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ORIGINAL ARTICLE

# *Acacia auriculiformis* oil fractions: promising tool for the control of *Callosobruchus maculatus* (F.)

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## Abstract

*Acacia auriculiformis* (Linn, Benth.) is a medicinal plant whose insecticidal potential has been established. This work investigated the biopesticidal potential of different fractions of the plant oil against *Callosobruchus maculatus* at laboratory temperature and humidity. The oil of the plant was extracted using ethanol as solvent. Different fractions were made from the oil using column chromatography and fractions on the same band on the TLC plate were merged. The fractions were then tested against the adult beetle at 50 µl. mortality, oviposition, adult emergence, seed weight loss and damage, weevil perforation index, and inhibition rate were observed. The GC–MS analysis of the most active fractions was done to determine the active compounds contain in them. The result obtained showed that fractions of the plant oil was more effective than the crude oil of the plant. F1 was the most effective against the insect and was able to protect the cowpea against beetle infestation. Moreover, F2 and F4 also appeared potent against the insect as they both significantly affected the infestation of the insect. Hexadecanoic acid ethyl ester, phytol, alpha Amyrin, propanamide, methylpent 4-enylamine, cysteine, DL-Cystine, octadecanoic acid ethyl ester and phenylephrine were found to be present in F1, F2, F4 and crude oil. Since, F1 of the oil of *A. auriculiformis* has proven insecticidal in nature, it could be incorporated into pest management strategies while further research could be done to establish mode of action of its mode of action and its toxic level to human is needed to be done. Also, its long term protectability potential should be evaluated.

**Keywords** *Acacia auriculiformis* · Active compounds · *Callosobruchus maculatus* · GC–MS · Oil fractions · *Vigna unguiculata*

## Introduction

Up till now, in term of production and storage of *Vigna unguiculata* in Africa and other developing countries where insect pest management and control is still very low, *Callosobruchus maculatus* remains the number one enemy of human being. This popular insect pest of cowpea has for ages being known for its destructive activities both on the field and in the storage where it seems to have more favorable condition that supports its activities (Ashamo et al. 2013, 2021; Obembe and Ogungbite 2016; Tedela et al. 2017; Niranjana and Karunakaran 2019; Nisar et al. 2022; Ebadollahi et al. 2022). The reports of Kosini and Nukenine (2017), Yusuf et al. (2019), and Umeanaeto et al. (2020) showed that the infestation by this pertinent beetle is still very prominent in Africa as this insect can render tons of cowpea useless within 3–6 months if proper protection is not being provided during storage.

Since it has been well established that botanical based insecticides could stand a better chance of replacement for synthetic chemical insecticides which have been linked with numerous setbacks including adverse effects on both human and environmental health, many works have been done to establish different plant species with high insecticidal potential (Isman 2006; Ogungbite and Oyeniyi 2014; Ashamo et al. 2021; Nisar et al. 2022; Ebadollahi et al. 2022). This is because botanicals are believed to be readily available, biodegradable, and being eco-friendly. Hence, they have low or no adverse effect on non-target organisms, human and environmental health. It is a known fact that many of these botanicals are endowed with numerous active compounds that are insecticidal in nature (Zibaee 2011). However, many

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of these compounds that are contained in different plant oils may not be effective at the crude state of the plant extract except the plant oil is being separated into different fractions (Sannigrahia et al. 2010; Zibaee 2011; Srinivasula and Chinnaeswaraiah 2017). In order to support the biopesticide companies in commercializing the usage of plant-based insecticides against insect pests, there is a need to fractionate plant crude extracts into different fractions and then establish their effectiveness against different insects, and determine the active compounds contained in each fractions.

*Acacia auriculiformis* is a medicinal plant whose bactericidal efficacy has been established against *Staphylococus aureus, Pseudomonas aeruginosa and Bacillus subtilis* as well as *Aspargillus niger and Candida albican* (Sravanthi et al. 2014). Also, Kaur et al. (2014) and Tedela et al. (2017) reported the insecticidal potential of *A. auriculiformis* crude extract against fruit fly, *Bactrocera cucurbitae* and *Callosobruchus maculatus* respectively. Since the insecticidal potential of the crude extract of this plant has been established, there is a need to test different fractions of the plant extract for their insecticidal potential and establish the active compounds contained in each fraction. Therefore, this work evaluated insecticidal efficacy of different fractions of *A. auriculiformis* crude oil against cowpea beetle and determined the active compounds that may be responsible for the effectiveness of the fractions as this could go a long way in the production and commercializing *A. auriculiformis*-based insecticide.

## Materials and methods

### Insect culture

The initial culture of *C. maculatus* used was obtained from an already infested cowpea from the storage entomology laboratory, Biology Department, Federal University of Technology Akure. The insects were reared on clean uninfested Ife-Brown cowpea variety to ensure the removal of effect of maternally inherited dietary of previous food eaten by the insects. The insects were cultured at 12 light:12 dark regime, temperature of 28 ± 2 °C and relative humidity of 75 ± 5% inside containers covered with muslin cloth for good aeration and to disallow the escape of the insect and as well disallow the entry of intruding insects that may act as natural enemy of the insect. The culture was maintained by ensuring infested seeds are being replaced by uninfested cowpea. The insects were allowed to pass through five generations before being used as this will ensure pure culture.

### Collection of cowpea seeds and *A. auriculiformis* leaves

The cowpea variety used was collected from National Seed Service, Ibadan, Oyo State, Nigeria. The seeds were disinfested before use by placing them inside freezer at − 7 °C for 12 weeks and the seeds were exposed to air in the laboratory to avoid moldiness. The leaves of *A. auriculiformis* used were collected fresh in an open field around sport complex, Federal University of Technology, Akure. The collected leaves were identified and validated by the taxonomists in the plant bank laboratory in the Department of Plant Science and Biotechnology, Ekiti State University, Ado-Ekiti and then air dried under shade and pulverized into fine powder using an electric blender and kept inside air-tight container for further use.

### Fractionation of the plant oil

After the oil of the plant has been extracted as described by Tedela et al. (2017), the oil was fractionated into different fractions using 60–120mesh (coarse) column grade made into slurry to ensure uniform column packing. The slurry was prepared by ensuring that the weight of the silica gel used was 50 times heavier than the weight of the *A. auriculiformis* oil and the column was packed in such a way that the slurry had a height that was 10 times more than the diameter of the column. The column used was cleaned with dichloromethane and was allowed to dry. After this, the slurry was gently poured into the column with the openings of the column opened to allow solvent to flow through the column. To ensure uniform packing, the side of the column was tapped with a pencil as the slurry was poured into the column gently. After 3–5 min of collection of solvents from the slurry, the position of the slurry in the column was observed for changes. When no changes observed, the remaining solvent on the slurry was allowed to pass through on till little amount of the solvent remained on the slurry. This is necessary to avoid the slurry being dried, as dryness of the slurry may cause uneven or poor separation of the fractions. Pasteur pipette was used to place 3-mm-thick band of the oil to the top of the column and the oil was allowed to trickle down to the surface of the slurry. This is necessary in order to prevent the disturbance of the slurry. The side of the column was washed with the solvent used and this process continued until the fractions are collected into different bottles. In order, to ensure the collection of both polar and non-polar compounds present in the oil, the fractions were made into three groups. The first group was made with ethanol alone, the second group was made with ethanol and chloroform

in ratio 1:1 and the third group was made with chloroform alone.

