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Original Research Article

Comparative efficacy of single compounds and the synthetic blends of extracts from A. *melegueta* and X. *aethiopica* in controlling *S. zeamais* in stored maize in Calabar, Nigeria

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Abstract

Laboratory bioassays were conducted in Calabar Nigeria to compare the efficacies of single compounds and the synthetic blends of extracts from *A. melegueta* and *X. aethiopica* in controlling *Sitophilus zeamais,* a primary pest of stored maize in sub-Saharan Africa. Insect culture of the adult *S. zeamais* was prepared in the laboratory at the University of Calabar to obtain fresh insects for the experimental works. Dried seeds of *A. Melegueta* (R. schum) and fruits of *X. aethiopica* (Dunal), A. Rich were procured from a local market in Obudu for the purpose of the work. 100g each of the spice plants were pounded separately using laboratory pestle and mortar for the extraction of essential oils (EOS). The essential oils extracted were tested for repellence and toxicity against *S. zeamais* at 10µl per essential oil in a 4 –way olfactometer. The chemical constituents of the essential oils (Eos) were isolated and identified and then tested for repellence and toxicity against the *S. zeamais*. Synthetic blends of the chemical constituents were prepared based on their natural rations. The synthetic blends were also tested for repellence and toxicity against the insect pest. Results indicated that the essential oils and their constituents as individual compounds were significantly ($p<0.05$) repellent and toxic to *S. zeamais.* However, the synthetic blends of the chemical constituents were highly repellent and toxic to the insect pest than the single compounds of the essential oils (Eos), thereby providing a broad spectrum of bioactivity against *S. zeamais*. The synthetic blend of *A. melegueta* was more repellent to the insect than that of *X. aethiopica*. This action of the synthetic blends demonstrated their potential for development in Stored Products Protection especially at the small scale resource poor farmer's level in Nigeria.

Keywords: Laboratory bioassay, efficacy, Sitophilus zeamais, A. melegueta, X. aethiopica, Synthetic blends, Repellency, Toxicity, 4-way olfactometer.

INTRODUCTION

In Nigeria and many African countries, farmers harvest, dry and store their grains in a traditional manner, which is to store in open storage facilities that are only capable of holding 1000 to 1500kgs of the total grains harvested (Duke *et al.,* 2003). Much of the stored grains are often damaged by insect pests that infest them. Several figures have been estimated in literatures on the extent of stored grains damage resulting from insect pests attacks. Example, Duke *et al.* (2003) reported that about 15 to 20% losses were recorded worldwide and 30 to 40% in the tropical regions. In West Africa, 25 to 30% of stored maize have been destroyed only within few months of storage (Holst *et al.,* 2000; Meikle *et al.,* 2002). The maize attack by the insect usually occur when the Moisture content of the grains is between 30% and 40% (Adda *et al*., 2002).

The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is one of the most important

economic insect pests of stored maize in the tropical and Sub-tropical regions in the world. Both the adult and the lavae of the insect, attack the grains by feeding on the endosperm of the grain, thereby resulting in total weight loss, nutrient content reduction and overall deterioration of the grain's quality (Duke *et al*., 2003). More so, the specific gravity of the grain is often reduced and its market value lowered. The grains ability to germinate is drastically reduced as the germ is eaten up by the insect (Adda *et al*., 2002).

The consequences of insect pests infestations is great, 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 Penincillium vevrucosum (Hubert *et al*., 2008).

The control of insect pests of crops usually involves the use of synthetic pesticides such as the organophosphates and organochlorines, which are associated with adverse effects such as the development of resistance by the insect pests, destruction of the ecosystem and mammalian toxicity (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 non volatile oils) as possible substitute to fumigants or synthetic residual pesticides (Ogendo *et al*., 2008). However, these plant base pesticides are specific to the target species and have local availability (Ismah, 2006; Umoetok *et al*., 2009). Research has shown that single compounds of extracts from *A. melegueta* and *X aethiopica*, can to a certain level successfully repel and control *S. zeamais* in stored maize. Therefore the objective of this research work was to evaluate and compare the efficacy of the single major compounds and the synthetic blends of extracts from *A. melegueta* and *X. aethiopica* in a 4-way olfactometer bioassay.

