__Original Research Article__

Activities of organic extracts of *Cassia occidentalis* leaves on the mortality and fecundity of *Callosobruchus maculatus* (main insect pest of *Vigna unguiculata* seed stocks)

Abstract

Callosobruchus maculatus Fab (Coleoptera: Chrysomelidae) is the most formidable pest of cowpea (*Vigna unguiculata)* in the field. The damage continues even in stocks intended for food. The present work was carried out to evaluate the insecticidal effect of organic leaf extracts of *Cassia occidentalis* on mortality and emergence of F1 progeny in storage. The extracts were tested in contact in 90 mm diameter petri dishes with different doses. Phytochemical screening was carried out using coloring and precipitation tests. Mortality of *C. maculatus* was assessed between 1 and 17 days after infestation. The data were processed according to the General Linear Model procedure and using Minitab 19 software.

The results of the statistical analysis showed that the methanolic extract gives a better mortality rate on *Callosobruchus maculatus.* These results are corroborated by phytochemical tests with the identification of molecules (alkaloids, flavonoids, tannins, polyphenols, etc.) likely to be responsible for this insecticidal activity.

Key words: Organic extracts, *Cassia occidentalis,Callosobruchus maculatus*, cowpea.

Introduction

Callosobruchus maculatus causes significant damage to cowpea (*Vigna unguiculata*) seeds, which worries farmers about how to store this commodity, which represents a predominant source of plant protein. This damage, which can reach more than 800g/kg of seeds stored after seven (7) months, affects the quality and commercial and agronomic values of the seeds [1].

To counter this pest, many farmers use synthetic insecticides, which are often expensive and dangerous for public and environmental health [2]. In addition, several insects have developed resistance to insecticides [3], [4].

Faced with the harmful effects of the use of synthetic insecticides, several authors have sought alternative means by strengthening traditional pest control techniques in order to protect the environment. Thus, many researchers wanted to reduce the harmful effect of *C. maculatus* on cowpea stocks (*Vigna unguiculata*) using native plants with insect repellent and/or insecticide activities [5].

This study, which is part of developing strategies to protect cowpea stocks in Senegal, attempts to evaluate the bioactivity of organic extracts of *Cassia occidentalis* on the survival of eggs and adults of *C. maculatus* under controlled conditions.

I. Materials and methods

I.1. Materials

I.1.1. Plant material

The leaves of *Cassia occidentalis* studied are harvested in NGUENIENE (department of Mbour/Senegal) on June 8, 2022. The samples are dried in the shade and away from the sun for 1 month then reduced to powder using an electric grinder fitted with a sieve in order to have fine powders.

The cowpea used is purchased at the Tilène market (Dakar/Senegal).

I.1.2. Animal material

The strain of *Callosobruchus maculatus* used is provided by the Directorate of Plant Protection of Senegal (DPV).

I.2. Methods

I.2.1. Insect breeding

To obtain a sufficient number of individuals to carry out the tests, mass breeding of the strain is set up in a room at room temperature. This breeding was carried out in glass jars with a capacity of 500ml. In each pot, cowpea seeds are introduced with 25 insects without distinction of sex. For a better temperature and humidity condition which ensures the survival of the insect, hydrophilic cotton impregnated with water is introduced into each jar whose cover is perforated and covered with mosquito net. After 17 to 21 days, emergences were observed.

Emerged adults were used either for adulticide testing or to maintain mass reproduction. In this way, the *C. maculatus strain* was preserved in the laboratory.

I.2.2. Extraction

Three types of organic extracts are prepared from the powder obtained: a cyclohexane extract (cyclo), a chloroform extract (chloro) and a methanol extract (meth). For this purpose, 100 g of sample powder are weighed then subjected to a succession of extractions with solvents of increasing polarity. The powder is mixed with 750 mL of cyclohexane in a screw cap bottle then left to macerate for 72 h at room temperature.

After filtration and evaporation of the solvent on a rotary evaporator, the cyclohexane extract obtained is kept cool (around 4°C). The residue dried at room temperature is taken up in 750 mL of chloroform for 72 h, after filtration and concentration, the chloroform extract obtained is preserved. Finally, the dried residue is mixed in 750 mL of methanol for 72 h. the concentrated filtrate allows us to obtain the methanolic extract.

I.2.3. Phytochemical screening

The standard methods described by Kamsi et *al*., [6]are used with some modifications. The chemical groups identified are: alkaloids, flavonoids, polyphenols and tannins.