## TLC products of the fractions

To get the products of the fractionation, a 2 × 6.5 cm strip of a silica gel chromatogram sheet was used. The sheet was marked lightly at 1 cm at the origin. Then different spots of the same size and evenly spaced were made at the line origin of the sheet for each fraction. Two drops of the same fraction were applied on top of each other. The first drop was allowed to dry before the second drop was applied. After the drops of the fractions on the sheet were well dried, the sheet was placed inside a TLC chamber. 1% MeOH in $CH_2Cl_{22}$ as a developing solvent. The chromatograph was allowed to develop to about 1 cm from the top of the sheet. The sheet was removed from the chamber, the solvent front was marked with pencil, and the plate was allowed to dry. The plate was examined under UV light to see the components of each fractions. The spots of the fraction on the plate was outlined with pencil. Fractions with the same bands were considered to have the same active compounds present in them. However, to have accurate result the recovery factor (Rf) of each fraction was calculated using the formula below:

$$\mathrm{Rf} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Fractions with the same or almost the same Rf were merged together as a fraction. The picture of the chromatogram sheet is presented in Plate 3a and b. Moreover, from the chromatogram sheet, it should be noted that fraction 1–7 were the ones eluted with ethanol alone, fraction 8, 9, 10, 15, 16 and 17 were eluted with chloroform alone while fraction 7, 11, 12 and 13 were eluted with ethanol-chloroform. Base on their Rf, the following fractions were made: fraction 1, 4, 5, 6 and 10 while fraction 2, 3, 11, 12 and 13 were merged together to be fraction 2 and also, fraction 7 and 14 were merged as fraction 7 while fraction 8, 9, 15, 16 and 17 were merged to be fraction 8. Therefore, the research was continued with fractions 1, 2, 4, 5, 6, 7, 8 and 10.

## Effect of contact toxicity of the fractions of A. auriculiformis on survival of C. maculatus

Twenty grams of clean uninfested cowpea was weighed into plastic containers (250 ml) and 5% concentration of the fractions were mixed thoroughly with the cowpea inside containers at dosage of 50 µl. After the fractions have been well mixed with the cowpea in the containers, they were left open for 1 h to allow the solvent used as carrier to evaporate. Cowpea seeds that were not treated with neither plant oil nor solvent and those treated with only ethanol were used as the controls. Then 10 pairs of less than 24 h old *C. maculatus* was introduced topically to the treated cowpea and mortality was recorded at 24, 48, 72 and 96 h post treatment and percentage insect mortality was calculated. The experiment was setup in a complete randomized design with each sample replicated 5 times. On the fifth day, both live and dead insects were removed and oviposition was recorded while the samples were left for another 20 days after mortality has been observed. The cowpea seeds were observed for adult emergence and records were taken until no insects were found for 5 consecutive days. Percentage adult emergence of the insect was calculated.

## GC–MS characterization for alkaloid in fractions of A. auriculiformis oil

Fraction 1 *A. auriculiformis* was characterized for its active compounds using GC–MS. These fractions were selected because they were found to be more effective than other fractions of the plant oil. The aliquot used for the GC–MS qualitative characterization analysis was prepared by dissolving 500 µl of the sample extract in 500 µl of Methanol. The fractions and the crude oil extract of the plant was characterized using the method of Zhifeng et al. (2014) as described by Ademiluyi et al. (2016). A qualitative characterization analysis of possible alkaloids present in alkaloid extracted fraction was carried out using GC–MS using scan mode. This analysis was performed using 7820A gas chromatograph coupled to 5975C inert mass spectrometer (with triple axis detector) with electron-impact source (Agilent Technologies). The stationary phase of separation of the compounds was HP-5 capillary column coated with 5% Phenyl Methyl Siloxane (30 m length × 0.32 mm diameter × 0.25 µm film thickness) (Agilent Technologies). The carrier gas was Helium used at constant flow of 1.6 mL/min at an initial nominal pressure of 2.84 psi and average velocity of 46 cm/sec. One microliter of the samples were injected in splitless mode at an injection temperature of 260 °C. Purge flow was 21.5 mL/min at 0.50 min with a total flow of 25.8 mL/min; gas saver mode was switched on. Oven was initially programmed at 60 °C (1 min) then ramped at 4 °C/min to 110 °C (3 min) then 8 °C/min to 260 °C (5 min) and 10 °C/min to 300 °C (12 min). Run time was 56.25 min with a 3 min solvent delay. The mass spectrometer was operated in electron-impact ionization mode at 70 eV with ion source temperature of 230 °C, quadrupole temperature of 150 °C and transfer line temperature of 280 °C. Scanning of possible alkaloid compounds was from *m/z* 30 to 550 amu at 2.62 s/scan scan rate and were identified by comparing measured mass spectral data with those in ***NIST 11 Mass Spectral Library*** and literature. Prior to analysis, the MS was auto-tuned to perfluorotributylamine (PFTBA) using already established criteria to check the abundance of *m/z*

69, 219, 502 and other instrument optimal and sensitivity conditions. Analysis validation was conducted by running replicate samples in order to see the consistency of the constituent compound name, respective retention time, molecular weight (amu), Quality ion (Q-Ion) and % Total.

$$\% \text{ Total} = \frac{\text{Abundance of individual constituents}}{\text{Total Abundance of all consituents in extract}} \times \frac{100}{1}$$

These abundances were outputs from the *NIST 11 Library search report* of the extract and fractions constituents. Each compound identified via the NIST 11 Library Search report has a corresponding mass spectrum showing the abundance of the possible numerous *m/z* peaks per compound.

### Data analysis

Abbott (1925) formula was used to correct data on mortality counts using control mortality. All the data obtained were subjected to one-way analysis of variance, ANOVA at $p < 0.05$ and means were separated with Duncan's Multiple Range Test (DMRT). Also, the data obtained on mortality were subjected to Probit regression analysis to calculate the $LD_{50}$ and $LD_{95}$ of the treatments (Finney 1971). Linear regression analysis was done to reveal the relationship between the insect mortality and oviposition as well as between adult emergence and seed weight loss. All analysis was done with SPSS version 20.

## Results

### Mortality of C. maculatus exposed to cowpea treated with 50 µl different fractions of A. auriculiformis

The effect of crude oil and fractions of *A. auriculiformis* on the survival of adult *C. maculatus* is presented in Table 1. The survival of the insect varied with the treatments and period of exposure. Statistically significant differences existed between the treatments at $F = 65.441$, $df = 10, 44$, $p < 0.0001$ (24 h), $F = 96.115$, $df = 10, 44$, $p < 0.0001$ (48 h), $F = 185.434$, $df = 10, 44$, $p < 0.0001$ (72 h) and $F = 104.898$, $df = 10, 44$, $p < 0.0001$ (96 h). Regardless of the period of observation, the fractions were statistically significantly ($p < 0.05$) different from the crude oil extract and the two controls except at 24 and 48h post treatment where F8 and F10 recorded below 40% mortality of the insect. Within 24 h post treatment, F1 recorded 61.67% mortality of the insect and was significantly ($p < 0.05$) different from others except F2 and F4 that recorded 60 and 58.33% beetle mortality respectively. At 72 h of exposure, F1 and F2 recorded 100% beetle mortality and were significantly different from others except F4 that recorded up to 98.33% insect mortality. Nevertheless, all the treatments achieved above 50% insect mortality and were significantly different from the two controls.

