



IJSRM

INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY

An Official Publication of Human Journals



Human Journals

Research Article

October 2019 Vol.:13, Issue:4

© All rights are reserved by Toffène DIOME et al.

Biocidal Effect of the Leaves of *Azadirachta indica* A. Juss on A Resistant Strain of the Groundnut Bush *Caryedon serratus* (Olivier)



Aminata GNINGUE¹, Toffène DIOME*², Khady
FALL³, Mbacké SEMBENE⁴

^{1,2,3,4} Genetic Team and Population Management,
Department of Animal Biology, Cheikh Anta Diop
University, Faculty of Sciences and Technology, Dakar,
Senegal

Submission: 23 September 2019

Accepted: 28 September 2019

Published: 30 October 2019



HUMAN JOURNALS

www.ijsrm.humanjournals.com

Keywords: Groundnut, Pests, *Azadirachta indica*, Biological parameters.

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 bruchidea beetle, *Caryedon serratus*. 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. The purpose of this study is to test, the biological impact of leaf-based formulations of native plants of Senegal (*Azadirachta indica*) on the external forms of *C. serratus*. In this study, analysis of the biological parameters of strain *C. serratus* showed low adult mortality. This trend of mortality has been reversed from the second day to the ninth day when we have a mortality of 66.67% (C1) but for C3 we have a mortality of 33.33% on the third day. The extracts also affected female fecundity, which is 52% for C1 and 47% for C2. Nevertheless, the effect is more pronounced with Deltamethrin (36.5% for C1 and 35.7% for C2). For the lowest C3 concentration, the opposite is true: a 41.4% fecundity with Deltamethrin and 35.9% with *A. indica*. On the other hand, the extract affects the viability of eggs which depends on the dose and the fertility of the rescued females is reduce. On the other hand, there is an extension of the total development time.

INTRODUCTION

Peasant agriculture in Senegal occupies 60% of the active population and contributes 20% of GDP. It is dominated by several sectors including the peanut sector (Kouadio, 2007). It 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 (Sembène, 2006). High in protein and calories (50% fat, 25% protein), this oil seed is also a very important nutrient supply for local populations (Ndiaye, 1991). 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 damage it causes on peanuts can go up to 83% of quantitative loss for a storage period of 4 months (Sembène, 2000). 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* Link.) 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. 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 (Benhalima *et al.*, 2004), poisoning, environmental pollution, environment but also the reluctance of consumers to consume products treated with pesticides (Guèye *et al.*, 2011). 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 harvests without endangering the population and the environment (Bambara and Tiemtoré, 2008 ; Faye *et al.* 2014 ; Mbaye *et al.* 2014). The application of extracts obtained from organic solvents is the most practiced, while it is difficult to apply by farmers. This because of the high cost of these solvents, their delicate handling, which often requires precautions to establish, which can be commonly ignored by peasant populations. We propose to use tap water as a solvent because it is more accessible and less expensive and also easy to handle without danger (Faye, 2015). It is in the context of the reduction of the post-harvest losses of peanuts by alternative methods of traditional control that which is part of this study which aims at evaluating the biocidal effect of the plant *Azadirachta indica* on the biggest parasite of peanut, *C. serratus*. The aim of this

study is to evaluate the biocidal effect of the *Azadirachta indica* plant on the largest pest of peanuts, *C. serratus*.

MATERIALS AND METHODS

Biological material

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 leaves of *A. indica* 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.

The leaves of *A. indica* 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. 200g of fresh leaves of each of the two plants are extracted in 1L 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.

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.

Biocidal substance extraction

After harvest, the leaves of each of the two plants are crushed freshly and used for aqueous extractions by maceration for biological testing. 200g of fresh leaves of each of the two plants are extracted in 1L 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.

Experimental protocol of tests with aqueous extracts

We have a Petri dish, an aqueous solution for Neem and a synthetic pesticide solution (deltamet 25 EC) of different concentrations. $C_1 = 200\text{g} / 500\text{ml} = 0.4\text{g} / \text{ml}$ is the concentration of the mother solution (with the extraction 200g of fresh leaves in 500ml of water which is the solvent used) and from which the other concentrations are obtained by dilution (Ndiaye, 2015). $C_2 = 200\text{ g} / \text{L} = 0.2\text{ g} / \text{mL}$ and $C_3 = 200\text{ g} / 1.5\text{L} = 0.13\text{ g} / \text{mL}$.

