



Toxicity and Synergistic Effect of Native Plants Extracts against the Cowpea Weevil (*Callosobruchus maculatus* Fab.) (Coleoptera: Bruchidae)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors EM and NO designed the study and wrote the protocol and the first draft of the manuscript. Authors RS, DDAKS and BO collected the data. Author RAO managed and performed the statistical analysis. Authors PAEDS and SN supervised the study, wrote, reviewed and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Callosobruchus maculatus causes loss of cowpea seeds and its weight after six months of unprotected storage. This study aimed to evaluate the toxicity and synergistic effects of plant extracts on the biological parameters of *C. maculatus* from Bobo Dioulasso in order to reduce its damage on cowpea stocks in Bobo Dioulasso city. Seven treatments including an untreated control and doses of three powdery extracts of *Cassia nigricans*, *Zanthoxylum zanthoxyloides* and *Hyptis suaveolens*, and their combinations were compared in a Fischer block with four completely randomized repetitions. One hundred healthy cowpea seeds were mixed with doses of each extract in Petri dishes then submitted as food substrates to ten pairs of *C. maculatus* for 15 days. The synergistic effect was evaluated following the formula of Chou and Talalay. Extracts of *C. nigricans*, *Z. zanthoxyloides* and *H. suaveolens* was toxic against the weevil. The extract of *Z. zanthoxyloides* was more toxic to adults (21.16 mg/g) followed by *H. suaveolens* (51.84 mg/g) and *C. nigricans* (52.09 mg/g). The infestation rates of batches treated with *C. nigricans* and *Z. zanthoxyloides* extracts did not exceed 1.00% compared to 2.00% for *H. suaveolens* at 20 mg/g. All combinations were synergistic and resulted in 100% mortality and less than 14.98 eggs/female in *H. suaveolens* coupled with *C. nigricans*. The application of *Z. zanthoxyloides* extract alone or the combination of *H. suaveolens* and *C. nigricans* extracts would be an alternative for the sustainable management of *C. maculatus* in cowpea stocks.

Keywords: *Zanthoxylum zanthoxyloides*; *cassia nigricans*; *hyptis suaveolens*; insecticidal activity; stored seed.

1. INTRODUCTION

Cowpea *Vigna unguiculata* L. (Walpers) is a legume with nutritional values. It contains 60 to 65% carbohydrate and 26% protein. It contributes to fight hunger, especially during lean periods, and to correct food and nutritional imbalances in the world [1]. In Burkina Faso, it is the 5th legumes, it contributes with around 2 billion CFA francs of the national gross product with a production estimated at 454,840 tons during the 2021-2022 agricultural season [2].

Cowpea stocks in West Africa are mainly threatened by early pest attacks beginning during growing stage. Among these pests, the weevil *Callosobruchus maculatus* Fab. (Coleoptera: Bruchidae) can cause losses of nearly 80% of seeds and 30% of weight after six months of unprotected storage [3]; [4]. With an average development cycle of about 28 days, it can also cause economic damage at various stages of plant development in the field [5].

Aiming at protecting cowpea crops against this pest, systematic application of synthetic chemical insecticides aroused much satisfaction before becoming a threat to several ecosystems. The

harmful effects on the environment, along with the appearance of pest resistance to insecticides and the chronic poisoning caused to consumers have raised worries in the use of these chemicals [6]. They are involved in the unexpected impact on non-target organisms such as pollinating arthropods, predators or parasitoids [7] and the resistance to these insecticides can appear early and without delay [8].

This is why alternative of natural resources from plants which are both effective and sustainable because of their lower persistence compared to their synthetic counterparts are looked for [9]. The diversification of their active ingredients from various plant organs would constitute an efficient and sustainable strategy to limit post-harvest losses in a context of climate change.

A recent study of Mano et al. [10] was limited to the insecticidal and germinal activities of extracts from *Zanthoxylum zanthoxyloides* (Lam.), *Cassia nigricans* Vahl. and *Hyptis suaveolens* (L.) Poit. Given that bruchids adapt to insecticides as they are treated, this prompted this additional study to investigate low-dose synergistic combinations that could be more efficient and environment-friendly for insects and more economical to

rationalize the exploitation of environmental resources. Furthermore, knowing that bruchids reproduce sexually and asexually and that reducing the rejection of eggs by mated females (due to the absence of food or to poisoned undesirable food) extends their lifespan [11], lethal time of toxicity was considered in this study to prevent the active ingredients effective products from becoming ineffective because of the time parameter (extension of lifespan).

