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Synergistic effect of *Balanites aegyptiaca* essential oil and storage materials on cowpea seeds

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Abstract:
The cowpea (*Vigna unguiculata* L.) is a legume produced and consumed all over Africa and especially in Nigeria. These beans are a major source of protein in the region. The cowpea weevil (*Callosobruchus maculatus* L.) is a major pest that affects cowpea seeds. Therefore, cowpea farmers need effective non-toxic pesticides to replace synthetic chemicals. The present research tested the effect of *Balanites aegyptiaca* L. essential oil on cowpea weevils.

This research quantified weevil proliferation and cowpea seed qualities. The samples were treated with 5, 10, and 15 mL of *B. aegyptiaca* essential oil diluted in 1 mL of acetone and stored in five storage materials, i.e., jute bags, polythene bags, sacks, plastic containers, and glass bottles. The study featured a completely randomized design with three replications of each treatment: treatment time – 90 days, storage temperature – 30 ± 5°C, check – 0.125 g of aluminum phosphide, control – acetone.

B. aegyptiaca essential oil proved to be an effective insecticide against cowpea weevils. The treatment achieved 100% mortality rate at 10 and 15 mL of *B. aegyptiaca* essential oil after 72 h of exposure in glass bottles, plastic containers, and jute bags. In addition, *B. aegyptiaca* essential oil demonstrated a potent activity against oviposition and survival of immature cowpea weevils. Cowpea seeds packaged in glass bottles, plastics containers, and jute bags showed significantly less damage than those stored in sacks and polythene bags. Glass bottles were the best storage material in terms of safety and shelf stability, followed by plastic containers and jute bags.

B. aegeptica essential oil has potent insecticidal properties and can be used as pest control during grain storage.

Keywords: Essential oil, *Callosobruchus maculatus*, *Vigna unguiculata*, storage, storage material

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INTRODUCTION

The cowpea, *Vigna unguiculata* (L.), is a legume of the *Fabaceae* family. It is one of the most important legume crops in the world. The plant thrives in temperate climate and requires little agricultural inputs for growth [1]. Cowpeas are popular and cheap in many developing countries. In Nigeria, for instance, they are a major staple crop. Small-holder farmers are the major producer of cowpea grains in Nigeria [2].

Cowpeas serve as a rich and affordable source of nutrients, especially protein, in sub-Saharan Africa and some parts of America and Asia [3]. Cowpea beans can be cooked, powdered, germinated, or even used as part of a weaning formula. As a dish, they complement tubers and cereals. Consequently, cowpea grains are present in the diet of many developing countries where population suffers from malnutrition and protein deficiencies.

Cowpeas are mainly cultivated by the local farmers for profits and satisfy the basic nutritional needs of the local population. However, farmers fail to meet the local demand as a result of drastic post-harvest losses caused by insects and other pests. In fact, these losses are considered as one of the underlying causes of food scarcity and poverty [4].

Insects damage cowpea grains by boring holes, thus causing weight loss, poor quality, and low market value Cowpea aphids (*Aphis craccivora* L.), leafhoppers

(*Empoasca* spp.), cowpea weevils (*Callosobruchus maculatus*), and witch-weed (*Striga gesnerioides*) are the main insect species that feed on cowpea seeds [5, 6].

Callosobruchus maculatus is the main pest that causes losses in cowpea grains. *C. maculatus* is a field-to-store pest that typically begins in the field, and the level of prior-harvest infestation determines the extent of damage to stored grains [7]. A range of insect pest control measures have been adopted over decades to reduce the prevalence of cowpea grain loss in the field and during storage.

According to Ogunfowokan *et al.*, Nigerian farmers use Lindane (Gammalin 20EC), Dichlorvos dichlorodiphenyltrichloroethane (DDT), Chlorpyrifos, Endosufan, and Aldrin to prevent pest infestation [8]. Synthetic chemicals are very effective in preserving grains and increasing their production yield. However, they have major drawbacks as improper use often results in environmental pollution, pesticide residue in food, and toxic poisoning of the ecosystem [9]. Hence, several attempts have been made to test pesticides that are environmentally friendly, harmless to people, and inexpensive.

Resourceful African farmers tried to use such natural preservatives as plant powder, ashes, and cow dung. Several authors have documented the insecticidal efficacy of plant products on different types of pests [10]. In fact, plant products with aromatic properties are known to prevent insect infestation of stored cowpeas [11]. Ikbal and Pavela made an extensive research to assess the use of aromatic plants as pesticides during storage [12]. They reported that essential oils of plant origin could serve as botanical insecticides as they contain a lot of bioactive compounds with insecticidal, nematicidal, larvicidal, and anti-feedant properties that inhibit insect oviposition [13, 14]. Essential oils are natural derivatives from aromatic plants which contain volatile and phenolic compounds with unique flavors. Several works have reported the insecticidal effect of essential oils during grain storage [15–19].

Balanites aegyptiaca fruits have quite a number of bioactive compounds with various medicinal properties [20]. The essential oil extracted from *B. aegyptiaca* possesses anticancer, antimicrobial, antioxidant, anticarcinogenic, antidiabetic, antifeedant, and antiviral activities [21, 22]. Natural fumigants developed from plants do not threaten the ecosystem. Insecticides based on essential oils are sold all over the world, but their production does not exceed 5% [23]. In Asia, Europe, and North America, natural extracts have been used as insecticides for more than a century, much longer than any synthetic insecticides.

Previous studies have investigated the insecticidal efficacy of oil extracted from *B. aegyptiaca*. For instance, Mokhtar *et al.* reported a strong effect of *B. aegyptiaca* seed oil on the mortality rate of red flour beetle (*Tribolium castaneum* Herbst) [17]. Similarly, Nwaogu and Yahaya investigated the insecticidal efficacy of oil extracted from *B. aegyptiaca* in stored cowpea seeds [18]. The studies provided evidence that *B. aegyptiaca* seed oil could be used as an insecticide against storage pest. However, very few publications feature the use of *B. aegyptiaca* essential oils in controlling insect infestation of stored grains.

