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Deterrent Effects of *Alstonia boonei* Oil on Oviposition and Progeny Development of *Callosobruchus maculatus* (Fab.) [Coleoptera: Bruchidae].

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ABSTRACT

BACKGROUND: Botanicals insecticides has remained the major weapons amongst tropical zones farmers to combat hexapods infestation of stored cowpea seeds in lieu of expensive synthetic chemical insecticides that have toxic effects on our environment.

OBJECTIVES: Deterrence of cowpea bruchid oviposition and emergence on seeds treated with Cheese wood, *Alstonia boonei* stem bark oil extracted with different solvents was evaluated in dual- and multiple-choice laboratory tests at 28 ± 2^{o}C and 75 ± 5% relative humidity.

METHODS: Four rates of oil (1%, 2%, 3% and 4%) or the solvents and an untreated control treatment were replicated four times in Complete Randomized Design.

RESULT: The results of the dual-choice test shows that cowpea bruchid laid fewer eggs on cowpea seeds treated with petroleum ether and n–hexane oils of *A. boonei* stem bark oil compared to the other solvent treated seeds and untreated seeds. The numbers of eggs laid on cowpea seeds treated with 1.0 % oil extracted with methanol, ethanol, acetone, petroleum ether and n-hexane were 3.75 3.00 6.25, 2.25 and 2.00 respectively while the corresponding values for solvents-treated seeds were 9.00, 11.50, 12.50, 6.75 and 4.25, respectively. Generally, oviposition decreased as the concentration (1%, 2%, 3%, 4%) of the oils increased. The results of the multiple choice test shows that untreated cowpea seeds had significantly higher number of eggs than treated seeds. Among the latter, those treated with n– hexane had least numbers of eggs. The numbers of eggs laid on cowpea seeds treated with 2% oil extracted with methanol, ethanol, acetone, petroleum ether and n-hexane were 3.25, 4.00, 7.00, 2.00 and 1.50, respectively. The number of egg laid decreased as the concentration (1%, 2%, 3%, 4%) of the oils increased. Methanol, ethanol, petroleum ether and n-hexane extracts completely inhibited the progeny development of *C. maculatus* in dual and multiple choice tests.

CONCLUSION: *A. boonei* stem bark oil can control cowpea bruchid infestation on stored cowpea by deterring oviposition and suppressing development.

KEYWORDS: *Cowpea*, *Dual and multiple tests, Ethanol, Legume*.

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1. BACKGROUND

The significance of legumes as the world's major source of protein is fast increasing especially in the tropics as a consequence of escalation of human population (Ofuya, 2001). The cowpea bruhid, *Callosobruchus maculatus*, is a cosmopolitan field-to-store insect pest and multivoltine (Ofuya, 2001). It is one of the most destructive insect pests of stored legumes in the tropics (Adedire, 2002, Adedire and Ajayi, 2003). Infestations begin on mature pods on the field and serious damage is done in the store leading to considerable quantitative and qualitative loss of produce as well as loss of viabilities (Ofuya, 2001). Infested seeds were completely tunnelled by larval feeding and characteristic emergence holes are noticeable after the newly adults emerge from the seeds (Adedire *et al*., 2011). The efficient control as well as removal of insect pests from stored food products has long been the goal of many Entomologists throughout the world (Ileke *et al*., 2012, 2013). Botanical insecticides have long touted as substitute to avert the danger associated with synthetic chemical insecticides as a result of they are medicinal and eco-friendly properties (Akinkuolere *et al*., 2006). In the tropical region such as Nigeria, farmers intentionally use this hazardous synthetic chemical insecticides based on its quick action not considering the human and environmental health hazards. The utilization of botanicals with insecticidal properties to defend stored products against insect pest attack has a very long history (Oni and Ogungbite, 2015). Botanicals of various species have been investigated and suggested as promising alternative in the control of insect pests. Plant materials such as leaves, bark, roots, twigs, flowers and latex of locally available plants that are biodegradable have been used from time immemorial as protectants of stored seeds against storage pests in different parts of the world (Isman, 2006). Many of the plant species that have been investigated are often those used as culinary spices or in traditional medicine by local communities (Lale, 1992). Some researchers conclude that these plant materials are therefore safe to use as insecticides (Oni and Ogungbite, 2015). Many researchers are trying to validate the efficacy of ethnobotanicals which are readily available in the local environment for farmer use at village level (Adedire and Lajide, Adedire *et al*., 2011, Ileke *et al*., 2013). *Alstonia boonei* De Wild (Apocyanaceae) is an evergreen deciduous tree. The plant is about 45 m tall and 1.2 m in diameter that sheds it leaves annually. It has roots, barks, stems, leaves, fruits, seeds, flowers, and saps. The plant have medicinal values in some cultures in African countries (Moronkola and Kunle, 2012). In literature, there appears to be scarcity of experimental information on the utilization of *A. boonei* stem bark oils for ovipositional deterrent and progeny development inhibition, though Ileke *et al*. (2012, 2013, 2014a, b, c) reported the efficacy of *A. boonei* powders and oils for the control of *C. maculatus.*

