

Repellency Activity of Synthetic Blends of Semio-Chemicals from *Xylopia aethiopica* and *Dennittia tripetala* against *Prostephanus truncatus* (Horn)

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Abstract

Laboratory bioassays were conducted in Calabar, Nigeria, to evaluate repellencies of the essential oils extracted from *Xylopia aethiopica* and *Dennittia tripetala* fruits, chemical constituents of the oils and their blends as well as compare repellencies of the blends with those of the constituents using a 3-day old adult *Prostephanus truncatus*, a primary pest of stored maize and dried cassava chips, cultured in the laboratory. Fresh fruits of *X. aethiopica* and *D. tripetala*, 100 g each was collected, washed and dried, then pounded separately using laboratory pestle and mortar for the extraction of essential oils. The synthetic blends were prepared with 0.3 mg/ml of 1,8-Cineole, 0.4 mg/ml of b-Phellandrene and 0.3 mg/ml of b-Pinene, extracts of *X. aethiopica*, and the extracts of *Dennittia tripetala* (0.3 mg/ml of 1,3- Cyclopentadien, 0.5 mg/ml of 1,6- Cyclodecadien and 0.2 mg/ml of 2-Undecene. The essential oils extracted were tested for repellence and toxicity against *P. truncatus* at 10 µl per essential oil in a 4-way Olfactometer. Gas chromatography-Mass spectrometry (GC-MS) was used to isolate and identify the chemical constituents of the essential oils. Synthetic blends of the chemical constituents were prepared on the basis of their natural ratios and were also tested for bioactivity against the insect pest. Results indicated that the essential oils were significantly ($P < 0.05$) repulsive and toxic to *P. truncatus*. However, the synthetic blends of the chemical constituents of the essential oils were more repellent and toxic to the insect pest than the individual component of the essential oils. The blends provided a broad spectrum of bioactivity against the insect pest and demonstrated the potential for their development in stored product protection especially at the small scale resources poor farmer's level in Nigeria.

Keywords: Essential oils (EOs); *Xylopia aethiopica*; *Dennittia tripetala*; *Prostephanus truncatus*; 4-way Olfactometer

Introduction

In Nigeria, and indeed in most African countries, farmers usually dry and store their grains traditionally in open storage facilities that are capable of accommodating only about 1000 to 1500 kg of the total harvested grains [1]. A lot of the stored grains are often destroyed by insect pest attack culminating in varied levels of food and economic loss. Ukeh *et al.* (2010) reported 10 to 20 % crop loss world wide and 25 to 40 % loss in the tropics; in West Africa, stored grain insects inclusive of *Prostephanus truncatus* (Horn), *Rhizopertha dominica*, *Sitophilus spp*, *Trogoderma granarius*, *Sitotroga cerealella* and *Tribolium castaneum* have been reported to cause losses as high as 30 % [2-4]. *P. truncatus* is an exotic pest whose larvae and adults feed on stored grains and cassava resulting in losses in weight, quality, nutrient, market and seed value besides food poisoning arising from mycotoxins of fungal contaminants [5-7]. Over a short duration of storage, this pest caused the loss of 20-30 % of stored grains in Ghana, Togo, and Tanzania [5, 8-10]. The control of this pest in developing countries will foster their drive towards food security and self-sufficiency.

The use of synthetic insecticide is the most prevalent method of controlling stored product pests; the benefits have been overshadowed by negative impacts such as development of resistance by the insect pests, destruction of the ecosystem and mammalian toxicity [1]. There is therefore a need to adopt and integrate other methods of pest control that are less hazardous. The main objective of this research was to evaluate the potential for controlling *P. truncatus* using essential oils extracted from the fruits of guinea pepper (*X. aethiopica*) and pepper fruit (*D. tripetala*), constituents of the oils and their blends.

Materials and Methods

Insect Culture and the Collection of Materials

Insect culture was established in the Laboratory of Department of Crop Science, University of Calabar, with adult *P. truncatus*, collected from infested maize in a food shop in Obudu Local Government Area main market. The plant materials used, undamaged dry seeds of maize seeds, fresh fruits of *X. aethiopica* and *D. tripetala*, were purchased from the same market.

