

Phytochemical screening and effectiveness of *Calotropis procera* Ait. R. Br. (Asclepiadaceae) leaf powder against cowpea bruchid (*Callosobruchus maculatus* Fab.) (Coleoptera: Chrysomelidea) in Bertoua, East-Cameroun

Abstract

Cowpea grain is a legume that plays an important role in the diets of many populations. However its production is limited by several biotic and abiotic constraints, including the attack of *Callosobruchus maculatus* beetles. In this way, *Calotropis procera* leaf powder extract was evaluated in the ambient laboratory conditions ($t \approx 25.74 \pm 1.03^\circ\text{C}$; r.h. $\approx 71.48 \pm 2.04\%$) for adult mortality, F_1 progeny reduction and seed damage, as well as on seed viability. Leaf powder was tested at 2, 4, 8 and 16 g/kg with four replications. Seed viability was assessed using seeds preserved for two months at a single concentration of 16 g/kg. Results showed that significant mortalities of *C. maculatus* on treated cowpea grains was recorded with leaf powder at all the concentrations, and they increased with the increasing of concentrations used and exposure periods. At the lowest concentration of 2 g/kg, *C. procera* leaf powder recorded more than 85% of mortality 1 day after exposure. The highest concentration (16 g/kg) caused almost complete adult mortality. The leaf extract used in this study showed complete inhibition in the F_1 progeny emergence of *C. maculatus* within in the concentration of 8 g/kg, and considerably reduced grain damage caused by *C. maculatus*. Seeds viability were not affected by *C. procera* leaf extract used. Considering these results, *C. procera* leaf powder extract could be a good alternative insecticide for cowpea grains protection during storage.

Keywords: Cowpea, *Callosobruchus maculatus*, *Calotropis procera*, mortality, damage, viability, Bertoua

1. Introduction

Cowpea, *Vigna unguiculata* (L.) Walp. is the most important legume in tropical Africa [1], which is grown on different types of soil, and can improve soil fertility and prevent erosion [2]. Cowpea grains are also used to fight against malnutrition thanks their high protein content (19-25%) [3]. It is therefore an affordable source of plant protein, particularly for low-income people in many tropical countries in Africa and Asia, where it is mainly consumed [4]. In addition to its nutritional value, *V. unguiculata* is also used for livestock feed [5]. Global production of *V. unguiculata* amounted to more than 5.7 million tons of dry seeds per year from 5 to 7.5 million

ha in 2008 [6]. In sub-Saharan Africa, the total production is around 70% per year [7]. In Cameroon, its production is estimated at 1% of world production (112,501 tons of cowpea) [8]. Cowpeas are only grown once a year, but they are needed throughout the year, so they need to be kept in stock to maintain food security and seeds for future use.

Stored grain infestation is a very serious problem, as various life stages of insect pests cause cost-effective loss and deteriorate the quality of grains. There are numbers of stored grain insect pests that infest food grains in farmer stores and public warehouses and massively surge due to ambient environmental conditions and poor warehousing technology used [9]. Hence, insect pests are responsible of grain damage to stored foodstuffs and cause major economic losses in food storage [10]. Among these insects is *C. maculatus*, which is the primary field to stored pest that cause considerable losses to cowpea grains without any insecticidal protection when the insect population reaches harmful levels [11]. Kpatinvoh et al. [12] state that the damage caused by this beetle to cowpea seeds in storage results in deterioration in the physical appearance of the seeds, weight loss, reduced nutritional value, and grain germination ability. In fact, in the early stages of its attack, the only symptoms are the existence of eggs covered to the surface of the cowpea grains. As insect growth occurs completely within the seed, the immature larval and pupal stages are not normally seen. The adult insects emerge through holes in the grains, leaving round holes that are the main evidence of damage [13].

