

# Biocidal Effect of Leaves of *Crataeva religiosa* Forst on a Resistant Strain of Groundnut Bean *Caryedon serratus* (Olivier)

Aminata Gningue, Toffène Diome, Khady Fall, Mbacké Sembène

Genetic Team and Population Management, Department of Animal Biology, Faculty of Sciences and Technology, Cheikh Anta Diop University, Dakar, Senegal

Email: mbacke.semben@ucad.edu.sn

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## Abstract

Groundnut (*Arachis hypogaea* L.) is a legume that is highly coveted by West African populations, particularly those in Senegal. However, it suffers enormous damage caused by a bruchidae beetle, *Caryedon serratus*. This pest rests on its richness in proteins, calories and the absence of major constraints for its production. Losses recorded can reach 83% for a period of 4 months of storage. To counter this damage, several authors have looked for alternative methods to the use of synthetic insecticides, often harmful to animal populations and the environment. In this logic, we tested the biological impact of a leaf-based formulation of plants indigenous to Senegal (*Crataeva religiosa*) on the external forms of *C. serratus*. In this study, analysis of the biological parameters of strain *C. serratus* showed low adult mortality of *C. serratus*. On the other hand, the extract affects the viability of eggs and larvae and fertility is reduced. The effect of the *C. religiosa* plant also results in reduced fertility of surviving females and a sex ratio in favor of the males causing a risk of decreasing population growth. On the other hand, there is an extension of the total development time.

## Keywords

Groundnut, Biological Parameters, Devastator, Senegal

## 1. Introduction

Peasant agriculture occupies 60% of the active population and contributes 20% of GDP. It is dominated by several sectors including the peanut sector [1]. Groundnut (*Arachis hypogaea* L.) is a legume native to South America, probably from the eastern part of Bolivia. It currently occupies a prominent place in the

economic system of Senegal where its culture covers more than half of the cultivable surface. This leguminous crop yields around 80 billion FCFA each year, which represents 40% of the country's total exports [2]. High in protein and calories (50% fat, 25% protein), this oilseed is also a very important nutrient supply for local populations [3]. However, groundnut cultivation in Senegal faces many constraints. Indeed, few insects are able to attack in shell peanuts; among these, the one that causes the most damage in production is a Coleopteran insect belonging to the family Bruchidae: *Caryedon serratus* (Olivier), commonly called peanut brush. The holes left in the hull by the larvae of this pest promote the attack of other pests and facilitate the development of a mold *Aspergillus flavus* producing a carcinogenic substance: aflatoxin. All these losses that occur at all stages, from harvesting to consumption, not only harm farmers but also cost the national economy [4]. In the face of the threat posed by insects, which are the main pests of stocks, farmers often resort to synthetic insecticides, which have a great deal of adverse effects, including the selection of resistant strains [5], poisoning, environmental pollution, environment but also the reluctance of consumers to consume products treated with pesticides [6]. In the face of the perverse effects of synthetic insecticides, several authors today will rely on traditional methods of insect control, by the search for natural substances of plant origin adapted to the reduction of insect-induced damage without endangering the population and the environment [7] [8] [9] [10]. It is in the context of reducing groundnut postharvest losses by alternative methods of traditional control, that we put in place an effective and easily applicable pest management method. The aim of this study is to evaluate the biocidal effect of *Crataeva religiosa* on the main pest of peanuts, *Caryedon serratus*.

## 2. Materials and Method

### 2.1. Harvesting and Conservation of Plant Material

The *C. serratus* strain used in the experiment comes from an infested peanut sample purchased from the weekly market in Kébémér (Louga Region). Groundnuts used for breeding were also purchased in this same market. These peanut seeds are brought back to the Laboratory of Entomology and Acarology of the Faculty of Science and Technology of the University Cheikh Anta Diop of Dakar where they are put in bags and kept in the freezer for 96 hours to eliminate any infestation hidden. The seeds are then placed in glass jars 16 cm high and 8 cm in diameter hermetically sealed to prevent any further infestation. The leaves of *C. religiosa* are harvested around the Department of Animal Biology of the Faculty of Science and Technology of the Cheikh Anta Diop University of Dakar. The harvest is done early in the morning before sunrise to have a concentration of active molecules that act on the insect. After harvest, the leaves of each of the two plants are crushed freshly and used for aqueous extractions by maceration for biological testing. 200 g of fresh leaves of each of the two plants are extracted in 1 L of tap water which is the solvent used. The solutions obtained are placed

in the refrigerator for 5 days to overcome any fermentation and then filtered using a household sieve reinforced with muslin. The aqueous extracts are stored in one-liter bottles. These are placed in the refrigerator and used as needed.

