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# Effectiveness of different formulations leaves of *Senna occidentalis* on the external stages of *Callosobruchus maculatus* Fabricius main pest of cowpea (*Vigna unguiculata* Walp) stored

**Ablaye Faye[1*], Cheikh Thiaw[2], Malick Sarr[1], Mbacké Sembène[1-3]**

[1]*Department of Animal Biology, University Cheikh Anta Diop, Faculty of Sciences and Technology, Dakar, Senegal*

[2]*Department of Entomology Regional Centre for Studies on the Improvement of Plant Adaptation to Drought (CERAAS) Thies, Senegal*

[3]*Laboratory BioPASS CBGP, IRD, Bel Air B.P.1386 Dakar, Senegal*

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**Abstract**

The biocidal activity of *S. occidentalis* on the external forms of *C. maculatus* was assessed in the laboratory. Several formulations (contact, fumigation and aqueous extract) have been tested on the eggs and adults of this insect. The greater embryonic mortality (90.95% with the highest dose, D4 (0.0216 g/cm$^3$)) was recorded with fumigation with crushed fresh leaves. Fumigation always causes the highest mortality of adults. It thus induces a maximum elimination (100%) of adults with the D2 (0.00546 g/cm$^3$) dose from the 8$^{th}$ day of application. Other formulations (aqueous extract and contact) gave all consequent mortalities on the external forms of *C. maculatus*. Bioactive molecules responsible for the death of insects suggest us to put steroids in evidence and anthraquinones in the plant.

* **Corresponding Author:** Ablaye Faye ✉ fablaye 82@ yahoo.fr

## Introduction

*Callosobruchus maculatus* Fabricius has long hampered the farmers store their crop of Cowpea with ease, because it is the major pest. Since the Cowpea is a predominant source of protein for farmers who have difficulty accessing the proteins of animal origin, they are obliged to protect their harvest. For this protection, they often use insecticides of syntheses often very expensive and are real dangers to public health and the environment. Furthermore several insects developed resistance to insecticides.

To counter the adverse effects of the use of synthetic insecticides, several authors looked enhancement of traditional pest control techniques, thus constituting an alternative to chemical control for protection crops. So many researchers have wanted to reduce the harmful effect of *C. maculatus* on stocks of Cowpea *Vigna unguiculata* Walp by using native plants equipped with insect repellent activities and or insecticides (Kellouche and Soltani, 2004; Seri-Kouassi and al, 2003; Kiendrebeogo and al., 2006; Appleby and Credland, 2004; Olubayo and Port, 1997; Lale and Abdulrahman, 1999; Faye and al 2012) This study is intended to assess the effectiveness of several formulations (contact, fumigation and aqueous extract) leaves of *Senna occidentalis* on the two external stages of *C. maculatus* (eggs and adults). The choice of this plant lies in the fact that it is the subject of several studies biocides on other insects such as *Carryedon serratus* (Thiaw and al., 2004; Liénard and al., 1993). Besides the research of formulations easily applicable by the peasants, pushed us to test the efficiency of some formulations to basis of leaves *S. occidentalis*, plant already used in struggle against the devastating; giving satisfactory enough results thus but hardly applicable by the peasants.

## Materials and methods

Biological material

The biological material used in the experiment, comes from Sandiara (Department of Mbour, Senegal). *S. occidentalis* fresh leaves picked the evening to the lying of the Sun and are forwarded to the laboratory, where the following day they will be used. Crops are made in the months of September, October and November. Upon arrival at the laboratory, a part of the leaves is used once for testing of fumigation and contact; the other party is dried on trays from sunlight in the store of the laboratory of Entomology and Acarology of the Faculty of science the University Cheikh Anta Diop of Dakar. After drying, the leaves are transformed into powder then preserved in glass jars. This powder will be used for aqueous extractions by maceration for biological testing.

The specimen of *C. maculatus* used in this work comes from a sample of infested Cowpea bought Sandiara weekly market. The seeds of Cowpea used in mass and as support for biological tests are cleared of any infestation by an extended stay in the freezer.