MATERIALS AND METHODS

Insect Culture and the Collection of Materials

Insect culture was established with adult *S. zeamais* collected from infested maize in a food shop in Uwatt market Calabar, Cross River State. The insect culture was kept at room temperature in a laboratory at the Crop Science Department, University of Calabar, Calabar Nigeria where the bioassay experiments were conducted. The culture was sieved after three days to obtain fresh insects for the experiment. Some quantity of dry, clean and uninfested maize seeds and ripe, fresh fruits of *A. melegueta* and *X. aethiopica* were procured from the same market for the purpose of the bioassay experiments, the fruits were washed and sun dried.

Extraction of the Essential Oils (Eos) from *A. melegueta* and *X. aethiopica*

One hundred grammes (100mg) each of dried fruits of *A. melegueta* and *X. aethiopica* were separately ground into powder, using laboratory pestle and mortar. The powdered *A. melegueta* was dissolved in a 50 ml of redistilled diethyl ether. The container was inmmersed 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 shaped 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 ^{0}C. The residue extracted was then distilled for 24 hours at a pressure of 0.05 mmHg. *X. aethiopica* powder was vacuum distilled in a similar manner as explained above. The ether distillates of these substances were then ipette 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 ($MgS0_4$), then filtered and concentrated in order to obtain 4 ml each of *A. melegueta* and *X. aethiopica* essential oils (Eos) (Bonda *et al*., 2001). Each of the vacuum distilled extract was sealed under nitrogen, labeled accordingly and placed in different ampoules, pending when they were used for the laboratory olfactometer bioassay experiment.

Laboratory Bioassay with the Essential Oils (Eos)

Essential oils extracted from *A. melegueta* and *X. aethiopica* were each impregnated into a filter paper and placed alongside with maize seed in one arm of the olfactometer, while the other three arms were reserved as control. An olfactometer consists of a 6 mm thick transparent Perspex held together. A four pointed exposure chamber shaped like a star is fixed to a circular plate measuring 12 cm x 12 cm with a hole (3 mm) drilled into the walls at each point of the four cardinal glass frames. Another plate 10.2 cm x 10.2 cm x 0.6 cm with a hole of 4 mm in diameter at the centre is used as a cover. In order for the insect pest to work easily in the olfactometer, a sheet of fisher brand QL 100 filter paper (Springfield Mill, Maidstone, Kent England) was placed on the floor covering. An air stream was passed into

the olfactometer through a Telfon tubing of size 3.2 mm (Cam/ab/Ltd UK) from an Air entrainment chamber to keep the insect mobile (Ukeh *et al.,* 2010).

In the bioassay experiment involving the insect pest and the essential oils from the spice plants, the parameters assessed were the time spent by the insect (*S. zeamais*) in the different arms of the olfactometer and

the number of times the insect visited the different odour zones. All treatments were replicated ten (10) times on twelve minutes duration using a three day old insect (*S. zeamais*). A fresh (three day old) insect (*S. zeamais*) and a fresh stimulus source were used in each single choice test.

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 of the two spice plants (*A. melegueta* and *X. aethiopica*), and to test them for bioactivity against *S. zeamais*. 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 ^{0}C. Helium gas was used as a carrier gas while Hp-5ms (30 mm x 0.25 mm x 0.320 mm) was used as the stationary phase. The oven temperature was maintained at 75 ^{0}C per minute for six minutes. 1 ml essential oil (EO) each of *A. melegueta* and *X. aethiopica* 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 non-polar compound, while florisil ® diethyl ether fractions were the polar compounds. The major polar compounds isolated from the florisil ® diethyl ether of the two spice plants were *A. melegueta*: 0.1 mg/ml (S)-2-heptanol, 0.6 mg/ml (S)-2-heptyl acetate and 0.3 mg/ml ®- linalool, *X. aethiopica*: 0.3 mg/ml 1,8-Cineole, 0.4 mg/ml b-Phellandrene and 0.3 mg/ml b-Pinene in their natural ratios of 1:6:3 and 3:4:3 respectively.