Storage materials are also important for seed treatments and grain quality. Buleti *et al.* conducted an experiment in the Northeast of Nigeria to assess the effect of *B. aegyptiaca* oil on weevil growth in cowpea grains stored in various packing materials [15]. The present research provides some new data on using plant products as storage pesticides.

STUDY OBJECTS AND METHODS

Plant material. Fruits of *Balanites aegyptiaca* L. were acquired from the Gashua market in Yobe State, Nigeria. The fruits were authenticated at the Department of Agronomy, Faculty of Agriculture, Federal University Gashua, Nigeria.

Preparing the seeds. The mesocarp of *B. aegyptiaca* was scraped with a clean sharp knife and dried in an oven at 45°C for 24 h to reduce stickiness. The endocarp was broken down with a hammer to obtain seeds. Then, the seeds were dried to constant weight in an airtight oven (45°C, 72 h). Subsequently, the seeds were milled into fine particles using an electric blender and stored in zip lock bags.

Extracting *B. aegyptiaca* essential oil. The method developed by Nguefack *et al.* was used to extract essential oil from *B. aegyptiaca* seeds [24]. The experiment began by placing 500 g of pulverized seeds of *B. aegyptiaca* in a 5 L flask. After that, distilled water was added to cover the sample. Essential oil was obtained by hydrodistillation using a modified Clevenger-type apparatus at normal atmospheric pressure and 96–97°C for 4 h. The resulting essential oil was collected by drying it out with anhydrous sodium sulfate and kept at 4°C in Eppendorf tubes until the gas chromatography-mass spectrometry (GC/MS) analysis.

Evaluating the effect of *B. aegyptiaca* essential oil against *Callosobruchus maculatus*.

Experimental site. The experiment was carried out in the Agronomy Laboratory of the Department of Agriculture, Federal University, Gashua, after the growing season of 2021.

Experimental design and treatment. The research involved five storage materials (jute bags, polythene bags, sacks, plastic containers, and glass bottles), experimental samples (5, 10, and 15 mL of *B. aegyptiaca* essential oil diluted in 1 mL of acetone), a check sample (with 0.125 g of aluminum phosphide), and control (acetone). The experiment was laid out in a completely randomized design with three replications of each treatment.

Insect culture: source and rearing. *C. maculatus* was first cultured from a cowpea seed infested at the local market in Gashua. Weevils multiplied in fresh and

previously uninfested cowpea varieties in the laboratory at an ambient temperature (27–30°C) and relative humidity (70–75%).

Treatment and maintenance of cowpea seeds. Forty kilograms of pristine cowpea seeds was purchased directly from the local farmers in Gashua, immediately after harvest. To destroy and/or prevent any initial infection, they were placed in a plastic container and maintained in the freezer below 0°C for five days. After that, the seeds were taken out of the freezer and placed on a laboratory bench, covered with a screen, and left to equilibrate for 72 h [25].

Adult mortality of C. maculatus. Mortality contact effect of *B. agyptiaca* essential oil on adult *C. maculatus* was determined using the method developed by Obeng *et al.* [26]. According to the procedure, 200 g of cowpea seeds was held in different storage materials and then thoroughly mixed with: (a) 5, 10, and 15 mL of essential oil diluted in 1 mL of acetone; (b) 0.125 g of aluminum phosphide; and (c) acetone (control). After that, the storage materials were left open for 2 h at room temperature to disperse acetone. Thereafter, 20 unsexed pairs (10 males and 10 females) of three-day-old *C. maculatus* beetles were introduced into the storage materials. They were kept on laboratory benches. Dead insects were counted after 24, 48, and 72 h after infestation using Abbott's equation [27]. To confirm mortality, insects were probed three times with a sharp pin [28]. The data were subjected to Probit analysis [29].

Oviposition. For this part of the experiment, 100 g of cowpea seeds was held in the varying storage materials and thoroughly mixed with 5, 10, and 15 mL of essential oil diluted in 1 mL of acetone, 0.125 g of aluminum phosphide, and control (acetone). After that, 10 males and 10 females of three-day-old newly sexed *C. maculatus* beetles were introduced into the storage materials, where they paired and laid eggs. Following egg deposition, 100 seeds were randomly selected on days 7, 30, 60, and 90, and the number of eggs deposited on the cowpea seeds was counted and recorded in each treatment and replicate [30].

Egg hatchability. At this stage, 100 g of cowpea seeds was infested with 20 (10 males and 10 females) sexed pairs of five-to-seven-day-old *C. maculatus* beetles in a transparent plastic container. The insects paired and laid eggs for six days. After oviposition, 100 seeds (27 g) bearing eggs were chosen and placed in various storage materials that contained pure and uninfested cowpea seeds (73 g). They were thoroughly mixed with 5, 10, and 15 mL of essential oil diluted in 1 mL of acetone, 0.125 g of aluminum phosphide, and control (acetone).

In each treatment and replicate, the cowpea seeds were stored on the laboratory bench until adult beetles emerged. The number of emerged adults was recorded on days 30, 60, and 90 after the exposure. The percentage of adult emergence was calculated conversely from each of the treatments and replicates according to the method developed by Adesina and Ofuya [31], with a slight modification (1):

$$\text{Egg hatching} = \frac{Number\ of\ progeny\ emerged}{Number\ of\ eggs\ in\ treatment} \times 100 \quad (1)$$

Seed perforation. This test included 100 g of cowpea seeds held in the varying storage materials and thoroughly mixed with 5, 10, and 15 mL of essential oil, 0.125 g of aluminum phosphide, and control (acetone). After that, 10 pairs of three-day-old newly sexed *C. maculatus* beetles were introduced into the storage materials and kept in the laboratory for 90 days. Every four weeks for three months, the number of exit holes was assessed by counting in each seed from a random sample of 100 seeds.

