academicJournals

Vol. 14(36), pp. 2662-2671, 9 September, 2015
DOI: 10.5897/AJB2015.14785
Article Number: E52037055410
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Essential oil composition of different fractions of *Piper guineense* Schumach. et Thonn from Cameroon using gas chromatography-mass spectrometry and their insecticidal effect on *Sitophilus oryzae* (L.)

Esther Obi Oben[1]*, Robyn McConchie[2], Kim Phan-Thien[2] and Nelson Neba Ntonifor[3]

[1]Laboratory of Zoology, Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé 1, Cameroon.
[2]Department of Food Science, Faculty of Agriculture and Environment, University of Sydney, Australia.
[3]Department of Zoology and Animal Physiology, Faculty of Science, University of Buea, Cameroon.

Received 9 June, 2015; Accepted 2 September, 2015

Essential oil fractions from dried seed powder of *Piper guineense* were analyzed by gas chromatography-mass spectrometry (GC-MS) and evaluated for their insecticidal effects on *Sitophilus oryzae* L. The GC-MS analysis showed quantitative and qualitative differences between the oil fractions. Chromatographic results revealed chemical constituents like eugenol, piperanol, pinene, carene, copaene with insecticidal properties. New chemotypes were seen in the different fractions. Instead of β-caryophyllene reported in literature, α-caryophyllene was found in all the different fractions. Caryophyllene oxide, an oxygen-containing sesquiterpene was present in all fractions except n-hexane. In addition to α-phellandrene present in all, β-phellandrene, a monoterpene hydrocarbon was found in the n-Hexane fraction. Contact toxicity on wheat grains showed that all fractions caused significant ($P < 0.001$) mortality of the weevils. The oil fractions also showed variable contact toxicity on impregnated filter paper. All doses of the n-hexane fraction were very toxic to the test insect than the control, causing 100% mortality after five days of exposure. All the fractions produced a strong repellent activity against the test insect. These results suggest that *P. guineense* has potentials for development as an organic insecticide against *S. oryzae* and other pests of stored grains.

Key words: *Piper guineense* Schum. et Thonn., essential oil fractions, chemotypes, toxicity, repellency, *Sitophilus oryzae* (L.).

INTRODUCTION

Stored maize is infested by several important cosmopolitan pests such as weevils which cause considerable economic losses (Ndemah, 1999). Weevils have been reported to cause up to 30% grain damage in Cameroon, where

*Corresponding author. E-mail: estherobio1@yahoo.com, estherbesong@gmail.com. Tel: (+237) 677427933, 697799121.

stored grains constitute the most important food crop (Nukenine et al., 2010). Control of these insect pests is primarily dependent upon applications of synthetic insecticides (Bekele et al., 1996). Sometimes, insecticides may seem effective, but repeated application has disrupted biological control by natural enemies, leading to outbreaks of other pest species and also the development of resistance (Park et al., 2003). The problems caused by synthetic pesticides and their residues have increased the need for effective biodegradable pesticides of plant origin with greater selectivity and history of traditional use. Botanicals plants have been successful against a number of pests in Africa (Bekele et al., 1997; Ogendo et al., 2008).

Botanical products are based on powders, extracts or purified substances of plant origin and are promising alternatives to synthetic residual pesticides in Africa, where subsistence farming practice predominates. In many parts of Cameroon, farmers use different kinds of plant products for insect control (Tapandjou et al., 2000; Ngamo et al., 2007). Although, very little use is known by farmers in the South West region of Cameroon on plant products for insect pests' control. Plant products are known to have negligible effects on beneficial insects and lower environmental impacts. They are easily affordable, available and play useful role in integrated pest management IPM programs in developing countries. Since most developing nations suffer from high cost of synthetics, crop protection products with modest efficacy are preferred if they are readily available and less expensive than the conventional pesticides. Plants powders or extracts could be produced with limited skills and knowledge and their use entails little or no financial expenditure.

Of the 700 species of *Piper,* only three are recognized to be indigenous to Cameroon (Hutchinson and Dalziel, 1963). The most widely distributed, *Piper guineense*, is a native to tropical Africa, ranging from Guinea to Kenya and South Zambia. It is a forest liana with branchlets spiralling up to shrubs to about 10 m. The leaves are elliptical in shape and have a pleasant aroma when crushed. The spherical fruits (berries) are yellow becoming orange, red and finally black (Iwu, 1993). The fruits are commonly called "West African Black pepper" or "Poivrie" in French. The fruits are sold in the market as edible medicinal plant or food additives due to their strong, pungent aroma and flavour. In the South West region of Cameroon, the leaves (fresh or dried) are eaten as vegetables in 'Soups', while the ground powder is used as additives in sauce. Powders obtained from ground seeds are used as stimulants (Sofowora, 1982).