Tests of deltamethrin

Deltamethrin is applied at the recommended dose of 40ml per 30L of water, based on 1L of water, which makes it possible to determine the C_x concentration from which the other concentrations are drawn: $C_x = 40\text{ml} \times 1\text{L} / 30\text{L} = 1.3\text{ml} / \text{L}$; $C_1 = C_x \times 2 = 2.6\text{ml} / \text{L}$; $C_2 = C_x = 1.3\text{ml} / \text{L}$ and $C_3 = C_x / 2 = 0.65\text{ml} / \text{L}$.

Adulticide 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 (1ml) 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 (1ml) of tap water is sprayed onto the peanut seeds. Insects are exposed to aqueous extracts for ten days. Dead bruchles are counted every 24 hours and eggs laid are followed until emergence.

Calculated parameters and Statistical analyzes

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 Abott formula (Abott, 1925) is used :

$$M_c = \frac{M_o - M_t}{100 - M_t} \times 100$$

Where M_o = mortality in the treated lots, M_t = mortality in the control and M_c = calculated mortality

Calculation of fertility rate

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

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

Calculation of embryonic and larval mortality of offspring

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

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

Where ME = embryonic mortality

The larval mortality rate is calculated by the formula:

$$\%ML = \frac{N_{mo}}{N_l} \times 100$$

Where ML = larval mortality; N_{mo} = Number of dead larvae; N_l = Total number of larvae

Development period

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

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.

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

Statistical analyzes

Mean repetition calculations and graphs were performed on Excel 2013. Statistical analyzes of the measured variables were performed with R software. The resulting data were subjected to a non-parametric test analysis and averages were compared by the Fisher test at the 5% threshold.

RESULTS AND DISCUSSION

Calculates adult mortality of *C. serratus*

The aqueous extract of *Azadirachta indica* applied to adults of *Caryedon serratus* gave mortalities from the first day of application for the concentrations C1 (0.4 g / ml) and C3 (0.13 g / ml) with respectively 33, 33% and 66.67% mortality. This mortality trend was reversed from the second day of application with no mortality until the ninth day when we have a mortality of 66.67% for C1, whereas for C3 it is until the third day with a mortality of 33.33% that will be zero from the fourth day. Concentration C2 (0.2 g / ml) gave no mortality. None of the concentrations gave 100% mortality. The statistical analysis of the mortality test is not significant $p > 0.05$ (figure 1).

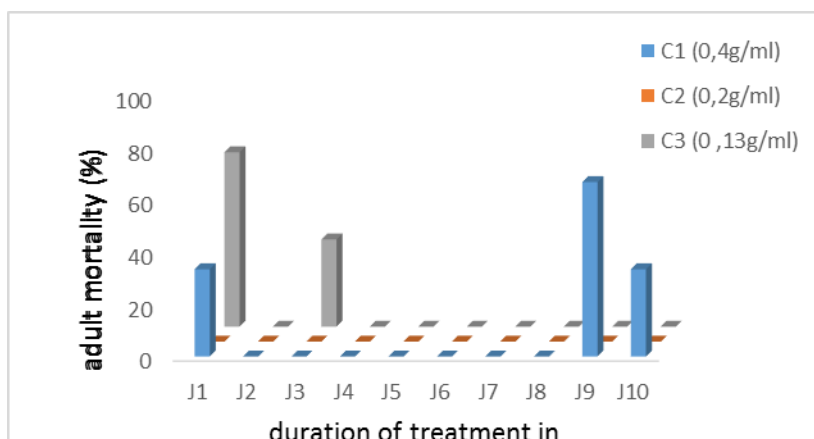


Figure No. 1 : Percent corrected adult mortality of *C. serratus* induced by aqueous extract of *A. indica* leaf powder

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 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 ($p < 0.05$).