The weevil perforation index (WPI), which measured the protective ability of the storage material, was calculated according to standard methods. If the weevil perforation index was ≥ 50%, it indicated an increase in weevil infestation or a low efficacy of the plant material. To obtain the percent protection ability (PPA), the weevil perforation index was subtracted from 100 using the following equation (2):

$$\text{WPI} = \frac{Percent\ of\ infested\ seeds}{Percent\ of\ infested\ seeds + Percent\ of\ infested\ Seeds\ in\ Control} \times 100 \quad (2)$$

where WPI is the weevil perforation index; WPI > 50 is the negative protectant of plant material, i.e., low anti-weevil activity; WPI < 50 is the positive protectant, i.e., high anti-weevil activity.

Seed weight loss. To calculate the seed weight loss, 100 g of cowpea seeds was randomly selected after 30, 60, and 90 days of storage. To obtain the final seed weight for the sample, all dead insects and other debris in the cowpea seeds were removed, and the cowpea seeds were weighed. As described by Sibakwe and Donga [32], the percentage of seed weight loss was calculated using the following equation (3):

$$\text{Weight loss} = \frac{Initial\ seed\ weight\ of\ cowpea\ seeds - Final\ seed\ weight}{Initial\ weight\ of\ cowpea\ seed\ sample} \times 100 \quad (3)$$

Seed damage. After 30, 60, and 90 days of storage, 100 g of cowpea seeds were randomly selected from the lots. We divided seeds into two groups, damaged and undamaged, and counted seeds with exit holes. Adenekan *et al.* [30] described how to quantify the percentage of damaged seed using the following equation (4):

$$\text{Seed damage} = \frac{Number\ of\ seeds\ damaged}{Total\ number\ of\ seeds} \times 100 \quad (4)$$

Seed germinability. After the storage period, 15 seeds were randomly picked from the various storage materials to test the effect of the essential oil concentrations on the germinability of cowpea seeds. A seed from each treatment was placed in 9-cm Petri dishes with moistened Whatman filter paper on laboratory benches at room temperature (27–30°C) and relative humidity (70–75%) [33]. Each treatment was triplicated. To avoid contamination, the seeds were watered (23 mL) twice a day (morning and evening) with distilled water from a wash bottle. According to Olisa *et al.* [34], the germination percentage of cowpea seeds was estimated from germination data on day 7 after sowing according to the following equation (5):

$$\text{Germination percentage} = \frac{Number\ of\ emerged\ seedlings\ at\ the\ final\ count}{Total\ number\ of\ seeds\ planted} \times 100 \quad (5)$$

Data analysis. Natural mortality in the control samples was corrected using Abbott's formula [27]. The acquired numerical data was square root transformed $\sqrt{n+1}$, and the adjusted mortality and other data in percentages was transformed *arc sine* before being subjected to the analysis of variance using JMP 13 Computer Software (2016). The Student Newman-Keuls (SNK) test was used to differentiate significant treatment means at the 5% level of probability. With the SPSS statistical software (version 19), the data were subjected to a two-way analysis of variance (ANOVA) at the 5 % significance level, and Duncan's Multiple Range Test was used to separate the means.

RESULTS AND DISCUSSION

Mortality of *Callosobruchus maculatus* L. exposed to *Balanites aegyptiaca* L. essential oil stored in different storage materials after 24, 48, and 72 h. The treatment under discussion provided appropriate protection to cowpea seeds against *C. maculatus*. Table 1 shows the effect of *B. aegyptiaca* oil extract and such storage materials as jute bags, polythene bags, sacks, plastic containers, and glass bottles on cowpea weevil mortality.

The experiment showed a statistically significant correlation between the effects of *B. aegyptiaca* essential oil and storage materials ($F(16,75) = 41.813$, $P = 0.000$) after 24 h of exposure. A simple main effect analysis showed that *B. aegyptiaca* essential oil had a statistically significant effect on mortality of cowpea weevils at 24, 48, and 72 h, respectively ($P < 0.000$).

Glass bottles, plastic containers, and jute bags proved to be the most effective storage material, while samples stored in polythene bags had the lowest beetle mortality rate at all the *B. aegyptiaca* essential oil concentrations. Our results confirmed those obtained by Buleti *et al.*, who reported a higher weevil mortality rate in grains stored in glass bottles compared to other storage materials [15]. On the other hand, the results can be explained by the techno-functional properties of the storage materials, e.g., water vapor permeability, the interaction between the plant extracts and the material, etc. [35]. Thus, such vapor proof containers as glass bottles, plastic containers, and jars can provide good insulation against weevils, thereby inhibiting their survival: insects suffocate as soon as they run out of oxygen.

In addition, the abrasive effect and contact toxicity of essential oils on the pest cuticle interferes with insect respiratory mechanism, thereby causing a knock down effect. This study is similar to the research conducted by Karimzadeh *et al.*, who reported that the abrasive effects of combined insecticides may cause abrasion of insect cuticle and dehydration of the insect body, thus leading to insect mortality [36].

Evidently, the *B. aegyptiaca* essential oil treatments had a noticeable effect on the population growth rate and mortality of the weevils. Low quantity of the essential oil (5 mL) resulted in a lower mortality rate, while high quantities (15 mL) provided the highest mortality rate, irrespective of the storage material used.

Similarly, the population of the weevils decreased as the treatment intervals progressed from 24 to 72 h. For instance, at the same essential oil dose, plastic containers and glass bottles caused 62.5 and 70% mortality rate, respectively, after 24 h. Likewise, 80 and 90% mortality rate were recorded after 48 h. However, 100% mortality was recorded for glass bottles, plastic containers, and jute bags after 72 h. Most importantly, all the three doses and storage materials showed high mortality rates of *C. maculatu*s after 72 h of exposure, if compared to the control samples.

