

## **Comparative analysis of the repellency activity of single compounds and the synthetic blends of semiochemicals from *Aframomum melegueta* (R.schum) and *Dennittia tripetala* (Bak.F) against *Rhizopertha dominica* in Calabar, Nigeria**

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### **Abstract**

Bioassay experiments were carried out in Calabar to compare the repellency activity of single compounds and the synthetic blends of Semiochemicals extracted from *Aframomum melegueta* and *Dennittia tripetala*. The aim of the experiment was to determine the efficacy of single compounds and the synthetic blends in suppressing the reproductive potential and adult emergence of *Rhizopertha dominica*, (Fabricius), a primary pest of stored cassava pellets and cereal grains in Sub-Saharan Africa. Insect culture of the adult *R. dominica* was set up in the laboratory at Federal College of Education Obudu to obtain fresh insects for the bioassay experiments. Dried seeds of *A. melegueta* (R. Schum) and *D. tripetala* (Baker f.) Schatz were procured from the main market in Obudu for the purpose of the research work. 100g each of the dried fruits of the spice plants were pounded separately with the laboratory pestle and mortar for the extraction of the essential oils (EOs). The oils extracted were tested for toxicity and reproductive potential deterrence against *R. dominica*. The chemical constituents of the essential oils were isolated, identified and tested against *R. dominica*. Synthetic blends of individual compounds were prepared based on their natural ratios and were also tested for toxicity and reproductive potential deterrence against the insect pests in the laboratory. The result showed that both the single compounds and the synthetic blends were toxic and repellent to the insect pest, and could significantly suppress the reproductive potential thereby inhibiting the emergence of adult insect. However, synthetic blends exhibited a stronger efficacy in their toxicity against the pest thereby providing a broad spectrum of insecticidal bioactivity against *R. dominica* than the single compounds. This action of the synthetic blends of extracts from the spice plants demonstrated their potential for development in stored products protection especially at the small scale resource poor farmer's level in Nigeria.

**Keywords:**Toxicity, Reproductive potential, Synthetic blend, Bioactivity, Oviposition, Deterrence, Essential oils (EOs).

### **1. Introduction**

In Nigeria and in most African countries, farmers harvest their crops, dry them and then store in traditional manner, which are open storage facilities that are only capable of holding just about 1000 to 1,500 kgs of the total grains harvested for the year (Duke *et al.*, 2003). Greater part of this stored grains are often times damaged by insect pests that infest them. Several figures have been estimated in literatures on the extent of damage of stored grains resulting from insect pest attacks. For instance, Duke *et al.* (2003) reported that about 15 to 20 % losses were recorded the world over, and 30 to 40 % in

the tropics. In West Africa. 25 to 30 % of stored of maize have been destroyed only within few months of storage. (Holst *et al.*, 2002; Meikle *et al.*, 2002). These attacks on maize by the insect pests, usually occur when the moisture content of the grains is between 30 % and 40 % (Adda *et al.*, 2002)

The lesser grain borer, *Rhizoperth dominica* (Fabricius) Bostrichidae is one of the most important economic insect pests of dried cassava chips and pellets and also attack stored grains in the tropical and sub-tropical regions of the world. The insect is also known to be a pest of whole cereals, even of rough rice which is resistant to the attack of most

storage pests. (Adda *et al.*, 2002). The females prefer to lay their eggs in crevices or on the rough surfaces of a seed. On emergence, the larvae will make use of even minute husk defects to bore into the grain (Add *et al.*, 2002). In undamaged grains, adult most frequently attack the grain. Beside whole cereals, *R. dominica* will develop on milled rice and cereal flours but not on highly polished rice. Dried cassava can be severely damaged by *R. dominica*, which is a major pest of the commodity.