## 2.2. Mass Rearing

Bruchids are raised in the laboratory. Mass rearing is done in cylindrical glass jars (approximately 16 cm in diameter and 8 cm in height), perforated and covered with muslin cloths to allow insects to breathe. Peanut seeds serve as a breeding ground for insects. In each jar, peanut seeds are introduced until its base is completely hidden and a sufficient number of male and female insects. The jars are left in the dark at room temperature. After 48 hours, the seeds that have been laid, are placed in glass Petri dishes where the egg will continue its development cycle until the emergence of the adult. The emergence of adults is noted and monitored every two days in order to respect the cohort and to avoid mixed cohorts of generations. The biological tests were carried out on adults (adulticidal effect) of *C. serratus* resulting from this breeding.

## 2.3. Experimental Protocol of Tests with Aqueous Extracts

We have a Petri dish, an aqueous solution for *Crataeva religiosa* and a synthetic pesticide solution (deltamethrin 25 EC) of different concentrations. C1 = 200 g/500 ml = 0.4 g/ml is the concentration of the mother solution (with the extraction 200 g of fresh leaves in 500 ml of water which is the solvent used) and from which the other concentrations are obtained by dilution (Ndiaye, 2015). C2 = 200 g/L = 0.2 g/mL and C3 = 200 g/1.5L = 0.13 g/mL.

### 2.3.1. Deltamethrin Tests

Deltamethrin is applied at the recommended dose of 40 ml per 30 L of water, based on 1L of water, which makes it possible to determine the Cx concentration from which the other concentrations are drawn:  $C_x = 40 \text{ ml} \times 1 \text{ L}/30 \text{ L} = 1.3 \text{ ml/L}$ ; C1 =  $C_x \times 2 = 2.6 \text{ ml/L}$ ; C2 =  $C_x = 1.3 \text{ ml/L}$  and C3 =  $C_x/2 = 0.65 \text{ ml/L}$ .

### 2.3.2. *Crataeva religiosa* Tests

The adults treated come from mass culture carried out in the laboratory in glass jars; they are older than 72 hours. In each petri dish, 20 g of peanut seed are added. The seeds are then infested with 6 adults of *C. serratus* (3 males and 3 females). For each solution and each concentration, one milliliter (1 ml) is sprayed on the peanut seeds contained in each box. The latter is then slightly shaken for 2 to 3 minutes to ensure the distribution of the solution on the substrate. Three repetitions and two controls (white control and solvent control) are performed for each given concentration. In the white control, adults are in no way in contact with the solutions and in the solvent control, one milliliter (1 ml) of tap water is sprayed onto the peanut seeds. Insects are exposed to aqueous extracts for ten days. Dead bruchids are counted every 24 hours and eggs laid are followed until emergence.

## 2.4. Calculates Parameters and Statistical Analyzes

### 2.4.1. Calculates Adult Mortality of *C. serratus*

After the treatments, daily monitoring is performed for each batch. With manual sieves dead insects are recorded. To correct the natural mortality rate observed in our experimental conditions, the Abbott formula (Abbott, 1925) is used:

$$Mc = \frac{Mo - Mt}{100 - Mt} \times 100$$

where Mo = mortality in the treated lots, Mt = mortality in the control and Mc = calculated mortality.

### 2.4.2. Calculation of Fertility Rate

Fertility rate (TF): This is the ratio between the number of emerged adults and the total number of larvae

$$(TF) = \frac{\text{Number of emerged adults}}{\text{Total number of larvae}} \times 100$$

### 2.4.3. Calculation of Embryonic and Larval Mortality of Offspring

The embryonic mortality rate will be calculated by the following formula:

$$ME = \frac{\text{Number of eggs not hatched}}{\text{Number of total eggs}} \times 100$$

where ME = embryonic mortality.

The larval mortality rate is calculated by the formula:

$$\%ML = \frac{Nmo}{NI} \times 100$$

where ML = larval mortality; Nmo = Number of dead larvae; NI = Total number of larvae.

### 2.4.4. Development Period

This is the time between the hatching of the egg on a seed and the formation of the cocoon.

