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Research Article

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Isolation and Characterization of *Eugenia Aromatica* Oil Extract Against Tropical Warehouse Moth *Ephestia cautella* [Lepidoptera: Pyralidae] In Cocoa Beans

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Abstract

Cocoa bean is a raw material used for the production of chocolate and other confectionaries. Ephestia cautella is the major pest of dried cocoa beans in storage and synthetic insecticide like organochlorides and organophosphates are the major insecticides used to control this pest in storage which further post health hazard to man and his environment. This then necessitate the search for insecticide of plant origins which are bio-degradable and non-toxic to man. This study investigates the contact and fumigant efficacy of the powder and oil extract of Eugenia aromatica on the developmental stages of E. cautella. Powders of E. aromatica were administered at different concentrations (0.5g, 1.0g, 1.5g, 2.0g, and 2.5g). The oil from E.aromatica was extracted with ethanol using soxhlet extractor and redistilled using rotary evaporator and tested as fumigant insecticidal against development stages of E. cautellaat 0.5ml, 1.0ml, 1.5ml, 2.0ml, and 2.5ml. Egg hatchability, adult emergence, larvae and adult mortality of E.cautella were used as indices of insecticidal activities at 24hrs, 48hrs, 72hrs, and 96hrs post-treatment. Essential oil obtained from the plant was purified using thin layer chromatography and analysed by Gas Chromatography -Mass Spectrometer (GC-MS). Result obtained shown that E. aromatica powder and oil completely inhibited egg hatchability and adult emergence both as contact and fumigant. Except the 0.5g of E. aromatica powder that recorded 50.00% larva mortality and 51.67% adult mortality, other treatment concentrations recorded 90-100% larva and adult mortality. At 2.5ml oil extract tested as contact and fumigant larvicides after 96hrs recorded 92.98% and 98.23% mortality respectively. Results from phytochemical analysis of the oil showed that the major components were eugenol (82.044%) and Caryophyllene (11.716%). These findings suggested that E aromatica extract could be a potential source of insecticide which may be used for the production of biopesticide.

Keywords: *Ephestia cautella*, *Eugenia aromatica*, Organophosphates, Insecticidal, Phytochemical.

Introduction

The tropical warehouse moth, *Ephestia cautella* (Walker), is a major pest of a wide range of commodities including cereals and cereal products, cocoa and oilseed. *E. cautella* caused zero germination and 60% weight loss of wheat after 7 days post-infestation periods [1- 2]. It is found throughout the tropics and subtropics; but it can develop during summer in temperate countries and throughout the year in food industry environments with high temperature [3]. Its larvae which are the destructive stage are found as primary pests infesting whole grains and feeding on the germ. Its development from larva to adult takes approximately 29-31 days and the estimated rate of increase is about 50 times per lunar month [3]. The webbing and the frass produced by this insect affect the aesthetics and handling process of infested produce [4].

Cocoa (*Theobroma cacao*) is the most popular cash crop in West African countries. In Nigeria, before the discovery of petroleum in the early 1970s, cocoa was the major cash crop exported to foreign countries [5]. The nutrition and health benefits of cocoa beans include the provision of adequate proteins and vitamins and rich source of antioxidant such as phenol. Cocoa product when added to food are said to inhibit plasma lipid oxidation, lowered cholesterol, reduced high density lipoprotein, enhance blood flow, facilitate nitric oxide synthesis and inhibit platelet activation [6]. Dried cocoa bean is the principal raw material for chocolate, cocoa powder which served as a major host for *E. cautella* [6]. The control of these pests by synthetic insecticide has serious drawbacks [7]. The continuous use of chemical pesticides has resulted in various measurable problems to the environment as a whole. Some of these problems include, genetic resistance by pest species, toxic residues; pollution of storage environment and ever-increasing costs of pesticides use with disastrous consequences of phytotoxicity to agricultural crops [8]. *Eugenia aromatica* (Clove) is an unopened flower bud growing on a tree belonging to the family of Mytaceace which is same as that of guavas. Clove buds has numerous medicinal properties. It has been reported to have anti-oxidant, antimicrobials, anti-inflammatory, antiseptic, anti-algesic and anti-convulsant

properties [9]. Some bioactive compounds such as the terpenoids, monoterpenes, sequienterpenes and other compounds have been reported to be present in the clove oil essential oil [10]. This research work is sought to reveal the insecticidal activity of *Eugenia aromatica* against *Ephestia cautella* (Lepidoptera: Pyralidae) and its toxicological effect on Albino rat.

Materials and methods

Study site

Laboratory experiments were conducted at the Department of Biology, Federal University of Technology, Akure (Longitude 8.49° N and Latitude 5.13°E), at ambient temperature of (28 ± 2°C) and relative humidity (75 ± 5%).

Insect Rearing

Naturally infested cocoa beans were collected from Coop Cocoa Nigeria Limited, Akure, Ondo State and placed in a two litres plastic container containing 300g of uninfested cocoa powder obtained from Ile-Oluji Cocoa Product Company, Ondo State to start *E. cautella culture*. The plastic containers were covered with muslin cloth, fastened with rubber bands, and placed inside wire mesh cage of dimension 75cm×50cm and 60cm (L×W×H) with its four stands dipped in water-kerosene mixture contained in the plastic container to prevent entry of predatory ants into the cages. The whole setup was left inside the postgraduate research laboratory of the Department of Biology, Federal University of Technology, Akure.

