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Study of The Comparative Effectiveness of The Aqueous Solution, Shredded Material and Fumigation of *Eucalyptus alba* Leaves on The Development of *Callosobruchus maculatus* in Cowpea Stocks

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ABSTRACT

The aim of present study was to highlight the type of formulation (aqueous solution, shredded material and fumigation) of *Eucalyptus alba* leaves that was most effective in constraining the development of *Callosobruchus maculatus*, through some of the barometers of its reproduction (rate emergence and adult mortality). Thus three doses (D1ea= 0.0315 g/cm3, D2ea= 0.0629 g/cm3 and D3ea= 0.0944 g/cm3) or concentrations (C1ea= 0.2 g/ml, C2ea= 0.04 g/ ml and C3ea= 0.02 g/ml) of *Eucalyptus alba* leaves were treated so as to obtain for each dose an aqueous extract, a crushed material (both sprayable and fumigant). Cowpea seeds infested with eggs or adults of the insect were associated with these doses or concentrations of the formulations, with an insecticide or with water, as a control. The exploitation of the results showed that considered individually, each formulation significantly compromises the development of the insect. But the already high egg and adult mortality rate of *Callosobruchus maculatus* did not increase significantly with the three formulations of *Eucalyptus alba* leaves, as evidenced by the P-values of the eggresults (0.332) and on adults (0.642).

INTRODUCTION

Cowpea is a plant of hot regions, which has been cultivated in Senegal for centuries (Ng and Maréchal, 1985). It represents a valuable source of protein whose high rate (22 to 24%) and excellent quality, destines it to play an important role in the nutritional balance of rural populations and more particularly in the protein deficiency of children (Ndiaye, 1986). However, like other market gardening crops which occupy an important place for human nutrition and contribute significantly to family incomes in West Africa in general, it is faced with pressure from pestssuch as *Callosobruchus maculatus*, which limits their productivity (Yarou et al, 2017). Many control methods are implemented to limit or even eradicate pest attacks. But the most fashionable alternative is the use of pesticides. Senegalese agriculture uses an average of 598 tons of solid pesticides and 1,336,560 liters of liquid pesticides per year, for a value of nearly 11 billion FCFA (PAN AFRICA, 2005). However, the use of these insecticides is not without damage to humans and the environment. Pesticides and waste water contribute to the pollution of groundwater, the main resource used in Dakar as irrigation water with real healthrisks (Cissé et al, 2003 ; Ndiaye et al 2010). In Cameroon, the excessive use of active ingredients has led to a loss of pest sensitivity (Achaleke et al, 2009). In the permanent quest for sustainable development and environmental protection, it is therefore urgent to develop control methods that combine both efficiency and respect for living beings and the environment. Our study falls within this perspective, it aims to highlight, among the various already insecticidal formulations of *Eucalyptus alba* leaves (aqueous solution, ground material and fumigation) the most effective in constraining the development of *Callosobruchus maculatus* through barometers of its reproduction. To achieve this goal, three doses (D1ea= 0.0315 g/cm3, D2ea= 0.0629 g/cm3 and D3ea= 0.0944 g/cm3) or concentrations (C1ea= 0.2 g/ml, C2ea= 0.04 g/ml and C3ea= 0.02 g/ml) of *Eucalyptus alba* leaves were treated so as to obtain for each dose or solution an aqueous extract, a crushed material (both sprayable and fumigant). Cowpea seeds infested with eggs or adults of the insect were associated with these doses/concentrations of the formulations, with an insecticide or with water, as a control. The results of the experiments were translated into graphics by the R software, then interpreted, in relation to the afore mentioned objective.

MATERIALS AND METHOD

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)

- three concentrations (C1= 0.2 g/ml, C2= 0.13 g/ml, C3= 0.1 g/ml), for each formulation of the plant (ground material, fumigation and aqueous solution) were used. For the chemical insecticide (Deltamethrin) a single dose repeated 3 times was used.

The deltamethrin used was applied at the recommended concentration, which is 40ml for 30L of water, dosage reported at 500ml of water, i.e. 0.66ml for 500ml of water (whose mass concentration C= 0.0035g/l).

