Original Research Article

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

ABSTRACT

Callosobruchus maculatus causes loss of nearly 80% of cowpea seeds and 30% of 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 Dioulassoin 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 eight days. The synergistic effect was evaluated following the formula of Chou and Talalay. Extracts of *C. nigricans, Z. zanthoxyloides and H. suaveolens*contain toxic active ingredients 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* extractswould 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 FCFA 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]. 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 lossesin a context of climate change.The objective of this study was therefore to evaluate the toxicity and synergistic effects of plant extracts on the biological processesof *C. maculatus* aiming at reducing its damage on cowpea stocks.

2. MATERIAL AND METHODS

2.1. Insects

Mass breeding of egg, male and female adults of the F1 generation of *C. maculatus* on healthy anduntreated 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 NaFaso. The adults were collected using bags in the city of Bobo Dioulasso in April 2023.

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 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 eight 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.

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

Treatments	**Products**	**Doses (mg/g)**
Simple	Control	-
	C. nigricans	20, 40, 60, 80
	Z. zanthoxyloides	20, 40, 60, 80
	H. suaveolens	20, 40, 60, 80

	C. nigricans + H. suaveolens	20 (1/2 ratio)
Combination	*H. suaveolens + Z. zanthoxyloides*	20 (1/2 ratio)
	Z. zanthoxyloides + C. nigricans	20 (1/2 ratio)

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 in each batch was used to determine the infestation rates after 15 days of treatment.

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[10]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.

$Syn(\%) = \left(\frac{a-b}{b}\right) x100$ (F1)

2.6. Data collection and analysis

The number of *C. maculatus adults* that died after eight 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.

3. RESULTS AND DISCUSSION

3.1. Toxicity of extracts on adult *C. maculatus*

The plant extracts were toxic against the brucids 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; $R^2 \geq 0.831$).

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

Extract	**Dose (mg/g)**			**Time (h)**		
	DL90 ± SE	**95% CI**	**R²**	**TL90 ± SE**	**95% CI**	**R²**
Z. zanthoxyloides	21.16 ± 2.62	13.54 - 24.78	0.900	90.87 ± 4.00	80.44 – 99.06	0.915
H. suaveolens	51.84 ± 5.01	44.83 – 59.80	0.911	108.85 ± 6.04	90.00 – 128.60	0.954

C. nigricans	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

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 ≥ 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 3: Analysis of seed infestation rates by *C. maculatus*

	Infested seed rate (%)			
Treatments	**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
C. nigricans	1,000 c	0.500 c	0.500c	0.250 bc
Z. zanthoxyloides	0.500 d	0.250 c	0.000 d	0.000 c
H. suaveolens	2,000 b	1,750 b	1,000 b	0.500 b
F.	705.9	796.1	862.7	923
P (>F)	< 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)**
Treatment _	12	142.46	11,872	321	<2nd-16***
Residuals	39	1.44	0.037		

Df: Degree of freedom

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%).

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(C. nigricans + Z. zanthoxyloides)*	86.25	-	28.97	-
	Σ(Z. zanthoxyloides + H. suaveolens)	83.75	-	38.04	-
	Σ (H. suaveolens + C. nigricans)	78.75	-	42.97	-
Combined effects	*C. nigricans _ Z. zanthoxyloides*	95.00	+ 10.15	18.45	+ 36.31
	Z. zanthoxyloides_H. suaveolens	97.50	+ 16.42	18.45	+51.50
	H. suaveolens_C. nigricans	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: nomber

3.4. 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 [11] 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, [12]obtained high mortalities in 48 hours using extracts of *Z. zanthoxyloides* ascontact 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 [13] 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* [14].

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 [15], 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 [16]who obtained insecticide tolerance synergetic of the same pest *C. maculatus*.

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 [17],[18] and[19] who attested that essential plant oils significantly inhibit the oviposition of females of *C. maculatus*. They corroborate those varied of [14]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 [20]. Several other studies addressing the same subject have concluded that bruchid eggs suffer high mortalities after the application of extracts of *Kigeliaafricana* (Lam.) Benth [21], *Eucalyptus globulus*, *Thymus vulgaris*, *Laurus nobilis* and *Juglans regia* [22] and alkaloids extracted from the leaves of *Moringa olievera*[23].

4. CONCLUSION

The aims of this study was to evaluate the toxicity and synergistic effects of plant extracts on the biological processesof *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 ofcowpea seeds's stocks.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable

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