Alkaloid test: Approximately 2 mg of each extract is diluted in 3 ml of hot water in a test tube then a few drops of H_2SO_4 (10%) are added. The whole thing is put in a water bath then 2 to 3 drops of Dragendorff's reagent are added. The formation of a red-orange precipitate indicates the presence of alkaloids.

Tannin test: Two types of tannins are highlighted: condensed tannins and hydrolyzable tannins. After diluting 1 mg of extract in 3 mL of hot water in a test tube, a few drops of Stiasny's reagent are added and then heated in a water bath for approximately 10 min. The formation of a precipitate indicates the presence of condensed tannins. This mixture is filtered using cotton and then saturated sodium acetate and a few drops of $FeCl_3$ (2%) are added to the filtrate. The blackish brown color indicates the presence of hydrolyzable tannins.

Flavonoid testing(cyanidin reaction): 2 to 3 fragments of magnesium shavings and a few drops of concentrated hydrochloric acid are added to the extract previously diluted in hot water. The appearance of a pink-orange color allows the flavonoids to be identified.

Polyphenol test: The extract diluted in hot water is mixed with ferric chloride ($FeCl_3$ 2%). A blue-violet, red-orange, pink-violet or red color indicates the presence of polyphenols [7].

I.2.4. Biological screening

The evaluation of insecticidal activities is carried out with contact tests in petri dishes with a diameter of 90 mm. The performance of several preliminary biological tests made it possible to select five concentrations (dose1: 16mg/ml, dose2: 8mg/ml, dose3: 4mg/ml, dose4: 2mg/ml and dose5: 1mg/ml) for the extracts. Twenty-five (25) adults (approximately 24 hours old) were introduced into each petri dish containing 25 grams of cowpea seeds treated with 35 µl of each dose after 30 minutes of evaporation at room temperature. The controls are treated only with the extraction solvent. Dead insects are sorted and collected using flexible tweezers every 24 hours for 17 days. Each concentration was the subject of four (4) repetitions. The number of dead, alive and emerged insects are then counted. Abboth 's formula: Mc = (Mo-Mt)/(100-Mt)*100; (with Mc: calculated mortality, Mo: observed mortality and Mt: mortality in the control batches) is used to correct the observed mortality[8].

I.2.5. Statistical analysis

The data obtained were subjected to the Minor General Method (ANOVA) to observe the effect of time, extract and concentration on mortality and emergence using Minitab 19 software.

II. Results and discussion

II.1. Results

II.1.1. Phytochemical screening

The results of the phytochemical screening are presented in **Table 1**. The sign (+) indicates a positive reaction and reflects the presence of the group of compounds sought while the sign (-) indicates a negative reaction. The intensity of the coloring observed was assimilated to the proportion of each compound and translated on a scale ranging from one to three by signs (+), (++) and (+++).

Table 1: Phytochemical screening results

Phytochemicals	Cyclohexane	Chloroform	Methanol
Polyphenols	+	-	+++
Flavonoids	-	+	+
Alkaloids	+	-	+++
Condensed tannins	-	-	-
Hydrolyzable tannins	-	-	+++

+++ very present ++ moderately present+ little present - absent

The results of the phytochemical screening show a strong presence of polyphenols, alkaloids and tannins in the methanolic extract of the plant. Flavonoids are only present in the methanolic and chloroform extract. Cyclohexane extracts contain polyphenols and alkaloids.

II.1.2. Biological screening results

The results of analysis of variance (Table **2) relating to the insecticidal effect of** *Cassia occidentalis* leaf extracts on *Callobruchus maculatus* reveal that the mortality rate has a very highly significant variance depending on the exposure time and the interactions. time extract. This shows that mortality is dependent on the extract and the exposure time.

Emergence presents a very highly significant variance depending on the factors extracted, time and time-dose interactions have a highly significant effect.

These results show that the insecticidal activity depends on the extract and time.

Table 2: Results of analyzes of variance

SOURCES OF VARIANCE	MORTALITY			EMERGENCE		
	D.L.	F	P	D.L.	F	P
Excerpts	2	1.07	0.345	2	29.28	0.000
Doses	4	0.07	0.992	4	0.73	0.569
Time	16	59.35	0.000	16	20.17	0.000
Dose times	64	1.00	0.487	64	1.56	0.004
Time extracts	32	5.44	0.000	32	5.37	0.000
Dose extracts	8	0.06	1,000	8	0.77	0.542
Total	1019			1019		

Very highly significant effect ($P < 0{,}001$) **Highly significant effect (** $P < 0{,}01$**)**

Significant effect ($\mathbf{0,01} < P < 0{,}05$) **Non-significant effect (**$P > 0{,}05$)

Figures 1 and 2 respectively study the main effects and extract-dose interactions of mortality as a function of time, doses and extracts. The analysis shows that the methanolic extract has a more effective mortality rate. This mortality depends on time and reaches its peak between the 2nd [and] 6th days.