### Amount of fractions of *A. auriculiformis* required to achieve 50 and 95% mortality of *C. maculatus* within 48 h post treatment

The lethal dosage that will achieve 50 and 95% mortality of adult *C. maculatus* by the oil and fractions of *A.*

**Table 1** Mortality of *C. maculatus* exposed to 50 µl dosage of *A. auriculiformis* fractions

| Treatments | % Mortality in hours | | | |
|---|---|---|---|---|
| | 24 | 48 | 72 | 96 |
| F1 | 61.67 ± 4.41d | 98.33 ± 1.67f | 100.00 ± 0.00 g | 100.00 ± 0.00e |
| F2 | 60.00 ± 0.00d | 83.33 ± 1.67e | 100.00 ± 0.00 g | 100.00 ± 0.00e |
| F4 | 58.33 ± 4.41d | 81.67 ± 4.41e | 98.33 ± 1. 67 g | 100.00 ± 0.00e |
| F5 | 16.67 ± 4.41b | 41.67 ± 4.41c | 58.33 ± 4.41d | 85.00 ± 7.64 cd |
| F6 | 38.33 ± 1.67c | 56.67 ± 1.67d | 70.00 ± 2.89e | 95.00 ± 2.89de |
| F7 | 41.67 ± 1.67c | 73.33 ± 4.41e | 88.33 ± 1.67f | 100.00 ± 0.00e |
| F8 | 15.67 ± 1.67b | 28.33 ± 1.67b | 45.00 ± 2.89c | 61.67 ± 7.26b |
| F10 | 16.67 ± 4.41b | 33.33 ± 7.26bc | 53.33 ± 4.41d | 75.00 ± 5.00c |
| Crude | 11.00 ± 2.89b | 25.00 ± 2.89b | 36.67 ± 4.41b | 55.00 ± 2.89b |
| Control 1 | 0.00 ± 0.00a | 0.00 ± 0.00a | 0.00 ± 0.00a | 0.00 ± 0.00a |
| Control 2 | 0.00 ± 0.00a | 0.00 ± 0.00a | 0.00 ± 0.00a | 0.00 ± 0.00a |

Each treatment is a mean ± standard error of five replicates. Values followed by the same letter in the same column are not significantly ($p > 0.05$) different from each other using New Duncan's Multiple Range Test

*auriculiformis* are presented in Table 2. Low amount of the oil extract and fractions of *A. auriculiformis* was required to achieve high mortality of the insect. However, F1 appeared to be the most effective as only 1.32 and 8.32 µl of it were required to achieve 50 and 95% mortality of the insect within 48h and was highly significant ($p < 0.0001$) compared to other treatments. The Chi-square values of the treatments also reflected the level of their effectiveness as many of them recorded a Chi-square value that was above 3.81. However, only F2, F8, F10 and crude oil extract recorded Chi-square value below 3.81 and they were not significant ($p > 0.05$). The slope and intercept of the treatments showed that the treatments are very effective as their values are very low. Nevertheless, the order of effectiveness of the treatments could be arranged as follow F1 > F2 > F4 > F7 > F6 > F5 > F10 > F8 > oil extract.

**Table 2** The lethal dosage (µl) required to achieve 50 and 95% mortality of *C. maculatus* exposed to fractions of *A. auriculiformis*

| Treatments | Slope ± SE | Intercept ± SE | $X^2$ | $LD_{50}$ (95% FL) | $LD_{90}$ (95% FL) | Sign |
|---|---|---|---|---|---|---|
| F1 | 2.05 ± 0.25 | −0.25 ± 0.11 | 21.359 | 1.32(0.89–1.94) | 8.32(6.97–10.21) | 0.0001 |
| F2 | 1.92 ± 0.24 | −0.48 ± 0.11 | 2.030 | 1.77(1.46–2.05) | 10.35(7.97–15.25) | 0.192 |
| F4 | 2.40 ± 0.25 | −0.79 ± 0.12 | 4.736 | 2.13(1.87–2.39) | 12.748(8.01–22.37) | 0.045 |
| F5 | 1.84 ± 0.27 | −1.29 ± 0.14 | 4.819 | 5.00(4.17–6.63) | 39.24(21.53–115.44) | 0.018 |
| F6 | 2.42 ± 0.34 | −1.89 ± 0.18 | 8.884 | 6.05(4.09–49.33) | 28.89(10.81–58.46) | 0.031 |
| F7 | 2.19 ± 0.26 | −1.09 ± 0.13 | 9.086 | 3.14(2.13–5.59) | 17.75(9.09–28.16) | 0.028 |
| F8 | 2.34 ± 0.41 | −2.24 ± 0.23 | 1.161 | 9.05(6.83–15.67) | 45.69(23.13–183.89) | 0.762 |
| F10 | 2.55 ± 0.41 | −2.25 ± 0.23 | 0.498 | 7.67(6.13–11.49) | 33.90(19.32–98.95) | 0.919 |
| Crude | 2.95 ± 0.57 | −2.79 ± 0.34 | 2.009 | 8.85(6.80–15.47) | 57.98(54.49–120.94) | 0.571 |

*LD* lethal dosage; *SE* standard error; $x^2$ Chi-square; *FL* Fiducial limits

**Table 3** Correlation between the adult mortality and number of egg laid of *C. maculatus* exposed to different fractions and crude oil of *A. auriculiformis*

| Treatments | *R* | $R^2$ | $AR^2$ | K ± SE | RC ± SE | RE | *t*-test | Sign |
|---|---|---|---|---|---|---|---|---|
| F1 | 0.983 | 0.967 | 0.965 | 113.91 ± 4.01 | −1.21 ± 0.05 | O = 113.91–1.21(M) | −23.553 | 0.0001 |
| F2 | 0.957 | 0.917 | 0.912 | 109.80 ± 5.38 | −1.12 ± 0.08 | O = 109.80–1.12(M) | −14.448 | 0.0001 |
| F4 | 0.949 | 0.900 | 0.895 | 108.16 ± 6.44 | −1.19 ± 0.09 | O = 108.16–1.19(M) | −13.086 | 0.0001 |
| F5 | 0.874 | 0.764 | 0.752 | 99.50 ± 7.48 | −1.09 ± 0.14 | O = 99.50–1.13(M) | −7.852 | 0.0001 |
| F6 | 0.934 | 0.872 | 0.865 | 105.69 ± 7.40 | −1.15 ± 0.10 | O = 105.69–1.15(M) | −11.361 | 0.0001 |
| F7 | 0.949 | 0.900 | 0.895 | 109.18 ± 5.68 | −1.13 ± 0.09 | O = 109.18–1.13(M) | −13.070 | 0.0001 |
| F8 | 0.732 | 0.537 | 0.512 | 87.54 ± 8.69 | −1.15 ± 0.25 | O = 87.54–1.15(M) | −4.690 | 0.0001 |
| F10 | 0.832 | 0.692 | 0.676 | 95.92 ± 7.92 | −1.18 ± 0.18 | O = 95.92–1.18(M) | −6.538 | 0.0001 |
| Crude | 0.846 | 0.715 | 0.700 | 100.56 ± 4.73 | −1.06 ± 0.15 | O = 100.56–1.15(M) | −6.912 | 0.0001 |

*AR* adjusted *R*-square; *K* constant; $R_C$ regression coefficient; $R_E$ regression equation; *SE* Standard error; *O* oviposition and *M* Mortality