Preparation of Synthetic Blends

Synthetic blend of (S) -2-heptanol, (S)-2-heptyl acetate and ®-linalool florisil ® diethyl ether fractions of *A. melegueta* was prepared by dissolving 0.1 mg/ml of (S)-2-heptanol, 0.6 mg/ml of (S)-2-heptyl acetate and 0.3 mg/ml of ®- linalool in 10 ml flask, then 1 ml of each compound was combined in a 10 ml volumetric flask filled up with the hexane. Similarly, blend of the major components of florisil ® diethyl ether fractions of *X. aethiopica* was prepared based on their natural ratios of 3:4:3 (v/v). The blend was prepared by dissolving 0.3 mg/ml 1,8- Cineole, 0.4 mg/ml b-Phellandrene and 0.3 mg/ml b-Pinene in 10 ml flask, then 1 ml of each of the compounds was combined in a 10 ml volumetric flask filled up with hexane (Ukeh *et al*., 2010). The synthetic solutions were sealed in ampoules under nitrogen for storage prior to the bioassay experimental work with the olfactometer.

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. Data on the number of entries or visits made by the test insect to the test arm and control were analyzed using t-test at (0.05) level of probability. Minitab 15 statistical soft ware was used for the analysis of data.

RESULTS

The essential oils (EOs) extracted from the two spice plants were tested individually for bioactivity against the insect pest (*S. zeamais*) of stored grains.

The time spent by the insect in the test arm containing maize seed plus essential oil from *A. melegueta* and *X. aethiopica*, impregnated in a filter paper was significantly ($p<0.05$) different compared on separate occasions to the time spent in the control arms (Table 1).

The number of visits made by the insect to the test arm containing maize seed plus essential oils of *A. melegueta* and *X. aethiopica* on different occasions was significantly ($p<0.05$) different compared to the control arms (Table 1b).

The time spent by the insect in the test arm containing maize seed plus vacuum distilled hexane fractions of *A. melegueta* and *X. aethiopica* each impregnated into filter paper was not significantly ($p>0.05$) different compared to the time spent in the control arms (Table 2).

The number of visits or entries by the insect to the test arm containing maize seed plus vacuum distilled hexane fractions of *A. melegueta* and *X. aethiopica* was also not significantly ($p>0.05$) different compared to the number of entries into the control arms.

The time spent by *S. zeamais* in the test arm of the olfactometer containing on separate occasions maize seed plus vacuum distilled diethyl ether fractions of *A. melegueta* and *X aethiopica* impregnated in a filter paper was significantly ($p<0.05$) different, compared to the time spent by the insect in the control arms Table 3a).

Similarly, the number of entries or visits by the insect to the test arms containing maize seed plus the vacuum distilled diethyl ether fractions of *A. melegueta* and *X aethiopica* on separate occasions, was significantly ($p<0.05$) different, compared to the number of entries or or visits by the insect to the control arms (Table 3b).

The result of the bioassay experiment involving florisil ® diethyl ether extracts from *A. melegueta* showed that, the time spent by *S. zeamais* in the text arm containing maize seed and the florisil ® diethyl ether impregnated in

Table 1: Behavioural responses of *S. zeamais* to volatiles from maize seed plus essential oils (EOS) of *A. melegueta* and *X. aethiopica* in a 4-way olfactometer.
(a) Mean Time Spent by the Insect (Minutes ± SE).