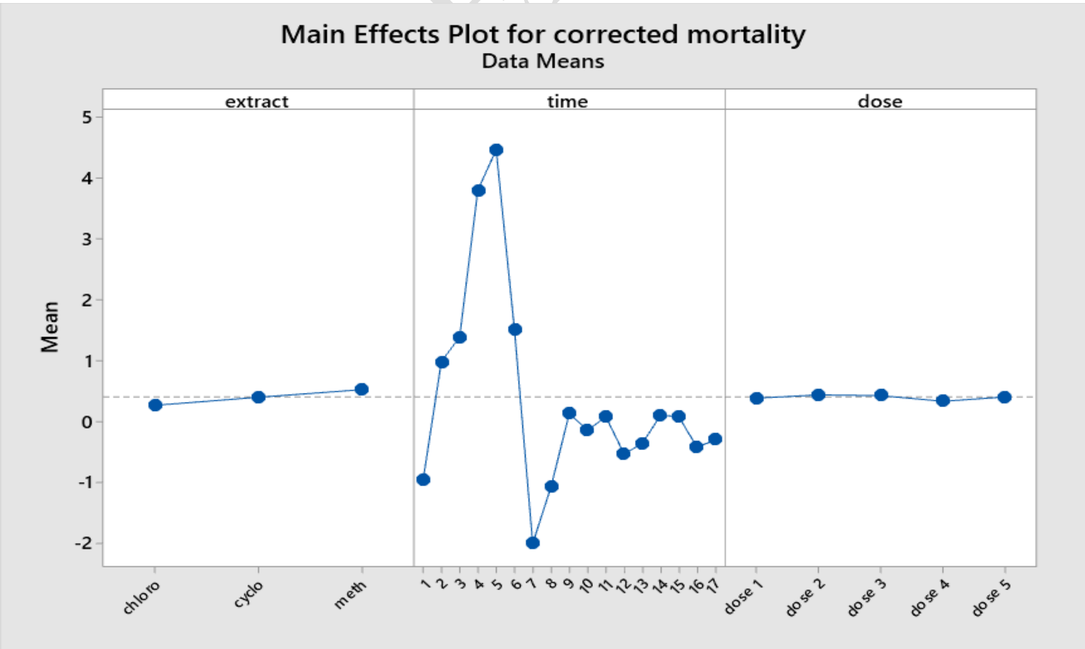

Figure 1: Mortality curve depending on doses, extracts and time

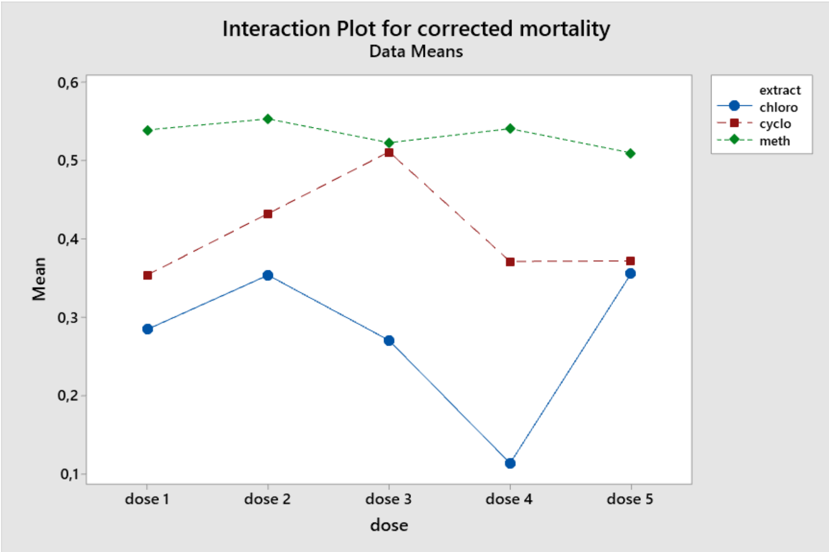

Figure 2: Interaction between extracts and doses of *Cassia occidentalis* for mortality

The evolution of emergence studied as a function of time, doses and extracts in Figures 3 and 4 shows that the cyclohexane extract ensures better blocking of insect reproduction with the doses.

