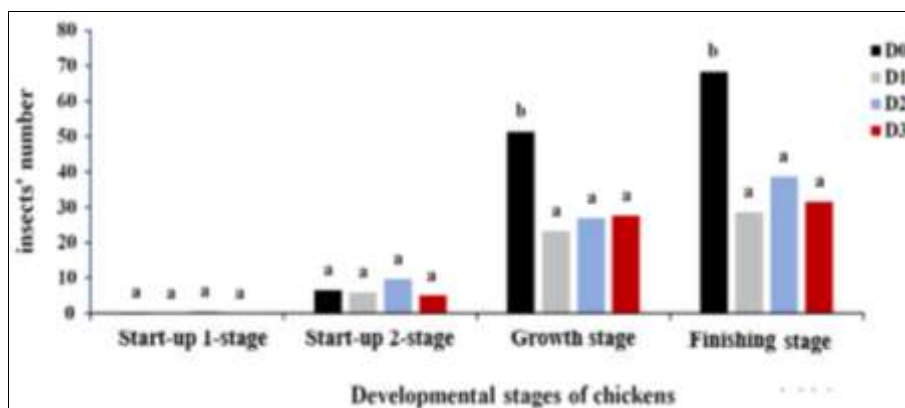
**3.1.2.1. Effect of *Eucalyptus citriodora* essential oil on the general appearance and behavior of chickens**

The chickens showed no change in general physical appearance. Apart from the fact that the chickens moved away

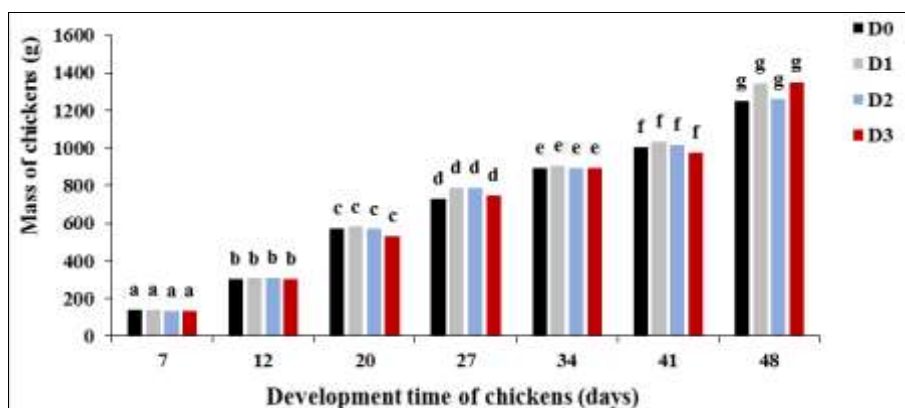
when sprayed, no abnormal behavior was observed during the rearing period.

**3.1.2.2. Effects of *Eucalyptus citriodora* essential oil on the weight growth of poultry**

Monitoring the variation in body mass of the animals in each block during the treatment of the litter with *E. citriodora*'s essential oil revealed that there is an increase in the mass of the birds at the end of their growth both for the treated blocks (D1, D2 and D3) and for the controls (D0). The mean masses of the chickens in the different blocks at the end of rearing were  $1248.762 \pm 32.14$  (D0);  $1343.78 \pm 32.53$  (D1);  $1259.23 \pm 35.87$  (D2) and  $1350.57 \pm 37.59$  (D3), respectively ( $F = 2.43$ ;  $df = 79$ ;  $> 0.05$ ) (Figure 4).



**Fig 3:** Effect of doses of *E. citriodora* essential oil on insects at different stages of chicken's development



**Fig 4:** Effect of different doses of *E. citriodora* essential oil on the weight growth of chickens

D0: Control treatment; D1: Single dose; D2: Single + half dose; D3: Double dose.

**3.1.2.3. Effects of *Eucalyptus citriodora* essential oil on relative organ weights of chickens**

The mean kidney and heart weights of the controls were relatively low compared with those of the treated. The average organ weights (kidney, heart, liver, spleen and lung)

did not vary significantly in the different blocks. The analysis performed showed no significant difference ( $p > 0.05$ ) in organ weights of the chickens treated at the different doses (Table 2).

**Table 2:** Effects of *E. citriodora* essential oil doses on the relative weight of internal organs of chickens in breeding

Doses	Weight in gram (g)				
	Kidney	Heart	Liver	Spleen	Lung
D0	$0.56 \pm 0.03^a$	$0.42 \pm 0.01^a$	$2.89 \pm 0.10$	$0.12 \pm 0.007^a$	$0.55 \pm 0.03^a$
D3	$0.6 \pm 0.01^a$	$0.45 \pm 0.01^a$	$2.69 \pm 0.12$	$0.12 \pm 0.004^a$	$0.63 \pm 0.008^a$
D2	$0.53 \pm 0.04^a$	$0.48 \pm 0.03^a$	$2.71 \pm 0.10$	$0.1 \pm 0.01^a$	$0.49 \pm 0.06^a$
D1	$0.62 \pm 0.03^a$	$0.47 \pm 0.12^a$	$2.35 \pm 0.06$	$0.11 \pm 0.009^a$	$0.6 \pm 0.04^a$
df	3	3	3	3	3
F	1.18	0.13	4.51	1.09	1.88
P	0.37	0.93	0.05	0.40	0.21

