EVALUATION OF *Cassia sieberiana* (DC) AND *Vernonia amygdalina* (Del.) AGAINST *Callosobruchus maculatus* (F.) INFESTING STORED BAMBARA GROUNDNUT *Vigna subterranea* (L.) Verdc.)[1]

[EVALUACIÓN DE *Cassia sieberiana* (DC) Y *Vernonia amygdalina* (Del.) CONTRA *Callosobruchus maculatus* (F.) INFESTANDO MANI BAMBARA (*Vigna subterranea* (L.) Verdc.) ALMACENADO]

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SUMMARY

The insecticidal activity of *Cassia sieberiana* (DC) and *Vernonia amygdalina* (Del.) aqueous extract against *Callosobruchus maculatus* (F.) in treated bambara groundnut was evaluated under laboratory conditions (25 – 29°C and 56 – 70% r.h.). Seeds were treated separately at four concentrations (2, 4, 6 and 8 ml/100g of seeds) of both plants aqueous extracts. Pirimiphos methyl applied at 8 mg a.i./kg was included as positive control. Data collected were adult mortality, number of adult progeny (F_1 and F_2), percentage seed damage, percentage weight loss and germination capacity. Results showed that increasing concentration of both plant extracts and period of exposure to treated seeds significantly (P<0.05) increased adult mortality levels from 16.9±2.9 to 100%. Furthermore, both plant extracts applied at 8 ml/100 g of seeds were comparable to pirimiphos-methyl after 96 h of exposure. The plant extracts significantly (P<0.05) reduced number of adult progeny, percentage seed damage and percentage weight loss, which all progressively declined with increasing concentration from 2 to 8 ml. Both plant aqueous extracts had no effect on germination capacity of treated seeds. For all the parameters measured, the plant extracts tested showed significantly higher biological activity in treated seeds compared to the untreated control. The present results suggest that these plant aqueous extracts have the potential to control *C. maculatus* populations in stored bambara groundnut.

Keywords: *Callosobruchus maculatus*; stored bambara groundnut; *Cassia sieberiana*; *Vernonia amygdalina*; insect control

RESUMEN

Se evaluó la actividad insecticida de *Cassia sieberiana* (DC) y *Vernonia amygdalina* (Del.) contra *Callosobruchus maculatus* (F.) en el maní bambara tratado en condiciones de laboratorio (25 - 29° C y 56-70% H.R.). Las semillas se trataron por separado a cuatro concentraciones (2, 4, 6 y 8 ml/100 g de semillas) de ambos extractos acuosos de plantas. Pirimifos metilo aplicado a 8 mg/kg se incluyó como control positivo. Los datos recogidos fueron la mortalidad de adultos, el número de progenies adultas (F1 y F2), el porcentaje de daño de las semillas, el porcentaje de pérdida de peso y la capacidad de germinación. Los resultados mostraron que el aumento de la concentración de ambos extractos de plantas y el período de exposición a las semillas tratadas aumentó (P <0.05) los niveles de mortalidad de adultos de 16.9 ± 2.9 a 100%. Además, ambos extractos de plantas aplicados a 8 ml/100 g de semillas eran comparables a pirimifos-metilo después de 96 h de exposición. Los extractos de planta redujeron (P <0.05) el número de progenies adultas, el porcentaje de daño a las semillas y el porcentaje de pérdida de peso, que disminuyó progresivamente con una concentración creciente de 2 a 8 ml. Ambos extractos acuosos de plantas no tuvieron efecto sobre la capacidad de germinación de las semillas tratadas. Para todos los parámetros medidos, los extractos de plantas probados mostraron actividad biológica significativamente mayor en semillas tratadas en comparación con el control no tratado. Los presentes resultados sugieren que estos extractos acuosos de plantas tienen el potencial de controlar las poblaciones de *C. maculatus* en el maní bambara almacenado.

