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ASSESSMENT OF CLOVES, *Eugenia aromatic* (Baill.) AS AN ENTOMOCIDE AGAINST THE BEAN BRUCHID BEETLE, *Callosobrucus maculatus* (Fabricius) AND THE MAIZE WEEVIL, *Sitophilus zeamais* (Motschulsky)

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Abstract
The potency of the medicinal plant, Eugenia aromatica as a biocide against storage insect pests of cowpea and maize was assessed in the laboratory. Different treatment doses 0.0g (Control), 0.5g, 1.0g, 1.5g and 2.0g of E. aromatica were applied on maize seeds infested with Sitophilus zeamais and cowpea seeds infested with Callosobruchus maculatus. The treated samples were monitored at different hours of exposure, 24hrs, 48hrs, 72hrs, 96hrs and 120hrs.The lethal time LT_{50} for each dosage level for both S. zeamais and C. maculatus were determined. The earliest LT_{50} for S. zeamais was observed in 48hrs at 1.5g (51.07± 12.38) while the LT_{50} for C. maculatus was observed in 48hrs at 1.5g (51.07± 2.23). The percentage (%) mortality for S. zeamais and C. maculatus were also determined. It was observed that for S. zeamais, 2.0g at day 5 gave the highest % mortality (88.87%) while for C. maculatus, 2.0g at day 5' gave the highest % mortality (100%). The result shows that E. aromatic is more effective on C. maculatus than S. zeamais. It is concluded that E. aromatica is an effective plant material that can be used to control insect pests.

Key Words: *Eugenia aromatic, Lethal time, Sitophilus zeamais, Callosobruchus maculatus, Cowpeas, Maize*

Introduction

Maize (*Zea mais*) is known to be one of the oldest, most important and most cultivated cereals in the world (Longe, 2016). Many insect pests are known to infest maize including the beetles – *Sitophilus zeamais* (Maize weevils), *Sitophilus oryzea* (Rice weevils), *Callosbruchus maculatus* (Beans weevils), *Lasioderma serricorne* (Cigarette beetle), *Araoecerus fasicilatus* (Coffee bean weevil), and *Tribolium castaneum* (Red rust beetle). *Sitophilus zeamais* (Maize weevils) is the major insect pest of maize in the field and store.

Cowpea (*Vigna unguiculata*) is reported to be a major core food crop and chief source of protein in sub-Saharan Africa, especially in the dry savanna regions of West Africa where animal protein is rarely available (Adebayo *et al.*, 2017). *Callosobruchus maculatus* (Coleoptera;

Bruchidae) also known as cowpea bruchid, is a major pest of cowpea (Kamara *et al*., 2017). Insects such as *Rhyzopertha dominica*, *Callosobruchus chinensis*, *Sitophilus granarius* and *Callosobruchus maculatus* are some of the insect pests that attack food commodities during storage, of these, *Callosobruchus maculatus* is a major stored cowpea insect pest distributed in Asia and Africa, ranging from tropical to subtropical world (Fatima *et al*., 2016). Adebayo *et al.,* 2017, stated that protection of cowpea and maize in storage against *C. maculatus* and *S. zeamais* had been mainly through the use of synthetic chemicals. However, synthetic chemicals are known to have adverse effects on non-target organisms and the environment. Resistance and toxicity problems of associated with the use of synthetic insecticides have resulted in the necessity of finding more effective and healthier alternative control methods (Idoko and Adesina, 2013; Gbaye *et al.,* 2015). The use of botanical base insecticides is one of several methods being giving attention. Through several screening studies, extracts of parts (e.g., stem, bud and seed) of some plants are known known to have an insecticidal property (Adesina *et al*., 2019). The use of plant parts in the control of storage insect pests is considered safer for man and the environment because they little or no adverse effect. Such plants include: *Piper Guineense* (African Black Pepper), *Acalypha godseffiana*, *Alstonia boonei* and *Eugenia aromatica* (Cloves) (Ofuya *et al*., 2010). Cloves is a medicinal plant (Ganiyu *et al.,* 2015) known to have antidiabetic properties when taken orally (Norafida *et al.,* 2015), and can serve as spices in different parts of the world. Several research studies have also been conducted on *Eugenia aromatica* both in the field of Entomology and Medical Science; for diabetes test (Norafida *et al.,* 2015), and for metabolic change analysis (Fernanda *et al*., 2012).

