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BIOCHEMICAL FRACTIONS ACTIVITY OF *ANNONA SENEGALENSIS* PERS. EXTRACT LEAVES TO PROTECT GROUNDNUT AGAINST THE SEED-BEETLE *CARYEDON SERRATUS* OL. (COLEOPTERA, CHRYSOMELIDEA, BRUCHINAE).

GUEYE Sabelle* ♠, DIOP Mamadou Thiam ♣, SECK Dogo• & SEMBENE Mbacké*

* Département de Biologie animale, faculté des sciences et techniques BP 5005 Dakar
♠ Laboratoire de chimie des produits naturels, faculté des sciences et techniques BP 5005 Dakar
• Fondation CERES-LOCUSTOX, Km 15 Route de Rufisque, BP 3300 Dakar
♣ Université de Thiès Km 7,5 Route de Khombole, B.P. 3320, Thiès Escale

Corresponding author: SEMBENE Mbacké : mbacke.sembene@ird.fr

ABSTRACT : Biological activity of biochemical fractions of *Annona senegalensis* leaves was evaluated in the laboratory on different stages of *Caryedon serratus* development. The extracts tested were obtained by soaking the powder with three solvents (methanol, hexane and ethyl acetate). The acetate fraction eliminates 100% of eggs with 0.1 g / ml, whereas the methanol fraction kills 33% with 0.01 g / ml. The crude extract and the hexane fraction proved ineffective but lead to a reduction in the duration of larval life. Pupal stage duration and the fecundity of survivors are not affected by the different extracts; an increase in longevity is denoted with all extracts. In adults, the effect depends on extract and dose. At about 72 hours, at high concentration, the crude extract and the methanol fraction eliminate respectively 75 and 94% of the groundnut seed-beetle. With a concentration of 0.01 g / ml, crude extract and the hexane fraction kill respectively 75.86 and 47.22% of insects. At low concentration (0.001 g / ml), acetate fraction eliminates 94%. Acetate and methanol fractions may be used in the protection of stocks by applying at the beginning of the infestation and at the time of emergence of adult survivors.

Keywords: peanut, *Arachis hypogaea*, *Caryedon serratus, botanicals insecticides,* extracts*, Annona senegalensis*.

INTRODUCTION

Senegal, like all Sahelian countries, is facing a very high rate of destruction of crops particularly that of peanut. During storage, the peanut crops are mainly attacked by *Caryedon serratus* Olivier (Olivier, 1790), a beetle belonging to the Bruchidae family, commonly known as the groundnut bruchid. This pest can attack groundnut in shell from the field (Sembene, 2010) creating quantitative losses that can range up to 83% over a period of 4 months [17]. Moreover, the holes left on the hull by the larvae infestation promote other minor species such as *Trogoderma* spp. *Ephestia cautella* (Walker), *Tribolium* spp., facilitate the development of a fungus, *Aspergillus flavus* Link., producer of aflatoxin [10]. Among the methods used to limit the *C. serratus* damage there generally use chemical insecticides that can induce a poisoning of farmers consumers, resistance to pests or have a negative impact on the environment. To achieve effective protection, an alternative that would not create health problems or nuisance to consumers the environment must be found. So many are offered for stored products including: modified atmosphere (use of CO_2, the reduction of oxygen by use of nitrogen other gases, temperatures of storage) [8, 16], the mixture of contact insecticides growth regulators, plant extracts ... The use of these as biopesticides in the protection of legume seeds or stored grain against insects, has been the subject of numerous studies [5], especially in tropical areas. In Africa, they have focused on the application of substances of plant origin (fresh crushed leaves, powders, essential oils, extracts) against bruchid beetles among them *Callosobruchus phaseoli* (Gyllenhal), *C. chinensis* (Linn.), *C. maculatus* (Fab) . [27; 26; 13].

In *C. serratus*, such studies are less advanced compared to other weevils. However, some plants were tested for efficacy against the bruchid peanuts: it is *Pachyrhizus erosus* (L.), *Boscia senegalensis* (Pers) Lam [6], *Calotropis procera, Senna occidentalis* [25] In this study, we tested the effect of *A.. senegalensis* extracts on *C. serratus* whose fresh or dried leaves have a repellent insecticide against weevils pests of sorghum millet [12].

