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Experimental study of the effects of an aqueous solution of *Eucalyptus alba* leaves on the development of *Callosobruchus maculatus*, a pest of bean (Cowpea) stocks in Senegal (West Africa)

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Abstract
The article aims to highlight a constraining effect of the aqueous solution of *Eucalyptus alba* leaves on the development of *Callosobruchus maculatus*, the main pest of cowpea (Bean) stocks. The long-term objective is to offer an alternative to the use of pesticides by producers which, in the process, cause a lot of damage to the environment, biodiversity and man. To achieve this objective, cowpea grains infested with adults or eggs of the insect were treated on the one hand with three different concentrations (C1ea=0.2 g/ml, C2ea=0.04 g/ml and C3ea=0.02 g/ml) of aqueous solution of leaves of the plant and on the other hand with an insecticide or with water as a control. Compared to the control (water), the results revealed significantly (all P-values are less than 0.05) a high ovicide rate for each of the concentrations, especially with C1ea (69.44%) compared to adulticidal rates the highest of which was 34% and the lowest 16%. The solution also delays the development of the insect, especially at the C1ea concentration, which lengthens its average development cycle to 26 days compared to the normal, which is 24 days.
Thus, due to its high levels of ovicide and adulticide, the aqueous solution proves to be an effective solution for the elimination of insect pests from cowpea stocks and is respectful of living beings, unlike chemical insecticides which present many dangers.

Keywords: Bean, *Eucalyptus alba*, *Callosobruchus maculatus*, ovicide, adulticide

Introduction
The bean or cowpea in the vernacular, known by the scientific name of *Vigna unguiculata* is a legume playing an essential economic function in sub-Saharan Africa, particularly in Senegal, due to its high protein content and its strong resilience to water shortage [11]. However, this important place is certainly threatened by climatic and edaphic factors, but above all by heavy losses. Indeed, cowpea grains can suffer losses that can vary from 25% to 30% [5]. In order to limit or even eradicate these losses, farmers mainly use pesticides. Senegalese agriculture uses an average of 598 tonnes of solid pesticides and 1, 336, 560 liters of liquid pesticides per year [13]. The use of insecticides is unfortunately accompanied by significant damage to humans and the environment. Indeed, pesticides and wastewater contribute to the pollution of groundwater, the main resource used in Dakar as irrigation water with real health risks [7]. Faced with these excesses, it is essential to find other effective and healthier remedies. The purpose of this article falls within this perspective. It aims to highlight an insecticidal effect of the solution of *Eucalyptus alba* leaves on *Callosobruchus maculatus*, through several factors of its development (laying, development cycle, adult mortality). To achieve this objective, seeds infested with adults or eggs of the insect were associated with different concentrations (C1ea=0.2 g/ml, C2ea=0.04 g/ml and C3ea=0.02 g /ml) solutions of the leaves of the plant, an insecticide (deltamethrin) or water. The R software allowed us to graphically exploit the results of the experiments and then to interpret them in relation to the purpose of the research.

Materials and Methods
Materials
The equipment used during all the experiments consists of:

- A 0.0001 g EXACTA type precision chamber balance.
- Binocular magnifier and hand magnifier.
- Flexible pliers.
- Petri dishes 9 cm in diameter and 1 cm in height, i.e. a volume of 63.585 cm^3.

Methods

Experimental device and solutions

The experimental device includes:

- three (3) white controls (Seeds + adults or eggs)
- three (3) solvent controls (Tap water + adults or eggs)
- three concentrations (C1ea, C2ea, C3ea) of aqueous solution were used and for the chemical insecticide (Deltamethrin), a single dose repeated 3 times. (D1ea).

Contact test of cowpea seeds soaked in aqueous solutions of fresh leaves of the plant with adults and eggs of *Callosobruchus maculatus*

Preparation of solutions and doses

The concentrations of the solutions of *Eucalyptus alba* leaves and doses of deltamethrin were prepared as follows:

- 200 g of crushed leaves of E. alba were mixed with 1 liter of tap water and left for 2 days of maceration. This mixture was strained through muslin. The aqueous extract obtained was put in bottles for storage in a fridge. All three concentrations that were used in our tests were obtained from the stock solution by the following method:

C1ea = 40 ml of the solution (C1ea = 1 g / 5 mL);
C2ea = 40 ml of the solution + 20ml of tap water (C2ea = 1 g / 25 mL);
C3ea = 40 ml of the solution + 40ml of tap water (C3ea = 1 g / 45 mL).

