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The Efficacy of *Alstonia boonei* Stembark Oil as a Long-term Storage Protectant against Cowpea Bruchid, *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae)

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Abstract

This study was conducted to assess the efficacy of *Alstonia boonei* stembark oil extracted with five solvents (methanol, ethanol, acetone, petroleum ether, and n-hexane) as a long-term storage protectant (after thirty, sixty, and ninety days of treatment) against *Callosobruchus maculatus* in the Laboratory. The mortality of adult insects, oviposition, percentage of adult emergence, progeny development, seed damage, weight loss, and beetle perforation index were measured and studied in this research. The results showed that the n-hexane oil extract of the *A. boonei* stembark was the most toxic, which caused 45 %, 57.5 %, 67.5 % and 75 % of adult mortality of *C. maculatus* at the rates of 1 %, 2 %, 3 % and 4 %/ 20g of cowpea seeds after thirty days of treatment respectively. It was followed by the petroleum ether oil extract, while the least toxic oil was the acetone extract. Generally, the percentage of adult mortality of *C. maculatus* decreased with the increase of storage periods (sixty and ninety days). The *Alstonia boonei* stembark oil extracted with non-polar and polar solvents completely inhibited the perforation potential of bruchids. The extracted oil can definitely serve as a biopesticide for the protection of cowpea seeds in storage up to ninety days against infestation by *C. maculatus*.

Keywords: *Alstonia boonei*, Perforation index; Progeny development, *Callosobruchus maculates*, cowpeas, Protectant.

1. Introduction

Postharvest losses of cowpea seeds by their major coleopteran insect pest, *Callosobruchus maculatus,* has led to seed perforation, reductions in weight, loss of nutritional value, market value, and viability (Ofuya, 2001; Akinkurolere, 2012; Idoko, 2016). Cowpea seeds are considered by farmers of poor resources in the tropical regions as the poor man's meat to combat malnutrition in young children instead of expensive protein sources such as meat, fish, and eggs, Cowpea seeds can face up to 100 % losses in terms of qualities and quantities as a result of *C. maculatus* infestation (Singh, 1985; Ogunwolu and Odunlami, 1996; Akinkurolere *et al*., 2006; Akinkurolere, 2012; Ileke, 2014).

Cowpea bruchid is a field-to-store coleopteran insect pest. Their eggs are laid on the cowpea pods by adult females before harvest and these can develop into larvae that feed exclusively on the pods after they penetrate through the pod covers and remain concealed within the seeds (Southgate, 1978, Akinkurolere, 2012). During harvest, the seeds infested with bruchid developmental stages are conveyed to store where infestation continues, and the emergence of adult *C. maculatus* leads to secondary infestation such as fungi causing a total destruction of the seeds' viability within three to four months (Singh and Jackai, 1985; Akinkurolere, 2012).

In order to reduce the qualitative and quantitative losses, the management of *C. maculatus* by Nigerian farmers has been dominated by the use of synthetic chemical insecticides and fumigants (Park *et al*., 2003; Akinkurolere, 2012; Idoko and Adesina 2012; Ileke *et al*., 2016). The use of synthetic chemical insecticides in the developing countries is restricted by environmental, financial, and safety contemplations. The high cost of chemical insecticides has led to the indiscriminate use of cheap pesticides of high mammalian toxicity to grains by farmers and traders in most Nigerian markets, which exposes the consumers of such products to chronic toxicity (Akinkurolere, 2012). The indiscriminate use of synthetic pesticides by untrained local farmers and traders has been a major concern for agricultural and storage entomologists all over the world who wish to find alternative methods that are readily available, eco-friendly, and cheap in order to replace the chemical insecticides (Adedire and Lajide 1999; Ogunwolu and Odunlami, 1996; Odeyemi *et al*., 2006; Ileke *et al*., 2012; 2013; 2016). The use of botanicals as pesticides in order to solve the problems of high cost, environmental hazards and the killing of the natural enemies of the pests is gaining more attention. Recently, researches have revealed that plant powders, ashes, oils, extracts, and the latex of different plant parts are effective protectants of stored cowpeas (Adedire and Lajide 1999; Lale and Abdulrahman, 1999; Akinkurolere *et al*., 2006;

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Akinkurolere, 2007; Ileke 2014; Ileke *et al*., 2013; 2014; Okosun and Adedire 2010; 2017).

Alstonia boonei belongs to the family Apocyanaceae. It is an African large evergreen deciduous crude medicinal tree that sheds its leaves annually. The plant is about 45 m tall, and its trunk is 1.2 m in diameter. The plant stembark and latex are applied in traditional medicine for treating many diseases (Moronkola and Kunle, 2012). In traditional African medicine, *A. boonei* is a medicinal plant used extensively for the treatment of malaria, fever, intestinal helminths, rheumatism, hypertension (Terashima, 2003; Betti, 2004; Abel and Busia, 2005). The insecticidal activity of *A. boonei* has been reported by several workers (Ileke and Oni 2011; Ileke *et al*., 2012; Ileke *et al*., 2013; 2014). Ileke and Oni (2011) reported the insecticidal potential of *A. boonei* stembark powder against *Sitophilus zeamais*. Ileke *et al*. (2012 and 2013) reported the insecticidal activity of *A. boonei* powder against *C. maculatus* and the response of cowpea bruchid to a 2 % of *A. boonei* stembark oils extracted with methanol, ethanol, acetone, petroleum ether, and n-hexane using cold extraction methods. Ileke *et al*. (2014) reported the insecticidal activity of *A. boonei* latex against *C. maculatus*.

