International Journal of Zoology Studies
www.zoologyjournals.com
ISSN: 2455-7269
Received: 07-03-2021, Accepted: 08-04-2021, Published: 18-05-2021
Volume 6, Issue 3, 2021, Page No. 19-25

Insecticidal activity of *Annona senegalensis* leaves essences against *Caryedon serratus* Ol. (*Coleoptera, bruchidae*) pest of groundnut stocks

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Abstract

Biological activity of essences from *Annona senegalensis* leaves was evaluated in laboratory, on various stages of development of *Caryedon serratus*, a pest of groundnut stocks. The tested essences are obtained by steeping the powder with three solvents (methanol, hexane and ethyl acetate). The acetate fraction kills 100% of the eggs with 0.1g/ml whereas the methanol fraction kills 33% with 0.01g/ml. The crude extract and the hexane fraction have been shown to be ineffective but lead to a reduction of larval lifetime. Chrysalis lifetime and fecundity of the survivors are not modified by the various extracts; however, an increase in longevity is noted with all extracts. On adults, the effect depends on the extract and the dose used. After 72 h at high concentration, the crude extract and the methanol fraction eliminate 75% and 94% of insects, respectively; with a concentration of 0.01g/ml, the crude extract and the hexane fraction kill 75.86% and 47.22% of the insects, respectively, and at low concentration (0.001 g/ml) the acetate fraction acts and eliminates 94 %. Methanol and acetate fractions could be used in stocks protection, by applying them at the early infestation and at the period of emergence of the surviving adults.

Keywords: *Annona senegalensis*, *Caryedon serratus*, groundnut, plant essences

Introduction

Senegal, like all Sahelian countries, is confronted with a very high rate of crop destruction, especially that of groundnuts. During storage, groundnut crops are mainly attacked by *Caryedon serratus* Olivier, 1790, a beetle belonging to the Family Chrysomelidae, commonly known as grounnut bruchid. This pea beetle can attack husk groundnuts from fields [20], creating, in this way, quantitative losses up to 83 % over a period of four months [16]. Moreover, holes left on the husk by larvae favour (the) infestation of other side species such as *Trogoderma spp*., *Ephestia cautella* (Walker), *Tribolium spp*., and facilitate the development of a fungus, *Aspergillus flavus* Link., productive of aflatoxin [10]. Among the methods used to limit *Caryedon serratus* damages, we generally notice the use of chemical insecticides that can induce intoxication of farmers and consumers, resistance from pests or have a negative impact on the environment. To achieve effective protection, an alternative that would not bring about health problems or harm to consumers and the environment must be found. Thus, several ways are proposed for stored commodities in particular: modification of the atmosphere (use of CO2, reduction of oxygen by use of nitrogen and other gases, storage temperatures) [7] [15], mixture of contact insecticides and growth regulators, growth regulators, plant essences. The use of the last as bio pesticides, in the protection of stored vegetable or cereal seeds against insects, has been the subject of numerous studies [5], particularly in tropical areas. In Africa, they have focused on the application of substances from plants (fresh crushed leaves, powders, essential oils, extracts) against beetles, including bruchids *Callosobruchus phaseoli* (Gyllenhal), *C. chinensis* (Linn.), *C. maculatus* (Fab)... [12]. In *C. serratus*, such studies are less advanced compared to other bruchids. However, a few plants have been tested with efficacy against groundnut bruchid: *Pachyrhizus erosus*, (L.), *Boscia senegalensis*, (Pers) Lam. [6], *Calaotropis procera*, *Senna occidentalis* [6]. In the present study, we tested the effect of *A. senegalensis* essences against *Caryedon serratus* whose dried or fresh leaves have a repellent and insecticidal effects against bruchids as well as sorghum and millet pests [11]. The genus *Annona* is characterized by the presence of acetogenins [18], alkaloids and other classes of compounds including carbonhydrates, lipids, amino acids, polyphenols, essential oils and terpenoids [13]. The acetogenins, isolated from *Annona senegalensis* especially, have antihelminthic, cytotoxic and antitumor, antimicrobial, antimalarial, antiparasitic, antiprotozoal, immunosuppressive properties, and are a source of models for potential anticancer drugs and pesticides [25]. The methanolic extract of the roots of *A. senegalensis* showed antiparasitic activity on the chloroquine-resistant strain of *Plasmodium falciparum* [8].