**Table 4** Correlation between the adult emergence and weight loss of cowpea seeds treated with 50µl dosage of *A. auriculiformis* fraction

| Treatments | *R* | $R^2$ | $AR^2$ | K ± SE | RC ± SE | RE | *t*-test | Sign |
|---|---|---|---|---|---|---|---|---|
| F1 | 0.999 | 0.998 | 0.998 | 0.01 ± 0.26 | 0.49 ± 0.01 | W = 0.01 + 0.49(AE) | 95.373 | 0.0001 |
| F2 | 0.999 | 0.998 | 0.998 | 0.01 ± 0.26 | 0.49 ± 0.01 | W = 0.01 + 0.49(AE) | 95.373 | 0.0001 |
| F4 | 0.999 | 0.998 | 0.998 | 0.01 ± 0.26 | 0.49 ± 0.01 | W = 0.01 + 0.49(AE) | 95.373 | 0.0001 |
| F5 | 0.995 | 0.990 | 0.989 | −4.48 ± 0.64 | 0.54 ± 0.01 | W = −4.48 + 0.54(AE) | 43.237 | 0.0001 |
| F6 | 0.993 | 0.985 | 0.985 | −3.50 ± 0.75 | 0.53 ± 0.02 | W = −3.50 + 0.53(AE) | 35.870 | 0.0001 |
| F7 | 0.996 | 0.991 | 0.991 | −1.59 ± 0.56 | 0.51 ± 0.01 | W = −1.59 + 0.51(AE) | 46.888 | 0.0001 |
| F8 | 0.993 | 0.986 | 0.985 | 22.38 ± 1.08 | 1.55 ± 0.04 | W=22.38 + 1.55(AE) | 36.072 | 0.0001 |
| F10 | 0.991 | 0.982 | 0.981 | −13.61 ± 1.09 | 0.63 ± 0.02 | W = −13.61 + 0.63(AE) | 31.996 | 0.0001 |
| Crude | 0.977 | 0.955 | 0.952 | −3.47 ± 1.62 | 0.518 ± 0.03 | W = −3.47 + 0.03(AE) | 19.998 | 0.0001 |

*AR* adjusted *R*-square; *K* constant; $R_C$ regression coefficient; $R_E$ regression equation; *SE* Standard error; *O* oviposition and *M* Mortality

## Relationship between the mortality and oviposition of C. maculatus exposed to fractions of A. auriculiformis

Correlation between insect mortality at 96 h post treatment and oviposition rate is presented in Tables 3 and 4. The *R* values of the treatments that tend to 1 reflected high correlation between the mortality of the insects and their oviposition rate. Nevertheless, F1 recorded the highest *R*-value (0.983) while F8 recorded the lowest *R*-value (0.732). The $R^2$ value of F1 showed that mortality of the insect explains 96.7% oviposition rate of the insect. However, after the adjustment of the $R^2$ values, only 96.5% of the insect oviposition rate can be determined by the mortality rate of the insect. The *t*-values of the treatments that were greater than 1.98 indicated that there was a statistically significant relationship between the mortality and oviposition rate of the insect at $F = 554.728$ $df = 1,19$, $p < 0.0001$ (F1), $F = 129.063$, $df = 1,19$, $p < 0.0001$ (F2), $F = 171.241$, $df = 1,19$, $p<0.0001$ (F4), $F = 61.658$, $df = 1,19$, $p < 0.0001$ (F5), $F = 170.817$, $df = 1,19$, $p<0.0001$ (F6), $F = 208.745$, $df = 1,19$, $p < 0.0001$ (F7), $F = 21.996$, $df = 1,19$, $p < 0.0001$ (F8), $F = 42.740$, $df = 1,19$, $p < 0.0001$ (F10), $F = 47.771$, $df = 1,19$, $p < 0.0001$ (crude).

## Effect of 50 µl of different fractions of A. auriculiformis on number of eggs laid and adult emergence of C. maculatus

The number of eggs laid and percentage adult emergence of *C. maculatus* exposed to different dosages of oil extract and fractions of *A. auriculiformis* are presented in Fig. 1. The number of eggs laid and percentage adult emergence were directly proportional to the crude oil extract and fractions of the plant and the dosage used. Statistically significant differences existed between the treatments at $F = 2113.100$, $df = 10, 44$, $p < 0.0001$ (oviposition) and $F = 2113.100$, $df = 10, 44$, $p < 0.0001$ (adult emergence). Both F1 and F2 prevented the oviposition of the adult beetle. The adult emergence of the insect was totally prevented at F1, F2 and F4 and were significantly different from other treatments except F6 and F7. Nevertheless, regardless of the dosage used, the highest mean oviposition rate (117) was recorded in the two controls and they were significantly different from other treatments. Also, C1 recorded the highest percentage adult

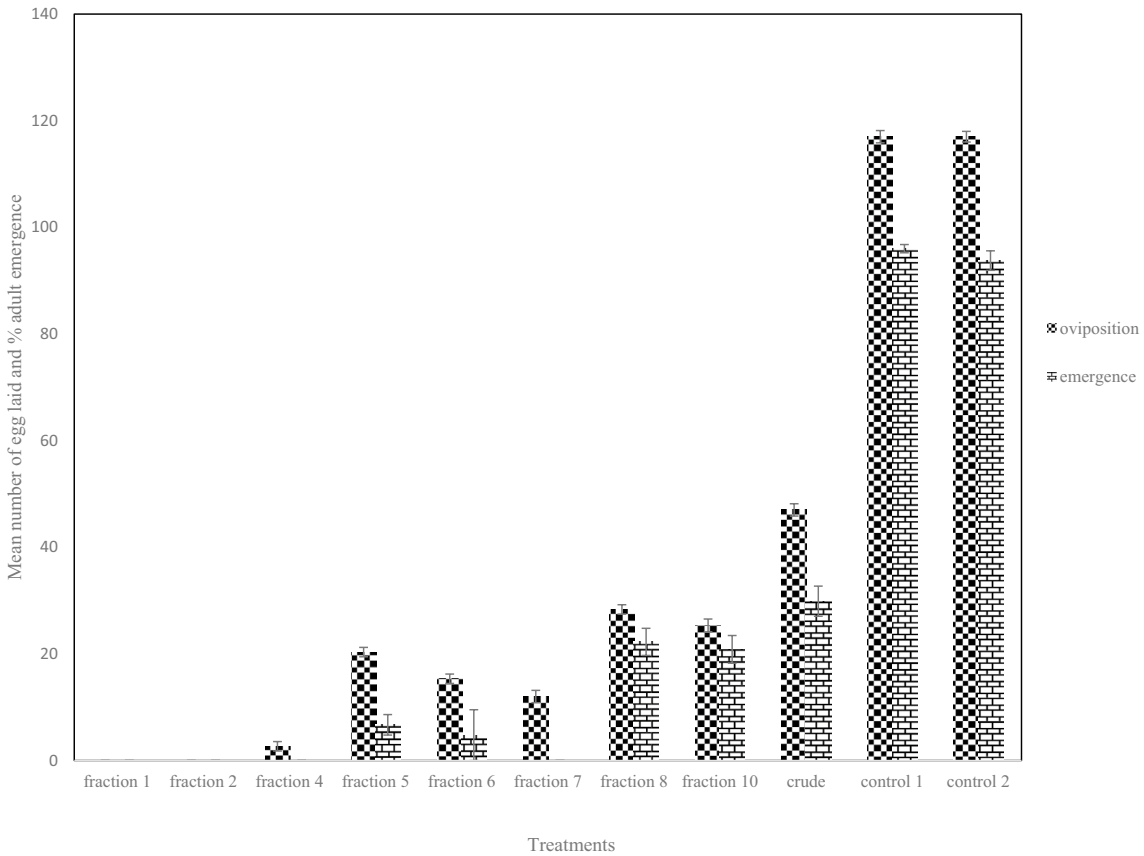


**Fig. 1** Ovipositioion and adult emergence of *C. maculatus* exposed to 50 µl of fractions of *A. auriculiformis*

emergence of 96% but was not significantly ($p > 0.05$) different from C2 which recorded up to 93.33% adult emergence.