Extraction of Essential Oils (EOs) from *Xylopia aethiopica* and *Dennittia*

One hundred grams (100 g) of the dried fruits of *X. aethiopica* and *D. tripetala* were separately ground into powder using laboratory pestle and mortar and vacuum-distilled in 50 ml of redistilled diethyl ether. The container was immersed in an ultrasonic wave device for 5 mins 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 section of the apparatus was strongly heated with a hot air blower to remove any less volatile contaminants from its internal surface. The U-tube and the pear-shaped distillate collection vessel were submerged completely in nitrogen at a temperature of 196 °C. The residue was then distilled for 24 h at pressure of 0.05 mmHg. The distillates were then pipetted into 50 ml separation funnels to remove water. The extracts were dried, using Magnesium sulphate (MgSO₄), filtered and concentrated to obtain 4 ml of each of *X. aethiopica* and *D. tripetala* essential oils [11]. Each of the vacuum distilled extract was sealed under nitrogen, labelled accordingly and placed in different ampoules, pending when needed for the laboratory bioassay experiment.

Laboratory Bioassay with the Essential Oils

Essential oils extracted from *X. aethiopica* and *D. tripetala* were each impregnated into 10 µl x cm² filter paper and placed alongside with maize seed in one arm of the Olfactometer, while the other three arms were reserved as control. The 6 mm thick Perspex Olfactometer used had a four-pointed exposure chamber shaped like a star was 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. The floor plate measured 10.2 cm x 10.2 cm x 0.6 cm) and a similar sized plate with a 4 mm diameter hole at the centre served as cover. In order for the insect 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 Teflon tubing of size 3.2 mm (can/ ab/ Ltd, U.K.) from an air entrainment chamber, to keep the insect mobile [12]. In the bioassay experiment, the parameters assessed were the time spent by a 3-day old adult *P. truncatus* in the different arms of the olfactometer and the number of times the insect visited the different odour zones over a 12-minute observation period. All treatments were replicated 10 times using a new insect and a fresh essential oil. It was observed that each time an insect is placed in an Olfactometer and air stream passed in from an air entrainment chamber through a Teflon tubing, the insect becomes active and mobile and begin to respond to the emissions from the spice plants by moving away from them and moving round the based of the Olfactometer, entering the different arms and coming out [11]. Soon after about eleven (11) or twelve (12) minutes of the commencement of the experiment, the insect gradually becomes tired, sluggish and suffocated from the toxic emissions coming from the spice plant extract, thereby affecting the insect's ability to respond actively to the emissions, while the strength of the emissions also becomes weaker and weaker with time. Therefore, there is need to change the insect and the plant extract for fresh ones to ensure vigour in the response and full emission of the somio-chemicals [2].

Also assessed was toxicity and adulticidal effect of the plant (EOs) extracts and their synthetic blend on *P. truncatus*. Each plant synthetic blend extract (5,10 &15 ml) was impregnated separately into filter paper and placed at the bottom of a plastic container, while the same quantity of the EO was also separately applied round the inside of the same container. Twenty (20) pairs of the 3 day old *P. truncatus* were introduced into the containers for oviposition to take place. All treatment were replicated 4 times and arranged in complete randomized design (CRD). Mortality count was done every 24 hourly for 72 hrs after the introduction of the insect 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 containers and then recorded until no more insects emerged.

Data obtained were analysed 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.

Isolation of the Chemical Constituents of the Essential Oils

The chemical constituents of the polar and non-polar fractions of the essential oils were isolated and identified using Gas chromatography-Mass spectrometry (GC-MS). 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 anion temperature of 250 °C. Helium gas was used as a carrier gas while Hp-5 ms (30 mm x 0.25 mm x 0.32 mm) were used as the stationary phase. The oven temperature was maintained at 75 °C for five minutes and ramped to 250 °C at the rate of 3.5 °C per minute for six minutes. One ml of the essential oil of *X. aethiopica* and *D. tripetala* was separately injected into the chromatographic column for analysis. Hexane was identified as the non-polar compound, while Florisil® diethyl ether fractions were identified as the polar

compounds. Polar compounds isolated from the Florisil® diethyl ether fraction of *X. aethiopica* were 0.3 mg/ml 1,8-cineole, 0.4 mg/ml b-phellandrene, and 0.3 mg/ml b-Pinene (natural ratio 3:4:3); those for *D. tripetala* were 0.3 mg/ml 1,3-cyclopentadiene, 0.5 mg/ml 1,6-cyclodecadiene, and 0.2 mg/ml 2-undecene (natural ratio 3:5:2). The constituents were tested for repellency as described above.