This research investigates the possibility of using *Eugenia aromatica* as an entomocide for the management of *Sitophilus zeamais* and *Callosbruchus maculatus*.

Materials and Methods

All experiments were carried out in the Biology Department Laboratory, School of Sciences, Federal University of Technology, Akure, Ondo State.

Preparation of insect Culture

Infested Cowpea, *Vigna unguiculata* and infested maize, *Zea mays*, purchased from Oja Oba market, Akure, Ondo State were used to prepare the cultures in 100L plastic jars. Clean *Vigna unguiculata* (cowpea) and maize seed samples were also purchased from the market. The samples were kept in the refrigerator at 7ºC for 96hours to disinfest them (eliminate the egg, larva, pupa or adult present) and damaged seed were sorted out. The disinfested cowpea and maize samples were transferred into the two plastic Petri plates (8.5cm diameter) containing 20g each of cowpea and maize respectively. Twenty unsexed adults of *Callosobruchus maculatus* and *Sitophilus zeamais* were added to each jar, labeled and covered with muslin cloth for adequate ventilation. These culture jars were kept in the insect rearing cage under laboratory condition (24ºC - 30ºC, 65% - 75% R.H.). After fifteen days, all adult insects were removed from the cultures. Newly emerging adults were used for subsequent experiments.

Preparation of Material

Dried buds of *Eugenia aromaticum* (cloves) purchased from Oja Oba market were further air dried under ambient temperature in the laboratory, then pulverized into powder form using a mortar and pestle, sieved of 1mm^2 size mesh and kept in air-tight plastic containers in the refrigerator for subsequent use.

Experimental Procedure

Exposure of Sitophilus zeamais to plant powder: Twenty grams of un-infested maize were weighed into in separate plastic Petri plates (8.5 cm diameter) using a high precision balance model KD-CN (Serial No: 100828072) and different dosage of *E. aromatica* bud powder 0.0g (control) 0.5g, 1.0g, 1.5g and 2.0g were introduced into each of the plastic Petri plates (8.5 cm diameter) and thoroughly mixed with the maize seed. Fifteen unsexed adults of *S. zeamais* were then introduced into each experimental set up, the control set up had only the insect introduced (no treatment.)

Exposure of C. maculatus to plant powder: Twenty grams of non-infested cowpea seed were weighed into different plastic plastic Petri plates (8.5 cm diameter) using a weighing meter. Different dosage of the treatment *Eugenia aromatica* powder (0.0g (control) 0.5g, 1.0g, and 2.0g) were introduced into each of the containers and thoroughly mixed with the cowpea seed. Fifteen unsexed adult of *C. maculatus* were then introduced into each experimental set up, the control set up had only the insect introduced (no treatment). Each experiment set up had three replicates.

Data Collection and Analysis

Number of dead *Sitophilus zeamais* and *Callosbruchus maculatus* (mortality) were counted and recorded after 24hrs, 48hrs, 72hrs, 90hrs and 120hrs of the experiments. The LT_{50} (the time at which mortality of adult insects is 50%) at specified dosage was calculated. Furthermore, data were subjected to statistical analysis using one way ANOVA of Statistical Package for Social Sciences (SPSS) version 21 at 95% of confidence interval. The mean of the values was separated using Tukey's test.