The genus *Annona* is characterized by presence of acetogenins [19], alkaloids other classes of compounds including carbonhydrate, lipids, amino acids, polyphenols, essential oils terpenoids [14]. Acetogenins isolated from *Annona senegalensis* particularly have anthelmintic properties, cytotoxic antitumor, antimicrobial, antimalarial, antiparasitic, antiprotozoal, immunosuppressive are a source of models for potential anticancer drugs pesticides. The methanol extract of the roots of *A. senegalensis* showed antiparasitic activity on chloroquine-resistant strain of *Plasmodium falciparum* [7]

MATERIALS AND METHODS

Collect and maceration

The fresh leaves of *A. senegalensis* are harvested Sangalkam, a village in the Niayes the region of Dakar, in June 2007 to 17h. They are then dried at room temperature and then pulverized with an electric grinder. Hundred (100) grams of powder are macerated in 500 ml of methanol for 24 h at room temperature. The filtrate is evaporated to a residue called crude extract. Part of this crude extract was separated successively by the method of differential solubility in three solvents of different polarity: hexane, ethyl acetate and methanol. The crude extract was macerated in 500 ml of hexane for 24 h and then evaporated. A hexane phase and marc (1) are recovered. Marc (1) is dried in the open air and then soaked in 500 ml of ethyl acetate for 24 h and then evaporated; phase acetate and marc (2) are also recovered. Marc (2) is finally taken up in 500 ml of methanol to recover the polar compounds in the methanolic phase. Insects used.

The strain used for the rearing and testing comes from *Piliostigma reticulatum* pods and *A. hypogaea*; this choice is based on work morphometric and genetic strains of *C. serratus* [21]. It has been demonstrated in this work that the strain that infects peanut comes precisely from the natural host *P. reticulatum*. The insects are reared in the laboratory in glass jars fitted with screen cover. In each jar, we introduce peanuts or *P. reticulatum,* a sufficient number of males and females, and cotton soaked in distilled water. Sexing adults is through observation of the last abdominal tergite which is curved in the male and the female elongated. After 48 h, the seeds are collected in glass Petri dishes and placed in an oven at 32 °C. After four generations of mass rearing, female *C. serratus* are used to lay on seeds of groundnut. After 24 h, each seed is carefully observed under a dissecting microscope to ensure that it has received only a single egg, where several eggs are laid on a seed, others are loose, one egg should develop into a seed for approximately the same amount of food each hatchling. The extracts were diluted in suitable solvents whose toxicity has already been tested on adults and eggs of *C. serratus*. Three doses calculated from the mass extracted were tested: C1 = 0.001 g/ml, C2 = 0.01 g / ml and C3 = 0.1 g / ml.

Ovicid tests

Groundnut seeds are placed in glass Petri dishes and sprayed with 2 ml of test solution. After 48 h, they are placed individually into wells of a rectangular plastic box with 4 rows of 6 dwellings. Two boxes are filled, thus corresponding to 48 repetitions for each sample. This device allows tracking individual insects. All the boxes are placed in the laboratory at room temperature. A white light in which the seeds are not treated and one control with the dilution solvent are introduced.

Calculated parameters

Parameters such as mortality rates are calculated embryonic and larval and are corrected using Abbott's formula [1] to assess the efficacy of insecticide products. The dates of cocoon formation and emergence are scored to determine the duration of the larval stage, pupal phase and that of the total development. Mortality rates of eggs and larvae are calculated using the following formulas:

Rate of egg mortality = [(number of eggs - number of eggs hatched) x100] / number of eggs laid
Rates of larval mortality = [(number of eggs hatched - number of adults emerged) x100] / number of eggs hatched
Adults that emerged despite the application of extracts (survivors) are coupled together to study some biological parameters such as fertility and longevity.
To assess fertility, each pair placed in a Petri dish containing numbered peanuts. Every day, the number of eggs emitted by each female was counted and the seeds are infested and replaced by other perfectly healthy. It should be noted that the conditions: lack of food and water are applied to these young adults. Track couples stops with their death.
Fertility calculated by averaging the number of eggs per female

Adulticid tests

The insecticidal substances are tested in Petri dishes of 90 mm diameter, Whatman filter paper as support. Two (2) ml of solution are used to treat insects with apolar extracts and intermediate polarity (hexane-ethyl acetate) and one (1) ml for the treatment of these with the polar extracts (crude extract and methanol) which are less volatile. This is to soak the paper with the solutions and allow solvents for 5 minutes before removing insects. The extracts were tested with three concentrations predefined; out of 12 insects aged 24 h at most, from the mass rearing. Adult mortality is relieved one hour after treatment and every 24 h for three days. Each treatment was repeated three times, and witnesses with insects treated with different solvents and dilution of a witness with untreated insects are put in place. To evaluate the insecticidal efficacy, we corrected mortalities obtained with the samples treated according to those of untreated samples using the formula of Abbott [1]. This correction allows excluding natural mortality observed in our experimental conditions.
Statistical analyses are performed with Statview. The raw data were subjected to analysis of variance or two factors (ANOVA), mean (± standard deviation) are compared with the multiple comparison test of Fischer.
P values less than 0.05 are considered significant. The LT50 is estimated using regression in Excel.