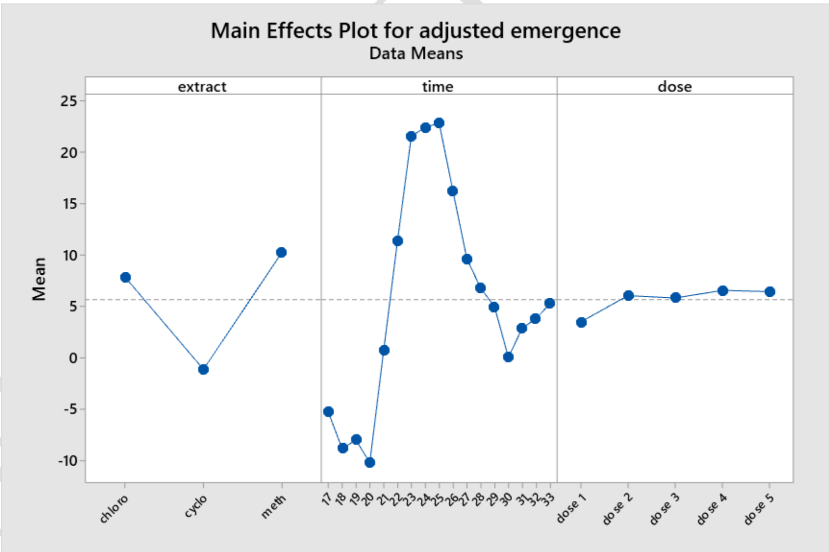

Figure 3: Emergence curve as a function of doses, extracts and time

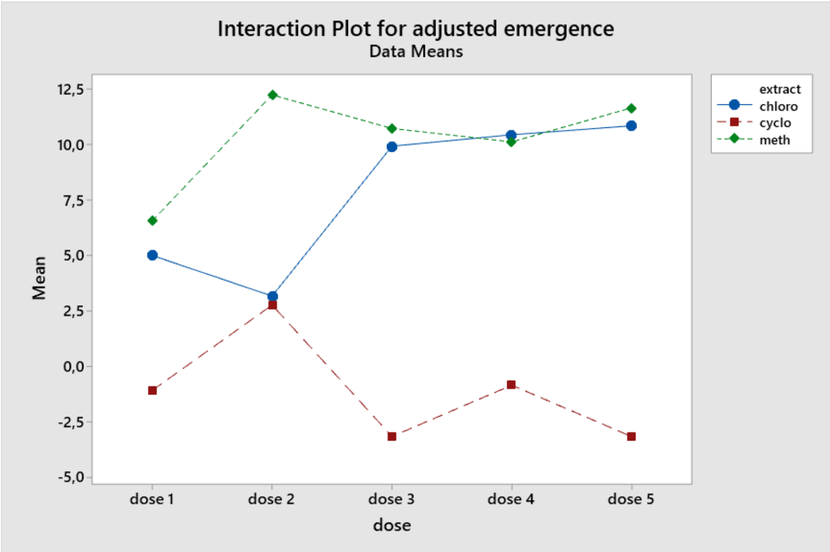

Figure 4: Interaction between extracts and doses of *Cassia occidentalis* for emergence

II.2. Discussion

Phytochemical screening shows the presence of secondary metabolites such as flavonoids, tannins, alkaloids and polyphenols in the plant. These results are corroborated by several previous studies such as those of Kamsi [6]who showed an abundant presence of total sugars, alkaloids, saponosides, phenols and flavonoids in cyclohexane, dichloromethane, methanol and deionized water from *S. occidentalis* leaves. In addition to these metabolites, tannins, sterols or triterpenes, quinones, reducing sugars and coumarins are found in the extracts. Thiaw et *al*. [9]showed that alkaloids, terpenoids, saponins or flavonoids are present in the different extracts (petroleum ether, methanol, hexane fraction, ethyl acetate fraction and methanol fraction) from the leaves of *S. occidentalis* tested. The work of Issa et *al*.,[10]reveal that the roots of *Senna occidentalis* are rich in alkaloids, coumarins, flavonoids, glycosides, saponins, tannins, sterol etc. The presence of these secondary metabolites could further explain the insecticidal activities of the *Cassia occidentalis* plant.