- 2, 4 and 6 g of fresh leaves were used, i.e. the following doses respectively:

M1 = 2 g equals D1ea = 2/63.585 = 0.0315 g/cm^3
M2 = 4 g equals D2ea = 4/63.585 = 0.0629 g/cm^3
M3 = 6 g equals D3ea = 6/63.585 = 0.0944 g/cm^3

- The deltamethrin used was applied at the recommended concentration which is 40ml for 30 L of water, dosage reported at 500 ml of water, i.e. 0.66 ml for 500 ml of water (Whose mass concentration C= 0.0035 g/ls). This dose is repeated 3 times.

The test

Twelve [12] couples of the same age (48 hours maximum) were placed in Petri dishes containing 25 g of cowpea seeds. Beforehand, two milliliters (2 ml) of each of the three concentrations were sprinkled on the seeds contained in each box. The latter is then strongly shaken for 2 to 3 minutes to ensure the distribution of the solution on the substrate before being infested with the 12 adults of C. maculatus. Three repetitions were carried out for each concentration, for the solvent control, for the blank control and for the deltamethrin control. The insects were exposed to the aqueous extracts for one week. Dead bruchids were counted every 24 hours.

Parameters evaluated and Statistical analyzes

Assessment of adult mortality of C. maculatus

After the treatments, a daily follow-up was carried out in 15 days for each batch then a certain number of parameters were evaluated.

➢ Daily mortality rate (% m_jour): % M_jour = $Nombre\_adultes\ mortsNombre\_adultes\ totals\ x$ 100

➢ Average mortality rate from the 5th day of treatment (% M_avg.) % $Mmoy.$ = $Nombreadultesmorts$ $(5jours)$ $Nombreadultestotals\ x$ 100

Evaluation of the pre-imaginal mortality rate (% M_emb)

% M_emb = 100− $Nombre\_adultes$ $émergés$ $Nombre\_oeufs\ total\ x$ 100

Evaluation of the effects of treatment on the development cycle of insects

The average bruchid development time for treated eggs (Dmd) is the time (d) elapsed between the midpoint of the laying period of the parent pairs and the time when 50% of the offspring have emerged.

Statistical analyzes

The calculations of the average of the repetitions were carried out on Excel 2013. The graphs and the statistical analyzes of the measured variables were carried out with the R software. The data obtained were subjected to parametric analyzes (One-factor ANOVA and Tukey test), after checking the normality and homogeneity of the variances of the data series to compare the means.

Results and Discussions

Results

Effect of *Eucalyptus alba* leaf solution on *Callosobruchus maculatus* eggs

The results show that the high ovicidal actions of the concentrations (Aqueous solution of *Eucalyptus alba* leaves) are significantly different (p-value < 0.001).

Table 1: Aqueous solution of *Eucalyptus alba* leaves) are significantly different

	Df	Sum Sq	Mean Sq	F-value	P-value
Formulations	5	14336	2867.3	74.32	<0.001
Residuals	12	463	38.6		

The highest egg mortality rate is obtained with the C1ea concentration (69.44%). Emergences with the two other concentrations of the aqueous solution of *Eucalyptus alba* leaves (C2ea and C3ea) are also low, with respective mortality percentages of 55.5% and 47.22%) (Figure I).

The white and control tubes where there is no leaf solution or insecticide naturally have the lowest ovicide rates (Respectively 16.66% and 22.22%).