There have been several reports of small populations of *R. dominica* on cereals in the field before harvest but infestation is mostly post-harvest. Considerable weight losses occur as a result of heavy attack and further damage may be done by the insects boring into the wooden structures of the store. Commodities infested by *R. dominica* rarely become mouldy. It would seem that this species does not raise the moisture content of the food to the same extent as *Sitophilus spp* which can cause a considerable increase (Duke *et al.*, 2003). The consequences of insect pests infestations is enormous and the economic damage they cause, relates to the physical loss of the commodity, spoilage and loss of quality of the product including the encouragement of mould growth such as Mycotoxin, Ochratoxin A and Citrinin produced by *Penicillium vevrucosun* (Hubert, Munzbergova, and Santino, 2008).

The control of insect pests of crops usually involves the use of synthetic pesticides namely; the Organo-phosphate and Organochlorines which are associated with adverse effects such as the destruction of the ecosystem, mammalian toxicity and the development of resistance by the insect pests (Duke *et al.*, 2003). In modern times, research has emphasized the use of plants essential oils, their chemical constituents and other compounds (plant powders, plant extracts, and nonvolatile oils) as possible substitute to fumigants or synthetic residual pesticides

(Ogendoet *al.*, 2008). However, these plant base pesticides are specific to the target species and have local availability (Isman, 2006). Research has shown that single compounds of extracts from *A. melegueta* and *D. tripetala* can, to some certain level successfully repel *R. dominica* and suppress oviposition, hence the insect's reproductive potential. Therefore, the objective of the research work was to evaluate the efficacy of the synthetic blends of the extracts from *A. melegueta* and *D. tripetala* in repelling *R. dominica*, deterring oviposition and suppressing the reproductive potential of the insect in stored dry cassava chips.

## 2. Materials and methods

### 2.1 Insect culture and the collection of materials

Insect culture was established with adult *R. dominica* collected from infested cassava pellets in a food shop in Obudu market in Cross River State. The insect culture was kept at room temperature in a laboratory at the Agricultural Department, Federal College of Education, Obudu where the bioassay experiments were conducted. The culture was sieved after three days to obtain fresh insects for the experiments. Some quantity of dry, clean and uninfested cassava pellets were procured from the same market for use in the bioassay experiments. Also procured were fresh, ripe fruits of *A. melegueta* and *D. tripetala*. They were washed and dried under the sun for the purpose of the experiment.

### 2.2 Extraction of essential oils (EOs) from *A. melegueta* and *D. tripetala*

A hundred gramme (100g) each of dried fruits of *A. melegueta* and *D. tripetala* were separately ground into powder using laboratory pestle and mortar. The powder of *A. melegueta* was dissolved in a 50 ml of redistilled diethyl ether. The container was immersed in an ultrasonic wave device for 5 minutes to disperse and homogenize the

contents. The vacuum distillation apparatus was then connected to a high vacuum pump (ES 50 vacuum pump. Edwards, England). The glass sections of the apparatus were strongly heated with a hot air blower to remove any less volatile contaminants from its internal surface. The tube which is in U-form and the pear shape vessel meant for the collection of the distillate were submerged completely in nitrogen at a temperature of  $-196^{\circ}\text{C}$ . The residue extracted was then distilled for 24 hours at a pressure of 0.05 mmHg. *D. tripetala* powder was vacuum distilled in a similar manner as explained above. The ether distillates of these substances were then pipetted from the vacuum distillation apparatus through long drawn Pasteur pipettes into 50 ml separation funnels to remove water. The extracts were dried using Magnesium Sulphate ( $\text{MgSO}_4$ ), then filtered and concentrated in order to obtain 4 ml each of *A. melegueta* and *D. tripetala* essential oils (EOs) according to the work of Bouda, Tapondjou, Fontam, and Gumedzoe (2001). Each of the vacuum distilled extract was sealed under nitrogen, labeled accordingly and placed in different ampoules, pending when they were needed for the laboratory bioassay experiments.