Materials and Methods

Harvesting and Steeping

Fresh leaves of *A. senegalensis* were collected in Sangalkam, a village located in the Niayes area of Dakar region, in June 2007 at about 17h. They are then dried at room temperature and ground to powder with an electric grinder. One hundred (100) grams of powder is steeped in 500 ml of methanol for 24 h at room temperature. The filtrate is evaporated to give a residue called crude extract. Part of this crude extract is successively separated, by the differential solubility method, in three solvents of different

polarity: hexane, ethyl acetate and methanol. The crude extract is steeped in 500 ml of hexane for 24 h then evaporated. A hexane phase and a mark (1) are recovered. The mark (1) is air-dried then macerated in 500 ml of ethyl acetate for 24 h then evaporated; an acetate phase and a mark (2) are also recovered. The mark (2) is finally taken back in 500 ml of methanol allowing to recover the polar compounds in the methanolic phase.

Insects used

The stock used for breeding and testing came from *Piliostigma reticulatum* and *Arachis hypogaea* pods; this choice was based on morphometric and genetic work on different stock of *C. serratus* [21]. It was shown in this work that the stock that infests groundnuts comes precisely from the natural host *P. reticulatum.* The insects are reared in laboratory in glass jars with wire mesh lids. In each jar, are introduced groundnut seeds or those of *P. reticulatum*, enough females and males, and cotton impregnated with distilled water. Sexing of adults is done by observing the last abdominal tergite which is curved in the male and straight in the female. After 48 h, seeds are recovered in glass Petri dishes and placed in an oven at 32 °C. After four generations of mass rearing, females of *C. serratus* are laid on groundnut seeds. After 24 h, each seed is meticulously observed with a binocular magnifying glass to ensure that it has received only one egg; if several eggs are laid on a seed, the others are taken off; only one egg must develop in a seed, to ensure approximately the same amount of food for each neonate larva. The extracts are diluted in appropriate solvents which have been previously tested for toxicity on eggs and adults of *C. serratus*. Three doses calculated on the extracted mass are tested: C1 = 0.001 g/ml; C2 = 0.01 g/ml, and C3 = 0.1 g/ml.

Ovicide tests

Groundnut seeds are placed in glass Petri dishes and sprayed with 2 ml of the test solution. After 48 h, they are put individually in wells of a rectangular plastic box with 4 rows of 6 lodges (wells). Two Petri dishes are filled corresponding to 48 repetitions for each extract. This device makes it possible to follow individually insects. All the Petri dishes are placed in the laboratory at room temperature. A white control in which the seeds are not treated and a control with the dilution solvent are set up.

- Calculated parameters

Parameters such as embryonic and larval mortality rates are calculated and corrected using [1] formula to evaluate the insecticidal efficacy of products. The dates of cocoon formation and emergence are noted to determine the duration of larval, pupal phases, and the total development. Egg and larval mortality rates are calculated according to the following formulas:

- Egg mortality rate = [(number of eggs laid - number of eggs hatched) x100] / number of eggs laid.
- Larval mortality rate = [(number of eggs hatched - number of adults emerged) x100] / number of eggs hatched.

Adults that emerged despite the application of the extracts (survivors) are paired together and monitored to study certain biological parameters such as fecundity and longevity. To assess fecundity, each pair is placed in a numbered Petri dish containing groundnut seeds. Every day, the number of eggs emitted by each female is counted and the infested seeds are replaced by perfectly healthy ones. However, it should be noted that conditions: absence of water and food are applied to these young imagos. The follow-up of couples ends with their death. The fecundity of females and the lifetime of adults can then be calculated.