Various tests carried out

1. Contact test between shredded fresh leaves of Eucalyptus alba and adults and eggs of *Callosobruchus maculatus*.

In each Petri dish containing 25g of cowpea seeds, 12 adults of *Callosobruchus maculatus* aged 48 hours maximum were introduced. Three doses of fresh leaves of the plant (2g, 4g and 6g) were used. For each dose, three repetitions were made with three blank controls.

Dead bruchids were counted at intervals of 24 hours up to 15 days. All bruchids were counted dead if they did not move when touching their legs and antennae for three to four minutes.

The ovicidal tests were carried out with the same device as before. The shredded material was mixed with cowpea seeds each carrying an egg, on the order of 12 seeds per Petri dish. The seeds each carrying an egg were obtained by the following process: healthy cowpea seeds were taken and introduced into a jar containing couples of bruchids. 24 hours after introduction, the bruchids were removed from the jar and the infested seeds were observed under a magnifying glass to ensure the presence of a single egg on each seed. If a seed carried more than one egg, it was put aside to serve as breeding.

2. Fumigation of fresh leaves of *Eucalyptus alba* in the presence of adults and eggs of *Callosobruchus maculatus*.

2, 4 and 6 grams of fresh leaves of *Eucalyptus alba* are crushed. Each shredded material was automatically placed in muslin cloth, placed in the center of a Petri dish containing the cowpea seeds and 12 adults of *C. maculatus*. After the introduction, each Petri dish was immediately closed so that the fumigant substances could not escape. For each dose used, three repetitions were carried out and a blank control without crushed leaves. The Petri dishes were then kept in the laboratory insectary at ambient temperatures and relative humidity. Dead insects were counted daily.

The ovicidal tests were also carried out with the same process, where the seeds each carrying an egg replaced the adults of the insect.

3. 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:

- 200g of ground 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 (C1, C2 and C3) that were used in our tests were obtained from the stock solution by the following method:

C1ea = 40ml of the solution (C1ea = 1g / 5mL);

C2ea = 40ml of the solution + 20ml of tap water (C2ea = 1g / 25mL);

C3ea = 40ml of the solution + 40ml of tap water (C3ea = 1g / 45mL).

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

M1 = 2g equals D1ea = 2/63.585 = 0.0315g/cm3

M2 = 4g equals D2ea = 4/63.585 = 0.0629 g/cm3

M3 = 6g equals D3ea = 6/63.585 = 0.0944 g/cm3

➢ **The test**

Twelve (12) couples of the same age (48 hours maximum) were placed in Petri dishes containing 25g of cowpea seeds. Before hand, 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 analysis

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 = $Nombre\_adultesmortsNombre\_adultestotalsx$ 100

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

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

% M_emb = 100− $Nombre\_adultes$ $émergésNombre\_oeufstotalx$ 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 analysis

The calculations of the average of the repetitions were carried out on Excel 2013. The graphs and the statistical analysis of the measured variables were carried out with the R software. The data obtained were subjected to parametric analysis (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 DISCUSSION

Results

Table 1: Results of the effect of 3 formulations of *Eucalyptus alba* leaves on adults and eggs of *Callosobruchus maculatus*

	Adults	**Eggs**
shredded	36.11 ± 20.97**a**	69.44 ± 4,81**a**
Fumigation	47.22 ± 9.62%**a**	63.88 ± 4.81**a**
aqueous solution	33.33 ± 22.05**a**	69.44 ± 4.81**a**

Effect of three (3) *Eucalyptus alba* leaf formulations on *Callosobruchu s*maculatus adults

The results on a possible mortality differential of the 3 formulations of *Eucalyptus alba* leaves show that there is no statistically significant difference in the lethal effect of the three formulations, namely the shredded material, the fumigation and the aqueous solution of leaves of *Eucalyptus alba* on adults of *Callosobruchus maculatus*. (P= 0.642>0.05).

Table 2: **Effect of three (3) *Eucalyptus alba* leaf formulations on *Callosobruchu s*maculatus adults**

	Df	Sum Sq	Mean Sq	F-value	P-value
Formulations	2	324.1	162.0	0.477	0.642
Residuals	6	2037.0	339.5		

However, The figure 1 shows that, despite there being no significant difference between these 3 formulations, the fumigation has the highest average adult mortality rate (47.22 ± 9.62), followed by ground material (36.11 ± 20.97) and finally the aqueous solution (33.33 ± 22.05).