This study aimed therefore to evaluate the toxicity and synergistic activities of extracts from *Z. zanthoxyloides*, *C. nigricans*, and *H. suaveolens* on the biological processes of *C. maculatus* to reduce its damage on cowpea stocks. *Z. zanthoxyloides* is a species of perennial tropical shrubs of the Rutaceae family widespread in the western zone of Burkina Faso. *C. nigricans* and *H. suaveolens* are non-cultivated herbaceous plants that grow in rural and urban areas during the wintering period from June to September. These plants contain several molecules with insecticidal properties against several insects: anthraquinone, emodic acid, citreorosein, flavonoid luteolin, hydroxyestraneic acid, ethyl ester, etc. Indeed, previous studies have already proven the bioactivity and insecticidal properties of extracts of *Z. zanthoxyloides* against *Sitophilus oryzae* L [12], of *C. nigricans* against *Bemisia tabaci* G. [13,14] and *H. suaveolens* against Anopheles species and *Aedes aegypti* [15,16]. This justifies the choice of these plants in this study.

2. MATERIALS AND METHODS

2.1 Insects

Mass breeding of egg, male and female adults of the F1 generation of *C. maculatus* on healthy and untreated seeds of *V. unguiculata* (L.) (Walp.) in the laboratory (T°: 35°C; RH: 70% and PP: 12h) were tested in this study. These seeds were of the *Koumkalé* variety supplied by the

company NaFaso. The adults were collected using bags in the field of Bobo Dioulasso in April 2023. A binocular microscope was used for observations and morphological identification by comparison of laboratory samples.

2.2 Extraction of Insecticides

Leaves of *Zanthoxylum zanthoxyloides* (Lam.), *Cassia nigricans* Vahl. and *Hyptis suaveolens* (L.) Poit. were collected in the peri-urban area of Bobo Dioulasso in April 2023. They were dried completely under shade at room temperature (38±2°C) on ventilated racks and reduced to homogenized powder using a BLG 450 electric grinder.

2.3 Experimental Set-up

One hundred healthy cowpea seeds were introduced into Petri dishes, of 16 cm x 2 cm dimensions, and treated with the extracts by mixing at different doses of 20, 40, 60, and 80 mg/g of cowpea. Ten (10) pairs of *C. maculatus* were introduced into each of the Petri dishes and observed for 15 days in laboratory conditions (T°: 38 ± 2 °C; RH: 70%; PP: 12 h). The seven treatments including an untreated control (Table 1) were arranged in completely randomized Fischer blocks provided in four doses and four repetitions.

2.4 Toxicity Test

Ten couples of weevil were introduced into the mixture of seeds in doses of powdered extracts in each Petri dish and the mortalities were counted every 24 hours. The dose-dependent mortality data was collected into Petri dishes and analyzed through a probit analysis to find the values and times of lethal doses. The number of infested seeds, having a perforation under binocular microscope, in each batch was used to determine the infestation rates after 15 days of treatment.

Table 1. Products and doses of insecticide treatments against *C. maculatus*

Treatments	Products	Doses (mg/g)
Simple	Control	-
	<i>C. nigricans</i>	20, 40, 60, 80
	<i>Z. zanthoxyloides</i>	20, 40, 60, 80
	<i>H. suaveolens</i>	20, 40, 60, 80
Combination	<i>C. nigricans</i> + <i>H. suaveolens</i>	20 (1/2 ratio)
	<i>H. suaveolens</i> + <i>Z. zanthoxyloides</i>	20 (1/2 ratio)
	<i>Z. zanthoxyloides</i> + <i>C. nigricans</i>	20 1/2 ratio)

2.5 Synergistic Assay

To examine the synergistic or antagonistic effect of extract combinations, a quantitative definition of sum was necessary since synergy involves more sum of effects while antagonism involves less. The average mortality and fertility rates induced by the combinations were therefore compared to the sum of the average rates of the simple treatments according to the formula (F1) of Chou and Talalay [17] where “a” is the mortality of the combined doses and “b” the arithmetic sum of the mortalities of the doses of separated extracts. Positive results indicate synergy and negative results indicate antagonism.

$$Syn (\%) = \left(\frac{a-b}{b}\right) \times 100 \quad (F1)$$

2.6 Data Collection and Analysis

The number of *C. maculatus* adults that died after 15 days of exposure to the treatments allowed the determination of synergistic effects after meeting the requirements for analysis of variance. The collected data were analyzed statistically in R 4.2.1 software and the means were separated by the Tukey test with a 5% threshold. Probit analysis was used to obtain LD 90 and LT 90.

3. RESULTS

3.1 Toxicity of Extracts on adult *C. maculatus*

The plant extracts were toxic against the bruchids in this study. The toxicity analysis (Table 2) shows that the extract of *Z. zanthoxyloides* was more toxic to adults of the bruchid (LD 90: 21.16 mg/g; TL 90: 90.87 h; $R^2 \geq 0.900$) followed by that of *H. suaveolens* (LD 90: 51.84 mg/g; TL 90: 108.85 h; $R^2 \geq 0.911$) and *C. nigricans* (LD 90: 52.09 mg/g; TL 90: 112.24 h; $R^2 \geq 0.831$).