**Table 3:** Effects of *E. citriodora* essential oil doses on biochemical parameters of chickens in breeding

Doses	URE (mmol/l)	CR (mg/l)	AST (UI/L)	ALT (UI/L)
D0	0.103 ± 0.003 <sup>a</sup>	03 <sup>a</sup>	325.66 ± 9.95 <sup>a</sup>	06 ± 0.00 <sup>a</sup>
D3	0.10 ± 0.00 <sup>a</sup>	04 <sup>a</sup>	371 ± 2.64 <sup>a</sup>	06 ± 0.00 <sup>a</sup>
D2	0.103 ± 0.003 <sup>a</sup>	03 <sup>a</sup>	319.33 ± 9.35 <sup>a</sup>	06 ± 0.33 <sup>a</sup>
D1	0.103 ± 0.003 <sup>a</sup>	03 <sup>a</sup>	287.66 ± 31.57 <sup>a</sup>	05 ± 0.33 <sup>a</sup>
df	3	3	3	3
F	0.3	-	3.9	3
P	0.80	-	0.05	0.09

D0: Control treatment; D1: Single dose; D2: Single + half dose; D3: Double dose; URE: Urea; CR: Creatinine; AST: Aspartate aminotransferase; ALT: Alamine - Aminotransferese

### 3.1.2.4. Effects of *Eucalyptus citriodora* essential oil on biochemical parameters of chickens: Analysis of

biochemical parameters showed that the mean value of urea, creatinine, AST and ALT varied little in treated chickens (D1, D2 and D3) compared to the control (D0). Statistical analysis showed no significant difference ( $p > .05$ ) (Table 3).

### 3.1.2.5. Effects of *Eucalyptus citriodora* essential oil on hematological parameters of chickens

Hematological analysis showed no significant changes in white blood cell count, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular rate, hemoglobin concentration, platelet, neutrophil and lymphocyte. No significant differences were observed ( $df = 3$ ;  $p > .05$ ) (Table 4).

**Table 4:** Effects of *E. citriodora* essential oil doses on hematological parameters of chickens of breded chickens

Treatment	GB (10 <sup>3</sup> /μl)	GR (10 <sup>6</sup> /μl)	HGB (g/dl)	HCT (%)	VGM (fl)
D0	214.41 ± 6.73 <sup>a</sup>	2.27 ± 0.13 <sup>a</sup>	6.6 ± 0.40 <sup>a</sup>	27.4 ± 0.62 <sup>a</sup>	120.43 ± 0.08 <sup>a</sup>
D3	214.3 ± 22.06 <sup>a</sup>	2.28 ± 0.17 <sup>a</sup>	6.23 ± 0.63 <sup>a</sup>	26.74 ± 1.86 <sup>a</sup>	123.13 ± 2.06 <sup>a</sup>
D2	208.64 ± 19.70 <sup>a</sup>	2.36 ± 0.21 <sup>a</sup>	6.42 ± 0.67 <sup>a</sup>	27.5 ± 2.38 <sup>a</sup>	120.45 ± 1.18 <sup>a</sup>
D1	215.16 ± 29.7 <sup>a</sup>	2.31 ± 0.19 <sup>a</sup>	6.48 ± 0.46 <sup>a</sup>	26.65 ± 1.87 <sup>a</sup>	117.1 ± 1.95 <sup>a</sup>
df	3	3	3	3	3
F	0.02	0.04	0.07	0.05	2.57
P	0.99	0.98	0.97	0.98	0.12

**Table 4: (continued):** Effects of *E. citriodora* essential oil doses on hematological parameters of breded chickens

Treatment	TGM (PG)	TCCH (g/dl)	PL (10 <sup>3</sup> /μl)	PN (10 <sup>3</sup> /μl)	LYM (10 <sup>3</sup> /μl)
D0	28.06 ± 1.0 <sup>a</sup>	24.13 ± 0.24 <sup>a</sup>	5.66 ± 0.33 <sup>a</sup>	194.86 ± 17.65 <sup>a</sup>	10.82 ± 0.88 <sup>a</sup>
D3	28.94 ± 0.23 <sup>a</sup>	23.9 ± 0.49 <sup>a</sup>	5.00 ± 0.00 <sup>a</sup>	197.05 ± 22.88 <sup>a</sup>	9.33 ± 0.44 <sup>a</sup>
D2	27.99 ± 0.23 <sup>a</sup>	23.23 ± 0.44 <sup>a</sup>	5.33 ± 0.88 <sup>a</sup>	191.76 ± 22.29 <sup>a</sup>	8.61 ± 0.37 <sup>a</sup>
D1	27.87 ± 0.38 <sup>a</sup>	23.43 ± 0.23 <sup>a</sup>	4.66 ± 1.20 <sup>a</sup>	203.92 ± 29.59 <sup>a</sup>	8.31 ± 0.39 <sup>a</sup>
df	3	3	3	3	3
F	0.65	1.24	0.31	0.05	3.81
P	0.60	0.35	0.81	0.98	0.05