RESULTS

Ovicidal and monitoring of survivors (Table 1)

With the crude extract low mortalities of eggs are stored. By splitting this excerpt, we get a very significant ovicidal effect with the fraction of acetate that eliminates 100% of eggs. It appears, with the follow-up analysis of the survivors, a significant difference between treatments ($p < 0.05$). Eggs treated with the crude extract and the hexane fractions of *A. senegalensis* realize short-lived larval stage, it is not affected if the eggs are treated with the fraction of acetate and methanol. The pupal larvae and fecundity of surviving females from these nymphs were not affected by the extracts of *A. senegalensis*. The life of survivors from the various treatments is, however more important than that of untreated insects (Table 2).

Table 1: ovicidal efficacy of different extracts on *Caryedon serratus*

	Extracts			
concentrations	crude extract	Hexane fraction	Acetate fraction	Méthanolic fraction
0.001g/ml	7,14 [a]	2,33 [a]	86,65 [a]	26,18 [a]
0.01g/ml	14,28 [a]	2,33 [a]	86,65 [a]	33,32 [a]
0.1g/ml	0 [a]	6,97 [a]	100 [a]	23,8 [a]

For the same column, values having the same superscript alphabet letter, do not differ statistically.

Table 2 : parameters of life survivors

extracts	Larval phase	Pupal phase	Fecondity	Longevity
Crude extract	37.91 [a]	21.15 [a]	55.94 [a]	46.69 [ab]
Hexane fraction	37.76 [a]	19.33 [a]	53.6 [a]	53. 67 [b]
Acetate fraction	39.5 [ab]	32.5 [a]	-	-
Méthanolic fraction	68.66 [b]	21.11 [a]	51.3 [a]	53.13 [b]
Witness	62.26 [b]	19.30 [a]	57.67 [a]	30.9 [a]

For the same column, values having the same superscript alphabet letter, do not differ statistically

Adulticid activity of extracts

Adult mortality varies according to excerpts; they seem to be effective at a given concentration of their polarity. The polar extracts (crude extract and methanol) was diluted in water, are effective at high doses C_2 and C_3 (0.01 and 0.1g/ml), the acetate fraction acts at low doses (0.001g/ml) while the fraction hexane is effective on *C. serratus* at a dose C_2 (0.01g/ml). Indeed, the crude extract causes low concentration (0.001g/ml) of mortalities were not significantly different from those recorded with insects treated with water. By increasing the concentration, insect mortality reached 66% in 24 h of contact and believes up to 85% after 72 h. With the hexane fraction, the contact time does not affect the mortality of insect activity levels did not differ significantly (P = 0.27). The high mortality rates are however recorded with 0.01 g / ml; it kills 47% of the insects in 72 h. When insects are treated with the acetate fraction with low dose (0.001g/ml) 41.6% die within the first hour of contact after 24 h, the mortality reached 97.4% and remains unchanged until the end of the experiment (72 h). However, by increasing the concentration a decrease in activity is noted with a mortality of 13.8 and 11.1 respectively for 0.01% and 0.1 g / ml after 72 h of contact. The purpose of the adulticid methanol fraction is low when dealing with concentrations 0.001 and 0.01 g/l are respectively about 25 and 8.33% after 72 h of contact, increasing the concentration (0.1g / ml) caused 94.4% mortality after only 24 hours of contact (Table no 3 to 7)..