Preparation of Plant Materials

The clove of *Eugenia aromatica* was purchased at the Oja Oba market Ondo town, Ondo. This plant part was brought into the laboratory, washed thoroughly with water and air-dried in the laboratory for 21 days. The clove was pulverized into fine powder using Binatone electric blender (Model 373). The powder was further sieved to pass through 1mm^2 perforation. The fine powder was kept in airtight plastic container to avoid the absorption of moisture and then stored at ambient temperature of 28 ± 2°C and 75 ± 5%.rh.

Extraction of the Plant Oil

Thirty grams of pulverized plant material was put in a muslin cloth of dimension 20cm by 4cm and then transferred into the thimble and extracted with ethanol in a soxhlet apparatus model 77-520 (Hospital Equipment Manufacturing Co, Limited India). The extraction was carried out for 3-4hrs and the extraction was terminated when the solvent in the thimble became clear. Then, the thimble was removed from the unit and the solvent recovered by distilling in the soxhlet extractor. Rotary evaporator was used to separate the solvent from the oil after collection of the resulting extracts from the soxhlet, after which the oil was exposed to air so that traces of the volatile solvent evaporates, leaving the oil extract.

Chromatography

Column chromatography was used to purify individual chemical compounds from mixtures of compounds. The mobile phase or eluent used is a mixture of four different solvents (Ethanol, Methanol, N-hexane and Chloroform) which are categorized into polar and non-polar solvents. The solvents were mixed at different ratio (6:2:1:1) in a pyrex separating funnel. Furthermore, Thin-layer chromatography (TLC) is a chromatography technique was used to separate each compound from the mixture.

$$\text{Retardation factor (Rf)} = \frac{\text{Distance traveled by substance}}{\text{Distance traveled by Solvent}}$$

Experimental Procedure For Insecticidal Activity

In this experiment, fumigant and contact toxicity for 24 hours, 48 hours, 72 hours and 96 hours of *Eugenia aromatica* powders and oil extracts were tested on developmental stages of *Ephestia cautella* to assess them for bioinsecticidal potentials. All the experiments were conducted at room temperature of 28 ± 2°C and relative humidity of 75 ± 5%.

Contact toxicity of E. aromatica powders on eggs, larval and Adult of E. cautella

Twenty freshly laid eggs (0-24hrs old) were placed on 20g of cocoa beans treated with 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5g clove powder of *E. aromatica* inside plastic container (8cm diameter and 4cm depth). The treated and the control (untreated) were replicated three times. Daily observation was made with dissecting microscope to determine the number of egg that hatch from the total number of eggs incubated and the experiment was left inside breeding wire mesh cage measuring (75 × 50 × 60) cm and after 41 days and the number of adult emerged was determined and the percentages calculated.

For the larva, ten third instars larva were introduced into the treated and uninfested cocoa beans and were replicated three times. The numbers of dead larvae were counted after 24hrs, 48hrs, 72hrs and 96hrs post treatment and mean determined. Ten pairs of newly emerged adult of *E. cautella* were introduced into plastic containers, and the same procedure was used for adult mortality. The same procedure was repeated for third instar larvae and adults obtained from the stock culture. At the end of the 96 hours post treatment data on percentage larva and adult mortality were recorded and corrected using Abbott formula [11].

Contact toxicity of *E. aromatica* oil on eggs, larval and Adult of *E. cautella*

Different concentration of 5%, 10%, 15%, 20% and 25% oil extract of *E. aromatica* clove was prepared. A concentration of 5% was prepared by dilution of 0.5ml of plant extracts in 9.5ml of ethanol (solvent). 10% concentration was made by 1.0ml of plant extracts in 9.0ml ethanol. Also, 15%, 20% and 25% concentration were obtained by diluting 1.5ml, 2.0ml and 2.5ml of the plant extract with 8.5ml, 8.0ml and 7.5ml of the solvent respectively. The oil extract of the plant was measured with the aid of graduated syringe at different concentrations were administered on plastic container containing 20g of cocoa beans and the oil were thoroughly mixed with the beans using glass rod in order to ensure uniform coating of the bean with the oils. The treated Cocoa bean were exposed to air for 30 minutes to allow the escape of the volatile solvent. Twenty eggs were placed on the treated samples in the plastic container. Also, twenty eggs were introduced into untreated samples to serve as control. The set up were replicated three times. The eggs were examined daily with dissecting microscope to determine the number of eggs that hatch from the total number of eggs incubated and the experiment was left inside the insect breeding wire mesh cage pending adult emergence. 2ml of ethanol solvent control treatments were also set up to see the effect of solvent on hatchability and adult emergence.