2. OBJECTIVES

The goals of this research was to evaluate the ovipositional deterrent and progeny development inhibition of cowpea bruchid, *C. maculatus* (F.) on cowpea seeds treated with *A boonei* stem bark oils extracted with methanol, ethanol, acetone, n–hexane and petroleum ether solvents.

3. MATERIALS AND METHODS

3.1. Test Insect

Newly emerged *C. maculatus* adults used for this study were obtained from existing culture maintained at 28±2°C and 75±5% relative humidity in the Storage Entomology Research Laboratory of the Department of Biology, Federal University of Technology, Akure, Ondo State. They were sorted by sex, at the Storage Entomology Research Laboratory, Department of Biology, Federal University of Technology, Akure, Ondo State, under a binocular microscope based on the characteristics described by Halstead (1963).

3.2. Plant Material

Fresh stem bark of *A. boonei* stripped from a tree at Akola farm at Igbara-Odo Ekiti, Ekiti State, Nigeria was washed thoroughly with clean water, air-dried in the laboratory for one month, pulverized into fine powder using an electric blender (Supermaster ®, Model SMB 2977, Japan) and there after sieved to pass through 1mm^2 perforation. The fine powder was kept in separate plastic containers with tight lids and stored in a refrigerator at 4°C prior to oil extraction. Five hundred (500g) powder was weighed into a beaker, packed in a thimble using muslin cloth, and extracted with 500ml of methanol, ethanol, acetone, petroleum ether, or n-hexane in a Soxhlet apparatus at 40 – 60°C. Excess solvent was recovered using rotary evaporator vacuum. The resulting stock oils were concentrated by air drying to remove traces of the solvent. Different concentrations (1%, 2%, 3% and 4%) of the stock oils were made by dilution of each with the appropriate solvent following the method of Ileke *et al*. (2013).

3.3. Oviposition Deterrence

Dual-choice Test

The method of Adedire and Lajide (1999) was used for the dual-choice test with little modification. Cowpea seeds (20 g) were treated with 2 ml of 1%, 2%, 3%, or 4% *A. boonei* oil in Petri dishes and air dried for 1 hour. Each Petri dish was infested with two pairs of bruchids in four replicates and arranged in insect rearing cages. At 7 and 14 days post-infestation, the eggs laid were counted and recorded; the latter count was used to compute percentage inhibition of progeny development. From 30 - 37 days post-infestation, newly emerged adult cowpea bruchids were counted and percentage progeny emergence was calculated as:

$$\%\ \text{Adult emergence} = \frac{\text{Total number of adult emergence}}{\text{Total number of eggs laid}} \times \frac{100}{1}$$

3.4. Multiple-choice Test

The method of Okosun and Adedire (2010) was used for the multiple-choice to compare oil-treated and solvent-treated cowpea seeds as substrate for oviposition by *C. maculatus*. The seeds were treated as described for the dual-choice test, arranged alternatively in a circle in a Petri dish, and infested with two pairs of adult cowpea bruchids. Four replicates were set up and oviposition and adult emergence data were taken as previously described.

