



Research Paper

Bio-efficacy of some tropical plant extracts as storage protectants against *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae)

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ABSTRACT

A study was conducted to evaluate the insecticidal activity of oil extracts and essential oil extracts of six tropical plants, namely: *Eugenia aromatica*, *Hyptis suaveolens*, *Eucalyptus citriodora*, *Cymbopogon citratus*, *Azadirachta indica* and *Citrus sinensis* against cowpea beetle and *Callosobruchus maculatus* Fabricius using insect mortality, oviposition and adult emergence as indices. Result obtained revealed that all test plant extracts exhibited high mortality action against the cowpea beetle, *C. maculatus* showing 100% beetle mortality with concentrations of 2, 3 and 4% v/w in day 4 of exposure to the extracts. All extracts significantly suppressed oviposition by the insects and totally stopped adult emergence while the essential oil caused 100% mortality at the first exposure and hence, prevented oviposition and adult emergence. All extracts used have potentials for use as alternative crop protectants against *C. maculatus*.

Key words: Insecticidal, essential oil, *Eugenia aromatica*, *Hyptis suaveolens*, *Eucalyptus citriodora*, *Cymbopogon citratus*, *Azadirachta indica*, *Citrus sinensis*, *Callosobruchus maculatus*, mortality, oviposition, adult emergence.

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INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is a food and animal feed crop grown in semi-arid tropic, covering Africa, Asia, Europe, United States, Central and South America. The high protein content (25%) of cowpea with vitamins and mineral salts makes it an important economic crop in the sub-Saharan regions like Nigeria. Cowpea is the most important food crop after cereal crops (Uzoehina, 2009). Cowpea weevil (*C. maculatus*) is a major storage insect pest of legumes and peas, especially cowpea (Beck and Bulmet, 2014). *C. maculatus* is very destructive on account of its short life cycle. The activities of this insect pest result in reduction in quality and quantity of the seed (Dike, 1994) most especially in the store.

The protection of post-harvest losses against insect pest has been a major constraint facing agricultural development in the world at large. As a result of huge losses suffered by farmers over time, a variety of pest management options are available to farmers to reduce losses associated to farm products. These include various cultural practices such as

sun-drying and storage in air-tight container etc. It has also been recorded that storing cowpea seeds by admixture with dry sand reduced insect damage because the sand fills inter-granular spaces and prevent free movement of adult to lay eggs (Schmutterer, 1985).

Ash has been suggested for use as grain desiccant (Akpaetok, 1974) in which case it may desiccate and cause the drying up of insect eggs, larva as well as, causing adult mortality as a result of the abrasion of insect cuticle resulting in dehydration and consequently death (De Lima, 1987). Also, particles could clog insect spiracles and trachea causing suffocation (Wolfson et al., 1991).

Control of cowpea bruchid in the field and storage is to be considered in relation to the economic importance of the crop, since it is evident that these weevils are capable of attacking cowpea both in the field and store. The use of synthetic insecticides is one of the methods used to control bruchids (Adedire et al., 2011). Insecticides may be applied as liquid or fumigant formulation. However, continuous use

Table 1: Test plants evaluated for insecticidal activities.

Scientific name	Family	Part of plant used	Common name
<i>H. suaveolens</i>	Lamineae	Leaves	Pignut or Chan
<i>E. citriodora</i>	Mytaceae	Leaves	Lemon scented gum
<i>C. citratus</i>	Gramineae	Leaves	Lemon grass
<i>A. indica</i>	Meliaceae	Leaves	Neem
<i>C. sinensis</i>	Rutaceae	Peels	Sweet orange
<i>E. aromatica</i>	Mytaceae	Leaves	Cloves

of chemical insecticides may lead to serious problems, such as insect resistance, environmental pollution, ozone layer depletion, affordability, and health hazard etc (Obembe and Kayode, 2013).

The aforementioned problems associated with chemical insecticides have led to the search for safe biological agent that can be used as insecticides for stored food products (Adedire et al., 2011). Hence, the need to discover and develop botanicals that are ecologically friendly and do not constitute any harm to the non-target organisms as crop protection agents. The use of botanical insecticides is one of the important approaches to the management of stored grain pest and it has shown many benefits over the use of chemical insecticides (Weinzieri, 1982).