Preparation of Synthetic Blends

Synthetic blends of 1,8-cineole, b-Phellandrene and b-Pinene florisol diethyl ether fractions of *X. aethiopica* was prepared by dissolving 0.3 mg/ml of 1,8-cineole, 0.4 mg/ml b-Phellandrene and 0.3 mg/ml b-Pinene in 10 ml flask, then 1ml of each compound was combined in a 10 ml volumetric flask and filled up with hexane. Blends of the major compounds of Florisil® diethyl ether fractions of *D. tripetala* were similarly prepared based on their natural ratios. The synthetic solutions were sealed in ampoules under nitrogen for storage prior to the bioassay experiments.

Data Analysis

All data generated were subjected to analysis of variance and means were compared using Tukey's simultaneous means separation, according to (Zar, 1999) or least significant difference (LSD) at 5 percent level of probability [13]. 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 software was used for the analysis of data.

Results

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

The time spent by the insect in the test arm containing maize seed plus essential oils from *X. aethiopica* and *D. tripetala*, impregnated in a filter paper was on separate occasions significantly ($p < 0.05$) different compared to the time spent in the three control arms (Table 1). The number of visits the insect made to the test arm containing maize seed plus essential oils from *X. aethiopica* and *D. tripetala* on separate occasions, was significantly ($p < 0.05$) different compared to the number of entries into the three control arms (Table 1).

Treatments	Number of entries in each arm of the Olfactometer	
	<i>X. aethiopica</i>	<i>D. Tripetala</i>
Maize with essential oils	2.84±0.21	1.74±0.20
Control	6.42±0.23	8.64±0.26
Means	4.63±0.22	5.69±0.23
T (0.05)	0.57	1.23*
	Mean time of permanence by the insects (min±SE)	
Maize with essential oils	0.66	0.83
Control 1 (maize without essential oils)	3.68	2.56
Control 2 (maize without essential oils)	3.73	3.69
Control 3 (maize without essential oils)	3.67	3.18
Means	2.94	2.56
SEM±	0.19	0.53
CV	20.4	22.8
LSD (0.05)	0.56	0.67

*Significant at $P < 0.05$ level of probability

Table 1: Responses of *Prostephanus truncatus* to volatiles from essential oils of *Xylopia aethiopica* and *Dennettia tripetala* in filter paper with maize seeds inside arm of the Olfactometer

The time spent by the insect in the test arm of the Olfactometer containing on separate occasions, maize seed plus non polar vacuum distilled hexane fractions of *X. aethiopica* and *D. tripetala* impregnated in a filter paper was not significantly ($p > 0.05$) different, compared to the time spent in the control arms (Table 2). Similarly, the number of entries made by the insect to the test arms containing maize seed plus the non polar hexane fractions of *X. aethiopica* and maize seed plus the non polar hexane fraction of *D. tripetala*, was not significantly ($p > 0.05$) differently compared to the number of entries or visits made to the control arms of the Olfactometer; signifying that the non polar hexane compounds of *X. aethiopica* and *D. tripetala* were not toxic and repellent to the insect pest (*P. truncatus*). The time spent by the insect in the test arm containing maize seed plus 1,8-cineole florisol® diethyl ether, maize seed plus b-Phellandrene florisol® diethyl ether and maize seed plus b-Pinene florisol® diethyl ether extracts of *X. aethiopica* was significantly ($p < 0.05$) different compared to the time spent in the control arms (Table 3). The number of visits made by the

insect to the test arm containing maize seed plus 1,8-cineole florasil[®] diethyl ether, maize seed plus b-Phellandrene florasil[®] diethyl ether extract of *X. aethiopica* was significantly ($p < 0.05$) different compared to the number of entries into the control arms of the 4-arm Olfactometer (Table 3).