During storage, to improve the quality of their products, farmers frequently use different methods to reduce the losses induced by insect pest on grains [14], with synthetic insecticides as the most popular control method and found to be the most effective [15]. Despite their effectiveness, synthetic insecticides cause various health and environmental adverse effects. Their repetitive use induces the development of pest resistance, destruction of ecosystems, environmental pollution, health problems, destruction of natural enemies, and also and non-targeted organisms [16, 17]. Their use is a source of health risks, water and soil pollution, and the development of resistance in targeted pests [18, 19]. Additionally, these synthetic pesticides are imported from African countries and are very expensive [20]. Using insecticidal products based on plant materials with insecticide potential is one of the approaches currently explored, based on prospecting secondary metabolites produced by plant species [21, 22]. In this way, chemical products derived from plants considered as insecticides are among the best alternative methods to synthetic insecticides because of their lesser impact on the environment and their

biodegradability [23]. Many researchers have turned to finding alternative approaches to the use of synthetic chemical control methods. Several investigations on the control of stored product pests have begun to accentuate the use of natural products of plant origin [24, 25, 26, 27]. Among the insecticidal plants used as insecticides is *Calotropis procera*, which belongs to the Asclepiadaceae family, including more than 280 genera and around 2,000 species [28]. It is originally from India and Africa, with wide geographical distribution in tropical and subtropical regions [29]. Different studies reported that different parts of *Calotropis* have abundant phytochemical components such as flavonoids, tannins, sterols, alkaloids, cardiac glycosides, sterols and triterpenes [30]. In many countries, leaf from *C. procera* is used in traditional medicine to reduce blood glucose in patients suffering from diabetes mellitus [31]. In pest management, many findings showed the use of *C. procera* in stored products as a repellent against *C. maculatus* [32], as toxic plant against *Sitophilus zeamais* in Ivory Coast [33]. In the same way, Abubakar *et al.* [34] showed the insecticidal effects of leaf powder from this plant against *S. zeamais* in storage and recorded good results in Nigeria. The current study was aimed to evaluating the efficacy of powdered leaves from *Calotropis procera* in controlling cowpea grains in storage against *Callosobruchus maculatus* in Bertoua, Cameroon.

2. Materials and methods

2.1. Presentation of the study site

The present study was carried out at the Department of Life Sciences, Higher Teacher Training College, University of in Bertoua (Lom-et-Djerem department, Eastern Region, Cameroon), from January to April 2023. The geographical localization of this region are as follows: 4°34' and 4°38" North latitude between 13°41' and 13°04" East longitude. The altitude in relation to sea level is 665 m [35]. It is located in a contact zone between savannah to the North and East, and forest to the South and West, with a Guinean equatorial climate. Rainfall is generally around 1,450 mm per year. It is characterized by four seasons, two dry seasons and two rainy seasons [35].

2.2. Cowpea grains

Vigna unguiculata grains used in this study are the “Fekem variety” obtained from farmers in the Gobo subdivision, Mayo Danay division, Far North region, Cameroon. This genotype is one of the most widely grown and consumed varieties in this locality because of its good yield

and seed size [36]. Before use in the experiment, damaged grains and impurity materials were removed from the cowpea stock, and the cleaned cowpea grains were kept in the freezer at -20°C for disinfestation [36]. After 14 days, grains were removed from the freezer and stored under ambient conditions for another 14 days for acclimatization [36]. The grain moisture content was determined using an electronic moisture tester (Pfeuffer HE 50 Mess-und prüfgeräte, Hoh-express, Germany); it was 12.1%.

2.3. Insect rearing

Callosobruchus maculatus parents used for this experiment were obtained from infested cowpea grains from traders in storage facilities at the market in Bertoua, Cameroon. The insects were reared in 900 ml glass jars containing cleaned and untreated cowpea grains. The glass jars were covered with cotton clothes to avoid the escape of insects and closed with perforated lids for sufficient aeration. The insects were allowed to reproduce in ambient laboratory conditions. The insects used for the experiment were those obtained from the second generation, in ambient laboratory conditions ($t \approx 25.74 \pm 1.03^{\circ}\text{C}$; r.h. $\approx 71.48 \pm 2.04\%$). *C. maculatus* adults used for all the experiments were no more than 3 days old [36].

2.4. Collection and preparation of insecticidal plant

Green leaves of *Calotropis procera* (Asclepiadaceae) were harvested in Maroua-Cameroon in January 2023, precisely at latitude $10^{\circ}35'20.3''\text{N}$; longitude $014^{\circ}19'07.2''\text{E}$, altitude of 401 m. The local name of this plant was obtained from the farmers of the Maroua region, and it is known under the name of “Babambé” in the Peulh language or “kulfaya” among the Guiziga. The scientific name was confirmed by the National Herbarium of Yaoundé-Cameroon as *Calotropis procera*. The harvested leaves were dried at room temperature for 14 days and ground using a wooden mortar until the powder passed through a 0.20 mm mesh sieve. The powder was then stored in a freezer at -4°C until needed for insect bioassays.