3.5. Data Analysis

Data were subjected to analysis of variance and treatment means were separated using the New Duncan's Multiple Range Test.

4. RESULTS

4.1. Effect of A. boonei Stem Bark Oils on Oviposition Deterrence: Dual choice and Multiple – choice Tests

The results of the dual-choice test shows that cowpea bruchid laid fewer eggs on cowpea seeds treated with petroleum ether and n–hexane oils of *A. boonei* stem bark compared to the other solvent treated seeds and the untreated seeds (Table 1). The numbers of eggs laid on cowpea seeds treated with 1% oil extracted with methanol, ethanol, acetone, petroleum ether and n-hexane were 3.75 3.00 6.25, 2.25 and 2.00, respectively, while the corresponding values for solvent-treated seeds were 9.00, 11.50, 12.50, 6.75 and 4.25, respectively. The numbers of eggs laid on cowpea seeds treated with 4% oil extracted with methanol, ethanol, acetone, petroleum ether and n-hexane were 3.00 2.50 5.50, 1.25 and 1.00, respectively while the corresponding values for solvent-treated seeds were 8.50, 10.25, 11.50, 5.75 and 3.25, respectively. Generally, oviposition decreased as the concentration (1%, 2%, 3%, 4%) of the oils increased (Table 1). The results of the multiple choice test shows that untreated cowpea seeds had significantly ($P<0.05$) higher number of eggs laid than treated cowpea seeds. Among these, those treated with n-hexane extracted oil – had least number of eggs at all concentrations (Table 2). The eggs laid on cowpea seeds treated with methanol, ethanol, acetone, petroleum ether and n-hexane oils of *A. boonei* at rate 2% concentration were 3.25, 4.00, 7.00, 2.00 and 1.50, respectively. At rate 4% concentration, the eggs laid on cowpea seeds treated with methanol, ethanol, acetone, petroleum ether and n-hexane oils of *A. boonei* were 2.50, 4.00, 625, 1.50 and 1.00, respectively. Oviposition decreased as the concentration (1%, 2%, 3%, 4%) of the oils increased (Table 2).

Table 1. Oviposition deterrent effect of *Alstonia boonei* oils on female *Callosobruchus maculatus* in dual-choice test

Extracting Solvent	Mean number of eggs laid/20g seeds at indicated oil concentration (%)			
	1	2	3	4
Methanol	3.50±0.01^{*a}	3.25±0.027^{a}	3.00±0.03^{a}	2.50±0.01^{a}
Ethanol	4.25±0.27^{a}	4.00±0.30^{a}	4.25±0.27^{a}	4.00±0.30^{a}
Acetone	7.25±0.27^{ab}	7.00±0.30^{ab}	6.75±0.03^{ab}	6.25±0.27^{ab}
Petroleum ether	2.00±0.30^{a}	2.00±0.07^{a}	1.75±0.03^{a}	1.50±0.01^{a}
N – hexane	1.75±0.03^{a}	1.50±0.01^{a}	1.25±0.27^{a}	1.00±0.30^{a}
Untreated (0.00)	15.25±0.05^{b}	15.25±0.05^{b}	15.25±0.05^{b}	15.25±0.05^{b}
	Mean number of eggs laid on Solvent treated cowpea seeds			
Methanol	9.00±1.30^{a}	9.25±0.27^{a}	9.00±0.30^{a}	8.50±0.01^{a}
Ethanol	11.50±0.01^{a}	11.00±0.30^{a}	10.75±0.03^{a}	10.25±0.27^{a}
Acetone	12.50±0.01^{a}	12.25±0.27^{a}	12.00±0.30^{a}	11.50±0.01^{a}
Petroleum ether	6.75±0.03^{a}	6.50±0.01^{a}	6.00±0.30^{a}	5.75±0.03^{a}
N – hexane	4.25±0.27^{a}	4.00±0.30^{a}	4.00±0.30^{a}	3.75±0.03^{a}
Untreated (0.00)	15.25±0.05^{b}	15.25±0.05^{b}	15.25±0.05^{b}	15.25±0.05^{b}