P. guineense has demonstrated wide potentials against major noxious insect pests of arable crops (Ukeh et al., 2008; Ntonifor, 2011). A laboratory assessment of the repellent and anti feedant properties of both aqueous extracts and powders of *P. guineense* against *Callosobruchus maculatus* and *Putella xylostella* revealed potent repellent and anti feedant activities of this plant (Ajayi and Wintola, 2006; Ntonifor et al., 2010). Insecticidal activities of the essential oil have been tested (Asawalam et al., 2007). Several studies have been done on the essential oil composition of *P. guineense* seeds from different geographic origins (Amvam et al., 1998; Jirovtez et al., 2002). Many investigators have worked on whole fruit powder of *P. guineense* from Cameroon (Jirovtez et al*.,* 2002; Tchoumgougnang et al., 2009). Their essential oil composition depended on the geographic locations and climatic conditions where the seeds were taken. Essential oil composition of different fractions of this plant by GC-MS was not reported. Therefore, the purpose of the present study was: (1) to identify the aroma compounds of different fractions of this plant responsible for the characteristic odour and taste, (2) to evaluate the insecticidal activity of the different fractions against *Sitophilus oryzae*, a pest of stored grains found in Cameroon.

MATERIALS AND METHODS

Plant material and extract preparation

Dried fruits (berries) of *P. guineense* were purchased from a local market in Yaounde, Cameroon. The spices were authenticated by comparing with herbarium specimens at the Limbe Botanic Garden (Limbe, Cameroon). Samples were air-dried for 3 days at room temperature and crushed to a fine powder using a mechanical blender. The powder was then packed in air-tight bags and exported to Faculty of Agriculture and Environment (University of Sydney, Australia). Extracts of the spice were prepared in a sequential manner as follows: 100 g of seed powder was dissolved in 200 ml methanol, vortexed for 15 min and sonicated for 1 h under high frequency in an ultrasonic cell disruptor (Microson™). This process was to break and disrupt the plant cells for easy extraction. The process was repeated 3 times. The resulting solution was filtered with Whatman No. 1 filter paper using a vacuum pump. The filtered solution was evaporated in a BUCHI (R-114) rotavapor under reduced pressure at a temperature of 60°C to concentrate the samples. The sample was re-dissolved in 50 ml methanol and transferred into a separating funnel and eluted successively in hexane, chloroform, ethyl acetate and methanol. Each solvent was evaporated in a BUCHI rotavapor under reduced pressure at a temperature of 60°C. The resultant residues were re-dissolved in acetone, to give a stock volume of 10 ml for each fraction. The solutions were refrigerated prior to application and GC-MS analysis.

Gas chromatography- mass spectra analysis

The essential oil fractions of *P. guineense* were analyzed by GC-MS and identification of their constituents was achieved based on their retention indices determined with reference to standards and by comparing with those reported in the literature (Jirovtez et al., 2002; Adams, 2007; Tchoumbougnang et al., 2009). Analytes were tentatively identified by reference to the NIST 2008 mass spectral library. An Agilent 7890A series GC with 5975C inert MSD triple axis detector (Agilent Technologies, USA) was used for GC-MS analysis. A Markes Series 2 ultra-unity system (Markes International Ltd., UK) was used for automated thermal desorption. Extract (3 µL) was injected onto sampling tubes containing Tenax TA (Supelco Inc., Bellefonte, PA, USA) adsorbent resin via a stream

of zero air (100 mL/min). The samples were desorbed by heating the sample tubes for 6 min at 300°C and focused onto a Tenax TA cold trap at - 30°C for 6 min. The cold trap was then flash-heated to 300°C for 5 min and the sample injected onto a HB5 column (30 m × 250 µm × 0.25 µm ID) via a heated transfer line held at 260°C. The GC oven was initially held at 40°C for 2 min, heated to 160°C at 5°C min^{-1}, 320°C at 10°C min^{-1}, then held for 2 min.

Insecticidal activity of the oil fractions against *S. oryzae*

Insect culture

S. oryzae adults were obtained from a stock maintained at the Faculty of Agriculture and Environment (University of Sydney) and reared on whole wheat grains in a constant temperature and humidity chamber (26°C, 65% RH) in darkness.

Bioassays

Bioassays were conducted using one month old adults of *S. oryzae*. Doses of 0, 0.5, 1 and 2 ml of the stock solution of each oil fraction were serially diluted in acetone to make up 10 ml, giving a series of dilutions 0, 0.05, 0.1 and 0.2 of each solution.

Grain contact toxicity

One millimeter from the new preparation was mixed with 40 g of wheat grains and stirred thoroughly for 5 min to allow even distribution over the grains. The control grains were treated with acetone only. Treated grains were kept for 20 min to allow the solvent evaporation. Twenty (20) adults of one month-old from the laboratory culture were introduced to the grains in a 250 ml glass vial with perforated lids. Each treatment and control was replicated four times. Mortality was evaluated after 24 h for up to five days.

Filter paper contact toxicity

Contact effect of extract fractions was evaluated on filter paper, 7 cm Whatman no.1. The filter paper was placed in Petri dishes and 0.5 ml of the serially diluted extract fractions was applied on the filter paper disc. The control filter papers were sprayed with acetone only. The acetone was allowed to evaporate for 20 min, after which, 20 unsexed one month-old adult insects were introduced at the center of each disc. This was kept under laboratory conditions (25±1°C, 60% RH). The treatments and control were replicated 4 times. Insect mortality was recorded after 24 h for up to five days. Percent mortality was calculated using Abbott correction formula for natural mortality in untreated controls (Abbott, 1925) (PT), as follows:

$$PT = (PO-PC)/(100-PC) \times 100$$

Where, PO = observed mortality of treated adults (%), PC = control mortality.