Table 3: mean mortality (± SD) of *Caryedon serratus* adults treated with the crude extract of *Annona senegalensis*

	Concentration (g/ml)			
Hours after treatement	solvent	0.001	0.01	0.1
1h	2.78 ± 0.58	0 ± 0 [a A]	36.11 ± 4.04 [a A]	0 ± 0 [a A]
24h	11.11 ± 0.58	0 ± 0 [a A]	66.67 ± 1 [b A]	66.67 ± 2.08 [b B]
48h	13.89 ± 0.58	11.11 ± 0.58 [b B]	75 ± 1 [b A]	80.56 ± 1.73 [b B]
72h	19.44 ± 0.58	13.88 ± 1.15 [a B]	80.56 ± 0.58 [A]	80.56± 0 [b B]

a, b, within a row values with same superscript lowercase alphabetic not statistically different. A B within a row values with same superscript capital letter alphabet do not differ statistically

The calculation of LT50 reveals that the efficiency is not linearly dependent on concentration of different extracts tested. With the crude extract the time it takes to eliminate 50% of the insects is respectively 11, 7 and 29.7 h with concentrations 0.01 g / l and 0.1 g / ml (figure 1).

Table 4: mean mortality (± SD) of *Caryedon serratus* adults treated with the hexane fraction of *Annona senegalensis*

	Concentration (g/ml)			
Hours after treatement	solvent	0.001	0.01	0.1
1h	0	0 ± 0 $^{a\,A}$	5.56 ± 0.58 $^{a\,A}$	5.56 ± 1.25 $^{a\,A}$
24h	0	5.56 ± 0.58 $^{a\,AB}$	33.33 ± 2.65 $^{a\,A}$	22.22 ± 2.52 $^{a\,A}$
48h	0	5.56 ± 0.58 $^{a\,AB}$	47.22 ± 4.62 $^{a\,A}$	27.78 ± 3.06 $^{a\,A}$
72h	0	8.33 ± 0 $^{a\,B}$	47.22 ± 4.62 $^{a\,A}$	27.78 ± 3.06 $^{a\,A}$

a, b: within a row values with same superscript lowercase alphabetic not statistically different. A B within a row values with same superscript capital letter alphabet do not differ statistically

Table 5: mean mortality (± SD) of *Caryedon serratus* adults treated with the acetate fraction of *Annona senegalensis*

	Concentration (g/ml)			
Hours after treatment	solvent	0.001	0.01	0.1
1h	0	41.67 ± 1.73 $^{a\,A}$	0 ± 0 $^{b\,A}$	0 ± 0 $^{b\,A}$
24h	0	94.44 ± 1.15 $^{a\,B}$	11.11 ± 0.58 $^{b\,B}$	8.33 ± 0 $^{b\,B}$
48h	0	94.44 ± 1.15 $^{a\,B}$	11.11 ± 0.58 $^{b\,B}$	11.11 ± 0.58 $^{b\,B}$
72h	0	94.44 ± 1.15 $^{a\,B}$	13.89 ± 1.15 $^{b\,B}$	11.11 ± 0.58 $^{b\,B}$

a, b, within a row values with same superscript lowercase alphabetic not statistically different. A B within a row values with same superscript capital letter alphabet do not differ statistically

Table 6: mean mortality (± SD) of *Caryedon serratus* adults treated with the methanolic fraction of *Annona senegalensis*

	Concentration (g/ml)			
Hours after treatment	solvent	0.001	0.01	0.1
1h	2.78 ± 0.58	0 ± 0 $^{a\,A}$	0 ± 0 $^{a\,A}$	52 .78 ± 0.58 $^{b\,A}$
24h	11.11 ± 0.58	22.22 ± 0.58 $^{a\,B}$	5.56 ± 0.58 $^{b\,B}$	94.4 ± 0.58 $^{c\,B}$
48h	13.89 ± 0.58	25 ± 1 $^{a\,B}$	8.33 ± 0 $^{b\,B}$	94.4 ± 0.58 $^{c\,B}$
72h	19.44 ± 0.58	25 ± 1 $^{a\,B}$	8.33 ±0 $^{b\,B}$	94.4 ± 0.58 $^{c\,B}$

a, b, within a row values with same superscript lowercase alphabetic not statistically different. A B within a row values with same superscript capital letter alphabet do not differ statistically

Table 7: efficacy of the extracts after 72 h of contact

Concentration (g/ml)	Crude extract	Hexane fraction	Acetate fraction	Methanolic fraction
0.001	- ^{a}	8,33 ^{b}	94,44 ^{c}	6,9 ^{a}
0.01	75,86 ^{bc}	47,22 ^{b}	13,89 ^{a}	- ^{a}
0.1	75,86 ^{b}	27,78 ^{a}	11,11 ^{a}	93,04^{b}

On the same line (for the same concentration) values with the same superscript alphabet letter did not differ significantly at $p < 0.05$.