### 2.3 Isolation of the chemical constituents of the essential oils (EOs)

Gas-Chromatography-Mass Spectrometry (GC-MS) was conducted in order to identify and isolate the chemical constituents of the polar and non-polar fractions of the essential oils from the two spice plants (*A. melegueta* and *D. tripetala*) and to test them for bioactivity against *R. dominica*. The GC-MS analysis was carried out using agilent technology Model 7890A, interfaced with mass selective Detector (MSD) Model 5975C. An electron ionization was at a 70 eV with an ion source temperature of  $250^{\circ}\text{C}$ . Helium gas was used as a carrier gas while Hp-5ms (30mm x 0.25 mm) was used as the stationary

phase. The oven temperature was maintained at  $75^{\circ}\text{C}$  for five minutes and ramped to  $250^{\circ}\text{C}$  at the rate of  $3.5^{\circ}\text{C}$  per minute for 6 minutes. 1 ml essential oil (EO) each of *A. melegueta* and *D. tripetala* were separately injected into the chromatographic column for analysis of the chemical constituents, which were later separated out for the two spice plants.

However, hexane was identified as the nonpolar compound while florisol diethyl ether fractions were the polar compounds. The major compounds isolated from the florisol diethyl ether of the two spice plants were 0.1 mg/ml (S) – 2- heptanol, 0.6 mg/ml (S) – 2 – heptyl acetate and 0.3mg/ml (R)-linolool for *A. melegueta* and 0.3 mg/ml 1, 3 – Cyclopentadiene, 0.5 mg/ml 1, 6 – Cyclodecadien, 0.2 mg/ml 2 – Undecene for *D. tripetala*, in their natural ratios of 1:6:3 v/v *A. melegueta* and 3:4:3 v/v for *D. tripetala* respectively.

### 2.4 Preparation of synthetic blends from the diethyl ether compounds of the two spice plants

Synthetic blend of 0.1mg/ml (S) – 2 – heptanol, 0.6 mg/ml (S) – 2 – heptyl acetate, and 0.3mg/ml (R)- linolool diethyl ether fractions of *A. melegueta* was prepared by dissolving 0.1mg/ml of 2-heptanol, 0.6 mg/ml of (2) – heptyl acetate and 0.3 mg/ml of (R)-linolool in 10ml flask, then 1ml of each compound was combined in a 10ml volumetric flask filled up with hexane. Similarly, blend of the major components of diethyl ether fractions of *D. tripetala* was prepared by dissolving 0.3 mg/ml of 1, 3 Cyclopentadien, 0.5 mg/ml of 2-Undecand and 0.2 mg/ml Cyclodecadien in 10 ml flask, then 1 ml of each of the compound was combined in a 10ml volumetric flask filled up with hexane according to the methods of (Ukeh, Birkett, Bruce, Allan, Pickett and Mordue, 2020). The synthetic solutions were sealed in ampoules under

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nitrogen for storage pending when they were needed for the bioassay experiments.

### 2.5 Toxicity effect of the plant essential oil (EO) extracts and their synthetic blend on *R. dominica*

Each plant essential oil (EO) extract (5, 10, and 15 µl) was impregnated separately into filter paper and placed at the bottom of a plastic container, while the same quantity of the essential oil was also separately applied round the inside of the same container. Twenty (20) pairs of three day old *R. dominica* were introduced into the containers for oviposition to take place. All treatments were replicated four (4) times and arranged in Complete Randomized Design (CRD). Mortality count was done every 24 hours for 72 hours after the introduction of the insects into the separate containers. On each count, after every two days, dead insects were removed by sieving, counted and discarded while the emerging new insects were also counted and returned to the container and then recorded. Until no more new insect emerged. The same procedure was carried out when the chemical constituents of the

$$\frac{\text{Mean No. emerged adult in control} - \text{mean no. emerged adult in treated}}{\text{Mean no of emerged adult in control}} \times 100$$

According to the method of Ukeh *et al.* (2010).

### 2.7 Data Analysis

All data generated were subjected to analysis of variance (ANOVA) procedure and means were compared using Tukey's simultaneous means separation, according to Zar (1999) or least significant difference (LSD) at 0.05 level of probability. Mentab 15 statistical software was used for the analysis of data.

## 3. Results

The essential oils (EOs) extracted from the two spice plants were tested individually for bioactivity (Toxicity and oviposition

essential oils were isolated and the single compounds and their synthetic blends were separately impregnated into filter paper and some quantities of the oils applied on the inside of the containers in order to conduct the mortality test on the insect pest, *R. dominica*. The mortality experiment was to study the efficacy of the synthetic blends in suppressing the oviposition potential of the insect thereby preventing the emergence of the F<sub>1</sub> progeny insects from the eggs laid. Ten insects were randomly selected from each of the four replicates during the first time that counting was done, and then weighed using Sartorius weighing balance.