### 2.4.5. Evaluation of the Sex Ratio (*R*)

The sex ratio (*R*) which gives the percentage of females compared to all the descendants is determined for each test product.

$$R = \frac{\text{Number of emerged females}}{\text{Total number of individuals emerged}} \times 100$$

### 2.4.6. Statistical Analyzes (*R*)

The raw data are recorded in microsoft the Excel spreadsheet as a database for the analyses. Statistical analyzes of the measured variables were performed with *R* software. The resulting data were subjected to a non-parametric test of Wilcoxon and Kruskal-Wallis rank sum test. The first one allowed to determine the significant differences between the calculated means of two samples. The second was used to test the significant differences between the calculate means of many

samples. The difference between two values is considered significant when the p-value is less than 5% ( $p < 0.05$ ).

### 3. Results

#### 3.1. Calculates Adult Mortality of *C. serratus*

The effectiveness of the aqueous extract of the *Crataeva religiosa* powder is noticeable until the second day of application for the C3 concentration (0.13 g/ml) with a mortality of 33%. For the other concentrations, there was no mortality (Figure 1). The statistical analysis of the mortality test is not significant ( $p > 0.05$ ).

Figure 2 highlights a very disproportionate efficacy of Deltamethrin on adults of *C. serratus*. Thus, on the first day of application, the C1 and C2 concentrations gave the highest mortality. This trend reverses from the second day of testing with zero effects for all concentrations (C1, C2 and C3). On the third day only the highest C3 concentration is effective. The lowest concentration is more effective than the others at the fourth and sixth days, with greater efficiency on the sixth day of application (100%). On the fifth and seventh days, only C1 and

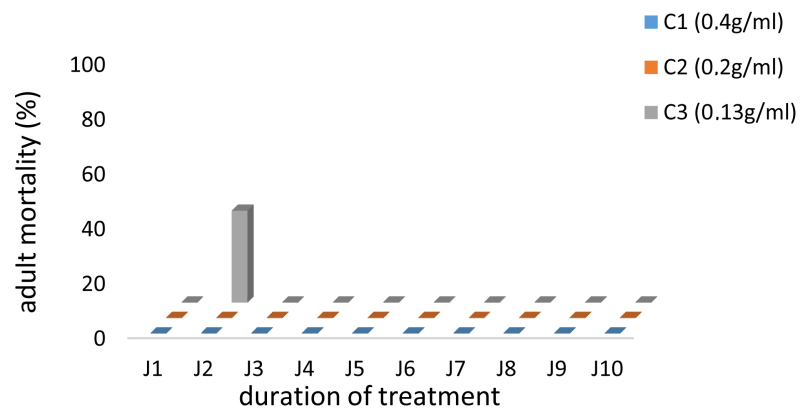


Figure 1. Percent corrected adult mortality of *C. serratus* induced by aqueous extract of *C. religiosa* leaf powder.

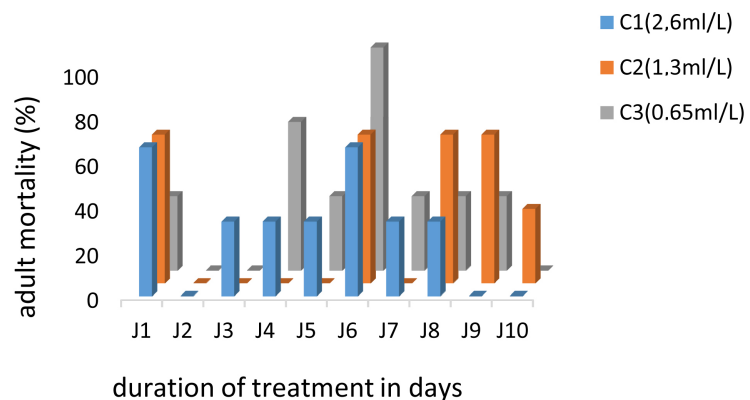


Figure 2. Percent corrected adult mortality of *C. serratus* Induced by deltamethrin insecticide.

C2 concentrations show an efficiency of 33%. Even at the eighth and ninth days, C2 was more effective than other concentrations. On the tenth day alone the C2 concentration is effective on adults of *C. serratus*. The mortality test is statistically significant ( $p < 0.05$ ).

### 3.2. Calculation of the Fertility Rate

The fertility rate of female survivors of *C. serratus* is greater with the treatment of the aqueous extract *C. religiosa* for all C1 concentrations (41%), C2 (46%) and C3 (44%) compared to Deltamethrin which has a rate of 36.5% for C1, 35.7% for C2 and 41% for C3. There is also evidence that controls have a higher fertility rate with more than 50% fertility. The statistical analysis of the fertility test is highly significant ( $p = 0.034$ ) (Figure 3).

