

Phytochemical Composition and Insecticidal Property of Native Plants against the Cowpea Weevil *Callosobruchus maculatus* Fab. (Coleoptera: Bruchidae) for Natural Preservation of Cowpea Seeds

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Abstract

About 80% of cowpea seeds are lost along with 30% of their weight after six months of unprotected storage due to attacks by *Callosobruchus maculatus*. This study aimed at evaluating the effect of applying bioinsecticides on the biological parameters of *C. maculatus* to reduce its damage to cowpea stocks. Four treatments including an untreated control and three doses of powdery extracts of *C. nigricans*, *Z. zanthoxyloides* and *H. suaveolens*, were compared in a Fischer block with four completely randomized repetitions. One hundred undamaged cowpea seeds were mixed with doses of each extract in Petri dishes and then fed as food substrates to ten pairs of *C. maculatus* for eight days. The extracts of *H. suaveolens*, *C. nigricans* and *Z. zanthoxyloides* all contain active ingredients including polyphenols, flavonoids, anthocyanins and alkaloids, etc. The extract of *Z. zanthoxyloides* was found to be more active on adults (100.00% mortality) followed by *C. nigricans* (98.75% mortality) and *H. suaveolens* (97.50% mortality) at 80 mg/g. Fecundity per female decreased significantly in the treated batches of 13.82 eggs for *H. suaveolens* at 10.12 eggs for *C. nigricans* at a dose of 80 mg/g. Germination rate of seeds



was statistically raised to 100% at 60 and 80 mg/g by *C. nigricans* powder and lowered to 83.00% at 20 mg/g by *Z. zanthoxyloides* extracts. The application of *Z. zanthoxyloides* extract would be a less expensive alternative for the sustainable management of *C. maculatus* in cowpea stocks.

Keywords

Cassia nigricans, *Hyptis suaveolens*, *Zanthoxylum zanthoxyloides*, Biological Activities, *Vigna unguiculata* Pest, Burkina Faso

1. Introduction

The preservation of cowpea seeds, *Vigna unguiculata* (L.) Walpers) in traditional storage systems in West Africa is of crucial importance for the nutrition of the rural population [1]. This legume has nutritional benefits of up to 26% protein and 60% to 65% carbohydrates. It is recommended to populations to correct dietary imbalances throughout the world. It is a vital crop during lean periods. In Burkina Faso, the market value of cowpea is high; it contributes to 2 billion FCFA of the GDP and is ranked as 5th among plant crops with a production of 454,840 tons during the 2021-2022 agricultural cropping season [2].

However, this plant is often attacked during growing and storage stages. The most fearsome of these pests is the *Callosobruchus maculatus* Fab weevil (Coleoptera: Bruchidae) which can cause losses of nearly 80% of seeds and 30% of weight after six months of unprotected storage [1] [3]. It can also cause economic damage at various stages of plant development [4].

In order to efficiently protect cowpea harvests against this insect pest, conservationists systematically resort to the application of synthetic insecticides despite evidence of chronic poisoning caused to consumers, pest resistance and negative impact on the environment [5]. Their high cost and short availability hinders African farmers' businesses [6] [7]. The use of these synthetic insecticides has become a threat to several ecosystems. They could also have a negative impact on predatory arthropods or parasitoids that prey on pests [8] and it is well known that resistance to these insecticides can develop rapidly [9].

This is why natural substances as alternatives, are considered effective and durable, less toxic and less polluting than synthetic insecticides [10] and give hope for several agricultural stakeholders. The objective of this study was to evaluate the effect of native plant extracts on the biological parameters of *C. maculatus* in order to reduce its damage to cowpea seeds during storage.

2. Material and Methods

2.1. Animal Material

Eggs, male and female adults of the F1 generation of *C. maculatus* freshly emerged from mass breeding on undamaged and untreated seeds of *V. unguiculata* (L.) (Walp.) in the laboratory (T°: 35°C; RH: 70% and PP: 12 h) were tested

in this study. This seed variety was *Koumkalé* supplied by NaFaso.

2.2. Extraction of Insecticides

Cassia nigricans leaves Vahl., *Zanthoxylum zanthoxyloides* (Lam.) and *Hyptis suaveolens* (L.) Poit. were collected in the city of Bobo Dioulasso. They were dried completely under shade at room temperature ($38^{\circ}\text{C} \pm 2^{\circ}\text{C}$) on ventilated racks and reduced to powder using a BLG 450 electric grinder. One hundred grams of each powder was macerated in one liter of water (ratio 1/10; m/v) under mechanical stirring for 24 hours, then aqueous extracts were collected for phytochemical analysis. The essential oils were extracted for two hours by hydrodistillation using an alambic. They were dried with anhydrous sodium sulfate and stored in a refrigerator ($T^{\circ}: -4^{\circ}\text{C}$) until analysis.