Results

Determination of Lethal Time (LT_{50}) of Sitophilus zeamais Treated with Different Dosages of Eugenia aromatica Bud Powder after 4 Days

The lethal time of adult *S. zeamais* treated with different dosages of *E. aromatic* bud powder at varied hours is shown on Table 1. There was significant difference in LT_{50} among the adult *S. zeamais* treated with 0.5g for different hours of exposure ($F=8.725$, $p>0.003$). The LT_{50} was achieved in the 120 hours (55.53±4.47). Also, there was significant difference in LT_{50} among adult *S. zeamais* treated with the same dosage 1.0g for different hours of exposure ($F=21.329$, $p=0.000$) and the LT_{50} was achieved in the 96 hours (64.43±4.43).

At the 1.5g dosage, there was significant difference in LT_{50} among the group of adult *S. zeamais* treated for different hours of exposure ($F=8.496$, $p=0.003$). However, the LT_{50} was observed in the 48 hours (51.07±12.38). There was significant difference in LT_{50} among the group of adult *S. zeamais* treated with the same dosage of 2.0g for different hours of exposure ($F=9.991$, $p=0.002$). However, the LT_{50} was observed at 48 hours (59.93±13).

Table 1: Lethal time (LT_{50}) of *Sitophilus zeamais* treated with different dosage of *Eugenia aromatic* bud powder at 96 hours.

LT_{50}	Dosage (g) of *Eugenia aromatica* bud powder			
Time (Hrs)	0.5	1.0	1.5	2.0
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
24	11.07±8.00[a]	11.07±2.23[a]	19.93±6.67[a]	19.97±3.84[a]
48	33.33±6.67[ab]	33.30±3.87[ab]	51.07±12.38[ab]	59.93±13.33[ab]
72	35.53±4.47[ab]	48.87±5.89[bc]	62.17±8.02[ab]	77.77±9.68[b]
96	48.87±4.43[b]	64.43±4.43[c]	79.97±10.17[b]	82.17±8.02[b]
120	55.53±4.47[b]	71.07±8.00[c]	88.87±8.02[b]	88.87±5.88[b]

Each value is a means ± standard error of three replicates
Mean followed by same superscript alphabet along the same column are not significantly different ($p>0.05$) using Tukey's Test

Determination of LT_{50} of Callosobruchus maculatus treated with different Eugenia aromatica dosages for 4 days

The lethal time of *C. maculatus* adult*s* with same dosage for different hours is shown on Table 2. Significant difference was observed among the group of adult *C. maculatus* treated at 0.5g dosages for different hours of exposure ($F=9.732$, $p=0.002$). However, the LT_{50} was achieved in the 120 hours (51.07±2.23). Also, significant difference was observed among adult *C. maculatus* treated with the same dosage 1.0g for different hours of exposure ($F=13.411$, $p=0.000$) and the LT_{50} was achieved in the 72 hours (57.73±4.43).

Table 2: The Lethal Time (LT_{50}) of *Callosobruchus maculatus* treated at different dosage of *Eugenia aromatica*

LT_{50}	Dosage (g) of *Eugenia aromatica*			
Time (Hrs)	0.5	1.0	1.5	2.0
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
24	15.53±2.23[a]	19.97±3.84[a]	28.83±2.23[a]	35.53±8.00[a]
48	28.87±8.02[ab]	39.93±6.67[ab]	51.07±2.23[b]	57.73±5.88[b]
72	37.73±4.43[bc]	57.73±4.43[bc]	71.07±2.23[c]	82.20±2.20[c]
96	44.40±2.20[bc]	71.07±8.00[c]	84.43±4.43[cd]	97.77±2.23[c]
120	51.07±2.23[c]	77.73±8.02[c]	93.33±6.67[d]	100.00±.00[c]

Each value is a means ± standard error of three replicates.
Mean followed by same superscript alphabet along the same column are not significantly different ($p>0.05$) using Tukey's Test

Significant difference was observed in adult *C. maculatus* treated with the same dosage 1.5g for different hours of exposure ($F=42.951$, $p=0.000$). However, the LT_{50} was observed in the 48 hours (51.07±2.23). At 2.0g dosages significant difference was observed in adult *C. maculatus* treated for different hours of exposure ($F=35.179$, $p=0.000$). The LT_{50} was observed or achieved in the 48 hours (57.73±5.88).