By splitting the crude extract, we have an LT 50 of 64 h with 0.01 g / ml of the hexane fraction (Figure 2) while acetate fraction eliminates over 50% of the insects from the first hour of application with 0.001g / ml (calculated Tl 50 - 11.06 h) (Figure 3), the same result is recorded when the insects are treated with the highest concentration of the methanol fraction with Tl 50calculated equal to- 28.8 h (Figure 4)

After 72 h of treatment, the results of the ANOVA showed a significant difference between the extracts for the same concentration tested. The lowest concentration of acetate fraction caused more deaths on insects. At high concentration (0.01g/ml), the efficiency is registered with the crude extract causing 75.86% mortality. The effect of acetate fraction is not significantly different from that of the crude extract (e.g. P <0.005).

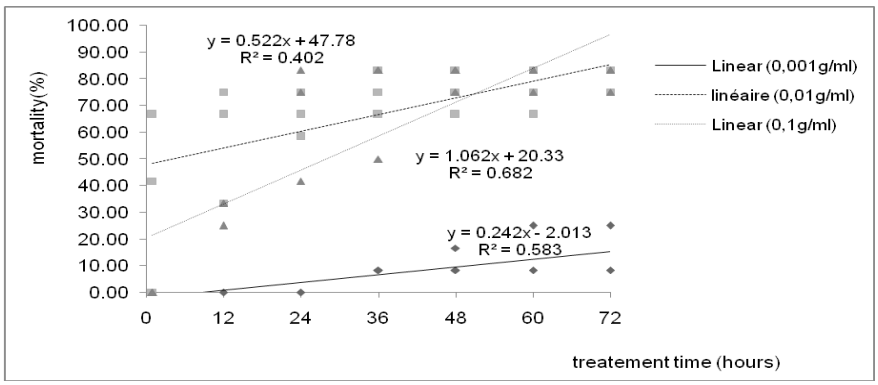

Figure 1: time trends in *C. serratus* adult mortality treated with crude extract.

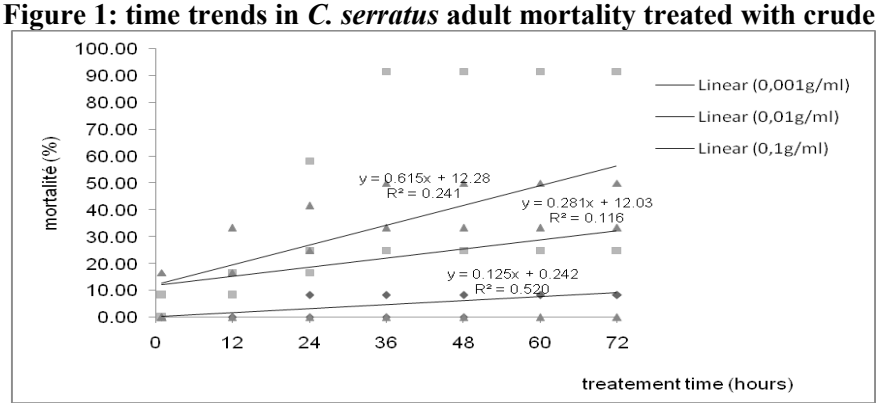

Figure 2: time trends in *C. serratus* adult mortality treated with hexane fraction.

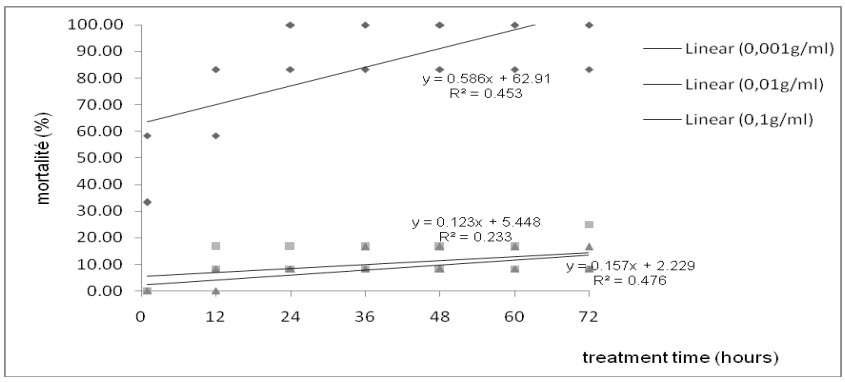

Figure 3: time trends in *C. serratus* adult mortality treated with acetate fraction