Adulticide tests

The insecticidal effect of substances is tested in Petri dishes of 90 mm diameter, with a Whatman filter paper as support. Two (2) ml of solution are used to treat the insects with the essence without polarity and the one with intermediate polarity (hexane and ethyl acetate); and one (1) ml to treat them with the polar essences (crude and methanolic essences) which are less volatile. This consists in impregnating the paper with the solutions and letting the solvents evaporate for 5 minutes before depositing the insects. The essences are tested, at the three predefined concentrations, on 12 insects aged up to 24 hours, from mass rearing. Adult mortality is recorded one hour after treatment and every 24 h for three days. Each treatment is repeated three times; controls with insects treated with the different dilution solvents and a control with untreated insects are set up. To evaluate the insecticidal efficacy, we corrected the mortalities obtained with the treated samples to those of the untreated samples according to the formula of [1]. This correction allows us to exclude the natural mortality observed in our experimental conditions.

Statistical analysis

Statistical analyses are performed with the Stat view software. The raw data are put through a variance analysis (ANOVA) with one factor or two, the means (± standard deviation) are compared using Fischer's multiple comparisons test. P-values less than 0.05 are considered significant. Tl50 are estimated using regression by Excel software.

Results

Ovicidal activity and follow-up of survivors (Table 1)

With the raw extract low egg mortality is recorded. In fractionating this extract, a very important ovicidal effect is obtained with the acetate fraction which eliminates 100 per cent of the eggs. It appears, with the analysis of the follow-up of the survivors, a significant difference between the treatments ($p < 0.05$). Eggs treated with the crude extract and hexane fraction of *A. senegalensis* achieve short larval life spans, which are not affected if eggs are treated with the acetate and methanolic fractions. The pupal life of the surviving larvae and the fecundity of the females from these pupae were not modified by the extracts of *A. senegalensis*. On the other hand, the life span of the survivors from the different treatments is longer than that of the untreated insects (Table 1).

Table 1: Ovicidal efficacy of the various essences on *Caryedon serratus*

	Essences			
Concentrations	**Crude essence**	**Hexane fraction**	**Acetate fraction**	**Methanol 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 with the same exponent alphabetical letter do not differ statistically (Table 2).

Table 2: Life parameters of survivors

Essences	Larva duration	Nymph duration	Fecundity	Longevity
Crude essence	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]	-	-
Methanol fraction	68.66 [b]	21.11 [a]	51.3 [a]	53.13 [b]
Control	62.26 [b]	19.30 [a]	57.67 [a]	30.9 [a]

For the same column, values with the same exponent alphabetical letter do not differ statistically.

