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## Biopesticide activity of crude extracts and fractions of *Calotropis procera* Ait. towards the groundnut seed-beetle *Caryedon serratus* Ol. (Coleoptera, Bruchidae)

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### ABSTRACT

In preparation for designing a strategy of integrated management associated with post-harvest products in Senegal, the bioactivity of crude and extracts of *Calotropis procera* Ait. on the survival of eggs and adults *Caryedon serratus* (Ol.) pest of groundnut, *Arachis hypogaea* L., was studied under controlled conditions. The crude extracts (ether and methanol) and fractions of extract (hexane, ethyl acetate and methanol) were applied through contact with *C. serratus* at different concentrations C<sub>1</sub> (0.1 g of dry residue / ml of solvent dilution), C<sub>2</sub> (0.01 g/ml) and C<sub>3</sub> (0.001 g/ml). The action of each biocid extract of *C. procera* on the survival of treated is dependent on the concentration and varies with the polarity of the extract. All concentrations induce high mortality of *C. serratus* eggs and adults regardless of the sample in use. The most important (86.14 ± 4.21%) adulticidal action was noted in 10 days of treatment with the methanol fraction. The adulticidal effect of crude methanol extract on *C. serratus* was in the range of 74.10 ± 2.82%. The biological activity of extracts of *C. procera* on the insect is also expressed by an imbalance of sex-ratio in favor of males, an extension or a reduction in the duration of the phases of development, reduced fecundity and fertility limited. The fecundity averages, less important, were obtained with the hexane fraction (44.83 ± 3.01 eggs) and high concentrations of ethyl acetate fraction (35 ± 0.71 eggs) or a respective rate of reduction of spawning 52.93% and 63.25% compared to the control. After characterization of reactions, it is clear from this study that the bioactivity of extracts of *C. procera* is likely related to suspected active compounds (alkaloids, flavonoids, tannins, anthracene derivatives, saponins, sterols and terpenes) as revealed from staining or precipitation using specific reagents.

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**Keywords:** *Caryedon serratus*, groundnut, *Calotropis procera*, biocides plants, extraction, ether, methanol, hexane, ethyl acetate.

### INTRODUCTION

Groundnut (*Arachis hypogaea*) is a herbaceous, annual legume, originating from Peru. Currently, it is cultivated in sub-tropical savanna regions in sub-saharan Africa, USA,

Middle East and Asia. It was introduced into Africa from Brazil by the Portuguese at the end of the 15th century (Adrian and Jacquot, 1968 ; Perhaut, 1976). The grain contains 38-50% oil and is also rich in protein. It is

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consumed by humans as grain, flour, paste and oil. Groundnut cake and foliage are used as animal fodder and the oil is also used in soap production.

The largest West African groundnut producer since 1840 is Senegal (Adrian and Jacquot, 1968). In Senegal, the crop is mostly rain fed with a maximum harvest of about one million tonnes of pods. Most groundnuts are marketed and stored as part of a campaign of groundnut commercialisation previously called "Traite" and which involves State and private organisations. Only a small part of the total groundnut production is stored by farmers and traders. Of the groundnut production in Senegal, 15% is used for domestic consumption and for artisanal oil production, 10% for seed grain and 75% for oil, processed industrially and destined mostly for export markets. Plant breeders have selected several varieties according to the length of their growing times, adaptation to different agro-ecological zones and final product, i.e. for grain consumption or oil production. The main constraints to groundnut storage are attack by rodents, especially *Xerus erythropus* Desmarest and *Cricetomys gambianus*, insects such as *Elasmolomus sordidus* (Heteroptera: Lygaeidae) and the groundnut bruchid *Caryedon serratus* (Coleoptera : Bruchidae) and mould, especially *Aspergillus flavus* which produces aflatoxin, a carcinogenic mycotoxin causing serious contamination of groundnut products.

The first infestations of stored groundnuts by the bruchid *Caryedon serratus* (Olivier, 1790) were reported in this country at the turn of the XXth century (Sembène et al., 2008). *C. serratus* has a wide distribution in Africa, from Senegal to South Africa, and in southern Asia (Johnson, 1986). Its larvae feed in the seeds of wild Caesalpiniaceae belonging to a small number of species in four genera: *Bauhinia*, *Cassia*, *Piliostigma*, and *Tamarindus* (Borowiec, 1987). Commonly known as the groundnut seed-beetle, *C. serratus* is nowadays responsible for important weight losses in stored groundnuts, reaching in Senegal up to 83% in four months storage (Sembène et al., 2010). About 60

years after its first record as a pest of groundnut in West Africa (Roubaud, 1916), it has become a major primary groundnut pest in Central Africa (Matokot et al., 1987) and Asia (Dick, 1987).

The syntheses of insecticides used to fight against pests' stocks present potential risks to human health, the environment and their high cost are forcing farmers to use new techniques for traditional control and to revive the interest of specialists to guide their thinking on the use of biocidal substances of plant origin.

African farmers have been using since ancient times, the homogenates of organs of certain plants repellent properties and / or insecticides. In *Caryedon serratus*, the use of products derived from plants repellent or insecticide lags behind other weevils such as *Callosobruchus maculatus* F. (Seck et al., 1993 ; Seck, 1994 ; Alzouma, 1995 ; Mahgoub, 1995), *Callosobruchus analis* F. (George and Patel, 1992), *Callosobruchus chinensis* L. (Pandey and Singh, 1995).

This study is part of the protection of groundnut, evaluates *Caryedon serratus* on the bioactivity of crude extracts and fractions of extract of a plant very common in Senegal: *Calotropis procera* Ait.

## MATERIALS AND METHODS

### *Caryedon serratus*

The specimen comes from pods of *Bauhinia reticulata* (Mabberly, 1993). The collection was made in the village of "Keur Baka" 22 km south of the region of Kaolack (14° 09' N - 16° 04' W) during the period from January to May where populations of Bruchids are more abundant. These pods are then kept in the laboratory in plastic bags at room temperature for at least two months. The cocoons formed outside are isolated in Petri dishes. The adults that emerge from these cocoons will be farmed.

Mass breeding is carried out in cylindrical glass jars (about 18 cm high and 7 cm in diameter) with a screen cover. The seeds of groundnut and *B. reticulata* serve as substrate for insect development. This choice is based primarily on their availability, accessibility and also on the results of

morphometric and genetic study obtained by Sembène and Delobel (1996, 1998) which showed that the infestation of newly harvested peanuts in Senegal was mainly due to females of *C. serratus* from seeds of *B. reticulata* and those that develop in residual peanut pods attics.