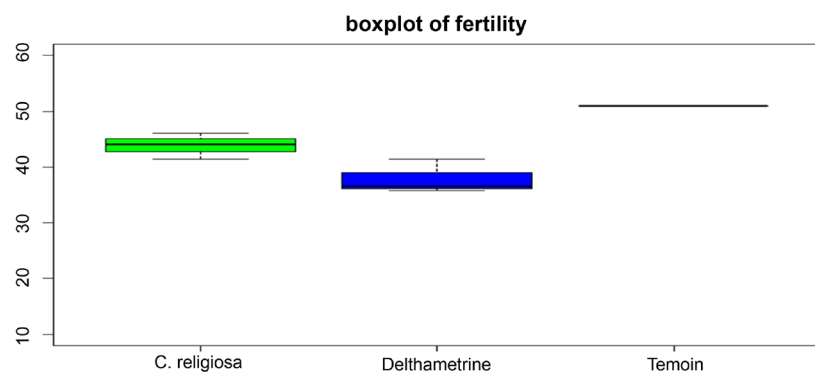
### 3.3. Calculation of Embryonic and Larval Mortality of Offspring

The effect of *Crataeva religiosa* on the viability of eggs from adult survivors of *Caryedon serratus* manifests as concentration-dependent mortality. The rate of unhatched eggs increases as the concentration decreases with 60.76% for C1, 62.53% for C2 and 73.69% for C3. The effect of Deltamethrin is more consistent with higher rates C1 (74.14%), C2 (62.46%) and C3 (75.83%). Statistical analysis is not significant  $p > 0.05$  (Figure 4).

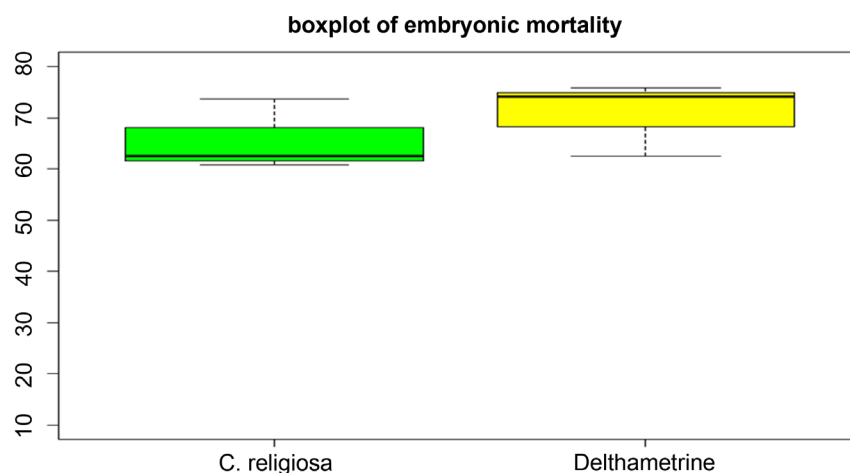
The effect of *Crataeva religiosa* on the larvae from the offspring of adults of *Caryedon serratus* already tested is shown by a mortality of 24.7% for the highest concentration. This level observed in C1 is higher than those observed for the C2 and C3 concentrations with a mortality of respectively 4.69% and 7%. The effect of Deltamethrin is more consistent with higher rates C1 (20.5%), C2 (13.9%) and C3 (24.7%). It is also observed that the highest concentration of *C. religiosa* and the lowest concentration of Deltamethrin have the same larval mortality rate of progeny derived from adulticidal *C. serratus* tests (Figure 5). This test is statistically insignificant ( $p > 0.05$ ).

### 3.4. Development Time

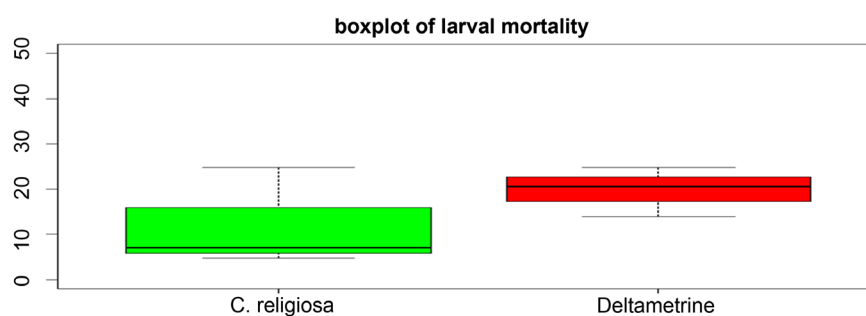
The extracts of *C. religiosa* have a mean spawning/weaving cocoon duration of



**Figure 3.** The fertility rate of *C. serratus* females rescued from adulticide tests of the aqueous extract of *C. religiosa* leaf powder and deltamethrin.