The breeding of the bruchid *C. maculatus* is made with seed free of any infestation in the glass jars (10, 5 cm high and 8 cm in diameter). Both males and females are introduced into jars containing Cowpea seeds until their base is completely covered.After 24 hours of contact with the seed, the bruchid beetles are recovered. Infested seeds are now tracked and adults who emerge in will be used either for tests adulticidal, or to keep livestock of mass. In this way remains the strain of *C. maculatus* in the laboratory.

## Methods

Contact tests

After their arrival at the laboratory, the fresh leaves are crushed with mortar and introduced in 10.5 cm in diameter and 8 cm jars with lids are screened. In each jar, 12 non-gendered adults of *C. maculatus* are introduced. Four weights of leaves are used: 2g, 4g, 8g et16g. For each weight, three replicates were made and a white witness always accompanied the rehearsals. For each repetition, crushed leaves and insects are mixed in a jar. Are considered as dead all the bruchid beetles who touched legs and antennae do no movement of legs or antennae. Bruchids dead are counted daily.

The same process was used with eggs. For testing ovicidal, it conducted a count of the eggs hatched and unhatched eggs. This occurs 2 weeks after the introduction of crushed leaves and seeds each carrying an egg into the jars. To be accomplished, leftovers of the eggs hatched and unhatched eggs are removed from the seed coat and then the seeds are crushed to see the status of the larvae if they exist. This process will allow us to calculate the rate of embryo mortality by the following formula:

ME = number of eggs not hatched X 100/number of eggs total. This setting is reported as a percentage and corrected by the Abbott formula (1925) next: Mc = (Mo - Mt) x 100 / (100 - Mt) ; MC = corrected mortality, MB = observed mortality, Mt =mortality witness. This same form is also used to quantify the rate of adult mortality.

*Fumigation tests*

After grinding, the fresh leaves are automatically put in small jars (8 cm in diameter and 5 cm high) with screen cover. Each small jar is then inserted in a box larger (10, 5cm in diameter and 8cm) containing 12 non-gendered adults of *C. maculatus* . After introduction, the large jars are immediately closed with a lid without toasting and the closure is reinforced by the scotch glue so that the fumigant substances cannot escape. Still four weights of crushed leaves are used. For each weight of leaves used, three repetitions are performed and a white witness without crushed leaves. Dead insects are counted daily. Is counted dead any insect lying on his back and doing no movement of legs or antennae after shaking.

Ovicidal tests are carried out in the same way as before. Seeds each bearing an egg replace adults in large jars. The count is 15 days after the introduction and are counted the eggs hatched and unhatched eggs. To get the jars are open and each seed is taken to remove the unhatched egg or the rest of the egg hatched. And after each seed was divided into to see if the hatched larva is alive or dead. This allows us to calculate the rate of embryo mortality by the Abbott formula. Mortality rates are presented in the form of tables or graphs.

*Aqueous extract tests*

The powdered leaves of *S. occidentalis* has been used. We have macerated 200 g of leaf powder with 1 L of tap water. The mixture is left to rest for 1 hour and after shaking, we filtered the mixture with a reinforced household sieve by the muslin. The aqueous extract is preserved in a liter to the laboratory fridge bottle and will be used if necessary. Three solutions of different concentrations are obtained by the following method:

$C_1$ = 40 ml of the solution obtained with extraction (0.2 g powder per $cm^3$)

$C_2 = C_1$ + 20 ml of tap water (0.13 g powder per $cm^3$)

$C_3 = C_1$ + 40 ml of tap water (0.1 g powder per $cm^3$).

Adults aged no more than 72 hours, from mass farming are used in experimentation; It is the same, for a box of Petri dishes. In each box of Petri dishes, we place paper Wattman. A micropipette is used to 1 ml of the solution prepared in a consistent manner on paper Wattman and 12 non-gendered adults are deposited. Three replicates and two witnesses (solvent and white controls) are made for each particular extract concentration. For the indicator solvent, 1 ml of tap water is spread on paper Wattman and 12 adults are deposited; as the white witness, adults of bruchids are directly introduced in the boxes of Petri dishes with paper Wattman without pre-treatment. The dead are recorded daily. The rate of adult mortality is calculated and corrected later with the Abbott formula.

Seeds of Cowpea each bearing an egg are sprayed per dozen with a micropipette of 1 ml of solution of each concentration. Three replicates and two witnesses (solvent and white controls) are made for each concentration. For the solvent control, seeds are sprinkled with the solvent (water), in contrast to the white light, they are not processed.