In each jar, we have introduced a handful of seeds, a sufficient number of adults (males and females), cotton soaked in distilled water and a paper folded in a zigzag pattern that allows insects can easily move inside the jar. After 48 hours, seeds receiving the egg, are placed in Petri dishes in glass where the egg will continue its development cycle until the emergence of adults. The adult emergence was recorded and monitored every two days to observe the cohort and avoid lots of mixed generations. Maintaining the cleanliness of farms and determination of their duration in the laboratory were provided. The optimum temperature and humidity are respectively 32-33 °C and 45-50% (Gueye, 2000). Biological tests performed on adults (adulticid effect) and eggs (ovicidal effect) of *C. serratus* are from the 5th and 6th generation of this breeding.

#### ***Calotropis procera* Ait.**

We chose to test the biocidal activity of *Calotropis procera* on pest *C. serratus*. The reasons for this choice are based partly on the results of a survey conducted in rural areas that showed that farmers use the shredded bodies of the plant to protect their crops and secondly, on the fact that it is very common in Senegal and easily accessible.

Harvesting is done during the period from October to January, in several areas in the region of Dakar including the cornice along the West, the North Channel Clearing (VDN), borders the highway behind the stage "Léopold Sedar Senghor" where *C. procera* is present in abundance. Leaf samples are collected, processed, dried at room temperature away from sunlight (greenhouse) and then transformed into powder by crushing and sieving. The drying time of leaves of each species varied depending on their size. *C. procera* leaves are cut into small pieces, allowing the reduction of water content during

the drying process, which lasted 15 days. The powders obtained using a power grinder used to extract the biocides.

#### **Extraction of biocides *C. procera***

For biocidal substances (crude extracts and fraction of extract) of *C. procera*, we conducted a cold maceration.

##### ***Crude extract***

For the crude extracts of *C. procera*, a cold maceration is done either with petroleum ether or with methanol. It was to put into prolonged contact with 100 g of powder to 500 ml solvent (petroleum ether or methanol) in a sealed jar with foil and lid. The mixture is then placed at 25 °C. After a maceration time (48 h), the contents of each jar were filtered through a funnel and filter paper. The filtrate is evaporated using a rotary evaporator to obtain a dry residue called crude extract. Each crude extract was divided in pillboxes then placed in a desiccator. Their contents will be used to implement concrete testing biological and phytochemical analysis.

##### ***Fractions of crude extract in methanol***

This step is to take successively the crude methanol extract, obtained directly from leaf powder, in three solvents of increasing polarity which are hexane, ethyl acetate and methanol. To do this, first maceration was done with hexane. It involved mixing the dry residue with methanol 500 ml of hexane for 24 h and then shaking and straining. The hexane phase is evaporated to dryness at 45 °C using a rotary evaporator. The dry residue was placed in a desiccator to remove all traces of solvent. The remaining sample form of dry pulp was kneaded a second time with 500 ml of ethyl acetate for 24 hours under the same conditions as before. A dry residue called ethyl acetate fraction was recovered after filtration and evaporation of the mixture. What remained of the crude extract to look more and more dry paste was dissolved in a volume of 500 ml of methanol, solvent polarity higher than hexane and ethyl acetate. The final solution was then evaporated to give a residue called dried methanol fraction, different from the crude extract in methanol. Each fraction extract (hexane, ethyl acetate or methanol) was collected in pillboxes which

were then placed in a desiccator to remove any trace of solvent. Their contents will be used to implement the bioassays.

After screening, the extracts of biocides having shown a strong insecticidal efficacy were characterized to identify the main chemical families of bioactive natural products responsible for ovicidal and adulticidal effects.

### **Biological testing**

The objective of the bioassay is to assess the biocidal activity of extracts of *C. procera* on egg and adult stages of *C. serratus* commonly known as groundnut bruchid and descent "survivor" first generation. Similarly, the reproductive activity of adult "survivors" of the insect pest will be discussed including that of females hatched from eggs treated with extracts of *C. procera*. This is the crude extract with light petroleum, crude methanol extract and its fractions hexane, acetate and methanol obtained by cold maceration.

### **Preparation of test solutions**

After a preliminary test which revealed that water and diethyl ether are not toxic to *C. serratus* (less than 5% mortality), diethyl ether was chosen as solvent for the dilution of the crude petroleum ether extract, hexane and ethyl acetate fractions. The crude methanol extract and the methanol fraction were diluted in water.

For each dry residue, three doses ( $C_1 = 10^{-1}$  gram of dry residue / ml of solvent dilution;  $C_2 = 10^{-2}$  g/ml;  $C_3 = 10^{-3}$  g/ml) are applied (Thiaw, 2004). Two control groups were used: one white witness and a witness solvent. In the control blank (TB), the weevils have undergone no treatment. By cons in the solvent control (TS), weevils were treated with solvent dilution testing products. The adulticidal and ovicidal effects of *C. procera* were evaluated on the basis of these test solutions.

### **Ovicidal effect of extracts of *Calotropis procera***

In these bioassays, the test solutions are applied by contact with the eggs laid by females of *C. serratus* and aged 24 hours at most.

### **Choice of eggs**

After more than four generations of mass rearing, females of *C. serratus* were made to lay on peanuts, which previously cleared of all infestation through a prolonged stay in the freezer. After 24 h, each seed is carefully observed under a binocular microscope to ensure that it has received only one egg. If a seed receives more than one, the others are detached with fine forceps so that there is no larval intraspecific competition.

### **Operative study**

To assess the ovicidal effect of *C. procera*, the same experimental design was adopted. The crude extract with light petroleum or crude methanol extract and fractions of hexane, ethyl acetate and methanol were used; three doses and two control groups were predefined. For each concentration and each control, 48 healthy peanut seeds, each bearing an egg under the age of 24 hours are introduced in a Petri dish 9 cm in diameter and 1.5 cm in height. We added 2 ml of the solution of the extract and then shook the box to impregnate uniformly seeds. These are dried for several minutes under a stream of air to evaporate the solvent dilution (Gbolade and Adebayo, 1994). In total, the ovicidal tests with extracts of *C. procera* were based on 1200 eggs of *C. serratus*.

The next day, the infested seeds were placed in rectangular plastic boxes. Each of them has 4 rows of 6 wells (home) numbered with letters and numbers in index from 1 to 6. For each dose, two boxes are filled. All the boxes are placed on the laboratory bench and checked daily. The experience took place at room temperature between 29 °C and 35 °C and 47 to 92% Relative Humidity.