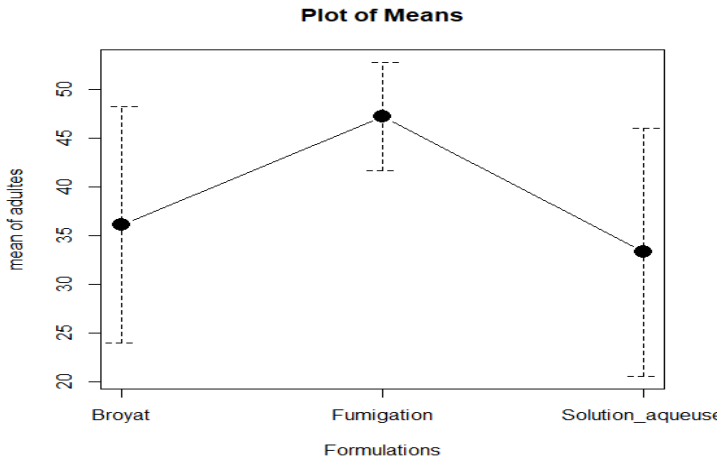

Figure 1: Mean mortality rate of 3 *Eucalyptus alba* leaf formulations on *Callosobruchus maculatus adults*

Effect of three (3) *Eucalyptus alba* leaf formulations on *Callosobruchus maculatus* eggs

The comparison of the results of the 3 formulations of *Eucalyptus alba* leaves show that there is no statistically significant difference in ovicide of *Callosobruchus maculatus* of the three formulations, namely the crushed, the fumigation and the aqueous solution of *Eucalyptus alba leaves* (P= 0.332>0.05).

Table 3: Effect of three (3) *Eucalyptus alba* leaf formulations on *Callosobruchus maculatus* eggs

	Df	Sum Sq	Mean Sq	F-value	P-value
Formulations	2	61.73	30.86	1.33	0.332
Residuals	6	138.89	23.15		

The figure 2 however reveals that, despite there being no significant difference between these 3 formulations, the aqueous solution and the ground material have the highest average rate of ovicide (69.44 ± 4.81), compared to that of the fumigation (63.88 ± 4.81).

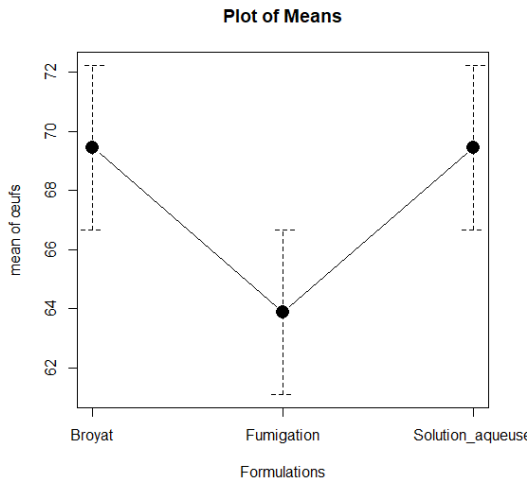

Figure 2: Average level of *Callosobruchus maculatus* ovicide of the 3 *Eucalyptus alba* leaf formulations