Data obtained in this experiment were analyzed using analysis of variance (ANOVA) procedure, after which the data were further transformed using the formula  $\log_{10}^x + 1$  in order to remove the aspect of zero and to ensure that they do not conflict with the analysis of variance.

### 2.6 Reproductive potential deterrence effect

Based on the data obtained from toxicity effect, the percentage reproductive potential deterrence effect was calculated as follows:

deterrence) against the insect pest (*R. dominica*) of stored cassava pellets.

### Toxicity effect

There was no significant ( $P > 0.05$ ) difference in the mortality count of adult *R. dominica* at 24, 48 and 72 hours at 5µl compared to the control treatment in all the essential oils of the two spice plants. There was however a significant ( $P < 0.05$ ) difference within the plant essential oil treatments and between 10µl and 15µl of the plant essential oil compared to the control. *Aframomum melegueta* essential at 10 µl and 15µl

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exhibited the highest toxicity against the insect by recording the highest mortality of the insects compared to the untreated and at 5  $\mu$ l of the oil. *Dennittia tripetala* essential oil

was equally toxic to the insect pests (*R. dominica*) at 10  $\mu$ l and 15  $\mu$ l but not as toxic as essential oil from *A. melegueta* (Table 1).

**Table 1: Effect of essential oil (EO) extracts of *A. melegueta* and *D. tripetala* on the mortality count of *R. dominica* at 24, 48 and 72 hours post treatment in the laboratory**

Treatments	Cumulative mean % mortality		
	24 hours	48 hours	78 hours
Control	9.35 $\pm$ 1.25 <sup>a</sup>	9.62 $\pm$ 1.40 <sup>a</sup>	9.82 $\pm$ 1.20 <sup>a</sup>
<i>A. melegueta</i> EO (5 $\mu$ l)	9.44 $\pm$ 1.22 <sup>a</sup>	9.65 $\pm$ 1.50 <sup>a</sup>	10.01 $\pm$ 1.45 <sup>a</sup>
<i>A. melegueta</i> EO (10 $\mu$ l)	10.46 $\pm$ 1.20 <sup>b</sup>	11.84 $\pm$ 1.42 <sup>b</sup>	12.32 $\pm$ 1.26 <sup>b</sup>
<i>A. melegueta</i> EO (15 $\mu$ l)	12.21 $\pm$ 1.40 <sup>ab</sup>	13.60 $\pm$ 1.25 <sup>ab</sup>	14.62 $\pm$ 1.22 <sup>ab</sup>
Control	9.13 $\pm$ 1.72 <sup>a</sup>	9.75 $\pm$ 1.63 <sup>a</sup>	12.12 $\pm$ 1.42 <sup>a</sup>
<i>D. tripetala</i> EO (5 $\mu$ l)	9.28 $\pm$ 1.23 <sup>a</sup>	9.76 $\pm$ 1.52 <sup>a</sup>	10.73 $\pm$ 1.36 <sup>a</sup>
<i>D. tripetala</i> EO (10 $\mu$ l)	10.65 $\pm$ 1.35 <sup>b</sup>	11.16 $\pm$ 1.45 <sup>b</sup>	11.24 $\pm$ 1.68 <sup>b</sup>
<i>D. tripetala</i> EO (15 $\mu$ l)	12.52 $\pm$ 1.28 <sup>ab</sup>	14.22 $\pm$ 1.36 <sup>ab</sup>	14.52 $\pm$ 1.46 <sup>ab</sup>

Means in the same column followed by the same letter(s) are not significantly different at 0.05 level of probability as determined by Tukey's test.