This device allows the study to track individual eggs. For each seed, the laying date corresponds to the day before the start of the experiment is mentioned. The same applies to the dates of hatching to cocoon formation and emergence of adult survivors. It becomes easy to calculate some biological parameters such as percentages of mortality of eggs, larvae and the rate of total mortality.

These deaths are then corrected by Abbott's formula (Abbott, 1925), which gives

values corrected mortality percentage based on the mortality of treated samples and that of the control. This correction allows to exclude bias due to natural mortality observed in our experimental conditions.

#### **Follow eggs "survivors"**

The eggs of *C. serratus* managed to hatch after treatment were followed individually for calculating a number of developmental parameters such as:

- The egg-hatching period which represents the stage of embryonic development;
- The duration of the outbreak-woven cocoon or larval development taking place mainly within the seed;
- The weaving-emergence duration or pupal stage;
- The length-nesting emergence or development phase covers the total time between laying and the emergence of adults;
- The lifetime represents the time interval between egg laying and death in adults.

All these data are regularly recorded on forms developed for this purpose.

#### **Reproductive activity of adult "survivors"**

Adults are called "survivors" from insect eggs of *C. serratus* previously treated. Their follow-up was conducted to evaluate the bioactivity tests that the extracts could submit their reproductive activity. The study will examine a number of biological parameters, such as:

- The sex-ratio of adult "survivors";
- The duration of pre-oviposition period, oviposition and post-oviposition females "survivors";
- The fecundity and fertility of female "survivors".

The sex-ratio means the compared rate to males and females for a given generation. It is an important biological index, because the proportion of male and female can affect reproductive success. For this, a sexing of adults emerging is necessary and will be by observation of the last abdominal segment which is curved in the male and the female lying.

Mating between male and female is then performed. Each pair is placed alone in a Petri dish with numbered as spawning substrate peanuts healthy. The pre-oviposition period is the period between the emergences of females at the time of first spawning. The presence of eggs indicates the end of the pre-oviposition. The fertility of female "survivors" was followed to estimate the bioactivity of extracts biocides *C. procera* in the maintenance and evolution of populations of *C. serratus*. For the importance of laying, the number of eggs laid on the walls of the jars and the seeds from each female was counted ones daily under stereomicroscope. Thus infested seeds are replaced by other perfectly healthy. It should be noted that the conditions of lack of food and water are applied to these young adult survivors. The experiment is performed at room temperature in the laboratory. The couples stop with their death, and the total lifespan of adults of *C. serratus*, the reduction rate of egg laying compared to the control is given by the following formula:

$$Tx = 100 \times \frac{Nt - Ne}{Nt}$$

Where  $Tx$  = rate of reduction compared to the control;  $Nt$  = number of eggs in the cookie jar and  $Ne$  = number of eggs in the test.

#### **Adulticidal effect of extracts of *C. procera***

Different *C. procera* extracts are directly tested in young adults of *C. serratus* reared mass. The treatment was done in Petri dishes in glass 90 mm in diameter and 15 mm box which was placed in a Whatman filter paper previously soaked with 3 ml of test solution. For each sample (crude or fraction), the three predefined levels are applied, a white witness and a witness with the solvent dilution. For each concentration (0.1, 0.01 or 0.001g.ml<sup>-1</sup>) and for each control group, 12 adults (6 males and 6 females) aged less than 24 hours are introduced in a Petri dish. The device was repeated thrice.

Individuals living and the dead are counted two, six, twelve and twenty-four hours after the start of handling. Thereafter, mortality was assessed every 24 h. The proportion of adult deaths [(number of deaths/

number used) x 100] is calculated for each concentration. The results are corrected using the formula of Abbott (1925) and subsequently exploited in the form of graphs and tables. In total, the five biocides *C. procera* adulticid tests focused on 900 individuals.

### Statistical analysis

The results expressed in percentages of unhatched eggs, larvae or non-living and dead adults in the form of tables or graphs are constructed using the software EXCEL. The statistical analysis was performed with XLSAT software version 6.1.9, SPSS and Statview. The raw data were subjected to analysis of variance (ANOVA), averages ( $\pm$  sd) are compared with tests of multiple comparisons by Newman-Keuls, Fisher, Tukey and Bonferroni. P values below 0.05 were considered significant.

### Phytochemical analysis of extracts of *C. procera*

Following laboratory tests, the crude and fractions of extract of *C. procera*, which showed a high toxicity towards the insect *C. serratus* egg stages and/or adult, have been characterized to identify the main chemical families of bioactive natural products. The phytochemical analysis was carried out through precipitation reactions for alkaloids and staining for flavonoids, tannins, anthracene derivatives, sterols and terpenes. The saponin content of these drugs was made by measuring the foam index (Im) on the decoction to 1%. The results are classified according to: Positive result: + + +; Average result: + + ; Trace :  $\pm$  Negative test : --

## RESULTS

### Laboratory tests

#### Ovicidal effect of extracts of *C. procera*

Figure 1 shows that the share of each test sample from *C. procera* on the survival of embryos of *C. serratus* is an increasing function of concentration and varies from one sample to another. The rate of embryonic mortality obtained at different doses show that *C. procera* significantly reduces the hatching

of *C. serratus* compared to the control ( $13.54 \pm 1.47\%$  on average).

For the sample with light oil, embryo highest mortality (81.25%) is obtained with the highest concentration ( $C_1$ : 0.1g/ml). The doses  $C_2$  (0.01g/ml) and  $C_3$  (0.001g/ml) which cause embryonic mortality were not significantly different ( $p > 0.05$ ) respectively with 62.50 and 56.25%. This homogeneous group was statistically different from that  $C_1$  (Figure 1). Mortality of larval 22.22% and 11.11% respectively were also noted in 0.1 and 0.01g/ml while the low dose (0.001g/ml) produced no mortality to eggs survived (Figure 2).

The crude methanol extract killed an average of  $55.55 \pm 7.88\%$  of treated eggs. Indeed, embryonic mortality of 64.58% was obtained with the highest concentration 0.1 g/ml. The concentrations  $C_2$  and  $C_3$  respectively induce ovicidal activity of about 52.08 and 50%, which is statistically different ( $p < 0.05$ ) from that recorded in  $C_1$  (Figure 1). Larval mortalities of 5.88 and 12.5% respectively are also noted in  $C_1$  and  $C_3$  while the intermediate dose of  $C_2$  did not induce larval mortality on eggs which have come to hatch (Figure 2).