Generally, comparison of *E. aromatica* treatment of *C. macullatus* and *S. zeamais* shows that the lethal time (LT_{50}) of *C. maculatus and S. zeamais* are observed to reduce as the dosage increases (Table 3). *C.maculatus* earliest LT_{50} was observed at 1.5g after 48hrs (51.07%), and that of *S. zeamais* at 1.5g after 48hrs 51.07%.

Table 3: Comparison of Lethal Time (LT_{50}) of *C. maculatus* and *S. zeamais* treated at different dosage of *E. aromatica*

Dosage (g)	Lethal Time (LT_{50})	
E. aromatica	*C. maculatus*	*S. zeamais*
0.5	120(51.07± 2.23)	120(55.53±4.47)
1.0	72 (57.73±4.43)	96 (64.43±4.43)
1.5	48 (51.07±2.23)	48 (51.07±12.38)
2.0	45 (57.73±5.88)	48 (59.93±13.33)

Percentage mortality of *Sitophilus zeamais* in maize seeds treated *Eugenia aromatic* bud powder

The percentage mortality of *S. zeamais* increased as the concentration of dosage increased (Table 4). For the control set up, there was no significant difference in percentage insect mortality for all the recorded hours of exposure. After 24hours, there was no significant difference in the percentage mortality in all the treatment at pvalue (>0.05). The lowest percentage mortality after 24hrs was observed in 0.5g and 1.0g (11.07) while the highest was observed at 2.0g (19.97).

After 48hrs, mean of the mortality at all the dosages are not significantly different from one another (p>0.05). The lowest percentage mortality at 48hrs was observed in 1.0g (33.30%) while the highest was observed in 2.0g (59.93%). After 72hours, mortality in 0.5g dosage of was not significantly different from those of dosages 1.0g and 1.5g, but significantly different from that of 2.0g. Dosage 1.0g is not significantly different from 0.5g, 1.5g and 2.0g. Dosage at 1.5g is not significantly different from 0.5, 1.0 and 2.0g. Dosage at 2.0g is not significantly different 1.0g and 1.5g, but significantly different from 0.5. The lowest percentage mortality after 72hrs was observed in 0.5g (35.53) while the highest was observed at 2.0g (77.77). After 96hours, dosage at 0.5g is not significantly different from dosages 1.0g and 1.5g but significantly different from 2.0g, dosage at 1.0g is not significantly different from 0.5, 1.5 and 2.0g. Dosage at 1.5g is not significantly different from 0.5, 1.0 and 2.0g. Dosage at 2.0g is not significantly different 1.0g and 1.5g, but significantly different from 0.5g. The lowest percentage mortality after 96hrs was observed in 0.5g (48.87) while the highest was observed at 2.0g (82.17). After 120hours, dosage at 0.5g is not significantly different from dosages 1.0 and 1.5 but significantly different from 2.0g, dosage at 1.0 is not significantly different from 0.5, 1.5 and 2.0g. Dosage at 1.5 is not significantly different from 0.5g, 1.0 and 2.0g. Dosage at 2.0g is not significantly different 1.0g and 1.5g, but significantly different from 0.5g. The lowest percentage mortality after 120hrs was observed in 0.5g (55.53) while the highest was observed at 2.0g (88.87).