**Effect of essential oil extracts from *A. melegueta* and *D. tripetala* on the reproductive potential and adult emergence of *R. dominica***

The result in Table 1 showed a significant (P<0.05) difference between the plant essential oils and the control in the mean number of (F1) emergence. The (F1) progeny emergence was significantly suppressed at

15 $\mu$ l of the essential oils of the two spice plants with the highest suppression in the application of 15  $\mu$ l of *A. melegueta* essential oil (Table 2).

**Table 2: Effect of essential oil extracts from *A. melegueta* and *D. tripetala* on the reproductive potential and adult emergence of *R. dominica***

Treatments	Mean adult emergence	Mean body weight (mg) at 5 weeks	Reproductive potential determance (%)
<i>A. melegueta</i>			
Control	3.255 <sup>a</sup>	5.22 <sup>a</sup>	0.00 <sup>a</sup>
Essentialoil (5 $\mu$ l)	1.321 <sup>b</sup>	5.24 <sup>a</sup>	15.55 <sup>b</sup>
Essential Oil (10 $\mu$ l)	1.122 <sup>b</sup>	5.21 <sup>a</sup>	16.58 <sup>b</sup>
Essential oil (15 $\mu$ l)	1.110 <sup>c</sup>	5.10 <sup>a</sup>	20.46 <sup>c</sup>
<i>D. tripetala</i>			
Control	2.254 <sup>a</sup>	5.36 <sup>a</sup>	0.00 <sup>a</sup>
Essential oil (5 $\mu$ l)	1.722 <sup>b</sup>	5.23 <sup>a</sup>	4.56 <sup>b</sup>
Essential oil (10 $\mu$ l)	1.332 <sup>b</sup>	5.22 <sup>a</sup>	13.82 <sup>a</sup>
Essential oil (15 $\mu$ l)	1.291 <sup>c</sup>	5.20 <sup>a</sup>	14.77 <sup>c</sup>

Mean in the same column followed by the same letter(s) are not significantly different at 0.05 level of probability as determined by Tukey's test.

**Effect of diethyl ether fraction of *A. melegueta*, *D. tripetala* and their synthetic blend on the mortality count of *R. dominica* at 24, 48 and 72 hours post treatment in the laboratory**

The result showed a significant ( $P < 0.05$ ) difference between the treatments with the plants essential oils and control and also a significant ( $P < 0.05$ ) difference between the treatments with the single compounds and their synthetic blend, in terms of the mortality count of the insect pest at 24, 48, 72 hours post treatment. The mortality count of the

insect in the treatment with diethyl ether from *A. melegueta* at 48 hours and 72 hours was higher than that of diethyl ether from *D. tripetala*. However, the synthetic blend of diethyl ether from the two spice plants recorded the highest mortality count of the insect pest at 48 hours and 72 hours than the single compounds (Table 3).

**Table 3: Effect of diethyl ether fractions of *A. melegueta*, *D. tripetala* and their synthetic blend on the mortality count of *R. dominica* at 24, 48, and 72 hours post treatment in the laboratory**

	24 hours	48 hours	72 hours
<b><i>A. melegueta</i></b>			
Treatments			
Control	6.63 ± 1.53 <sup>a</sup>	6.82 ± 1.42 <sup>a</sup>	6.68 ± 1.55 <sup>a</sup>
0.1 mg/ml(S)-2-Heptanol	8.52 ± 1.50 <sup>b</sup>	8.65 ± 1.08 <sup>b</sup>	9.55 ± 1.83 <sup>b</sup>
0.6 mg/ml (S)-2-Heptyl acetate	8.60 ± 1.63 <sup>b</sup>	10.62 ± 1.21 <sup>c</sup>	12.68 ± 1.82 <sup>c</sup>
0.3 mg/ml (R)-linolool	9.53 ± 1.56 <sup>b</sup>	12.81 ± 1.36 <sup>b</sup>	15.65 ± 1.84 <sup>b</sup>
Synthetic blend (10 µl)	10.62 ± 1.55 <sup>c</sup>	15.84 ± 1.42 <sup>d</sup>	18.34 ± 1.55 <sup>d</sup>
<b><i>D. tripetala</i></b>			
Control	7.73 ± 1.52 <sup>a</sup>	6.65 ± 1.56 <sup>a</sup>	6.45 ± 1.54 <sup>a</sup>
0.3 mg/ml 1, 3 Cyclopentadien	8.82 ± 1.43 <sup>b</sup>	9.55 ± 1.60 <sup>b</sup>	10.63 ± 1.56 <sup>b</sup>
0.5 mg/ml 1, 6-Cyclodecadien	7.56 ± 1.55 <sup>c</sup>	9.65 ± 1.83 <sup>b</sup>	11.12 ± 1.46 <sup>b</sup>
0.2 mg/ml 2-Undecene	7.66 ± 1.54 <sup>c</sup>	10.24 ± 1.67 <sup>c</sup>	15.63 ± 1.45 <sup>c</sup>
Synthetic blend (10 µl)	10.64 ± 1.83 <sup>d</sup>	12.46 ± 1.52 <sup>c</sup>	16.42 ± 1.50 <sup>c</sup>