Palabras clave: *Callosobruchus maculatus*; Bambara almacenado; *Cassia sieberiana*; *Vernonia amygdalina*; Control de insectos

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INTRODUCTION

Bambara groundnut [*Vigna subterranea* (L.) Verdc.] is the third most important pulse legumes in Africa, after groundnut and cowpea. It has beneficial nutritional effects and serves as a low cost protein in most African diet. Nutritionally, the bambara groundnut is a valuable food, containing 54.5 - 69.3% carbohydrates, 17 - 24.6% protein and 5.3 -7.8% fat. It is also a good source of fiber, calcium, iron and potassium (Hillocks *et al*., 2012).

Like cowpea, bambara groundnut is susceptible to infestation by bruchids which are field-to-store pests (Ajayi and Lale, 2001). Bambara groundnut is often infested simultaneously by *Callosobruchus subinnotatus* (Pic.) and *C. maculatus* (Mbata, 1993). *Callosobruchus maculatus* is more destructive because of its shorter life cycle and higher intrinsic rate of increase. *Callosobruchus* is one of the genera in the subfamily Bruchinae (seed beetles) that is in the family Chrysomeloidae. This group is part of the order of beetles, Coleoptera. They are agricultural pest, insects of Africa and Asia that presently range throughout the tropical and subtropical world (Kergoat *et al.*, 2007).

These insects infest seeds in the field and continue to multiply and cause serious damage during storage (Boureima *et al*., 2015). Grain damage and losses (both quantitative and qualitative) are usually caused by larvae which bore inside grains, feeding on cotyledons, consuming the seed and rendering it unsalable or inconsumable (Haines, 1991; Allotey and Oyewo, 1993). The level of bambara groundnut losses due to bruchids in West Africa is not well documented and figures available are inconsistent. For instance, Amuti and Larbi (1981) recorded an average loss of 3.7% after 5 months of storage in Ghanaian local conditions. According to Golob *et al*. (1996), average seed losses of about 10% per month are common. In North Western Benin, bambara groundnut was the most attacked and damaged crop by insects, with half of the harvest lost in 2011 (Rochat and Guenat, 2013). In Niger, Baoua *et al*. (2015) reported losses of 61.8±2.3% due to *C. maculatus* and *C. subinnotatus* after 7 months of storage without treatment. In addition, in the same country, Boureima *et al.* (2015) reported that loss rates up to 100% were recorded in some cases under traditional storage methods.

Although synthetic insecticides have been very effective in suppressing insect populations in stored commodities, their use is on the declined due to increasing serious problems of insects’ genetic resistance to insecticides, toxic residues in food and contamination of the biosphere (Osipitan *et al*., 2010; Adeniyi *et al.*, 2010). Another reason is the withdrawal of subsidy on pesticides by governments in developing countries (Lale and Yusuf, 2001). Consequently, in many developing countries research focus has shifted to evaluation and application of plant-derived insecticides for the protection of stored grains. Accordingly, botanical insecticides have long been recommended as alternatives to synthetic insecticides. The reasons are: they cause little risk to human health, the cost of production is low, and processing and use by farmers and by small enterprises is easy (Klys and Przystupinska, 2015). Furthermore, they can be easily cultivated and are generally used as powders, extracts or oils (Oliveira *et al*., 1999). More than 100,000 secondary metabolites with insecticidal properties have already been identified, such as alkaloids, terpenoids, flavonoids and quinones, in approximately 200,000 species of plants worldwide (Potenza et al., 2004). Hence, they have potential to play an important role in the production and post-harvest protection of food grains, which are seriously damaged by pests (Boeke *et al.*, 2004; Tooba *et al.*, 2005).