2.3. Experimental Set-Up

One hundred healthy cowpea seeds were first introduced into Petri dishes, of $16\text{ cm} \times 2\text{ cm}$. These seeds were then 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 finally introduced into each Petri dish and observed for eight days in laboratory conditions ($T^{\circ}: 38^{\circ}\text{C} \pm 2^{\circ}\text{C}$; RH: 70%; PP: 12 h). The four treatments (*C. nigricans*, *Z. zanthoxyloides*, *H. suaveolens* and the control) were arranged in completely randomized Fischer blocks distributed through four doses and four repetitions. The germination test of the seeds of each batch treated with insecticides was carried out on Petri dishes covered with cotton soaked in water for 4 to 5 days.

2.4. Data Collection and Analysis

The number of *C. maculatus* adults that died after eight days of treatments was used for the determination of mortality rates after meeting the requirements for analysis of multiple treatment effects. The eggs laid on the seeds were counted under a binocular microscope after 15 days of treatment. Counting the number of germinated seeds from each batch enabled to calculate the germination rates after 15 days of treatment. Data collected were analysed with R 4.2.1 software. The homogeneity of variances was checked with the Bartlett test. Comparison of mortality and germination rates and the number of eggs laid per female of *C. maculatus* was carried out with the Pairwise test. The non-parametric Kruskal Wallis test was used to check the dose effect of the extracts on the variables “mortality rate”, “seed germination rate” and “number of eggs/female” of *C. maculatus*.

2.5. Phytochemical Analysis

Chemical screening of plant extracts was carried out by the qualitative method described by [11]. The objective was to highlight the bioactive phytochemical groups involved in the insecticidal activities observed. The essential oils were obtained by hydrodistillation.

3. Results and Discussion

3.1. Results

3.1.1. Insecticidal Activity on Adults of *C. maculatus*

Figure 1 indicates significant differences between the doses of studied extracts and the untreated control after 8 days of treatment ($df = 12$, $p\text{-value} = 1.776e-06$). Doses of *Z. zanthoxyloides* were found to be more active with 90.00%; 91.25%; 98.75% and 100.00% mortality respectively for doses of 20; 40; 60 and 80 (mg/g). Those of *H. suaveolens* and *C. nigricans* followed with respectively 97.50% and 98.75% mortality at 80 mg/g of cowpea seeds. The lowest mortalities of bruchid adults were induced by the control (5.00%). **Table 1** indicates significant differences between doses with the exception of 20 mg/g and 40 mg/g in *Z. zanthoxyloides* and *C. nigricans*. This table shows that there is no significant difference in mortality between doses of 60 mg/g and 80 mg/g in all extracts.

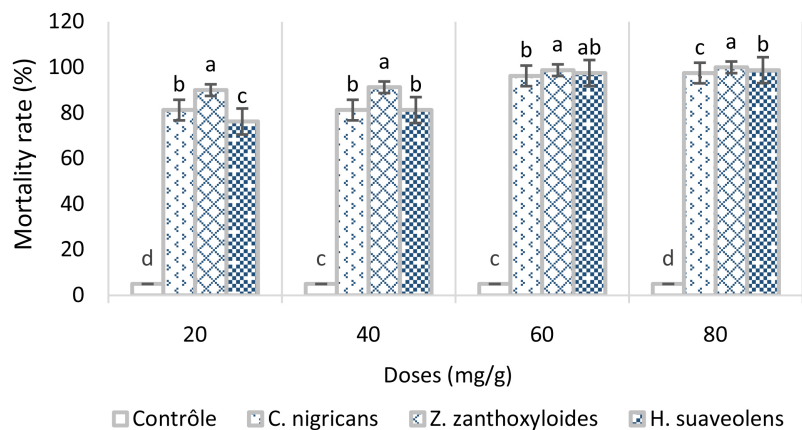


Figure 1. Analysis of mortality rates of *C. maculatus*.

Table 1. Comparison of *C. maculatus* mortalities between treatment doses (Pairwise-t-test).