Adulticidal activity of essences

Adult mortality varies with essences; these appear to be effective at given concentrations depending on their polarity. Polar essences (crude essence and methanol) suspended in water are effective at high doses C2 and C3 (0.01 and 0.1g/ml), the acetate fraction acts at low dose (0.001g/ml) while the hexane fraction is effective on *Caryedon serratus* at dose C2 (0.01g/ml). In fact, the crude essence causes, at low concentration (0.001g/ml), mortalities which are not significantly different from those recorded with insects treated with water. By increasing the concentration, the mortality of insects reaches 66% in 24 hours of contact, and it increases to 85% after 72 hours. With the hexane fraction, the duration of contact does not influence insect mortality; the activity of concentrations does not differ significantly (p = 0.27). However, high mortalities are recorded with 0.01 g/ml, which kills 47% of the insects in 72 h. When insects are treated with the acetate fraction at low dose (0.001g/ml) 41.6 % die from the first hour of contact after 24 h, the mortality reaches 97.4% and remains unchanged until the end of the experiment (72 h). However, by increasing the concentration a decrease of the activity is noted with a respective mortality of 13.8 and 11.1 % for 0.01 and 0.1 g /ml after 72 h of contact. The adulticidal effect of the methanol fraction is low when treated with concentrations 0.001 and 0.01 g/l; they are respectively of the order of 25 and 8.33 % after 72 h of contact; an increase in concentration (0.1g/ml) causes 94.4 % mortality after only 24 h of contact. The calculation of Tl 50 reveals that the efficiency is not linearly dependent on the concentration of the various essences tested. With the crude essence, the time required to eliminate 50% of insects is respectively 11. 7 and 29.7 h with the concentrations 0.01 g/l and 0.1 g /ml (figure 1). By fractionating the crude extract, we have a TL 50 of 64 h with 0.01 g/ml of the hexane fraction (figure 2), while the acetate fraction eliminates more than 50% of insects from the first hour of application with 0.001g/ml (Tl 50 calculated - 11.06h) (figure 3). The same result is recorded when (the) insects are treated with the highest concentration of the methanol fraction with a calculated Tl 50 equal to (- 28.8 h) (Figure 4).

Table 3: Mean mortality (± standard deviation) of adults of *Caryedon serratus* treated with the crude essence from *Annona senegalensis*

	Concentration in g/ml			
Hours after treatment	**Solvent indicator**	**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 line, values having in exponent the same alphabetical small letter do not differ statistically. A, B within a line, values having the same alphabetical capital letter as exponent do not differ statistically.

Table 4: Mean mortality (± standard deviation) of adults of *Caryedon serratus* treated with the hexane fraction of *Annona senegalensis*

	Concentrations in g/ml			
Hours after treatment	**Solvent indicator**	**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 line, values having in exponent the same alphabetical small letter do not differ statistically. A, B within a line, values having the same alphabetical capital letter as exponent do not differ statistically.

Table 5: Mean mortality (± standard deviation) of adults of *Caryedon serratus* treated with the acetate fraction of *Annona senegalensis*

	Concentrations in g/ml			
Hours after treatment	**Solvent indicator**	**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 line the values having in superscript the same alphabetical lowercase letter do not differ statistically. A, B Within a line, the values having the same alphabetical capital letter as superscript do not differ statistically.

Table 6: Mean mortality (± standard deviation) of adults of *Caryedon serratus* treated with the methanol fraction of *Annona senegalensis*

	Concentrations in g/ml			
Hours after treatment	**Solvent indicator**	**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 line, values having in exponent the same alphabetical small letter do not differ statistically. A, B within a line, values having the same alphabetical capital letter as exponent do not differ statistically.