### Effect of *A. auriculiformis* fractions on ability of *C. maculatus* to cause seed damage and weight loss as well as percentage weevil perforation index (WPI) and inhibition rate (IR)

The effect of *A. auriculiformis* fractions on the ability of *C. maculatus* to cause seed damage and weight loss of protected cowpea and the weevil perforation index as well as percentage inhibition rate are presented in Fig. 2. The percentage seed damage and weight loss as well as the percentage WPI and IR varied with the treatments. Significant differences existed among the treatments at $F = 2284.616$, $df = 10,44$, $p < 0.0001$ (damage), $F = 2289.892$, $df = 10,44$, $p < 0.0001$ (weight loss), $F = 2578.240$, $df = 10,44$, $p < 0.0001$ (WPI) and $F = 5934.188$, $df = 10,44$, $p < 0.0001$ (IR). F1, F2 and F4 prevented the damage and weight loss of the cowpea seed by the beetle and as well recorded 0% WPI and inhibited the emergence of the adult beetle completely (100%) and were significantly ($p < 0.05$) different from other treatments except F6 and F7. Furthermore, the highest percentage seed damage (87.78%), weight loss (47.72%), WPI (100%) and IR (0%) were observed in C2. However, C2 was not significantly ($p > 0.05$) different from the C1 that recorded 85.36% seed damage, 46.15% seed weight loss, 97.24% WPI and 0.97% IR.

### Relationship between adult emergence of *C. maculatus* and weight loss of cowpea seed treated with 50 µl of *A. auriculiformis* fractions

The correlation between the adult emergence and the seed weight loss is presented in Table 5. There was a great correlation between the adult emergence of the beetle and weight loss of the seed caused by the insect as *R*-values of the treatments tend toward 1. Nevertheless, F1, F2 and F4 recorded the highest *R*-value (0.999) while the lowest *R*-value of 0.977 was recorded by the crude oil extract of the plant. The $R^2$ value of F1, F2 and F4 showed that 99.9% of the seed weight loss was determined by the emergence of the adult beetle. Nevertheless, after the adjustment of the $R^2$ values, adult emergence of the insect determined up to 99.8% of the seed weight loss. The *t*-values of the treatments that were greater than 1.98 indicated that there was statistically significant relationship between the adult emergence and seed weight loss at $F = 9095.949$, $df = 1,19$, $p < 0.0001$ (F1), $F = 9095.949$, $df = 1,19$, $p < 0.0001$ (F2), $F = 9095.949$, $df = 1,19$, $p < 0.0001$ (F4), $F = 1869.412$, $df = 1,19$, $p < 0.00091$ (F5), $F = 1287.107$, $df = 1,19$, $p < 0.0001$ (F6), $F = 2179.771$, $df = 1,19$, $p < 0.0001$ (F7), $F = 1301.172$, $df = 1,19$, $p < 0.0001$ (F8), $F = 1023.718$, $df = 1,19$, $p < 0.0001$ (F10), $F = 399.916$, $df = 1,19$, $p < 0.0001$ (crude).

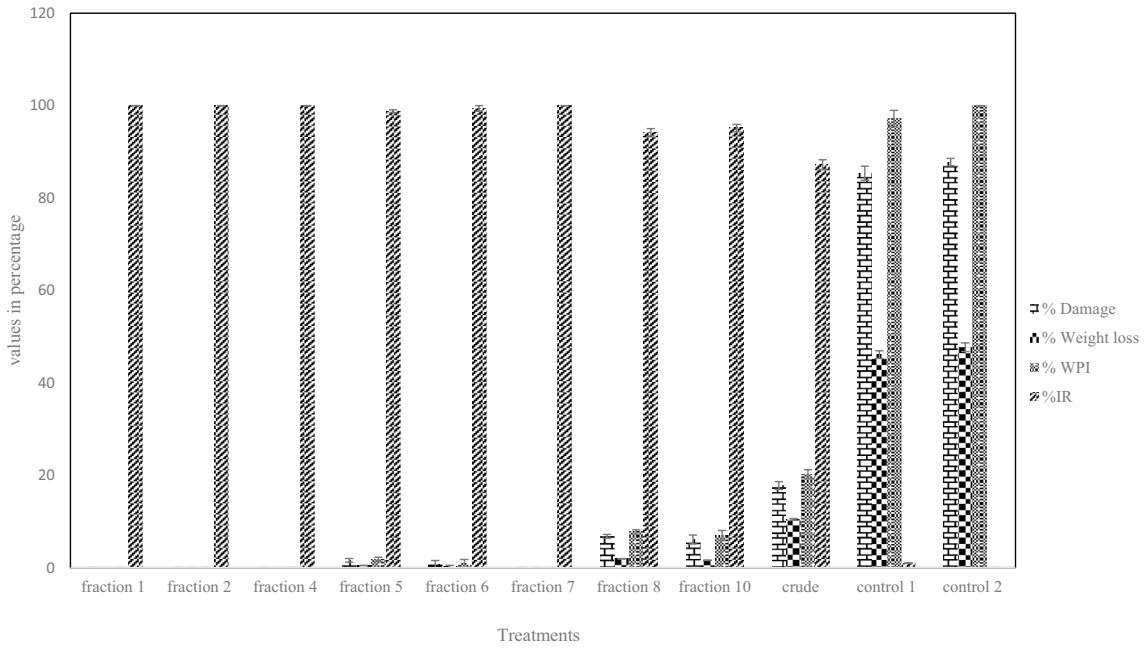


**Fig. 2** Percentage damage, weight loss and WPI of the protected cowpea seed and IR of *C. maculatus* at 50 µl *A. auriculiformis* fractions