	Treatments	
	A. melegueta	***X. aethiopica***
Test Arm	0.630	1.499
Control 1	3.056	3.382
Control 2	2.243	3.421
Control 3	2.382	3.663
X	2.077	2.991
SEM±	0.286	0.642
CV%	22.38	20.22
LSD (0.05)	0.532	0.622

(b) Mean Number of Entries into each Arm of the Olfactometer.

	Treatments	
	A. melegueta	***X. aethiopica***
Test Arm	0.625 ± 0.22	2.821 ± 0.30
Control	6.421 ± 0.24	3.016 ± 0
X	1.335 ± 0.20	2.918 ± 0.25
t(0.05)	0.22*	0.62*

* = Significant at ($p<0.05$) level of probability

Table 2. Behavioural responses of *S. zeamais* to volatiles from maize seed plus 10µl vacuum distilled hexane fraction of *A. melegueta* and *X. aethiopica* in a 4-way olfactometer.
(a) Mean time spent by the insect (minutes ± SE).

	Treatments	
	A. melegueta	***X. aethiopica***
Test Arm	0.749	1.450
Control 1	3.717	3.850
Control 2	3.079	3.470
Control 3	2.827	3.560
X	2.593	3.083
SEM±	0.179	1.356
CV%	21.90	12.00
LSD (0.05)	NS	NS

NS = Not significant at ($p>0.05$) level of probability

(b) Mean number of entries into each arm of the olfactometer.

	Treatments	
	A. melegueta	***X. aethiopica***
Test Arm	1.800 ± 0.25	2.900 ± 0.33
Control	5.463 ± 0.37	3.031 ± 0.29
X	3.632 ± 0.31	2.965 ± 0.31
t(0.05)	NS	NS

NS = Not significant at ($p>0.05$) level of probability

Table 3. Behavioural responses of *S. zeamais* to volatiles from maize seed plus 10µl vacuum distilled florisil ® diethyl ether fractions of *A. melegueta* and *X. aethiopica* in a 4-way olfactometer

(a) Mean time spent by the insect (Minutes ± SE).

	Treatments	
	A. melegueta	***X. aethiopica***
Test Arm	1.004	1.278
Control 1	3.176	1.924
Control 2	3.042	1.695
Control 3	3.042	2.474
X	3.049	1.843
SEM±	2.567	0.166
CV%	22.50	27.80
LSD (0.05)	0.524	0.835

(b) Mean number of entries into each arm of the olfactometer.

	Treatments	
	A. melegueta	***X. aethiopica***
Test Arm	1.900 ± 0.05	4.900 ± 0.23
Control	3.963 ± 0.27	5.070 ± 0.35
X	2.93 ± 0.17	4.985 ± 0.29
t(0.05)	1.83*	2.62*

* = Significant at ($p<0.05$) level of probability

a filter paper on separate occasions, was significantly ($p<0.05$) different compared to the time spent in the control arms of the olfactometer (Table 4a).

Similarly, the number of entries or visits made by the insect to the test arm containing the different extracts of the florisil ® diethyl ether of *A. melegueta,* on different occasions was significantly ($p<0.05$) different compared to the number of entries or visits to the control arms of the olfactometer (Table 4b).

The result of the experiment involving florisil ® diethyl ether extracts from *X. aethiopica* also showed that, the time spent by the insect (*S. zeamais*) in the test arm containing maize seed plus the florisil ® diethyl ether extracts was on separate occasions significantly ($p<0.05$) different when compared to the time spent by the insect in the three control arms (Table 5a).

In the same manner, the number of entries or visits made by the insect to the test arm containing maize seed plus the different florisl ® diethyl ether extracts on separate occasions, was significantly ($p<0.05$) different compared to the number of entries or visits to the control arms of the olfactometer (Table 5b).

Result of the above bioassay experiment involving synthetic blends of *A. melegueta* and *X. aethiopica* against *S. zeamais*, showed that the time spent by the insect in the test arm containing on different occasions, maize seed plus the synthetic blend each of *A. melegueta* and *X. aethiopica* impregnated into filter paper, was highly significant ($p=0.01$), compared to the time spent by the insect in the control arms of the olfactometer (Table 6a).