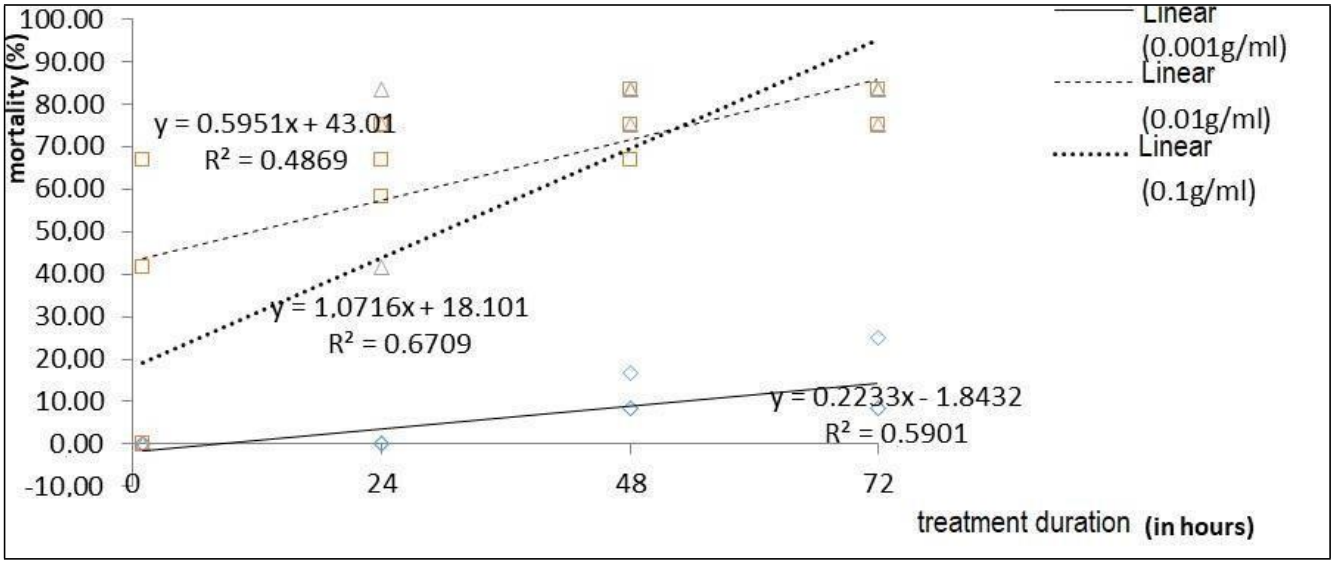

Fig 1: Chronological evolution of mortality of *C. serratus* adults treated with the crude extract