Treatments	Number of entries in each arm of the Olfactometer	
	<i>X. aethiopica</i>	<i>D. Tripetala</i>
Test arm	2.00±0.26	1.82±0.30
Control	4.83±0.32	2.03±0.42
Means T(0.05)	3.42±0.29 NS	3.43±0.36 NS
	Mean time of permanence by the insects (min±SE)	
Maize with essential oils	0.69	2.03
Control 1 (maize without essential oils)	3.01	2.11
Control 2 (maize without essential oils)	3.32	2.22
Control 3 (maize without essential oils)	3.12	2.20
Means	2.54	2.14
SEM±	0.19	0.19
CV	22.4	22.8
LSD (0.05)	NS	NS

*Significant at $P < 0.05$ level of probability

Table 2: Behavioural responses of *Prostephanus truncatus* to volatiles from maize seed plus 10 µl vacuum distilled hexane fractions of *X. aethiopica* and *D. tripetala* in a 4-way Olfactometer bioassay

	Mean number of entries into each arm of the Olfactometer		
	1,3- Cyclopentadien (0.3 mg/ml)	1,6- Cyclodecadien (0.5 mg/ml)	2-Undecene (0.2 mg/ml)
Maize with volatiles	2.29±0.20	1.36±0.21	3.28±0.24
Control	8.22±0.32	7.52±0.22	5.67±0.30
Means	5.57±0.26	4.44±0.22	4.50±0.27
t(0.05)	0.56* 0.68*	0.66*	
	Mean time of permanence by the insects (min±SE)		
Maize with volatiles of oils	1.62±0.22	1.20±0.30	1.80±0.20
Control 1 (without oil volatiles)	2.52±0.21	3.00±0.21	2.82±0.22
Control 2 (without oil volatiles)	2.81±0.33	2.46±0.30	3.21±0.21
Control 3 (without oil volatiles)	2.62±0.20	2.30±0.20	2.81±0.24
Means	2.39±0.24	2.40±0.25	2.66±0.22
SEM	0.520	0.559	0.872
CV	6.50	11.60	18.60
LSD(0.05)	0.534	0.723	0.687

*Significant at $P < 0.05$ level of probability

Table 3: Behavioural Responses of *Prostephanus truncatus* to Volatiles from Maize Seed Plus 10µl Vacuum Distilled Florasil[®] Diethyl Ether Fractions of *X. aethiopica* in a 4-way Olfactometer Bioassay

The time spent by the insect in the test arm containing on different occasions, maize seed plus 1,3-cyclopentadiene florasil[®] diethyl ether, maize seed plus 1,6-cyclodecadiene florasil[®] diethyl ether and maize seed plus 2-undecene florasil[®] diethyl ether extracts of *D. tripetala* was significantly ($p < 0.05$) different compared to the time spent in the different control arms (Table 4). Similarly, entries by the insect to the Olfactometer arms containing maize seed plus 1,3-cyclopentadiene florasil[®] diethyl ether, maize seed plus 1,6-cyclodecadiene florasil[®] diethyl ether and maize seed plus 2-undecene florasil[®] diethyl ether extracts from *D. tripetala* was significantly ($p < 0.05$) different compared to number of entries to the control arms of the Olfactometer (Table 4). The result above showed that the time spent by *P. truncatus* in the test arm of the Olfactometer containing on separate occasions, maize seed plus synthetic blends from *X. aethiopica* and *D. tripetala* was significantly ($p = 0.01$) higher compared to time spent in the different control arms (Table 5). Similarly, the number of entries or visits made by the insect to the test arm containing on separate occasions maize seed plus synthetic blends of *X. aethiopica* and *D. tripetala* was highly significant ($p = 0.01$) compared to the number of entries or visits to the control arms [14]. The result in table 5 showed a high significance ($p < 0.05$) in the repellence and toxicity of the synthetic blend of *X. aethiopica* against *P. truncatus* compared to the single compounds in terms of the mean time spent by the insect