The same procedure was used for larval and adult. Ten larvae were introduced into a plastic container containing 20g of treated cocoa

beans at concentrations 5%, 10%, 15%, 20% and 25% of the oil extract placed inside a plastic container containing the cocoa beans sample and replicated thrice. While ten pairs of newly emerged adult of *E. cautella* were introduced into plastic containers, and the same procedure was used for adult mortality. The control experiment i.e. untreated cocoa beans and solvents treated cocoa beans were also prepared and replicated three times. Then, larvae and adult mortality were counted at 24hrs, 48hrs, 72hrs and 96 hrs after application. The same procedure was repeated for third instar larvae obtained from the stock culture. At the end of the 96 hours post treatment data on percentage larva and adult mortality were corrected using Abbott formula [11].

Fumigant effect of plant oil on the eggs, larval and Adult of *E. cautella*

Oil extract of the plant was measured with the aid of graduated syringe at concentration of 0.0, 5%, 10%, 15%, 20% and 25% were administered on the filter papers and were allowed to air dried for four hours and placed inside plastic container containing 20g of cocoa beans maintained in an air tight condition. Twenty eggs were placed on the treated samples in the plastic container. Also, twenty eggs were introduced into untreated samples to serve as control. The set up were replicated three times. The eggs were examined daily and the total number of eggs that hatched was recorded. Solvent control treatments were also set up to see the effect of solvent on hatchability and adult emergence. At the end of 41 days post-treatment period the total number of emerged adult was determined and percentage calculated.

The same procedure was used for larval and adult. Ten larvae were introduced into a plastic container containing 20g of cocoa beans with filter paper treated at concentrations 5%, 10%, 15%, 20% and 25% of oil extract placed inside a plastic container containing the cocoa beans sample in an air tight condition and replicated thrice. While ten pairs of newly emerged adult of *E. cautella* were introduced into plastic containers, and the same procedure was used for adult mortality. The control experiment i.e. untreated filter paper and solvents treated filter paper were also prepared and replicated three times. Then, larvae and adult mortality were counted at 24hrs, 48hrs, 72hrs and 96 hrs after application. The same procedure was repeated for third instar larvae and adults obtained from the stock culture. At the end of the 96 hours post treatment data on percentage larva mortality was corrected using Abbott formula thus [11].

$$P_T = \frac{P_0 - P_C}{100 - P_C}$$

Where P_T= Corrected mortality P_O= Percentage observed mortality P_C= Percentage control mortality

Toxicity of *E. aromatica* fractions on eggs and larval of *E. cautella*

Different fraction of the oil extract of *E. aromatica* were prepared. From the fraction, 2.5ml was obtained using graduated syringe and was administered on the filter papers and were allowed to air dried for four hours and placed inside plastic container containing 20g of cocoa beans. Ten eggs were placed on the samples in the plastic container. The setup was replicated three times for each fraction with different retardation factor while untreated paper strips were used as the control experiment. The eggs were examined daily for four days and the total numbers of eggs hatched were recorded. The same procedure was used for larval mortality. Ten larvae were introduced into a plastic container containing 20g of cocoa beans with filter paper treated with 2.5ml of oil fraction. The fraction was obtained using graduated syringe and then administered on the filter papers. The papers were allowed to air dried for four hours and placed inside anairtight plastic container containing the cocoa beans sample in an air tight condition. The setup was replicated three times for each fraction with different retardation factor while untreated paper strips were used as the control experiment. Then, larvae mortality was recorded at 48h and 96 hours after application. Chemical compounds present in fractions that caused the highest larva mortality and that prevented egg hatchability were determined using Gas Chromatography coupled with Mass Spectrum (GC-MS)

GC-MS analysis of extract

Gas chromatography coupled with Mass Spectrometer (GC-MS) analysis was used to reveal profiles of compounds contained in the extracts. One µl of each extract were analyzed using Agilent Technologies. The models of machine are as follows: Mass spectrum (5975C VLMSD), Injector (7683B Series) and GC (7890A). The capillary column was HP-5MS. The column has dimensions of: 30cm in length, 0.320mm internal diameter, and a film thickness was 0.25µm. Helium was used as the carrier gas. The GC oven temperature was set at 80°C for 2 minutes. The temperature increased steadily at 6°C per minutes to 240°C and was held for 6 minutes. The running time of each sample was 36 minutes. The peak of each chemical compound is expressed based on its retention time and balance.

Results

Contact toxicity of *E. aromatica* powders on egg hatchability and adult emergence of *Ephestia cautella*

The effect of *E. aromatica* powder on egg hatchability and adult emergence of *E. cautella* is presented in Table 1. All the rates of the plant powder completely inhibited egg hatching and adult emergence of *E. cautella*. There was no significant different ($P>0.05$) in the percentage egg hatched and percentage adult emerged at all application rates of the plant powder when compared but differ significantly ($P<0.05$) from the control.

Contact toxicity of *Eugenia aromatica* on larva of *E. cautella*

Toxic effect of *E. aromatica* on the larva mortality of *E. cautella* is presented in Table 2. There was 50% and 76.67% larva mortality at 0.5g and 1.0g rate at 24hrs post-treatment using the plant powder respectively. Total (100%) moth larva mortality was recorded at 1.5g – 2.5g rate at 24hrs post-treatment period. At 48hrs, 72hrs and 96hrs; 100% larva mortality were recorded at all concentration and there was significant different ($P<0.05$) at all rate when compared with the control.