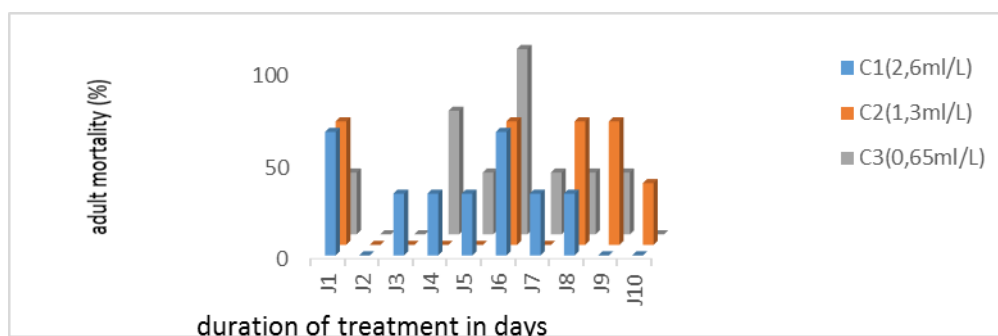


Figure No. 2 : Percent Corrected Adult Mortality of *C. serratus* Induced by Deltamethrin Insecticide

Calculation of the fertility rate

The fecundity rate of the female survivors of *C. serratus* is greater for the concentrations (C1 (52%) and C2 (47%)) with the aqueous extract of *A. indica* compared to Deltamethrin which has a rate of 36.5% for C1 and 35.7% for C2. By cons for the lowest concentration C3, the rate obtained for Deltamethrin is higher with 41.4% against 35.9% for *A. indica*. It is also found that the controls have a fertility rate of about 50%, hence the highest concentration of *A. indica* has the highest fertility rate for this test with a non-significant p-value $p > 0.05$ (Figure 3).

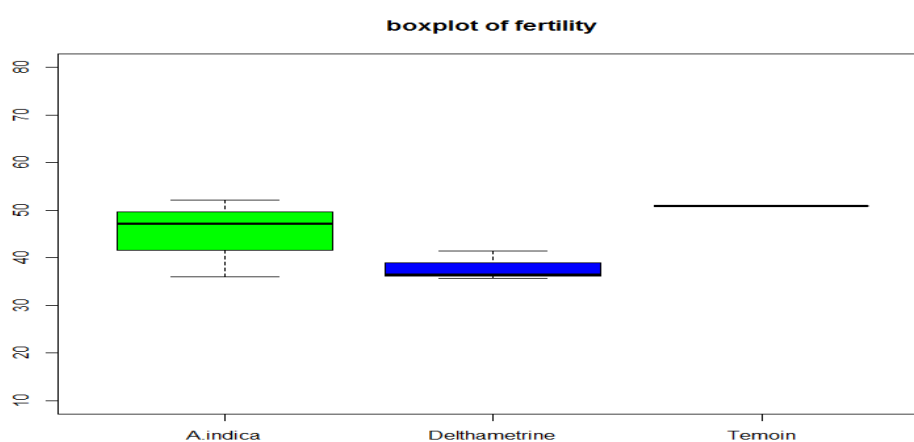


Figure No. 3 : The fecundity rate of *C. serratus* females rescued from adulticide tests of the aqueous extract of leaf powder *A. indica* and Deltamethrin

Calculation of embryonic and larval mortality of offspring

The effect of *Azadirachta indica* 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 30.99% for C1, 31.97% for C2 and 33.51% for C3. The effect of Deltamethrin is more consistent with higher rates C1 (74.14%), C2 62.46%) and C3 (75.83%). The statistical analysis of this test is significant $p < 0.05$ (Figure 4).