The hexane fraction causes  $C_1$  a mortality rate of 62.50% of the eggs of *C. serratus*, whereas  $C_2$  and  $C_3$  egg mortalities were respectively about 56.25 and 52.08%. This homogeneous group was statistically different from that of  $C_1$  (Figure 1). Regarding the dead larvae, against all odds, it is at the intermediate concentration ( $C_2$ ) that we observed larval mortality of about 9.52% (Figure 2).

The same pattern obtained with ovicid effect with petroleum ether, methanol extract and the hexane fraction was observed with the ethyl acetate fraction of *C. procera*. The dose causing maximum mortality (54.17%) in eggs was also there in 0.1 g/ml. The concentrations  $C_2$  and  $C_3$  cause the same rate of embryonic mortality (45.83%) which was significantly different from that observed in  $C_1$ . A larval mortality of 3.85% is noted at  $C_3$  (Figure 2). The doses of  $C_1$  and  $C_2$  did not induce mortality on larvae.

*C. procera* methanol fraction causes embryonic mortality averaged about  $38.19 \pm 5.24\%$ . 16.67% and 25% of larval mortality were also recorded respectively with low doses of  $C_2$  and  $C_3$ . We can assume that methanol was the fraction that caused most larval mortality but also the least mortality of eggs. It would take longer before arresting larval development (Figure 2).

Comparative analysis of embryonic mortality, induced by different extracts of *C. Procera*, shows greater effectiveness of ether extracts ( $p < 0.05$ ) whatever the dose of the usual solution (Figure 3). The methanolic fraction considered as the most polar product induced the lowest embryonic mortality. However, it caused the highest larval mortality. Moreover, whereas both subsets of crude extracts and fractions from the crude extract methanol other hand, we find that the apolar biocidal products were more toxic to eggs of *C. serratus* than polar. Thus we can say that the ovicidal effect would depend on the polarity of the extracts applied as it was with the usual dose (Figure 3). These results also show a more pronounced larvicidal action with the polar extracts of which suggests the possibility of the existence of a positive correlation between efficiency of the applied sample and stage of development.

#### **Follow eggs "survivors"**

Table 1 shows that the bioactivity of each sample of *C. procera*, on the average durations of different phases of egg development "survivors" of the insect *C. serratus*, is not a function of increasing concentration but varies from one sample to another. Compared to controls, the extracts of *C. procera* cause either a lengthening or shortening of the average duration of life stages from egg survivors (pretreated and succeeded in hatching).

The study parameters of the development cycle of these individuals has survived in the control of the average time laying/hatching ( $6.1 \pm 0.3$  days), hatching/weaving of the cocoon ( $34.27 \pm 5$  days), weaving the cocoon/emergence ( $19.1 \pm 8.67$  days) and life cycle of the adult ( $119.55 \pm 18.79$  days).

For the parameter laying/hatching, this is the stage of embryonic development, the crude extract in methanol and the methanol fraction induced by the longer average duration which was  $10.88 \pm 0.18$  days, while the ethyl acetate fraction caused an average embryonic development for a shorter duration (6 days).

The average duration outbreak/cocoon weaving the longest were obtained with the hexane fraction ( $54.88 \pm 1.36$  days) and ethyl acetate ( $57.34 \pm 1.36$ ). The ether extract oil induces larval development time is shorter, which was  $29.71 \pm 3.14$  days.

Regarding the pupal stage, extracts of low polarity (ether, hexane and acetate) induce the longer development times ( $31.35 \pm 2.35$  days) while the other polar extracts only provoke durations of  $21.58 \pm 0.71$  days average.

Regarding the whole life of these surviving eggs, the methanol fraction induced the longest period ( $152.66 \pm 15.12$  days). However, the shortest periods are marked with the ether extract ( $115.02 \pm 8.13$  days) and the hexane fraction ( $115.02 \pm 4.23$  days).

#### **Reproductive Activity of adult "survivors"**

The study on the reproductive activity of adult survivors has led us determine the sex-ratio in the first generation, fecundity and fertility of female survivors.

#### **The sex-ratio of adult survivors**

Compared with the control indicating a sex-ratio of 0.03, *C. procera* induces sex-ratio nearer to 1, but more often in favor of males. This rate compared to male and female for the first generation of survivors varies from one sample to another. The ether extract induces the sex-ratio of the highest ( $0.40 \pm 0.16$ ) while the ethyl acetate fraction caused a sex-ratio lower  $0.09 \pm 0.01$ . The methanol extract and its fractions hexane and methanol reports induce sex-ratio statistically homogeneous around  $0.11 \pm 0.2$  (Figure 4). The analysis of Figure 5 shows, in general the effect of each extract of *C. procera* on the sex-ratio of adult survivors of *C. serratus* that is not a function of increasing concentration. However, the extract petroleum ether  $C_1$  shows a report sex-ratio of 0.40 significantly higher than those recorded in  $C_2$  (0.14) and  $C_3$  (0.11).

### The fertility of female survivors

Table 2 shows that all extracts biocides *C. procera* significantly reduce the fertility of female survivors of *C. serratus* compared to the control. The average fertility of the least important were obtained with the hexane fraction ( $44.83 \pm 3.01$  eggs) and ethyl acetate ( $35 \pm 0.71$  eggs in  $C_1$  and  $C_2$ ), a respective rate of reduction of egg 52.93% and 63.25% compared to the control. This reduction of egg laying is not a function of increasing concentration but varies from one sample to another. However, the ethyl acetate fraction showed a more decisive effect concentration because the number of eggs was inversely proportional to the applied dose. The concentrations  $C_1$  and  $C_2$  cause puntits respective averages of  $35.5 \pm 12.02$  and  $34.50 \pm 0.71$  eggs, a rate reduction of spawning respective 62.73% and 63.78% while the low-dose  $C_3$  induced the highest fertility ( $85 \pm 2.83$  eggs), a rate reduction of 10.76% for a period of oviposition approximately 7 days (Figure 6).

The crude extract with light petroleum gave an average of eggs from  $63.83 \pm 4.65$ , with a maximum nesting of  $69 \pm 2.83$  eggs in  $C_2$  for a oviposition period of 72 hours and spawning at least  $60 \pm 5.66$  eggs in  $C_1$  for a period of oviposition than 120 hours. For the crude methanol extract, the average fertility was  $67.33 \pm 9.41$  eggs, with a maximum nesting  $73.50 \pm 0.71$  eggs in  $C_2$  and minimum of  $56.50 \pm 0.71$  eggs in  $C_1$ . The methanol fraction caused an average fecundity of  $63.17 \pm 2.36$  eggs, with a maximum nesting of  $65 \pm 8.19$  eggs in  $C_3$  and minimum of  $60.5 \pm 2.12$  in  $C_1$  for a period of oviposition 72 h. The longest oviposition duration was observed in the hexane fraction  $C_1$  where the laying produced only the 2nd day ( $34.5 \pm 17.68$  eggs) and the 4th day evening after treatment ( $10 \pm 1.41$  eggs). The witness gives an average fecundity of  $95.25 \pm 11.87$  eggs an oviposition period of 11 days (Table 2 and Figure 6).