Literature on the use of *A. boonei* stembark oils extracted with methanol, ethanol, acetone, petroleum ether, and n-hexane as long-term storage protectants against cowpea bruchid is relatively scarce. The aim of this research is to evaluate the *A. boonei* stembark oil extracted with five solvents as a long-term storage protectant (after thirty, sixty and ninety days of treatment) against *C. maculatus.*

2. Materials and Methods

2.1. Insect Rearing

The adults of the cowpea bruchid, *C. maculatus,* were supplied by Storage Entomology Research Laboratory, Department of Biology, Federal University of Technology, Akure, Nigeria. Eighty pairs of *C. maculatus* were introduced into a 1L glass kilner jar containing 300g of *Vigna unguiculata* (cultivar Ife brown) obtained from the International Institute for Tropical Agriculture, Ibadan, Nigeria. The beetle colony was maintained under constant insectary conditions of $28 \pm 2^{o}C$ and 75 ± 5 % relative humidity.

2.2. Plant Materials

The fresh stembark of *A. boonei* stem was obtained from Akola farm, Igbara Odo Ekiti, Nigeria. The plant stembark was first authenticated by a plant taxonomist at the Department of Crop, Soil, and Pest Management, Federal University of Technology, Akure, Nigeria. The stembark was air- dried in the Laboratory for four weeks before it was pulverized into fine powder using an electric blender, and was sieved using a $1mm^2$ perforation sieve. The powder was kept in plastic containers with tight lids and was stored in a refrigerator at $4^{o}C$ prior to use.

2.3. Soxhlet Extraction of A. boonei Stembark.

Three hundred grams (300g) of the powdered stembark was separately extracted with methanol, ethanol, acetone, petroleum ether, and n-hexane using the Soxhlet extraction method. The excess solvent was recovered using a rotary evaporator vacuum. The resulting oil was concentrated by air-drying to remove the traces of the solvent. From this stock solution, different oil concentrations (1 %, 2 %, 3 % and 4 %) were prepared separately.

2.4. Contact Toxicity of A. boonei Stembark Oil

Twenty grams (20g) of cowpea seeds that have been previously treated for thirty (30), sixty (60) and ninety (90) days with different concentrations (1 %, 2 %, 3 % and 4 %) of *A. boonei* stembark oils were used for this study. Ten pairs of two – three-day-old adults of *C. maculatus* were introduced to each of the containers and covered. Four replicates of the treated and untreated controls were laid out in Complete Randomized Design. The adult mortality was assessed after twenty-four hours. The adults were considered dead when probed gently with a fine needle and showed no response. At the end of day one, all insects, both dead and alive, were removed from each container, and the eggs were counted and recorded before returning the seeds to their respective containers.

The experimental setup was kept inside the insect-rearing cage for thirty more days for the emergence of the first filial (F_1) generation. The containers were sieved out, and the newly- emerged adult cowpea bruchids were counted and recorded. The percentage of adult emergence was calculated as described by Odeyemi and Daramola (2000):

$$\% \text{ Adult emergence} = \frac{\text{Total number of adult emergence}}{\text{Total number of larvae introduced}} \text{ x } \frac{100}{1}$$

The percentage of reduction in adult emergence of F_1 progeny or inhibition rate (IR) was calculated according to the method described by Tapondju *et al*. (2002):

$$\% \text{ IR} = \frac{C_n - T_n}{C_n} \text{ x } \frac{100}{1}$$

where C_n is the number of emerged insects in the control. and T_n is the number of emerged insects in the treated container.

The percentage of weight loss of the cowpea seeds was also determined:

$$\% \text{ Weight loss} = \frac{\text{Change in weight}}{\text{Initial weight}} \text{ x } \frac{100}{1}$$

The numbers of damaged cowpea seeds were also evaluated by counting wholesome seeds and the seeds with bruchid emergence holes:

$$\% \text{ Seed damage} = \frac{\text{Number of seeds damaged}}{\text{Total number of seeds}} \text{ x } \frac{100}{1}$$

The percentage of seeds' damage was calculated using a standard method. Beetle Perforation Index (BPI) used by Fatope *et al.* (1995) was adopted for the analysis of damage. Beetle perforation index (BPI) was defined as follows:

$$\text{BPI} = \frac{\% \text{ treated cowpea seeds perforated}}{\% \text{ control cowpea seeds perforated}} \text{ x } \frac{100}{1}$$

BPI value exceeding fifty has been regarded as enhancement of infestation by the weevil or negative protectability of the extract tested.

2.5. Statistical Analysis

The mortality percentages were calculated and corrected relative to the associated controls using Abbott's (1925) formula**.** Data were subjected to analysis of variance (ANOVA), and means were separated using the new Duncan's Multiple Range Test.

3. Results

3.1. Mortality of Adult C. maculatus in Treated Cowpeas

Table 1 presents the toxicity of *A. boonei* stembark oils after thirty, sixty, and ninety days of treatment of adult mortality of *C. maculatus*.

Table 1. Dose response mortality % of *C. maculatus* adults treated with *A. boonei* stembark oils after 30, 60 and 90 days of treatment.