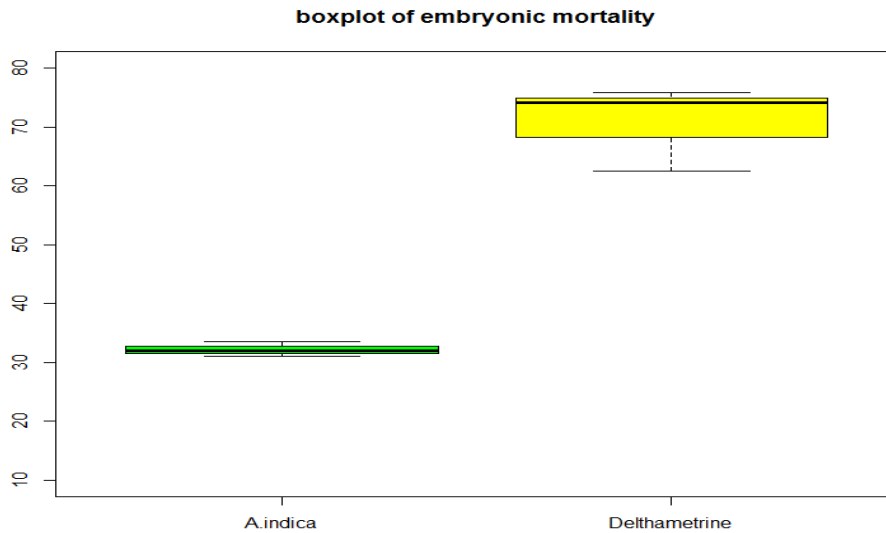


Figure No. 4 : Embryal mortality of progeny of *C. serratus* adults tested by aqueous extract of leaf powder *A. indica* and deltamethrin

The effect of *Azadirachta indica* on larvae from offspring of previously tested adult *Caryedon serratus* is shown by downward mortality as a function of concentration C1 (10.4%), C2 (4.7%) and C3 (1, 9%). These observed rates for *A. indica* are lower than those observed for Deltamethrin with C1 (20.5%), C2 (13.9%) and C3 (24.7%). It is also observed that the lowest concentration of Deltamethrin has the highest rate of larval mortality of progeny derived from adulticidal *C. serratus* tests (Figure 5). This test is statistically significant with $p < 0.05$.

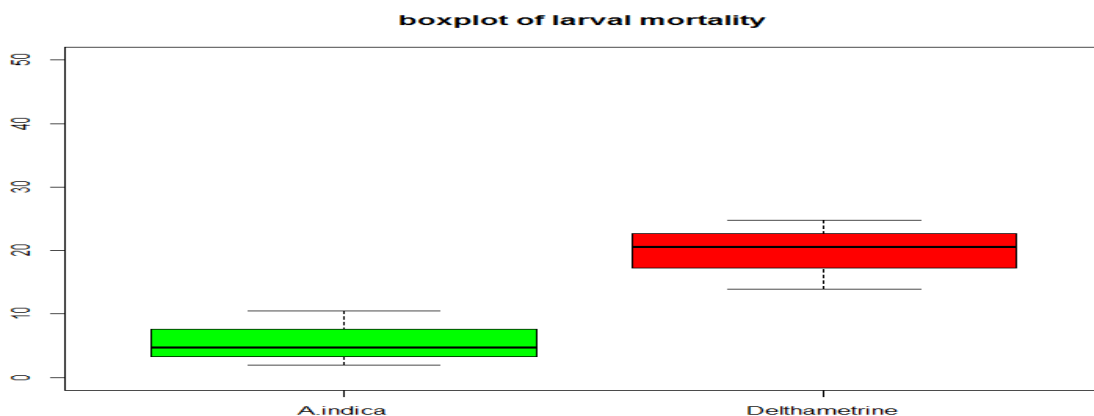


Figure No. 5 : Larval mortality of offspring of *C. serratus* adults tested by the aqueous extract of leaf powder *A. indica* and Deltamethrin

Development time

The extracts of *A. indica* have a mean spawning weaving time of 59.05 ± 4.28 days with a minimum duration of 54.5 ± 14.8 days in C3 and a maximum of 63.67 ± 3 days in C1. For pupation, we have an average of 24.5 ± 2.5 days with a minimum of 22 ± 3.46 days for C1 and a maximum of 27 ± 5.56 days for C2 (Table 1 and 2).

Table No. 1 : Larval development time

Larval development time (days)± standard deviation				
Products	C1 (0,4g/ml)	C2 (0,2g/ml)	C3 (0 ,13g/m)	Average
Azadirachta indica	63 ± 3	59.67 ± 5.03	54.5 ± 14.8	59.05 ± 4.28
Deltamethrine	62 ± 0	54 ± 0	67 ± 16.97	61 ± 6.56

Table No. 2 : Nymphal development time

Nymphal development time (days) ± standard deviation				
Products	C1 (0,4g/ml)	C2 (0,2g/ml)	C3 (0 ,13g/m)	Average
Azadirachta indica	22 ± 3.46	27 ± 5.56	24.5 ± 0.7	24.5 ± 2.5
Deltamethrine	29 ± 0	46 ± 0	38 ± 2.8	38 ± 2

Evaluation of the sex ratio (R)

The analysis of the effect of different plants on the nature of 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 females of *C. serratus* with the application of *A. indica* for concentrations C1 and C2 at the time when C3 the sex ratio is in favor of males. We notice that this favor eases with the decrease in concentration. Thus, the highest concentration gives a sex ratio of 60% at the moment when the other concentrations give respectively 52% (C2) and 39% (C3) of sex ratio. Deltamethrin induced a sex ratio in favor of groundnut mussel males for the application of all concentrations. Only *A. indica* gave with C1 (60%) 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 (Figure 6).

