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Bioefficacy of *Alstonia boonei* leaf extract against cowpea beetle *Callosobrochus maculatus* infesting stored cowpea seeds in storage

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Abstract. This study was conducted to investigate the efficacy of the oils of *Alstonia boonei* leaf extracted with n-hexane, petroleum ether, methane and acetone as contact insecticides against the activities of C*allosobrochus maculatus* in stored cowpea seed. The oils were incorporated at rates 2.0 mL per 20 g of cowpea seeds. The parameters assessed include, mortality of adult insects, oviposition and adult emergence to ascertain the control of the beetle. All concentration of the extracts used evoked 100% mortality of *C. maculatus* after 72 h of post treatment. The development of C*allosobrochus maculatus* was inversely proportional to the concentration of the oil. As the ratio of *Alstonia boonei* leaf oil extract increased, the mortality of the beetle increased. Therefore, complete protection of seeds and complete inhibition of adult emergence in the oils extracts of *Alstonia boonei* leaf were effective in controlling cowpea bruchid in stored cowpea seed.

Keywords: *Alstonia boonei*; Leaf oil extracts; Cowpea seed; *Callosobrochus maculates*; Seed protection.

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Introduction

Cowpea *Vigna unguiculata* L. (Walp), is an important crop in the tropics especially in West Africa where it is a cheap source of dietary protein (Boeke et al., 2004). Cowpea is widely cultivated and consumed in Nigeria; the most important producing areas in Nigeria are located in the savanna (Agboola, 1979). According to Blade et al. (1997), Nigeria accounts for 70% of the world's production. Cowpea is an extremely valuable crop both as a source of revenue and an important source of cheap dietary protein for the third world where meat is expensive (Ileke and Olotuah, 2012). High protein and lysine contents of cowpea make it a natural supplement to staple diets of cereals, roots, tuber and fruits (Bressani, 1985). In Nigeria, the production of cowpea is carried out largely by peasant farmers and cowpea production is limited by insect pests which cause serious post-harvest losses to the cowpea grain. The value of cowpea lies in its high protein content and ability to tolerate drought and also its compatibility as an intercrop with maize, millet, sorghum, sugarcane and cotton (Caswell, 1984). This makes the grain legumes an important component of traditional intercropping systems especially in the complex and elegant subsistence farming systems of the dry savanna in sub-Saharan Africa.

Cowpea bruchid, *Callosobruchus maculatus* depreciates stored cowpea (Jackai and Adalla 1997). The huge post-harvest losses and quality deterioration caused by this insect pest are threat to food security in a developing nation like Nigeria (Boeke et al., 2004).

The infestation of cowpea seeds by Callosobrochus maculates (F.) accounted for the major loss of cowpea both in store and at fields (Adedire, 2001; Udo, 2005). The females of the bruchid lay their eggs on the seed surface which hatch into larvae within 5 days. The larvae bore through the seed coat into the underlying cotyledons where development continues until adult emergence; the adults are already sexually mature on emergence (Credland and Wright, 1989; Ofuya and Credland, 1995).

Effective and efficient controls of storage insect pests are centered mainly on synthetic insecticides. The use of these synthetic chemicals is hampered by many attendant problems like development of resistance insect strain, toxic residues in foods and humans; workers safety and high cost of procurements (Adedire and Lajide 2001, Ileke and Oni, 2011, Ileke and Olotuah, 2012). These have necessitated research on the use of alternative eco-friendly insect pest control methods among which are the use of plant products (Ileke and Oni, 2011). Lale (1995) reported that plants materials and local traditional methods are safer than synthetic insecticides and suggested that their use needed exploitation. Small scale farmers in the tropics have reported the need to protect cowpea against pest damage during storage (Lale, 1995; Golob et al., 1999). The potential of using plant products such as leaf, bark, root powder from certain plants such as Capsicum spp, Piper spp, and citrus peels have been investigated by Rajapakse and Van Emden (1997) and Ileke and Bolus (2012).

Alstonia boonei De wild is a medicinal plant of West and Central African origin which is extensively used for the treatment of malaria fever, intestinal helminthes, snake bite, arrow, impotence, toothache and oedema (Terashima, 2003; Akinmoladun et al., 2007). The various ethnomedical, chemical, pharmacological and toxicological properties of *Alstonia boonei* were recently reviewed and the profile further revealed that it is useful in the treatment and management of several illnesses (Adotey et al., 2012). In spite of its vast acceptability as a medicinal plant, the insecticidal potential of *A. boonei* has not been fully explored compared to other botanicals used in storage. The objectives of this study is to investigate the efficacy of *A. boonei* leaf extract against the biological activities of *Callosobruchus maculatus* in storage.