The results are exploited in the form of tables and graphics and the use of Student tests allowed to make comparisons between the induced mortality.

**Results of the ovicidal and adulticidal tests**

*Ovicidal effect*

Contact with crushed fresh leaves of *S. occidentalis* with eggs due to mortalities which are around 40% for all doses. The lowest dose gave more deaths than the others, or 45 per cent. Doses D2 (0.00546 g/cm3), D3 (0.0108 g/cm3) and D4 (0.0216 g/cm3) respectively cause of mortality of 40%, 33.3% and 40%. Mortalities led by all doses are statistically the same to $p < 0.05$.

**Table 1.** % mortality of eggs for testing contact. The consistent values as exposing the same alphabetic letter are statistically equal.

| Doses (g/cm3) | Mortality of eggs (in %) |
|---|---|
| D1 (0.00273 g/cm3) | 45[a] |
| D2 (0.00546 g/cm3) | 40[a] |
| D3 (0.0108 g/cm3) | 33.3[a] |
| D4 (0.0216 g/cm3) | 40[a] |

Fumigation has been proportional to the dose administered egg mortality. That is how one gets the greatest mortality (90.95%) with D4 (0.0216 g/cm3), while D1 (0.00273 g/cm3) trained 14.33% of deaths among the eggs. Mortalities caused by the D2 doses and D3 are statistically equal and defer the one induced by the D4 dose; who on his/her/its turns gives different mortalities to those provoked by D1 to $p < 0.05$.

**Table 2.** % of mortality corrected for fumigation testing eggs**.** The consistent values as exposing the same alphabetic letter are statistically equal.

| Doses (g/cm3) | Mortality of eggs (in %) |
|---|---|
| D1(0.00273 g/cm3) | 14.33[a] |
| D2(0.00546 g/cm3) | 54.87[b] |
| D3(0.0108 g/cm3) | 63,92[b] |
| D4 (0.0216 g/cm3) | 90.95[c] |

The effectiveness of the concentration of aqueous extract powder of *S. occidentalis* on the eggs of *C. maculatus* has been tested. Tests reveal most great mortality (44.83%) with C2, while C1 and C3 give 24.14% and 8%, respectively. Mortalities led by the two bigger concentrations (C1 and C2) are statistically equal and defer those provoked by the C3 concentration to $p < 0.05$.

**Table 3.** % mortality of eggs for the tests with aqueous extract. The consistent values as exposing the same alphabetic letter are statistically equal.

| Concentration | Mortality of eggs (in %) |
|---|---|
| C1(0,2g/cm3) | 24.14[b] |
| C2(0,13g/cm3) | 44.83[b] |
| C3(0,1g/cm3) | 8[a] |

*Adulticidal effect*

The analysis of figure 1 shows a differential effectiveness of contact with the crushed leaves of *S. occidentalis* on *C. maculatus* adults with different doses. The first day that only the largest dose (D4 (0, 0218 g/cm3)) presented mortality (5.18%). Two immediately lower doses showed mortalities on the sixth day of tests, while the lowest dose always gives 0% mortality. This last dose will give mortality on the ninth day of application (7.22%) at the time where the other doses reveal respectively from the smallest to the largest dose, mortality of 81,11% and 100% for the last two doses. On the tenth day of contact, single dose (D1 (0, 00273 g/cm3)) did not give 100% mortality.

## Discussion

The effectiveness of *S. occidentalis* on *C. maculatus* was highlighted in this study. Therefore, several formulations (contact, fumigation and aqueous extract) leaves of this plant are applied on the external forms (adults and eggs) of the insect.

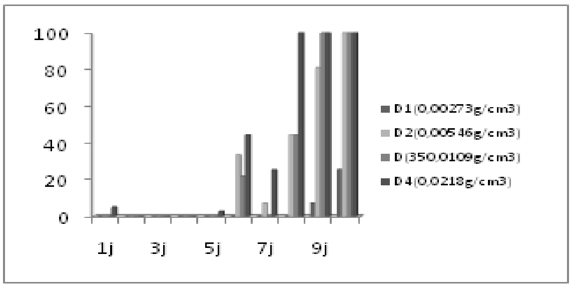


**Fig. 1.** Percentage of mortality corrected for testing contact adult Fumigation on adult shows mortality with all doses on the first day of application, but the dose (D2 (0, 0728 g/cm3)) has proved to be more efficient with 19.49% of mortalities. We note that the highest dose is effective on the sixth day of application with 55.33% of mortalities; at the time where the lowest dose (D1 (0, 00364 g/cm3)) gives 66.67% of mortalities. On the eighth day of test, only

the latter did not 100% mortality and reveals 88,67%. On the ninth day of the application all insects have been killed by different doses.