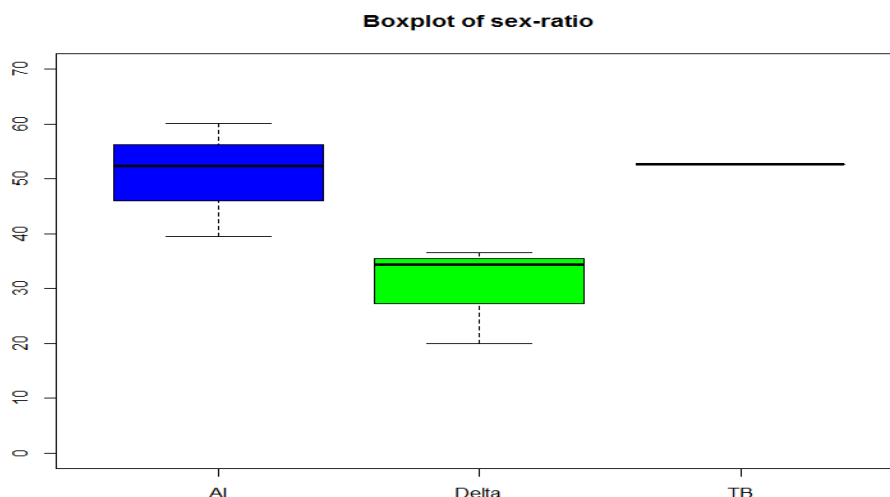


Figure No. 6 : Sex ratio of adults from eggs treated with the aqueous leaf extract of the plant *A. Indica* and Deltamethrin Insecticide

DISCUSSION

The purpose of this study is to evaluate the biocidal effect of aqueous extracts formulation *A. indica* on eggs and adults of *C. serratus*. The results indicate that *A. indica* induces low mortality of *C. serratus* at the adult stage. The extracts of *A. indica* caused 12.87% mortality of *C. serratus* adults in 10 days of exposure. On the other hand synthetic insecticide, Deltamethrin caused less than 50% in 10 days. The weak activities of the Neem extracts could be explained by the fact that the adults come from resistant strains on the one hand and by the fact that the egg-laying substrate (the peanut sheaths) absorb completely 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, 1996), which show that the solid products of Neem (powder, leaves and almonds) did not show 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 (Saxena, 1989) reports that females of *C. serratus* in contact with Neem extracts have greatly reduced fecundity and spawning. According to Kellouche and Soltani (2004) 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 (2002) 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 *Camaldulensus* (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, extracts from *A. indica* 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 have been due to the temperature and humidity conditions in which the tests were performed.

Indeed, with the extracts of *A. indica* we obtained a mean spawning / weaving cocoon duration of 58.88 ± 7.33 days with a minimum duration of 51.66 ± 7.76 days in C3 and maximum of 66.33 ± 2.51 days in C1. The work of Ndiaye (1991) and those of Delobel and Tran (1993) indicate larval development between 40 and 58 days depending on temperature and relative humidity conditions. Gueye (2002) reveals in his studies a larval stage duration of about 45 days on average at 35°C. With regard to pupation, although there is no significant difference between plants and controls, these results corroborate those of Thiaw (2008) who show a nymphal development time that varies from 21,33 to 33,43 days with extract and methanolic fraction of *Calatropis 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 extract of *A. indica* on the sex ratio remains in favor of the females for the concentrations C1 and C2 whereas at the other concentration it decreases and turns in favor of the males which would decrease the risks of increase of the population.