An affect on the normal behavior of nesting female survivors was also noted. Indeed, these females tend to make their eggs not on the peanut seeds available for them but on the sides and bottom of Petri dishes. In

addition, the punters were sometimes grouped by location, where you can have up to more than 10 eggs agglutinated. These constraints were not conducive to the monitoring of eggs laid making it impossible to estimate fertility.

### Adulticid effect of extracts of *Calotropis procera*

Figure 7 shows that the extracts of *C. procera* significantly affect the viability of adult *C. serratus* compared with controls. This effect of adulticide *C. procera* is an increasing function of the polarity of the applied sample and varies from one concentration to another, depending on the duration of exposure. Indeed, whatever the usual concentration, the crude extract in methanol and the methanol fraction had a significantly higher share compared to other extracts and cause respectively  $74.10 \pm 2.82\%$  and  $86.14 \pm 4.21\%$  mortality. The polar extracts showed statistically similar rates of mortality at all concentrations. By cons, for apolar extracts, the effectiveness adulticid increases with the concentration applied. The rate of adult mortality induced by the high concentration (0.1 g/ml) of crude extract to petroleum ether (45.95%) and the hexane fraction (26%) are significantly higher than those of other concentrations (0.01 and 0.001 g/ml).  $C_2$  and  $C_3$  induced statistically similar adulticid effect for ether extract ( $24.4 \pm 0.42\%$ ) and the hexane fraction ( $17.1 \pm 2.78\%$ ). The ethyl acetate fraction of intermediate polarity induced an adult mortality rate in the range of  $50.16 \pm 1.3\%$ .

The comparative study of the level of adult mortality of *C. serratus* depending on the exposure time (12 h, 24 h, 48 h, 72 h, 96 h, 120 h, 6 days, etc.) shows that each sample biocide *C. procera* has a decisive effect concentration which varies depending on the duration of treatment (Figure 8: a, b, c, d and e). The analysis revealed that for exposure time between 0 to 6 days of treatment, the high concentration (0.1 g/ml) of each extract of *C. procera* showed adulticidal efficacy greater than that of other concentrations (0.01 and 0.001 g/ml), while an opposite effect was noted between the 6th and 10th days of treatment. This observation was more



pronounced with the extract in methanol, the ethyl acetate fraction and the methanol fraction (Figures 8b, 8d and 8e). For example, low concentrations (0.01 and 0.001g/ml) of the hexane fraction does not cause mortality within the 6th day of treatment when it is sufficient to apply a high concentration (0.1g/ml) to meet the adults 26.6% mortality between 24 h and 96 h of treatment (Fig. 8c). Therefore, for smaller exposure times, the doses would act more effectively on adult survival. The  $13.54 \pm 1.47\%$  of untreated adults occurred beyond 10 days of treatment.

### Phytochemistry of crude extracts and fraction extracts

#### Extraction efficiency

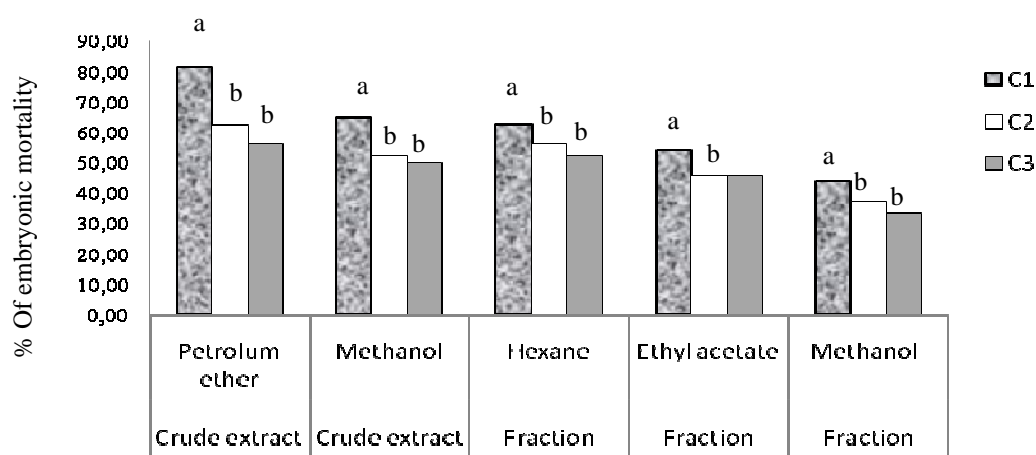
The extraction yields compared to the weight of the material used (powder or crude extract) vary depending on the polarity of the solvent used. The polar extracts gave the highest returns. The powdered leaves of *C. procera* gives 1.2% with petroleum ether and 12.5% with methanol. Regarding extract fractions on a mass of 9.5 g of dry methanol

residue, *C. procera* gave yields of 10.53% with hexane, 20% with ethyl acetate and 25.26% with methanol (Table 3).

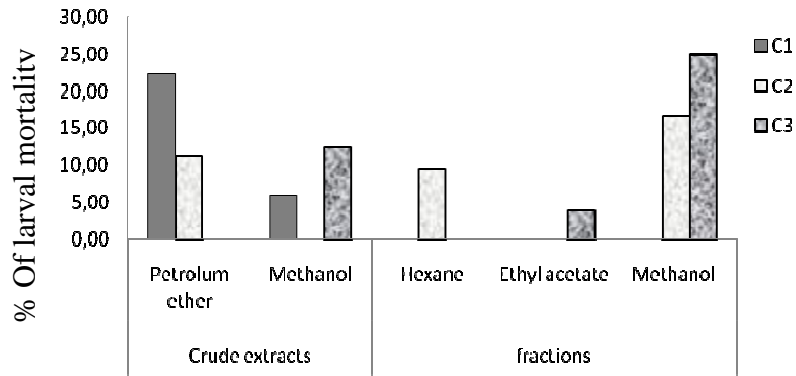
#### Characterization of chemical groups

Reactions characterization showed the presence of a large number of phytochemicals found in the form of discoloration or precipitation using specific reagents. Among these groups of compounds, we mentioned alkaloids, flavonoids, tannins, anthracene derivatives, terpenes, sterols and saponins. Alkaloids, flavonoids, sterols and terpenes represent groups of compounds the most abundant (+ + +). The characterization of reaction was moderately positive (+ +) for anthracene derivatives and low ( $\pm$ ) for tannins and saponins (Table 4). The height of the foam in *C. procera* was less than 1 cm in all tubes.