Table 4: Mean (± S.E.) percentage mortality of *Sitophilus zeamais* in maize seeds treated with *Eugenia aromatic* bud powder

E. aromatica	Period of exposure (hrs) of *S. zeamais*				
Dosage (g)	24	48	72	96	120
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.5	11.07± 8.00[a]	33.33±6.67[a]	35.53±4.47[a]	48.87±4.43[a]	55.53±4.47[a]
1.0	11.07± 2.23[a]	33.30±3.87[a]	48.87±5.88[ab]	64.43±4.43[ab]	71.07±8.00[ab]
1.5	19.93± 6.67[a]	51.07±12.38[a]	62.17±8.02[ab]	79.97±10.17[ab]	88.87±8.02[ab]
2.0	19.97± 3.84[a]	59.93±13.33[a]	77.77±9.68[b]	82.17±8.02[b]	88.87±5.88[b]

Each value is a means ± standard error of three replicates.

Mean followed by same superscript alphabet along the same column are not significantly different (p>0.05) using Tukey's Test

Percentage Mortality of Callosobruchus maculatus in Cowpea Seeds Treated Eugenia aromatica Bud Powder

The percentage (%) mortality of *C. maculatus* increased as the concentration of dosage increased (Table 5). For the control set up, there was no significant difference in mortality for all the recorded hours of exposure. After 24hours, all the dosage used are not significantly different from one another at p value of >0.05. The lowest percentage mortality after 24hrs was observed in 0.5g (15.53±2.23) while the highest was observed in 2.0g (35.53±8.00). After 48hours, mortality at dosage 0.5g was not significantly different from those of other dosages (1.0g and 1.5g) but significantly different from dosage 2.0g. Dosage at 1.0 is not significantly different from 0.5 and 1.5 but significantly different from 2.0. Dosage at 1.5 is not significantly different from 0.5 and 1.0 but significantly different from 2.0. Dosage at 2.0g is significantly different from the other dosages (0.5, 1.0 and 1.5). The percentage mortality ranges from 28.87±8.02 - 57.73±5.88.

After 72hours, significant difference was observed among the group, dosage at 0.5g is not significantly different from the other dosages 1.0 and 1.5 but significantly different from 2.0. Dosage at 1.0 is not significantly different from 0.5 and 1.5 but significantly different from 2.0. Dosage at 1.5 is not significantly different from 0.5 and 1.0 but significantly different from 2.0. Dosage at 2.0g is significantly different from the other dosages (0.5, 1.0 and 1.5).

The percentage mortality ranges from 37.73±4.43 - 82.20±2.20. After 96hours, significantly difference was observed among the group, dosage at 0.5g is significantly different from the other dosages (1.0, 1.5 and 2.0), and dosage at 1.0 is not significantly different from 1.5 but significantly different from 0.5 and 2.0, Dosage at 1.5 is not significantly different from 1.0 and 2.0 but significantly different from 0.5. Dosage at 2.0g is not significantly different from 1.5 but significantly different from 0.5 and 1.0. The percentage mortality ranges from 44.40±2.20 - 97.77±2.23. After 120hours, Dosage at 0.5 is significantly different from all other dosage level (1.0, 1.5 and 20), dosage at 1.0 is significantly different from 0.5 dosage level but not significantly different from 1.5 and 2.0 dosage level, dosage at 1.5 is significantly different from 0.5 dosage level but not significantly different from 1.0 and 2.0 dosage level, dosage at 2.0 is significantly different from

0.5 dosage level but not significantly different from 1.0 and 1.5 dosage level. The lowest percentage mortality after 120hrs was observed in 0.5g (51.07±2.23) while the highest was observed at 2.0g (100.00±0.00).

Table 5: Percentage (%) Mortality (Mean± S.E) of *Eugenia aromatica* treated seeds on *Callosobruchus maculatus*

Dosage (g)	Hours of Exposure (hrs) of *C. maculatus*				
E. aromatica	24	48	72	96	120
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.5	$15.53±2.23^{a}$	$28.87±8.02^{a}$	$37.73±4.43^{a}$	$44.40±2.20^{a}$	$51.07±2.23^{a}$
1.0	$19.97±3.84^{a}$	$39.93±6.67^{ab}$	$57.73±4.43^{ab}$	$71.07±8.00^{b}$	$77.73±8.02^{b}$
1.5	$28.83±2.23^{a}$	$51.07±2.23^{ab}$	$71.07±2.23^{ab}$	$84.43±4.43^{bc}$	$93.33±6.67^{b}$
2.0	$35.53±8.00^{a}$	$57.73±5.88^{c}$	$82.20±2.20^{c}$	$97.77±2.23^{c}$	$100.00±0.00^{b}$