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

Table 2. Oviposition deterrent effect of *Alstonia boonei* oils on female *Callosobruchus maculatus* in multiple-choice test

Extracting Solvent	Mean number of eggs laid/20g seeds at indicated oil concentration (%)			
	1	2	3	4
Methanol	3.50±0.01^{*a}	3.25±0.027^{a}	3.00±0.03^{a}	2.50±0.01^{a}
Ethanol	4.25±0.27^{a}	4.00±0.30^{a}	4.25±0.27^{a}	4.00±0.30^{a}
Acetone	7.25±0.27^{ab}	7.00±0.30^{ab}	6.75±0.03^{ab}	6.25±0.27^{ab}
Petroleum ether	2.00±0.30^{a}	2.00±0.07^{a}	1.75±0.03^{a}	1.50±0.01^{a}
N – hexane	1.75±0.03^{a}	1.50±0.01^{a}	1.25±0.27^{a}	1.00±0.30^{a}
Untreated (0.00)	15.25±0.05^{b}	15.25±0.05^{b}	15.25±0.05^{b}	15.25±0.05^{b}

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

4.2. Effect of Solvent-extracted Oil of A. boonei Stem Bark on Cowpea Bruchid Emergence

Tables 3 and 4 show the effects of *A. boonei* stem bark oils on the progeny development of *C. maculatus*. There was significant (P<0.05) reduction in the percentage adult emergence compared with untreated control. The results obtained on cowpea seeds treated with oils of *A. boonei* stem bark were significantly different from those of solvent-treated seeds (Table 3). The results revealed that *A. boonei* stem bark oils at different dose level were very effective against the viability of the eggs of *C. maculatus* on stored cowpea. At rate 4% concentration, oils completely inhibited the adult emergence of *C. maculatus* in dual choice test. Oils inhibited the development of *C. maculatus* eggs in multiple choice tests based on the percentage adult emergence. At rate 2% concentration, the % progeny emergence on cowpea seeds treated with methanol, ethanol, acetone, petroleum ether and n-hexane oils of *A. boonei* were 0.00 0.00 11.50, 0.00 and 0.00, respectively (Table 4). The progeny development gradually decreased with increase in the treatment dose level of acetone extract. Acetone oil of 4% dosage rate *of A. boonei* stem bark completely protected cowpea seeds.

Table 3. Effect of *A. boonei* stem bark extracts on adult emergence of *C. maculatus* in dual-choice test

Extracting Solvent	**Mean number of adult emergence/20g seeds at indicated oil concentration (%)**			
	1	**2**	**3**	**4**
Methanol	0.00±0.00*a	0.00±0.00a	0.00±0.00a	0.00±0.00a
Ethanol	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
Acetone	15.90±0.85b	11.14±0.66b	8.69±1.25b	0.00±0.00a
Petroleum ether	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
N – hexane	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
Untreated (0.00)	85.25±2.50c	85.25±2.50c	85.25±2.50c	85.25±2.50b
	Mean % adult emergence on Solvent treated cowpea seeds			
Methanol	86.05±2.72a	84.80±2.42a	82.59±2.41a	83.33±2.41a
Ethanol	86.67±2.54a	84.21±2.81a	82.65±2.47a	82.31±2.64a
Acetone	86.15±2.82a	87.50±2.22a	80.00±4.08a	80.00±4.08a
Petroleum ether	86.70±2.41a	83.33±2.97a	83.33±2.41a	83.33±2.41a
N – hexane	85.60±2.49a	85.00±2.89a	83.33±2.49a	82.01±2.05a
Untreated (0.00)	85.25±2.50a	85.25±2.50a	85.25±2.50a	85.25±2.50a