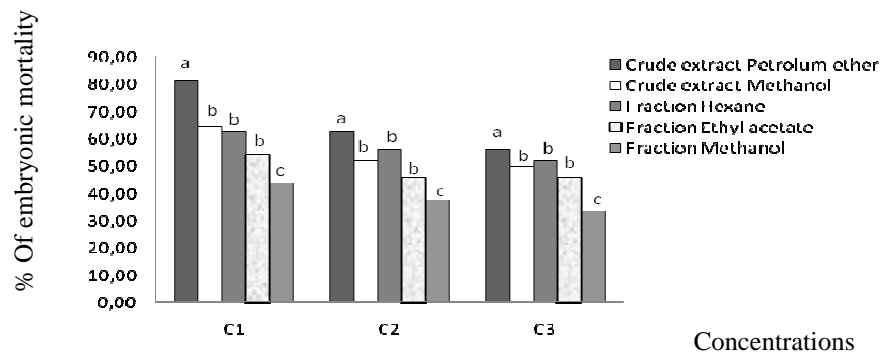
The presence of any compound varies depending on the polarity of the product. Sterols, terpenes and saponins are easier to characterize in apolar biocides while alkaloids, flavonoids and tannins are easily characterized in the polar products.



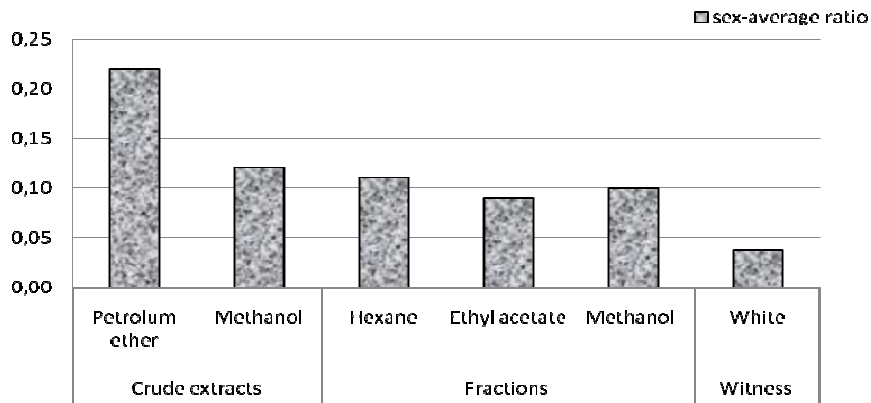
**Figure 1 :** mortality of *Caryedon serratus* eggs treated with *Calotropis procera* extracts at different concentrations.



**Figure 2 :** Larval mortality obtained with biocides products of *Calotropis procera* at different Concentrations.



**Figure 3:** Comparative mortality of eggs treated with biocides extracts of *Calotropis procera* at different concentrations.



**Figure 4 :** Comparative effects of reporting sex-ratio means induced by different extracts of *Calotropis procera*.

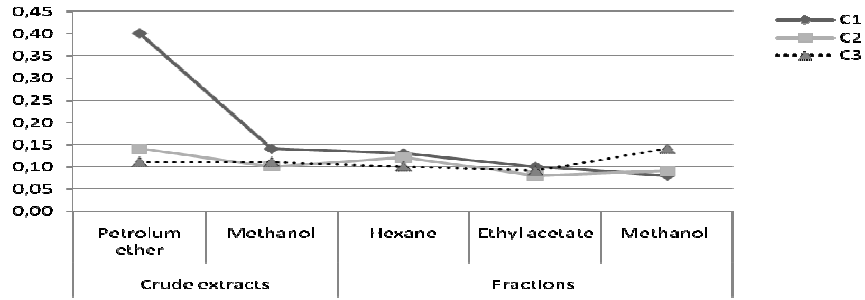


Figure 5: Sex-ratio of adults from eggs *Caryedon serratus* treated with extracts of *Calotropis procera* at different concentrations

Figure 5 : Sex-ratio of adults from *Caryedon serratus* eggs treated with *Calotropis procera* extracts at different concentrations.

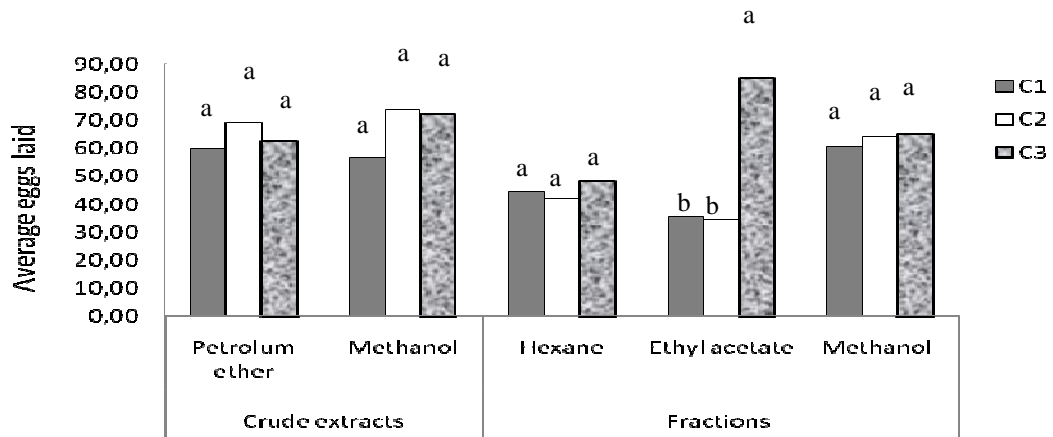


Figure 6: Effects of *Calotropis procera* extracts on fecundity of female offspring of *Caryedon serratus* eggs treated with different concentrations.