Means in the same column followed by the same letter(s) are not significantly different at 0.05 level of probability as determined by Tukey's test.

**Effect of Diethyl ether fractions of *A. melegueta*, *D. tripetala* and their synthetic blend on the reproductive potential and adult emergence of *R. dominica***

There was a significant ( $P < 0.05$ ) difference between the control and the treatments of diethyl ether fraction from the two spice plants, and between the single compound of the diethyl ether fractions and their synthetic blend, in terms of the mean adult emergence and the percentage reproductive potential deterrence. In the control experiment involving the two spice plants, the mean adult emergence was higher than in the other treatments, since no plant extract was applied. The control in the two spice plants, also recorded zero percentage reproductive

deterrence compared to the main adult emergence and the percentage reproductive potential deterrence for the other treatments with the plants extracts. The mean adult emergence in the treatment with synthetic blends of the two spice plants was zero recording between 30% reproductive potential deterrence for *A. melegueta* and 26% for *D. tripetala*. The suppression of the mean adult emergence by the synthetic blend of *A. melegueta* was higher than that of *D. tripetala*. Also the percentage reproductive potential

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deterrence of *A. melegueta* was higher than that of *D. tripetala* (Table 4).

**Table 4: Effect of Diethyl ether fractions of *A. melegueta*, *D. tripetala* and their synthetic blend on the reproduction potential and adult emergence of *R. dominica* in the laboratory**

	24 hours	48 hours	72 hours
Treatments		<i>A. melegueta</i>	
Control	3.831 <sup>a</sup>	3.62 <sup>a</sup>	0.00 <sup>a</sup>
0.1 mg/ml (S)-2-Heptanol	1.422 <sup>b</sup>	3.51 <sup>a</sup>	16.82 <sup>b</sup>
0.6 mg/ml (S)-2-Heptyl acetate	1.401 <sup>b</sup>	3.52 <sup>a</sup>	16.92 <sup>b</sup>
0.3 mg/ml (R)-Linolool	1.411 <sup>b</sup>	3.54 <sup>a</sup>	16.88 <sup>c</sup>
Synthetic blend (10 µl)	0.250 <sup>c</sup>	3.48 <sup>a</sup>	30.78 <sup>d</sup>
		<i>D. tripetala</i>	
Control	2.981 <sup>a</sup>	3.64 <sup>a</sup>	0.00 <sup>a</sup>
0.3 mg/ml 1,3-Cyclopentadien	1.521 <sup>b</sup>	3.48 <sup>a</sup>	11.52 <sup>b</sup>
0.5 mg/ml 1,6-Cyclodecadien	1.482 <sup>b</sup>	3.38 <sup>a</sup>	11.48 <sup>b</sup>
0.2 mg/ml 2-Undecene	1.382 <sup>b</sup>	3.39 <sup>a</sup>	18.52 <sup>c</sup>
Synthetic blend (10 µl)	1.441 <sup>c</sup>	3.55 <sup>a</sup>	28.24 <sup>d</sup>

Means in the same column followed by the same letter(s) are not significantly different at 0.05 level of probability as determined by Tukey's test.