**Table 5** Active compounds present in fraction 1 of oil of *A. auriculiformis*

| SN | Compound name/Hit name | Molecular weight (amu) | Quality ion (Relative Intensity, %) | Percentage total of all compound (% Total) | Entry number in NIST11 Library |
|---|---|---|---|---|---|
| 1 | 1H-Indol-5-ol | 133.053 | 47 | 1.594 | 14,711 |
| 2 | 4-Fluorohistamine | 129.07 | 72 | 0.061 | 12,838 |
| 3 | Oxirane, methyl-, (S)- | 58.042 | 2 | 1.636 | 230 |
| 4 | 2-(2,2-Dimethylvinyl)thiophene | 138.05 | 2 | 0.321 | 17,438 |
| 5 | 2-(4,5-Dihydro-3-methyl-5-oxo-1-phenyl-4-pyrazolyl)-5-nitrobenzoic acid | 367.092 | 64 | 0.210 | 194,638 |
| 6 | DL-Cystine | 240.024 | 59 | 0.643 | 94,416 |
| 7 | Furazan-3-carboxamide, oxime, 4-amino-*N,N*-dimethyl- | 171.076 | 45 | 1.099 | 39,157 |
| 8 | Acetamide, 2-cyano- | 84.032 | 45 | 0.186 | 1336 |
| 9 | Urea, *N,N'*-diethyl- | 116.095 | 59 | 0.837 | 7998 |
| 10 | Cystine | 240.024 | 45 | 0.096 | 94,414 |
| 11 | 5-Aminoisoxazole | 84.032 | 64 | 0.554 | 1334 |
| 12 | cis-Aconitic anhydride | 156.006 | 59 | 0.132 | 28,618 |
| 13 | 4,5-Bis(methylamino)-fluorene | 224.131 | 3 | 2.391 | 81,157 |
| 14 | 1,5-Hexadiene, 3,3,4,4-tetrafluoro- | 154.041 | 1 | 0.065 | 27,235 |
| 15 | Isoindole, 1-(hydrazinedicarboxylic acid, diethyl ester)-3-(hydrazinedicarboxylic acid, dimethyl ester)-2-(2,3-dimethylphenyl)- | 541.217 | 9 | 0.059 | 238,609 |
| 16 | Lanost-9(11)-en-18-oic acid, 3-(acetyloxy)-20,25-dihydroxy-16-oxo-,.gamma.-lactone, (3.beta.)- | 528.345 | 11 | 1.566 | 237,756 |
| 17 | *N*-Isopropyl-3-phenylpropanamide | 191.131 | 64 | 0.264 | 53,808 |
| 18 | 2-Pentenimidic acid, 3-methyl-*N*-phenyl-, methyl ester, (2E)- | 203.131 | 2 | 0.044 | 63,434 |
| 19 | Kryptogenin 2,4-dinitrophenylhydrazone | 790.365 | 7 | 0.063 | 243,463 |
| 20 | 7-Oxa-15,20,24,27-tetraazatetracyclo[13.9.6.2(8,11).1(2,6)]tritriaconta-2,4,6(33),8,10,12,31-heptaene-14,26-dione, 20-acetyl-5-methoxy-, [s-(Z)]- | 548.3 | 9 | 0.144 | 239,016 |
| 21 | 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)- | 180.115 | 78 | 0.609 | 45,538 |
| 22 | 2,4,7,9-Tetramethyl-5-decyn-4,7-diol | 226.193 | 72 | 0.240 | 82,809 |
| 23 | 4-Aminopyrimidine | 95.048 | 27 | 0.068 | 2652 |
| 24 | 7-Chloro-*N*-[[4'-chloro-5-[[diethylamino]methyl]-6-ethoxy]-1,1'-biphenyl-3-]-quinoline-4-amine | 493.169 | 91 | 0.054 | 234,426 |
| 25 | Phosphine, tridodecyl- | 538.561 | 18 | 0.250 | 238,406 |
| 26 | Orcinol | 124.052 | 64 | 3.991 | 10,406 |
| 27 | Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha.)- | 138.141 | 83 | 0.068 | 17,013 |
| 28 | 2-[.gamma.-Dimethylaminopropylamino]-3-methyl-4-diethylamino-5-[4-chlorophenyl]-8-chloro-9-methoxybenzo[h][1,6]naphthyzidine | 539.222 | 9 | 0.847 | 238,440 |
| 29 | .beta. Carotene | 536.438 | 10 | 1.019 | 238,269 |
| 30 | .psi.,.psi.-Carotene, 7,7',8,8',11,11',12,12'-octahydro- | 544.501 | 5 | 0.794 | 238,821 |
| 31 | 7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl- | 226.157 | 59 | 1.070 | 82,624 |
| 32 | .eta.-Pentamethylcyclopentadienyl-ethylisonitril-(*N,N,N',N'*-tetramethylethin-1,2-diamin)-molybdaeniodid | 527.07 | 20 | 7.273 | 237,653 |
| 33 | 1,5-Methano-1H,7H,11H-furo[3,4-g]pyrano[3,2-b]xanthene-7,15-dione, 3,3a,4,5-tetrahydro-8-hydroxy-1-(4-hydroxy-3-methyl-2-butenyl)-3,3,11,11-tetramethyl-13-(3-methyl-2-butenyl)-, [1R-[1.alpha.,1(Z),3a.beta.,5.alpha.,14as*]]- | 546.262 | 10 | 0.883 | 238,918 |
| 34 | .pi.-Pentamethylcyclopentadienyl-di(ethylthio)-diethylaminocarbin-wolfram(vi) | 525.172 | 53 | 2.506 | 237,518 |

**Table 5** (continued)

| SN | Compound name/Hit name | Molecular weight (amu) | Quality ion (Relative Intensity, %) | Percentage total of all compound (% Total) | Entry number in NIST11 Library |
|---|---|---|---|---|---|
| 35 | Phthalic acid, monoamide, *N,N*-diundecyl, propyl ester | 515.434 | 38 | 1.642 | 236,777 |
| 36 | Hexadecanoic acid, ethyl ester | 284.272 | 97 | 4.578 | 131,288 |
| 37 | Phytol | 296.308 | 87 | 10.038 | 141,393 |
| 38 | 2,4-Diamino-6,8-bis[3,4-dichlorophenyl]-5,6-dihydro-8H-thiapyrano[4',3'-4,5]thieno[2,3-d]pyrimidine | 525.941 | 15 | 1.061 | 237,565 |
| 39 | Nalmefene, bis(trifluoroacetate) | 531.148 | 4 | 1.421 | 237,890 |
| 40 | Ethyl 9,12,15-octadecatrienoate | 306.256 | 99 | 6.650 | 149,921 |
| 41 | Propanediamide, *N,N*''-1,8-octanediylbis[N'-heptyl-N'-methyl- | 538.446 | 11 | 0.140 | 238,345 |
| 42 | Octadecanoic acid, ethyl ester | 312.303 | 96 | 0.076 | 154,936 |
| 43 | Ethanone, 1-[4-[4,6-bis(2,2,2-trifluoro-1-trifluoromethylethoxy)-1,3,5-triazin-2-yl]amino]phenyl- | 546.056 | 9 | 0.123 | 238,870 |
| 44 | 2,6,10,14-Tetrabora-1,3,5,7,9,11,13,15-octaoxacyclohexadecane, 2,6,10,14-tetraethyl-4,8,12,16-tetrakis(2,2,2-trifluoromethyl)- | 616.165 | 1 | 4.453 | 241,467 |
| 45 | Didodecyl phthalate | 502.402 | 52 | 1.418 | 235,532 |
| 46 | dl-Phenylephrine | 167.095 | 68 | 0.498 | 36,229 |
| 47 | Phenylephrine | 167.095 | 76 | 0.160 | 36,222 |
| 48 | Benzeneethanamine, 4-fluoro-.beta.,3-dihydroxy-*N*-methyl- | 185.085 | 64 | 0.179 | 49,744 |
| 49 | 3-Ethoxyamphetamine | 179.131 | 64 | 0.463 | 44,803 |
| 50 | p-Hydroxynorephedrine | 167.095 | 64 | 0.128 | 36,230 |
| 51 | Squalene | 410.391 | 98 | 2.923 | 215,927 |
| 52 | Benzeneethanamine, 4-methoxy-.alpha.-methyl- | 165.115 | 64 | 2.300 | 34,376 |
| 53 | Metaraminol | 167.095 | 68 | 1.683 | 36,216 |
| 54 | Methylpent-4-enylamine | 99.105 | 64 | 13.060 | 3548 |
| 55 | Benzeneethanamine, 2-fluoro-.beta.,5-dihydroxy-*N*-methyl- | 185.085 | 64 | 3.602 | 49,745 |
| 56 | dl-.alpha.-Tocopherol | 430.381 | 90 | 0.075 | 222,353 |
| 57 | Azastreptonigrin, isopropylidene- | 545.191 | 64 | 0.113 | 238,827 |
| 58 | 1,2-Benzenediol, 4-[2-(methylamino)ethyl]- | 167.095 | 64 | 1.238 | 36,258 |
| 59 | 2-Butanamine, 3-methyl- | 87.105 | 64 | 0.310 | 1914 |
| 60 | 1-Octadecanamine, *N*-methyl- | 283.324 | 68 | 0.938 | 130,250 |
| 61 | Stigmasta-7,16-dien-3-ol, (3.beta.,5.alpha.)- | 412.371 | 56 | 0.382 | 216,719 |
| 62 | .alpha.-Amyrin | 426.386 | 76 | 0.121 | 221,182 |
| 63 | Desmethyldoxepin | 265.147 | 64 | 5.680 | 114,886 |
| 64 | 1,4-Benzenedicarboxamide, *N,N*'-bis(2-hydroxy-1-methyl-2-phenylethyl)- | 432.205 | 64 | 0.072 | 222,756 |
| 65 | *N*-Methyl-2-phenyl-1-propylamine | 149.12 | 64 | 0.076 | 23,375 |
| 66 | 3-(2-Pyridyl)-5-phenylisoxazoline | 224.095 | 5 | 0.139 | 80,984 |
| 67 | Benzenemethanol,.alpha.-[(methylamino)methyl]- | 151.1 | 43 | 0.195 | 25,033 |
| 68 | Betulin | 442.381 | 68 | 1.523 | 225,491 |
| 69 | Propanamide | 73.053 | 47 | 0.159 | 727 |
| 70 | Cyclobutanol | 72.058 | 46 | 0.241 | 655 |
| 71 | Phenethylamine, p,.alpha.-dimethyl- | 149.12 | 43 | 0.345 | 23,391 |
| 72 | Fluoxetine | 309.134 | 46 | 0.204 | 151,964 |