**Figure 4.** Mortalité embryonnaire de la descendance des adultes de *C. serratus* testés par l'extrait aqueux de la poudre de feuilles de *C. religiosa* et de la Deltaméthrine.



**Figure 5.** Larval mortality of adult progeny of *C. serratus* tested by the aqueous extract of *C. religiosa* leaf powder and deltamethrin.

$68.11 \pm 3.15$  days with a minimum duration of  $65.67 \pm 2.52$  days in C2 and maximum of  $71.67 \pm 10.26$  days in C3. For pupation, we have an average of  $22 \pm 3.71$  days with a minimum of  $18 \pm 3.60$  days for C3 and a maximum of  $25.33 \pm 2.51$  days for C2 (Table 1 and Table 2).

### 3.5. Evaluation of the Sex Ratio (*R*)

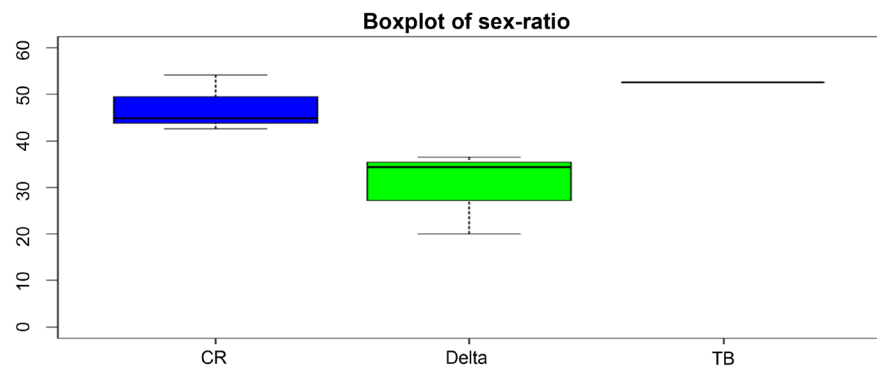
The analysis in Figure 6 highlights the effect of different plants on the sex of adult survivors from adulticidal tests with the application of all concentrations. It turns out that the sex ratio is in favor of the males of *C. serratus* with the application of *C. religiosa* for the C1 and C2 concentrations at the time when the C3 sex ratio is in favor of the females. We notice that this favor increases with the decrease of the concentration. Thus, the lowest concentration gives a sex ratio of 54% at the time when the other concentrations give respectively 44% (C2) and 42% (C1) of sex ratio. Deltamethrin induced a sex ratio in favor of groundnut mussel males for the application of all concentrations. Only C3 (54%) of *C. religiosa* gave a sex ratio higher than that given by controls (52%) in favor of females. The sex ratio revealed a significant difference with the application of different plants ( $p = 0.04$ ).

**Table 1.** Larval development time.

Products	Larval development time (days) $\pm$ standard deviation			
	C1 (0.4 g/ml)	C2 (0.2 g/ml)	C3 (0.13 g/ml)	Average
<i>C. religiosa</i>	67 $\pm$ 1.73	65.67 $\pm$ 2.52	71.67 $\pm$ 10.26	68.11 $\pm$ 3.15
Deltamethrine	46.67 $\pm$ 41.63	48 $\pm$ 43.26	44.33 $\pm$ 38.42	46.33 $\pm$ 1.85

**Table 2.** Nymphal development time.

Products	Nymphal development time (days) $\pm$ standard deviation			
	C1 (0.4g/ml)	C2 (0.2 g/ml)	C3 (0.13 g/ml)	Average
<i>C. religiosa</i>	22.67 $\pm$ 0.57	25.33 $\pm$ 2.51	18 $\pm$ 3.60	22 $\pm$ 3.71
Deltamethrine	29.5 $\pm$ 23.33	12 $\pm$ 2.82	23.5 $\pm$ 4.94	21.67 $\pm$ 8.89

**Figure 6.** Sex ratio of adults from eggs treated with aqueous extract of leaves of *C. religiosa* plant and deltamethrin insecticide.