Contact tests proved to be overall more effective on adults than on eggs. We thus record mortality of 100% from the 9th day of contact with the two higher doses in adults while for eggs, they show the mortality not exceeding 40% at the end of the application. The contact test biocidal activity has been spread in time for adults of *C. maculatus*. Our results are going the same way as those of Sarr (2010) and Faye and al. (2012). Sarr results show a greater spread of mortality (34 days to get 100% mortality), while those of Faye and al show more efficiency (100% mortality with dose 0, 0218 g/cm3 from 12 hours of contact). These authors have worked with the leaves of *C. religiosa* using the same application that we process. It could lead to think that the active molecules responsible for the insect mortality observed with the application of these two plants are different. Other authors have tested by contact, powders of leaves of some plants. Therefore, Kellouche and Soltani (2004) have used four plants powders: *Ficus carica* (Moraceae), *Eucalyptus globulus* (Myrtaceae), *Olea europaea* (Oleaceae) and Citrus limon (rutaceae). Their results show a significant reduction in the longevity of adults. For this purpose powders *C. Limon*, *E. globulus* and *O. europaea* by more than 50% the number of adults that emerge, at doses of 4 and 5%.

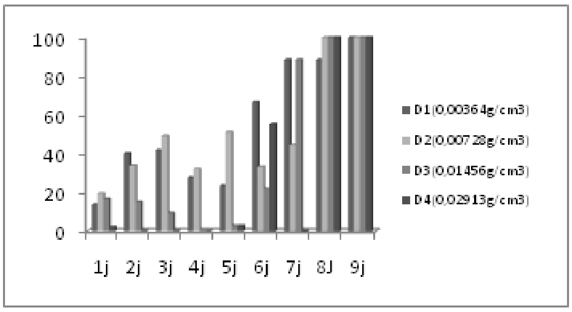


**Fig. 2.** Percentage of mortality corrected for fumigation testing adult extract is noticeable on the fourth day of application, with proportional to concentrations mortalities. This trend was reversed as soon as the 5th day and will reappear only to the 7th day. At the 10th day C1, C2 and C3 concentrations respectively give mortalities of 87.25%, for the first two concentrations and 74.87%.

Fumigation with crushed fresh leaves of S. occidentalis is proving less effective on the eggs of *C. maculatus* with the lowest dose, at a time when higher doses are very effective with greater than 50% mortality. We note also that the observed mortality are proportional to the dose administered. For adults, the fumigation gives mortality differential depending on the dose and spreading over time. We thus see more efficiency on adults than on eggs. Several researchers have worked in research of plants with insecticidal activities and or repellents. Therefore, Faye and al (2012), working with *C. religiosa*, demonstrate its effectiveness on *C. maculatus*. To this end, they get 100% of mortality from 6 hours of exposure of adult dose 0, 00728 g/cm3. One realizes that adults of *C. maculatus* are more susceptible to C. religiosa and *S. occidentalis*. While on eggs, it is s. occidentalis which has proved most effective. In this wake Bâ Kébé (2004) highlights a 100% mortality of adults of *C. serratus* after 24 hours of exposure with 1 g fruit crushed *Boscia senegalensis* while 1 to 2 g of crushed leaves give 3 deaths and 17% of adults. Several other authors such as Ketoh *et al.*, have shown very considerable mortality of adults of *C. maculatus* exposed to the essential oil of *Ocimum basilicum* (25µl/g) after 12 hours of fumigation. Very important ovicidal effects are highlighted by Ketoh and al. (2005), as well as by Kiendrebeogo and al. (2006).