in the different arms of the Olfactometer. The time spent by the insect in the test arm containing the synthetic blend of the spice plant was by far shorter than the time spent in the control and the other arms containing the individual compounds. Similarly, the number of entries or visits by the insect to the test arm containing the synthetic blend is significantly ($p < 0.05$) less than the number of times or visits the insect made to the control arms and the other arms containing the single compounds (Table 6). The result in table 7 also showed a significant difference in the main time spent by the insect pest in the test arm containing the synthetic blend compared to the time spent in the control arms and the single compounds. It was obvious that the insect made significantly shorter time spent in the test with synthetic blend compared to the time spent in the test arms containing the single compounds and the control arms. Also the number of visits to the test arm with the synthetic blend was highly significantly ($p = 0.01$) different compared to the number of visits to the control arms and the single compounds (Table 7). There was no significant ($p > 0.05$) difference in the mortality count of adult *P. truncatus* at 24, 28 and 72 hour at 5 ml, compared to the control treatment in the blends of the essential oil (EOs) of the two spice plants. However, there was a significant ($P < 0.05$) difference within the plants synthetic blends applied, and between 5 ml and 10-15 ml and the control experiment. Synthetic blend of *X. aethiopica* at 10 ml and 15 ml exhibited the highest mortality count of the insect (*P. truncatus*) compared to the untreated (control) and at 5 ml of the extract. Synthetic blend of *D. tripetala* was equally toxic to the insect (*P. truncatus*) at 10-15 ml, but only next to the toxicity of *X. aethiopica* (Table 8). On the whole, the synthetic blends were more toxic to the insect than the single compounds.

Mean number of entries into each arm of the Olfactometer			
	1,3- Cyclopentadien (0.3 mg/ml)	1,6- Cyclodecadien (0.5 mg/ml)	2-Undecene (0.2 mg/ml)
Maize with volatiles	2.89±0.22	2.60±0.30	2.50±0.32
Control	6.44±0.21	7.62±0.22	8.26±0.24
Means	4.36±0.22	5.11±0.26	5.38±0.28
t(0.05)	2.62* 0.55*	0.71*	
Mean time of permanence by the insects (min±SE)			
Maize with volatiles of oils	1.42±0.31	1.52±0.22	1.86±0.22
Control 1 (without oil volatiles)	2.26±0.25	3.22±0.23	2.81±0.23
Control 2 (without oil volatiles)	3.21±0.31	2.88±0.20	3.20±0.21
Control 3 (without oil volatiles)	2.88±0.22	3.10±0.23	3.11±0.22
Means	2.44±0.29	2.68±0.22	2.74±0.22
SEM	0.433	0.489	0.848
CV	6.60	8.10	7.90
LSD(0.05)	0.334	0.066	0.199

*Significant at $P < 0.05$ level of probability

Table 4: Behavioural Responses of *Prostephanus truncatus* to Volatiles from Maize Seed Plus 10µl Vacuum Distilled Florisil® Diethyl Ether Fractions of *D. tripetala* in a 4-way Olfactometer Bioassay

Mean time spent by the insect (Minutes ± SE)		
Treatments	<i>X. aethiopica</i>	<i>D. tripetala</i>
Maize with essential oils	0.628±0.22	1.500±0.20
Control	4.820±0.21	5.560±0.41
Means	3.224±0.22	3.530±0.31
t(0.05)	0.33**	0.24**
Mean time of permanence by the insects (min±SE)		
Test arm	0.824	0.427
Control 1	3.180	3.317
Control 2	3.281	3.817
Control 3	3.712	3.041
Means	2.753	2.650
SEM ±	0.372	0.165
CV %	22.50	20.40
LSD (0.05)	0.150	0.250

*Significant at $P < 0.05$ level of probability

Table 5: Essential oils plus 10 ml synthetic blends of chemical constituents of *X. aethiopica* and *D. tripetala* in maize and responses of pest *Prostephanus truncatus*