Many reports show the efficacy of locally available plants against various insect pest of stored-products. For example, the use of species of the genus *Cassia*, such as C. *nigricans* (Vahl.) C. *occidentalis* (L.) or C. *siamea* (Lam.) as protectants of stored legumes has been reported by Kestenholz *et al.* (2007). *Cassia sophera* (L.) is traditionally used by subsistence farmers in northern Ghana to protect stored cowpea (*Vigna unguiculata* (L.) Walp.) and bambara groundnuts (*V. subterranea*). Similarly, *C. sieberiana* is listed among the plants traditionally used to protect stored cowpea in Senegal (Toumnou *et al*., 2005). However, the authors did not find reports of systematic studies on its insecticidal value. Powders from the dried leaves of *Vernonia amygdalina* were also found to have insecticidal potency against the larvae of *C. maculatus* and *Sitophilus zeamais* (L.), insects that cause heavy losses of stored cow pea and maize, respectively (Kabeh and Jalingo, 2007). Asawalam and Hassanali (2006) reported that the essential oil of *V. amygdalina* was effective in the control of *S. zeamais.* There are no reports, however, on the efficacy of aqueous extracts of *C. sieberiana* and. *V. amygdalina* against *C. maculatus* on bambara groundnut. This study was, therefore undertaken to evaluate the insecticidal activity of these two botanical species towards *C. maculatus* in treated bamabara groundnut seeds.

MATERIALS AND METHODS

Source of the Insects

Callosobruchus maculatus adults were collected from cultures maintained on cowpea in the laboratory and were used to establish new cultures on bambara groundnut. Glass jars and bambara groundnuts seeds were sterilized at 60ºC for one hour (Murdock and Shade, 1991). The insects were then reared for two generations on bambara groundnut (cv. Bulmonu) before being used in the experiments. Culturing entailed placing 100 unsexed adults and introduced into 500 g of sterilized bambara groundnuts seeds placed in 1-liter capacity jar for five days and then sieved out and discarded.

Source of the Bambara Groundnut Seeds

A local cultivar of bambara groundnut 'Bulmono' was used in the study. Seeds were obtained from farmers' 2015 harvest in Damboa local Government Area, Borno State- Nigeria. It is the dominant variety grown in the major bambara groundnut producing area. Seeds were stored in a refrigerator for about 8 weeks prior to beginning the experiments.

Plant extracts formulation

Fresh leaves of *C. sieberiana* were collected from the University of Maiduguri, while that of V. *amygdalina* were purchased from Gamboru Market in Maiduguri. Each plant material was washed and dried under shade in the laboratory and ground to a fine powder. The powders were further sieved to pass through 1 mm^2 perforation. The aqueous extracts were obtained with the methodology of Prates *et al*. (2003) by placing 10 g leaf powder in a jar with 100 ml distilled water at boiling point, covering, filtering after 24 h through Whatman N°10 filter paper, and discarding the solid part. The filtrate was collected in a 200 ml capacity conical flask and later used for grain treatment. A synthetic commercial insecticide, Pirimiphos methyl (Actellic 25EC®, 25% Emulsifiable Concentrate) was included in the trials as a positive control.

Experimental Procedure and Data Collection

Bioassay was conducted under ambient laboratory conditions (25 – 29°C and 56 – 70% r.h.). Bambara groundnut seeds were treated at 2, 4, 6, 8 ml/100 g of seed of each plant extract. For each concentration, an appropriate amount of distilled water was added, so that each experimental unit receives 10 ml. The untreated control received distilled water only. Pirimiphos methyl was also diluted in distilled water and applied at the recommended concentration of 8 mg active ingredient (a.i) per kg of seed (Groot, 2004). This served as the positive control. The treated grains were then spread on 19 cm Petri dishes to dry off and then placed in 150 ml capacity bottles. Into each bottle 30 mixed-sex adult *C. maculatus* aged 1 – 24 h were added and capped with perforated lids lined with filter paper. Each treatment was replicated three times. Adult mortalities were measured after 24, 48, 72 and 96 h of exposure. Afterwards all life and dead adults were discarded and the seeds were returned to their respective bottles and kept on a laboratory shelf. Assessment of F_1 and F_2 progeny was conducted after additional 31 and 66 days, respectively, being the approximate time for completion of one and two generations under the experimental conditions. Progeny suppression in a treatment was calculated using the formula: (1 – no. of progeny in a treatment/no. of progeny in control) x100; % (Arthur and Throne, 2003).

Damage assessment was carried out by separating the seeds into damaged (grains with characteristic hole) and undamaged. The percentage of damaged seeds was calculated as: % Seeds damaged = Number of damaged grains /Total number of grains x 100.