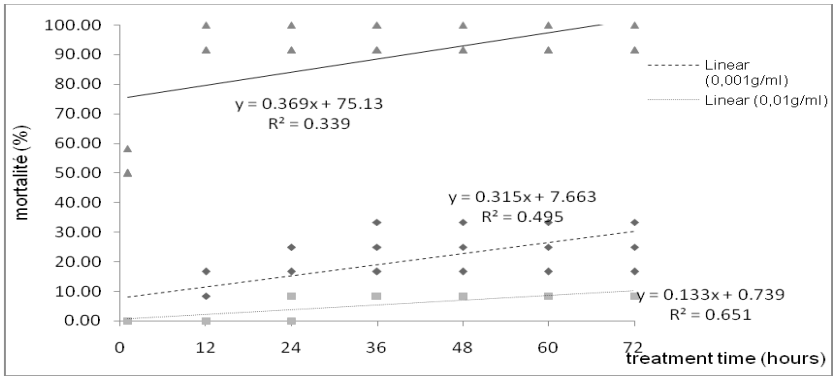

Figure 4: time trends in *C. serratus* adult mortality treated with methanolic fraction

DISCUSSION

Effect of treatments on eggs and the development of the survivors

It was noted that the ovicidal effect was obtained with acetate and methanol fractions. On the polar extract of *A. senegalensis* such activity has been demonstrated by other authors. For example, the aqueous fraction of the leaves has shown antiparasitic activity against *Trypanosoma brucei brucei*, with a dose of 200 mg / kg it completely eliminates the parasites from the bloodstream after 3 days [18] as well with a concentration of 7.1 g / ml, the aqueous extract of the stems induces a significant reduction of hatching eggs of this parasite [4]. [20], in diarrheal disorders find that a rabbit can be calmed with the methanol extract of stem bark of *A. senegalensis*; with an oral dose of 10 mg / kg, the spontaneous contractions are attenuated thereby reducing the rate of intestinal transit.

Mortality, development time and weight of larvae are the performance indices the largest and most analyzed in the studies related to the dynamics of insect populations. It is also recognized that the effect of secondary plant substances on the physiology of the insect is expressed mainly by increased mortality, longer development time, a limited fertility and birth when certain particular tissues are affected. This prompted us to pay attention to these parameters in *C. serratus* after application of the extracts.

After treatment, both groups reduced their larval development are distinguished: those obtained from treatments with low mortality (crude extract and hexane fraction of *A. Senegalensis*) and a second, having escaped from treatment with caused significant mortality. The extracts of *A. senegalensis* normally cause an elongation of adult longevity but do not involve changes in the fertility of females.

Adulticide effect

By analyzing the results obtained with extracts of *A. senegalensis*, it appears that the crude extract, acetate and methanol fraction, show an adulticid activity and that it depends strongly on the concentration of treatment. Indeed, with the crude extract, concentration 0.01 g / ml eliminates about 35% of adults over 1 h while it takes 24 hours to obtain the same mortality rate for treating 0.1 g/ml, for only the fraction acetate concentration 0.001 g / ml is effective only when the methanol fraction should be treated with 0.1 g / ml to obtain a mortality rate of about 50%. One could say that these are intermediate polarity and polar compounds of *A. senegalensis*, which showed activity on *C. serratus.* Of these extracts several authors have highlighted the presence of acetogenins that have some activity on multiple targets as well as alkaloids in polar fractions. For example, [8] isolated 5 acetogenins on neutral dichloromethane extract and one of them, the squamocine showed significant anthelmintic activity against *Rhabditea pseudoelongata*. With methylene chloride extract of the roots, [19] have isolated acetogenins and 5 showed a significant cytotoxicity on *Artemia salina* larvae.

[3] also found, with the methanol extract of leaves of *A. senegalensis* cytotoxic activity against ovarian cancer cells with an IC50 = 28.8 mcg / ml, its activity against *Plasmodium berghei* antimalarials is also tested in vivo by the same authors with 91% mortality for a dose of 800 mg / g of animal weight. Some effectiveness against the venom of *Naja nigricotlis nigricotlis* is also obtained with the methanol extract of the roots of *A. senegalensis* [2].

Conclusion

Crude extract, acetate and methanol fractions proved active against eggs, while the adulticide effect was obtained with acetate and methanol fractions of *A. senegalensis*. It would be interesting to turn these extracted powders for a better application of peanut stocks. It was noted that acetate and methanol fractions act on eggs and adults of the insect, which could be used in the protection of stocks by applying at the beginning of the infestation and at the time of emergence of adult survivors.

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