Mean number of entries into each arm of the Olfactometer				
	1,3- Cyclopentadien (0.3 mg/ml)	1,6- Cyclodecadien (0.5 mg/ml)	2-Undecene (0.2 mg/ml)	Synthetic blend
Maize with volatiles	2.89±0.22	2.60±0.30	2.50±0.32	0.628
Control	1.36±0.21	7.62±0.22	8.26±0.24	4.820
Means	3.28±0.24	5.11±0.26	5.38±0.28	2.22
t(0.05)	0.56*	0.68*	0.66*	0.33
Mean time of permanence by the insects (min±SE)				
Maize with volatiles of oils	1.62±0.22	1.20±0.30	1.80±0.20	0.824
Control 1 (without oil volatiles)	2.50±0.21	3.00±0.21	2.82±0.22	3.180
Control 2 (without oil volatiles)	2.81±0.33	2.46±0.30	3.21±0.21	3.281
Control 3 (without oil volatiles)	2.62±0.20	2.30±0.20	2.81±0.24	3.712
Means	2.39±0.24	2.40±0.25	2.66±0.22	2.753
SEM	0.520	0.559	0.872	0.372
CV	6.50	11.60	18.60	22.52
LSD(0.05)	0.53	0.72	0.69	0.15

*Significant at P<0.05 level of probability

Table 6: Interaction between the bioactivity of individual Compounds and the synthetic blends of *X. aethiopica* against *P. truncatus* in a 4-way Olfactometer bioassay

Mean number of entries into each arm of the Olfactometer				
	1,3- Cyclopentadien (0.3 mg/ml)	1,6- Cyclodecadien (0.5 mg/ml)	2-Undecene (0.2 mg/ml)	Synthetic blend
Maize with volatiles	2.28±0.22	2.60±0.30	2.50±0.32	1.500
Control	6.44±0.21	7.62±0.22	8.26±0.24	5.560
Means	4.36±0.22	5.11±0.26	5.38±0.28	3.530
t(0.05)	2.62*	0.55*	0.71*	0.24*
Mean time of permanence by the insects (min±SE)				
Maize with volatiles of oils	1.42±0.31	1.52±0.22	1.86±0.22	0.427
Control 1 (without oil volatiles)	2.26±0.25	3.22±0.23	2.81±0.23	3.317
Control 2 (without oil volatiles)	3.21±0.31	2.88±0.20	3.20±0.21	3.187
Control 3 (without oil volatiles)	2.88±0.22	3.10±0.23	3.11±0.22	3.041
Means	2.44±0.27	2.68±0.22	2.71±0.22	2.650
SEM	0.433	0.489	0.848	0.165
CV	6.60	8.10	7.90	20.40
LSD(0.05)	0.334	0.066	0.199	0.250

* Significant at P < 0.05 level of probability

Table 7: Interactions between the bioactivity of individual Compounds and the synthetic blend of *D. tripetala* against *P. truncatus* in a 4-way Olfactometer bioassay

Cumulative mean % mortality			
Treatment	24hrs	48hrs	72hrs
Control	9.26±25a	9.80±1.42a	9.86±1.20a
<i>X. aethiopica</i> (5 ml)	9.46±1.24a	9.65±1.52a	10.06±1.48a
<i>X. aethiopica</i> (10 ml)	10.56±1.23b	12.82±1.55b	12.46±1.27b
<i>X. aethiopica</i> (15 ml)	12.72±1.52ab	14.72±1.24ab	15.63±1.23ab
Control	9.12±1.72a	9.73±1.63a	11.14±1.42a
<i>D. tripetala</i> (5 ml)	9.27±26a	9.66±1.58a	10.72±1.35a
<i>D. tripetala</i> (10 ml)	10.79±1.36b	11.15±1.5b	11.28±1.62b
<i>D. tripetala</i> (15 ml)	12.10±1.28ab	13.45±1.38ab	14.24±1.46ab

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.

Table 8: Effects of the plant extracts from *X. aethiopica* and *D. tripetala* on the mortality count of *P. truncatus* at 24, 28, and 72 hour post treatment in the laboratory