Materials and method

Insect cultures

The starter culture of *Callosobruchus maculatus* was obtained from an infested cowpea seeds at Environmental Biology and Fisheries laboratory, Adekunle Ajasin University, Akungba Akoko, Nigeria. The cowpea were first sorted to eliminate stones, chaff and other unwanted material, and were later disinfected by keeping them in a freezer at -5 °C for 7 days. The disinfected seeds were then air dried in the laboratory to prevent mouldiness before the introduction of insects from infected ones (Adedire et al., 2011). They were placed in kilner jars and covered with muslin cloth. The jars were placed in insect rearing cages at ambient temperature of 30±3 °C and 70%±5% relative humidity.

Plant collection

Fresh leaves of *Alstonia boonei* were collected once from Ijiro, Ilasa Ekiti, Ekiti State. The plant leaves were air dried in an open laboratory and ground into very fine powder using an electric blender (Supermaster, Model SMB 2977, Japan). The powders were further sieved to pass through 1 mm^2 perforations prior to storage in a plastic with tight lids in a refrigerator at 4 °C before use (Ileke and Oni, 2011).

Oil extract

Extracts of *Alstonia boonei* was carried out using cold extraction method. About 250 g of *A. boonei* powders were soaked separately in an extraction bottle containing absolute acetone, petroleum ether, methanol and N-hexane. The mixture was stirred occasionally with a glass rod and extraction was terminated after 72 h. Filteration was carried out using a double layer of Whatman No. 1 filter paper and solvent evaporated using a rotary evaporator at 30 °C to 40 °C with rotary speed of 3 to 6 rpm for 8 h (Ileke and Olotuah, 2012). The resulting extract was air dried in order to remove traces of solvents.

Toxicity of *A. boonei* leaf oil extract on mortality, oviposition, adult emergence of *Callosobruchus maculatus,* weight loss, damage and weevil perforation index of cowpea seed

The toxic effect of plants oil on adult *C. maculatus* was accomplished using 250 mL plastic containers containing 20 g of cowpea seeds with concentration of 2 mL of solvent extract of *Alstonia boonei* leaf. The oil and seeds were thoroughly mixed with the aid of a glass rod and agitated for 5-10 min to ensure uniform coating. The containers were left open for 30 min so as to allow trace of solvent to evaporate off. Ten copulating pairs of *C. maculatus* were introduced into the container and mortality was observed daily for 4 days. Cowpea seeds that were solvent treated served as the control experiment (Arannilewa et al., 2006). Similar preparation was set up for *Alstonia boonei* leaf extracted with N-hexane, petroleum ether and methanol. Three replicates per treatment were prepared. The adult mortality was assessed every 24 h for 96 h. Adult insects were considered dead where no response was observed after probing them with forceps. After 96 h, all insects both dead and alive were then removed. Number of eggs laid in each container was counted and recorded.

Progeny emergence (F1) was then recorded at 6 weeks. The containers were sieved out and newly emerged adult bruchid were counted with an aspirator. The percentage adult emergence was calculated according to the method described by Odeyemi and Daramola (2000):

$$\% \text{ Adult emergence} = \frac{\text{No of adults emerged}}{\text{No of eggs laid}} \times 100$$

The cowpea were re-weighed by using Metler weighing balance and percentege loss in weight was determined as described by Odeyemi and Daramola (2000):

$$\% \text{ Weight loss} = \frac{\text{Difference in weight}}{\text{Initial weight}} \times 100$$

After re-weighing, the numbers of damaged cowpea seeds were evaluated by counting wholesome and bored or seed with bruchid emergent holes. Percentage seed damaged was calculated according to the method described by Ileke and Olotuah (2012) as follows:

$$\% \text{ seed damage} = \frac{\text{Number of seeds damaged}}{\text{Total number of seeds}} \times 100$$

Weevil Perforation Index (WPI) were adopted from the analysis of damage using the method of Adedire and Ajayi (1996). The weevil perforation is defined as follows:

$$\text{WPI} = \frac{\% \text{ treated cowpea seeds perforated}}{\% \text{ control cowpea seeds perforated}} \times 100$$

Statistical analysis

Data were subjected to analysis of variance (ANOVA) in SPSS 21.0 software and treatment means were separated using the New Duncan's Multiple Range Test.