Also, the number of entries or visits made by the insect to the test arm containing on different occasions, maize seed plus synthetic blend of each of *A. melegueta* and *X. aethiopica* extracts was highly significant ($p=0.01$) compared to the number of entries or visits to the control arms (Table 6b).

The interaction between the single compounds and their synthetic blends showed that, the main time spent by the insect in the test arm containing the synthetic blends of *A. melegueta* and *X. aethiopica* differ at highly significant ($p=0.01$) level than the time spent in the control arms containing the individual compounds. The result also showed that, the time spent by the insect in the test arm containing synthetic blend of *A. melegueta* differ significantly ($p<0.05$) from the time spent in the test arm containing synthetic blend of *X. aethiopica* (Table 6).

DISCUSSION

Insects usually and frequently locate their food source/host through the detection of volatile chemical cues emanating from it, through olfactory receptors situated on the antenna (Duke *et al*., 2003). This means that insect species are able to detect a suitable host while

Table 4. Behavioural responses of *Sitophilus zeamais* to volatiles from maize seed plus 10µl vacuum distilled florisil ® diethyl ether fractions of *A. melegueta* in a 4-way olfactometer.
(a) Mean time spent by the insect (Minutes ± SE).

	Treatments		
	(R)-linalool (0.3 mg/ml)	**(S)-2-heptyl acetate (0.6 mg/ml)**	**(S)-2-heptanol (0.1 mg/ml)**
Test Arm	1.22 ± 0.21	1.82 ± 0.29	1.24 ± 0.26
Control 1	2.37 ± 0.20	2.65 ± 0.26	2.53 ± 0.19
Control 2	3.05 ± 0.22	2.46 ± 0.21	3.02 ± 0.25
Control 3	2.81 ± 0.22	2.48 ± 0.22	2.92 ± 0.24
X	2.36 ± 0.21	2.35 ± 0.24	2.43 ± 0.24
SEM±	0.185	0.524	0.162
CV%	22.80	21.90	20.10
LSD (0.05)	0.532	0.632	0.464

(b) Mean number of entries into each arm of the olfactometer.

	Treatments			
	Test Arm	**Control**	**X**	**t(0.05)**
(R)-linalool (0.03 mg/ml)	1.21 ± 0.22	6.23 ± 0.22	3.74 ± 0.22	0.68*
(S)-2-heptyl acetate (0.6 mg/ml)	2.00 ± 0.28	5.82 ± 0.22	3.91 ± 0.25	0.51*
(S)-2-heptanol (0.1 mg/ml)	1.21 ± 0.24	7.22 ± 0.21	4.22 ± 0.23	0.96*

* = Significant at ($p<0.05$) level of probability

Table 5. Behavioural responses of *Sitophilus zeamais* to volatiles from maize seed plus 10µl vacuum distilled florisil ® diethyl ether fractions of *X. aethiopica* in a 4-way olfactometer.
(a) Mean time spent by the insect (Minutes ± SE).

	Treatments		
	1,8 Cineol (0.3 mg/ml)	**b-Phellandrene (0.4 mg/ml)**	**b-Pinene (0.3 mg/ml)**
Test Arm	1.50 ± 0.21	1.83 ± 0.25	1.60 ± 0.22
Control 1	2.45 ± 0.24	2.33 ± 0.21	2.81 ± 0.24
Control 2	2.52 ± 0.22	2.72 ± 0.23	2.64 ± 0.22
Control 3	2.46 ± 0.21	2.62 ± 0.22	2.74 ± 0.21
X	2.23 ± 0.22	2.37 ± 0.23	2.44 ± 0.22
SEM±	0.159	0.202	0.653
CV%	18.30	24.60	20.40
LSD (0.05)	0.522	0.580	0.265

