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Research article

Efficacy of *Kigelia africana* Lam. (Benth.) leaf and stem bark ethanolic extracts on adult cowpea seed beetle, [*Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae)] affecting stored cowpea seeds (*Vigna unguiculata*)

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ABSTRACT

Callosobruchus maculatus is the most damaging insect pest of stored cowpea (*Vigna unguiculata*) seeds in Nigeria. Thus, this present research work was put in place to assess the insecticidal activities of the extracts obtained from the leaf and stem bark of *Kigelia africana* (Lam.) Benth against the cowpea seed beetle, *C. maculatus.* The parameters that were assessed were adult mortality, oviposition and adult emergence of *C. maculatus.* The experiments were conducted under laboratory conditions of 28±2 °C temperature and 75 ± 5% relative humidity. The extracts were applied at dosages of 0.3, 0.6, 0.9 and 1.2 mL per 20 g of cowpea seeds. The two extracts of *K. africana* were found to be toxic to the survival of the *C. maculatus.* However, the extract obtained from the leaf was more potent to the beetle than the extract obtained from the stem bark of the same plant. The two extracts also reduced ovipositipon and completely suppressed adult emergence at the highest dose of 1.2 mL per 50 g of cowpea seeds. The results obtained in the present research work showed that the two extracts of *K. africana* were effective in suppressing the population of the infamous stored pest of cowpea seed beetle, *C. maculatus* and could therefore be recommended to replace the harmful synthetic chemical insecticides in protecting cowpea seeds in storage.

1. Introduction

Cowpea, *Vigna unguiculatal* (L.) Walp is an important tropical crop of the family Fabaceae. It is widely grown in sub-saharan Africa (Adedire et al., 2011; Mensah et al., 2017). It is an important edible crop in most nations of the world especially in the tropical and subtropical parts of the world (Ileke et al., 2013; Obembe and Ojo, 2018). It is consumed by human due to its high protein content and also used as feed for livestocks (Fouad et al., 2020). It is regarded as one of the cheapest sources of protein to many families where assess to animal protein and other means of getting protein are difficult (Nta et al., 2013). Cowpea also constitutes the main source of revenue to resource-poor farmers (Musa and Olaniyan, 2015).

One of the major constraints to large scale production of cowpea seeds has been the problem of insect pests (Mailafiya et al., 2014; Adesina and Ofuya, 2015). This has led to serious economic losses both in the field and in storage (Uyi and Igbinoba, 2016). The cowpea seed beetle, *Callosobruchus maculatus* is a field-to-store pest of cowpea (Alabi and Adewole, 2017). Infestation usually commences in the field and continues in the store where the population is being built up (Ojo et al., 2018). *C. maculatus* causes a great deal of losses to stored cowpea seeds which has led to serious problems to peasant farmers all over the world (Obembe and Ojo, 2018). The beetle is known to cause quantitative and qualitative losses manifested by seed perforation, reduction in weight, market value and viability (Iloba et al., 2016; Adarkwah et al., 2018). Infestation enhances a substantial reduction in grain value. Infested cowpea seeds are unattractive to consumers and usually reduce the agricultural and commercial purposes (Adesina and Ofuya, 2015; Iloba et al., 2016). Losses caused by *C. maculatus* to untreated seeds falls between 40 to 100% (Akami et al., 2017; Mensah et al., 2017).

In order to overcome the problem of insect pests damage in the store, different control measures have been developed and used around the

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world. The use of conventional insecticides has been in place for many years (Ojo et al., 2018). These conventional insecticides are associated with many problems, including high persistence, poor knowledge of application by the peasant farmers, pest resurgence, genetic resistance by the insects, ozone layer depletion potential and effects on non-target organisms in addition to environmental pollution and direct toxicity to users (Adedire et al., 2011; Obembe et al., 2020). With all the aforementioned limitations on the use of chemical insecticides, there is a greater need to explore suitable alternative methods of pest control against stored products (Suleiman and Suleiman, 2014; Gbaye et al., 2016). However, plant-derived insecticides which may offer a sustainable, environmentally friendly and safer alternative to synthetic insecticides have been extensively used to control these notorious insect pests (Ojo and Ogunleye, 2013). Tropical countries of the world are well endowed with these plants that possess insecticidal properties (Gbaye and Oyeniyi, 2015). It has also been established that many of these plant materials used in crop protection against insect pests are also safe for human consumption (Omotoso, 2014).