Repellency bioassay

A repellence effect was evaluated using the modified area preference method of McDonald et al. (1970) (Tapondjou et al., 2005). Test areas consisted of 7 cm Whatman no. 1 filter paper cut in halves. Test solutions were prepared by series of dilutions of the extract fractions in acetone as above. Each solution (0.5 ml) was uniformly applied to a half-filter paper disc using a micropipette. The other half disc was treated with acetone alone and served as control. Treated and untreated discs were air-dried for 10 min to evaporate the solvent completely. Full discs were remade by attaching treated halves to untreated halves with clear adhesive tape. Each remade disc was placed in a 7 cm Petri dish and 20 unsexed adult insects of one month-old were released at the center of the filter paper disc and the Petri dishes were covered. Each treatment and control was replicated 4 times. The number of insects present on the control (Nc) and treated (Nt) areas of the discs was recorded after 3 h. Percent repellency (PR) was calculated as follows:

$$PR= [(Nc-Nt)/ (Nc+ Nt)] \times 100$$

And assigned to repellency classes (0 - V) according to Talukder and Howse (1993) as follows: Class 0 (PR < 0.1%), class I (PR = 0.1 - 20%), class II (20.1 - 40%), class III (PR = 40.1 - 60%), class IV (PR = 60.1 - 80%), class V (PR = 80.1 - 100%).

Statistical analysis

The results of the experiments were analysed by one-way analysis of variance (ANOVA) using the General Linear Model Procedure (GLM) of GenStat 13th edition. Data were log-transformed before analysis. Duncan multiple range tests were used to compare the means. Percentage mortalities were calculated from the overall number of dead insects. Significant levels were set at 0.05.

RESULTS

Chemical composition of dried fruit of *P. guineense*

The screening results of the plant revealed the presence of various chemical groups such as terpenes and flavonoid (Table 1). The fruit essential oil of the different fractions had colours ranging from pale yellow (chloroform) to deep yellow (n-hexane) with the characteristic pungent and aromatic odor of *Piper* plants. These essential oils were composed (90%) mainly of terpenes (mono- and sesquiterpenoids) with the most important ones being identified as copaene (99%), caryophyllene (99%), eugenol (98%), α-cubebene (98%), γ-elemene (94%). In the n-hexane fraction the most abundant constituents were Longifolene (91%), copaene (97%), caryophyllene (99%), α -caryophyllene (97%) and α -cubebene (96%). In addition to these, the methanol fraction also had piperanol (97%), camphor (94%), and eugenol (98%). The chloroform fraction contained oleic acid (78%), while all the fractions contained β-myrcene instead of myrcene as seen in literature.

Insecticidal activities of *P. guineense* essential oil fractions

Contact toxicity by grain treatment

Insect mortality by grain treatment of the different oil fractions differed significantly (P < 0.05) (Table 2). The n-hexane fraction produced 100% mortality of *S. oryzae*

Table 1. Comparative percentage composition of essential oil major fractions from dried seeds of *Piper guineense* bought from Yaounde, Cameroon.

n-Hexane fraction			
Compound	**Retention time (min)**	**Quality**	**Molecular weight**
α-Phellandrene	10.06	83	136
4-Carene	10.06	50	136
(+)-4-Carene	20.21	83	136
β-Phellandrene	10.06	38	136
3-Carene	13.12	58	136
β-Pinene	13.12	49	136
α-Pinene	13.12	43	136
β-Myrcene	13.12	38	136
Copaene	21.36	97	204
α-Cubebene	21.36	96	204
Ylangene	21.36	18	204
Caryophyllene	21.75	99	204
α-Caryophyllene	23.584	97	204
(Z, Z) α-Farnesene	22.58	46	204
Ï-Neoclovene	22.58	42	204
γ-Elemene	22.83	58	204
Longifolene	24.44	91	204
Thujopsene	24.61	53	204
Seychellene	24.60	41	204
Aromadendrene	26.24	47	204
3-phenyl-Piperidine	27.03	25	161
Camphene	28.88	42	136
α-Santalol	28.88	42	220
α-Bisalolol	28.88	41	222
Cis- α –Bisabolene	28.88	42	204
Piperidine	31.6	33	161
2-mehtyl-Piperazine	33.18	36	100
Methanol fraction			
3-Carene	10.12	78	136
1R α-Pinene	10.12	42	136
1S α-Pinene	12.20	70	136
β-Pinene	13.44	43	136
4-Carene	10.45	95	136
β-Phellandrene	10.89	90	136
α-Phellandrene	10.89	72	136
β-Myrcene	13.44	46	136
Camphor	14.66	94	152
Isoborneol	15.17	58	154
endo-Borneol	15.17	47	154
Borneol	15.45	53	154
Ocimene	20.29	89	136
Piperanol	20.39	97	150
α-Cubebene	20.62	98	204
Copaene	21.47	99	204
Eugenol	20.85	98	164
Ylanglene	21.78	80	204
α-Muurolene	21.79	62	204
Seychellene	22.31	87	204

Table 1. Contd.