(b) Mean number of entries into each arm of the olfactometer

	Treatments			
	Test Arm	**Control**	**X**	**t(0.05)**
(1,8 Cineol (0.3 mg/ml)	1.48 ± 0.26	6.52 ± 0.22	4.00 ± 0.24	0.628*
(b-Phellandrene (0.4 mg/ml)	1.96 ± 0.25	6.72 ± 0.31	4.34 ± 0.28	0.92*
(b-Pinene (0.3 mg/ml)	1.98 ± 0.25	3.58 ± 0.25	2.78 ± 0.28	0.736*

* = Significant at ($p<0.05$) level of probability

Table 6. Behavioural responses of *S. zeamais* to volatiles from maize seed plus 10µl synthetic blends of the florisil ® diethyl ether extracts of *A. melegueta* and *X. aethiopica* in a 4-way olfactometer .

(a) Mean time spent by the insect (Minutes ± SE).

	Treatments	
	A. melegueta	***X. aethiopica***
Test Arm	0.66	0.58
Control 1	3.65	3.38
Control 2	3.12	3.28
Control 3	3.18	3.52
X	2.653	2.69
SEM±	0.21	0.37
CV%	24.90	22.50
LSD (0.05)	0.096	0.128

(b) Mean number of entries into each arm of the olfactometer.

	Treatments	
	A. melegueta	***X. aethiopica***
Test Arm	1.700 ± 0.20	1.622 ± 0.20
Control	5.520 ± 0.30	5.270 ± 0.34
X	3.11 ± 0.25	3.446 ± 0.27
t(0.05)	0.29**	0.22**

** = Highly significant at ($p<0.05$) level of probability

working or during flight, and also that host selection can depend on a lack of repellent odour. *S. zeamais* responded positively toward air plumes that emanated from the maize seed and negatively to those from the product of the spice plants (*A. melegueta* and *X. aethiopica*). The positive response of the insect to volatiles from maize seed proved that it was able to identify its food source by perceiving the odour emitted from the maize seed with the help of its antenna. This result is in agreement with Pike *et al.* (1994) who reported that *S. zeamais* was attracted to maize volatiles. The volatile aroma compounds from different rice flavour types have also been reported by Yang *et al.* (2008). Wakefield *et al.* (2005) reported the attraction of *S. oryzae* to carob volatiles from rice.

The volatile aroma compounds from the spice plants are commonly used domestically to flavour foods and in the industries to manufacture perfumes (Coppen, 1995). The essential oils from these aromatic plants are said to consist of complex mixtures of monoterpenes, diterpenes, triterpenes and sesquiterpenes hydrocarbons, aliphatic as well as aromatic compounds with a few major constituents (Rosell *et al.*, 2008). In an olfactometer experiment conducted with vacuum distilled essential oils from *A. melegueta* and *X. aethiopica* against *S. zeamias*, the insect was at separate times repelled by the aroma from the essential oils of the two spice plants. This result is in line with (Ukeh *et al.*, 2010) who reported the repellency of *A. melegueta* and *Zingiber officinale* against *S. zeamais*. There was no repellent effect against the insect (*S. zeamais*), when conducted with the non-polar hexane fraction of the essential oils from the two spice plants (Table 2). But there was a repellent effect against the insect in the olfactometer experiment conducted with the diethyl ether fractions of the essential oil (Table 3). It was therefore established that the repellency of the essential oils (EOs) was attributed to the polar florisil ® diethyl ether fractions of the essential oils (EOs), rather than the non polar hexane fractions. The repellency test with the diethyl ether fractions against *S. zeamais* was significantly different ($p<0.05$) in respect to the mean time spent by the insect in the test arm and control arms, as well as the number of entries by the insect to the test arm was significantly less compared to the number of entries to the control arms (Table 3). The isolation of the chemical components of the diethyl ether showed that *A. melegueta* contains 0.3 mg/ml (R)-linalool, 0.6 mg/ml (S)-2-heptyl acetate and 0.1 mg/ml (S)-2-heptanol, while *X. aethiopica* contains 0.3 mg/ml 1,8- Cineole, 0.4 mg/ml b-Phellandrene and 0.3 mg/ml b-Pinene. All the isolated compounds of the two spice plants showed significant ($p<0.05$) repellency and toxicity against the stored products insect pest (*S. zeamais*). (Table 4a & b, 5a & b). The isolation here was