## The active compounds present in the fractions of *A. auriculiformis* oil

The active compounds present in F1 of *A. auriculiformis* are presented in Table 4. F1, contained total number of 72 compounds. Methylpent-4-enylamine recorded the highest percentage (13.06%) of the total number of compounds present in F1 of the oil. Hexadecanoic acid ethyl ester, phytol, alpha Amyrin, propanamide, methylpent 4-enylamine, cysteine, DL-Cystine, octadecanoic acid ethyl ester and

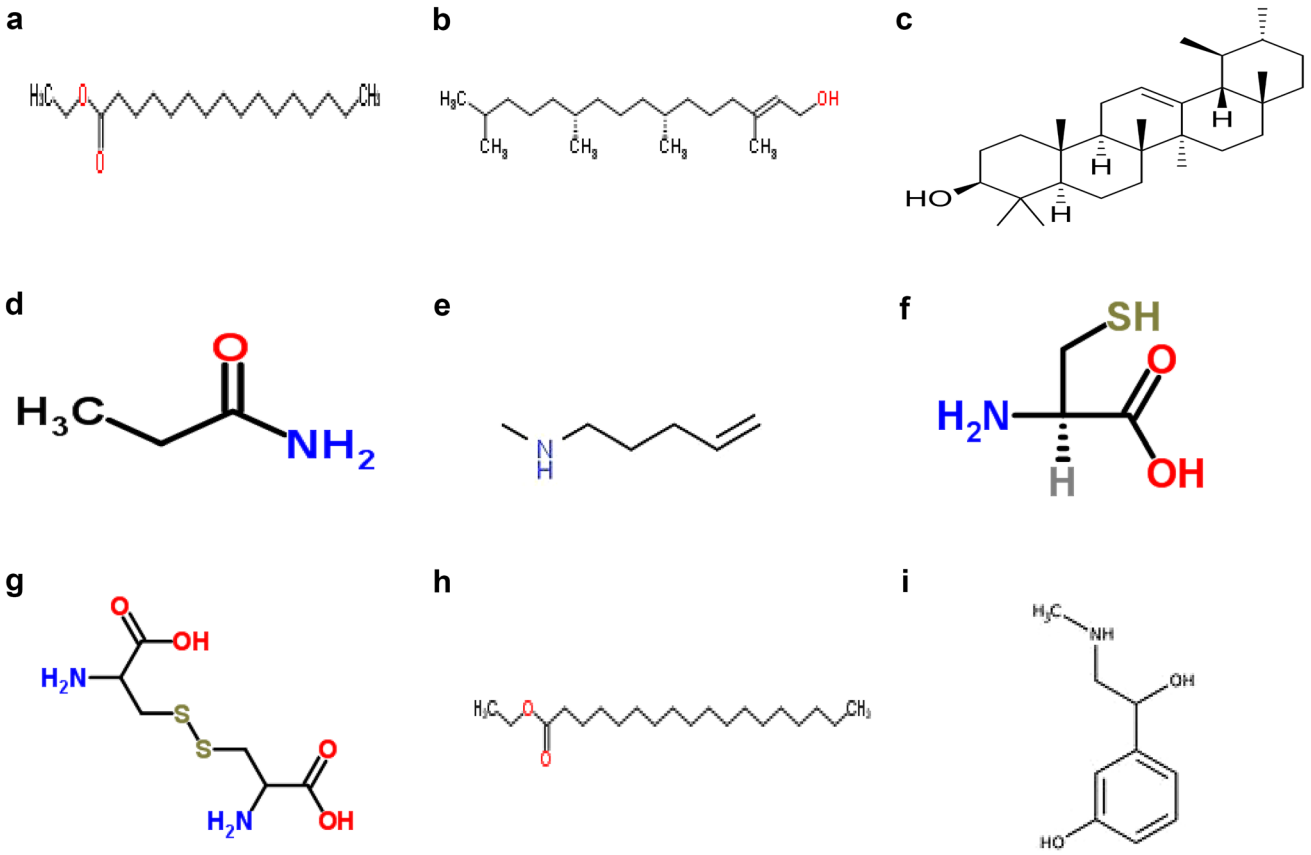


**Fig. 3** **a** Hexadecanoic acid ethyl ester. Molecular Formula: $C_{18}H_{36}O_2$. **b** Phytol. Molecular Formula: $C_{20}H_{40}O$. **c** Alpha amyrin. Molecular Formula: $C_{30}H_{50}O$. **d** Propanamide. Molecular Formula: $C_3H_7NO$. **e** Methylpent 4-enylamine. Molecular Formula: $C_6H_{13}N$. **f** Cysteine. Molecular Formula: $C_3H_7NO_2S$. **g** DL-Cystine. Molecular Formula: $C_6H_{12}N_2O_4S_2$. **h** Octadecanoic acid ethyl ester. Molecular Formula: $C_{20}H_{40}O_2$. **i** Phenylephrine. Molecular Formula: $C_9H_{13}NO_2$

phenylephrine were found to be present in F1of the plant. The molecular structures of the compounds that were in abundant in this fraction are presented in Fig. 3.