	C20	C40	C60	C80	Z20	Z40	Z60	Z80	H20	H40	H60	H80
C40	1.00000	-	-	-	-	-	-	-	-	-	-	-
C60	<2e-16	<2e-16	-	-	-	-	-	-	-	-	-	-
C80	<2e-16	<2e-16	0.33452	-	-	-	-	-	-	-	-	-
Z20	<2e-16	<2e-16	5.7e-13	2.0e-15	-	-	-	-	-	-	-	-
Z40	<2e-16	<2e-16	3.3e-10	5.7e-13	0.33452	-	-	-	-	-	-	-
Z60	<2e-16	<2e-16	0.00058	0.33452	<2e-16	2.0e-15	-	-	-	-	-	-
Z80	<2e-16	<2e-16	1.00000	0.00058	<2e-16	<2e-16	0.33452	-	-	-	-	-
H20	3.3e-10	3.3e-10	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	-	-	-	-
H40	1.00000	1.00000	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	3.3e-10	-	-	-
H60	<2e-16	<2e-16	0.33452	1.00000	<2e-16	<2e-16	0.33452	0.00058	<2e-16	<2e-16	-	-
H80	<2e-16	<2e-16	0.00058	0.33452	<2e-16	<2e-16	2.0e-15	<2e-16	<2e-16	<2e-16	0.33452	-
Cont	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16

3.1.2. Effects of Treatments on the Fecundity of *C. maculatus*

The results show that the reduction effect of the powdered extracts of the plants on females egg laying of *C. maculatus* on cowpea was significant (df = 12, p-value = 8.402e-05) (Figure 2). Fertility in the control batches was 33.3 eggs/female. It decreased significantly in the treated batches at 13.82 eggs for *H. suaveolens* at 10.12 eggs for *C. nigricans* at a dose of 80 mg/g of cowpea seeds. Among all the treatments, *C. nigricans* powder was found to be more effective in the majority of the tested doses. Table 2 shows significant differences in the numbers of eggs per female between all doses in *H. suaveolens*; no significant differences were noted in *Z. zanthoxyloides*. It was similarly observed in *C. nigricans* with the exception of 20 mg/g dose which was statistically different from the other doses.

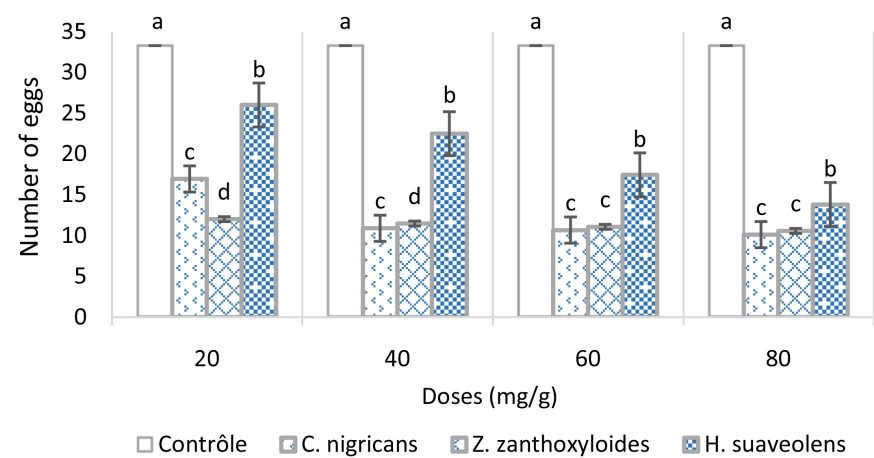


Figure 2. Analysis of the number of eggs laid per female of *C. maculatus*.

Table 2. Comparisons of fecundity (number of eggs/female) of *C. maculatus* between treatment doses (Pairwise-t-test).

	C20	C40	C60	C80	Z20	Z40	Z60	Z80	H20	H40	H60	H80
C40	5.2e-14	-	-	-	-	-	-	-	-	-	-	-
C60	1.7e-14	1.00000	-	-	-	-	-	-	-	-	-	-
C80	1.2e-15	1.00000	1.00000	-	-	-	-	-	-	-	-	-
Z20	2.2e-11	0.40033	0.13246	0.00560	-	-	-	-	-	-	-	-
Z40	1.1e-12	1.00000	1.00000	0.12482	1.00000	-	-	-	-	-	-	-
Z60	1.3e-13	1.00000	1.00000	0.81713	0.84284	1.00000	-	-	-	-	-	-
Z80	1.1e-14	1.00000	1.00000	1.00000	0.08352	0.94591	1.00000	-	-	-	-	-
H20	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	-	-	-	-
H40	7.2e-13	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	1.4e-07	-	-	-
H60	1.00000	4.5e-15	1.5e-15	<2e-16	1.3e-12	7.8e-14	1.1e-14	1.0e-15	<2e-16	1.1e-11	-	-
H80	1.7e-06	6.9e-06	1.7e-06	4.6e-08	0.01008	0.00033	2.3e-05	8.9e-07	<2e-16	<2e-16	7.0e-08	-
Cont	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16	<2e-16