Caryophyllene	22.74	99	204
α-Caryophellene	23.66	96	204
α-Farnesene	22.74	52	204
Camphene	22.91	38	136
β-Humulene	23.99	49	204
β-Guaiene	24.79	64	204
(E, Z) α-Farnesene	25.06	66	204
cis- α-Bisabolene	25.06	38	204
α-Calacorene	25.91	91	200
Caryophyllene oxide	27.69	50	220
Spathulenol	28.19	90	220
β-Panasinsene	28.55	93	204
γ-Elemene	33.53	15	205
Ethyl acetate fraction			
3-Carene	13.18	70	136
(+) 4-Carene	20.25	91	136
β-Pinene	13.18	64	136
β-Myrcene	13.18	58	136
Ocimene	20.25	93	136
α-Cubebene	20.58	98	204
Copaene	20.58	98	204
Ylanglene	20.58	68	204
α-Muurolene	21.44	45	204
α-Farnesene	22.46	58	204
Caryophyllene	22.68	99	204
α-Caryophyllene	23.61	97	204
Caryophyllene oxide	31.51	64	220
γ-Elemene	22.88	96	204
Cubenol	23.02	38	222
Thujopsene	23.02	30	204
Camphene	23.61	42	136
α-Muurolene	23.95	43	204
β-Humulene	23.95	43	204
Longifolene	24.54	95	204
γ-Neoclovene	24.82	72	204
α-Calacorene	25.82	94	200
Guaiene	28.34	83	204
Spathulenol	30.38	90	220
Limonene oxide cis	31.90	25	152
Chloroform fraction			
β-Myrcene	13.19	49	136
β-Pinene	13.19	48	136
α-Pinene	13.19	43	136
1S-α-Pinene	13.19	43	136
3-Carene	13.19	30	136
4-Carene (1S,3S,6R)	13.19	30	136
(+)-4-Carene	20.24	94	136
Ocimene	20.24	93	136
α-Cubebene	20.58	98	204
Ylanglene	21.78	83	204

Table 1. Contd.

Copaene	21.44	99	204
Neoisolongifolene	22.26	93	204
Seychellene	22.26	89	204
Patchoulene	22.26	86	204
β-Guaiene	22.26	86	204
β-Panasinsene	22.26	64	204
(Z,Z) α-Farnesene	22.47	89	204
Trans-α-Bergamotene	22.47	87	204
Caryophyllene	22.68	99	204
(-)-Aristolene	23.02	89	204
(+)-Epi-bicyclosesqui-phallendrene	23.42	30	204
α-Caryophyllene	23.61	96	204
α-Phallendrene	23.61	43	136
Camphene	23.61	38	136
α-Muurolene	23.98	52	204
Longifolene -(V)	24.54	94	204
Thujepsene-(12)	24.54	64	204
β-Humulene	24.54	46	204
γ-Elemene	26.32	55	204
Aromadendrene	26.32	35	204
γ-Himachelene	26.32	70	204
Caryophyllene oxide	26.84	87	220
Bergamotol-(Z)-α-trans	26.84	25	220
Epizonarene	27.13	80	204
δ-Selinene	28.32	90	204
cis-α-Bisabolene	28.89	90	204
Cis-α-Santalol	28.89	38	220
α-Bisabolol	28.89	38	222
Spathulenol	31.00	50	220
Trans-Longipinocarveol	31.63	60	220
Oleic acid	35.12	78	282

Table 2. Summary of analysis of contact toxicity by grain treatment.

Source	d.f.	deviance	Mean deviance	Deviance ratio	Approx. chi pr.
Regression	3	387.74	129.25	129.25	<0.001
Residual	256	693.81	2.71		
Total	259	1081.55	4.18		

after five days of grain treatment, while the aqueous methanol fraction produced less than 50% mortality after five days of treatment. The chloroform and ethyl acetate fractions showed very little mortality and were not significantly ($P < 0.05$) different from the control (Figure 1). All the different doses of n-Hexane fraction of the essential oil induced 100% mortality after five days of exposure. This mortality could have been due to the high percentages of sesquiterpenes hydrocarbons present in this fraction. Methanol fraction, on the other hand, had percentages of eugenol and piperanol which induced high mortality in the essential oil. The 0.2 ml/cm² of the methanol fraction induced more than 30% mortality different from the control.