Contact toxicity of *Eugenia aromatica* on the adult of *E. cautella*

The contact toxicity of *E. aromatica* on the adult mortality of *E. cautella* is presented in Table 3. At 24hrs post-treatment, 0.5g, 1.0g and 1.5g rate of *E. aromatica* clove powder caused 51.67%, 71.67% and 87.33% adult mortality respectively, while 2.0g and 2.5g rate caused 100% adult mortality. However, at 48hrs post-treatment with plant powder, all rate caused 100% adult moth mortality except 0.5g rate which caused 77% mortality. There was significant difference when comparing the control with 0.5g rate and 1.0g rate at 48hrs post-treatment but no significant different ($P>0.05$) between 1.0g – 2.5g rate. Mortality of 100% were recorded at all rate (0.5g – 2.5g) at 72hrs and 96hrs post-treatment period.

Table 1: Contact toxicity of *E. aromatica* powders on egg hatchability and adult emergence of *E. cautella*(M ± SE)

Rate (g/20g Cocoa bean)	Egg Hatchability (%)	Adult Emergence (%)
Control	88.33±4.41[b]	88.33±4.41[b]
0.5	0.00±0.00[a]	0.00±0.00[a]
1.0	0.00±0.00[a]	0.00±0.00[a]
1.5	0.00±0.00[a]	0.00±0.00[a]
2.0	0.00±0.00[a]	0.00±0.00[a]
2.5	0.00±0.00[a]	0.00±0.00[a]

Means followed by same alphabet are not significantly different at p<0.05 using Tukey's post hoc test.

Table 2: Contact toxicity of *E. aromatica* powders on larva mortality of *E. cautella* (M ± SE)

Rate (g/20g Cocoa bean)	Mean % Larva Mortality After			
	24 Hrs	48 Hrs	72 Hrs	96 Hrs
Control	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
0.5	50.00±2.89[b]	100.00±0.00[b]	100.00±0.00[b]	100.00±0.00[b]
1.0	76.67±1.67[c]	100.00±0.00[b]	100.00±0.00[b]	100.00±0.00[b]
1.5	100.00±0.00[d]	100.00±0.00[b]	100.00±0.00[b]	100.00±0.00[b]
2.0	100.00±0.00[d]	100.00±0.00[b]	100.00±0.00[b]	100.00±0.00[b]
2.5	100.00±0.00[d]	100.00±0.00[b]	100.00±0.00[b]	100.00±0.00[b]

Means followed by same alphabet are not significantly different at p<0.05 using Tukey's post hoc test.

Table 3: Contact toxicity of *E. aromatica* powders on Adult mortality of *E. cautella* (M ± SE)

Rate (g/20g Cocoa bean)	Mean %Adult Mortality After			
	24 Hrs	48 Hrs	72 Hrs	96 Hrs
Control	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
0.5	51.67±2.89[b]	77.00±5.00[b]	100.00±0.00[b]	100.00±0.00[b]
1.0	71.67±1.67[c]	100.00±0.00[c]	100.00±0.00[b]	100.00±0.00[b]
1.5	87.33±1.67[d]	100.00±0.00[c]	100.00±0.00[b]	100.00±0.00[b]
2.0	100.00±0.00[e]	100.00±0.00[c]	100.00±0.00[b]	100.00±0.00[b]
2.5	100.00±0.00[e]	100.00±0.00[c]	100.00±0.00[b]	100.00±0.00[b]

Means followed by same alphabet are not significantly different at p<0.05 using Tukey's post hoc test.

Contact toxicity of *ethanolic* oil extract of *E. aromatica* on egg hatchability and adult emergence of *E. cautella*.

The contact effect of ethanolic oil extract of *E. aromatica* on egg hatchability and adult emergence of *E. cautella* is presented in Table 4. All the concentration of the ethanolic oil extract of the clove of *E. aromatica* completely inhibited egg hatch and adult emergence of *E. cautella*. There was no significant difference (P>0.05) in the percentage egg hatch and percentage adult emergence at all application concentration of (5% – 25%) clove oil extract when compared but differ significantly from the untreated and solvent control.

Contact toxicity of ethanolic oil extract of *E. aromatica* on larva of *E. cautella*.

The contact effect of ethanolic oil extract of *E. aromatica* on larva mortality of *E. cautella* is presented in Table 5. Concentration 5% at 24hrs post-treatment shows no significant difference (P>0.05) when compared with the control. While, larva mortality at concentration 10% – 25% has significantly varied from each other (P<0.05) when compared to the control. No significant different (P>0.05) was observed at 5% and 10% larva mortality in relation to untreated and solvent control at 48hrs post-treatment. Moreover, 72hrs post-treatment revealed that concentration 5% and 10% caused 10% and 12% larva mortality while concentration 15%, 20% and 25% caused 37.33%, 62.67%, and 74.67% mortality respectively. Moreover, highest larva mortality, 81.00% and 91.33% were observed at concentration 20% and 25% at 96hrs post-treatment.

Contact toxicity of ethanolic oil extract of *E. aromatica* on the adult of *E. cautella*.