Percentage weight loss was calculated, using FAO (1985) method as follows:

% Weight loss = [UaN-(U+D)] / UaN X 100

Where: U = weight of undamaged fraction in the sample; N = total number of grains in the sample; Ua = average weight of one undamaged grain; and, D =weight of damaged fraction in the sample.

Germination test was conducted after assessment of damage and weight loss according to the method of Oparaeke and Bunmi (2006). From each experimental unit 20 randomly selected seeds were placed in a 9 cm diameter petri-dish lined with Whatman filter paper moistened with distilled water. Seven days later, the number of germinated seeds was counted and expressed as percentage germination.

Data Analysis

Where necessary corrections for control mortality were made using the Abbott's formula (Abbott, 1925). For the purpose of normalizing variances data on adult mortality, percentage seed damage, percentage weight loss and germination were arcsine transformed, while those relating to number of progeny were square root ($\sqrt{(x+0.5)}$ transformed. These were then subjected to ANOVA. Significant difference among means were separated using Bonferroni test at α = 5% level of probability using statistical software package (Statistix 8.0).

RESULTS

The effect of plant aqueous plants extracts on mortality of *C. maculatus* in treated bambara groundnut is shown in Table 1. Results indicate that all treatments significantly (P<0.05) reduced the survival of *C. maculatus* adults compared to the untreated control. The insecticidal effect of plant extracts increased with increasing concentration of extract and exposure interval. After 24 and 48 h of exposure, the highest mortality level was caused by pirimiphos-methyl, although the values were not significantly different from those recorded for 6 and 8 ml of plant extracts. Mortality further increased over time, so that 70 to 100% mortality were recorded in treated seeds after 72 h. After the 96 h exposure very few adults survived in treated seeds and even the lowest dose of plant extract caused >93% mortality.

The highest number of progeny was recorded in the untreated control and the number almost doubled in the F_2. In the treated seeds, the number of F_1 or F_2 progeny never exceed 24 weevils. All treatments (the tested plant extracts and actellic) applied at all rates significantly reduced progeny numbers relative to the untreated control, despite the significant (P<0.05) differences among dose levels. As shown in Table 2, increasing the concentration of the tested plant extracts reduced the emergence of *C. maculatus* adults even more. Pirimiphos-methyl was the most effective treatment, completely inhibiting progeny emergence. However, progeny production could not be entirely suppressed by application of plant extract. Generally, plant extracts applied at 4 - 8 ml resulted in about 48 – 90% F_1 progeny suppression, whereas the corresponding values in the F_2 were 71 – 93%. (Table 2).

Table 1. Mean mortality (%±SE) of *C. maculatus* adults exposed to bambara groundnut seeds treated with different doses of *C. sieberiana* and *V. amygdalina* aqueous extracts

Treatment	Dose of extract (ml/100 g of seed)	Exposure period (h) 24	48	72	96
Untreated control	0.0	2.0±1.0c	5.2±1.1e	6.2±0.2d	9.2±0.2c
C. sieberiana	2.0	17.0±4.1b	40.8±3.5d	71.6±4.1c	98.8±1.2ab
	4.0	18.1±2.0b	60.6±4.1cd	81.0±3.1bc	100±0.0a
	6.0	63.6±4.1a	93.3±6.3ab	98.8±1.2a	100±0.0a
	8.0	73.8±6.3a	98.8±1.1a	100±0.0a	100±0.0a
V. amygdalina	2.0	16.9±2.9b	38.3±5.2d	73.8±8.4c	93.7±2.6b
	4.0	16.1±4.1b	43.1±5.1d	70±3.2c	94.5±3.3ab
	6.0	54.4±6.0a	77.9±5.3bc	94.0±4.3ab	98.7±1.2ab
	8.0	73.8±3.0a	96.5±0.0ab	96.4±0.0ab	99.9±0.1a
Pirimiphos-methyl	PC	76.1±3.8a	100±0.0a	100±0.0a	100±0.0a

Means within a column followed by same letter are significantly not different from each other, Bonferroni Test at (P≤0.05). PC - positive control (pirimiphos methyl at 8 mg a.i./kg of seed).