Filter paper contact toxicity

The percentages of insect mortality recorded after five days of exposure to increasing concentrations of volatile

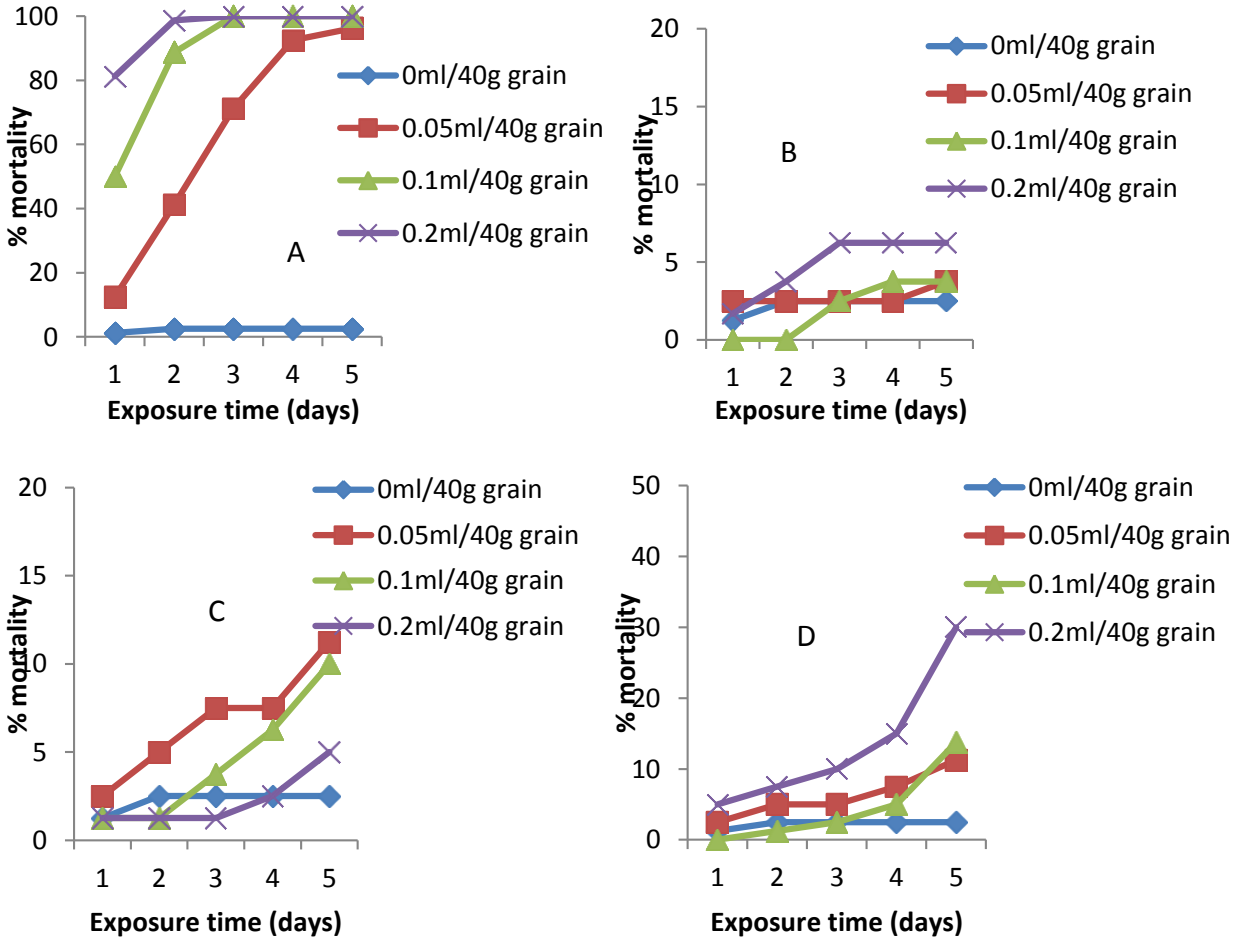

Figure 1. Percentage mortality of *Sitophilus oryzae* exposed to different fractions of *Piper guineense* essential oil coated on wheat grains (A- Hexane fraction; B- chloroform fraction; C- ethyl acetate fraction and D- methanol fraction).

oil on filter paper discs showed similar results. The n-Hexane fraction had a significantly ($P < 0.05$) higher mortality on *S. oryzae* and doses of 0.1 and 0.2 ml/cm² were able to induce 85 and 100% mortality, respectively. The Chloroform, Methanol and Ethyl acetate fractions were almost nil within the first three days, and were not significantly ($P < 0.05$) different from the control, causing less than 50% mortality after five days of exposure on impregnated filter paper disc (Figure 2). There was a significant correlation between treatments, duration and concentrations as seen below (Table 3).

Repellency testing

Repellency bioassay using the area preference test showed that *P. guineense* extract fractions significantly repelled *S. oryzae* with an overall repellency of 87.5% (Table 4). The effect of the volatile oil on repellency of *S. oryzae* after 3 h of exposure was not dose-dependent. The n-Hexane fraction produced the highest repellency at different concentrations, while chloroform, methanol and ethyl acetate had 50% repellency at the highest concentrations.