Results

Effects of *Alstonia boonei* leaf extracts on adult mortality of *Callosobruchus maculatus* (F.)

Table 1 shows the mortality rate of *C. maculatus* in 20 g of cowpea seeds treated with 2 mL of *Alstonia boonei* oil extracts. The effect of N-hexane was significantly ($P > 0.05$) higher at 24 h on mortality rate of *C. maculatus* and 72 h in all the cowpea seeds treated with 2 mL of leaf oils. At 24 h post treatment with 2 mL of extracts, the percentage mortality were 60%, 33%, 53%, 33%, 50%, 30%, and 0%, with N-hexane pet ether, acetone, methanol, and control, respectively. At 48 h the percentage mortality rate increases to 80%, 76.67%, 70%, 53.33%, 0%, in N-hexane, pet-ether, acetone and methanol, respectively, without any mortality in the control experiment while at 72 h post treatment, there was an increase in the percentage mortality. The percentage mortality rate at 96 h of post treatment was 100% in all treatment. The mortality rate of adult *C. maculatus* in the control was significantly higher than those in cowpea seeds treated *Alstonia boonei* oil. N-hexane extract of *A. boonei* leaf was observed to have the highest mortality effect on *C. maculatus*. The control was significantly higher than those in cowpea seeds treated with other oils.

Table 1. Mortality of adult *C. maculatus* in cowpea seeds treated with different solvents of *Alstonia boonei*.

***Alstonia boonei* leaf extract**	**% mortality at hours post treatment**			
	24	**48**	**72**	**96**
N-hexane	60.33±001^{c}	80.00±0.00^{d}	100.00±0.00^{c}	100.00±0.00^{b}
Petroleum-ether	53.33±0.01^{bc}	76.67±0.04^{cd}	100.00±0.00^{c}	100.00±0.00^{b}
Acetone	50.00±0.00^{bc}	70.00±0.00^{c}	100.00±0.00^{c}	100.00±0.00^{b}
Methanol	30.00±0.00^{b}	53.33±0.01^{b}	80.00±0.00^{b}	100.00±0.00^{b}
Control	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Each value is a mean ±standard error of three replicates. Means within the same column followed by the same letter(s) are not significantly different at $p > 0.05$ using New Duncan Multiple Range Test.

Effect of *Alstonia boonei* leaf extracts on oviposition and adult emergence of *Callosobruchus maculatus* (F.)

The number of eggs laid by adult C. maculatus varied with *A. boonei* leaf extract used and the concentration of the extracts added to the cowpea seeds, (Table 2). The mean number of egg oviposition by adult *C. maculatus* where 10.00, 10.33, 10.00, 23.33 and 87.67 in leaf extracted with n-hexane, pet ether, acetone, methanol and control respectively. The number of eggs laid in cowpea seeds treated with methanol leaf extracts while control having the highest number of eggs. There was a reduction in the numbers of eggs laid in cowpea seeds treated with n-hexane leaf extracts which proves to be the most effective among other treatments.

Table 2. Effects of *Alstonia boonei* leaf extracts on oviposition and adult emergence of *Callosobruchus maculatus*.

***Alstonia boonei* extracts**	**No. of eggs laid (oviposition)**	**% Adult emergence**
n-hexane	10.00±0.00^{a}	0.00±0.00^{a}
Petroleum-ether	10.33±0.01^{a}	0.00±0.00^{a}
Acetone	10.00±0.01^{a}	0.00±0.00^{a}
Methanol	23.33±0.01^{b}	0.00±0.00^{a}
Control	87.67±0.04^{c}	85.28±0.11^{b}

Each value is a mean ±standard error of three replicates. Means within the same column followed by the same letter(s) are not significantly different at $p>0.05$ using New Duncan Multiple Range Test.

There were no adult emergences of *C. maculatus* in all the treated cowpea seeds. The numbers of adult emerged in cowpea seeds treated with various *Alstonia boonei* leaf extracts was lower ($p < 0.05$) when compared with number of adult emerged in cowpea seeds in control that recorded 85.28%.