Essential oil from plants has proved to possess good potential for use as fumigants against stored product bruchids (Papachristos and Stamopoulos, 2002; Tapondjou et al., 2002). Raja et al. (2000) reported that when jute bags treated with different plant leaf extracts including *Azadirachta indica*, *Vitex negundo*, and *Cleistanthus collinus* were used for cowpea seed storage, the oviposition rate by *Callosobruchus maculatus*, adult emergence and seed damage reduced.

The aim of the present study is to evaluate the insecticidal activities of *Eugenia aromatica*, *Eucalyptus citriodora*, *Citrus sinensis*, *Cymbopogon citratus*, *Hyptis suaveolens* and *A. indica* on mortality, oviposition and adult emergence of cowpea weevil, *C. maculatus*.

MATERIALS AND METHODS

Plant materials such as *E. aromatica*, *E. citriodora*, *C. sinensis*, *A. indica*, *H. suaveolens* and *C. cytratus* were obtained from the surroundings of Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria. Table 1 shows test plants evaluated for insecticidal activities.

Preparation of insect culture

The parent culture of *C. maculatus* was obtained from infested grains in the food store of Ekiti Anglican Diocesan High School, Ado-Ekiti, Ekiti State, Nigeria. The insects were cultured in the laboratory under the temperature of $28\pm 2^{\circ}\text{C}$

and $75\pm 5\%$ relative humidity. This was done by weighing 800 g of cowpea seeds into Kilner jar. Twenty unsexed adult *C. maculatus* were then introduced into the Kilner jar and kept in the laboratory for one month for the insects to oviposit and multiply.

Preparation of plant extracts

The leaves of *E. aromatica*, *E. citriodora*, *A. indica*, *C. citratus* and the peels of *C. sinensis* were air-dried in the laboratory for a period of one month. They were pulverized into fine powder using an electric blender. Thereafter, 300 g of the plant materials were then exhaustively extracted with ethanol using a Soxhlet apparatus. These extracts were concentrated using a rotary evaporator and kept separately in different labeled containers and stored in the refrigerator until required for use.

Preparation of essential oil

The essential oil was extracted from the dry powder of the test plants by the use of volatile or essential oil steam distillation apparatus. During the steam distillation process, 100 g each of the plant powder was weighed into distillation flask and 300 ml of water added and heated for a period of 4 h on a heating mantle. The volatile oil deposit on the water was collected through the attached graduated measuring cylinder tube.

Insect mortality bioassay after treated with oil extracts

Clean, wholesome and un-infested cowpea seeds were obtained from Oba market, Ado-Ekiti, Ekiti State, Nigeria. They were kept in the refrigerator for 96 h to kill any available insect eggs and larva before commencing the experiment. Clean Ife brown variety cowpea seeds weighing 20 g were treated with 0.1, 0.2, 0.3 and 0.4 ml (1, 2, 3 and 4% v/w) of the oil extracts, respectively. The extracts and the cowpea seeds were properly mixed with a glass rod to ensure uniform coating of the extracts on the seeds. Thereafter, 5 pairs of adult weevils were introduced into Petri-dish containing the treated seeds. All

experiments were replicated four times in 9 cm inner diameter Petri-dish. The control experiment contained untreated seeds. The treatments were arranged in a Complete Randomized Design (CRD). The experimental set-up was observed for 4 days and the number of dead insects counted and recorded daily. On the fourth day all dead and live insects were removed. Insects that did not respond to pin probe on the abdomen were considered dead.

Insect mortality bioassay after treated with essential oil

The procedure for insect mortality bioassay as earlier mentioned is used for essential oil. No essential oil was obtained from *A. indica* and *E. citriodora*. Observations were made on daily basis during which the number of dead insects were counted, recorded and all the insects removed from the Petri-dishes.

Oviposition and adult emergence bioassay

For both the extracts and essential oil extracts, the number of eggs laid were counted and recorded after the mortality experiment. The eggs were allowed to stay (incubate) until new adults emerged.

Seed damage and weight loss

At the end of the incubation period, the number of damaged seeds were counted and recorded. Thereafter, the seeds were re-weighed to determine weight loss.