The plant powder were phytochemically screened using procedures for the detection of the chemical compounds as described in Dohou et al. [37], Bruneton [38], in Békro et al. [39], and N'Guessan et al. [40]

2.5. Mortality bioassay

The mortality test of *C. maculatus* on treated cowpea grains using *C. procera* was performed under ambient laboratory conditions ($t = 25.74 \pm 1.03^\circ\text{C}$; r.h. = $71.48 \pm 2.04\%$), recorded by a data logger (model EL-USB-2, LASCAR, China) [36]. In 500 ml glass jars, four dosages: 0.1, 0.2, 0.4 and 0.8 g (corresponding to 2, 4; 8 and 16 g/kg of cowpea) of *C. procera* leaf powder were mixed individually with 50 g of cowpea grains. Then, the glass jars were shaken manually for 2 minutes to allow uniform coating of the extract on the seed [25]. The negative control consisted in 50 g of cowpea grains without plant insecticide. After this, twenty *C. maculatus* adults aged ≤ 3 days old were added to the glass jars containing the treated or untreated cowpea grains. All glass jars containing treated, untreated and infested cowpea grains were covered with cotton clothes to prevent insects from escaping and closed with perforated lids for sufficient aeration [25]. The number of dead and alive insects was recorded 1, 3, 5 and 6 days after infestation. The insect was considered dead after several delicate contacts with entomological forceps without any movement of insect antennae and legs. According to Abbot the percentage of control mortality was corrected [41].

2.6. Population increased and cowpea seeds damage

After recording mortality in 6 days post-infestation of the previous experiment (mortality bioassay), the glass jars were maintained for further observations. After two months of storage, emerging bruchids, the number of damaged and undamaged cowpea seeds were counted and evaluated. The percentage of inhibition in progeny (IR) emergence was calculated using the formula below:

$$IR = \frac{(Nc - Ne)}{Nc} \times 100$$

Where Nc: the number of insects that emerged in the negative control; Ne: the number of insects that emerged in the treated jars.

The damaged seed rate is the ratio of the number of damaged seeds to the total number of seeds. It was estimated following the formula used by Fotso *et al.* [25]:

$$\%GE = \frac{Nd}{Nt} \times 100$$

Where: Nd is the number of damaged seeds and Nt is the total number of seeds.

The percentage weight loss (%PW) was evaluated as follows:

$$\%PW = \left[\frac{(Pu \times Nd) - (Pd \times Nu)}{Pu(Nd + Nu)} \right] \times 100$$

Where: Pu is the weight of undamaged seeds; Nu: the number of undamaged seeds; Pd: the weight of damaged seeds; Nd: the number of damaged seeds.

2.7. Seeds viability assessment

To assess seed viability, 50 g of cleaned cowpeas were placed in a 450 ml glass jar and mixed with the highest content of 16 g/kg *Calotropis procera* leaf powder with the highest content. Two different treatment batches were made; one was infested with adult *C. maculatus* and the other was uninfested. Three replications were made for each batch containing the treatment. After two months of storage, 30 unperforated seeds were taken randomly from each glass jar and placed on moistened filter paper in 9 cm Petri dishes and stored under ambient conditions ($t \approx 25.22^{\circ}\text{C} \pm 2.04^{\circ}\text{C}$; $\text{RH} \approx 72.53\% \pm 2.28\%$). Each petri dish was watered every day for 10 consecutive days [42]. After this period, the number of germinated and ungerminated seeds was recorded [21]. The percentage of germinated seeds (%PG) was calculated according the following formula:

$$\%PG = \frac{Ng}{Nt} \times 100$$

Where: Ng: the number of germinated seeds (infested or non-infested) in the treatment and Nt: the total number of seeds in the petri dishes.

2.8. Data analysis

The *C. procera* bio-efficacy study was conducted from January to April 2023, and data on various parameters were collected. Abbott's formula [41] was used to correct mortality relative to negative control before analysis of variance (ANOVA) and probit analysis. The corrected cumulative mortality data were log transformed ($x + 1$). The transformed data were subjected to the ANOVA procedure using Statgraphics Plus 5.0 software. Probit analysis [43] was performed to determine the lethal dose (LD_{50}) at 1, 3, 5 and 6 days post-treatment. Graphs were plotted using Excel (2016).