Kigelia africana (Lam.) Benth belongs to the family Bignoniaceae. It is commonly known as sausage or cucumber tree due to the presence of huge fruit that normally hangs from its long fibrous stalk (Inas, 2015; Obafemi et al., 2017). All the different parts of the plant have medicinal properties and most traditional healers used them to treat a wide range of diseases (Obafemi et al., 2017). A decoction of the fruit is used to induce the secretion of breast milk in nursing mothers (Gbadamosi and Okolosi, 2013). The root and stem bark are ground and boiled in clean water to treat diseases such as asthma, dysentery, pneumonia and stomach problems in children (Inas, 2015). Most of the medicinal plants have also been found to possess insecticidal properties (Ileke et al., 2013). For instance, the leaves of *K. africana* were found to be highly effective against the larvae of both the *Anopheles* and *Culex* mosquito (Inas, 2015). Despite the huge volume of research work on the medicinal and insecticidal properties of *K. africana*, little or no research work has been carried out on the insecticidal properties of this plant on stored products insects such as *callosobruchus maculatus* that infest cowpea seeds in storage. This present research was therefore designed to evaluate the effects of *k. africana* extracts on *C. maculatus* infesting cowpea seeds in the store.

2. Materials and methods

2.1. Insect rearing

The naturally infested cowpea seeds containing the seed beetles, *Callosobruchus maculatus* used in this study were bought from Oba market, Ado Ekiti, Ekiti State, Nigeria. The infested seeds were put into plastic containers and covered with muslin cloth and held tightly with rubber and then taken to the laboratory. The beetles were allowed to acclimatize to the laboratory conditions for four days before they were used for the research work. After the period of acclimatization, about 750 g of clean uninfested seeds were weighed into 2 L kilner jar. Then, twenty copulating pairs (20 males: 20 females) of *C. maculatus* were put inside the kilner jar containing the disinfested seeds (*Vigna unguiculata*). The females' *C. maculatus* have strong black markings on their elytra while males are less distinctly marked (Odeyemi and Daramola, 2000). In terms of size, females are larger than males (Lale, 2002). The kilner jar was then covered with muslin cloth and held tightly with rubber bands to allow aeration and disallow the beetles from escaping from the jar. The culture was then put inside the wooden cage to allow the beetles to lay eggs and multiply. The first filia (F_1) generations of the insects were then reared on another clean uninfested cowpea seeds in the laboratory and they served as the stock culture of the insects used for the whole experiments.

2.2. Collection of clean cowpea seeds

The clean uninfested cowpea seeds, Ife brown variety used for this study was obtained from the office of Agricultural Development Program (ADP) in Ikole Ekiti, Ekiti State, Nigeria. The seeds were first of all sterilized by putting it in a deep freezer maintained at 0 °C for 3 days to ensure that any existing insect eggs and larvae are killed. The disinfested seeds were later air dried in the laboratory for 3 days in order to prevent mouldiness (Adedire and Ajayi, 1996).

2.3. Preparation of the ethanolic extracts of Kigelia africana

Fresh leaves and stem bark of *K. africana* were obtained from the premises of Ekiti Anglican Diocesan High School, Ado Ekiti, Ekiti State, Nigeria. The leaves and stem barks were first of all washed in a clean water in order to remove the impurities. They were later air-dried for one (1) month in a clean place in the laboratory where the plant materials will not be contaminated with impurities. The plant materials were then packed and put inside Okapi grinder for pulverization. The powders obtained were later sieved using a sieve of 1 mm^2 mesh. This is done in order to obtain a fine powder. Thereafter, 200 g of the pulverized plant powders were weighed with an electronic weighing balance (Model JTC 2101N) (R) and packed into thimbles using muslin cloth separately. Then, 500 mL of the solvent (ethanol) was measured and poured in the Soxhlet apparatus set at 60 °C. The extracts were concentrated by removing the solvent using rotary evaporator. The oil extracts were stored in air-tight specimen bottles and kept in the refrigeration until ready for use.

2.4. Effect of K. africana ethanolic extracts of the leaf and stem bark on adult C. maculatus mortality

Exactly 0.3, 0.6, 0.9 and 1.2mL of the ethanolic extracts of the leaf of *K. africana* were thoroughly mixed with 20 g of clean and wholesome cowpea seeds in Petri-dishes. The seeds were air-dried in the laboratory for 1 h, after which twenty (20) newly emerged (about 2 days old) adults of *C. maculatus* were put into each Petri-dish containing the treated seeds. A control experiment which contained untreated seeds was also included in the set up. Each treatment including the control was replicated four times. The same procedure was used in determining the contact toxicity of the ethanolic extract from *K. africana* stem barks. The experiment was carried out at ambient temperature of 28±2 °C and relative humidity of

Table 1. Effects *K. africana* leaf ethanolic extracts on adult *C. maculatus* mortality.