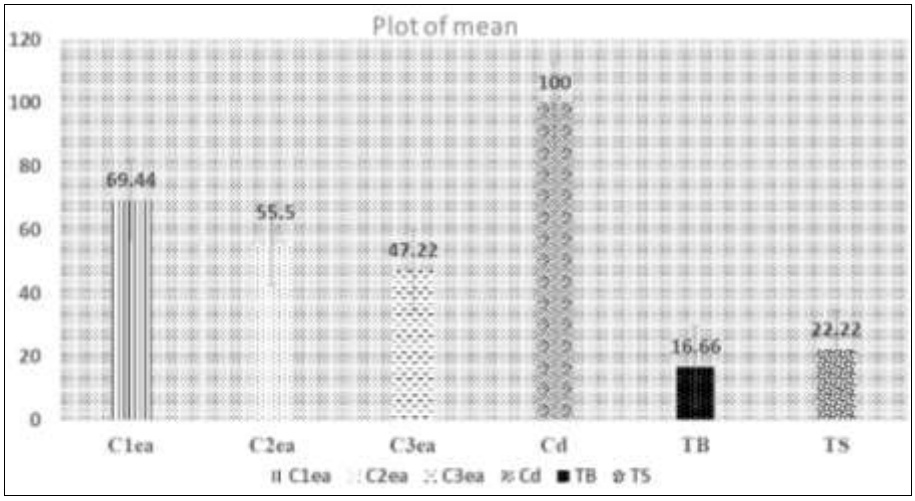

Fig 1: Mortality of different concentrations of *Eucalyptus alba* solution on *Callosobruchus maculatus* eggs.

Effect of *Eucalyptus alba* leaf solution on the development cycle of *Callosobruchus maculatus*.

The different concentrations of the aqueous solution of *Eucalyptus alba* leaves show significantly different negative effects on the development cycle of the insect (P-value= 0.01).

Table 2: Significantly different negative effects on the development cycle of the insect

	Df	Sum Sq	Mean Sq	F-value	P-value
Formulations	4	6.745	1.6862	11.04	0.01
Residuals	10	1.527	0.1527		

With an average duration of 26 days, the C1ea concentration further lengthens the development cycle of *Callosobruchus maculatus*. The other two concentrations (C2ea and C3ea) impose an average development cycle of 25 days on the insect.

Water has no effect on the development cycle of *Callosobruchus maculatus*.

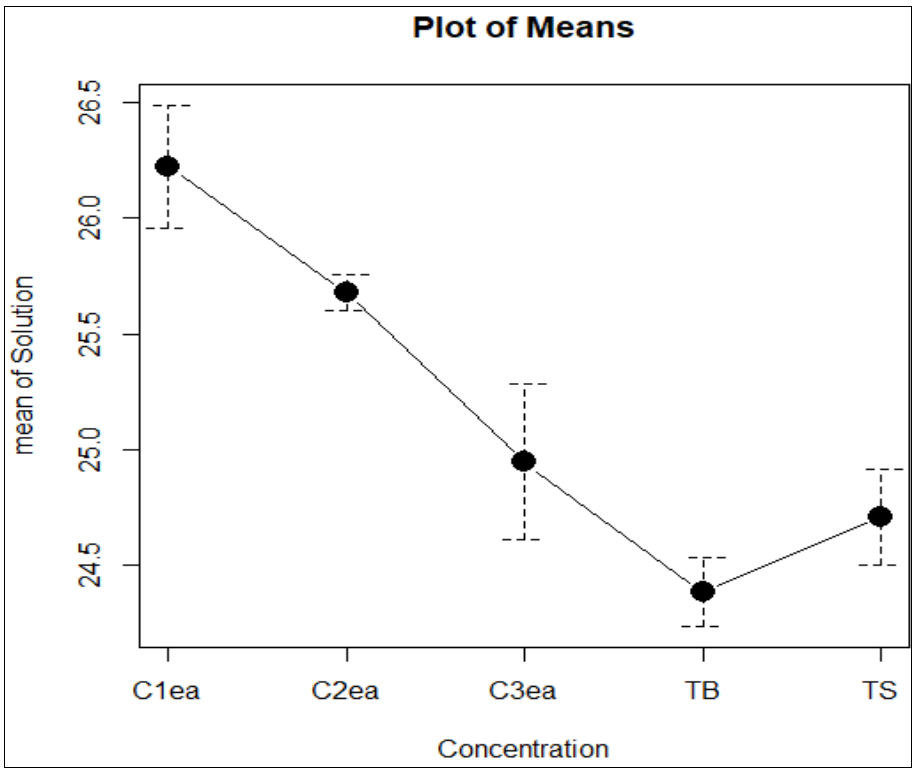

Fig 2: Effect of the aqueous solution of *Eucalyptus alba* leaves on the development cycle of *Callosobruchus maculatus*.

Effect of *Eucalyptus alba* leaf solution on insect adults

The concentrations of the aqueous solution of *Eucalyptus alba* leaves show high and significantly different mortality rates (p-value=0.001).