Oils of *A. boonei* extracted by	Concentration in %	Mean % Mortality ± S.E. after 30-90 Days		
		30	60	90
Methanol	1	27.50±2.50^{b}	25.00±2.89^{b}	20.00±4.08^{b}
Ethanol		25.00±2.89^{b}	22.50±7.50^{b}	17.50±2.50^{b}
Acetone		17.50±2.50^{b}	15.0±2.89^{b}	12.50±3.74^{b}
Petroleum ether		32.50±7.50^{b}	30.00±2.89^{b}	27.50±2.50^{b}
N-hexane		45.00±2.89^{b}	37.50±2.50^{b}	32.50±7.50^{b}
Control	0.0	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Methanol	2	47.50±2.50^{c}	40.00±4.08^{b}	30.00±4.08^{b}
Ethanol		37.50±2.89^{bc}	32.50±7.50^{b}	25.00±2.89^{b}
Acetone		30.00±4.08^{b}	27.50±2.50^{b}	22.50±7.50^{b}
Petroleum ether		55.00±2.89^{c}	42.50±7.50^{bc}	35.00±2.89^{b}
N-hexane		57.50±2.50^{c}	50.00±5.79^{c}	37.50±2.50^{b}
Control	0.0	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Methanol	3	50.00±5.79^{c}	40.00±4.08^{bc}	30.00±4.08^{b}
Ethanol		47.50±2.50^{bc}	35.00±2.89^{b}	27.50±2.50^{b}
Acetone		37.50±2.50^{b}	30.00±4.08^{b}	22.50±7.50^{b}
Petroleum ether		57.50±2.50^{cd}	47.50±2.50^{c}	37.50±2.50^{b}
N-hexane		67.50±2.50^{d}	52.50±7.50^{c}	40.00±4.08^{b}
Control	0.0	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Methanol	4	62.50±7.50^{bc}	42.50±7.50^{bc}	30.00±4.08^{b}
Ethanol		60.00±4.08^{bc}	37.50±2.50^{bc}	27.50±2.50^{b}
Acetone		47.50±2.50^{b}	32.50±7.50^{b}	25.00±2.89^{b}
Petroleum ether		57.50±2.50^{c}	50.00±5.79^{c}	37.50±2.50^{b}
N-hexane		75.00±2.89^{c}	55.00±2.89^{c}	42.50±7.50^{b}
Control	0.0	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Each value is a mean ± standard error of four replicates. Means followed by the same letter along the column are not significantly different (*P*>0.05) using New Duncan's Multiple Range Test.

The n-hexane oil extract of *A. boonei* stembark caused 45%, 57.5%, 67.5% and 75 % of adult mortality of *C. maculatus* at the rates of 1 %, 2 %, 3 % and 4 %/ 20g of cowpea seeds after thirty days of treatment respectively. This is followed by the petroleum ether oil extract of *A. boonei* stembark which evoked 32.5 %, 55 %, 57.5 % and 67.5 % of the mortality of cowpea bruchid at the rates of 1 %, 2 %, 3 % and 4 % / 20g of cowpea seeds after thirty days of treatment respectively. The least toxic oil was the stembark oil extracted with acetone which evoked 17.5 %, 30 %, 37.5 % and 47.5 % of the mortality of adult *C. maculatus* at the rates of 1 %, 2 %, 3 % and 4 % / 20g of cowpea seeds after thirty days of treatment respectively.

Sixty days post-treatment, 37.5 %, 50 %, 52.5 % and 55 % rates of adult mortality of the cowpea bruchids were recorded on cowpea seeds treated with the n-hexane oil extract of *A. boonei* stembark at the rates of 1 %, 2 %, 3 % and 4 % respectively. Ninety days post-treatment, the rates 32.5 %, 37.5 %, 40 % and 42.5 % of adult mortality of the cowpea bruchids were recorded on the seeds treated with the n-hexane oil extract of *A. boonei* stembark at the rates of 1 %, 2 %, 3 % and 4 % respectively. On the whole, the percentage of adult mortality of *C. maculatus* decreased with the increase of the storage period.

3.2. Effect of Treatments on C. maculatus Emergence

The effects of *A. boonei* stembark oils after thirty, sixty, and ninety days of treatment on oviposition, adult emergence, and reduction in progeny development of the adults of *C. maculatus* are presented in Tables 2, 3, 4, and 5. In all cases, the ANOVA results showed that the treatments had significant effects ($P < 0.05$) against the emergence of the first filial generation of *C. maculatus*, with the exception of the control groups. Thirty days post-treatment, the methanol, ethanol, petroleum ether, and n-hexane stembark oils reduced the number of eggs laid by cowpea bruchids showing a 100 % reduction in progeny development of adult bruchids at all of the tested concentration rates (Tables 2, 3, 4, and 5).

Sixty days post-treatment, the methanol, ethanol, petroleum ether, and n-hexane stembark oils at the rate of 4 % reduced the number of eggs laid by cowpea bruchid, showing a 100 % reduction rate in progeny development of adult bruchids, while the acetone extract of *A. boonei* stembark showed 12.12 % of adult emergence and a 95 % inhibition rate of progeny development of cowpea bruchid (Table 5). The number of eggs laid, the percentage of adult emergence, and progeny development of adult *C. maculatus* all decreased as the extract concentrations increased (Tables 2, 3, 4, and 5).

Table 2. Number of eggs laid, adult emergence and inhibition rate (IR) of adult *C. maculatus* in cowpea seeds treated with 1% oil of *A. boonei* stembark after 30, 60 and 90 days of treatment.