in line with Ajaieoba and Ekundayo (1999) who identified and isolated four aryldecanones and eight minor compounds from n-hexane and methanolic seed extract of *A. melegueta* obtained from South Western Nigeria.

Synthetic blends of the florisil ® diethyl ether fractions of the essential oils (EOs) of the two spice plants were prepared based on the identified natural ratios of 3:6:1 for *A. melegueta* and 3:4:3 for *X. aethiopica*. The synthetic blends showed a highly significant (p=0.01) toxicity and repellency level against the insect pest (Table 6). The interaction between the blends of the two spice plants showed that synthetic blend of *A. melegueta* was more repellent to the insect than that of *X. aethiopica* (Table 7). The toxicity and repellency of the extracts of the essential oils (EOs) of the spice plants was probably as a result of the interaction of the diethyl ether fractions with the insect nervous system, either by inhibiting the release of the enzyme acetylcholinesterase or by antagonizing the function of octopamine receptors (Rosell *et al*., 2008). Octopamine is a biogenic amine that acts as neurotransmitter, neurohormone and neuromodulator in invertebrates (Orchard *et al*., 1993). The repellency of the extracts of the essential oils from the spice plants here, implies that if the synthetic blends are used in stored product protection such as maize or dried cassava chips in storage, will provide total protection of the grains or chips. The result here demonstrated that, the blends of the compounds in their natural ratios (Table 6a & b) were more toxic and repellent to the insect pest, and presented a broad spectrum of bioactivity against the insect pest than the individual compounds (Table 4a & b, 5a & b). However, the blends of the essential oils (EOs) presented percentage repellency (PR) which is equivalent to a class IV repellent with between 60-80 percent repellency of the pest species. This is in accordance with Juliana and Su (1983) percentage repellency class of 0 to V, class 0 (PR = 0.15), class 1(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%).

CONCLUSION

To keep stored products free of the destructive effects of insect pests, is a common practice in several countries of the world (Duke *et al*., 2003). However, there is great need to replace the synthetic pesticides with biospesticides. The option of replacing the toxic synthetic pesticides with plant base pesticides, at a time when there are heightened public concerns over the hazardous effect of the synthetic pesticides is now receiving serious attention amongst scientists the world over. The result of the laboratory experiment conducted here, showed that the blends of the florisil ® diethyl ether fractions of *A. melegueta* and *X. athiopica* have broad spectrum of bioactivity against *S. zeamais* than the individual compounds. Identifying and testing the repellent and toxic effects of the chemical constituents of the essential oils (EOs) from the two spice plants and their blends in this study, may provide further opportunities for their use in post harvest crop protection (Ukeh *et al*., 2010), especially by the resource poor small scale farmers in traditional African setting.

REFERENCES

Adda C, Borgemeister C, Biliwa A, Meikle WG, Markham RH, Peohling HM (2002). Integrated pest management in Post-harvest maize: a case study from the Republic of Togo (West Africa). Agriculture, Ecosystems and Environment, 93:305-321.

Ajaiyeoba EO, Ekundayo O (1999). Essential oil constituents of Afromomum melegueta (Roscoe) K. Schum Seeds (alligator pepper) from Nigeria. Flavour and Fragrance J., 14:109-111.

Bouda H, Tapondjou LA, Fonstam DA, Gumedzoe MYD (2001). Effects of essential oils from leaves of Ageratum conuzoides, Lantana camara and Chromolaena odorata on the mortality of Sitophilus zeamais (Coleoptera: Curculionidae). J. Stored Products Res., 37: 103-109.