REFERENCES

1. Bambara D. Tiemtoré J. Biopesticide Efficacy of *Hyptis spicigera* Lam., *Azadirachta indica* A. Juss. and *Euphorbia balsamifera* Ait. on the cowpea *Vigna unguiculata* L. Walp, In the Tropicultura. 2008 ; 26 : 53-55.
2. Benhalima H. Chaudhry MQ. Mills KA. Price N.R. Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. J. of stored prod. research. 2004 ; 40 : 241–249.
3. Delobel A. et Tran M. Les Coléoptères des denrées alimentaires entreposées dans les régions chaudes. ORSTOM/CTA. 1993 ; 312-316.
4. El Atta HA. Ahmed A. Comparative effects of some botanicals for the control of the seed weevil *Caryedon serratus* Olivier (Col., Bruchidae). J. of Applied Entomology. 2002. 126 : 577–582.
5. Faye A. « Biocidal activity of leaves of *Azadirachta indica* A. Juss, *Crataeva religiosa* Forst and *Senna occidentalis* L. against *Callosobruchus maculatus* Fabricius, main pest of cowpea stocks in Senegal, » PhD thesis at the University Cheikh Anta Diop University of Dakar (Senegal). 2015 ; 168p.
6. Faye A. Thiaw C. Sarr M. Sembène M. Effectiveness of different formulations leaves of *Senna occidentalis* on the external stages of *Callosobruchus maculatus* Fabricius main pest of cowpea (*Vigna unguiculata* Walp) stored. Int. J. of Biosciences, 2014 ; 4 (9) : 246-253.
7. Guèye M. Ecophysiology, structure and evolution of stands of *Caryedon serratus* (Olivier), peanut pest stored in Sahelian environment. PhD thesis at the University Cheikh Anta Diop University of Dakar (Senegal). 2000 ; 165p.
8. Guèye MT. Seck D. Wathelet JP. Lognay G. Lutte contre les ravageurs des stocks de céréales et de légumineuses au Sénégal et en Afrique occidentale : synthèse bibliographique. Biotechnol. Agron. Soc. Environ. 2011 ; 15 (1) : 183-194.
9. Kandji ST. Optimization of the use of neem in the protection of seed stocks of three legumes against caryedon beetle species (Coleoptera, Bruchidea). Agronomist's thesis ENSA de Thiès. 1996 ; 46p.
10. Kellouche A. Soltani N. Biological activity of the powders of five plants and the essential oil of one of them on *Callosobruchus maculatus* (F.) » Int. J. of Tropical Insect Science. 2004 ; 24 (2) : 184-191.
11. Kouadio AL. Interuniversity in Natural Risk Management: Prediction of National Groundnut Production. Senegal from the Agrometeorological Model AMS and NDVI. ULG-Gembloux. 2007 ; 54p.
12. Mbaye NN. Sarr M. Ndiaye AG. Samb A. Sembène M. Repulsive and biocide activities of leaves powder of *Crataeva religiosa* (Forst) on *Dermestes* spp. associated with the salty smoked-dried fish. Int. J. of Biosciences. 2014 ; 4 (1) : 306-312, 2014.
13. Ndiaye S. Groundnut bush in an agro-ecosystem of west-central Senegal: contribution to the study of contamination in the open field and in peanut stocks (*Arachis hypogaea* L.) by *Caryedon serratus* (Ol. (Coleoptera-Bruchidae); role of legumes wild hosts in the cycle of this burr. University thesis of Pau and the Adour countries. 1991 ; 96p.
14. Saxena RC. Insecticides from neem. In Insecticides of Origin Plant, Arnasson JT, Philogene BJR, Morand P (eds). American Chemical Society : Washington.1989 ; 150-163.
15. Sembene M. Variability of Transcribed Internal Spacer (ITS1) of ribosomal DNA and polymorphism of microsatellite loci in cedar *Caryedon serratus* (Olivier): Differentiation in host races and peanut infestation at ground level. Senegal. Ph.D. thesis at the Faculty of Science and Technology, Department of Animal Biology, University Cheikh Anta Diop of Dakar. 2000 ; 212p.
16. Thiaw C. Bioactivity of extracts of *Calotropis procera* AIT. and *Senna occidentalis* L. on *Caryedon serratus* (Ol.), a pest of peanut stocks and seeds in Senegal, » Thesis 3rd cycle, FST, UCAD. 2008 ; 182.