The insecticidal efficacy of *B. aegyptiaca* essential oil could be attributed to such active compounds as hexadecanoic acid, (9*Z*,12*Z*)-octadeca-9,12-dienoic acid, (*Z*)-octadec-9-enoic acid, ethyl hexadecanoate, 3,3-dihydroxypropyl hexadecanoate, and methyl hexadecanoate. All these compounds have been reported to possess repellent and insecticide activities [17]. This result confirms the findings obtained by Mokhtar *et al.*, who observed 100% mortality of *C. maculatus* after 24 h on cowpeas treated with chloroform extract of *B. aegyptiaca* seeds at 1.131 mg·cm^{-2} [17]. Various studies have also demonstrated the insecticidal effect of *B. aegyptiaca* essential oil against such pests as *C. maculatus*, *Tribolium casteneum*, and khapra beetle [17,18].

In our research, various doses of *B. aegyptiaca* essential oil and storage materials provided an excellent protection against *C. maculatus*, both independently and synergistically. *B. aegyptiaca* essential oil extract had a substantial impact on the longevity and survival of cowpea weevils. In addition, glass bottles, plastic containers, and jute bags caused 100% mortality at the highest dose (15 mL) after 72 h of exposure.

Table 1 Mortality of cowpea weevils exposed to *Balanites aegyptiaca* oil extract in different storage materials after 24, 48, and 72 h

Samples	Storage materials	Mortality ± SE, %		
		24 h	48 h	72 h
Balanites aegyptiaca essential oil (5 mL)	Glass bottle	20.00 ± 0.00	46.50 ± 4.79	90.00 ± 0.00
	Jute bag	12.50 ± 5.00	25.00 ± 4.08	70.00 ± 0.00
	Plastic container	20.00 ± 0.00	60.00 ± 0.00	61.20 ± 2.50
	Polythene bag	10.00 ± 0.00	25.00 ± 0.00	60.00 ± 0.00
	Sack bag	10.00 ± 0.00	30.00 ± 0.00	75.00 ± 0.00
Balanites aegyptiaca essential oil (10 mL)	Glass bottle	40.00 ± 0.00	65.00 ± 5.77	100.00 ± 0.00
	Jute bag	30.00 ± 0.00	60.00 ± 0.00	83.60 ± 2.50
	Plastic container	35.00 ± 0.00	75.00 ± 0.00	90.00 ± 0.00
	Polythene bag	23.70 ± 2.50	42.00 ± 5.00	70.00 ± 0.00
	Sack bag	32.50 ± 2.89	50.00 ± 0.00	90.00 ± 0.00
Balanites aegyptiaca essential oil (15 mL)	Glass bottle	70.00 ± 0.00	80.00 ± 0.00	100.00 ± 0.00
	Jute bag	50.00 ± 0.00	70.00 ± 0.00	100.00 ± 0.00
	Plastic container	62.50 ± 2.89	90.00 ± 0.00	100.00 ± 0.00
	Polythene bag	32.50 ± 2.89	55.00 ± 0.00	80.00 ± 0.00
	Sack bag	55.00 ± 0.00	70.00 ± 0.00	97.50 ± 5.00
Aluminum phosphide	Glass bottle	90.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	Jute bag	81.20 ± 2.50	90.00 ± 0.00	100.00 ± 0.00
	Plastic container	90.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	Polythene bag	85.00 ± 4.08	90.00 ± 0.00	95.00 ± 5.77
	Sack bag	90.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Control (acetone)	Glass bottle	0	0	20.00 ± 0.00
	Jute bag	0	0	0
	Plastic container	0	0	02.50 ± 2.89
	Polythene bag	0	0	0
	Sack bag	0	0	0
A		$P < 0.05$	$P < 0.05$	$P < 0.05$
B		$P < 0.05$	$P < 0.05$	$P < 0.05$
AB		$P < 0.05$	$P < 0.05$	$P < 0.05$

A – essential oil; B – storage material

Oviposition of *C. maculatus* exposed to *B. aegyptiaca* essential oil stored in different storage materials after 7, 30, and 90 days of storage. Figures 1–3 show the mean value of oviposition in both treated and untreated cowpeas. The analysis of variance showed that a statistically significant correlation between the effects of *B. aegyptiaca* oil extracts and storage materials on the oviposition of cowpea weevils ($F(16,75) = 3346.73$, $P = 0.000$). When compared to the control, the treated samples showed a much lower oviposition. At a seven-day interval, all the storage materials showed the same trend in the total oviposition of cowpea weevils as the essential oil doses increased, except polythene bags (Fig. 1).

B. aegyptiaca essential oil significantly reduced the number of eggs laid by weevils. The highest amount of eggs was recorded at 5 mL, and then it significantly decreased at 10 mL. The lowest amount of eggs was registered at 15 mL. The drastic reduction in the number of eggs laid by cowpea weevils might have been caused by the toxicity of the plant material active components to the weevils rather than by the prevention of oviposition.

The previous section that the *B. aegyptiaca* essential oil caused the highest mortality rate in the experimental samples, which was associated with its insecticidal effect. The chemical composition of plant oils and their phytocompounds is known to produce a toxic and repellent effect on insects that live in stored grain [37].

Grains stored in glass bottles had the fewest eggs, while those stored in polythene bags had the maximal number of eggs. Even though polythene bags had the lowest effect on weevil oviposition, they also showed a significant reduction in the number of eggs laid compared to the untreated samples. Buleti *et al.* also reported a reduction in the number of eggs laid in glass bottles [15]. Furthermore, the experimental samples revealed just a few eggs on day 90, with a mean fecundity of 1–4 eggs for glass bottles and 1–14 eggs for plastic containers. A slightly higher value was recorded for polythene and sack bags with a mean of 18–45 and 8–30 eggs, respectively. However, the values recorded