Coppen JJW (1995). Flavours and Fragrances of plant origin. Food and Agricultural Organization (FAO) Report, Rome, Italy.

Duke SO, Baerson SR, Dayan FE, Rimando AM, Schoffler BE, Tellaz MR, Wedge DE, Schrader KK, Ahey DH, Arthur FH, De Lucca AJ, Gibson DM, Harrison HF, Peterson JK, Gealy DR, Tworkoski T, Wilson CL, Morris JB (2003). United States Department of Agriculture, Agricultural Research Service, research on natural products for pest management. Pest Management Science, 59: 708-717.

Holst N, Meikle WG, Markham RH (2000). Grain injury models for Prostephanus truncatus (Coleoptera: Bostrichidae) and Sitophilus zeamais (Coleoptera: Curculionudae) in rural maize stores in West Africa. J. Econ. Entomol., 93: 1338-1346.

Hubert J, Munzbergova Z, Santino A (2008). Plant volatile aldehydes as natural insecticides against stored product beetles. Pest Management Science, 64, 57-64.

Juliana G, Su HCF (1983). Laboratory studies on several plant materials as insect repellents for protection of cereal grains. J. Econ. Entomol., 76: 154-157.

Meikle WG, Markham RH, Nansen C, Hoist N, Degbey P, Azoma K, Korie S (2002). Pests Management in traditional maize stores in West Africa: a farmer's perspective. J. Econ. Entomol., 95: 1079-1088.

Ogendo JO, Kostyukovsky M, Ravia U, Mtasyoh JC, Deng AT, Omolo EO, Kariuki ST, Shaaya E (2008). Bioactivity of Ocimum gratissinum L. Oil and two of its constituents against five insect pests attacking stored food products. J. Stored Products Res, 44: 328-334.

Orchard I, Ramirez TM, Lange AB (1993). A multifunctional role for Octopamine in locust flight. Annual Review of Entomology, 38: 227-249.

Pike V, Smith JL, White RD, Hall DR (1994). Studies of responses of stored products pest, Prostephanus truncatus (Horn) and Sitophilus zeamais (Motch), to food volatiles, In: Highley, E., Wright, E.J., Banks, H.J. and Champ, B.K. (eds). Proceedings of the 6th International Working Conference on Stored Products Protection, Canbera, Australia. Wallingford, UK, CAB International, pp.566-569.

Rosell G, Quero C, Coll J, Guerrero A (2008). Biorational insecticides in pest management. J. Pesticide Sci., 33: 103-121.

Sman MB (2006). Botanical Insecticides, Deterrents and Repellents in Modern Agriculture and an increasingly regulated world. Annual Review of Entomology, 51: 45-66.

Ukeh DA, Birkett MA, Bruce TJA, Allan EJ, Pickett JA, Mordue (Luntz) AJ (2010). Behavioural responses of the maize weevil, S. zeamais to host (maize grain) and non-host plant volatiles. Pest Manag. Sci., 66: 44-50.

Umoetok SBA, Osuagwu AN, Udo IA, Idiongette MI, Ukeh DA (2009). Effects of Azadirachta indica products on the management of Ootheca mutabilis on Telfairia occidentalis in Calabar, Southern Nigeria. Crop Protection, 28: 583-587.

Wakefield ME, Bryning GP, Chambers J (2005). Progress towards a lure to attract three stored product weevils, Sitophilus zeamais Motschulsky, S. oryzae (L.) and S. granarius (L.) (Coleoptera: Curculionidae). J. Stored

Product Res., 41: 145-161.
Yang DS, Shewfelt RL, Lee KS, Kays SJ (2008). Comparism of odour active compounds from six distinctly different rice flavour types. J. Agric. Food. Chemistry, 56: 2780 – 2787.
Zar JH (1999). Biostatistical analysis. 4th edition, Prentice Hall, Upper Saddle River, New Jersey.