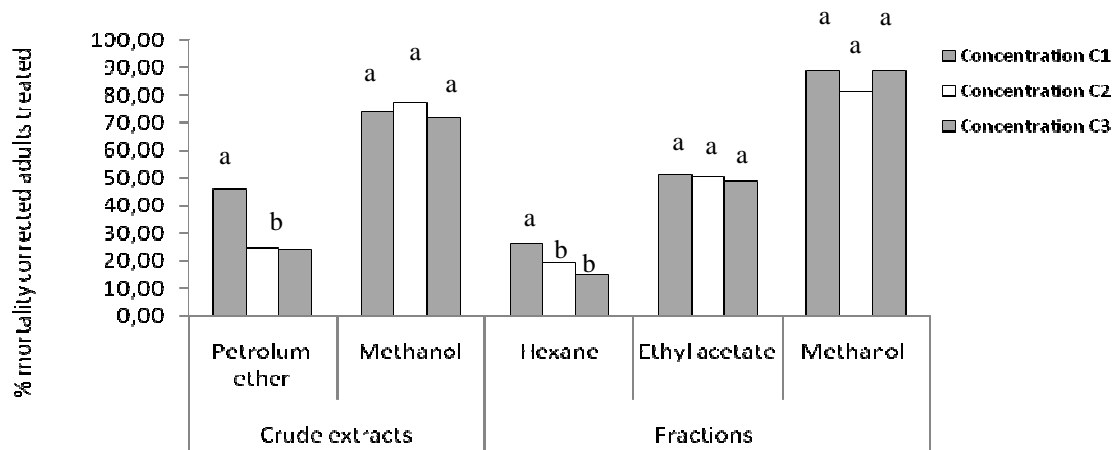
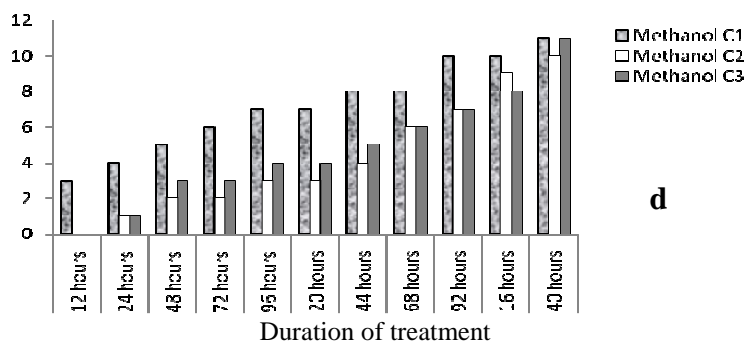
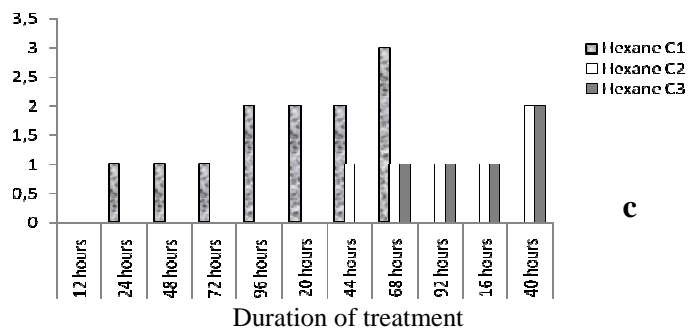
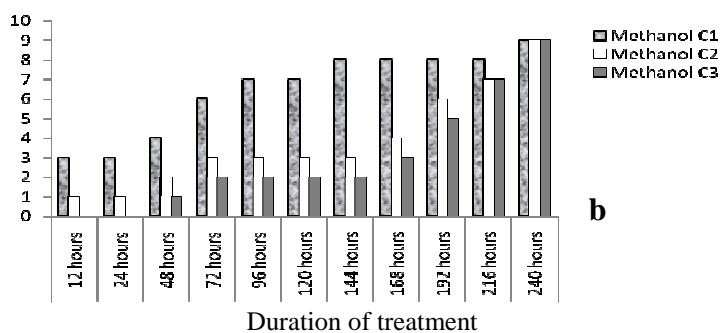
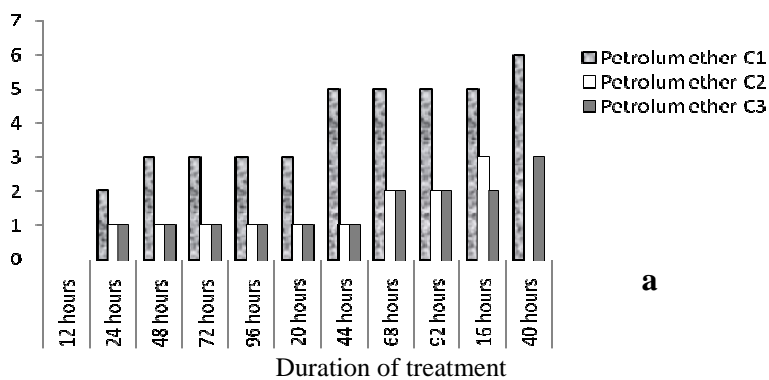
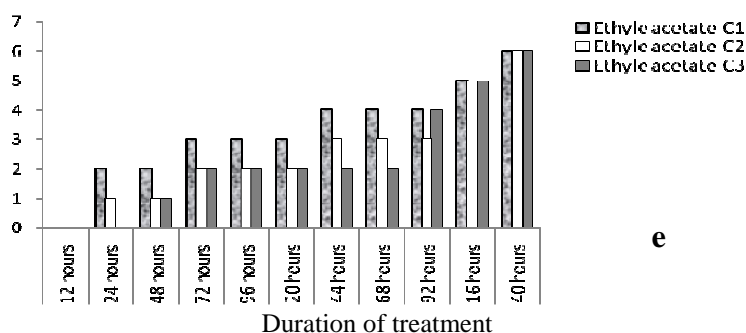


Figure 7 : Adult mortality of *Caryedon serratus* treated with biocides extracts of *Calotropis procera* at different concentrations.





**Figure 8:** Kinetics of mortality of adult *Caryedon serratus* obtained with three concentrations of each extract of *Calotropis procera* (a: crude extract to petroleum ether ; b: crude methanol extract ; c: hexane fraction ; d: ethyl acetate fraction ; e: methanol fraction).

**Table 1:** Comparison of effects induced by the crude extracts and fractions of extracts of *C. procera* on the average durations ( $\pm$  SD) of different stages of development of treated eggs of the insect pest *C. serratus*.