Days after treatment	1% oil of *A. boonei*	Mean number of eggs laid ± SE	% adult emergence ± SE	% IR ± SE
30	Methanol	8.50±1.23^{a}	0.00±0.00^{a}	100.00±0.00^{g}
	Ethanol	8.75±0.85^{a}	0.00±0.00^{a}	100.00±0.00^{g}
	Acetone	10.25±1.70^{ab}	29.27±1.40^{c}	82.35±3.50^{cde}
	Pet-ether	8.50±1.23^{a}	0.00±0.00^{a}	100.00±0.00^{g}
	N-hexane	8.00±0.91^{a}	0.00±0.00^{a}	100.00±0.00^{g}
	Untreated	22.25±2.70^{b}	76.41±3.25^{f}	0.00±0.00^{a}
60	Methanol	9.00±0.91^{a}	11.11±0.43^{b}	95.00±2.89^{fg}
	Ethanol	9.25±1.70^{a}	21.62±1.67^{bc}	90.00±4.08^{efg}
	Acetone	11.00±0.91^{ab}	27.27±1.40^{c}	85.00±2.89^{def}
	Pet-ether	9.00±0.91^{a}	11.11±0.43^{b}	95.00±2.89^{fg}
	N-hexane	8.75±0.85^{a}	11.43±0.74^{b}	95.00±2.89^{fg}
	Untreated	25.00±2.89^{b}	80.00±4.08^{f}	0.00±0.00^{a}
90	Methanol	10.00±0.91^{a}	40.00±4.08^{de}	77.78±3.12^{cd}
	Ethanol	10.50±1.23^{ab}	47.62±2.53^{e}	72.22±3.41^{c}
	Acetone	11.25±1.70^{ab}	71.11±3.43^{f}	55.56±2.65^{b}
	Pet-ether	10.00±0.91^{a}	40.00±4.08^{de}	77.78±2.12^{cd}
	N-hexane	9.75±0.85^{a}	30.77±4.12^{cd}	83.33±3.45^{cde}
	Untreated	23.00±2.96^{b}	78.26±3.38^{f}	0.00±0.00^{a}

Each value is a mean ± standard error of four replicates. Means followed by the same letter along the column are not significantly different (*P*>0.05) using New Duncan's Multiple Range Test.

Table 3. Number of eggs laid, adult emergence and inhibition rate (IR) of adult *C. maculatus* in cowpea seeds treated with 2% oil of *A. boonei* stembark after 30, 60 and 90 days of treatment.

Days after treatment	2% oil of *A. boonei*	Mean number of eggs laid ± SE	% adult emergence ± SE	% IR ± SE
30	Methanol	7.50±1.23^{a}	0.00±0.00^{a}	100.00±0.00^{e}
	Ethanol	7.75±0.85^{a}	0.00±0.00^{a}	100.00±0.00^{e}
	Acetone	9.50±1.23^{ab}	21.05±1.40^{bc}	88.35±3.20^{d}
	Pet-ether	7.50±1.23^{a}	0.00±0.00^{a}	100.00±0.00^{e}
	N-hexane	7.25±1.70^{a}	0.00±0.00^{a}	100.00±0.00^{e}
	Untreated	22.25±2.70^{b}	76.41±3.25^{f}	0.00±0.00^{a}
60	Methanol	8.75±0.85^{a}	11.43±0.74^{b}	95.00±2.89^{de}
	Ethanol	9.00±0.91^{a}	11.62±0.43^{b}	90.00±4.08^{de}
	Acetone	10.75±0.85^{ab}	37.21±2.39^{d}	85.00±2.89^{d}
	Pet-ether	9.75±0.85^{ab}	10.26±1.02^{b}	95.00±2.89^{e}
	N-hexane	8.25±1.70^{a}	0.00±0.00^{a}	100.00±0.00^{e}
	Untreated	25.00±2.89^{b}	80.00±4.08^{f}	0.00±0.00^{a}
90	Methanol	9.75±0.85^{a}	30.77±4.16^{cd}	83.33±3.35^{cd}
	Ethanol	10.25±1.70^{ab}	39.02±3.91^{de}	72.78±2.12^{bc}
	Acetone	11.00±0.91^{ab}	54.55±2.83^{e}	66.67±2.31^{b}
	Pet-ether	10.00±0.91^{a}	30.00±4.08^{cd}	83.33±3.35^{cd}
	N-hexane	9.75±0.85^{a}	20.51±4.20^{bc}	88.89±3.27^{de}
	Untreated	23.00±2.96^{b}	78.26±3.38^{f}	0.00±0.00^{a}

Each value is a mean ± standard error of four replicates. Means followed by the same letter along the column are not significantly different (*P*>0.05) using New Duncan's Multiple Range Test.

Table 4. Number of eggs laid, adult emergence and inhibition rate (IR) of adult *C. maculatus* in cowpea seeds treated with 3% oil of *A. boonei* stembark after 30, 60 and 90 days of treatment.