Each value is a means ± standard error of three replicates
Mean followed by same superscript alphabet along the same column are not significantly different ($p>0.05$) using Tukey's Test

Discussion

Lethal Time (LT_{50}) of Callosobruchus maculatus and Sitophilus zeamais Treated with Different Dosages of Eugenia aromatica

The lethal time (LT_{50}) of *C. maculatus and S. zeamais* decreased as the dosage increased, *C. maculatus* earliest LT_{50} was observed at 1.5g after 48hrs (51.07%), and that of *S. zeamais* at 1.5g after 48hrs 51.07%, this correlates with the report of Oyeniyi *et al.*, 2015, that tolerance of *C. maculatus* to *E. aromatica* at a low dosage result to increase in exposure time. At reduced dosage, *C. maculatus* is able to withstand the *E. aromatica* treatment due to the presence of phenolic acid produced by cowpea which gives the *C. maculatus* more ability to withstand long exposure time (Oyeniyi *et al.*, 2015), *C. maculatus* at 0.5g for 120hrs (51.07%), *S. zeamais* at 0.5g for 120hrs (55.53%), Similar to what was reported by Morrison *et al.* (1995), that cowpea possess phenolic compounds, which plants use as defense against pest such as *S. zeamais*. Increase in treatment dosage bringing about increased mortality rate of the insect pests correlates with Oyeniyi *et al.* (2015), which reported that regardless of treatment, increasing dose of botanical powder results in an increase in adult bruchid mortality.

This result shows that *E. aromatica* is effective in the control of *C. maculatus and S. zeamais.* In a previous study Ofuya *et al*. (2010) had reported that *E. aromatica* dry flower buds have entomocidal properties against *C. maculatus*. The active ingredient in *E. aromatica* is the Eugenol (Bhuiyan *et al*., 2010).

Percentage mortality of Callosobruchus maculatus in cowpea seeds treated Eugenia aromatic bud powder

Plant powder is an effective means of controlling insect pest, as reported in Gbaye *et al*. (201)5. *E. aromatica* and several other plants have been found to be effective against *C. maculatus*. It was reported in Adebayo *et al.* (2017) that *Hura crepitan* (L.) possess an insecticidal qualities which are useful in the control of *C. maculatus*. Several entomologists have also tested different botanicals in the control of *S. zeamais* such as *Artemisia* *capillaris (*Chineese worm wood), *Hyptis spicigera* (Lam) - Black sesame, *Carum carvia*- Caraway, *Erythrophleum suaveolen*- Red water tree (Niber *et al.*, 1992; Liu *et al*., 2010; Wekesa *et al.*, 2011).

It was observed that *E. aromatica* produced the highest mortality of *C. maculatus* adult after 24hrs of exposure, and *S. zeamais* adult after 48hrs. This contradicts the report of Olotuah 2014 that *E. aromatica* produce highest mortality of *C. maculatus* adult after 48hrs of exposure and *S. zeamais* highest mortality after 72hrs of exposure. The effectiveness of this plant material is due to the presence of Eugenol as observed in Olotuah, 2014.

Conclusion and Recommendation

The results of this study show that *E. aromatic* is an effective botanical in the control of *S. zeamais* and *C. maculatus* although the botanical is more effective on *C. maculatus* than *S.zeamais* Since, the use of plant based insecticides appears to be promising in combating insect pests, mass production of the crop *E. aromatica* should be taken into consideration.. Active ingredients of different botanicals should be extracted by the Chemical Industries and used to formulate more effective products having greater insecticidal properties.

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