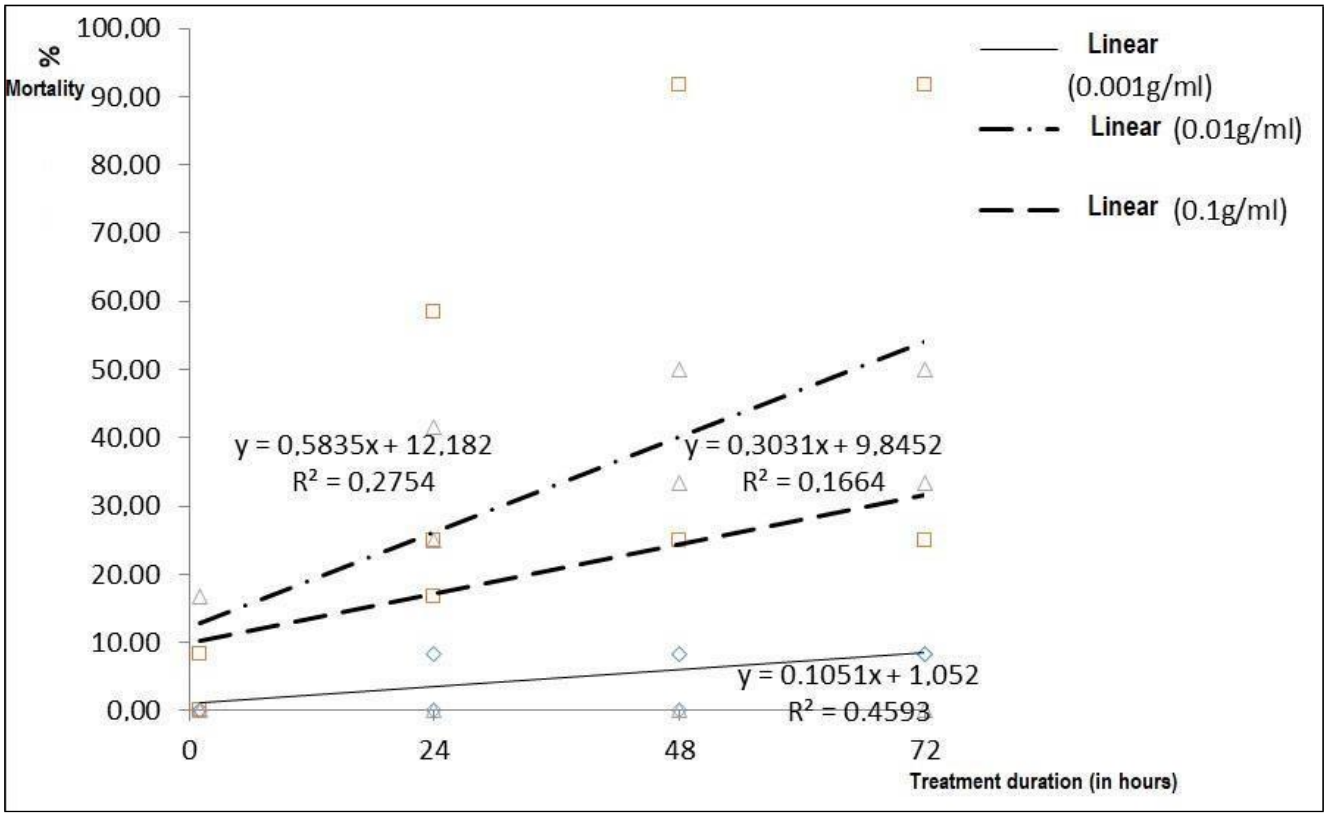

Fig 2: Chronological evolution of mortality of *C. serratus* adults treated with the hexane fraction