3. RESULTS

3.1. Phytochemical constituents of *Calotropis procera*

The qualitative phytochemical screening revealed the presence of various compounds such as alkaloids, phenolic compounds, anthocyanins, flavonoids, terpenoids, saponins, tannins and Glycosides in the leaf powder from *Calotropis procera* (Table 1).

Table 1: Phytochemical analyses of *Calotropis procera* leaf powder

Statut	Compounds							
	Alkaloids	Phenolics	Anthocyanins	Flavonoids	Saponins	Tannins	Terpenoids	Glycosides
+ Present	++	++	+	+	+	++	+	+

3.2. Insecticidal activity of *Calotropis procera* against *Callosobruchus maculatus* in cowpea protection

3.2.1. Effect of *C. procera* leaf powder on the mortality of *C. maculatus*

The recorded results showed that the mortality rate ranged from 0 to almost 100% respectively for D0<<<D1<<D2<D3<D4 corresponding to 0, 2, 4, 8 and 16 g/kg of cowpea grains respectively (Figure 1). This mortality increased with the increasing content of leaf plant powder used in and according to the days of exposure. At the lowest content D1 (2g/kg), the mortality rate was significant, and when the content and the exposure period were increased; D4 (16g/kg) caused almost complete mortality of *C. maculatus* after days 1, 3, 5 and 6.

Figure 1 below shows the variation in the mortality rate as a function of the different doses on exposure days 1, 3, 5 and 6. It was observed that, among these doses, there was a significant difference between doses D0 and D1; D0 and D2; D3; D4 and also between doses D1 and D2; D3; D4 at $P < 0.05$. However, doses D2; D3; D4 were not significant.

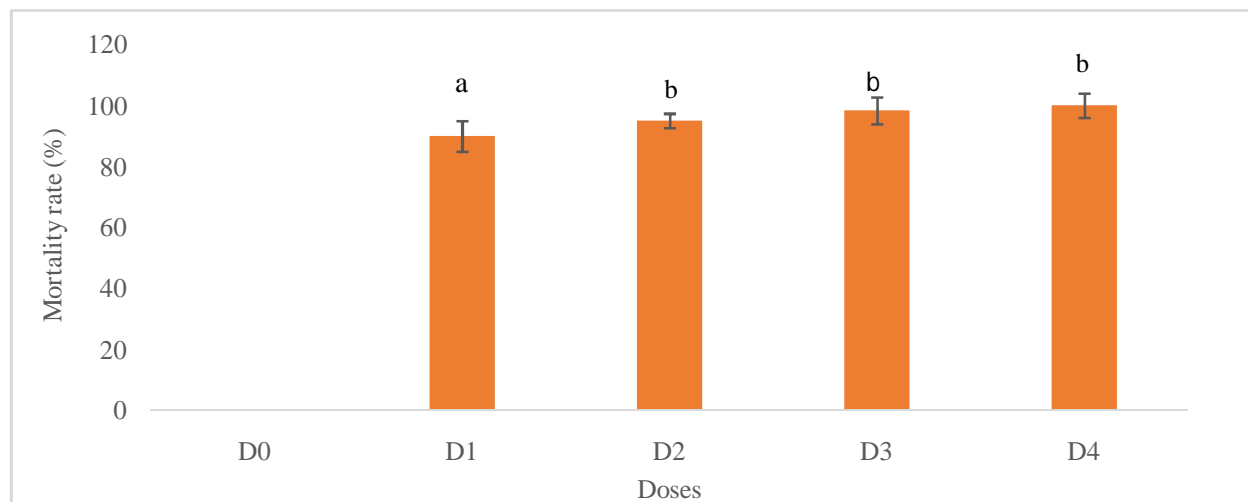


Figure 1: Variations in the mortality rate according to the different doses, expressed as a % relative to the D0 dose, of *Callosobruchus maculatus* during 6 days of treatment. Negative control not treated with leaf powder and containing cowpea and *Callosobruchus maculatus* (D0); cowpea treated with *Calotropis procera* powder extract at 2 g/kg (D1), 4 g/kg (D2), 8 g/kg (D3) and 16 g/kg (D4). Significant differences: ^a $P < 0.05$; ^b $P < 0.01$ compared with the negative control (D0). n = 20 insects/jar.