The contact effect of ethanolic oil extract of *E. aromatica* on adult mortality of *E. cautella* is presented in Table 6. No adult mortality was noticed at 24hrs post-treatment in concentration 5% and has no significant difference (P>0.05) when compared to the control. Moreover, at 72hrs post-treatment, concentration 5% and 10% caused 12.67% and 35.33% adult mortality while concentration 15%, 20% and 25% caused 63%, 77%, and 96.67% mortality respectively. Therefore, highest (100%) larva mortality was observed at concentration 25% at 96hrs post-treatment.

Table 4: Contact toxicity of ethanolic oil extract *E. aromatica* on egg hatchability and adult emergence of *E. cautella*(M ± SE)

Concentration (%)	Egg Hatchability (%)	Adult Emergence (%)
5	0.00±0.00[a]	0.00±0.00[a]
10	0.00±0.00[a]	0.00±0.00[a]
15	0.00±0.00[a]	0.00±0.00[a]
20	0.00±0.00[a]	0.00±0.00[a]
25	0.00±0.00[a]	0.00±0.00[a]
Control	99.33±1.67[c]	88.33±6.01[b]
Solvent	81.67±1.67[b]	80.00±2.89[b]

Means followed by same alphabet are not significantly different at p<0.05 using Tukey's post hoc test.

Table 5: Contact toxicity of ethanolic oil extract *E. aromatica* on larva of *E. cautella* (M ± SE)

Concentration (%)	Mean %Larva Mortality After			
	24 Hrs	48 Hrs	72 Hrs	96 Hrs
5	0.00±0.00[a]	5.00±2.89[a]	10.00±5.77[a]	10.33±6.06[a]
10	1.67±1.67[a]	5.00±2.89[a]	12.00±1.53[a]	14.00±3.00[a]
15	8.33±1.67[ab]	23.67±4.10[b]	37.33±1.45[b]	49.33±2.33[b]
20	10.33±3.18[ab]	34.67±4.00[bc]	62.67±2.67[c]	81.00±4.16[bc]
25	13.67±3.18[b]	37.33±1.45[c]	74.67±0.33[c]	91.33±4.48[c]
Control	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Solvent	5.00±2.89[ab]	8.33±1.67[a]	10.00±2.89[a]	10.67±4.67[a]

Means followed by same alphabet are not significantly different at p<0.05 using Tukey's post hoc test.

Table 6: Contact toxicity of ethanolic oil extract of *E. aromatica* on adult of *E. cautella* (M ± SE)

Concentration (%)	Mean %Adult Mortality After			
	24 Hrs	48 Hrs	72 Hrs	96 Hrs
5	0.00±0.00[a]	6.67±1.67[ab]	12.67±1.67[b]	21.00±2.89[b]
10	3.33±1.67[a]	18.33±1.67[b]	35.33±1.67[c]	50.67±3.67[c]
15	18.33±1.67[b]	40.00±2.89[c]	63.00±8.19[cd]	80.67±1.67[d]
20	21.67±3.33[b]	46.67±4.41[cd]	77.00±6.24[de]	89.33±3.18[de]
25	25.00±2.89[b]	58.33±3.33[d]	96.67±1.67[e]	100.00±0.00[e]
Control	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Solvent	1.67±1.67[a]	1.67±1.67[a]	1.67±1.67[a]	3.67±3.67[a]

Means followed by same alphabet are not significantly different at p<0.05 using Tukey's post hoc test.

Fumigant toxicity of ethanolic oil extract of *E. aromatica* on egg hatchability and adult emergence of *E. cautella*.

The effect of *E. aromatica* ethanolic oil extract on egg hatchability and adult emergence of E. cautella is presented in Table 7. All the concentration of the ethanolic oil extract of the clove of *E. aromatica* completely inhibited egg hatch and adult emergence of *E. cautella*. There was no significant difference (P>0.05) in the percentage egg hatch and percentage adult emergence at all application concentration of (5% – 25%) clove oil extract when compared but differ significantly(P<0.05) from untreated and the solvent control.

Fumigant toxicity of ethanolic oil extract of *E. aromatica* on larva of *E. cautella*.

Table 8 shows the fumigant effect of the oil on the third instar larva of *E. cautella*. There was no mortality observed at 24hrs post-treatment at 5% concentration of the oil extract, while 27% larva mortality was observed at the same concentration at 96hrs post-treatment. At 48hrs post-infestation period, all concentrations, 5% – 25% concentrations of the plant oil extract caused 3.33% – 46.67% larva mortality compared to the control which had 0.00% mortality. At 72hrs post-treatment, more than 50% mortality was recorded at concentration 20% and 25%. So, at 96hrs post-treatment, high larva mortality of 57.33%, 89.33% and 98.33% were recorded at 15%, 20% and 25% respectively. Significant different (P<0.05) existed between all the treatment rate at 96hrs when compared with the untreated control and the solvent treated samples.

Fumigant toxicity of ethanolic oil extract of *E. aromatica* on adult of *E. cautella*.