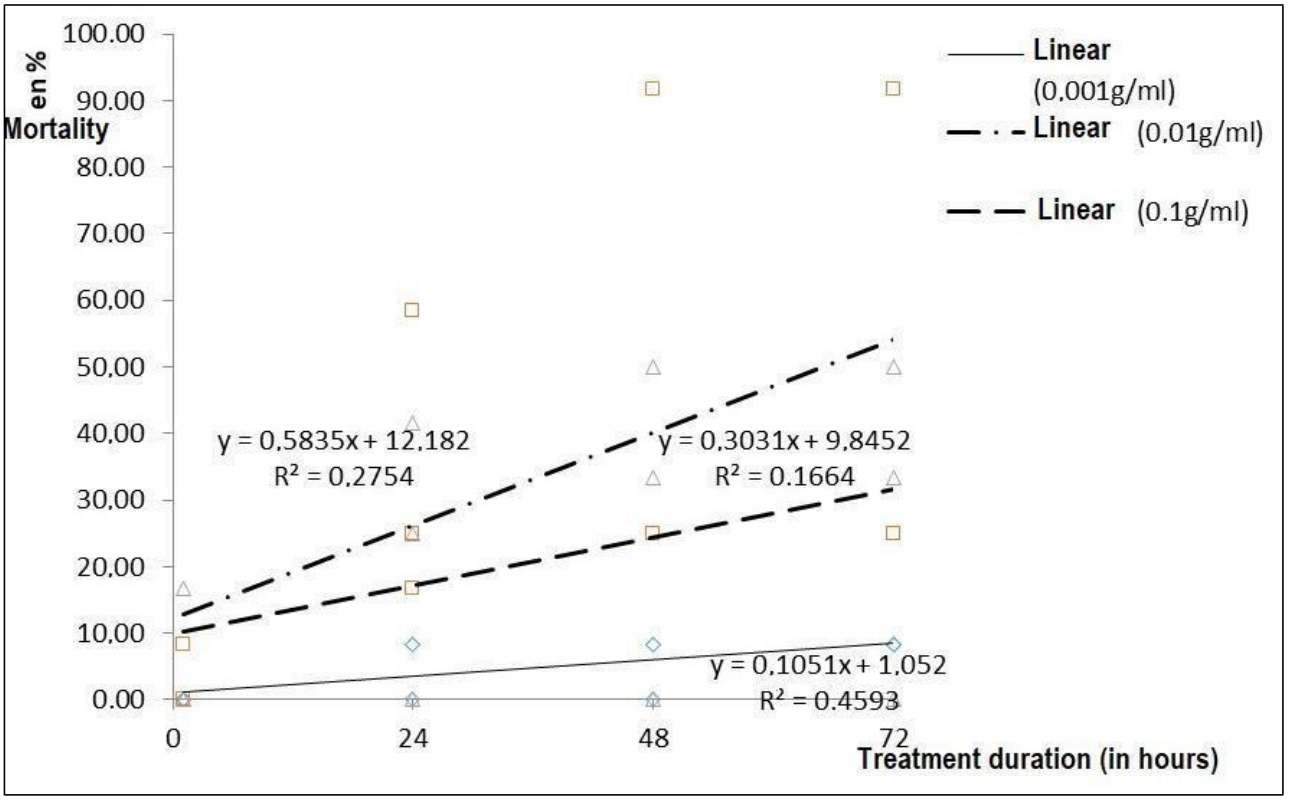

Fig 3: Chronological evolution of mortality of *C. serratus* adults treated with the acetate fraction

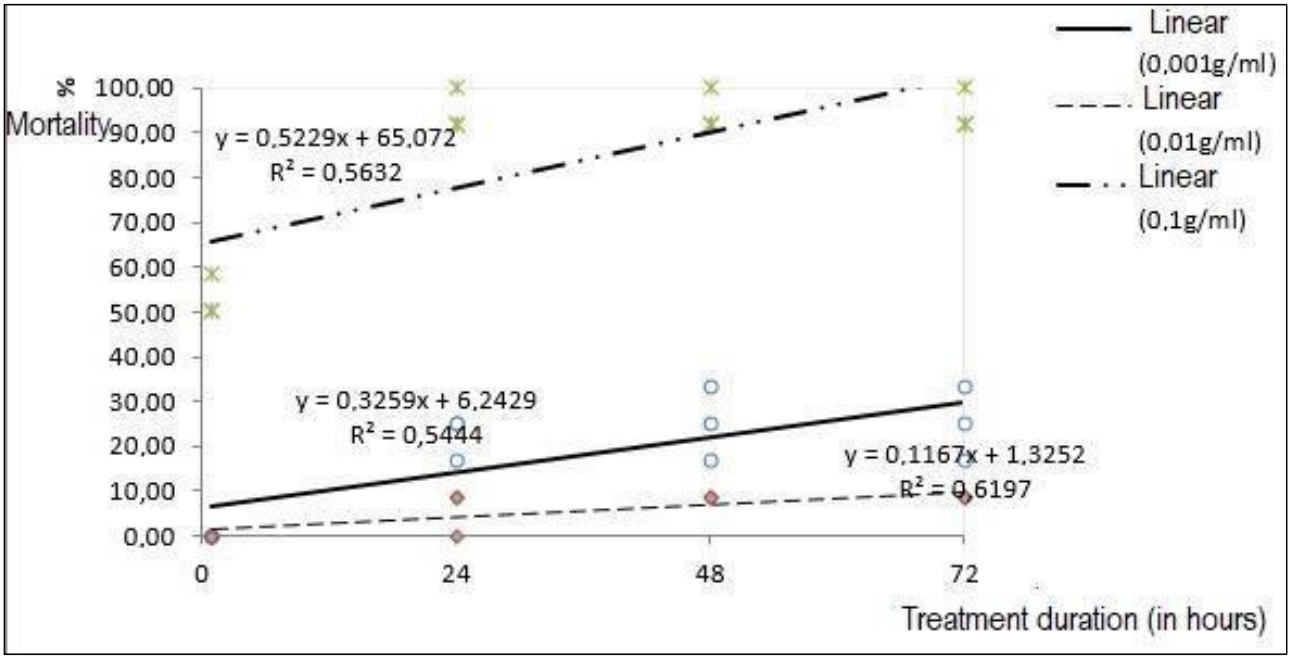

Fig 4: Chronological evolution of mortality of *C. serratus* adults treated with the methanol fraction

Table 7: Efficiency of essences after 72 h of contact

Concentration (g/ml)	Crude essence	Hexane fraction	Acetate fraction	Methanol 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 (i.e. for the same concentration) values with the same exponent letter do not differ significantly at $p<0.05$.