Dosage (mL)	Percentage of mortality at hours post- treatment			
	24	48	72	96
Untreated	0.00 ± 0.00^e	0.00 ± 0.00^d	1.50 ± 1.22^d	3.10 ± 1.12^d
0.3	30.00 ± 3.15^d	50.20 ± 2.34^c	54.25 ± 3.29^c	56.10 ± 3.14^c
0.6	40.25 ± 1.14^c	52.25 ± 3.28^b	70.20 ± 2.22^b	90.10 ± 2.12^b
0.9	48.24 ± 1.34^b	55.25 ± 2.88^b	75.15 ± 1.73^b	100.00 ± 0.00^a
1.2	52.30 ± 2.53^a	78.25 ± 2.32^a	100.00 ± 1.15^a	100.00 ± 0.00^a

Each value is a mean ± standard error of four replicates. Means within the same column follow by the same letter(s) are not significantly different (p > 0.05) using Tukey's test.

75 ± 5%. The experiment was conducted in a Complete Randomization Design (CRD). Beetle mortality was observed daily for 4 days at 24 h interval. Dead beetles were counted and recorded after every 24 h. The insects were confirmed dead by not responding to probing with sharp needle on their abdomen. At the end of 96 h of exposure, all data on percentage mortality was corrected using the formula (Abott, 1925): thus:

$$P_T = \frac{P_O - P_C}{100 - P_O} \times \frac{100}{1}$$

where P_T = corrected mortality (%)

P_O = observed mortality (%)
P_C = control mortality (%)

2.5. Effect of K. africana ethanolic extracts on oviposition and adult emergence of C. maculatus

Exactly 50 g of clean and wholesome seeds were measured separately into 100 mL volume conical flasks. Different dosages of 0.5, 1.0, 1.5 and 2.0 mL of ethanolic extracts of the leaf of *K. africana* were measured with the aid of pipette and added to the clean cowpea seeds in each of the conical flasks. The extracts and the seeds were thoroughly mixed together with the aid of a glass rod to enhance uniform coating of the extracts on the seeds. Untreated bean seed was also set up to serve as the control experiment. Two copulating pairs (2 males: 2 females) of newly emerged (1 day old) adult *C. maculatus* were put into each conical flask, which were covered with muslin cloth fixed with rubber band. This is to allow free flow of air and prevent the escape of insects from the conical flasks. Four replicates were prepared for both the treated and untreated control were conducted in Complete Randomized Design (CRD). This same method was used to determine the effects of the ethanolic extracts of the stem bark of *K. africana* on oviposition and adult emergence of *C. maculatus* using the same concentrations. The experiment was carried out at ambient temperature of 28±2 °C and relative humidity of 75 ± 5%. The set up was left in the laboratory for 12 days after which both dead and live insects were removed and the numbers of eggs laid were counted and recorded. The whole set up was kept undisturbed in the wooden cage for 30 days and examine daily until adults emergence. Emerged adults were counted and recorded on a daily basis until there was no more adult emergence. The percentage of adult emergence was calculated using the method of Odeyemi and Daramola (2000).

$$\%\ \text{Adult emergence} = \frac{\text{Total number of adult emergence}}{\text{Total number of eggs laid}} \times \frac{100}{1}$$

2.6. Data analysis

All data obtained in this study except the number of eggs laid were converted to percentages. The data were then transformed using arcsin transformation for the percentage values and square root transformation for number of eggs laid values. The data were later subjected to one-way analysis of variance (ANOVA) and means were separated using Tukey's test. Statistical package for social sciences (SPSS) software version 22 was used for the data analysis.

3. Results

3.1. Effect of K. africana leaf ethanolic extracts on adult C. maculatus mortality

Beetle mortality in extract treated seeds differed significantly ($p < 0.05$) from beetle mortality in the untreated seeds as indicated in Table 1. Mortality increased as the exposure time of insects to the extracts was prolonged. None of the dosages was able to achieve 100% insect mortality within 24 h of exposure. However, the highest mortality of 52.30% was recorded with cowpea seeds treated with 1.2 ml dosage of the extract after 24 h post-treatment and this was significantly different ($P < 0.05$) from the control and other treatment levels. The effects of extracts applied at 0.6 ml and 0.9 ml were not significantly different ($P > 0.05$) from each other after 48 and 72 h of exposure respectively. Cowpea seeds treated with the extracts at the dosage rates of 0.9 and 1.2 ml evoked 100% beetle mortality after 96 h of exposure. This level of effectiveness was followed by extract applied at 0. 6 ml where 90.10 % beetle mortality was achieved within the same period.