Discussion

One of the most important sources of repellants is the essential oil extracts from aromatic plants commonly used domestically to flavor foods and in the industries to manufactured perfumes [14]. These aromatic plants' essential oils consists of complex mixtures of monoterpenes, diterpenes, triterpenes and sesquiterpenes hydrocarbons, aliphatic as well as aromatic compounds, with a few major constituents [15]. In an olfactometer experiment, vacuum distilled essential oils from *X. aethiopica* and *D. tripetala* were repellant to adult *P. truncatus* when tested in combination with maize seed. It was therefore observed that the repellency of the essential oils (EOs) was facilitated by the polar florisil[®] diethyl ether fractions from the spice plants rather than from the non-polar hexane fractions, as the repellency test was not significant ($p > 0.05$) at 5 % level of probability (Table 2). The toxicity of the essentials oils (EOs) and their synthetic blends to the Insects pest was probably as a result of the interactions of the polar florisil[®] diethyl ether with the insect nervous system, either by inhibiting the release of the enzyme acetyl cholinesterase or by antagonizing the functions of octopamine receptors [15]. Octopamine is a biogenic amine that acts as neurotransmitter, neurohormone and neuromodulator in invertebrates [16]. Effects of essentials oils (EOs) of *X. aethiopica* and *D. tripetala* on the insect, may suggest their mode of actions such as repellency and feeding deterrence. Application of essential oil of Citroneller in stored –product protection, mosquito repellency and domestic pest (cockroaches, ants and flea) control, and the application of cinnamon oil in mites and urban pests control have been reported [17,18].

Gas chromatography-Mass spectrometry (GC-MS) conducted to isolate the chemical components of the essentials oils (EOs) from the two spice plants, identified the presence of monoterpenes, sesquiterpenes, oxygenated monoterpenes and alcohols as the components found in the essential oils (EOs). The major volatile constituents of the Diethyl ether fractions of *X. aethiopica* include; 1,8-cineole, b-Phellandrene and b-Pinene, while those of *D. tripetala* were 1,3-cyclopentadiene, 2-undecene and 1,6-cyclodecadiene. This result was in line with the report of Ajaiyeoba and Ekundayo (1999) who indentified and isolated four aryldecanones and eight minor compounds from n-hexane and methanoic acid extracts of *A. melegueta* obtained from South Western Nigeria [19].

The synthetic blends of the isolated florisil[®] diethyl ether compounds from *X. aethiopica* and *D. tripetala* were very repulsive to *P. truncatus*, therefore both the number of entries and the time spent in the test arm of the Olfactometer containing maize seeds and the organic compounds was highly significant ($p = 0.01$) compared to the time spent and the number of entries into the control arms. Time spent and the number of entries into the test arm containing maize seed plus synthetic blend of *X. aethiopica* was shorter than the time spent and the number of entries into the test arm containing maize seed plus synthetic blend of *D. tripetala*, meaning that the synthetic blend of *X. aethiopica* was more toxic and highly repellant to *P. truncatus* than the synthetic blend of *D. tripetala*. Each time the insect was entering the test arm having been attracted by volatiles from maize seed, it was in turn confronted by volatiles from the synthetic blends which were repellant and toxic to the insect, therefore it will run back to the control arms where there was no such toxic emission. This phenomenon is described as the “Pull Push” strategy (Ukeh *et al.* 2010), where the insect is pulled in, by volatiles from maize seed, it is pushed back by the toxic volatiles from the spice plants [2]. The repellence of the produce of the spice plants have 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. The result here showed that the blends of the compounds in their natural ratios were more toxic and repellant to the insect pest and provides a broad spectrum of bioactivity against the insect pest than the individual compound (Tables 6 and 7). Also in terms of mortality count of the insect (*P. truncatus*) there was a high mortality level in the applied, compared to the control and also between the application of 5 ml and 10-15 ml of the synthetic blends from the two spice plants. The high toxicity level of the insect of 10 to 15 ml of the synthetic blends from both *X. aethiopia* and *D. tripelela*, showed that the toxicity increases as the concentration of the blends of the spice plants increase (Table 8). The blends of the essential oils (EOs) produced a percentage repellency (PR), equivalent to a class iv repellant with between 60-80 percent repellency of the spices. This is in accordance with Juliana and Su (1983) percentage repellency class of 0 to v; class 0 (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 %) [20].

Conclusion

Both the individual components of the extracts (essential oils) from the spice plants and the synthetic blends of the extract have broad spectrum of bioactivity (repellent effect) against *P. truncatus*, However, the synthetic blends were more toxic to the insect than the single or individual compounds.

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