3.2.2. Relationship between plant product dose and *Callosobruchus maculatus* mortality rate (LD₅₀)

C. procera leaf powder used in the present findings proved to be toxic to adult cowpea bruchids and this toxicity increased with the doses used. The regression line $y = 1.7573x + 5.66$ is used to determine the LD₅₀ dose; the absolute value of X when Y equals 5 corresponds to the desired dose. Calculation of the LD₅₀ gave a value of 0.42g/kg. The correlation coefficient $R^2 = 0.973$ is close to 1, indicating a strong correlation between the two quantitative variables (dose and mortality rate) (Figure 2).

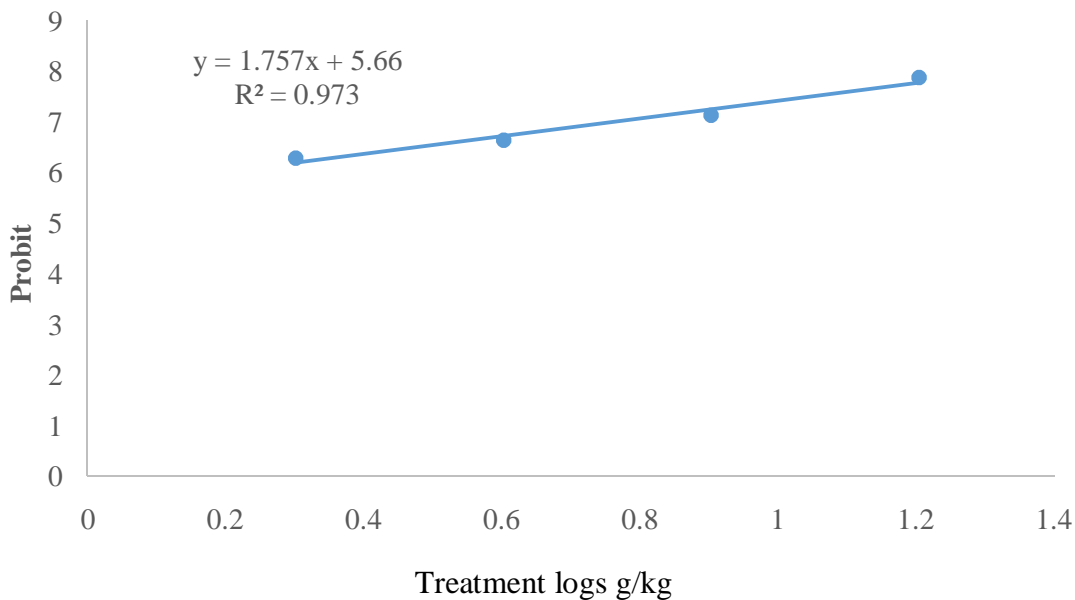


Figure 2: Fit of a regression line of the mortality rate of *Callosobruchus maculatus* adults as a function of the logarithm of the doses submitted to the *Calotropis procera* leaf powder function.

3.3. Emergence of *Callosobruchus maculatus* on cowpea and damage

3.3.1. Effect of plant powder on the reduction of F₁ progeny emergence

Figure 3 shows that there was a significant reduction in F₁ adult emergence at all the treatments used compared to the negative control. This inhibition of emergence is dose-dependent and decreases when the treatment is increased. In terms of adult inhibition, the different treatments ranked as follows: D0 >>D1>>D2>>D3>D4. At D4 (16g/kg) and D3 (8 g/kg), there were a complete inhibition in the F₁ progeny emergence of *C. maculatus*. However, a significant difference ($P < 0.05$) was observed among these contents compare to the negative control. *C. procera* leaf powder at D0 (0 g/kg), D1 (2 g/kg) and D2 (4 g/kg), recorded respectively 77.71, 13.17 and 8.13% of F1 progeny inhibition of *C. maculatus* (Figure 3).