DISCUSSION

The composition of different fractions of *P. guineense* essential fruit oil in this study was remarkably different from those reported earlier in Nigeria (Oyedeji et al., 2005; Owolabi et al., 2013) and Cameroon (Jirovetz et al., 2002; Tchoumgougnang et al., 2009 In all the different fractions, α-caryophyllene was present in high amounts instead of β-caryophyllene reported in literature. Such high content has not been found in *P. guineense* essential oil from Cameroon until now. Eugenol was abundant only in the Methanol fraction. These and others represented a number of new chemotypes revealed from the GC-MS analysis. The presence of constituents such as aromadendrene, piperazine and piperidine, though in low amount in the n-Hexane may have accounted for its

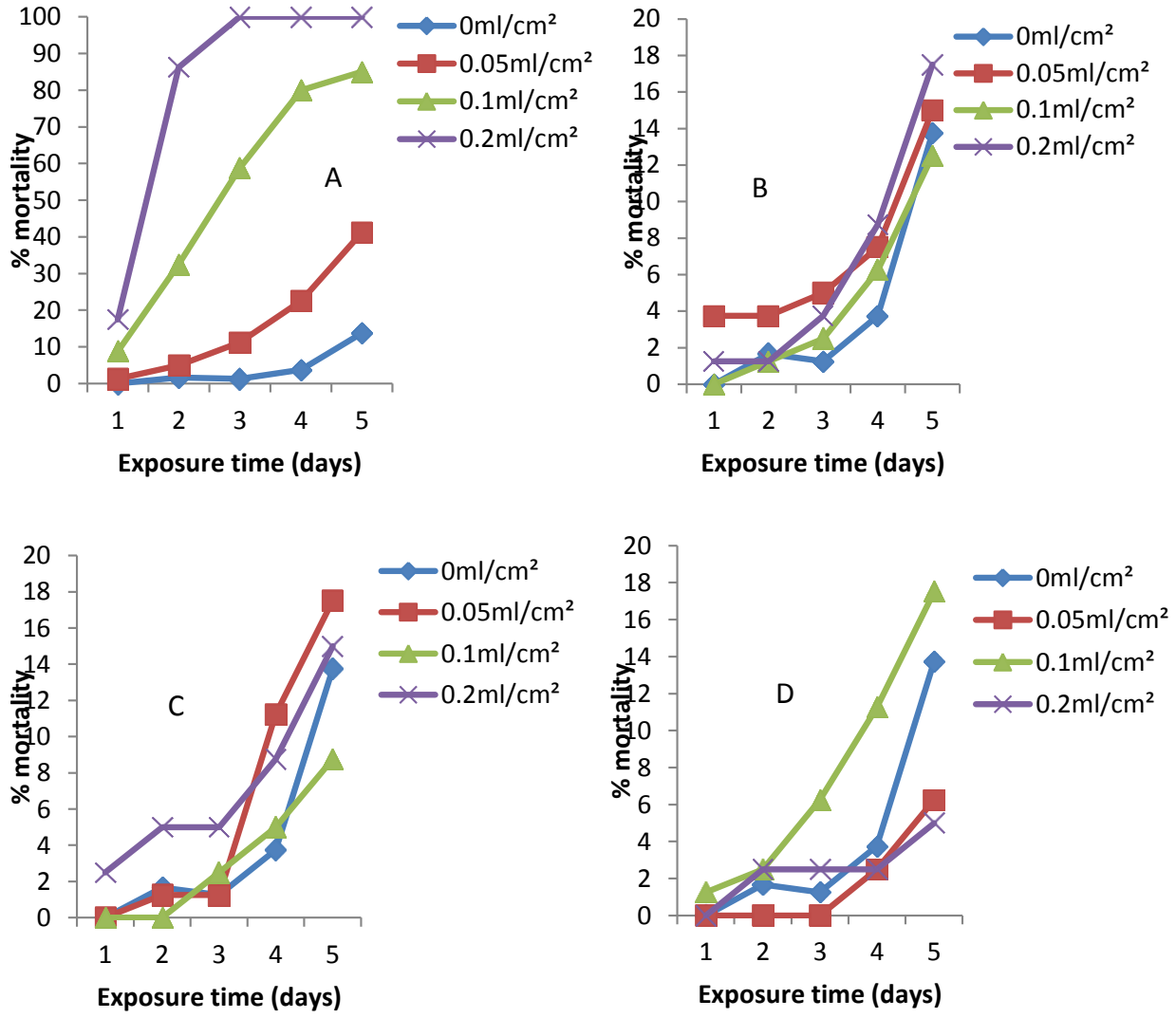

Figure 2. Percentage mortality of *Sitophilus oryzae* exposed to different fractions of *Piper guineense* essential oil impregnated on filter paper discs (A- Hexane fraction; B- Chloroform fraction; C- Ethyl acetate fraction and D- Methanol fraction).

Table 3. Correlation parameters for contact toxicity by grain treatment and impregnated filter paper assay.

	Parameter	1	2	3	4
Contact toxicity by grain treatment					
1	Constant	1.000	-		
2	Treatment	-0.592	1.000	-	
3	Concentrations	-0.558	0.030	1.000	-
4	Durations	-0.554	0.064	-0.006	1.000
Impregnated filter paper assay					
1	Constant	1.000	-		
2	Treatment	-0.518	1.000	-	
3	Concentrations	-0.547	0.043	1.000	-
4	Durations	-0.612	-0.012	0.003	1.000

Table 4. Repellent effects of different fractions of essential oil from *Piper guineense* on *Sitophylus oryzae*.