#### 4. Discussion

The specific purpose of this study is to evaluate the biocidal effect of aqueous extracts formulation of *C. religiosa* on adults of *C. serratus*. The results indicate that *C. religiosa* induces low mortality of *C. serratus* in the adult stage. The extracts of *C. religiosa* caused a mortality of 5.55% with the C3 concentration. The highest concentrations of C1 and C2 did not give mortality. On the other hand synthetic insecticide, Deltamethrin caused less than 50% in 10 days. The weak activities of extracts of *C. religiosa* could be explained by the fact that adults are derived from resistant strains on the one hand and secondly by the fact that the laying substrate (peanut sheaths) completely absorbs the solution after only a few minutes of application. In any case, by analyzing the adulticidal effects, it is clear that the nature of the product (solid, solution or oil) plays a preponderant role in the results obtained. For example, the results of Kandji [11] show that Neem solids (powder, leaf and almond) do not have adulticidal activity in *C. serratus*. The effects of plant extracts on the fecundity of female survivors of *C. serratus* depend on the dose applied. The results obtained show that the plants reduce the fertility of *C. serratus* females at the low C3 dose. Indeed the average is reduced compared to the witnesses. These results are in line with those of many authors.



For example, Saxena [12] reports that females of *C. serratus* in contact with Neem extracts have greatly reduced fecundity and spawning. According to Kel-louche and Soltani [13] on chickpea seeds, the leaf powders of four plants rich in essential oils (fig, olive, lemon and eucalyptus) reduce the fertility of female *Callosobruchus maculatus*, while that the essential oils extracted from the clove completely inhibit the oviposition. We also note that Deltamethrin has a great influence on the number of eggs laid, which results in a large reduction, or a complete absence of spawning at certain rehearsals during treatment regardless of the concentration used. Extracts of *C. religiosa* also affect the viability of *C. serratus* eggs. Results on egg-laying mortality by females show that there is a concentration-dependent effect. With the extract of *C. religiosa*, the percentage of unhatched eggs increases as the concentration decreases. The highest embryonic mortality is noted in C3 with 73.69%. These results are similar to those of El Atta and Ahmed [14] who observed in the same species, the hatching of the eggs of the peanut shrub was significantly reduced by the oil extracts of the Eucalyptus leaves. *Camaldulensis* (Dehn) and oil of *Azadirachta indica* seed (A. Juss). The effects of the extracts also influenced the viability of the larvae. Thus, they induce larval mortality of offspring which decreases as the concentration decreases with a rate of 24.7% for C1. The same trends are noted for deltamethrin, which has levels below 25%. In our study conditions, the extracts of *C. religiosa* affect the viability of *C. serratus* eggs from treated adults. The monitoring of eggs from adults treated through their different developmental phases revealed no difference compared to controls, however there is an increase in the duration of development of different stages of *C. serratus* compared to those listed in Literature. This could be due to the temperature and humidity conditions in which the tests were performed. Indeed, with the extracts of *C. religiosa* we obtained a mean spawning/weaving cocoon duration of  $68.11 \pm 3.15$  days with a minimum duration of  $65.67 \pm 2.52$  days in C2 and maximum of  $71.67 \pm 10.26$  days in C3. The work of Ndiaye [3] and those of Delobel and Tran [15] indicate larval development between 40 and 58 days depending on temperature and relative humidity conditions. Gueye [16] reveals in his studies a larval stage duration of about 45 days on average at 35°C. Regarding pupation, although there is no significant difference between plant extracts and controls, we have an average of  $22 \pm 3.71$  days with a minimum of  $18 \pm 3.60$  days for C3 and a maximum of  $25.33 \pm 2.51$  days for C2, these results corroborate those of the work of Thiaw (2008) [17] which shows a duration of nymphal development which varies 21.33 and 33.43 days with the extract and the methanolic fraction of *Calotropis procera* and *Senna occidentalis* on the same insect. In this study, there is a spread of emergences, the interval between the first and last outings being able to reach more than one month. This difference could be a quiescent mechanism inside the cocoon. The effect of the *C. religiosa* extract on the sex ratio decreases but remains in favor of the males for the C1 and C2 concentrations while the lower concentration increases and turns in favor of the females which would increase the risks of increase in population. These results corroborate those of

Thiaw and Sembene [18] which showed the effect of extracts of *C. procera* on the insect *C. serratus* expressing a sex ratio in favor of the males which causes a decrease of the increase of the population.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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