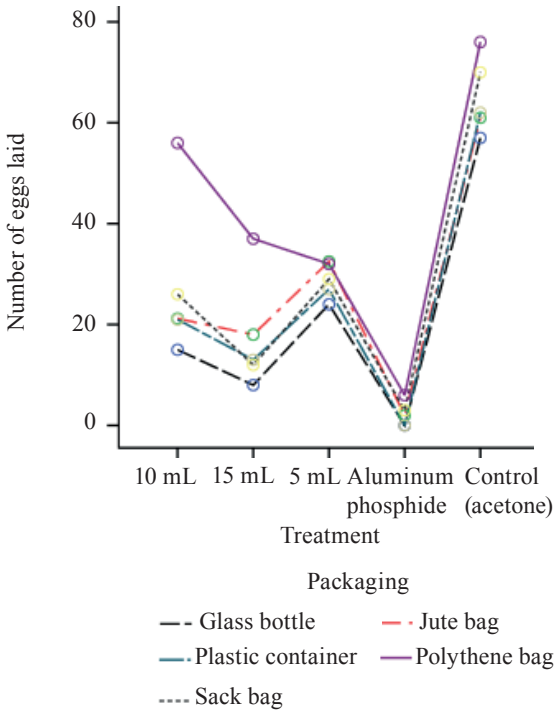

Figure 1 Effect of *Balanites aegyptiaca* on *Callosobruchus maculatus* oviposition at various treatments in different storage materials after 7 days of storage

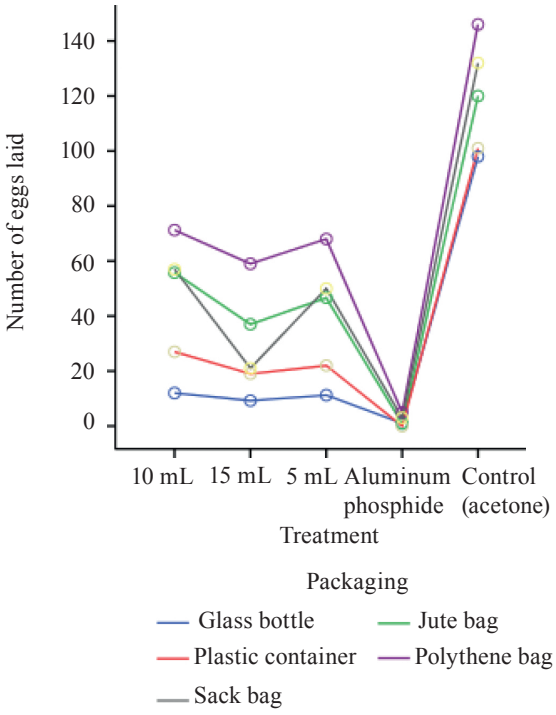

Figure 2. Effect of *Balanites aegyptiaca* on *Callosobruchus maculatus* oviposition at various treatments in different storage materials after 30 days of storage

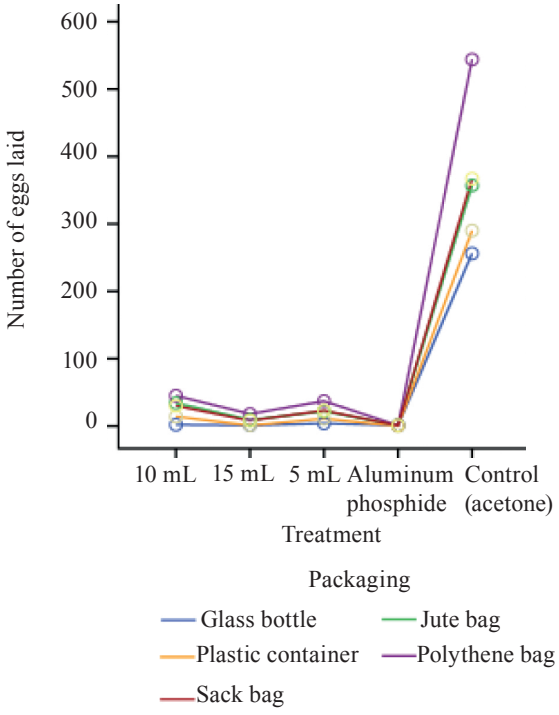

Figure 3. Effect of *Balanites aegyptiaca* on *Callosobruchus maculatus* oviposition at various treatments in different storage materials after 90 days of storage

in polythene and sack bags were lower than in the control samples with a mean fecundity of 256–544 eggs per female after 90 days, which implied a significant difference in the oviposition of treated cowpeas.

The synergic treatment of *B. aegyptiaca* essential oil and storage materials killed more than 50% of the total eggs laid at various stages of development from 7 to 90 days. Likewise, the high numbers of eggs laid in polythene and sack bags could be explained by the porous surface of these materials that allowed moisture and air circulation. Such conditions encouraged weevils present in the seed lot to lay eggs and proliferate continuously.

The lower oviposition rates observed in this study suggested that *B. aegyptiaca* essential oil could be useful as cowpea protectants. This finding confirms that made by Alves *et al.*, who discovered that lemon grass essential oil extract reduced *C. maculatus* oviposition significantly [38]. Previous studies by a Nwaogu and Yahaya and Aous *et al.* reported the effect of essential oil of *Cymbopogon schoenanthus* (L.) on the development of freshly laid eggs and newborn larvae of *C. maculatus* [18, 39]. The extract probably contained a powerful oviposition deterrent. Also, cowpea seeds that are packaged in glass bottles and plastic containers showed a low oviposition, which made them the optimal ovipositional deterrents in this study.