Days after treatment	3% oil of *A. boonei*	Mean number of eggs laid ± SE	% adult emergence ± SE	% IR ± SE
30	Methanol	7.25±1.70^{a}	0.00±0.00^{a}	100.00±0.00^{f}
	Ethanol	7.50±1.23^{a}	0.00±0.00^{a}	100.00±0.00^{f}
	Acetone	9.25±1.70^{ab}	10.81±1.40^{b}	94.12±2.63^{cdef}
	Pet-ether	7.00±0.91^{a}	0.00±0.00^{a}	100.00±0.00^{f}
	N-hexane	6.75±0.85^{a}	0.00±0.00^{a}	100.00±0.00^{f}
	Untreated	22.25±2.70^{bc}	76.41±3.25^{e}	0.00±0.00^{a}
60	Methanol	8.00±0.91^{a}	0.00±0.00^{a}	100.00±0.00^{f}
	Ethanol	8.00±0.91^{a}	12.50±1.23^{b}	90.00±4.08^{d}
	Acetone	9.00±0.91^{a}	22.22±2.41^{bc}	85.00±2.89^{cde}
	Pet-ether	7.75±0.85^{a}	0.00±0.00^{a}	100.00±0.00^{f}
	N-hexane	7.25±1.70^{a}	0.00±0.00^{a}	100.00±0.00^{f}
	Untreated	25.00±2.89^{c}	80.00±4.08^{e}	0.00±0.00^{a}
90	Methanol	9.00±0.91^{a}	22.77±2.41^{bc}	88.89±3.22^{cd}
	Ethanol	9.25±1.23^{a}	32.43±2.74^{c}	83.33±3.35^{bcd}
	Acetone	10.00±0.91^{ab}	50.00±5.79^{d}	72.22±3.41^{b}
	Pet-ether	9.00±0.91^{a}	11.11±0.58^{b}	94.44±3.62^{cdef}
	N-hexane	8.75±0.85^{a}	11.43±0.74^{b}	94.44±3.62^{cdef}
	Untreated	23.00±2.96^{bc}	78.26±3.38^{e}	0.00±0.00^{a}

Each value is a mean ± standard error of four replicates. Means followed by the same letter along the column are not significantly different (*P*>0.05) using New Duncan's Multiple Range Test.

Ninety days post-treatment, the petroleum ether and n-hexane stembark oils at the concentration rate of 4 % reduced the number of eggs laid by cowpea bruchids showing a 100 % reduction rate in the progeny development of adult bruchids, while the methanol, ethanol, and acetone extracts of *A. boonei* stembark oils allowed 11.43 %, 11.11 %, and 32.43 % of adult emergence and 94.44 %, 94.44 % and 83.33 % inhibition or reduction rates of progeny development of cowpea bruchid respectively (Table 5).

Table 5. Number of eggs laid, adult emergence and inhibition rate (IR) of adult *C. maculatus* in cowpea seeds treated with 4% oil of *A. boonei* stembark after 30, 60 and 90 days of treatment.

Days after treatment	4% oil of *A. boonei*	Mean number of eggs laid ± SE	% adult emergence ± SE	% IR ± SE
30	Methanol	6.25±1.70^{a}	0.00±0.00^{a}	100.00±0.00^{c}
	Ethanol	6.25±1.70^{a}	0.00±0.00^{a}	100.00±0.00^{c}
	Acetone	7.25±1.70^{a}	0.00±0.00^{a}	100.00±0.00^{c}
	Pet-ether	6.00±0.91^{a}	0.00±0.00^{a}	100.00±0.00^{c}
	N-hexane	5.75±0.85^{a}	0.00±0.00^{a}	100.00±0.00^{c}
	Untreated	22.25±2.70^{bc}	76.41±3.25^{d}	0.00±0.00^{a}
60	Methanol	7.00±0.91^{a}	0.00±0.00^{a}	100.00±0.00^{c}
	Ethanol	7.50±1.23^{a}	0.00±0.00^{a}	100.00±0.00^{c}
	Acetone	8.25±1.70^{a}	12.12±1.63^{b}	95.00±2.89^{bc}
	Pet-ether	7.00±0.91^{a}	0.00±0.00^{a}	100.00±0.00^{c}
	N-hexane	6.75±0.85^{a}	0.00±0.00^{a}	100.00±0.00^{c}
	Untreated	25.00±2.89^{c}	80.00±4.08^{d}	0.00±0.00^{a}
90	Methanol	8.75±0.85^{a}	11.43±0.74^{b}	94.44±3.62^{bc}
	Ethanol	9.00±0.91^{a}	11.11±0.58^{b}	94.44±3.62^{bc}
	Acetone	9.25±1.70^{ab}	32.43±2.74^{c}	83.33±3.35^{b}
	Pet-ether	8.25±1.70^{a}	0.00±0.00^{a}	100.00±0.00^{c}
	N-hexane	8.00±0.91^{a}	0.00±0.00^{a}	100.00±0.00^{c}
	Untreated	23.00±2.96^{bc}	78.26±3.38^{d}	0.00±0.00^{a}

Each value is a mean ± standard error of four replicates. Means followed by the same letter along the column are not significantly different (*P*>0.05) using New Duncan's Multiple Range Test.

3.3. Beetle Perforation Index caused by C. maculatus

The percentage of seeds' damage, weight loss, and Beetle Perforation Index caused by *C. maculatus* in cowpea seeds treated with *A. boonei* stembark oils after thirty, sixty and ninety days of treatment are shown in Table 6, 7, 8, and 9. Thirty days post-treatment, the methanol, ethanol, petroleum ether, and n-hexane of stembark oils completely protected the seeds from being damaged by cowpea bruchids at all the concentrations tested. There was neither seed damage nor weight loss observed in the cowpea seeds treated with the acetone oil of *A. boonei* stembark and BPI was zero for the concentrations tested after thirty days of application (Tables 6, 7, 8, and 9).