### Discussion

There was no significant ( $P > 0.05$ ) difference in the mortality count of the adult *R. dominica* at 24, 48 and 72 hours between 5 µl and control. This is because the plants essential oils (EOs) concentration was too low at 5 µl to cause any serious mortality of the adult *R. dominica*. However, at between 10 µl and 15 µl, there was a significant ( $P < 0.05$ ) difference in the mortality count of *R. dominica* compared to the control. The significant ( $P < 0.05$ ) difference was attributed to the high concentrations, higher enough to cause the mortality of the insect pest. The cumulative mortality values at 10 µl and 15 µl at 72 hours post treatments, showed that essential oil (EO) from *A. melegueta* caused higher mortality of *R. dominica* compared to 10 µl and 15 µl of *D. tripetala* essential oil at 48 hours and 72 hours. The mortality of the insect however, appeared to increase as the concentration increases and as the number of days of exposure to all treatments except in the control. This result is in line with the

report of Oparaeke and Kuhiep (2006) that the number of emerging *S. zeamais* in untreated (control) was significantly higher than those in the maize treated with 10 µl and 15 µl concentration of powders of *Aframomum melegueta* and *Zingiber officinale* in an experiment.

On the reproductive potential and adult emergence, there was a significant ( $P < 0.05$ ) difference between the treatments and control, in terms of the number of new adult emergence. Essential oil (EO) from *A. melegueta* at 10 µl and 15 µl were significantly ( $P < 0.05$ ) toxic and repellent to *R. dominica* compared to control and at 5 µl of the essential oil (EO). Essential oil of *A. melegueta* at 10 µl and 15 µl were more toxic to the insect than essential oil of *D. tripetala* at the same concentration of 10 µl and 15 µl. However, the essential oils (EOs) were seen to suppress oviposition to a certain level by the reproductive potential deterrence of the insect pest resulting in better protection of the stored cassava pellets from infestation and damage.

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The mean number of F<sub>1</sub> progeny insects produced by *R. dominica* in the untreated (control) was significantly higher (P<0.05) than those treated with 10 µl and 15 µl doses of the essential oils (EOs) of the spice plants (Tables 2). This result is also in support of the report of Operaeké and Kuhiep (2006) that the number of emerging F<sub>1</sub> *S. zea* in untreated (control) was significantly higher than those emerging from the seeds treated with 10 and 10% (w/w) of powders of *A. melegueta* and *Z. officinale*, and that the efficacy of the powders depended on the dosage with higher doses providing greater protection and significantly fewer numbers of insect emerging. However, the treatments did not influence the weight of the emerged adults of the *R. dominica* which was determined by weighing with Sartorius weighing balance (Table 2). This finding is in agreement with Danho *et al.* (2001) who reported that emergent *S. zea* adult weight was not affected by competition on different quantities of host grains.

The result of the effect of diethyl ether fractions from *A. melegueta*, *D. tripetala* and their synthetic blends on the mortality of *R. dominica* showed a significant (P<0.05) difference between the treated and the untreated (control) and also a significant (P<0.05) difference between the other treatments and the synthetic blend. The application of 0.6mg/ml (S)-2- heptyl acetate and 0.3 mg/ml (R) – linalool of *A. melegueta* for 72 hours was more toxic to the insect pest than 0.1mg/ml (S)-2- heptanol of the same *A. melegueta*. It was also higher than the application of 0.3 mg/ml 1, 3-Cyclopentadiene, 0.2 mg/ml-2- Undecene and 0.5 mg/ml 1, 6 Cyclodecadien of *D. tripetala* at 72 hours. However, application of synthetic blend of *A. melegueta* for 48 and 72 hours was highly significantly (P=0.01) different than all other treatments including the application of single compounds and synthetic blends of *D. tripetala* (Table 4).

The bioactivity of diethyl ether fractions of the two spice plants against the insect pest here in terms of mortality count showed that they were toxic to the insect. It is clear from this result that the bioactivity of the essential oils against the insect is attributed majorly to the diethyl ether fractions of the polar compound. The result is in agreement with the views of (Takabayashi and Dicke, 1996; Van Tolet *et al.*, 2007) who reported that the specific ratios of the behaviourally active compounds from florasil (R) diethyl ether fractions of *A. melegueta* and *Z. officinale* were responsible for the efficacy of their oils to repel maize weevil from stored maize.