Table 2. Effect of plant extracts on progeny production of *C. maculatus* in treated bamabara groundnut seeds

Treatment	Dose of extract (ml/100 g)	No. of emerged F_1 progeny (mean +SE)	Progeny reduction (%)	No. of emerged F_2 progeny (mean±SE)	Progeny reduction (%)
Untreated control	0.0	45.7±4.3a	-	84.7±6.2a	-
C. sieberiana	2.0	23.0±0.6b	47.9	23.0±1.7bc	72.9
	4.0	21.0±0.9bc	54.1	22.0±1.5b-d	71.1
	6.0	15.3±0.9c	66.5	15.0±2.7de	82.3
	8.0	8.7±0.3d	90.0	6.3±3.3f	92.6
V. amygdalina	2.0	26.0±1.6b	43.1	27.3±0.9b	67.8
	4.0	21.3±1.0bc	53.4	21.7±1.5b-d	74.4
	6.0	15.3±1.0c	66.5	18.3±1.2c-e	78.4
	8.0	10.0±0.6d	78.1	12.0±0.6e	85.8
Pirimiphos-methyl	PC	0.0±0.0e	100±0.0	0.0±0.0g	100

Means within a column followed by same letter are significantly not different from each other, Bonferroni Test at (P≤0.05). PC - positive control (pirimiphos methyl at 8 mg a.i./kg of seed).

Differences in seed damage were significant (P<0.05) among treatments. Seed damage was very high (>90%) in the untreated control and declined to 36.2 and 33.1% in seed treated with 8 ml of *C. sieberiana and V.*

amygdalina, respectively. No damage was recorded in seeds treated with pirimiphos-methyl. Accordingly, weight loss was also highest in the untreated control but declined with increasing concentration of plant extracts. Weight loss of 4.7 – 8.8 % could not be avoided event at the highest concentration of *C. sieberiana and V. amygdalina* (Table 3).

Germination capacity was severely reduced in the untreated seeds infested by *C. maculatus*, where more than 50% failed to germinate. followed by pirimiphos-methyl treated seeds had the highest level of germination percentage, although those treated with plant extracts at 6 or 8 ml were not significantly different (P>0.05) (Table 3).

DISCUSSION

The data presented in this study demonstrate insecticidal activity of *C. sieberiana and V. amygdalina* aqueous extracts. The plant extracts performed significantly (P < 0.05) better than the untreated control against *C. maculatus* in stored bambara groundnut. Effectiveness of botanical products against stored-product insects in the laboratory including the plant genera tested in the present study is frequently reported (Akinkurolere *et al*., 2006; Saidana *et al*., 2007; Denloye *et al*., 2010; Ileke *et al.* 2013). In the present study, extracts of both plant species caused high adults mortality and progeny suppression in *C. maculatus*. Botanical compounds present several modes of action on insects, mainly acute toxicity, repellency and the inhibition of feeding, growth, development and reproduction (Coats, 1994). The observed high adult mortality might be due to direct toxicity to insects. Previous reports have indicated the efficacy of the tested botanical species against stored-product insects. For example, it was reported that *C. sophera* leaf powder mixed with different commodities at 1% and 5% concentrations significantly reduced F_1 emergence of *C. maculatus*, *S. zeamais* and *R. dominica* in laboratory experiments (Belmain *et al*., 2001). The essential oil of *V. amygdalina* induced 82% mortality of *S. zeamais* adults when applied at 750 mg/250 g application rate after 7 days of treatment (Awasalam and Hassanali, 2006).

The tested plant extracts reduced the population of progenies of *C. maculatus* in treated grains. The reduced progeny might be due to reduced egg production or inhibition of egg laying or ovicidal activity. Ofuya (1990) reported weakening of adults by plant powder may cause insects to lay fewer eggs than normal. It is interesting to note that the extracts could not completely prevent progeny development even in treatments where complete adult mortality was recorded. Hence, these results imply that higher dose rate of the plant extracts might be required for progeny inhibition than for adult mortality. Progeny production could be contained by increasing concentration of the extracts in treated seeds. The reduced percentage weight loss and damage in the plant extract treated seeds suggests potency of the two plants extracts. In addition, the results suggest residual effect against *C. maculatus*, given that the number F_2 individuals almost doubled in the untreated control while progeny reduction was increased in the treated grains.