3.1.3. Effect of Extracts on the Germination Power of Cowpea Seeds

The germination power (**Figure 3**) of the treated cowpea seeds shows a significant difference at 20; 40, 60 and 80 mg/g ($df = 12$, $p\text{-value} = 2.407e-06$). *Cassia nigricans* powder promoted the highest germination rates reaching 100% at doses of 60 and 80 mg/g. The lowest germination rates were recorded by the extracts of *Z. zanthoxyloides* with 83.00% at 20 mg/g of cowpea. **Table 3** indicated significant different germination rates between all doses of *Z. zanthoxyloides*. Germination rates of doses of 20; 40, and 60 mg/g in *H. suaveolens* were statistically identical. If the doses of 60 mg/g and 80 mg/g caused statistical differences in *H. suaveolens*, this was not the case in *C. nigricans* where these two doses were statistically similar.

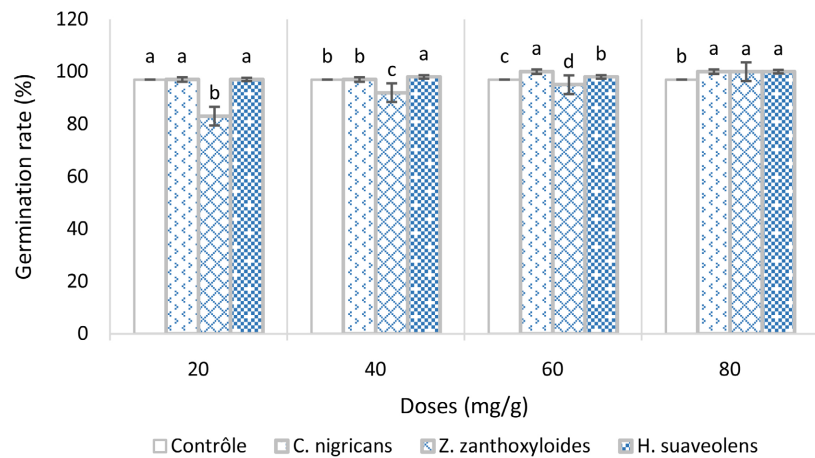


Figure 3. Analysis of germination rates of treated seeds.

Table 3. Comparisons of germination power (germination rate) of seeds between treatment doses (Pairwise-t-test).

	C20	C40	C60	C80	Z20	Z40	Z60	Z80	H20	H40	H60	H80
C40	1.00	-	-	-	-	-	-	-	-	-	-	-
C60	1.0e-08	1.0e-08	-	-	-	-	-	-	-	-	-	-
C80	1.0e-08	1.0e-08	1.00	-	-	-	-	-	-	-	-	-
Z20	<2e-16	<2e-16	<2e-16	<2e-16	-	-	-	-	-	-	-	-
Z40	2.1e-15	2.1e-15	<2e-16	<2e-16	<2e-16	-	-	-	-	-	-	-
Z60	3.7e-05	3.7e-05	2.7e-15	2.7e-15	<2e-16	7.2e-09	-	-	-	-	-	-
Z80	1.0e-08	1.0e-08	1.00	1.00	<2e-16	<2e-16	2.7e-15	-	-	-	-	-
H20	1.00	1.00	1.0e-08	1.0e-08	<2e-16	2.1e-15	3.7e-05	1.0e-08	-	-	-	-
H40	0.14	0.14	5.2e-05	5.2e-05	<2e-16	<2e-16	7.2e-09	5.2e-05	0.14	-	-	-
H60	0.14	0.14	5.2e-05	5.2e-05	<2e-16	<2e-16	7.2e-09	5.2e-05	0.14	1.00	-	-
H80	1.0e-08	1.0e-08	1.00	1.00	<2e-16	<2e-16	2.7e-15	1.00	1.0e-08	5.2e-05	5.2e-05	-
Contact	1.00	1.00	1.0e-08	1.0e-08	<2e-16	2.1e-15	3.7e-05	1.0e-08	1.00	0.14	0.14	1.0e-08

Note: C: *Cassia nigricans*; Z: *Zanthoxylum zanthoxyloides*; H: *Hyptis suaveolens*.