On the effect of extracts from single compounds and their synthetic blend on the reproductive potential and adult emergence of *R. dominica*, the significant (P<0.05) difference between the control and the treatment with the single compounds was as a result of the fact that extracts from the single compounds of *A. melegueta* and *D. tripetala* were toxic to the insect pest compared to the untreated (control). There was also a significant (P<0.05) difference between the treatments with single compounds and their synthetic blend. The separate synthetic blend of the two spice plants were highly toxic to the insect pest than the single compounds. The synthetic blend of *A. melegueta* was more toxic to the insect than that of *D. tripetala*. However, the synthetic blend from each of the two spice plants, were able to suppress the adult emergence of the insect pest to zero thereby deterring the reproductive potential of the pest, compared to the single compounds (Table 5).

It was also observed in the treatments with the single compounds that the pungent odours from the essential oils could cause the insect to climb to the walls of the container soon after introduction thereby limiting adequate feeding and oviposition. This result is in agreement with the views of Adler,

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Ojimelukue, and Leon (2000) who observed that *S. zeamais* and *R. dominica* react to emissions from the non-host plants by making oriented movement away from the source of the emissions, and this could be deployed in stored products protection.

The toxicity of the essential oils (EOs) to the insect pest is as a result of the interaction with the insect nervous system, either by inhibiting the release of the enzyme acetylcholinesterase or by antagonizing the function of Octopamine receptors (Rosell, Quero, coll, and Guerrero, 2008). Octopamine is a biogenic amine that acts as neuro transmitter, neuro hormone and neuro modulator in invertebrates (Orchard, Ramirez and Lange, 1993). It is widely used in energy demanding behaviours such as flying, courting, egg-laying and jumping by all insects including crustaceans and spiders (Adanio, Linn and Hoy, 1995).

The single compounds individually could not have produce total protection of the stored cassava pellets, but the blend of each essential oil (EO) in their natural ratios gave significantly higher repellency and toxicity against *R. dominica* in the laboratory studies than the individual compounds. The blend produced percentage repellency (PR) equivalent to a class (iv) repellent with between 60-80 percent repellency (PR) classes of Juliana and SU (1983) from O to V, class O (PR=0.15), class I, (PR=0.15-20%), class II (PR=20.1 – 40%), class III (PR=40.1-60%), class iv (PR=60.1-80%) and class v (PR=80.1-100%).

The synthetic blend can be prepared and applied as protective bands around grain bulk or bags of cassava pellets or incorporated into packaging materials, such as sacking and paper to mask odours from stored cassava pellets or cereals or evoke non-host avoidance and repellent behaviours in the insects. The synthetic blends could also be used to treat the structure of an empty store to flush out hidden infestation before fresh

cassava pellets or cereals are introduced (Emana, 1999). Also Bekeleand Hassanali (2001) observed that blend effects were responsible for the bioactivity of essential oil constituents of *Ocimum kilimandscharius* and *O. Kenyense* against stored products pests such as *S. zeamais* and *R. dominica* in Kenya.

### Conclusion

To protect stored products especially cassava pellets, flour, seeds and grains from the destructive effects of insect pests is a common phenomenon in many countries of the world (Duke *et al.*, 2003). However, there is great need to replace the inorganic pesticides with biopesticides such as extracts from plants' roots, seeds, fruit etc. the option of replacing the toxic synthetic inorganic pesticides, which are toxic to the user, consumers and the environment with plant base pesticides at a time when there are heightened public concerned over the hazardous effect of the synthetic pesticides is now receiving serious attention amongst scientists all over the globe. The result of the laboratory experiment conducted here, showed that the blends of the diethyl ether fractions of *A. melegueta* and *D. tripetala* have broad spectrum of bioactivity against *R. dominica* than the individual compounds. Identifying and testing the repellence and toxic effects of the chemical constituents of the essential oils (EOs) of the two spice plants and their blends in this study may provide further opportunities for their use in postharvest crop protection.

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