3.2. Effect of K. africana stem bark ethanolic extracts on adult C. maculatus mortality

Mortality increased with the increase in insect exposition time to extracts. The highest mortality of 100 % was recorded on exposure of *C. maculatus* to 1.2 ml of the stem bark extract by 96 h, this was followed by 78.10 %, 75.25 %, and 55.14 % with 0.9 ml, 0.6 ml, and 0.3 ml extracts dosages respectively (Table 2). There are significant differences ($p < 0.05$) in insect mortality in treated and untreated cowpea seeds.

3.3. Effect of K. africana leaf ethanolic extracts on oviposition and adult emergence of C. maculatus

The ethanolic extracts of the leaf of *K. africana* effectively reduced oviposition by the weevils (Table 3). Oviposition by the female beetles and the emerged adults decreased with the increase in the extract dosages applied. There was a significant reduction ($P < 0.05$) in the number of eggs laid on the treated seeds when compared with the control. Seeds treated with 2.0 mL extract dosage showed no sign of oviposition and hence no adult emerged. Also, no adult emerged in seeds treated with 1.5 mL leaf extract. The highest percentage adult emergence of 90% was recorded in the control experiment.

3.4. Effect of K. africana stem bark ethanolic extracts on oviposition and adult emergence of C. maculatus

The results obtained on oviposition and adult emergence on seeds treated with the ethanolic extracts of the stem bark of *K. Africana* shows

Table 2. Effects of *K. africana* stem bark ethanolic extracts on adult *C. maculatus* mortality.

Dosage (mL)	Percentage of mortality at hours post- treatment			
	24	48	72	96
Untreated	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	1.50 ± 1.22^{e}	3.12 ± 1.22^{e}
0.3	28.20 ± 3.05^{d}	$30.22 \pm 2.3.34^{d}$	46.14 ± 2.35^{d}	55.14 ± 2.24^{d}
0.6	35.25 ± 1.23^{c}	55.20 ± 3.18^{c}	66.22 ± 2.22^{c}	75.25 ± 3.12^{c}
0.9	42.40 ± 1.14^{b}	50.20 ± 4.33^{b}	62.15 ± 1.71^{b}	78.10 ± 2.15^{b}
1.2	48.20 ± 2.13^{a}	62.15 ± 4.35^{a}	88.25 ± 1.25^{a}	100.00 ± 0.00^{a}

Each value is a mean ± standard error of four replicates. Means within the same column follow by the same letter(s) are not significantly different ($p > 0.05$) using Tukey's test.

Table 3. Effects of *K. africana* leaf ethanolic extract on oviposition and adult emergence of *C. maculatus*.

Treatment (mL)	Number of eggs laid	Percentage adult emergence
Untreated	30.10 ± 1.66[a]	90.00 ± 1.35[a]
0.5	12.15 ± 1.40[b]	15.10 ± 3.15[b]
1.0	10.20 ± 2.13[c]	10.00 ± 1.10[c]
1.5	4.10 ± 0.81[d]	0.00 ± 0.00[d]
2.0	0.00 ± 0.00[e]	0.00 ± 0.00[d]

Each value is a mean ± standard error of four replicates. Means within the same column follow by the same letter(s) are not significantly different (p˃0.05) using Tukey test.

that oviposition and adult emergence decreased with increased in dosages of extracts applied (Table 4). The highest number of eggs (32) was laid on untreated seeds while the lowest number of eggs was laid on seeds treated with the highest dose of 2.0 mL used in this experiment. The highest percentage adult emergence of 92.10 was observed on untreated seeds while there was no adult emerged in seeds treated with 2.0 mL extract.

4. Discussion

Peasant farmers still rely on chemical insecticides despite the numerous problems associated with their usage (Alabi and Adewole, 2017; Ojo et al., 2020). These problems range from environmental pollution and toxicity on the users and the non-targeted useful organisms (Mgbemena et al., 2016). The most widely used chemical fumigant for infested legume such as cowpea in Nigeria is aluminum phosphide. The fumigation tablet of aluminium phosphide emits toxic phosphine gas (PH_3) in contact with moist atmosphere under hermetic storage condition (Gbaye et al., 2016). The sales and use of adulterated and expired pesticides are part of the problems militating against the use of chemical pesticides in Nigeria (Ogunwolu et al., 2001). Even after the purchase of the conventional insecticide, the illiterate and poor resource farmers find it very difficult to read and interpret the labels, therefore resulting in the wrong or excessive application of insecticides (Gbaye et al., 2016; Mensah et al., 2017).