Data analysis

Data obtained were converted to percentage. Arcsin transformation was carried out on the percentage values. ANOVA was performed on transformed data and the means separated using the Tukey's test.

RESULTS

Toxicity of oil extracts against *C. maculatus*

The essential oil from *C. citratus* and *C. sinensis* showed high mortality rate. *C. citratus* achieved 100% weevil mortality with all concentrations after 2 h of exposure to the treated seed, while extract from *C. sinensis* caused 100% mortality on seeds treated with 3.0% extract concentration starting from the first hour of exposure (Table 3). Table 2 shows extracts from *E. aromatica* and *H. suaveolens* evoked between 74 and 100% mortality by the 8th hour of exposure

to the treated seeds, respectively.

Generally, weevil mortality increased with increase in extract concentrations and time of exposure to the extracts. All extracts demonstrated high potency on mortality of *C. maculatus* as compared to the control. Extract of *C. sinensis* showed high weevil mortality within 2 days of exposure at concentration of 2 and 4% (v/w). All extracts showed 100% mortality with concentrations of 2, 3 and 4% of exposure except for *C. citratus* which showed 37.43 and 75.37% at the concentration of 1 and 2% oil extract, respectively by day 4 of exposure. All the tested plant extracts significantly ($p < 0.05$) reduced the population of adult *C. maculatus* on the treated cowpea seed.

Toxicity of oil extract on oviposition and adult emergence of *C. maculatus*

All the extracts significantly suppressed oviposition by the weevils as compared to the control. The most effective extract is that of *E. aromatica* followed by *H. suaveolens* which totally suppressed oviposition in seeds treated with 3 and 4% oil extract, respectively (Table 4). Adult emergence was only recorded in the control experiment while the other treatment did not show any weevil emergence (Table 4).

Toxicity of essential oil on oviposition and adult emergence of *C. maculatus*

Table 5 shows no egg was laid and hence there was no adult emergence.

DISCUSSION

Insect pests are controlled worldwide using chemical insecticides. The use of these chemical insecticides is impregnated with some short comings. The most widely used chemical fumigant for infested cereals such as cowpea in Nigeria is aluminum phosphide. The fumigation tablet of aluminium phosphide emits toxic phosphine gas (PH_3) on contact with moist atmosphere under hermitic storage condition. The sales and use of adulterated and expired pesticides are part of the problems militating against the use of chemical pesticides in Nigeria (Ogunwolu et al., 2001). Even after the purchase of the conventional insecticide, the illiterate and poor resource farmers find it very difficult to read and interpret the labels, therefore, miss-using the insecticides.

Insecticides derived from plants have been discovered to serve as alternatives to the widely used conventional insecticides as many of them have been used in the control of number of species of stored product in the past, including Coleoptera and Lepidoptera (Nathan et al., 2007).

Table 2: Mean percentage mortality of *C. maculatus* treated with oil extracts of the test plants.

Tested plants	Concentration (% v/w)	Day 1	Day 2	Day 3	Day 4
<i>C. citratus</i>	1	0.00±0.00 ^e	0.00±0.00 ^e	12.15±0.05 ^e	37.43±1.60 ^c
	2	35.32±0.55 ^c	70.15±0.00 ^{bc}	72.00±1.00 ^c	75.37±0.12 ^b
	3	88.55±1.22 ^b	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
	4	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
<i>E. aromatic</i>	1	28.12±0.51 ^{cd}	40.00±1.30 ^{cd}	70.00±1.20 ^c	100.00±0.00 ^a
	2	32.22±0.62 ^c	68.01±0.20 ^{bc}	90.00±1.11 ^b	100.00±0.00 ^a
	3	70.27±0.20 ^{bc}	88.11±0.10 ^b	100.00±0.00 ^a	100.00±0.00 ^a
	4	92.18±0.60 ^{ab}	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
<i>H. suaveolens</i>	1	70.12±22.2 ^{bc}	90.33±0.01 ^b	100.00±0.00 ^a	100.00±0.00 ^a
	2	85.18±1.22 ^b	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
	3	92.23±0.13 ^{ab}	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
	4	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
<i>E. citriodora</i>	1	25.27±0.12 ^d	38.16±0.01 ^{cd}	60.22±0.21 ^{cd}	80.18±0.00 ^b
	2	33.15±0.21 ^c	62.28±1.20 ^c	83.40±0.22 ^{bc}	100.00±0.00 ^a
	3	80.33±1.20 ^b	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
	4	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
<i>C. sinensis</i>	1	95.22±0.11 ^a	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
	2	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
	3	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
	4	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
<i>A. indica</i>	1	37.53±0.71 ^{bc}	40.00±0.10 ^{cd}	40.00±2.15 ^d	41.22±1.66 ^c
	2	62.17±0.02 ^b	95.22±0.11 ^{ab}	100.00±0.00 ^a	100.00±0.00 ^a
	3	90.00±0.12 ^{ab}	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
	4	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
Control		0.00±0.00 ^e	0.00±0.00 ^e	0.00±0.00 ^f	0.00±0.00 ^d