## Discussion

Despite the public concern of the adverse effect of synthetic chemical insecticides that have adversely affected both human and environmental health, billions of dollar are being spent every year to procure these chemicals in order to ensure security of farmer produce. This is because the biopesticide market is still very low compared to chemical pesticides that have been widely advocated for in the past (Isman 2006; Begum et al. 2013; Oni et al. 2019). Though, millions of botanicals have been reported of being insecticidal but the adequate information that could help pesticides manufacturers to produce botanical based biopesticides in large quantity are still very limited; the reason why more works need to be done beyond the usage of crude plant powders or extracts. Therefore, the need for identifying the fractions of plants whose insecticidal potential have been established become a matter of importance. More so, that these fractions contain numerous active compounds that could be responsible for the insecticidal potential of these plants (Ching et al. 2012; Tata et al. 2020).

The result obtained in the work showed that the fractions from the oil of *A. auriculiformis* have both abilities to control *C. maculatus* and as well protect cowpea, *V. unguiculata* from the infestation of the insect as they were able to cause high mortality of the insect, low oviposition rate and adult emergence of the insect, reduced seed damage and weight loss as well as low WPI and high inhibition rate. It was observed that the mortality of the insect increased with increase in the dosages of the treatments. The Probit analysis showed that the F1 of the plant oil was required at very low dosage to caused high mortality of the insect within short period of exposure. Hence, the F1 of the plant oil was the most effective fraction against the survival of the insect. It is known that *C. maculatus* do not usually feed at adult stage and therefore don't live more than 14 days under normal conditions. However, if

supplied with honey or sugary substance, their life span could be increased by another 4–7 days. Therefore, the high mortality of the insect could be due to inability of the insect to feed on the cowpea seeds that have been coated with the treatments. Thus, this reflected that the treatments were not contained with sugary substance on which the insect can feed and thereby led to starvation of the insect (Tedela et al. 2017; Obembe and Ogungbite 2017). In addition, Schmutterer (2002) reported that botanical based insecticides are known for their negative effect on respiratory organ of insects, leading to hyperactivity and convulsion and total knockdown of insects (Zibaee 2011; Rajashekar et al. 2014). Furthermore, respiration has been reported has an important factor necessary to produce energy requires for physiological process that leads to production of defense mechanism against insecticides and other toxic substances (Guedes et al. 2006). Consequently, the mortality of cowpea beetle recorded in this work showed that treatments may have blocked the voltage-gated sodium channels in the nerve axons or electron transport chain (in the mitochondrion, leading to inhibition of energy production) as suggested by Schmutterer (2002), Isman (2006), Zibaee (2011) and Obembe and Ogungbite (2017).

Different secondary metabolites were found to be present in the crude oil extract and fractions of *A. auriculiformis* as shown in the GC–MS analysis. The analysis showed that the main active compounds present in the crude oil extract and the three fractions analyzed were mainly alkaloids. Hexadecanoic acid ethylester, phytol, apha amyrin, propanamide and many of the major active compounds present in the fractions and crude oil extract of this plant have been reported of being insecticidal in nature by different authors (Lucie et al. 2013; Fernandes et al. 2014; Céspedes et al. 2015; Cáceres et al. 2015). This agreed with the findings of Chew et al. (2011) and Sravanthi et al (2014) in which alkaloid was found to be in abundance in the leaf of *A. auriculiformis.* Mordue-huntz and Nibet (2000), Yang et al. (2006) and Oigiangbe et al. (2010) reported that alkaloids have high level of toxicity against wide range of insect pests and reduce their life span. Therefore, the high mortality rate of *C. maculatus* recorded by the fractions of *A. auriculiformis* oil may be linked with these active compounds present in them. However, it was noted that the crude oil extract of this plant was unable to cause high mortality of the insect as did by F1, F2 and F4 of the plant oil despite the fact that it contains all the active compounds present in these fractions. This reflected that some of the compounds present in the oil of *A. auriculiformis* may not be synergistically active against the survival of the insect. Thus, this may be responsible for the low mortality of *C. maculatus* caused by the crude oil of the plant compared to the fractions. The result of this research acquiesced with the findings of Tak and Isman (2015) in which 1,8-cineole and camphor from rosemary oil were individually active than when they were used together against *Trichoplusia ni.*

The oviposition rate of the adult *C. maculatus* exposed to different dosages of crude oil and fractions of *A. auriculiformis* was prevented or significantly reduced. The low oviposition rate of the insect may be because of the high mortality rate of the insect, caused by the treatments. Linear regression analysis done for the oviposition and mortality of the insect showed that the two variables were negatively correlated. Thus, this indicated that mortality and oviposition are inversely proportional to each other. That is, the more the mortality of the insect caused by the fractions, the lower the number of eggs laid by the insect. Also, it could be that the insects were unable to mate before death as suggested by Obembe and Ogungbite (2017). Isman (2006) and Zibaee (2011) reported that botanical insecticides cause sterility of insects and thereby make the male sperm infertile. Therefore, the reduced oviposition may be that the treatments have caused sterility of the insect male sperm. The result obtained agreed with the findings of Nenaah et al. (2015) and Smedt et al. (2016) in which insecticides were found to cause reduced oviposition rate of insect. Mbata and Payton (2013) have also reported inhibition of oviposition of mated *C. maculatus* by some monoterpenoids.

The oviposition of insect pests is not as important as their emergence because increase in the emergence of adult insect pests is directly proportional to damage and weight loss of stored commodities. The result obtained in this work revealed that increase in dosage of the fractions caused decrease in the emergence of the adult *C. maculatus.* Furthermore, it was observed that the higher the number of adult that emerged from the treated cowpea the more the percentage damage of the protected cowpea seeds and the weight loss of the seeds. The linear regression analysis done for adult emergence and the weight loss of the treated cowpea seeds showed that there was positive correlation between the adult emergence and weight loss of the cowpea seeds. Thus, the higher the number of adult that emerged the more the seed weight loss. The low number of adult emergence recorded could be due to the low number of eggs laid by the insects which may have in turn caused reduce number of larvae that could have caused the damage and weight loss of the protected cowpea grains. In addition, botanical insecticides have been noted for their ability to inhibit the synthesis and release of ecdysteroids from their prothoracic gland. Thus, this causes the incomplete ecdysis in their larvae (Isman 2006; Zibaee 2011). The reduction in the adult emergence of *C. maculatus* by the treatments could be due to inability of the insect larvae to castoff their exoskelecton that remained attached to their posterior abdomen (Begum et al. 2013; Tedela et al. 2017). Martins et al. (2012) reported that botanical extracts affect the activity of primary protein,

trypsin by inhibiting its secretion from the mid-gut epithelial cell. Therefore, the prevention or reduction in the emergence of the adult beetle that led to low seed damage and weight loss as well as low WPI and high inhibition rate could mean that the larvae which are the main feeding stage in the life cycle of *C. maculatus* may have been affected by the treatments. Since the result of our research have shown that the fractions of *A. auriculiformis* was potent against the infestation of *C. maculatus*, they could be incorporated into pest management system. However, further research is required to test each of the active compounds found in the fractions of the plant. Also, it is necessary to find out the mode of action of this fractions and their long term protectability efficacy as these could serve as valid information for biopesticide manufacturers to produce *A. auriculiformis* based insecticides against *C. maculatus* in large quantity.

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