3.1.4. Chemical Profile of Water-Soluble Metabolites

The aqueous extracts of *H. suaveolens*, *C. nigricans* and *Z. zanthoxyloides* contains individually potential bioactive secondary metabolites, namely: polyphenols, flavonoids, Leucoanthocyanidin and alkaloids. **Table 4** shows the absence of terpene compounds in *H. suaveolens*, tannins in *Z. zanthoxyloides* and essential oils in *C. nigricans*. The presence of anthraquinones was only evidenced in the extract of *H. suaveolens*.

3.2. Discussion

This study revealed that extracts of *C. nigricans*, *H. suaveolens* and *Z. zanthoxyloides* all contain polar active ingredients, namely polyphenols, flavonoids, anthocyanins and alkaloids. These same compounds have already been highlighted by [12] and [13]. The presence of anthraquinones and the absence of terpene compounds in the extract of *H. suaveolens* as well as the absence of tannins in the extract of *Z. zanthoxyloides* have already been presented by [14] and [15]. These secondary metabolites are responsible for several insecticidal activities: repulsion, mortality, anti-feeding, anti-oviposition, anti-morphogenesis, etc. Some of these metabolites identified in *Z. zanthoxyloides* include: zanthoxylol [16], benzophenanthridine, furoquinoline, aporphine alkaloids and several aliphatic amides [17], pellitorin, [18]; [19] and several chemical compounds in oils of the genus *Zanthoxylum* [20].

The toxicity of the extracts against the development and egg laying of *C. maculatus* in this study supports [14] and [21] results who reported an influence on the bioactivity of several *C. maculatus* enzymes. The latter obtained with the hexanic extract of *Z. zanthoxyloides* 100% mortality of *C. maculatus* in 24 hours, which is consistent with 100% mortality obtained by [19] using crude powder

Table 4. Chemical composition of extracts.

Chemical groups	Aqueous extracts		
	<i>H. suaveolens</i>	<i>C. nigricans</i>	<i>Z. zanthoxyloides</i>
Sterols and terpenes	(–)	+	+
Polyphenols	+	+	+
Flavonoids	+	+	+
Leucoanthocyanins	+	+	+
Catechical tannins	+	+	(–)
Tannins gallic	+	+	(–)
Alkaloids	+	+	+
Saponins	+	+	(–)
Quinones and anthraquinones	+	(–)	(–)
Oil essential	+	(–)	+

Note: (–): absence; +: presence.

and methanolic extract of *Z. zanthoxyloides* as contact insecticide against *C. maculatus*, *Sitophilus oryzae*, *Oryzaephilus mercator* and *Rhyzopertha dominica*. The volatile compounds and essential oils highlighted in this study would have led to antifeedant activity; these results are already mentioned against the larvae of *Spodoptera littoralis* [22] and [23].

The fecundities of adults of *C. maculatus* were significantly affected by the effects of the different powders treated individually as well as those of the combinations. The results of our work show an average of 33.30 eggs per female in the control batches which significantly decreased in the treated batches by 13.82 eggs for *H. suaveolens* at 10.12 eggs for *C. nigricans* at a dose of 80 mg/g of cowpea seeds. *Cassia nigricans* powder was found to be more effective at all doses. Our results conform to those of [24] and [25] whose results show a difference between the extracts. Authors [26] and [27] attested that treatments based on essential oils of plants considerably inhibit the egg laying of females of *C. maculatus*.

Application of extracts improves germination power seeds. Indeed, cowpea seeds treated with different powders were significantly less damaged compared to untreated controls. Doses of extracts significantly reduced the damage of *C. maculatus*, confirming the results of [28] [29] which demonstrated considerable protection of *H. suaveolens* extracts.

4. Conclusion

This study aimed at evaluating the effect of applying bioinsecticides on the biological parameters of *C. maculatus* to reduce its damage to cowpea stocks. Extracts of *C. nigricans*, *H. suaveolens* and *Z. zanthoxyloides* possess active ingredients against *C. maculatus*, an insect pest of cowpea stocks. *Zanthoxylum zanthoxyloides* extract is more active on adults of the bruchid followed by that of *H. suaveolens* then *C. nigricans*. The latter reduced the fecundity of females. Application of extracts improves germination power of cowpea seeds. The application of *Z. zanthoxyloides* extract would be an alternative for sustainable management of *C. maculatus*, an insect pest of stored cowpea seeds.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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