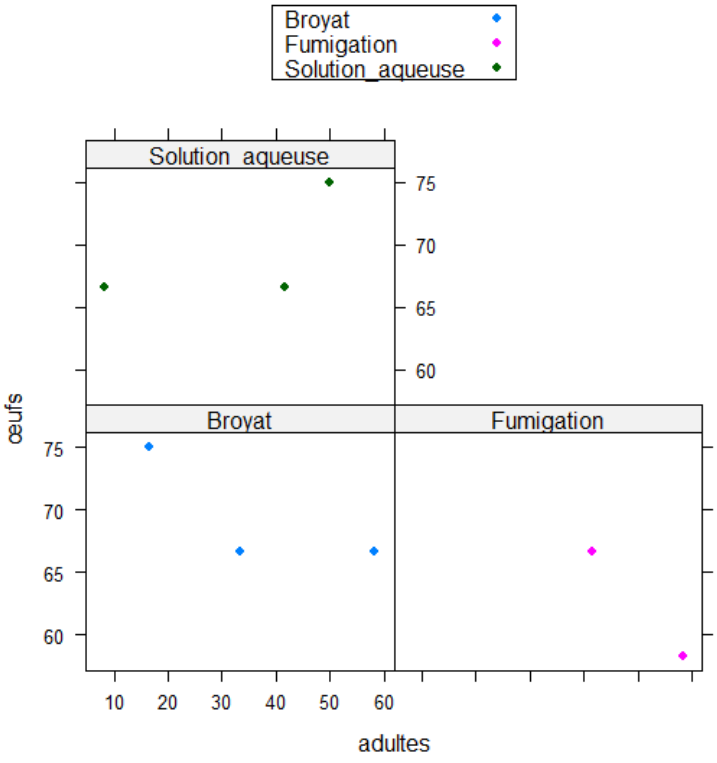

Figure 3: Comparative influence of the 3 formulations of *Eucalyptus alba* on *Callosobruchus maculatus*

The figure 3 shows that in adults, fumigation is more restrictive than aqueous solution and ground material. On the other hand, at the egg level, the shredded material and the aqueous solution have a more negative influence than fumigation.

DISCUSSION

The aim of present study was to test a differential of binding effects of three (3) formulations of *Eucalyptus alba* leaves on development barometers of *Callosobruchus maculatus*, namely the percentage of emergence and the adulticidal rate. Whether it is the aqueous solution, the crushed material or the fumigation, each of these types of treatment of the leaves of the plant has had a significant negative impact on the reproduction of the insect. Many works on at least one of these formulations of other plants have confirmed this state of affairs. Thiaw et al (2004) who highlighted the insecticidal effects of the ethereal extract of *Calotropis procera* on *Caryedon serratus*. Bambou et al (2011) also demonstrated the efficacy of the fumigant action of two fruit ecotypes on five species (*Callosobruchus maculatus*, *P. truncatus*, *Sitophilus zeamais*, *Tribolium castaneum and Caryedon serratus*). Thus any formulation of leaves, plant fruits can be an alternative to the fight against crop pests. Especially since chemical control, which is the method most used by producers to eliminate pests, is fatal to living beings and the environment. In fact, the intensive use of pesticides is today accompanied by numerous abuses and damage: The habituation of insects and the selection of resistant strains (Benhalima et al, 2004), poisoning, environmental pollution and ecological disorders (Regnault-Roger, 2002).

If therefore the aqueous solution, the shredded material and the fumigation individually impact the development of the insect in a negative way, it is clear on the basis of the results that the high rate of egg and adult mortality does not vary significantly depending on the formulations. The P-values of the results on the hatching rate (0.332) and the adulticide rate (0.642) are well above the reference (0.05). Nevertheless, we noticed for emergence that the aqueous solution and the ground material have the highest average rate of ovicide (69.44 ± 4.81), compared to that of fumigation (63.88 ± 4.81), while for adulticide mortality, fumigation has the highest average adult mortality rate (47.22 ± 9.62), followed by ground material (36.11 ± 20.97) and finally aqueous solution (33.33 ± 22.05). This differential effect, here not significant, of formulations of a plant species on pests was significantly highlighted by the study by Diane et al (2010). Indeed, treatment with *Azadirachta indica* leaf solutions of the same concentrations on the following

medicinal plants generated 80% incidence for *Cassia italica*, 41.17% for *Hibiscus esculentus* and 38.835% for *Solanum melongena* while treated with solutions this time from seeds of *Azadirachta indica*, the pest attack rates are 10.52%, 15% and 5% respectively for *Cassia italica*, *Hibiscus esculentus* and *Solanum melongena*.

CONCLUSION

The study revealed that all three formulations of *Eucalyptus alba* leaves, in the event the aqueous solution, the mash and the fumigation, are effective in eliminating *Callosobruchus maculatus*, the main pest of bean stocks, but that none of them is more effective than the other.

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