The fumigant effect of ethanolic oil extract of *E. aromatica* on adult mortality of *E. cautella* is presented in Table 9. At 24hrs post-treatment, concentration 5% caused 0% mortality of the adult insect. There was no significant different (P>0.05) between the control and concentration 5% at 24hrs post-treatment while, concentration 10% – 25% has significant different (P<0.05) on *E. cautella* adult mortality when compared with the control. No significant different (P>0.05) was observed at 5% and 10% adult mortality when compared with untreated and solvent control at 48hrs post-treatment. At 72hrs post-treatment, concentration 5% and 10% caused 10.62% and 33.82% adult mortality while concentration 15%, 20% and 25% caused 40.84%, 78.56%, and 85.87% mortality respectively. Moreover, highest larva mortality of 88.99% and 98.25% were observed at concentration 20% and 25% at 96hrs post-treatment respectively.

Table 7: Fumigant toxicity of ethanolic oil extract of *E. aromatica* on egg hatchability and adult emergence of *E. cautella* (M ± SE)

Concentration (%)	Egg Hatchability (%)	Adult Emergence (%)
5	0.00±0.00^{a}	0.00±0.00^{a}
10	0.00±0.00^{a}	0.00±0.00^{a}
15	0.00±0.00^{a}	0.00±0.00^{a}
20	0.00±0.00^{a}	0.00±0.00^{a}
25	0.00±0.00^{a}	0.00±0.00^{a}
Control	95.00±2.89^{c}	93.33±1.67^{c}
Solvent	91.67±1.67^{b}	88.33±1.67^{b}

Means followed by same alphabet are not significantly different at p<0.05 using Tukey's post hoc test.

Table 8: Fumigant toxicity of ethanolic oil extract of *E. aromatica* on larva of *E. cautella* (M ± SE)

Concentration (%)	Mean %Larva Mortality After			
	24 Hrs	48 Hrs	72 Hrs	96 Hrs
5	0.00±0.00^{a}	3.33±1.67^{a}	12.67±3.21^{a}	27.00±3.21^{b}
10	3.33±1.67^{ab}	14.00±3.00^{ab}	35.33±1.67^{b}	50.33±4.26^{c}
15	10.00±2.87^{bc}	19.00±5.69^{b}	42.33±7.97^{b}	57.33±8.67^{c}
20	16.67±1.67^{bc}	36.33±0.67^{c}	78.67±6.06^{c}	89.33±5.67^{d}
25	23.33±1.67^{c}	46.67±4.91^{c}	86.00±4.73^{c}	98.33±1.67^{d}
Control	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Solvent	1.67±1.67^{a}	3.33±1.67^{a}	3.33±1.67^{a}	5.67±3.18^{ab}

Means followed by same alphabet are not significantly different at p<0.05 using Tukey's post hoc test.

Table 9: Fumigant toxicity of ethanolic oil extract of *E. aromatica* on adult of *E. cautella* (M ± SE)

Concentration (%)	Mean %Adult Mortality After			
	24 Hrs	48 Hrs	72 Hrs	96 Hrs
5	0.00±0.00^{a}	3.42±1.71^{ab}	10.62±2.95^{a}	25.34±4.47^{b}
10	3.33±1.67^{ab}	13.68b±3.16^{b}	33.82±3.02^{b}	49.22±3.90^{c}
15	8.42±3.29^{b}	18.77±5.82^{c}	40.84±8.92^{b}	56.34±9.65^{c}
20	15.26±0.26^{bc}	36.23±0.61^{d}	78.56±6.09^{c}	88.99±5.60^{d}
25	22.02±1.52^{c}	46.49±4.88^{d}	85.87±4.55^{c}	98.25±1.75^{e}
Control	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Solvent	1.67±1.67^{a}	3.42±1.71^{ab}	3.51±1.75^{a}	5.36±3.04^{ab}

Means followed by same alphabet are not significantly different at p<0.05 using Tukey's post hoc test.

Toxicity of *E. aromatica* Fractions on egg and larva of *E. cautella*

The toxic effects of the fractions collected were tested on the egg and the larva of *E. cautella* and is presented in Table 10 and 11. Seven fractions with different retardation factor (Rf) labelled S1, S2, S3, S6, S7, S12 and S17 were tested on the egg and larva of the insect. All the fractions completely inhibited egg hatchability of *E. cautella* except S2 which caused 76.67% inhibition after 96 hrs of exposure. Faction S6 and S12 caused 100% larva mortality at 48 hrs post-treatment while S1 and S17 caused 90% larva mortality at same time of exposure. Whereas, at 96 hrs post-treatment, all fractions caused 100% mortality except sample S2 which caused 83.33% larva mortality.

Table 10: Toxicity of *E. aromatica* factions on egg hatchability of *E. cautella*

Samples	No of Eggs	% Eggs Hatched	% Unhatched Eggs after 96 hours
Control	30	93.33	6.67
S1	30	0.00	100.00
S2	30	23.33	76.67
S3	30	0.00	100.00
S6	30	0.00	100.00
S7	30	0.00	100.00
S12	30	0.00	100.00
S17	30	0.00	100.00

Table 11: Toxicity of *E. aromatica* factions on larva mortality of *E. cautella*

Samples	No of Larva	% Larva mortality after 48 Hours	% Larva mortality after 96 Hours
Control	30	0.00	6.67
S1	30	90.00	100
S2	30	63.33	83.33
S3	30	86.67	100
S6	30	100.00	100
S7	30	83.00	100
S12	30	100.00	100
S17	30	90.00	100