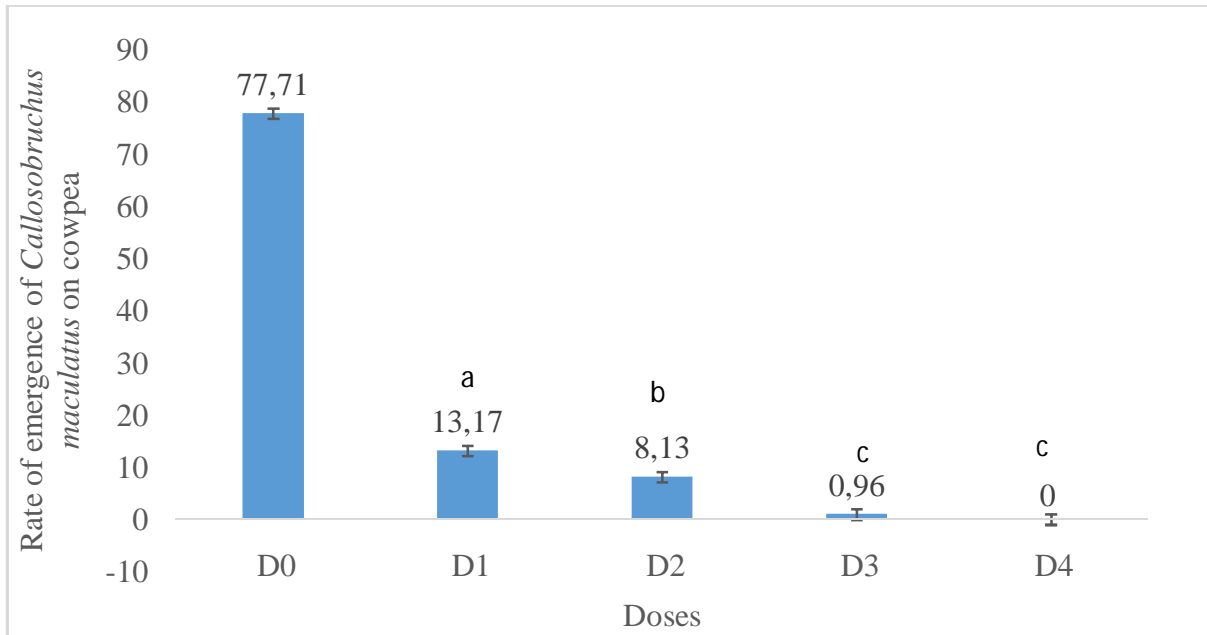


Figure 3: Variation in the rate of emergence of *Callosobruchus maculatus* on cowpea as a function of dose, expressed as a % relative to the D0 dose, during 58 days of treatment. Negative control not treated with leaf powder and containing cowpea and *Callosobruchus maculatus* (D0); cowpea treated with *Calotropis procera* powder extract at 2 g/kg (D1), 4 g/kg (D2), 8 g/kg (D3) and 16 g/kg (D4). Significant differences: ^a $P < 0.05$; ^b $P < 0.01$; ^c $P < 0.001$ compared to the negative control (OD). n = 20 insects/jar.

3.3.2. Effect of *Calotropis. procera* leaf powder on the seed perforation

According to Table 2, the rate of perforated seeds decreased significantly in cowpea seeds treated with *Calotropis. procera* leaf powder when the dose increased. The rate of perforated seeds evolved in proportion to the rate of weight loss and inversely proportional to the rate of non-perforated seeds. The effect of the treatments used was classified according to the

percentage of perforated seeds as follows: D0<D1<D2<D3<D4. However, in terms of weight loss, there was a significant difference ($P < 0.05$) between the negative control, which recorded $94.66 \pm 1.3\%$ of seeds weight loss, and the other powder treatments; there was no significant difference between powder doses D1, D2 and D3 which recorded 11 ± 3.4 , 8.06 ± 0.91 and $1.32 \pm 0.36\%$ of seeds weight loss. At the highest dose of 16 g/kg (D4) there was not seeds damage and no seeds weight loss was recorded in the seeds treated with *C. procera* compare to the negative control (Table 2).

Table 2: Parameters of stored seeds

Parameters	Doses (g/kg)				
	Percentage of perforated seeds – unperforated seeds				
	Weight loss rate (%)				
	D0 (0 g/kg)	D1 (2 g/kg)	D2 (4 g/kg)	D3 (8 g/kg)	D4 (16 g/kg)
Rate of perforated seeds (%)	100 ± 0.00	13.17 ± 1.34^a	8.53 ± 0.97^b	3.87 ± 0.27^c	0.00 ± 0.00^d
Rate of unperforated seeds (%)	0.00 ± 0.00	86.82 ± 1.34^a	91.47 ± 0.96^b	96.12 ± 0.57^c	100.00 ± 0.00^d
Weight loss rate (%)	94.66 ± 1.30	11.00 ± 3.40^a	8.06 ± 0.91^a	1.32 ± 0.36^a	0.00 ± 0.00^b