Egg hatchability of *C. maculatus* exposed to *B. aegyptiaca* essential oil stored in different storage materials after 24, 48, and 72 h. Table 2 shows the effect of *B. aegyptiaca* essential oil on *C. maculatus* egg development and hatchability. The main significant effect of *B. aegyptiaca* essential oil and storage materials ($P < 0.05$) was observed after 30, 60, and 90 days. Similarly, the study revealed no significant

Table 2 *Callosobruchus maculatus* egg hatchability treated with *Balanites aegyptiaca* essential oil in different storage materials after 30, 60, and 90 days of storage

Samples	Storage materials	Mean number of hatched eggs ± SE		
		30 days	60 days	90 days
Balanites aegyptiaca essential oil (5 mL)	Glass bottle	4.10 ± 0.17	2.40 ± 0.25	1.00 ± 0.00
	Jute bag	7.00 ± 0.00	4.70 ± 0.14	1.30 ± 0.08
	Plastic container	4.20 ± 0.68	3.10 ± 0.78	1.10 ± 0.03
	Polythene bag	8.60 ± 0.79	7.70 ± 0.17	5.50 ± 0.05
	Sack bag	6.40 ± 0.36	4.60 ± 0.09	1.60 ± 0.00
Balanites aegyptiaca essential oil: (10 mL)	Glass bottle	1.50 ± 0.78	1.00 ± 0.00	1.00 ± 0.00
	Jute bag	5.10 ± 0.15	3.60 ± 0.79	1.20 ± 0.02
	Plastic container	2.20 ± 0.15	1.20 ± 0.96	1.00 ± 0.01
	Polythene bag	5.40 ± 0.34	6.30 ± 0.24	3.30 ± 0.28
	Sack bag	3.20 ± 0.00	2.00 ± 0.02	1.00 ± 0.00
Balanites aegyptiaca essential oil: (15 mL)	Glass bottle	0.80 ± 0.09	1.00 ± 0.00	1.00 ± 0.00
	Jute bag	3.00 ± 0.00	2.20 ± 0.11	1.00 ± 0.00
	Plastic container	1.10 ± 0.05	1.10 ± 0.10	1.00 ± 0.00
	Polythene bag	2.90 ± 0.12	6.10 ± 0.13	1.90 ± 0.01
	Sack bag	2.90 ± 0.17	1.90 ± 0.07	1.00 ± 0.00
Aluminum phosphide	Glass bottle	0.20 ± 0.06	1.00 ± 0.00	1.00 ± 0.00
	Jute bag	1.00 ± 0.13	1.00 ± 0.00	1.00 ± 0.00
	Plastic container	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
	Polythene bag	1.10 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
	Sack bag	0.50 ± 0.04	1.00 ± 0.00	1.00 ± 0.00
Control (acetone)	Glass bottle	16.60 ± 0.00	20.40 ± 0.67	42.10 ± 1.14
	Jute bag	23.40 ± 0.84	27.30 ± 1.51	52.60 ± 31.1
	Plastic container	18.20 ± 0.56	21.90 ± 0.89	52.00 ± 0.04
	Polythene bag	31.30 ± 0.76	38.90 ± 0.93	72.40 ± 1.47
	Sack bag	26.80 ± 1.30	26.20 ± 0.46	66.40 ± 0.28
A		$P < 0.05$	$P < 0.05$	$P < 0.05$
B		$P < 0.05$	$P < 0.05$	$P < 0.05$
AB		$P > 0.05$	$P > 0.05$	$P > 0.05$

A – essential oil; B – storage material

($P > 0.05$) interactive effect of *B. aegyptiaca* essential oil and storage materials on *C. maculatus* egg hatchability. The results demonstrated that the productivity in the experimental samples was extremely low at all intervals and doses, with the mean values ranging from 1 to 8.6.

Hence, over 65% of the total eggs laid in the experimental samples died at different stages of development in all the trials. *B. aegyptiaca* oil extracts obviously had a strong larvicidal effect on the development of immature weevils. Similarly, the high mortality of *C. maculatus* in the experimental samples implied that the plant had some phytochemical properties, which reduced egg production [40].

Furthermore, the egg hatchability reduced as the storage interval progressed from 30 to 90 days. Among the experimental samples, the highest mean values of egg hatchability were observed in cowpeas stored in polythene bags at all the essential oil concentrations. However, the values observed in polythene bags were lower in comparison to the control samples. Therefore, the low egg hatchability was caused by the effectiveness of *B. aegyptiaca* essential oil with its poisonous component and physical properties, which affected the surface and oxygen tension of eggs.

The essential oils of *Borago officinalis*, *Melissa officinalis*, *Carapichea ipecacuanha*, and *Laurus nobilis* have also been reported to reduce hatchability [41]. Similarly, *Piper gaudichaudianum* essential oil showed a better insecticidal activity against *Lucilia cuprina* third instar larvae under laboratory conditions [42].

Weevil Perforation Index, weight loss, damage, and germinability of cowpea seeds treated with *B. aegyptiaca* essential oil stored in different storage materials after 30, 60, and 90 days. These parameters of cowpea seeds indicate its suitability for consumption and other aesthetic values because damaged seeds with holes and flour dust are not marketable. Figures 4 and 5 show the weevil perforation index for cowpea seeds treated with different concentrations of *B. aegyptiaca* essential oil packaged in different storage materials on days 30, 60, and 90.

After 30 days, the highest weevil perforation index was observed in polythene and sack bags. When compared to the control treatment, the cowpea seed treated with *B. aegyptiaca* essential oil showed a

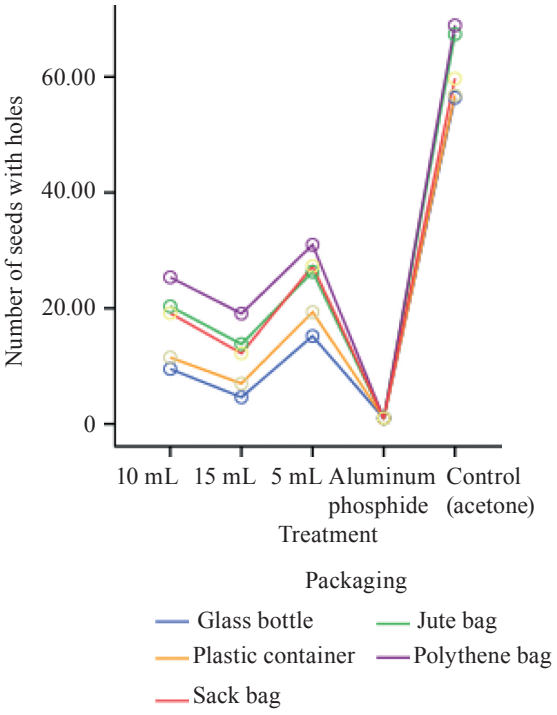

Figure 4 % Weevil perforation index of cowpea seeds treated with *Balanites aegyptiaca* in different storage materials after 30 days of storage