Extract fraction	Concentration (ml/cm²)	% Mean Repellency	Repellency class
n-Hexane	0.05	80±7.1	IV
	0.1	87.5±2.5	V
	0.2	72.5±13.8	IV
Chloroform	0.05	20±14.7	I
	0.1	10±10.8	I
	0.2	50±16.8	III
Ethyl acetate	0.05	45±15.6	III
	0.1	28.7±15.6	II
	0.2	50±23.8	III
Methanol	0.05	22.5±24.3	II
	0.1	42.5±9.3	III
	0.2	50±18.7	III

'heat' and aroma. *P. guineense* have been demonstrated to have 5 to 8% Piperine which gives them their 'heat' (Oparaeke, 2006). It also contains about 31-mono-sesquiterpenoids that have high insecticidal properties due to their pungent nature (Taponjou et al., 2005). The variability of the different constituents of *P. guineense* is important in light of its use as flavouring and medicinal agent. Therefore the efficacy varies depending on the chemotypes used. In the present study, the essential oil fractions of *P. guineense* exhibited different levels of toxic and repellent effects on *S. oryzae* at various doses. These effects may be due to factors such as the chemical composition of the plant as well as the insect susceptibility. The different doses of the n-Hexane fraction caused 100% mortality of *S. oryzae* on filter paper fumigation toxicity and grain contact after five days of exposure. The insects appeared to avoid the treated areas. The mortality of weevils on treated grains and filter paper varied with dosage of essential oil fractions. High mortality rates were recorded with the n-Hexane fraction at all doses compared to the other fractions. The toxicity of volatile oil from *P. guineense* is generally attributed to the presence of the alkaloids, piperine, chavicine and piperidine which are reported to be the major active components in *P. guineense* seeds (Lale and Alaga, 2000). This suggests that the toxicity of *P. guineense* was not due to ingestion of treated grains, but due to suffocation. The oil fractions evoked a very high repellency against the insect, suggesting that the oils could have contained a very pungent substance that caused high repellency of the insects. n-Hexane again demonstrated the highest level of repellency probably due to presence of constituents like Piperazine and Piperidine, though in very low quantity. Many other researchers have demonstrated the toxic and repellent effects of constituents of this oil such as 1S-α-Pinene, Copaene, β-caryophyllene, Eugenol, Piperanol and many others (Amvam Zollo et al., 1998; Owolabi et al., 2013). They are similar to those reported by Jirovetz et al. (2002) and Tchoumbougnang et al. (2009), in *P. guineense* obtained from Cameroon and Nigeria.

The results demonstrated a scientific rationale for the traditional incorporation of the dried seeds oils of *P. guineense* into grain protection practices in rural communities of Cameroon. However, there is need for further investigations into such practices to improve their efficacy and reliability in rural communities. With the right dosage and proper formulations this essential oil could be exploited at the small scale farmers' level against insect infestations in the fields and in storage. Most of the oils are more effective, less cumbersome and are not particularly dangerous to consumers because they are used in pharmaceutical preparations (Bauer et al., 1990). They are also less expensive, safe to the environment and harmless to humans and other mammals. This study concludes that all of the fractions from the seeds of *P. guineense* demonstrated toxic and repellent effect on *S. oryzae*, a storage pest in Cameroon. The study also showed that the n-Hexane fraction of the essential oil was more toxic to *S. oryzae.*

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENTS

This research was funded by the Australian Leadership

Awards Fellowship- round 13 and the Australian Government. The authors are grateful to the Faculty of Agriculture and Environment of the University of Sydney and its entire staff for providing Laboratory space and equipment used. We also acknowledge Dr. Sevilor Kekeunou for proof reading several draft of the manuscript.

REFERENCES

Ajayi FA, Wintola HU (2006). Suppression of the Cowpea Bruchid *Callosobruhus maculatus* (F.) infesting stored Cowpea *Vigna unguiculata* (L.) Walp. seeds with some edible plant product powders. Pak. J. Biol. Sci. 9(8):1454-1459.

Amvam Zollo PH, Biyiti L, Tchoumbougnang F, Menut C, Lamaty G, Bouchet PH (1998). Aromatic Plants of Tropical Central Africa XXXII, Chemical composition and antifungal activity of thirteen Essential oils from Aromatic Plant of Cameroon. Flavour Frag. J.13:107-114.

Asawalam EF, Emosairue SO, Ekeleme F, Wokocha R (2007). Efficacy of *Piper guineense* (Schum&Thonn) seed extract against maize weevil, *Sitophilus zeamais* (Motschulsky) as influenced by different extraction solvents. Int. J. Pest Manage. 53(1):1-6.

Bauer K, Garbe D, Surburg H (1990). Common fragrance and flavour materials.2nd Edition. VCH Verlagsgesellschaft Ltd., Weinheim. pp. 153-155.

Bekele AJ, Obeng-Ofori D, Hassanali A (1996). Evaluation of *Ocimum suave* (Wild) as a source of repellents, toxicants and protectants in storage against three stored product insect pests. Int. J. Pest Manage. 42:139-142.

Bekele AJ, Obeng-Ofori D, Hassanali A (1997). Evaluation of *Ocimum kenyense* (Ayobangira) as source of repellent, toxicants and protectants in storage against three major stored products insect pests. J. Appl. Entomol. 212:169-173.