Sixty days post-treatment, the methanol, petroleum ether, and n-hexane of the stembark oil extracts completely protected the seeds from being damaged by cowpea bruchid at the rates of 2%, 3%, and 4%.

Ninety days post-treatment, only the n-hexane oil completely protected the cowpea seeds from being damaged by *C. maculatus*. The n-hexane oil effect was not significantly different from the petroleum oil extract of *A. boonei*. Generally, the percentage of seed damage, weight loss, and Beetle Perforation Index by *C. maculatus* increased with increase of the storage period. Conversely, the percentages of seed damage, weight loss, and Beetle

Perforation Index by *C. maculatus* decreased with the increase in the oil concentrations.

Table 6. Perforation Index caused by *C. maculatus* in cowpea seeds treated with 1% oil of *A. boonei* stembark oil after 30, 60 and 90 days of treatment.

Days after treatment	1% oil of *A. boonei*	Mean total number of cowpea seeds	Mean number of damaged cowpea seeds	Mean % cowpea seeds damaged	Mean % weight loss	Beetle perforation Index (BPI)*
30	Methanol	93.00	0.00	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
	Ethanol	94.75	0.00	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
	Acetone	94.00	3.25	3.46±0.11[ab]	6.24±0.68[b]	18.39±1.16[c]
	Pet-ether	95.25	0.00	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
	N-hexane	92.75	0.00	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
	Untreated	93.00	17.50	18.81±1.19[c]	62.52±2.21[c]	50.00±0.00[e]
60	Methanol	93.75	1.00	1.07±0.54[ab]	3.07±0.54[ab]	5.06±0.73[b]
	Ethanol	94.25	1.50	1.59±0.11[ab]	3.70±0.97[ab]	7.52±1.21[b]
	Acetone	94.00	4.00	4.26±0.57[b]	7.38±0.18[b]	20.15±4.74[c]
	Pet-ether	95.00	1.00	1.05±0.42[ab]	2.92±0.87[ab]	4.97±0.95[b]
	N-hexane	92.75	1.00	1.08±0.76[ab]	3.11±0.61[ab]	5.11±0.43[b]
	Untreated	94.75	20.25	21.14±2.66[c]	65.68±3.83[c]	50.00±0.00[e]
90	Methanol	94.50	4.25	4.50±0.23[b]	7.74±0.83[ab]	23.24±2.63[c]
	Ethanol	93.00	5.50	5.91±1.19[b]	7.80±0.23[ab]	30.53±4.74[cd]
	Acetone	94.00	8.00	8.51±1.91[b]	9.63±0.61[b]	43.96±2.96[de]
	Pet-ether	95.00	4.75	5.00±0.91[b]	7.29±0.46[ab]	25.83±2.82[c]
	N-hexane	93.75	3.50	3.73±0.86[ab]	6.34±1.44[ab]	19.27±3.40[c]
	Untreated	94.25	18.25	19.36±0.62[c]	63.67±3.08[c]	50.00±0.00[e]

Each value is a mean ± standard error of four replicates. Means followed by the same letter along the column are not significantly different (*P*>0.05) using New Duncan's Multiple Range Test.

*****Beetle Perforation Index (BPI). Value lower than 50 is an indication of positive protectant effect while BPI greater than 50 is an indication of negative protectability.

Table 7. Perforation Index caused by *C. maculatus* in cowpea seeds treated with 2% oil of *A. boonei* stembark after 30, 60 and 90 days of treatment

Days after treatment	2% oil of *A. boonei*	Mean total number of cowpea seeds	Mean number of damaged cowpea seeds	Mean % cowpea seeds damaged	Mean % weight loss	Beetle perforation Index (BPI)*
30	Methanol	94.25	0.00	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
	Ethanol	93.75	0.00	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
	Acetone	95.00	2.50	2.63±0.61[ab]	4.99±0.72[b]	13.39±1.67[bc]
	Pet-ether	93.50	0.00	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
	N-hexane	94.75	0.00	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
	Untreated	95.00	17.50	18.81±1.19[c]	62.52±2.21[c]	50.00±0.00[f]
60	Methanol	93.50	1.25	1.34±0.41[ab]	3.62±0.59[ab]	6.34±1.41[b]
	Ethanol	94.00	1.75	1.86±0.19[ab]	3.96±0.95[ab]	8.80±1.20[bc]
	Acetone	95.25	4.25	4.46±0.15[b]	7.89±1.22[b]	21.10±4.98[cd]
	Pet-ether	94.00	1.25	1.33±0.03[ab]	3.55±0.11[ab]	6.29±1.39[b]
	N-hexane	93.00	0.00	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
	Untreated	94.75	20.25	21.14±2.66[c]	65.68±3.83[c]	50.00±0.00[f]
90	Methanol	93.00	3.50	3.76±0.11[ab]	4.74±0.54[ab]	19.42±2.75[d]
	Ethanol	92.75	4.25	4.58±0.13[b]	4.71±0.97[ab]	23.66±3.79[cd]
	Acetone	94.25	6.50	6.90±1.29[b]	7.38±0.18[b]	35.64±2.59[e]
	Pet-ether	95.00	3.00	3.16±0.37[ab]	3.92±0.87[ab]	16.32±2.74[cd]
	N-hexane	94.25	2.25	2.39±0.61[ab]	3.19±0.61[ab]	12.35±3.20[bcd]
	Untreated	94.25	18.25	19.36±0.62[c]	63.67±3.08[c]	50.00±0.00[f]

Each value is a mean ± standard error of four replicates. Means followed by the same letter along the column are not significantly different (*P*>0.05) using New Duncan's Multiple Range Test.