Average duration (days)	Crude extracts		Fractions of extracts		
	Petroleum ether	Methanol	Hexane	Ethyl acetate	Methanol
Laying eggs / Hatching	7.33 <sup>b</sup> $\pm$ 0.58	10.75 <sup>a</sup> $\pm$ 0.21	8 <sup>b</sup> $\pm$ 0.00	6 <sup>c</sup> $\pm$ 0.00	11. <sup>a</sup> $\pm$ 0.00
Outbreak / weaving of cocoons	29.71 <sup>c</sup> $\pm$ 3.14	47.9 <sup>b</sup> $\pm$ 1.70	54.88 <sup>a</sup> $\pm$ 1.36	57.34 <sup>a</sup> $\pm$ 6.29	50.16 <sup>b</sup> $\pm$ 1.3
Weaving / emergence	29.64 <sup>a</sup> $\pm$ 2.72	21.08 <sup>b</sup> $\pm$ 1.19	30.84 <sup>a</sup> $\pm$ 2.67	34.18 <sup>a</sup> $\pm$ 1.53	22.08 <sup>b</sup> $\pm$ 2.27
Lifetime	115.02 <sup>c</sup> $\pm$ 8.13	133.82 <sup>b</sup> $\pm$ 5.07	115.02 <sup>c</sup> $\pm$ 4.23	135.54 <sup>b</sup> $\pm$ 3.59	152.66 <sup>a</sup> $\pm$ 15.12

Times are expressed in days, the values are averages followed by standard deviation. On a horizontal line means followed by one or the same (s) letters do not differ significantly between them (p less than 0.05)

**Table 2:** Comparative analysis of effects induced by the crude extracts and fractions of extracts of *C. procera* on fecundity averages ( $\pm$  SD) of females hatched from eggs "survivors" of the insect pest *C. serratus*.

Parameters studied	Petroleum ether	Methanol	Hexane	Ethyl acetate	Finam methanol	Witnesses
Average fecundity	63.83 $\pm$ 4.65 <sup>b</sup>	67.33 $\pm$ 9.41 <sup>b</sup>	44.83 $\pm$ 3.01 <sup>a</sup>	51.67 $\pm$ 28.87 <sup>a</sup>	63.17 $\pm$ 2.36 <sup>b</sup>	95.25 $\pm$ 11.87 <sup>c</sup>
Reduction of spawning (%)	19.75 <sup>b</sup>	17.17 <sup>b</sup>	35.99 <sup>a</sup>	29.66 <sup>a</sup>	20.25 <sup>b</sup>	-

For fecundity, values are averages expressed in eggs and followed the standard deviation. On a horizontal line means followed by one or the same (s) letters do not differ significantly between them (p less than 0.05).

**Table 3:** Yields of crude extracts and fractions of the crude methanol extract of powdered leaves of *C. procera* Ait.

Crude extracts or fractions		<i>Calotropis procera</i>
Crude extracts	Petroleum ether	1.2%
	Methanol	12.5%
Fractions	Hexane	10.53%
	Ethyl acetate	20%
	Methanol	25.26%

**Table 4:** Results of reactions characterization of crude extracts and fractions of extract powder leaves *Calotropis procera* Ait.

Phytochemical constituents Searched	Results of reactions
Alkaloids	+++
Flavonoids	++
Tannins	±
Anthracene derivatives	++
Sterols and Terpenes	+++
Saponosides	±

Very abundant (+++); moderately abundant (++); low concentration (±)

## DISCUSSION

The results showed that extracts of *C. procera* significantly affected the viability of eggs and adults of *C. serratus*. The ether extract showed the most pronounced ovicidal effect, since it killed 81.25% of eggs in C<sub>1</sub>. The crude methanol extract and the methanol fraction have the most important adulticidal effect since they involve, respectively,  $74.10 \pm 2.82\%$  and  $86.14 \pm 4.21\%$  of adults of *C. serratus*. This effective ovicidal and/or adulticidal effects would be mainly due to the biological action of chemical compounds such as alkaloids, terpenoids, saponins and flavonoids which act on the embryo or the adult cuticle, causing it to suffocate. This was confirmed by the results of Ketoh et al. (2000), Choi et al. (2003) and Bouchelta et al. (2005).

This study also shows that there is a positive correlation between mortality and the induced dose applied. High concentrations are the most effective insecticides, and the activity varies according to the duration of exposure. These results confirm those of Ketoh et al. (2000) who conclude that the choice of the applied dose affects the majority

of compounds responsible for the insecticidal action. Such results were also obtained by Bouchelta et al. (2003), Borges et al. (2003), Thiaw (2004). Furthermore, the developmental stage killed varies depending on the polarity of the test product. The nonpolar extracts are more effective on the eggs of *C. serratus* while adults are more sensitive to polar extracts. Such results have been obtained by Borges et al. (2003), Gueye (2004), Kiendrebeogo et al. (2006), Thiaw et al. (2007).

It appears from this study that the bioactivity of extracts of *C. procera* on the insect *C. serratus* expressed on the one hand, an extension or a reduction in the duration of egg development survivors and secondly, an unbalanced sex-ratio in the first generation in favor of males. This inhibition of development time is due to the action of triterpenes, saponins and sterols, which can cause cell lysis by affecting the permeability of the cell. These results support those of Strebler (1989), Kiendrebeogo et al. (2006). This unbalanced sex-ratio of between 0.4 and  $0.09 \pm 0.16 \pm 0.01$  could be an asset in the management of insect pests. This hypothesis was also issued

by the works of Nishimura (1993) and Gauthier (1996).

The bioactivity of *C. procera* is also reflected by a significant reduction in the number of eggs laid by female survivors. The ethyl acetate fraction caused by its heavy doses 0.1 and 0.01 g/ml of average fecundity respectively  $35.5 \pm 12.02$  and  $34.50 \pm 0.71 \pm 95.25$  eggs against 11.87 eggs in the witness, a respective spawning rate reduction of 62.73 % and 63.78%. The inhibition of fertility was due to the action of alkaloids, flavonoids and tannins, substances that inhibit digestion or reproduction of insects. Similar results were reported by Saxena (1989), Strebler (1989), Abbasi et al. (2003), Kellouche and Soltani (2004), Seri-Kouassi et al. (2004), Thiaw (2008).

### Conclusion

*C. serratus*, a species recognized as the most harmful and seed stocks of peanut in West Africa, continues to be controlled by overuse of synthetic insecticides. To preserve the environment, human health and animal, the use of biocidal substances of plant origin effect biodegradable insecticide or insect repellent is one of the alternatives and reasoned to help peasant farmers to increase production, provide crops, conserve stocks and seeds to the processing or use.

From a plant species tested, the results of this study showed that crude extracts and fractions of extracts of *C. procera* induced high mortality of *C. serratus* the egg or adult stage. The bioactivity of extracts of *C. procera* on the physiology of the insect was also expressed by an unbalanced sex-ratio, an extension or reduction of development time, reduced fecundity and limited fertility.

This work could be continued in order to lead to a practical use of these substances in biocidal protection and seed stocks peanut farmer in rural Africa.

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