Hutchinson J, Dalziel JM (1963). Flora of West Tropical Africa. Vol 2, 2nd ed., Crown Agents, London.

Iwu M (1993). Hand book of African Medicinal Plants of West Tropical Africa. CRC Press, Boca raton.

Jirovetz L, Buchbauer G, Ngassoum MB, Geissler M (2002). Aroma compound analysis of *Piper nigrum* and *Piper guineense* essential oils from Cameroon using solid-phase microextraction-gas chromatography, solid-phase microextraction-gas chromatography-mass spectrometry and olfactometry. J. Chromatogr. A. 976:265-275.

Lale NES, Alaga KA (2000). Exploring the insecticidal, larvicidal and repellent properties of *Piper guineense* (Schum and Thonn) seed oil for the control of rust-red flour beetle *Tribolium castaneum* (Herbst) in stored pearl millet *Pennisetum glaucum* (L.) R. Br. J. Plant Dis. Prot. 108(3):305-313.

McDonald LL, Guy RH, Speirs RD (1970). Preliminary evaluation of new candidate materials as toxicants, repellents and attractants against stored product insects. Marketing Research Report. No 882. Agricultural Research Service United State Department of Agriculture, Washington.

Ndemah R (1999). Towards an integrated crop management strategy for the African stalk borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in maize systems in Cameroon. Ph.D. thesis, University of Hannover, Germany, 136 pp.

Ngamo LST, Ngassoum MB, Mapongmetsem PM, Malaisse F, Haubruge E, Lognay G, Hance T (2007). Current post harvest practices to avoid insect attacks on stored grains in northern Cameroon. Agric. J. 2:242-247.

Ntonifor NN (2011). Potentials of Tropical African Spices as sources of reduced-risk pesticides. J. Entomol. 8(1):16-16.

Ntonifor NN, Mueller-Harvey I, Brown RH (2010). Extracts of tropical African spices are active against *Plutella xylostella*. J. Food Agric. Environ. 8(2):498-502.

Nukenine EN, Adler C, Reichmuth C (2010). Efficacy of *Clausena anisata* and *Plectranthus glandulosus* leaf powder against *Prostephanus truncatus* (Coleoptera: Bostrichidae) and two strains of *Sitophilus zeamais* (Coleoptera: Curculionidae) on maize. J. Pest Sci. 83:181-190.

Ogendo JO, Kostyukovsky M, Ravid U, Matasyoh JC, Deng AL, Omolo EO, Kariki ST, Shaaya E (2008). Bioactivity of *Ocimum gratissmum* L. oil and two of its constituents against five insect pests attacking stored food products. J. Stored Prod. Res. 44:328-334.

Oparaeke AM (2006). The sensitivity of Flower Bud Thrips, *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae), on Cowpea to three Concentrations and Spraying Schedules of *Piper guineense* Schum. & Thonn. extracts. Plant Prot. Sci. 42(3):106-111.

Owolabi MS, Lawal OA, Ogunwande AI, Hauser RM, Setzer WN (2013). Aroma Chemical Composition of *Piper guineense* Schumach.andThonn. From Lagos, Nigeria: A new chemotype. Am. J. Essent. Oils Nat. Prod. 1(1):37-40.

Oyedeji OA, Adeniyi BA, Ajayi O, König WA (2005). Essential oil composition of *Piper guineense* and its antimicrobial activity; another chemotype from Nigeria. Phytother. Res. 19:362-364.

Park IK, Lee SG, Choi DH, Park JK, Ahn YJ (2003). Insecticidal activities of constituents identified in the essential oil from leaves of *Chamaecyparis obtusa* against *Callosobruchus chinensis* (L.) and *Sitophilus oryae* (L.). J. Stored Prod. Res. 39: 375-384.

Sofowora A (1982). Medicinal plants and traditional medicine in Africa. John Wiley, Chichester.

Tapandjou AL, Bouda H, Fontem DA, Zapfack L, Lontsi D, Sondengam BL (2000). Local plants used for traditional stored product protection in the Menoua division of the Western highlands of Cameroun. IOBC Bull. 23:73-77.

Tapondjou LA, Adler C, Fontem DA, Bouda H, Reichmuth Ch (2005). Bioactivities of cymol and essential oils of *Cupressuss empervirens* and *Eucalyptus saligna* against *Sitophilus zeamais* Motschulsky and *Tribolium confusum* du Val. J. Stored Prod. Res. 41:91-102.

Tchoumgougnang F, Jazet Dongmo PM, Sameza ML, Fombotioh N, Wouatsa Nangue AV, Amvam Zollo PH, Menut C (2009). Comparative essential oils composition and insecticidal effect of different tissues of *Piper capense* L., *Piper guineense* Schum.etThonn., *Piper nigrum* L. and *Piper umbellatum* L. grown in Cameroon. Afr. J. Biotechnol. 8(3):424-431.

Ukeh DA, Arong GA, Ogban EI (2008). Toxicity and oviposition deterrence of *Piper guineense* (Piperaceae) and *Monodora myristica* (Annonaceae) against *Sitophilus zeamais* (Motsch) on stored maize. J. Entomol. 5 (4):295-299.