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

5. DISCUSSION

The use of botanicals has remained major weapons amongst the tropical zones farmers to combat insect pest infestation of stored product in lieu of expensive synthetic chemical insecticides. Results showed *A. boonei* stem bark oil was to varying degrees effective in deterring oviposition and progeny development by *C. maculatus*. This study shows that no plant oils completely prevented the females from laying eggs on treated seeds. So study also showed the plant oil reduce significantly, number of eggs laid per females compared to that obtained in untreated control seeds. The reduction in oviposition in extract treated seeds may be caused by extracted oil coating of seeds which became unsuitable for oviposition. Cowpea seeds treated with acetone-extracted oils were most preferred for oviposition followed by those treated with ethanol.

Table 4. Effect of *A. boonei* stem bark extract on % adult emergence of *C. maculatus* in multiple–choice test

Extracting Solvent	Mean number of adult emergence/20g seeds at indicated oil concentration (%)			
	1	2	3	4
Methanol	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Ethanol	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Acetone	15.14±1.76[b]	11.50±0.31[b]	9.84±1.15[b]	0.00±0.00[a]
Petroleum ether	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
N – hexane	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Untreated (0.00)	85.25±2.50[c]	85.25±2.50[c]	85.25±2.50[c]	85.25±2.50[b]

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

This may be ascribed to the polarity of acetone, which is intermediate between polar (methanol, ethanol) and non–polar (n–hexane, petroleum ether) solvents. Acetone might not have able to extract all the polar or the non–polar constituent of powdered of *A. boonei* (Ileke *et al.*, 2013). Many researchers have reported ineffectiveness of acetone as solvent. Mohrig *et al*. 1999, Su, 1989 for example, found that acetone extract of *M. fragrans* was less toxic to *C. maculatus, Lasioderma serricorne*and *T. castaneum* but it was moderately toxic to *S. oryzae*. The ability of *A. boonei* stem bark oils to reduce egg laying capability by the female beetles may be attributed to the obstruction of the normal embryonic development by suppressing hormonal and biochemical processes as opined by Raja and Williams (2008). Adebowale and Adedire (2006) observed that treatment of cowpea seeds with *Jatropha curcas* seed oil reduced the number of eggs laid by *C. maculatus* and prevented adult emergence at concentration between 0.5 and 2% (v/w). Credland (1992) and Tapondjou *et al*. (2002) suggested that oviposition inhibition property of plant powders on adult cowpea bruchids made them lay fewer eggs and killed the larvae hatching from eggs laid on seeds. Oviposition inhibitors have the advantage of attacking a pest at the start of its life cycle and deterred adult insects from laying its eggs on the seeds, thus preventing the pest population from escalating (Pandey *et al*., 2011). The *A. boonei* stem bark extract used in this study was found significantly superior in reducing adult emergence; as the concentrations of extracts increased, their inhibitory effect on adult emergence also increased (Ileke *et al*. 2012, 2013, 2014a, 2014b, 2014c, Jose and Adesina, 2014, Olaifa and Erhun, 1998, Paranagama *et al*., 2003). The egg mortality and failure to hatch on seeds treated with extract were probably attributed to toxic component of extracts and also to physical properties, which caused changes in surface tension and oxygen tension within eggs (Singh *et al*. 1978). The ovicidal effect of extracts on the bruchid may also be explained in terms of asphyxiation by blocking the major route of gas exchange between a thin area of the chorion and outside (Credland, 1992) which ultimately reduced the emergence of the insects from treated seed (Copping and Menn, 2000).

5. 1. CONCLUSION

The study demonstrated *A. boonei* stem bark oil can be utilized as potential substitute to synthetic insecticides for the management of *C. maculatus* infestation on stored cowpea seeds owing to the significant anti-oviposition and ovicidal activities observed from this study.

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