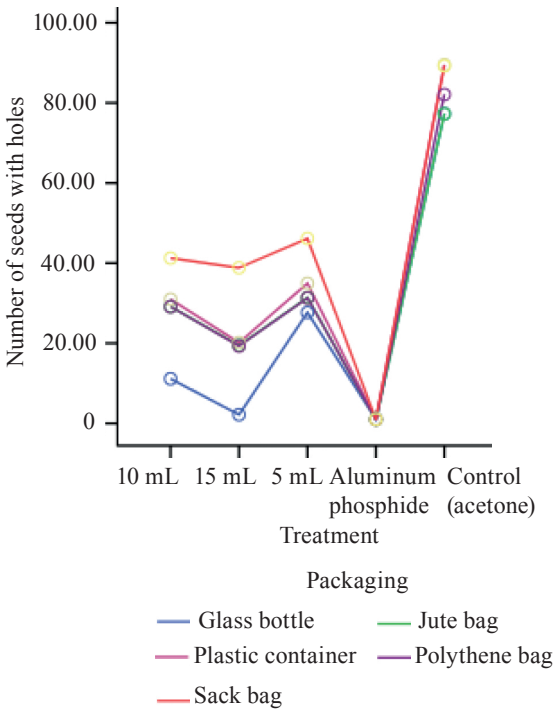

Figure 5 % Weevil perforation index of cowpea seeds treated with *Balanites aegyptiaca* in different storage materials after 90 days of storage

substantial reduction in seed damage. The analysis of variance showed a statistically significant effect of *B. aegyptiaca* oil extracts and storage materials on weevil perforation index ($F(16, 75) = 176.150$, $P = 0.000$). Exposure of weevil-infested cowpea seeds to *B. aegytiaca* essential oil in various storage materials resulted in a significant reduction in seed weight ($F(16,75 = 311.357$, $P = 0.000$).

The weevil perforation index decreased following the increase in *B. aegytiaca* essential oil concentration for all storage materials. The lowest weevil perforation index was observed in the seeds packaged in glass bottles followed by those stored in plastic containers. The highest weevil perforation index was recorded in the seeds stored in polythene bags throughout the storage period (Figs. 4 and 5).

Furthermore, exposure of weevil-infested cowpea seeds to *B. aegytiaca* essential oil in various storage materials resulted in a significant reduction in seed weight ($F(16,75) = 311.357$, $P = 0.000$). After 90 days of exposure, the seeds treated with *B. aegyptiaca* essential oil in all the storage materials showed a significantly low weight loss at all doses (5, 10, and 15 mL), except the cowpea seeds stored in polythene bags (Table 3). For these parameters, interactive treatment of *B. aegyptiaca* essential oil and storage materials had equally significant effects ($P < 0.05$) on cowpea weevils.

Taken together, *B. aegyptiaca* had a more detrimental effect on the weevils in comparison to the control. A similar research by Borzoui *et al.* also registered a significantly low amount of seed damage because the oviposition rate was reduced by the sublethal doses of essential oil [43]. Among all the storage materials, glass bottles and plastic bags showed the lowest weevil perforation index, seed weight loss, and seed damage. The low seed weight loss and damage observed in the seeds stored in these storage materials could be attributed to the significant reduction of weevils that could have caused seed damage.

B. aegyptiaca essential oil and storage materials demonstrated both individual and interactive effects on the germination rate of cowpea seeds. The germination rate of the treated seeds ranged from 46.7 to 93.3%, which was lower than the values reported for cowpea seed germination rate by Gad *et al.* [44].

An increased germination rate of cowpea seeds was observed with increased *B. aegyptiaca* essential oil concentration, which implied a strong relationship between the treatment and germination rate. Similar results were described by Bhavya *et al.* and Harshani and Karunaratne, who indicated that essential oils and their principal components affected seed germination. Storage materials also affected seed germination [45, 46].

In this study, glass bottles proved to be the optimal storage material as indicated by the maximal seed germination rate of 88.3, 91.6, and 93.3% after essential oil treatment of 5, 10, and 15 mL, respectively. Storing seeds in appropriate storage material retained higher germination capacity rate.

Table 3 Effect of *Balanites aegyptiaca* essential oil and storage material on cowpea seed damage, weight loss, and germination rate caused by *Callosobruchus maculatus* infestation