Table 3. Effect of *C. maculatus* infestation seed damage, weight loss and germination capacity in bambara groundnut seeds treated with *C. sieberiana and V. amygdalina* aqueous extracts

Treatment	Dose of extract (ml/100 g)	Seed damage (%) (mean +SE)	Weight loss (%) (mean +SE)	Seed germination (%) (mean +SE)
Untreated control	0.0	92.8±1.4a	31.1±0.3a	46.7±1.7d
C. sieberiana	2.0	75.8±0.5ab	10.6±0.2c-e	63.3±1.7cd
	4.0	60.1±1.4b-d	10.9±0.2cd	63.3±3.5cd
	6.0	47.2±1.7c-e	9.0±0.5de	78.3±1.7a-c
	8.0	36.2±0.2de	4.7±0.2f	83.3±3.3ab
V. amygdalina	2.0	53.5±11.9b-e	12.3±0.2bc	65.0±2.8c
	4.0	65.2±5.0bc	13.7±0.4b	71.7±6.0bc
	6.0	36.5±1.9de	10.8±0.5cd	76.7±1.7a-c
	8.0	33.1±4.3e	8.8±0.4e	81.7±1.7ab
Pirimiphos-methyl	PC	0.0±0.0f	0.0±0.0g	86.7±1.7a

Means within a column followed by same letter are significantly not different from each other, Bonferroni Test at (P≤0.05). PC - positive control (pirimiphos methyl at 8 mg a.i./kg of seed).

The germination percentage of over 80% recorded in plant-extract-treated seeds after infestation by *C. maculatus* suggests that plant aqueous extracts have no negative effect on the viability of seeds. In agreement with our results, Sighamony *et al*. (1990) showed that plant oils are relatively safe for the treatment of seeds meant for sowing, as they have no detrimental effect on the seed germination. Similarly, Enobakhare and Law-Ogbomo (2002) in a study with *Sitophilus zeamais* in three varieties of maize treated with *V. amygdalina* showed that the treatments have no negative effect on the viability and quality of the treated maize. Kasa and Tadesse (1995) investigated the use of crude powders of 17 plants to control *S. zeamais* on sorghum and indicated that the botanical had no effect on germination. The extracts could therefore be used to protect bambara groundnut seeds meant for sowing from *C. maculatus* infestation. The results of this study also showed that pirimiphos-methyl has the highest effectiveness at protecting the stored seeds compared to the plant extracts. This is consistent with the findings of Awasalam and Hassanali (2006), Osipitan *et al*. (2010) and Ahuchaogu and Ojioka (2015), who showed superiority of pirimiphos-methyl over different botanical products.

According to Silva *et al*. (2012) and Devappa *et al*., (2010) the insecticidal activity botanicals vary according to the part of the plant from which the toxic metabolite was synthesized. Phytochemical analysis revealed the presence of four insecticidal principles (tannins, quinines, alkaloids and saponins) in *C. sieberian*a and these appear to be present in the different organs but in varying concentrations (Karumi *et al*., 2008). Similarly, phytochemical screening of ethanol extracts of *V. amygdalina* leaf samples showed the presence of alkaloids, flavonoids, saponins, tannins, phlobatannins, terpenoids and cardiac glycosides (Adeniyi *et al*., 2010). A comparative study using various plant parts would help identify the most suitable plant part for use as botanical insecticide.

CONCLUSION

This study demonstrates the potential *C. sieberian*a and *V. amygdalina* aqueous extracts for use against *C. maculatus* infestation in stored bambara groundnut. These extracts induced high adult mortality and progeny suppression. For the containment of progeny, however, higher dose (>8ml/100 g of seed) of the extracts would be required. The extracts had no adverse effect on seed germination and therefore could be used to protect seeds meant for sowing. The results deserve further research with increased doses and target insect species, as well as field validation in order to substantiate their usefulness as safer and cost-effective alternatives to synthetic insecticides.

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