Variations in the rate of perforated seeds, rate of non-perforated seeds, and rate of weight loss according to the different doses, expressed in % to the D0 dose, of cowpea during 58 days of treatment. Negative control (NC) not treated with leaf powder and containing cowpea and *Callosobruchus maculatus* D0; cowpea treated with *Calotropis procera* powder extract at 2 g/kg (D1), 4 g/kg (D2), 8 g/kg (D3) and 16 g/kg (D4). Significant differences: ^a $P < 0.05$; ^b $P < 0.01$; ^c $P < 0.001$; ^d $P < 0.0001$ compared with the negative control (DO). n =215 seeds/ jar

3.4. Evaluate the post-storage germination capacity of seeds protected by *Calotropis procera* leaf powder after storage.

The percentage of seeds germination of cowpea seeds treated with *C. procera* and infested or not with *C. maculatus* are presented in Table 3. After 2 months of storage, the germination rate of the seeds varied according to whether or not the seeds were infested with bruchids. Non-infested cowpea seeds recorded a higher germination rate (91.66%) than infested seeds (38.33%) when they were treated with the *C. procera* compare to the negative control, which recorded in the infested seeds 0% of seeds germination and in non-infested seeds 80% of germination (Table 3).

Table 3: Effect of *C. procera* leaf powder on cowpea seed viability

Treatments	without insects	with insects	p-value
Control (%)	80 ± 4,71	0^a	0,0017
Powder (%)	91,66 ± 2,36	38,33 ± 2,36^a	0,0019

Viability test for seeds treated with *Calotropis procera* powder as a function of the D4 dose expressed as a % to the negative control (D0), cowpea during 58 days of treatment. Negative control was not treated with leaf powder and containing cowpea with insects and another jar without insects; cowpea treated with *Calotropis procera* powder extract at 16 g/kg (D4). Significant differences: ^a $P < 0.05$; compared with the negative control (D0). n = 30 seeds/jar

4. DISCUSSION

4.1. Insecticidal activity of *Calotropis procera* against *Callosobruchus maculatus* in cowpea.

Generally, in only 6 days of observation, almost complete mortality caused by *C. procera* leaf powder was recorded at the highest content of 16 g/kg. The mortality rate was proportional to the different doses of *C. procera* powder. This suggests that the increasing doses effectively reduces the lifespan of *C. maculatus*. The mortality rate would be due to the chemical compounds contained in the *C. procera* leaf powder. The insecticidal activity would therefore be due to cardenolides, toxic substances, present on almost all parts of the *C. procera* plant [44]. The death of the cowpea bruchids, which increases with the dose of the product, can be explained by the increase in the quantity of the active ingredients according to the contents used [45]. Several other studies have already been carried out on insects associated with insecticidal plants, both in Cameroon and elsewhere. In Cameroon, the work of Saotoing *et al.* [44] on the insecticidal effect of the acetone extract of dried *C. procera* leaves on *Anopheles gambiae* adults revealed 100% mosquito mortality after 24 hours of exposure at concentrations of 59.15 mg and 84.5 mg in the agro-ecological zone known as the western highlands. Goudougou *et al.* [36] showed that *Plectranthus kirbii* leaf powder was toxic at a dose of 16 g/kg and achieved over than 80% mortality of *C. maculatus* adults in 6 days. In Congo, the effect of *Tephrosia vogelii* powder in the preservation of cowpea seeds in stock against *C. maculatus* in Mbuji mayi showed that the longevity of *C. maculatus* adults was inversely proportional to the dose of the powder; 100% mortality in 6 days of observation was recorded for the dose 45 g/kg [46]. The present

results are similar to these authors regarding the high mortality rate within a few days of exposure only to insecticidal plants but differ by the insecticidal plant.

Faraway [47] reports that in biological sciences, when the coefficients of determination R^2 are less than 0.6, the favorable results found are not attributable to the products used. In our case, this assertion confirms the strong relationship between the mortality rate and the plant powder and doses used in the current study ($R^2 = 0.973 > 0.6$).