3.2 Effect of Extracts on Seed Infestation Rate

The analysis of variance (Table 4) indicates a significant variation in the average seed infestation rates ($F: 321; P < 0.001$). Table 3 reveals that the batches of cowpeas treated with plant extracts did not suffer significant damage. It shows a highly significant reduction in the infestation rates of the extract batches compared to those of the untreated controls ($F \geq 705.9; P < 0.001$). Infestations in batches treated with extracts of *C. nigricans* and *Z. zanthoxyloides* did not exceed the rate of 1.00% while in *H. suaveolens* this rate reached 2.00% at a dose of 20 mg/g.

Table 2. Analysis of the toxicity of the extracts on *C. maculatus*

Extract	Dose (mg/g)			Time (h)		
	DL90 ± SE	95% CI	R ²	TL90 ± SE	95% CI	R ²
<i>Z. zanthoxyloides</i>	21.16 ± 2.62	13.54 - 24.78	0.900	90.87 ± 4.00	80.44 - 99.06	0.915
<i>H. suaveolens</i>	51.84 ± 5.01	44.83 - 59.80	0.911	108.85 ± 6.04	90.00 - 128.60	0.954
<i>C. nigricans</i>	52.09 ± 4.27	40.86 - 66.33	0.831	112.24 ± 10.20	97.49 - 131.11	0.932

n = 10 pairs of *C. maculatus* / Petri dishes

Table 3. Analysis of seed infestation rates by *C. maculatus*

Treatments	Infested seed rate (%)			
	20 (mg/g)	40 (mg/g)	60 (mg/g)	80 (mg/g)
Control	6.500 a	6.500 a	6.500 a	6.500 a
<i>C. nigricans</i>	1.000 c	0.500 c	0.500c	0.250 bc
<i>Z. zanthoxyloides</i>	0.500 d	0.250 c	0.000 d	0.000 c
<i>H. suaveolens</i>	2.000 b	1.750 b	1.000 b	0.500 b
<i>F.</i>	705.9	796.1	862.7	923
<i>P</i> (> <i>F</i>)	< 9.36e-14	4.57e-14	2.83e-14	1.89e-14
Significance	***	***	***	***

Means in the same column, assigned with the same letter, are not significantly different at 5% threshold (Tukey test); ***: highly significant.

Table 4. Analysis of variance of average seed infestation rates

Source	Df	Sum Square	Mean Square	F. value	P (>F)
Treatments	12	142.46	11.872	321	<2nd-16***
Residuals	39	1.44	0.037		

Df: Degree of freedom

Table 5. Analysis of the synergistic effects of the extracts on the mortality and fertility of *C. maculatus*

Treatments		Mortality	Fertility		
Composition	Products	Mortality (%)	S. (%)	N eggs / female	- S. (%)
Sum of effects	Σ (<i>C. nigricans</i> + <i>Z. zanthoxyloides</i>)	86.25	-	28.97	-
	Σ (<i>Z. zanthoxyloides</i> + <i>H. suaveolens</i>)	83.75	-	38.04	-
	Σ (<i>H. suaveolens</i> + <i>C. nigricans</i>)	78.75	-	42.97	-
Combined effects	<i>C. nigricans</i> _ <i>Z. zanthoxyloides</i>	95.00	+ 10.15	18.45	+ 36.31
	<i>Z. zanthoxyloides</i> _ <i>H. suaveolens</i>	97.50	+ 16.42	18.45	+51.50
	<i>H. suaveolens</i> _ <i>C. nigricans</i>	100.00	+26.98	14.98	+65.14

Means of the same column assigned the same letter are not significantly different at the 5% threshold (Duncan test); S (%): synergistic rate; N: number

3.3 Synergistic Effect of Combinations on Mortality and Fertility of Bruchids

Analysis of the effects of combinations of extracts at a dose of 20 mg/g of cowpea seed indicated synergistic lethal effects (Table 5). The combination of *H. suaveolens* and *C. nigricans* was more synergistic (S: + 26.98%), followed by *Z. zanthoxyloides* combined with *H. suaveolens* (S: + 16.42%) and *C. nigricans* combined with *Z. zanthoxyloides* (S: + 10.15%) leading to 100.00%, 97.50% and 95.00% bruchid mortality respectively. In the same order, clutches varied from 14.98 eggs/female in *C. nigricans* combined with *Z. zanthoxyloides* (S: + 65.14%) to 18.45 eggs/female in the two other less synergistic combinations (S: + 51.50%; S: + 36.31%).