*****Beetle Perforation Index (BPI). Value lower than 50 is an indication of positive protectant effect while BPI greater than 50 is an indication of negative protectability.

Table 8. Perforation Index caused by *C. maculatus* in cowpea seeds treated with 3% oil of *A. boonei* stembark after 30, 60 and 90 days of treatment

Days after treatment	3% oil of *A. boonei*	Mean total number of cowpea seeds	Mean number of damaged cowpea seeds	Mean % cowpea seeds damaged	Mean % weight loss	Beetle perforation Index (BPI)*
30	Methanol	94.00	0.00	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$
	Ethanol	92.25	0.00	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$
	Acetone	94.00	1.25	$1.33±0.35^{ab}$	$3.55±0.11^{ab}$	$7.07±0.54^{b}$
	Pet-ether	93.75	0.00	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$
	N-hexane	92.75	0.00	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$
	Untreated	95.00	17.50	$18.81±1.19^{c}$	$62.52±2.21^{c}$	$50.00±0.00^{e}$
60	Methanol	95.00	0.00	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$
	Ethanol	94.25	1.00	$1.06±0.51^{ab}$	$3.47±0.14^{ab}$	$5.01±0.84^{b}$
	Acetone	92.75	2.50	$2.70±0.97^{ab}$	$4.96±0.94^{b}$	$12.77±1.81^{bc}$
	Pet-ether	94.75	0.00	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$
	N-hexane	93.25	0.00	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$
	Untreated	94.75	20.25	$21.14±2.66^{c}$	$65.68±3.83^{c}$	$50.00±0.00^{e}$
90	Methanol	94.75	2.25	$2.38±0.18^{ab}$	$3.11±0.43^{ab}$	$12.29±1.39^{bc}$
	Ethanol	94.00	3.75	$3.99±0.67^{ab}$	$3.96±0.23^{ab}$	$20.61±4.58^{cd}$
	Acetone	93.50	5.50	$5.88±1.23^{b}$	$6.59±0.09^{b}$	30.37±4.15d
	Pet-ether	92.75	1.50	$1.62±0.59^{ab}$	$3.80±0.21^{ab}$	$8.37±1.15^{b}$
	N-hexane	95.00	1.25	$1.36±0.07^{ab}$	$2.60±0.13^{ab}$	$7.03±1.64^{b}$
	Untreated	94.25	18.25	$19.36±0.62^{c}$	$63.67±3.08^{c}$	$50.00±0.00^{e}$

Each value is a mean $\pm$ standard error of four replicates. Means followed by the same letter along the column are not significantly different (P>0.05) using New Duncan's Multiple Range Test.

*****Beetle Perforation Index (BPI). Value lower than 50 is an indication of positive protectant effect while BPI greater than 50 is an indication of negative protectability.

Table 9. Perforation Index caused by *C. maculatus* in cowpea seeds treated with 4% oil of *A. boonei* stembark after 30, 60 and 90 days of treatment

Days after treatment	4% oil of *A. boonei*	Mean total number of cowpea seeds	Mean number of damaged cowpea seeds	Mean % cowpea seeds damaged	Mean % weight loss	Beetle perforation Index (BPI)*
30	Methanol	92.75	0.00	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$
	Ethanol	93.50	0.00	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$
	Acetone	93.00	0.00	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$
	Pet-ether	94.00	0.00	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$
	N-hexane	93.25	0.00	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$
	Untreated	95.00	17.50	$18.81±1.19^{b}$	$62.52±2.21^{c}$	$50.00±0.00^{d}$
60	Methanol	94.00	0.00	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$
	Ethanol	93.50	0.00	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$
	Acetone	93.00	1.50	$1.61±0.58^{a}$	$3.83±0.29^{ab}$	$7.61±1.58^{b}$
	Pet-ether	92.75	0.00	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$
	N-hexane	95.00	0.00	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$
	Untreated	94.75	20.25	$21.14±2.66^{b}$	$65.68±3.83^{c}$	$50.00±0.00^{d}$
90	Methanol	93.00	1.50	$1.61±0.58^{a}$	$3.83±0.29^{ab}$	$8.32±1.41^{bc}$
	Ethanol	94.00	1.75	$1.85±0.19^{a}$	$3.96±0.23^{ab}$	$9.61±1.58^{bc}$
	Acetone	95.00	3.50	$3.68±0.82^{a}$	$4.59±0.09^{b}$	$19.01±2.89^{c}$
	Pet-ether	93.00	1.00	$1.08±0.97^{a}$	$2.11±0.43^{ab}$	$5.58±0.13^{b}$
	N-hexane	93.75	0.00	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$
	Untreated	94.25	18.25	$19.36±0.62^{b}$	$63.67±3.08^{c}$	$50.00±0.00^{d}$

Each value is a mean $\pm$ standard error of four replicates. Means followed by the same letter along the column are not significantly different (P>0.05) using New Duncan's Multiple Range Test.