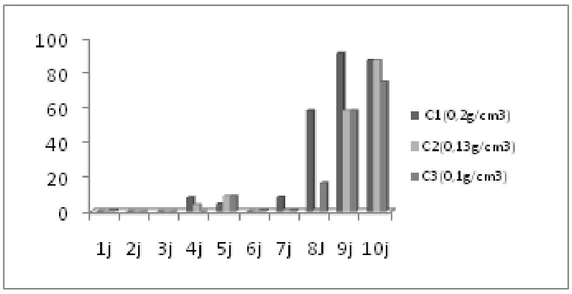


**Fig. 3.** Percentage of mortality corrected adult normal form for the tests with aqueous extract.

The aqueous extract by maceration of powdered leaves of *S. occidentalis* is proving less effective on adults of *C. maculatus*. Consequent mortalities are perceptible only to the 10th day. To this end, the two highest concentrations (C1 and C2) give all mortalities of 87.25%, while the lowest concentration (C3) reveals mortality not exceeding 74.87%. Ovicidal tests show most mortalities (44.83%) with C2, period during which C1 and C3 give respectively 24 deaths, 14 by 8%. The lower efficiency observed with the aqueous extract was linked to the polarity of the solvent used. In this sphere of use of substances biocides of vegetable origin, several studies have shown notable effects on insects. This is how Mr. Jayakumar (2010) gets a maximum reduction of the adult emergence of Cowpea with extraction bruche aqueous of the following plants, *Percularia daemia* (91.25%) *Cassia siamia* (82.08%) and *Acorus calamus* (92.28%), applied on Cowpea seeds. Ketoh and al. (1998) detect removal greater than or equal to 70% of *C. maculatus* fresh eggs at low concentrations of essential oils of *C. schoenanthus* who hold a large amount of piperitone. The essential oils ovicidal activity is explained by their penetrating power or direct toxicity of components (DON PEDRO, 1989). Thiaw and al. (2007) have highlighted the deaths of 66.67% and 50% of adults of *C. serratus*, respectively with ether and chloroform extracts; thus showing a more consistent efficacy on adults than on eggs.The trend of their results confirmed ours. The bioactive agents contained in powders of leaves of plants are flavonoids, steroids as well as the anthraquinones detected in extracts (Abdulahi, 2011). Abdulahi (2011) is part of a 100% mortality of adults of *C. maculatus* with the powder mixture of leaves of *Cassia occidentalis* and *Vittallaria paradoxa* andcassia with concentrations of 7.5 and 10%w/w in 24 hours of treatment. The biological activity of *c. occidentalis* on *C. maculatus* has been also highlighted by Liénard and al (1993); the leaves, seeds and the essential oil of this plant proved so very effective for the control of this insect. Toxicity of vapours of *Aeorus ealamus* on the eggs of HE of Callosobruchus chinensis L has been reportedby SCHMIDT and al..(1991) who indicated that HE had a sterilizing action eggs.

Our method of study meets the applicability by the peasants to fight effectively against insect pests of stored foodstuffs. Our different formulations give greater efficiency on the external forms of *C. maculatus*. Their combined application would give very satisfactory results for the protection of stocks of Cowpea. The observed differential action of different formulations would be linked to the nature of bioactive molecules and their mode of action on insects. This fact is confirmed by the results of Kandji (1996) and by those of Cardet and al (1998), which using the same plant with different formulations, detect discordant adulticidal mortalities on the insect *C. serratus*.

*Conclusion and perspectives*

This study is intended to implement effective storage techniques of foodstuffs stored and applicable by the peasants. These techniques have to combat pests of stored products such as *C. maculatus*, main pest of stored cowpea, *Vigna unguiculata* Walp. We had thus tested several formulations leaves of *Senna occidentalis* on the external forms of the beetle *C. maculatus*. It appears from this study that adults are more susceptible to applications. The adult mortality induced by applications (contact, fumigation and aqueous extract) is spread out in time. All these applications, it is the fumigation that proved to be most effective on adults as well as on the eggs of *C. maculatus*. The aqueous extract proved less decisive than contact with the fresh leaves crushed on the egg mortality as well as that of adults. We propose in the future to identify the impact of these applications on survival, as well as on the fertility of the ovicidal tests from survivors. We also verify the effectiveness of other parts of the plant on the insect, whilst developing their impact on the internal form (larvae) of C. maculatus.

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