Each value is the mean ± standard error of four replicates. Means in the same column followed by the same letter(s) are not significantly different at $p \geq 0.05$ by Tukey's test.

The advantages of botanical insecticides over the conventional ones are that, they are environmentally friendly and are believed to be easily bio-degradable and non-toxic to the non-target organisms.

Many Nigeria plant species are medicinal and very effective in the control of a wide range of insect pest (Akinkulore et al., 2006; Ileke and Olotua, 2012; Obembe and Ogunbite, 2017). The high mortality of adult *C. maculatus* exposed to oil extract treated seeds could be due to toxic effect of the oil. It may also be as a result of the oil

blocking the spiracle of the insect, thereby, causing suffocation and consequent death (Adedire et al., 2011). Oil extract of *C. sinensis* showed high weevil mortality within two days of exposure at the concentration of 2 and 4%, respectively. All other extracts showed weevil mortality within the concentration of 2, 3 and 4% in day 4 of exposure which is in line with the report of Obembe and Kayode (2013), who recorded high weevil mortality using extracts from some tropical plants.

All oil extracts from the tested plant drastically reduced

Table 3: Mean percentage mortality of *C. maculatus* after treated with essential oil from the test plants.

Tested plants	Concentration (% v/w)	2 h	4 h	8 h	10 h
<i>C. citratus</i>	1	100.15±0.02 ^a	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
	2	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
	3	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
	4	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
<i>E. aromatic</i>	1	0.00±0.00 ^d	0.00±0.00 ^c	72.00±0.12 ^c	88.02±0.15 ^b
	2	0.00±0.00 ^d	0.00±0.00 ^c	88.00±0.10 ^b	100.00±0.00 ^a
	3	66.34±0.12 ^b	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
	4	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
<i>H. suaveolens</i>	1	0.00±0.00 ^d	0.00±0.00 ^c	72.00±0.12 ^c	68.22±0.21 ^b
	2	0.00±0.00 ^d	0.12±0.31 ^c	63.12±0.21 ^c	74.12±0.10 ^b
	3	25.12±0.51 ^c	48.34±0.41 ^b	80.88±0.15 ^b	100.00±0.00 ^a
	4	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
<i>C. sinensis</i>	1	0.00±0.00 ^d	0.00±0.00 ^c	71.12±0.15 ^c	85.20±1.50 ^b
	2	0.00±0.00 ^d	0.00±0.00 ^c	75.13±0.21 ^c	92.12±0.21 ^{ab}
	3	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
	4	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
Control		0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^d	0.00±0.00 ^c

Each value is the mean ± standard error of four replicates. Means in the same column followed by the same letter(s) are not significantly different at $p \geq 0.05$ by Tukey's test.

Table 4: Mean oviposition count by adult *C. maculatus* after 4 days of application of oil extracts.