4. DISCUSSION

Analysis of the toxicity of the extracts showed that the extract of *Z. zanthoxyloides* is more toxic to bruchid adults with an LD 90 of 21.16 mg/g and a TL 90 of 90.87 h. This was followed by the extract of *H. suaveolens* (LD 90: 51.84 mg/g; TL 90: 108.85 h) and that of *C. nigricans* (DL 90: 52.09 mg/g; TL 90: 112.24 h). These results confirm those of Gbate et al. [18] who noted an influence of the bio-activity of several enzymes of *C. maculatus* treated with the extract of *Z. zanthoxyloides* which caused 100% mortality in 24 hours. Previously, [19] obtained high mortalities in 48 hours using extracts of *Z. zanthoxyloides* as contact insecticide against *C. maculatus*, *S. zeamais* et *T. castaneum*.

Application of extracts reduces infestation rates. Indeed, cowpea seeds treated with different powders had significantly reduced damage compared to untreated controls. The control batches had an infestation rate of 6.5% compared to a significant different rate of 0.25 to 2.19% for the extracts. Doses of extracts significantly reduced the damage of *C. maculatus*, confirming the results of Azeez et al. [20] indicating considerable protection with extracts of *H. suaveolens*. The high reduction of infestation would be due to the insecticidal action of several bioactive molecules contained in plant extracts. Indeed, recent research showed the presence of secondary metabolites such as polyphenols, flavonoids, anthocyanidin, and alkaloids in individual aqueous extracts of *Z. zanthoxyloides*, *H. suaveolens*, and *C. nigricans*. According to this study, anthraquinones were

also evidenced in the extract of *H. suaveolens* [10].

All combinations studied showed synergistic effects in increasing mortality and reducing egg laying, particularly in the combination of *H. suaveolens* and *C. nigricans*. According to recent studies by Swami et al. [21], mixtures of plant extracts have shown synergistic activity against harmful insects and disease vectors. This combination of *H. suaveolens* and *C. nigricans* demonstrated at a dose of 20 mg/g, a synergy rate of +26.98%, followed by *Z. zanthoxyloides* combined with *H. suaveolens* (+16.42%) and *C. nigricans* combined with *Z. zanthoxyloides* (+10.15%), respectively resulting in 100.00%, 97.50% and 95.00% bruchid mortality. These results are similar to those of Gbaye et al. [22,23] who obtained insecticide tolerance synergetic of the same pest *C. maculatus*. Synergists acting at low doses, have the advantage of being more economical because of the recommended low doses and increasing the number of molecules that act simultaneously against resistance to products [5]. The three plants are very available in the rainy season in Burkina Faso and West Africa.

The fertility of adults of *C. maculatus* was significantly affected by the effects of the different extracts treated individually as well as those of the combinations at a dose of 20 mg/g. The results of our work showed sum effects of 28.97 to 42.97 eggs per female in individual extract batches which significantly decreased to 18.45 eggs/female for combinations of *C. nigricans_Z. Zanthoxyloides* and *Z. zanthoxyloides_H. suaveolens* and 14.98 eggs/female for the combination of *H. suaveolens_C. nigricans*. Our results aligned to those of Musa [24,25] and [26] who attested that essential plant oils significantly inhibit the oviposition of females of *C. maculatus*. They corroborate those varied of Mano [10] which found, at a dose of 80 mg/g in the treated batches, that fecundity per female decreased significantly from 13.82 eggs for *H. suaveolens* at 10.12 eggs for *C. nigricans*. Treatment with the extracts would have led to oxygen depletion between the seeds. This will amplify egg mortality as stated by Obembe et al. [27]. Several other studies addressing the same subject have concluded that bruchid eggs suffer high mortalities after the application of extracts of *Kigelia africana* (Lam.) Benth [28], *Eucalyptus globulus*, *Thymus vulgaris*, *Laurus nobilis* and

Juglans regia [29] and alkaloids extracted from the leaves of *Moringa olievera*.

5. CONCLUSION

The aims of this study was to evaluate the toxicity and synergistic effects of plant extracts on the biological processes of *C. maculatus* in order to reduce its damage on cowpea stocks. Extracts of *C. nigricans*, *H. suaveolens* and *Z. zanthoxyloides* have active ingredients against *C. maculatus*, a pest of cowpea stocks. That of *Z. zanthoxyloides* is more toxic on adults of the bruchid followed by *H. suaveolens* and *C. nigricans*. Application of extracts reduces infestation rates seeds. All combinations studied have synergistic effects in increasing mortality and reducing egg laying, particularly in the couple *H. suaveolens* and *C. nigricans*. The application of *Z. zanthoxyloides* extract alone or the combination of *H. suaveolens* and *C. nigricans* would be an alternative for sustainable management of *C. maculatus*, a pest of cowpea seeds's stocks.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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