4.2. Emergence of *Callosobruchus maculatus* on cowpea and damage

The experiment on the emergence of *C. maculatus* in cowpea seeds treated with *C. procera* leaf powder was recorded after 2 months of storage. Therefore, all treatments containing *C. procera* powder significantly ($P < 0.05$) inhibited the bruchid population compared with the negative control. This result could be due to the action of the active compounds present in the powdered leaves from *C. procera* which increased with the increase of contents. The effect of the treatments on the emergence of *C. maculatus* can be explained by the fact that *C. procera* contains alkaloids that block ovarian development and vitellogenesis in females and prevent sexual maturity in males [48]. Other work has already been carried out on the effect of this insecticidal plant. Ben Hassan [49] states that the number of eggs per ootheca of females treated by ingestion with *C. procera* extract was 38 eggs; this reduction in egg-laying can probably be explained by a disruption in the insect's ovogenesis. The same author states that treatment with *C. procera* reduced the number of eggs hatched compared with the control series, whether by contact or ingestion. Ramos *et al.* [50] confirmed that a reduction in fecundity was observed in *C. maculatus* and *Zabrotis subfasciatus* after treatment with *C. procera* latex. According to Salunke *et al.* [51], flavonoids extracted from *C. procera* have an ovocidal action on *C. chinensis* eggs at a dose of 10 mg/ml. Our results corroborate those of these authors about the action of the treatments on the insect, resulting in a long-term reduction in the emergence of *C. maculatus*.

4.3 Evaluating the post-storage germination capacity of seeds protected by leaf powder.

According to these results, a significantly highest germination rate was recorded in uninfested seeds treated with *C. procera* leaf powder, while in these treated and infested seeds there was the lowest percentage of germination (38.33%) recorded after storage. This result obtained in the treated and infested cowpea seeds is because insect attack could alter or even destroy seed vigor and germination capacity. The non-perforated seeds selected from the infested

seeds, even though they had a normal appearance, had a low germination rate. This could be due to the development of larvae that consume the seed reserve. This result is similar to that recorded by Goudoungou *et al.* [36] on *P. kirbii*, which showed that in infested cowpea seeds, the highest germination rate was 37.78% when the cowpea seeds were treated with leaf powder, followed by the aqueous extract with 33.33% of seeds germination. On the other hand, when the variation in climatic conditions in the storage environment is poorly controlled, germination capacity is reduced. This was tested by Couturon (1980) cited by Younoussa [52] with *Coffea canephora* and *C. stenophylla* where less than 50% viability was observed after four months in fluctuating conditions compared with 90% in a controlled atmosphere after fifteen months of storage. During storage, the seeds increase their water content if the enclosure is not controlled. Hence the need for exposure to the sun to maintain an acceptable moisture content for storage [53].

CONCLUSION

The use of plant powder as insecticide could improve the biodegradability of insecticide treatments and therefore reduce the quantity of toxic insecticide remains. In the present study, *C. procera* leaf powder proved its effectiveness against *Callosobruchus maculatus* adults. After 6 days of exposure, the leaves powder from *C. procera* caused complete mortality of cowpea bruchid population its the highest dose (16 g/kg). At the two highest contents (8 and 16 g/kg), the powdered plant used in this study recorded complete inhibition in the progeny emergence of *C. maculatus* after 2 months. Inhibition of the *C. maculatus* population is therefore total with dose 4. The damage and losses caused by *C. maculatus* on cowpea seeds were progressively reduced with the increased of different contents used. Cowpea seeds treated with *C. procera* and uninfested at 16g/kg retained their viability after two months' storage. The results obtained show that *C. procera* leaves have a good insecticidal action against *C. maculatus*. Therefore with a view to promoting sustainable development and protecting the environment, *C. procera* leaf powder could be considered as a suitable insecticide to replace synthetic chemical. In Cameroon, more precisely in the Eastern region where cowpea farming is not strong, and more than half the population lives from storing this legume, *C. procera* leaves could be applied to protect cowpeas from bruchid attacks.

CONFLICT OF INTEREST

Authors have declared that no competing interests exist.

Authors' contributions

This work was carried out in collaboration among all authors. Authors RBB, AN and ENN designed the study and wrote the protocol. Authors MAE made the laboratory bioassays. Authors RBB and CN performed the statistical analysis. Authors MAE, TGF and OBRP managed the literature searches. Author MAE and TGF wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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- 1.
- 2.
- 3.

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