Tested plants	Concentration (% v/w)	Number of eggs laid	Mean number of adult emerged
<i>C. citratus</i>	1	10.95±1.22 ^{bc}	0.00±0.00 ^b
	2	10.12±0.21 ^{bc}	0.00±0.00 ^b
	3	4.02±0.22 ^d	0.00±0.00 ^b
	4	0.00±0.00 ^c	0.00±0.00 ^b
<i>E. aromatic</i>	1	8.14±0.40 ^{bc}	0.00±0.00 ^b
	2	6.22±1.11 ^{cd}	0.00±0.00 ^b
	3	0.00±0.00 ^e	0.00±0.00 ^b
	4	0.00±0.00 ^e	0.00±0.00 ^b
<i>H. suaveolens</i>	1	11.25±0.40 ^a	0.00±0.00 ^b
	2	7.10±1.21 ^c	0.00±0.00 ^b
	3	0.00±0.00 ^e	0.00±0.00 ^b
	4	0.00±0.00 ^e	0.00±0.00 ^b
<i>E. citriodora</i>	1	15.16±0.22 ^b	0.00±0.00 ^b
	2	13.20±0.31 ^b	0.00±0.00 ^b
	3	10.12±1.56 ^{bc}	0.00±0.00 ^b
	4	7.33±0.71 ^c	0.00±0.00 ^b
<i>C. sinensis</i>	1	8.22±0.11 ^{bc}	0.00±0.00 ^b
	2	6.12±0.71 ^{cd}	0.00±0.00 ^b
	3	5.00±1.00 ^{cd}	0.00±0.00 ^b
	4	2.12±1.30 ^d	0.00±0.00 ^b

Table 4: Conts. Mean oviposition count by adult *C. maculatus* after 4 days of application of oil extracts.

<i>A. indica</i>	1	14.15±0.22 ^b	0.00±0.00 ^b
	2	10.22±1.22 ^{bc}	0.00±0.00 ^b
	3	6.04±0.10 ^c	0.00±0.00 ^b
	4	3.01±0.20 ^d	0.00±0.00 ^b
Control		62.15±1.20 ^a	51.12±1.31 ^a

Each value is the mean ± standard error of four replicates. Means in the same column followed by the same letter(s) are not significantly different at $p \geq 0.05$ by Tukey's test.

Table 5: Mean oviposition count by adult *C. maculatus* in day one of application of essential oil.

Tested plants	Concentration (% v/w)	Number of eggs laid	Mean number of adult emerged
<i>C. citratus</i>	1	0.00±0.00 ^b	0.00±0.00 ^b
	2	0.00±0.00 ^b	0.00±0.00 ^b
	3	0.00±0.00 ^b	0.00±0.00 ^b
	4	0.00±0.00 ^b	0.00±0.00 ^b
<i>E. aromatica</i>	1	0.00±0.00 ^b	0.00±0.00 ^b
	2	0.00±0.00 ^b	0.00±0.00 ^b
	3	0.00±0.00 ^b	0.00±0.00 ^b
	4	0.00±0.00 ^b	0.00±0.00 ^b
<i>H. suaveolens</i>	1	0.00±0.00 ^b	0.00±0.00 ^b
	2	0.00±0.00 ^b	0.00±0.00 ^b
	3	0.00±0.00 ^b	0.00±0.00 ^b
	4	0.00±0.00 ^b	0.00±0.00 ^b
<i>C. sinensis</i>	1	0.00±0.00 ^b	0.00±0.00 ^b
	2	0.00±0.00 ^b	0.00±0.00 ^b
	3	0.00±0.00 ^b	0.00±0.00 ^b
	4	0.00±0.00 ^b	0.00±0.00 ^b
Control		6.12±0.22 ^a	5.10±0.10 ^a

Each value is the mean ± standard error of four replicates. Means in the same column followed by the same letter(s) are not significantly different at $p \geq 0.05$ by Tukey's test.

the process of oviposition and totally stopped adult emergence. Oviposition and adult emergence was totally stopped in seeds treated with essential oil from the tested plants in day one of application. This is because all the insects died within 8 h and so could not lay eggs and hence no adult emerged. The mechanism of its protective action against *C. maculatus* includes direct toxicity to the adult and eggs and inhibition and oviposition by female beetles.

The result from the present study shows that extracts from the tested plant are effective in the protection of cowpea seeds against *C. maculatus*. However, more study is required to extract, characterize and isolate the active ingredients which are potent on the insect pest and comparing its effectiveness with the ordinary oil extracts.

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