After 72h of treatment (the) results of the variance analysis (of variance) show a significant difference between (the) essences for the same tested concentration (tested). The lower concentration of the acetate fraction caused more mortality on (the) insects. At high concentration (0.01g/ml), the best efficacy is recorded with the crude essence which causes 75.86% mortality. However, the effect of the acetate fraction was not significantly different from that of the crude essence ($p < 0.005$).

Discussion

Effect of treatments on eggs and on the development of survivors

It has been noted that the ovicidal effect is obtained with the

acetate and methanol fractions. About the polar essence of *A. senegalensis* similar activity has been demonstrated by other authors. For instance, the aqueous fraction of (the) leaves showed antiparasitic activity against *Trypanosoma brucei brucei*; with a dose of 200 mg/kg, it completely eliminates parasites from the bloodstream after 3 days [17]. Similarly, at a concentration of 7.1 g/ml, the aqueous essence of stems induced a significant reduction in the eggs hatching (of eggs) of this parasite [4]. Suleiman *et al.* [15] found that diarrheal disorders in a rabbit could be soothed with methanolic essence from *A. senegalensis* stem barks; with an oral dose of 10 mg/kg, spontaneous contractions were attenuated, thus decreasing the rate of intestinal transit. Mortality, period of development, and larval weight are performance indices the most important and analysed in studies related to insect population dynamics. It is also recognized that the effect of secondary plant substances on insect physiology is mainly expressed by an increased mortality, prolonged period of development, limited fertility, and malformations when specific tissues are affected. This led us to pay particular attention to these parameters in *C. serratus* after application of the extracts. After treatment, two groups with reduced larval development can be distinguished; those from treatments that resulted in low mortality (crude essence and hexane fraction of *A. senegalensis*) and a second group with survivors from treatments that resulted in high mortality. Essences from *A. senegalensis* generally induce an elongation of the longevity of adults but do not lead to a modification of the fecundity of females.

Adulticidal effect

When we analyse results obtained with the essences from *A. senegalensis*, it appears that the crude ones, the acetate fraction as well as the methanol fraction, show an adulticidal activity and that this strongly depends on the treatment concentration. As a matter of fact, with the crude essence, the concentration 0.01 g/ml allows to eliminate approximately 35 % of the adults in 1 h, whereas it is necessary to wait 24 h to obtain the same rate of death for the treatment 0.1 g/ml; as for the acetate fraction, only the concentration of 0.001 g/ml is effective; when with the methanol fraction, it is necessary to treat with 0.1 g/ml to obtain a rate of mortality of about 50%. One could say that is polar compounds and those with intermediate polarity from *A. senegalensis* which have shown activity against *C. serratus*. About these essences, several authors have underlined the presence of acetogenins which have a certain activity on several targets as well as alkaloids in the polar fractions. For example, Fall *et al.* [9] isolated 5 acetogenins on neutral dichloromethane extract and one of them, squamocin showed significant anthelmintic activity against *Rhabditis pseudoelongata*. With the chloromethylene essence from roots, Sahpaz *et al.* [18] isolated 5 acetogenins and showed significant cytotoxicity on *Artemia salina* larvae. Ajaiyeoba *et al.* [3] also found, with the methanolic essence from leaves of *A senegalensis* a cytotoxic activity against ovarian cancer cells with an IC_{50} = 28.8 µg/ml, its antimalarial activity against *Plasmodium berghei* is also tested *in vivo* by the same authors with 91% mortality for a dose of 800 mg/g of animal weight. Some efficacy against the venom of Naja nigricotlis nigricotlis is also obtained with the methanolic essence from *A. senegalensis* roots [2].

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