Insecticides derived from plants have been discovered to serve as alternatives to the widely used conventional insecticides as many of them have been used in the management of a great number of species of stored product in the past, including Coleoptera and Lepidoptera (Nathan et al., 2007). Results from the present study, using extracts of *K. africana*, clearly revealed that there is a great potential in using plant extracts to reduce infestation by *C. maculatus*.

The result from this research work showed that the leaf extracts of *K. africana* invoked high percentage of beetle mortality and significant reduction in the fertility and percentage of adult emergence of *C. maculatus* when compared to those of untreated seeds. The result obtained in this research work is in agreement with the findings of Inas (2015) who reported that the aqueous extract of *K. africana* leaf evoked 100% mortality of the 3rd and early 4th instars of Anopheles and *Culex* larvae. Also, Poonia and Kaushik (2013) reported the larvicidal activities of the leaf of *K. africana* extracts against the 3rd and 4th instar larvae of the yellow fever mosquito, *Aedes aegypti*. The high percentage of beetle mortality observed in this present study could be connected with contact toxicity. Most insects respire by means of trachea which usually opens at the outer surface by structures called spiracles (Ajayi, 2012). The plant extracts used to treat the cowpea seeds might have adverse effects on these spiracles by blocking them thereby disturbing the free flow of air into these air chambers or spiracles and thereby leading to suffocation and death of the beetles as suggested by Adedire et al. (2011). The effectiveness of extracts of *K. africana* in controlling insect pests may also be connected with the presence of some phytochemicals such as glycoside saponin, alkaloid and flavonoid Abdulkadir et al. (2015), which have ovicidal, larvicidal, toxic and deterrent effects on coleopteran pests of stored products. The plant products can conveniently be recommended to peasant farmers to act as alternative to harmful synthetic insecticides in the control of *C. maculatus* in stored seeds. Even though the extracts obtained from stem bark had less negative effect on the bruchid when compared to the leaf extracts, it also caused high mortality and showed significant reduction in oviposition and hence the percentage of adults that emerged was drastically reduced when compared with the control experiment. The fact that plant extracts induce reduction in oviposition by the female bruchid and mortality of individuals along developmental stages had been reported by numerous authors (Boekel et al., 2001). The ability of extracts in reducing oviposition could be linked with high insect mortality of the insects especially the females which are involved in the egg laying. The observed reduction in oviposition could also be linked with the fact that the insects found it difficult to breathe due to blockage of their spiracles and which also affected their general metabolic activities, including sexual activities such as sexual communication and mating as suggested by Ileke et al. (2014). The complete prevention of adult emergence observed on cowpea seeds treated with *K. Africana* leaf at the highest dosage of 2.0 mL could be due to high insect mortality, even though the other dosages of the extracts could not prevent the insects from laying eggs but many of the eggs that were laid could not develop into adults. Also, the plant extracts may have compromised the eggs adherence to the seeds surfaces as a result of extracts coating the cowpea seeds thereby leading to egg mortality as suggested by Ileke (2014). Ileke and Olotuah (2012) observed that plant extracts have the ability of passing through the chorion of eggs thereby preventing further development.

The findings in this experiment agree with the result of Mofunanya and Nta (2016) who recorded high adult mortality, reduction in the

Table 4. Effects of *K. africana* stem bark extracts on oviposition and adult emergence of *C. maculatus*.

Treatment (mL)	Number of eggs laid	Percentage adult emergence
Untreated	32.20 ± 1.10[a]	92.10 ± 2.35[a]
0.5	28.15 ± 1.20[b]	18.30 ± 2.45[b]
1.0	22.30 ± 2.22[c]	13.45 ± 2.15[c]
1.5	15.30 ± 0.15[d]	10 .10 ± 0.20[d]
2.0	12.20 ± 2.20[e]	0.00 ± 0.00[e]

Each value is a mean ± standard error of four replicates. Means within the same column follow by the same letter(s) are not significantly different (p˃0.05) using Tukey test.

number of eggs laid and the number of emerged adults of *C. maculatus* when exposed to six indigenous plants extracts. The results obtained from this research work clearly revealed the potency of ethanolic extracts of the leaf and stem bark of *K. africana* as a control agent for *C. maculatus* infesting stored cowpea seeds and will serve as alternative to the chemical insecticides which are costly and cause adverse effects on the environment.

5. Conclusion

The results of the present investigation shows that the plant derived insecticides are very effective in protecting stored seeds from damage by *C. maculatus.* The ethanolic extracts obtained from the leaf and stem bark of *K. africana* were found to be very potent against the infamous cowpea beetle at all the concentrations tested.

Declarations

Author contribution statement

Olusola Michael Obembe: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Durojaye Olanrewaju Ojo, Kayode David Ileke: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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