Effect of *Alstonia boonei* leaf oil on short term storage of cowpea seeds

The mean percentage numbers of cowpea seeds by different oil extracts are significantly different (Table 3). The mean percentage damage by *C. maculatus* on cowpea seeds were not significantly different with the oil extracts of *Alstonia boonei* leaf except the control which have high number of seed damaged. The four oil extracts with n-hexane, pet ether, acetone and methanol from *Alstonia boonei* leaf are more effective in reducing the weight loss. The weevil perforation index (WPI) of *C. maculatus* on cowpea seeds with leaf oil extracts of *A. boonei* with different solvent were good protectant (less than 50) while control is not a good protectant (50.00±0.00).

Table 3. Effect of *Alstonia boonei* leaf oil on short term storage of cowpea seeds.

0.5% v/w *A. boonei* extracts	Mean total number of seeds	Mean number of damaged seeds	Mean % seed damage	Mean weight loss	Weevil perforation index
n-hexane	93.67	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Petroleum-ether	95.00	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Acetone	91.33	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Methanol	95.33	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Control	93.33	86.67±0.01[b]	92.86±0.14[b]	11.02±0.01[b]	50.00±0.00[b]

Each value is a mean ±standard error of three replicates. Means within the same column followed by the same letter(s) are not significantly different at p>0.05 using New Duncan Multiple Range Test.

Discussion

The resultant high mortalities of adults *C. maculatus* observed on cowpea treated with *A. boonei* leaf oil extracts could be due to high toxic effect of the product on adult *C. maculatus*. The *A. boonei* leaf oil extracts also prevented the emergence of *C. maculatus* adults, an effect that is in agreement with the observation of Osawe et al. (2007) who reported that the aqueous extracts of the stem bark and leaves of this plant adversely affected the survival and growth of *Sesamia calamists*. The results show that the leaf oil extracted with n-hexane, pet ether, acetone and methanol had varied degrees of effects on *C. maculatus* at 24 h, 48 h, 72 h and 96 h of post treatment. This study demonstrated that *A. boonei* oil is toxic to adults and eggs of *C. maculatus*. This is in agreement with the report of Daniel (1991) who reported that plant oils are toxic to young larval, adults and eggs of *C. maculatus*. Petroleum-ether and acetone oil extracts treatment on cowpea seeds show some degree effects against *C. maculatus* on oviposition and adult emergence but not as high as that of n-hexane leaf oil treatment which has highest degree of effect. This confirm the report of Benner (1993) who reported that extractions of insecticidal plants powders with appropriate solvent, often concentrates the active minerals and make their potency readily detectable.

In this study, the lethal effect of these oil extracts on cowpea beetle could be as a result of contact toxicity. Most insects breathe by means of trachea which usually opens at a surface of the body through spiracles (Adedire et al., 2011; Ileke and Olotuah, 2012). This spiracle must have been blocked by the oil extracts thereby leading to suffocation of the insects. The oils on application disrupt mating and sexual communication as well as deterring female from laying eggs (NRC, 1992). Also, on application to cowpea seeds, the oil covered the testa of the grains, serving as food poison to the adult's insects; while some of them penetrated into the endosperm and germ layer thereby suppressing oviposition and larval development. The non-protectant control had no effect on the mortality; oviposition and adult emergence of *C. maculatus* may be attributed to interference with normal respiration resulting in suffocation (Hall and Harman 1991). Out of the four solvents used, acetone has been found to give highest mean value, with no or low number of adult emergence in n-hexane. The lower the mean value observed, the more effective the solvent of leaf oil, n-hexane oil extract from the leaf at different concentration is the most effective on adult emergence of *C. maculatus*.

Conclusion

This study revealed the bioefficacy of Alstonia boonei lesf extract against cowpea beetle *Callosobruchus maculatus* infesting stored cowpea seeds in storage. Effective and efficient controls of storage insect pests are centered mainly on synthetic insecticides. The use of these synthetic chemicals is hampered by many attendant problems. These have necessitated research on the use of alternative eco-friendly insect pest control methods among which are the use of plant products. The plant (*Alstonia boonei*) is readily available, and has not been reported to be toxic to man. Therefore, this study suggest that *Alstonia boonei* leaf oil can be used as biopesticides against cowpea beetles in storage.

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Conflicts of interest

Authors declare that they have no conflict of interests.

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