Samples	Storage materials	Seed weight loss			Seed damage			Germination rate
		30 days	60 days	90 days	30 days	60 days	90 days	
Balanites aegyptiaca essential oil (5 mL)	Glass bottle	8.30 ± 0.00	3.50 ± 0.03	1.00 ± 1.63	1.00 ± 0.00	1.00 ± 0.05	3.00 ± 0.00	88.30 ± 3.30
	Jute bag	13.40 ± 0.16	6.00 ± 0.19	1.50 ± 0.25	2.00 ± 1.63	4.50 ± 0.14	7.50 ± 0.21	64.60 ± 4.91
	Plastic container	12.50 ± 0.29	3.90 ± 0.00	1.20 ± 0.19	1.00 ± 0.00	1.90 ± 0.22	3.30 ± 0.23	80.00 ± 0.00
	Polythene bag	15.60 ± 0.17	8.50 ± 0.25	3.00 ± 0.16	5.90 ± 0.25	7.00 ± 0.17	11.40 ± 1.49	46.70 ± 0.00
	Sack bag	13.60 ±0.47	6.80 ± 0.14	1.40 ± 0.25	2.20 ± 0.00	5.00 ± 0.16	7.70 ± 0.81	60.00 ± 0.00
Balanites aegyptiaca essential oil (10 mL)	Glass bottle	7.80 ± 0.09	2.00 ± 0.08	0.80 ± 0.00	0	1.00 ± 0.00	1.90 ± 0.17	91.60 ± 3.30
	Jute bag	11.70 ± 0.04	5.40 ± 0.32	1.50 ± 0.00	2.00 ± 0.00	4.50 ± 0.10	7.00 ± 0.08	60.00 ± 0.00
	Plastic container	10.00 ± 0.13	2.30 ± 0.34	0.90 ± 0.17	0	1.00 ± 0.00	2.00 ± 0.00	80.00 ± 0.00
	Polythene bag	13.00 ± 0.17	11.50 ± 0.57	2.50 ± 0.21	3.90 ± 0.41	7.00 ± 0.00	9.10 ± 0.87	55.00 ± 2.01
	Sack bag	12.00 ± 0.74	5.70 ± 0.19	1.30 ± 0.28	2.00 ± 0.16	5.40 ± 0.18	6.90 ± 0.01	60.00 ± 0.00
Balanites aegyptiaca essential oil (15 mL)	Glass bottle	2.80 ± 0.16	1.60 ± 0.02	0.30 ± 0.23	0	1.00 ± 0.00	1.50 ± 0.00	93.30 ± 0.00
	Jute bag	10.80 ± 0.48	3.10 ± 0.00	0.70 ± 0.19	0	2.00 ± 0.07	5.00 ± 0.16	66.70 ± 0.00
	Plastic container	3.10 ± 0.24	1.90 ± 0.10	0.30 ± 0.19	0	1.00 ± 0.00	2.00 ± 0.71	93.00 ± 0.00
	Polythene bag	11.70 ± 0.84	9.70 ± 0.22	1.40 ± 0.16	2.00 ± 0.16	5.00 ± 0.38	6.00 ± 0.00	65.00 ± 1.88
	Sack bag	10.50 ± 0.38	3.40 ± 0.13	0.80 ± 0.00	0	4.00 ± 0.16	5.00 ± 0.00	60.00 ± 0.00
Aluminum phosphide	Glass bottle	0	0	0.10 ± 0.00	0	0	0	100.00 ± 0.00
	Jute bag	0	0	0.10 ± 0.00	0	0	0	100.00 ± 0.00
	Plastic container	0	0	0.10 ± 0.00	0	0	0	100.00 ± 0.00
	Polythene bag	0	0	0.10 ± 0.00	0	0	0	100.00 ± 0.00
	Sack bag	0	0	0.10 ± 0.00	0	0	0	100.00 ± 0.00
Control (acetone)	Glass bottle	18.60 ± 0.26	37.40 ± 0.13	42.00 ± 0.08	15.70 ± 1.25	28.00 ± 0.16	71.80 ± 0.43	26.70 ± 0.00
	Jute bag	28.60 ± 0.36	42.40 ±1.77	48.50 ± 0.00	20.90 ± 0.68	30.80 ± 1.45	79.70 ± 1.39	20.00 ± 0.00
	Plastic container	20.80 ± 0.16	40.30 ± 1.57	45.70 ± 0.22	16.00 ± 0.16	29.20 ± 0.66	77.80 ± 0.44	26.70 ± 0.00
	Polythene bag	35.60 ± 0.77	56.00 ± 0.00	56.50 ± 0.25	23.70 ± 0.48	38.60 ± 0.41	91.50 ± 1.07	13.30 ± 0.00
	Sack bag	26.40 ± 0.16	44.30 ± 1.80	50.00 ± 1.63	18.90 ± 0.17	34.10 ± 1.57	84.10 ± 1.72	20.00 ± 0.00
A		$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
B		$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
AB		$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$

A – *Balanites aegyptiaca* essential oil; B – storage material

Therefore, the *B. aegyptiaca* essential oil could be used to protect cowpea seeds stored in glass bottles because the values observed were related to the international germination threshold of 90% required by seed exportation. This result is similar that obtained by Buleti *et al.*, who also used glass bottles as storage containers [15].

On the contrary, cowpea seeds stored in polythene bags displayed the lowest germination rate, which indicated a strong relationship between weight loss, damage score, and germination rate. However, the germination rates were not as highly related with seed damage traits. The lowest germination rate observed could be explained by weevil damage that occurred due to minimal weevil mortality rate in polythene bags, which, in its turn, led to nutrient exhaustion [15]. Storing seeds in inappropriate storage materials could significantly decline their germination rate and resulted in a rapid loss of seed viability.

Overall, even though *B. aegyptiaca* essential oil had a stronger effect on cowpea weevils, they failed to remove cowpea weevils completely; rather, they only lowered their numbers. Thus, hermetic storage could enhance the quality of cowpea seeds during storage.

CONCLUSION

The combination of essential oil of *Balanites aegyptiaca* L. and storage materials had significant effects on weevil proliferation in cowpea seeds ($P > 0.05$) during storage. *B. aegyptiaca* essential oil proved to possess insecticide properties that can help control *Callosobruchus maculatus* L. in stored cowpea seeds.

After 90 days of storage, mortality, oviposition, and egg hatchability fell down, following the increase in the concentration of *B. aegyptiaca* essential oil. In addition, such storage materials as glass bottles, plastic containers, and jute bags also reduced the population of cowpea weevils in cowpeas during storage. Hermetic storage material – glass bottles – had the greatest effecton weevil infestation and sustained the quality of cowpeas under storage conditions. These findings suggest that *B. aegyptiaca* essential oil could be useful as a botanical insecticide against cowpea pests. A large-scale trial is required to perform a toxicity assay of *B. aegyptiaca* essential oil.

CONTRIBUTION

Feyisola Fisayo Ajayi obtained the funds, designed the experiment, collected the data, and wrote the manuscript original draft, as well as performed the formal laboratory research. Akama Friday Ogori conducted the formal analysis and research, wrote the article, reviewed scientific publications, and edited the manuscript. Vivien O. Orede performed the formal analysis and research, reviewed and editing the manuscript. Emmanuel Peters performed the formal analysis and research, as well as provided the experimental design. All the authors were equally involved in reading and approving of the final manuscript before submission and are equally accountable for any potential cases of plagiarism.

CONFLICT OF INTEREST

The authors declare no conflict of interests regarding the publication of this article.

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