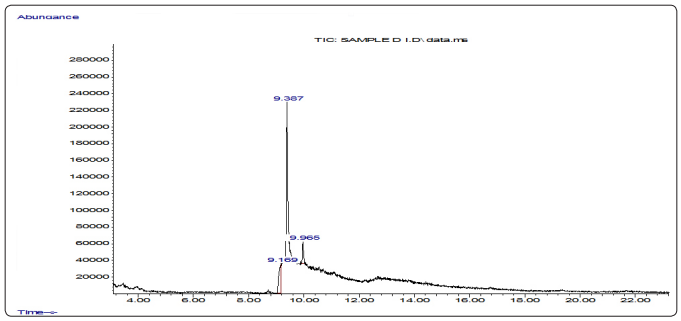

Figure 1: Chromatogram showing the chemical components of *E. aromatica* (Sample 1)

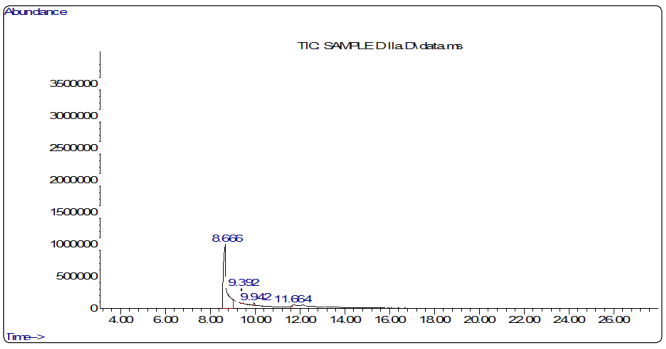

Figure 2: Chromatogram showing the chemical components of *E. aromatica* (Sample 2)

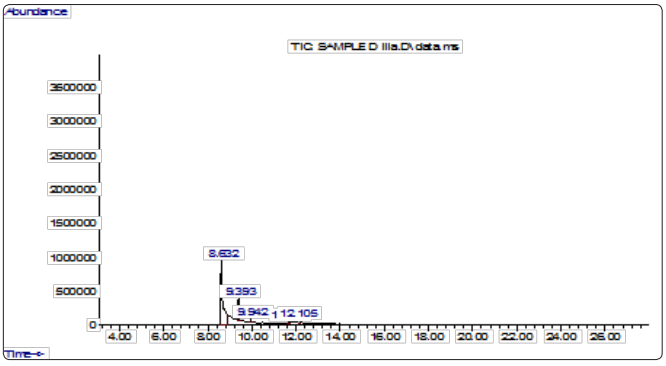

Figure 3: Chromatogram showing the chemical components of *E. aromatica* (Sample 3)

Table 12: Names of chemical components present in sample 1 of *E. aromatica* oil extract

RT (mins) Name of Components CAS no. % of total
9.169 Eugenol 000097-53-0 20.74 9.389 Caryophyllene 000087-44-5 71.72 9.965 2-methoxy-3-(2-propenyl), Phenol 001941-12-4 7.54

*RT: Retention Time
*CAS no: Chemical Abstract Service Registry Number.

Table 13: Names of chemical components present in sample 2 of *E. aromatica* oil extract

RT (mins) Name of Components CAS no. % of total
8.666 Eugenol 000097-53-0 90.216 9.392 Caryophyllene 000087-44-5 7.042 9.942 2-methoxy-3-(2-propenyl), Phenol 000093-28-7 1.470 11.994 2-methoxy-4-(2-propenyl), Phenol 001941-12-4 1.272

*RT: Retention Time
*CAS no: Chemical Abstract Service Registry Number.

Table 14: Names of chemical components present in sample 3 of *E. aromatica* oil extract

RT (mins) Name of Components CAS no. % of total
8.632 2-methoxy-3-(2-propenyl), Phenol 000097-53-0 82.044 9.393 Caryophyllene 000087-44-5 11.716 9.942 Humulene 006753-98-6 2.125 11.681 2-methoxy-4-(1-propenyl), Phenol 000097-54-1 2.757 12.105 2-methoxy-5-(1-propenyl), Phenol 019784-98-6 1.358

*RT: Retention Time
*CAS no: Chemical Abstract Service Registry Number.

Discussion

Contact and fumigant toxicity of *Eugenia aromatica* powder and oil on developmental stages of *Ephestia cautella*

The result of this research showed that powder and oil extracts of *Eugenia aromatica*, have significant effect on the survival of the egg, larvae and adult of *E. cautella*. The powder of *Eugenia aromatica* on contact toxicity was found to completely inhibit eggs hatching and emergence of adult of *E. cautella* at all levels of concentration. The inability of the egg to hatch to larval may be due to the fact that the powders inhibit gaseous exchange between the egg and external environment [12]. Katunku reported that saponnins that is found in *E. aromatica* affect the respiratory system of insects and causes expiratory effect due to their deterrent action on them [13]. The action of the powder treatments of *E. aromatic* amay also be attributed to their odours which corroborates the report of Lale and Abdulrahman that mortality of storage insects could be associated with the pungent odour produced by plants powders used against them [14].