D0: Control treatment; D1: Single dose; D2: Single + half dose; D3: Double dose; WB: White blood cell; RBC: Red blood cell; HGB: Hemoglobin; HCT: Hematocrit; MGV: Mean corpuscular volume; MGR: Mean corpuscular rate; HCCR: Hemoglobin concentration corpuscular rate; PL: Platelet; NP: Neutrophilic polymorphism; LYM: Lymphocyte

## 4. Discussion

For the control of *A. diaperinus*' population in chicken farms, the *E. citriodora* essential oil was used for treatments in different development phases of broilers (Start-up, growth and finishing). The number of insects collected after each treatment showed that *E. citriodora* essential oil was effective in controlling avian insects with a reduction of insects from 50%(D1) to 60%(D3) at the start-up stage and from 70%(D1) to 91%(D3) at the growth stage. This effectiveness would be related to the insecticidal properties of the compounds of this plant. During the applications, these compounds impregnate the litter and the insects with insecticidal properties contained in the *E. citriodora* essential oil.

These compounds would adhere to the insect cuticles, causing death by contact or consumption, thus reducing the insect population in the litter. These results are similar to those of Salin *et al.* [9], obtained during a study in a farm. This author reported that successive combined treatments of insecticides (Triflumuron + Cyfluthrin) controlled *A. diaperinus* populations throughout the rearing period. In farms trials, pyrethroids (Cyfluthrin and permethrin) exerted significant control on adult individuals throughout the growth period of chickens (42 days) according to Weaver [10]. The work of Bossou *et al.* [11], showed that the biopesticides of *E.*

*citriodora* have insecticidal and bactericidal properties. The main compounds in *E. citriodora*'s essential oil (Citronellal and Citronellol) have an insecticidal and repellent effect on insects [12]. These compounds have been reported to deter *Alphitobius diaperinus* individuals, preventing them from remaining in the litter or causing mortality in the population of this insect. Also, according to Stevenson [13], some plants contain bioactive agents such as phenolics, alkaloids, tannins, flavonoids, steroids and terpenes. These bioactive agents are believed to have insecticidal properties [13]. Gopalakrishnan [14] also showed that these compounds would also have repellent, toxic, deterrent and reproductive inhibitory properties.

However, the results of the work showed a high rate of recolonization of the litter by the insects. Indeed, this high number of individuals of this species at 15<sup>th</sup>, 30<sup>th</sup> and 45<sup>th</sup> day before the treatment because the *E. citriodora* essential oil would have a limited remanence in time, less than the 15 days chosen as treatment interval. In addition, it should be noted that the high treatment intervals (15 days) and abiotic parameters (temperature, relative humidity and atmospheric pressure) favored the reproduction of this insect in the treated blocks and more in the controls. According to Al Dam *et al.* [15], the reproductive cycle of this insect can last more than

two months and its success depends on temperature, relative humidity, moisture content of the litter, and presence of food. In this interval, at the peak of their breeding period, females lay an average of 5 eggs per day [15]. Because chicken coops farms multiple tenebrionids simultaneously, under optimal conditions, their reproductive potential can be enormous. In a chicken farms, the chances of tenebrionid eggs hatching are at their highest at a temperature of 30 °C and a relative humidity of 90% [15-16]. Under optimal conditions, the darkling beetle can reach the adult stage after only 29 days after egg laying and can reproduce almost immediately, which may explain the high numbers of individuals of this species at 30<sup>th</sup> and 45<sup>th</sup> day [15].

For the evaluation of the toxic effect of different doses of *E. citriodora* on chickens during litter treatment, body weight, vital organ analysis, biochemical and hematological parameters of chickens were determined. The treatment of the litter with *E. citriodora* essential oil did not influence the normal development of the poultry. The increase in body weight of the animals suggests that *E. citriodora* essential oil is not toxic. Regarding the organs of the chickens collected according to the treatments, no significant difference was observed in the relative weight of the organs in the treated animals compared to the controls. Biochemical and hematological parameters analyzed revealed no statistical differences. These results suggest that *E. citriodora* essential oil can be used in the control of avian insects without adversely affecting the health of poultry.

## 5. Conclusion

This study evaluated the effectiveness of *Eucalyptus citriodora* in controlling *Alphitobius diaperinus* and the toxic effect of the biopesticide on chickens. It was found that *E. citriodora* essential oil was effective against avian insects. The D3 dose resulted in the highest reduction rates with 60% at the start-up stage and 91% at the growth stage. The litter treatment don't affect negatively the growth of the chickens, vital organs, biochemical, hematological and histological parameters. These results suggest that the essential oil of *E. citriodora* could be used safely to effectively control *Alphitobius diaperinus*' populations in poultry farms. To be most effective, the user should have a good knowledge of the ethology and development cycle of *Alphitobius diaperinus*.

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## 7. Conflict of interest

The authors declare that there are no conflicts of interest between them regarding this work.

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