Table 3: Significantly different mortality rates

	Df	Sum Sq	Mean Sq	F-value	P-value
Formulations	5	17118	3424	24.65	<0.001
Residuals	12	1667	139		

The C1ea and C2ea doses, with an average percentage of 34%, kill insects much more than the C3ea dose (about 15%). Water has a low adult mortality rate for *Callosobruchus maculatus*: ten percent (10%) of insects die in the presence of water.

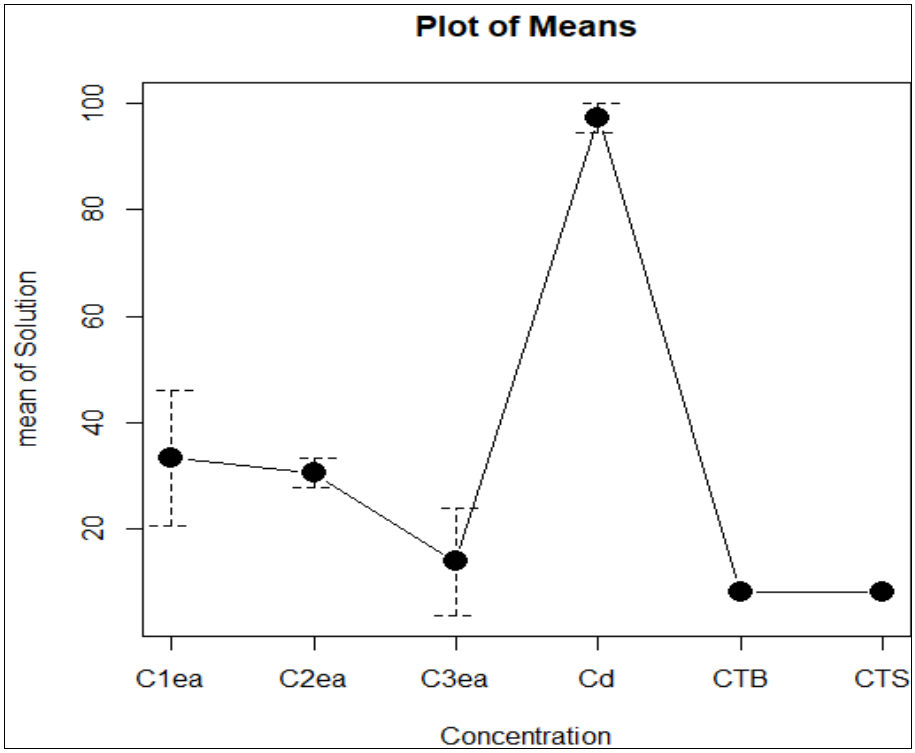

Fig 3: Effect of the aqueous solution of *Eucalyptus alba* leaves on adult mortality of *Callosobruchus maculatus*.

Discussions

The aqueous solution of *Eucalyptus alba* leaves has indeed constraining effects on the development of *Callosobruchus maculatus*, through several parameters of its development. The study revealed that the treatment of bean grains infested by the insect, with a concentration of 0.2 g per milliliter of solution, generates a low hatching rate, either an ovicidal percentage of approximately 70%. Morning and evening application of a solution of *Calotropis procera* leaves to larvae of *Plutella xylostella* [12] as equally effective. Even if the adulticidal effect of the aqueous solution of the plant is below the ovicidal one, the adulterous mortality rate (34%) of the solution remains substantial, like that of the study by Diamé [4] where the Azadirachta indica leaf solution treatment reduced pest attacks on Solanum melongena plantations by around 38.88%, although this rate reached 80% when the solution was applied to cultures of Cassia italica. Like aqueous solutions of leaves and stems of *Anogeissus leiocarpus* and *Mitragyna inermis* which attenuated the peak proliferation of caterpillars, potential pests of cotton fields, at D49 compared to the control, with concentrations of 0.208 kg/ l and 0.104 kg/l [8], the *Eucalyptus alba* leaf solution of 0.2 g per milliliter also retards the development of *Callosobruchus maculatus* by being able to lengthen the average development cycle of *Callosobruchus maculatus* at 26 days compared to normality (23 to 24 days). The use of the aqueous solution of this plant with a view to limiting the destruction of bean stocks is therefore effective, especially judicious in a context where the products intended for the chemical fight against crop pests are toxic to humans, pets, fish or game [14].

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