Ashamo and Ogungbite also reported that [15]. *E aromatica* was also able to prevent the emergence of the adult moth at concentration (2%). The results obtained in this study have corroborated reports of previous works that [16-17]. *E aromatica* powder has significant contact and fumigant action against *C. maculatus*. The ability of these extracts to prevent or reduce emergence of adult could be due to the death of the insect larval which may occur as a result of inability of the larval to fully cast off their exoskeleton which remained linked to the posterior part of their abdomen [18]. The results of this study indicated that the extracts of this plant had obvious effect on post embryonic survival of this insect which resulted in prevention of adult emergence at different concentrations. The contact toxicity of *Eugenia aromatica* to adult *Ephestia cautella* depends on concentration and exposure periods. Mortality varied with rate of application and time of exposure. This observation tallies with the findings of Adedire and Lajide that the pulverized powder of *Piper umbrellatum* seed and [16]. *E aromatica* were toxic to *C. maculatus* producing 100% mortality at 24hrs post-treatment across all concentrations. Olotuah [19] also reported that extract from *E. aromatica* significantly ($P < 0.05$) reduced the population of all the storage pests tested. The toxicity of *E. aromatica* powders used in this study suggest that they could serve as alternative to synthetic chemical insecticide to protect stored cocoa beans against *E. cautella*.

The oil extracts of this plant are used in control of stored product coleopteran and lepidoptera because of their relative high efficacy on all developmental stages of insect [19]. Result from this research showed that the ethanolic oil extract of *Eugenia aromatica* significantly inhibited egg hatchability and adult emergence at all concentration. The result obtained on the egg hatchability and adult emergence of *E. cautella* was supported by the findings of Akinneye and Ogungbite in which some botanical oils were found to prevent the hatching of the egg as well as emergence of adult [5, 20]. *E cautella*. The efficacy of the oils of these botanicals could be as a result of inability of the insect to feed on the oil coated cocoa beans which may in return leads to starvation. The oil extracts may have also disrupted the normal respiratory activity of the insect and this may lead to asphyxiation and death of the insect [5]. However, the secondary metabolites present in these plants could be responsible for the inability of the adult insect to emerge as opined by Mordue-luntz and Nisbet as well as Yang that secondary metabolites in botanicals are found to disrupt growth and reduced larva survival as well as disrupt life cycle of insects [21- 22].

Chemical composition of *Eugenia aromatica*

Essential oil from bud of clove was obtained from distillation, and their chemical constituents were determined by GC-MS. The findings indicated that the essential oils mainly contain Eugenol, Caryophyllene, Humulene, 2-methoxy-3-(2-propenyl), 2-methoxy-4-(2-propenyl) and 2-methoxy-5-(1-propenyl). Eugenol is the principal chemical component of essential oil from *Eugenia aromatica*, *Cinnamomum zeylanicum* and *Ocimum basilicum* and *Ocimum gratissium* [23]. The high concentration of eugenol in leaf and buds oil makes it potentially useful in the medicines because they exhibit antibacterial, antifungal, anti-inflammatory activity, antioxidant and insecticidal properties, and are used traditionally as flavouring agent and antimicrobial material in food [24-27]. The phytochemical results reveal that eugenol amount to over 80% of the chemical composition of the plant. These findings are in agreement with report of Alma, which revealed that 18 chemical components were found in the essential oil from Turkey clove bud, where major components are eugenol (87%), eugenyl acetate (8.01%) and β-caryophyllene (3.56%) [28]. The insecticidal potency of *E. aromatica* was suggested to be caused by eugenol, caryophyllene, humulene and other compounds which have been proven to be insecticidal. Research conducted by Miyazawa and Hisama revealed that toxicity and repellency activity of eugenol was concentration dependent and period of exposure [29]. Da Silva reported that the essential oil of *Commiphora leptophloeos* contain active compounds; (E)-caryophyllene and α-humulene which both act as oviposition deterrents against *Aedes aegypti* females [30]. This finding also agrees with the reports of Liu and Kim that (E)-Caryophyllene and its derivatives are widely distributed among plant oils, and reportedly possess acaricidal, insecticidal, repellent, attractant and antifungal properties [31-32]. Additionally, the presence of α-humulene gives rise to changes in mosquito behavior and exhibits high deterrent activity at low concentration (5 ppm), thus indicating a possible application in the control of A. aegypti. The practical use of (E)-caryophyllene and α-humulene could be used to avoid *E. cautella* oviposition in cocoa beans stored in warehouse.

Conclusion

The result obtained from this study suggests that ethanolic extract of essential oil of *E. aromatica* was effective for the control of *Ephestia cautella* on Cocoa beans since they completely inhibited development of the storage pest from eggs to adult stage at all concentration. However, the effect of essential oil of *E. aromatica* on this insect pest is dependent on concentration of oil administered and the period of exposure. Furthermore, *E. aromatica* also possesses the important property of being an anti-feedant, larvicidal and insecticidal. Result from phytochemical analysis of *E. aromatica* reveal the major active ingredient responsible for insecticidal action to be eugenol, caryophyllene and humulene. On the basis of the above findings it can be safely said that both the powder and the ethanol oil extract of *E. aromatica* are potential bio-pesticide and involves no risk in handling, in contrast to synthetics which requires many precautions.

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