Also, the statistical analysis shows that the methanolic extract gives a better mortality rate on *Callosobruchus maculatus* and the chloroform extract ensures better protection of the seeds. This can be explained by the presence in these extracts of certain secondary metabolites such as polyphenols and alkaloids (Table 2) whose toxicities towards insects have been shown in several works. The insecticidal effects against *C. maculatus* of *C. occidentalis* studied by Faye et *al* . [2]present similar results to our study. Indeed, contact with fresh leaves and doses caused mortality of approximately 40% ($P < 0{,}05$)on eggs and 100% in adults after 9 days, fumigation causes mortality proportional to the dose ranging from 14 to 90%. Deme et *al.*[11]showed that *Cassia alata* and *Cassia tora leaf powders* control *C. maculatus* with mortality percentages ranging from 40 to 90% and 30 to 70% respectively depending on the dose applied.

Conclusion

Cassia occidentalis extracts have proven insecticidal properties to control *C. maculatus* during cowpea storage. These extracts contain a wide range of compounds responsible for insecticidal activities. The variety of interactions of insecticidal properties would be interesting. They could therefore constitute an effective alternative in the conservation of cowpea, by replacing chemical insecticides which are not safe for the health of consumers.

REFERENCES

[1] LST Ngamo and Th. Hance, "Diversity of food pests and alternative control methods in tropical environments," *TROPICULTURA*, vol. 25, no. 4, pp. 215–220, 2007.

[2] Ablaye Faye, Cheikh Thiaw, Malick Sarr, and Mbacké Sembène, "Effectiveness of different formulations leaves of *Senna occidentalis* on the external stages of *Callosobruchus maculatus* Fabricius main pest of Cowpea (Vigna *unguiculata* Walp) stored," *International Journal of Biosciences (IJB)*, pp. 246–253, May 2014, doi: 10.12692/ ijb /4.9.246-253.

[3] JN Aubertot *et al.*, "Pesticides, agriculture and environment: reduce the use of pesticides and limit their environmental impacts. Summary of the expert report," 2020. [Online]. Available: https://hal.inrae.fr/hal-02587721

[4] NIYIBIZI GAKURU Patient, "Plant associations for pest control: from a synthesis of theoretical approaches to practical application NIYIBIZI GAKURU Patient," 2020.

[5] Soumaïla SOURABIE, Patrice ZERBO, Djibril YONLI, and Joseph I. BOUSSIM, "Traditional knowledge of local plants used against pests of agricultural crops and products among the Turka people in Burkina Faso," *Int. J. Biol. Chem.Sci.*, flight. 14, no. 4, pp. 1390–1404, 2020.

[6] LN Kamsi, LE Mengome, S. Aboughe-Angone, and PE Engonga, "Phytochemical study of Senna occidentalis (L.) Link and *Cissus quadrangularis* (Linn) two Gabonese

Medicinal Plants Used Against Loa Loa," *European Scientific Journal ESJ*, vol. 16, no. 21, Jul. 2020, doi: 10.19044/esj. 2020.v16n21p101.

[7] GR Feuya Tchouya *et al.*, "Ethnopharmacological surveys and pharmacological studies of plants used in traditional medicine in the treatment of HIV/AIDS opportunistic diseases in Gabon," *J Ethnopharmacol*, vol. 162, p. 306–316, Mar. 2015, doi: 10.1016/j.jep.2014.12.052.

[8] Abbott WS, "A Method of Computing the Effectiveness of an Insecticide," *J Econ Entomol*, vol. 18, no. 2, pp. 265–267, Apr. 1925.

[9] C. Thiaw *et al.*,"Senna *occidentalis* L., a promising plant in the fight against *Caryedon serratus*Ol. (Coleoptera, Bruchidae), insect pest of peanut stocks in Senegal," *Int J Biol Chem Sci*, vol. 9, no. 3, p. 1399, Sep. 2015, doi :10.4314/ijbcs. v9i3.24.

[10] TO Issa, AI Mohamed Ahmed, YS Mohamed, S. Yagi, AM Makhawi, and TO Khider, "Physiochemical, Insecticidal, and Antidiabetic Activities of *Senna occidentalis* Linn Root," *Biochem Res Int*, vol. 2020, 2020, doi: 10.1155/2020/8810744.

[11] Deme, YD Malann, ES Chundusu, and JA And Chup, "Insecticidal effects of *Cassia tora* and *Cassia alata* against cowpea weevil (*Callosobruchusmaculatus)*," *Science World Journal*, vol. 14, no. 1, pp. 144–147, 2019, [Online]. Available: www.scienceworldjournal.org