*****Beetle Perforation Index (BPI). Value lower than 50 is an indication of positive protectant effect while BPI greater than 50 is an indication of negative protectability.

4. Discussion

Entomologists employed many procedures to screen plant materials for their efficacy against cowpea bruchid, *C. maculatus* (Adedire and Lajide, 1999; Ogunwolu and Odunlami, 1996; Okonkwo and Okoye, 1996; Akinkurolere, 2016; Ileke, 2014). In all of the tested procedures, efficacious materials adversely affected the beetles by killing them, at the adult, pupal and larval stages, exterminated oviposited eggs, or prevented the full expression of oviposition through antifeedants, fumigants, repellents, attractants and contact poisoning (Ogunwolu and Odunlami, 1996; Boeke *et al*., 2001 Akinkurolere et al., 2006; Akinkurolere, 2012).

The results of this study show that the n-hexane oil extract from *A. boonei* stembark with the lowest beetle perforation index, was the most effective against *C. maculatus,* showing the highest bruchid mortality, suppressing F_1 emergence, causing low seed damage and weight loss as well as reducing the high residual toxicity thirty, sixty and ninety days after treatment. This is followed by the petroleum oil extract of *A. boonei* stembark, while the least effective was the acetone oil extract of *A. boonei* stembark. Significantly, less eggs were laid, at all of the tested concentrations by the bruchid on the cowpea seeds protected with the *A. boonei* stembark oils extracted with five solvents compared with the numbers of eggs laid on the untreated cowpea seeds. Previous studies have reported the insecticidal activity of *A. boonei* after four days of treatment (Ileke and Oni 2011; Ileke *et al*., 2012; Ileke *et al*., 2013; 2014). Ileke and Oni (2011) reported the insecticidal potential of *A. boonei* stembark powder after four days of treatment against *Sitophilus zeamais*. Ileke *et al*. (2012 and 2013) reported the insecticidal activity of *A. boonei* powder and latex after four days of treatment against *C. maculatus* and the response of cowpea bruchid to the treatment with a 2% of *A. boonei* stembark oils extracted with methanol, ethanol, acetone, petroleum ether, and n-hexane using cold extraction methods. Ileke *et al*. (2014) reported the insecticidal activity of *A. boonei* latex after four days of treatment against *C. maculatus*. The present study confirmed the earlier reports of the insecticidal potential of *A. boonei* stembark oils and the persistence of bioactive compounds present in the studied plant part. The oils were able to protect the seeds up to three months after treatment. The plant extracts contain some chemical compounds of the triterpenoids, indole and alkaloid groups such as alstonine, astondine, and porphine (Phillipson *et al*. 1987).

The greater effectiveness of n-hexane, petroleum ether (non-polar) oils over the methanol, ethanol (polar) oils may be a result of the more bioactive compounds in the non-polar oils than the polar oils Ho *et al*. (1994, 1995, 1996). The undamaged cowpea seeds treated with non-polar and polar oils of *A boonei* stembark may be attributed to the oil content of the plant part, which could have blocked the respiratory tracts (spiracles) of the insects, leading to their death and also reducing the F_1 generation and the seed damage (Dike and Mbah, 1992; Akinkurolere, 2012). The non-effectiveness of acetone oils compared to the non-polar and polar oils may be ascribed to the polarity of acetone which is intermediate between polar (methanol, ethanol) and non-polar (n-hexane, petroleum ether) solvents, which means it may not be able to extract all the polar or the non–polar constituents of the powdered *A. boonei* stembark. Okosun and Adedire (2010; 2017) reported the non-effectiveness of the acetone extract of *Monodora myristica* seeds against *C. maculatus*. Su (1989) reported a lesser toxicity of the acetone extract of *Myristica fragnans* to *C. maculatus, Lasioderma serricorne* and *T. castaneum*, though it was found moderately toxic to *Sitophilus oryzae.*

The *A. boonei* stembark oils did not completely prevent oviposition by *C. maculatus* on cowpea seeds. Nevertheless, the results indicate that *A. boonei* stembark oils manifested great anti-oviposition activity against the *C. maculatus* based on the insignificant percentage of adult emergence. At higher concentrations of 3 % and 4 %, the *A. boonei* stembark oils made the cowpea seeds immune to *C. maculatus* attacks even after three months of treatment. The oils may prevent the bruchids from moving freely thereby preventing mating among adult insects (Wolfson *et al*., 1991). The inability of the insect to oviposit resulted in insignificant weight and damage losses. The perforation index was also minimal compared with the negative protectant (above 50%) recommended by Fatope et al. (1995).

5. Conclusion

The novelties in the use of *A. boonei* stembark oils extracted with five solvents (methanol, ethanol, acetone, Petroleum ether, and n-hexane) using soxhlet extraction method as long-term storage protectants (30, 60 and 90 days) against *C. maculatus* have been highlighted in this study. The *Alstonia boonei* stembark oil extracted with non-polar and polar solvents could serve as biopesticides for the protection of cowpea seeds against infestation by cowpea bruchid, *C maculatus*. The anti-oviposition exhibited by the studied plant part was greatly reflected in the beetle perforation index which is insignificant compared with negative protectants (above 50%) recommended by Fatope *et al*. (1995). The plant is eco-friendly, biodegradable and readily available in the tropical region. The oils can be ranked in terms of their effectiveness as follows: n-hexane > Petroleum ether > ethanol > methanol > acetone.

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